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# AN ASSESSMENT OF THE STATUS OF CAPTIVE BROODSTOCK TECHNOLOGY FOR PACIFIC SALMON

FINAL REPORT

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# EXECUTIVE SUMMARY

This report provides guidance for the refinement and use of captive broodstock technology for Pacific salmon (*Oncorhynchus* spp.) by bringing together information on the husbandry techniques, genetic risks, physiology, nutrition, and pathology affecting captive broodstocks.

Captive broodstock rearing of Pacific salmon is an evolving technology, as yet without well defined standards. At present, we regard captive rearing of Pacific salmon as problematic: high mortality rates and low egg viability were common in the programs we reviewed for this report.

Egg-to-adult survival rates were also generally lower than expected, usually not ranging above 30-40%. In addition, viability of eggs from captive-reared spawners was commonly only 30-60% compared to viabilities of over 80% for the eggs from wild cohorts. The size of captive-reared adults was also generally smaller than that of wild fish.

The reasons for this generally poorer performance of captive-reared fish are not well understood. Most captive broodstock programs we reviewed used spawners collected from the wild population. Therefore, it seems intuitive that much of the poor performance, at least in first-generation offspring, would be related to interactions in the culture environment.

However, the potential for genetic change in the captive-reared population could not be ruled out. Overall, our review demonstrated that each of the areas of husbandry, genetics, physiology, nutrition, and disease represents a cornerstone for building the foundation of captive broodstock technology: each should be afforded high priority and all should be pursued concurrently if we are to produce comprehensive protocols in captive broodstock technology for Pacific salmon.

One of the most important elements in fish husbandry is the culture environment itself. Many captive broodstock programs for Pacific salmon have reared fish from smolt-to-adult in seawater net-pens, and most have shown success in providing gametes for recovery efforts. However, some programs have lost entire brood years to diseases that transmitted rapidly in this medium.

Current programs for endangered species of Pacific salmon rear most fish full-term to maturity in fresh well-water, since ground water is low in pathogens and thus helps ensure survival to adulthood. Our review suggested that captive rearing of fish in either freshwater, well-water, or filtered and sterilized seawater supplied to land-based tanks should produce higher survival than culture in seawater net-pens.

Anecdotal information suggests that full-term culture to maturity in freshwater will not compromise seawater adaptability of offspring; however, comprehensive life-history studies should be undertaken to assure this.

A number of studies have examined the quantitative genetic basis of life-history characters and their covariation with morphological and other characters. Increasingly, these studies have warned of the potential artifacts that artificial propagation may produce in a captive broodstock program.

The majority of research in quantitative fish genetics has emphasized the genetic and environmental components of variation only in traits important to aquacultural production, such as growth, maturation, and egg production. In general, the genetic basis for these characters is of a magnitude such that a reasonable response to selection can be realized, but individual responses depend strongly on the stock and environment involved.

It is apparent that the immediate genetic concerns of artificial propagation for conservation may differ substantively from those for enhancement and mitigation. For example, minimizing the genetic differentiation between hatchery fish and the natural fish they are intended to supplement may be as important as maximizing genetic variability in the hatchery population.

The absence of guidance on how to detect, monitor, and respond to domestication and other effects of selection undoubtedly result from the paucity of information about how adaptation operates on Pacific salmon in captive environments.

In general, however, quantitative genetic risks associated with captive broodstock programs have been grouped into three main categories:

1) loss of within-population variability by inbreeding depression produced by the mating patterns of a small, captive population.

2) domestication through rearing in protective culture.

3) genetic divergence of the captive fish from their natural source population, and the subsequent genetic consequences of genetic interactions between these groups.

To avoid these potential genetic losses, sound mating and rearing protocols are essential.

The major problems with reproduction of Pacific salmon species in captivity are inappropriate spawn timing, loss of gametes due to prespawning mortality, unreliable production of high quality gametes, and precocious maruration of male fish.

Most studies on factors affecting gamete quality and the age and seasonal timing of sexual maturation have been conducted on domesticated stocks of rainbow trout (0. *mykiss*) and Atlantic salmon (*Salmo salar*). Although some information generated from study of these species is applicable to Pacific salmon, further research is necessary to solve problems with poor reproductive performance of wild Pacific salmon stocks reared in captivity.

The need to adapt methods to monitor and control sexual maturation in Pacific salmon broodstocks has become obvious, both to ensure production of high-quality gametes and high survival of offspring, and to minimize asynchronous maturation of male and female fish.

Photoperiod and temperature are the most important enviromnental cues that affect seasonal spawn timing in salmonids. Alterations in photoperiod have been used extensively to manipulate both sexual maturation and spawn timing. In captive broodstock programs for depleted fish stocks, abnormal maturation and reproduction should be avoided: production should be timed in accordance with conditions in the natural habitat of the stock.

Therefore, photoperiods in captive-broodstock rearing facilities must be controlled, with inadvertent exposure of light during nighttime periods carefully avoided. Uncontrolled lighting could have catastrophic consequences for the stock in its natural environment: for example, it could artificially extend spawn timing into a period when ambient water temperatures are sufficiently high to impair gamete quality and offspring survival. Furthermore, exposure to continuous light can induce asynchronous maturation and atresia of oocytes.

The effects of temperature on reproductive performance in salmonids are not as well established as those of photoperiod. However, research on Atlantic salmon and rainbow trout has indicated that rearing temperatures can affect the timing of ovulation and gamete quality.

Further studies are necessary to develop better guidelines for rearing temperatures for Pacific salmon broodstock and to determine whether constant or seasonally fluctuating water temperatures affect reproductive performance. This research is particularly important because facilities for captive rearing of broodstock may lack the ability to control water temperature or to provide water of fluctuating versus constant temperatures.

While photoperiod and temperature influence spawn timing, the age of sexual maturity in salmonids is regulated primarily by genetic, environmental, and dietary factors affecting growth.

Reducing the age of maturity in both male and female fish in a captive broodstock program may seem advantageous, since generation times could be decreased and greater production achieved. However, in any manipulation of sexual maturation timing, there is substantial risk of producing mature males in the absence of mature females.

ln cultured chinook salmon (0. *tshawytscha*), the incidence of precocious male maturation are higher than in their wild cohorts and can be as high as 80%. In addition, selective mortality of precocious males could reduce the effective breeding population size of the captive broodstock. Thus, there is a critical need to develop methods to minimize precocious male maturation in captive broodstock programs for endangered fish species,

Evidence from studies of a variety of salmonid species indicates that the incidence of precocious male maturity is controlled by genetic factors influencing growth rate and stored energy (fat) levels, However, the relative importance of these factors and how they interact are

poorly understood. Research is necessary to determine how stored energy levels, growth rates, or rates of energy deposition at critical developmental stages either permit or prevent the onset of maturation in Pacific salmon species. In addition, diets and growth regimes that sustain somatic growth and provide sufficient stored energy for appropriate life-cycle transitions are needed.

In addition to the environmental cues mentioned above, maturation and the growth and development of gametes are controlled internally by the endocrine system. Current understanding of endocrine control of reproduction in salmonids is better than in most other fish species. Thus, it is now possible to use this information to develop technology for monitoring and controlling maturation in captive broodstock.

One of the most valuable tools that is applicable to captive broodstock programs is artificial induction of spawning. Technology for hormonal induction of ovulation and spermiation has several important applications to captive broodstock programs for endangered or threatened salmonid populations. This technology can be used to 1) prevent loss of gametes due to prespawning mortality, 2) synchronize spawning in wild and captive fish, 3) extend the period of spermiation and yield of sperm, and 4) synchronize and/or advance spawning in male and female broodfish.

Hatchery managers may not need to routinely induce spawning in broodstock, but the technology should be available: managers may need to adjust the spawning period of captive fish to align with that of their wild cohorts, or may need to advance spawn timing if there is a risk of prespawning mortality.

Work is needed to determine appropriate timing of hormone administration and to ensure that this technology does not impair the quality of gametes produced by broodstock. Artificial induction of spawning using hormones, such as gonadotropiu-releasing hormone analogues, should be further refined for general use in control of reproduction in Pacific salmon broodstock.

Successful fertilization of eggs and subsequent development of offspring depend greatly on the quality of gametes produced by the parent fish. Various biological and nonbiological factors have been implicated as determinants of gamete quality and subsequent survival of progeny. These include: composition and size of the egg, quality of the spenn, genetic makeup and nutritional status of the parents, husbandry procedures, and quality of the water supply. In many instances, it is difficult to separate the relative effects of these parameters because they are often interrelated.

Nevertheless, fish husbandry practices can have dramatic effects on egg quality. Probably the most profound effects are those that result from the timing of egg collection (stripping) and handling of the gametes, The timing of gamete collection is well characterized in domesticated stocks of rainbow trout. However, there are a number of problems with appropriate timing of egg collection from wild stocks of Pacific sahnon reared in captivity.

Pacific salmon spawning periods may be protracted, either due to the nature of the stock or to unintended effects of the captive rearing environment. Egg collection under these circumstances necessitates frequent handling of the broodstock to check for maturity status, which in turn may stress the fish. Both acute and chronic stress can have significant effects on the quality of eggs and sperm.

Thus, research is needed on the effects of rearing environment (water quality and rearing density) and on developing handling procedures that minimize stress in wild stocks of Pacific salmon. In addition, the effects of rearing temperature on maturation timing and gamete quality in captive broodstocks need to be evaluated in a variety of Pacific salmons species, as does the impact of diet on the quality of gametes.

The chemical composition of an egg is affected not only by the genetics of the male and female, but nutrition of the female and water quality upon spawning. Even if the genetic influences are isolated, environmental factors and husbandry practices that affect egg size, fecundity, and egg composition frequently also affect intake of food; therefore is it difficult to determine cause-effect relationships among these variables.

Little is known about the specific dietary requirements of Pacific salmon species designated for captive broodstock programs (i.e., spring chinook and sockeye salmon (0. *nerka*)). Commercial feeds intended for grow-out and broodstock fish are much higher in fat than feeds of 5-10 years ago, and are generally formulated for Atlantic salmon: their effects on Pacific salmon are unknown. However, it is known that diet composition and feeding level can influence precocious maturation and timing of adult maturation.

Optimum diet requirements for captive salmon broodstocks may be considerably different than those for commercial grow-out programs. For instance, the natural diet of sockeye salmon in the ocean suggests that supplementing their feeds with squid or krill may be beneficial.

Despite the lack of information needed to formulate feeds specifically for these species, enough information is available to develop feeds and feeding practices sufficient to rear them to maturation. Research is also needed to develop feeds and feeding practices for spring chinook and sockeye salmon that result in maximum fish health, survival to spawning, and reproductive performance.

Fish-rearing procedures which have been established for domesticated stocks of salmonids may not be applicable to wild stocks of fish. It is possible that wild stocks of fish may be more stressed by the rearing environment and handling, thus the impact of stress on the fish health and gamete quality may be more pronounced.

The major risk to captive broodstock health is from diseases transmitted vertically from parents to offspring. Ideally, the gametes of parent stocks should be free of vertically

transmissible diseases. However, with present techniques, it will be difficult to source disease-free broodstocks from relic populations.

Many viral and bacterial diseases and fungal, helminth, and protozoan parasites may affect captive broodstock culture in both freshwater and marine facilities. In our estimation, the most pernicious disease limiting culture of sockeye salmon and chinook salmon in the Pacific Northwest is bacterial kidney disease (BKD).

Some control of vertical transmission of ***Renibacterium salmoninarum*** (the causative agent of BKD) can be accomplished by injection of erythromyciu in maturing adults and gamete segregation after quantification of the disease in the spawning fish. At present, there is no known cure for BKD.

Development of an antibacterial drug that would eliminate ***R. salmoninarum*** from infected fish would be the preferred method to control horizontal transfer of BKD Other solutions to the problem of horizontal transmission may be realized with research on treatment of holding waters with chemicals, ozone, or other oxidizing agents (e.g., peroxides).

Continued research on BKD and other important Pacific salmon pathogens is required to develop a sound program of integrated fish health management for captive broodstock programs. Based on our analysis of survival and reproductive success of fish in past programs, we ranked Pacific salmon species by ease of captive culture as follows (starting with the least difficult): 1) coho salmon (0. *kisutch*) and steelhead ***(0. mykiss*), *2)*** chinook and sockeye salmon, 3) pink salmon ***(0. gorbuscha***) and chum salmon (0. *keta*). We conclude that captive broodstock technology for salmonids, although in its initial development stages, is sufficiently advanced to allow careful captive broodstock rearing to proceed.

However, husbandry methodologies for full-tenn culture of any Pacific salmon species remain poorly developed, and programs have not yet been thoroughly evaluated. Furthennore, supplementation with offspring from a captive broodstock has not yet been evaluated, and therefore its success is uncertain,

The authors of this report concur that captive broodstocks should be viewed as a short-term measure to aid in recovery; never as a substitute for returning naturally spawning fish to the ecosystem. Because the benefits and risks have not yet been sufficiently monitored and evaluated, captive broodstock programs should be considered experimental and used with caution.

Since a multitude of factors affect both the decline and potential recovery of a stock, exacting rules cannot be developed to generically determine the conditions that warrant implementation of a captive broodstock program. However, in general, these programs should be restricted to situations where the natural population is dangerously close to extinction.

In any case, proper precautions should be taken to minimize genetic change during collection, mating, and rearing: any alteration to the original genetic composition of the population may reduce the efficacy of supplementation stock in rebuilding the natural population. Furthermore, cultivation and release of fish from captive broodstocks should be based on the behavior of any remaining natural cohorts, or on the stocks known life-history characteristics if no natural fish remain.

Captive broodstocks can provide an egg-base to help "jump-start" a population, but these efforts must go hand-in-hand with habitat improvements to fully aid in recovery. Primary consideration should be to restore fish in the habitat. Nonetheless, in some cases, captive broodstocks may provide the only mechanism to prevent extinction of a stock and may be undertaken regardless of prospects for immediate habitat improvement.

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# PREFACE

Support for this research came from electrical rate payers of the Pacific Northwest through the Bonneville Power Administration.

ıidelines for source collection and breeding methods to reduce the
›tential for detrimental genetic consequences of captive broodstock
ograms and help ensure genetic stability and gamete quality.

'eport on current techniques in reproductive physiology which can be
ed to maximize gamete quantity and quality.

Ire-of-the-art nutrition and feeding strategies for Pacific salmon held to
ıturity.

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rrent practices for captive broodstock fish health management.

ıistory of the White River (Washington) spring chinook salmon captive
›odstock program.

ons of this report focus on chinook salmon (*Oncorhynchus tshawytscha*) and
3. *nerka*) because of their importance in ESA considerations in the Snake
ever, we believe that there are enough similarities in their culture requirements
›n in this report can be generally applied to all Pacific sahnon.

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## Introduction

Captive propagation is an internationally important component of species enhancement and the concept has won acceptance in restoration efforts for endangered species in the United States (Gipps 1991, Johnson and Jensen 1991, Olney et al. 1994). Currently, about 105 species of mammals, 40 species of birds, 12 species of reptiles, 29 species of fish, and 14 species of invertebrates worldwide are being maintained or enhanced through forms of captive breeding (CBSG 1991, Olney et al. 1994).

ln the United States, the U.S. Fish and Wildlife Service (USFWS) has used captive propagation to enhance depleted populations of a number of birds and mammals. These include the blackfooted ferret (*Mustela nigripes*), red wolf (*Canis rufus*), California condor (*Gymmogyps californianus),* and Peregrine falcon (*falco peregrinus*), listed as threatened or endangered under ESA (Gipps 1991, Johnson and Jensen 1991, Primack 1993, Olney et al. 1994). In addition, almost 30% of the nonanadromous North American fish species listed under U.S. Endangered Species Act (ESA) are being propagated by the USFWS using captive broodstock techniques (Johnson and Jensen 1991, Andrews and Kaufman 1994).

Many Pacific salmon stocks in the Pacific Northwest are currently in steep decline towards extinction. In 1991-1992, the National Marine Fisheries Service (NMFS) listed Snake River sockeye salmon (*Oncorhynchus nerka*) as endangered and Snake River spring, summer, and fall chinook salmon (*Oncorhynchus tshawytscha*) as threatened (now listed as endangered) under ESA (Waples et al. 1991a, Waples et al. 1991b, Matthews and Waples 1991). Another 200+ stocks of Pacific sahnon in Northwest States have recently been identified at risk of continued decline toward extinction (Nehlson et al. 1991). The NMFS is developing a recovery plan for Snake River sahnon that includes captive propagation to help rebuild listed Snake River salmon within their historic range.

The ESA recognizes that, in addition to other measures, conservation of listed species of anadromous Pacific salmon may be facilitated by artificial means (Hard et al. 1992). There are currently five formal captive broodstocks underway to aid recovery of anadromous Pacific salmon stocks:

1) Sacramento River (California) winter chinook salmon (0. *tshawytscha*), listed as threatened under ESA (B. Wyatt, Univ. Calif. Extension Office, 2604 Ventura Ave., Rm. 100, Santa Rosa, CA 95403-2894. Pers. commun., October 1992);

2) White River (Washington) spring chinook salmon. identified by the state of Washington as a stock of concern (Appleby and Keown 1995, this report);

3) Dungeness River (Washington) chinook salmon, identified by the state of Washington as a stock of concern (Shaklee et al. in press);

Pragmatically, captive broodstocks may offer the best chance for continued existence of endangered populations such as Snake River sockeye salmon. However, few attempts have been made to grow anadromous Pacific salmon to maturity in captivity, and little is known regarding techniques to maximize survival and reproduction.

Realistic levels of success of captive broodstocks are as yet undefined. In nature, egg-to-adult survival of Pacific salmon is often only a few tenths of one percent (Groot and Margolis 1991). Ideally, complete survival and total reproductive success in captivity may be required to "guard" against potential impacts of artificial propagation. While this is impractical, it is important to establish realistic target ranges for survival and reproductive performance to aid in determining levels of risk in implementing captive broodstocks.

In this study, we provide rationale and general husbandry guidelines for applying captive broodstock technology to anadromous Pacific sahnon. In addition, we review some current and past attempts at rearing fish to maturity to establish realistic expectations for future salmonid captive broodstock efforts. We also use this information to suggest areas of research that should be undertaken to improve captive broodstock performance.

## The Captive Broodstock Concept

Captive broodstock programs differ from conventional sahnon culture in that fish of wild origin are maintained in captivity throughout their life to produce offspring for the purpose of supplementing wild populations. Theoretically, the high fecundity of anadromous Pacific salmon and potentially high survival in protective culture should allow captive broodstocks to produce large numbers of juveniles for supplementation in a single generation. However, the complex life history of anadromous Pacific salmon provides survival challenges not faced with captive broodstocks for other species.

Many captive broodstock programs have established somewhat free-roaming breeding colonies on localized preserves (Gipps 1991, Johnson and Jensen 1991, Olney et al. 1994). Even in cases where adults from captive broodstocks of North American fish have been released to the wild, the species distribution normally occupies a geographically confined range, such as a discrete river basin or even a single tributary (Rinne et al. 1986, Johnson and Jensen 1991). These isolated breeding colony/preserve approaches undoubtedly aid in reducing the effects of domestication from confined culture. However, although wild anadromous Pacific salmon generally remain in geographically restricted habitats during their egg-to-smelt phase, they undertake long migrations through a diversity of habitats during their smolt-to-adult phase (Groot and Margolis 1991).

For instance, salmon from the Snake River traverse up to 1,450 km of the Columbia River system during both their downstream juvenile migration and their upstream adult migration. Few effective conservation measures can be proposed to totally protect anadromous salmon at sea. This limitation effectively eliminates the breeding colony/preserve approach favored by many conservation biologists and restricts captive broodstock strategies for Pacific salmon to full term captive culture.

The relatively short generation time and potential to produce large numbers of offspring make Pacific salmon ideal for captive broodstock rearing. For instance, sockeye sahnon normally have a 4- to 6-year life span (Foerster 1968, Burgner 1991) and often average 2,500 to 2,700 eggs per female (Mullan 1986, Flagg et al. 1991), and chinook salmon normally have a 4- to 8-year life span and may average over 5,000 eggs per female (Healey 1991). Survival advantages offered through protective culture of such large numbers of eggs can be profound. The potential benefits of captive culture over natural production can best be viewed in terms of two near-independent stages of anadromous Pacific salmon life history: the egg-to-smelt stage and the smelt-to-adult stage.

Pacific salmon generally have high natural mortality through the early life-history stage. For instance. Snake River sockeye salmon generally experience less than 6% egg-to-smelt survival (Bjomn et al. 1968). In contrast, the protective environment of hatcheries produces many more juveniles than are expected in the wild: egg-to-smelt survival for hatchery-reared sockeye salmon is generally at least 75% and frequently greater (Mullan 1986, Flagg et al. 1991).

Thus, successful hatchery rearing through the juvenile stages alone can easily provide a twelve-fold survival advantage compared with natural production.

The potential survival advantages of protective culture offer the greatest benefits during the smolt-to-adult phase. For instance, under current environmental conditions in the Cohunbia River Basin, wild smolt-to-adult survival of anadromous Snake River sockeye salmon has been estimated at about 0.2% (K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., December 1993.). However, potential smolt-to-adult survival of Pacific salmon in protective captive culture may exceed 40% (T. Flagg, W. Waknitz, and C. Mahnken, unpubl. data, NMFS): a two hundred-fold survival advantage over natural production during these life stages. Theoretically, captive culture of Pacific salmon through both egg-to-smelt and smolt-to-adult life stages could provide a survival rate more than 2,400 times higher than the rate of natural production.

Our calculations indicate that even if survival of Snake River sockeye salmon could be immediately increased to boost adult recruit/spawner ratios to 2:1 from the current 0.15:1 (0.3 returning adults from each spawning pair) (Flagg et al. in press), it would take 9 generations (over 50 years) for the population to recover to 1,000 adult fish (Fig. 1). It is doubtful that an immediate thirteen-fold increase in natural recruit survival can be achieved under existing environmental conditions in the Columbia River Basin and Pacific Ocean, and even if achieved, the population would remain at risk during most of the 9 generations leading to recovery of 1,000 adults. On the other hand, a captive broodstock founded on 2,500 eggs (ideally as a composite from as many spawners as possible) could easily produce 750 spawners in a single generation (Fig. 1). Juveniles from this captive brood would be available to amplify the natural population during the second generation, thereby markedly accelerating recovery.

Fish for captive broodstocks can be sourced from all available life stages: eyed eggs; fly; smolts captured from the wild; and prespawning adults, captured and artificially spawned. In describing the use of artificial propagation under ESA, Hard et al. (1992) stated:

> In choosing fish to make up broodstock for use in supplementing a listed species, a trade-off exists between maximizing the representativeness of the broodstock sample and minimizing the risks to the natural population that result from taking fish for breeding purposes.

As a practical matter, captures of adult Pacific salmon at weirs during their upstream migration and captures of smolts at weirs during their downstream migration are the easiest to accomplish. However, redd sampling and fry trapping may also be employed.

As discussed by Hard et al. (1992) and Hard and Hershberger (1995, this report) an important genetic consequence of captive broodstock programs is the potential artificial amplification of only a portion of a population through propagation and subsequent reduction in the effective population size ($N_e$) by dramatically increasing only a fraction of the available

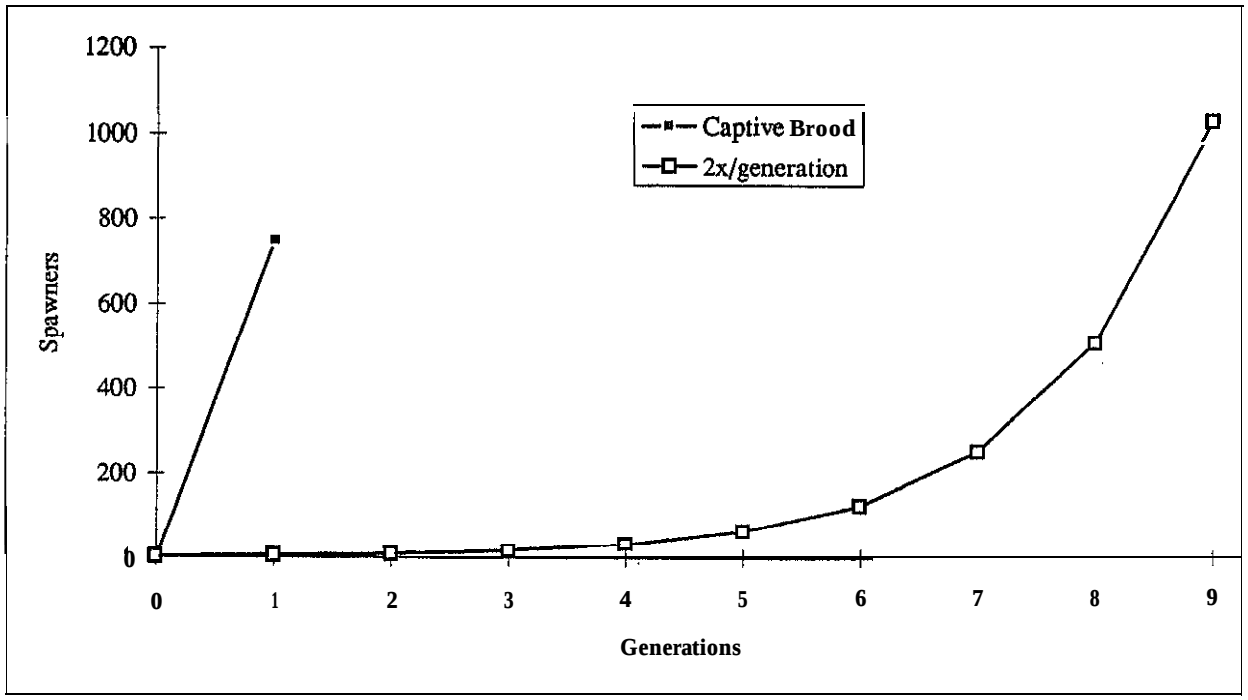


Figure 1. Comparison of production potential per spawner for: 1) captive broodstocks at 75% egg-to-smolt survival and 40% smolt-to-adult survival, and 2) theoretical natural production if barriers to survival could be relaxed to allow the population to immediately begin doubling each generation (2: I adult recruits/spawner).

genotypes in the parent population. In any event, each year-class of captive broodstock should be maintained for only a single generation or a limited number of generations to help assure that genetic integrity and adaptability to native habitats are preserved.

## Performance of Captive Broodstocks

Knowledge of survival, reproductive success, and offspring fitness is critical to determinign levels of risk in implementing a salmonid captive broodstock program. Unfortunately, detailed information on most of these factors has not been developed even though a number of attempts have been made to employ captive broodstocks as a stable source of eggs.

For instance, NMFS has reared over two dozen stocks and year-classes of Atlantic and Pacific salmon to maturity at the Manchester Marine Experimental Station near Manchester, Washington (Harrell et al. 1984, 1985, 1987; W. Waknitz, unpubl. data, NMFS). A number of other agencies in both the public and private sectors have also used captive broodstocks to maintain egg supplies for stocks of fish.

In this section, we present descriptions of some of these rearing programs in an attempt to establish the performance of past and present captive broodstock programs. Few controlled scientific studies on aspects of captive broodstock performance were found, and in many cases, information was presented only anecdotally. Furthermore, when survival, fecundity, and egg viability data were presented, they often represented only the select "best case" examples or were presented for just a portion of the life cycle. Nonetheless, we were able to compile enough data examples to provide useful information on both the potential success and the risk of implementing captive broodstocks.

Information regarding potential captive broodstock performance of coho salmon, chinook salmon, sockeye salmon, pink salmon (0. *gorbuscha*), and steelhead (0. *mykiss*) are presented in individual subsections of this report. No information is presented for chum salmon (0. *keta*) since effective captive broodstock culture of these species is considered impractical at this time (W. Waknitz, unpubl. data, **NMFS).** Subsections on relevant performance of captive broodstocks of other North American fish (e.g., trout and nonsalmonids) are included to provide the reader with a basic understanding of the problems encountered during the course of maintaining fish in full tenn captive culture.

### Review of Select Nonsalmonid Captive Broodstocks

For a number of years, the USFWS has been maintaining captive broodstocks of various nonsabnonid North American fish species listed under ESA (Johnson and Jensen 1991). The relative performance of some of these captive broodstocks is useful in gauging expected success for captive broodstocks of anadromous Pacific salmon.

Brandt et al. (1993) reviewed efforts at spawning and rearing endangered fountain darter (*Etheostoma fonticola*) as part of USFWS recovery investigations for this endangered species. Although overall survival was vnot given, the authors indicated that the 11.5% survival of fish during the first year of their experiment was much lower than normally experienced. The normal

phase. Johnston and Mercer (1977) indicated that between summer 1975 and fall 1977, total mortality was 80% for the broodstocks on hand. However, they did not detail causes of mortality.

From summer 1975 to fall 1977, the fish grew from about 60 to 1,000 g. All fish were spawned directly from seawater. Fecundity of age-2 spawners was about 500 eggs per female, and fecundity of age-3 spawners was about 1,200 eggs per female. The authors did not indicate relative body-size differences between age-2 and age-3 fish, nor did they present egg viability information. Johnston and Mercer (I 977) made no attempt to correlate performrmce of their captive broodstocks with expected wild performance.

Mercer and Johnston (1979) indicated that mortality often ranged between 30 to 80% per year for sea-run cutthroat trout captive broodstocks during the seawater rearing phase. However, again, causes of mortality were not discussed in detail. They reported that eyed-egg viability for a Hood Canal (Washington) stock of sea-run cutthroat trout was 93%; this suggested that spawning captive broodstock directly from seawater is a practical technique for sea-run cutthroat trout. The authors indicated that progeny from these captive broodstocks were outplanted for enhancement, but success of these supplementation efforts was not reported.

Kinunen and Moring (1978) and Barngrover (1990) presented information on origin and use of rainbow trout broodstocks in Oregon and California, respectively. Their papers detailed stock histories but presented no information on broodstock performance.

Varley and Gresswell(l988) reported using captive broodstocks as one of several methods used to maintain Yellowstone cutthroat trout (0. *clarki bouveri*). No details of captive broodstock husbandry were given. The authors reported evaluations of enzyme polymorphisms by electrophoretic techniques that suggested fish in their captive broodstock were genetically similar to the wild stock from which they were sourced. In this case, captive broodstocks may not have been beneficial to the wild stock, since the number of spawning cutthroat trout in Yellowstone Lake tributary streams increased dramatically after operations to mine wild adults for their eggs and sperm were discontinued (Gresswell and Varley 1988, Benhke 1992).

**Atlantic Salmon--**Captive broodstock methods have been an integral part of restoration efforts for Atlantic salmon (S. Salar) in U.S. East Coast states for a number of years(Harrell et al. 1984, Hendrix 1990). Atlantic salmon appear well suited to full-term rearing in captivity and have been noted to be extremely docile during culture (T. Flagg and W. Waknitz, NMFS, personal observation). Growth and survival of Atlantic salmon in culture can at times be excellent.

Mighell (1981) was one of the fist to evaluate the full-term culture of Atlantic salmon for broodstock. Fish were reared to smolt in freshwater at a hatchery and transferred to seawater net-pens in Puget Sound for rearing to maturity. Fry-to-smelt survival was 70 to 85% in top-performing freshwater groups, and over 90% for top-perfonning seawater groups (Mighell 198 I ). Fish averaged 2.5 to 9.0 kg at maturity and produced 4,000 to 6,000 eggs per female. Egg

Smolt-to-adult survival of broodstocks typically averages 75% or greater, and spawners typically range from 5 to 8 kg with egg viability generally greater than 80%. (C. Mahnken, NMFS, personal observation; D. Groves, Sea Springs AquaFarms, P.O. Box 870, Chemanus, B.C., Canada VOR1KO. Pers. commun., June 1994.)

Steelhead--The California Department of Fish and Game (CDFG) annual reports of Feather River Hatchery operation from the mid-1960s through the mid-1970s included information on groups of steelhead held full term in freshwater for broodstock (Groh 1971; Schlichting 1973; 1974a, b; 1976; 1978a, b). Details are not complete for most brood years; nevertheless, reports give insight into the problems encountered in attempting to rear these fish.

For instance, although beginning numbers are not presented for the 1967 brood, Groh (1971) indicated that a total of 8,750 1967-brood steelhead were being held at the CDFG Feather River Hatchery in June 1969, while less than 3,000 fish remained at spawning in spring 1970 (Schlichting 1974a), suggesting a potentially severe (60 to 70%) mortality during the last year of culture. High water temperatures, mechanical failures, and losses to disease caused by a myxosporidian protozoa parasite (*Ceratomyxa shasta*) and infectious hematopoietic necrosis (IHN) virus were listed as causes of this mortality (Groh 1971; Scblichting 1974a, b; Schlichting 1978a).

Egg production from captive-reared steelhead at the CDFG Feather River Hatchery ranged from about 1.9 to 3.2 million per year between 1970 and 1972 and 0.2 to 0.6 million per year from 1973 to 1975 (Schlichting 1973; 1974a, b; 1976; 1978a, b). From 1970 to 1975, overall egg production for captive-reared fish averaged 2,657 eggs/female compared to 2,984 egg/female for ocean-returned fish spawned at the hatchery the same years. From 1970 to 1972, eggs from captive-reared steelhead produced a majority (87 to 96%) of juveniles used to maintain the run to the hatchery (Schlichting 1973; 1974a, b).

Between 1973 and 1975, the adult contribution of captive-reared steelhead dropped to only 16-34% of juveniles reared for release at the hatchery (Schlichting 1976; 1978a,b). The authors did not, indicate whether steelhead captive brood production at the CDFG Feather River Hatchery contributed to the increased run size noted after 1973. Analysis of-data presented by Schlichting (1978a) indicated that in 1974, 0.8% of a group of marked juveniles from captive-reared parents returned to the hatchery as adults, compared with 1.4% of normal hatchery releases. This suggested that contribution from captive-reared fish was lower than from other sources.

More recently, Thrower (1993) compared performance of Alaskan steelbead captive-reared in seawater net-pens vs. ocean-ranched fish. Both 1986- and 1987-brood were included in the study, but survival during freshwater rearing was not reported for either group. Thrower (1993) indicated that losses during the first year in seawater for captive-reared fish were 39% for the 1986-brood and 20% for the 1987-brood. Survival of captive-reared steelhead during the final year(s) to maturation averaged 95% for the 1986-brood. Survival of the 1987-brood during

the final year(s) to maturation was similarly high (about 93%) until most of the fish were lost due to attacks on the net-pen by river otters (*Lutra canadensis*). Survival of ocean-ranched fish was not presented.

In Thrower's (1993) study, the 1986-brood, captive-reared steelhead averaged 3.0 kg for 4-year-old maturing fish and 4.6 kg for 5-year-old maturing fish compared to 2.7 kg for 4-year-old maturing fish and 4.1 kg for 5-year-old maturing ocean-ranched fish. The 1987-brood captive-reared fish averaged 2.4 kg for 4-year-old maturing fish compared to 2.3 kg for 4-year-old maturing ocean-ranched fish. These data suggests that steelhead captive broodstock have the potential to attain sizes similar or greater than wild fish.

The results of gamete-viability tests conducted by Thrower (1993) comparing captive-reared and ocean-ranched steelhead were comparable for both years: fanned males had lower gamete viability (60% average) than ranched males (7 1% average) and fanned females had slightly higher gamete viability (66% average) than ranched females (62% average). However, Thrower (1993) pointed out that "gamete quality of the adult steelhead varied more between years and between specific pairings than between treatment types".

Coho salmon--Noble and Ellis (1960) reported efforts by the Washington State Department of Fisheries (now WDFW) to rear coho salmon to maturity in seawater ponds during the mid-1950s. A total of 103 fish matured. The authors presented no information on starting numbers of fish in culture, so survival cannot be detennined. However, they indicated that age-3 fish were under 0.5 kg at spawning. Coho salmon normally range from 2 to 5 kg or larger at spawning, so it can be assumed that the fish in Noble and Ellis's (1960) studies did not thrive in culture.

Noble and Ellis (1960) reported that some eggs were hard and glass-like in appearance and did not fertilize. Egg loss during hatching was about 20%, fry loss after 32 days was less than 6%, and offspring appeared normal. Noble and Ellis (1960) state:

> At the time of release, these fingerlings did not show any abnormalities and in appearance were similar to fish that were offspring of naturally reared parents. [However] Confinement had a depressant effect upon growth, and limited the fecundity of the females to about one-tenth of normal ocean maturing adults.

West (1965) also provided an early report on full-tenn rearing of coho salmon. The California Department of Fish and Game successfully maintained a strain of coho salmon in freshwater for three generations in the late 1950s and early 1960s. These fish also did poorly in culture. Only 2 females from a group of 200 juveniles (1%) matured and only 30% of their progeny survived the first year of rearing. Causes of death were not indicated, and subsequent spawnings yielded poor-quality eggs that "exploded or collapsed when touched."

Fortunately, captive culture of coho salmon evolved. Mahnken and Waknitz (1979) indicated that by the mid-1970s, commercial production of coho salmon from seawater net-pens was feasible at densities ranging from 8 to 64 $kg/m^3$ with only a 10% growth reduction at the higher densities. The authors stated that survival was comparable among all groups and no major disease outbreaks occurred. However, they gave no indication of survival range, and these experiments were terminated when the fish reached "pan-size," approximately 9 months prior to spawning. Subsequent examination of records indicated that coho salmon survival to maturity typically ranged up to 35% during rearing experiments conducted in the 1970s (W. Waknitz, NMFS, unpublished data).

Clarke (198 1), discussing coho salmon grown to maturity at the Canadian Department of Fish and Oceans Experimental Fish Farm in Nanaimo, B.C., indicated that fish reached maturity about 18 months after introduction to seawater. However, the tune of ovulation and rate of fertilization of these fish was highly variable: fertilization ranged from 8 to 91% and averaged about 50%. Clarke's (1981) studies were conducted in an attempt to determine the influence of nutritional factors on fertility, but he was unable to reduce the variability in fertilization. Improvement in egg color (from a pale color) was noted with inclusion of euphausid shrimp mix in the diet.

McAuley (1981a) overviewed the efforts of Domsea Farms, Inc. to maintain coho salmon captive broodstocks. Reasons for developing these broodstocks were 1) to provide a stock suited for survival and growth in intensive net-pen culture, and 2) to establish a reliable source of eggs. Breeding protocols were established to select for domestication. Only 23% of the fish transferred to seawater survived to maturity (McAuley 1981a). Furunculosis, caused by *Aeromonas salmonicida*, was indentified as a major problem during seawater rearing (McAuley 1981b). Only about 20% of maturing fish were females (McAuley 1981a).

Initial coho salmon captive broodstocks reared by Domsea Farms, Inc. were spawned directly from seawater. Egg viability of the $F_1$ generation was only about 45% (McAuley 1981a), compared to 85-95% for the parent-generation eggs which had been received from state hatcheries for Domsea operations (McAuley 1981b). In response to these low egg viabilities from seawater spawned fish, Domsea Farms initiated protocols of returning fish to freshwater from the seawater net-pens 1 to 2 months prior to spawning, and egg viability was increased to an acceptable 82% (McAuley 1981a).

Hickey (1980) indicated that survival to hatch for eggs from coho salmon captive broodstocks held by Aquasea Farms, Inc. was also low, averaging only about 20%. Fish for spawning were selected from production groups. Unfortunately, Hickey (1980) gave no indication of survival during rearing. However. it is evident that these early attempts at rearing coho salmon to maturity were only partially successful.

Peterschmidt (1991) reported that by the mid-1980s Domsea Farms had adopted a dual rearing protocol for their coho salmon captive broodstocks. Some groups of fish were reared to

smolt in freshwater, placed in seawater net-pens for rearing to maturity, and transferred back to freshwater for spawning; other groups of fish were reared full-term to maturity in freshwater. Peterschmidt (1991) stated:

> During the 1986 and 1987 spawning seasons, eggs from broodstock reared entirely in freshwater had an average mortality of 20%, while eggs from sibling broodstock reared in salt water [and returned to freshwater prior to spawning] had a mortality rate of approximately 5%. The differential survival of eggs from broodstock reared under [these] two environments provided evidence, albeit empirically, that optimal reproductive potential may also be determined by the maturation environment.

However, since eggs from seawater-held fish were incubated at a different Domsea Farms facility (Domsea Gorst hatchery) than eggs from freshwater-held fish (Domsea Rochester hatchery), the differences could have been due to varying environmental conditions (water quality, temperature, etc.) (C. McAuley, NMFS, P.O. Box 130, Manchester, WA. 98353. Pers. commun., June 1994).

Full-term freshwater rearing of coho salmon was favored by Domsea Farms due to higher overall survivals (50 to 60%) compared to overall survival of fish reared in seawater (3 to 5%). In addition, Domsea Farms coho salmon, reared full-term to maturity in freshwater, attained about twice the size and fecundity of seawater-reared fish, averaging about 3.0 kg and 4,000 eggs/female in freshwater versus 1.5 kg and 2,700 eggs/female in seawater (C. McAuley, NMFS, P.O. Box 130, Manchester, WA. 98353. Pers. commun., June 1994).

In 1988, Peterschmidt (1991) conducted a study designed to compare characteristics of coho salmon captive broodstock reared entirely in fresh water to those of siblings with a 15-month residence in seawater. Adult broodstock were further subdivided during the final freshwater maturation period to compare the effects of constant-temperature well water and declining-temperature creek water on gamete maturation. Peterschmidt (1991) stated:

> Little research has been done comparing spawning success of sahnonids reared in salt water versus freshwater. Stauffer (1976) compared the fecundity of coho salmon reared in Lake Michigan and Lake Superior to that of coho from the Pacific Ocean, and found that the number of eggs was comparable for fish of similar size from the different environments. There was no mention of survival rates of fertilized eggs. Several studies have compared egg survival of fish undergoing final maturation in fresh water to that of fish undergoing final maturation in either estuarine or salt water (Allee 1981; McAuley 1981 b; Wertheimer 1981a, b, 1984; Sower and Schreck 1982; Sower et al. 1982). All of these studies concluded that eggs from fish which spent a final maturation period in fresh water, or estuarine water with the presence of a halocline, had significantly higher survival rates than eggs from broodstock maturing in seawater.

Furthermore, Peterschmidt (1991) also stated that:

> There have been conflicting reports on the effect of temperature on the viability of salmonid eggs. Studies involving coho salmon (Allee and Suzumoto 1981; Jensen 1981; Sower et al. 1982); brook trout (Hokanson et al. 1973); and cutthroat trout (Piper 1985) concluded that survival rates of eggs from females that matured in colder (<10°C) and/or fluctuating temperature water were higher than survival rates for eggs from females held in warmer (12.2 - 16°C) and/or constant temperature. However, Morrison and Smith (1986) found that whereas the timing of spawning was delayed by holding rainbow trout in declining temperature creek water, there was no significant difference in the survival rate of eggs from those females and eggs from females held in 10°C spring water.

Peterschmidt (1991) indicated that no differences were noted between treatments for size of fish at spawning: spawners averaged 1.2 to 1.4 kg and 45 to 50 cm. There was also no significant difference in egg viability between coho salmon captive broodstock reared entirely in fresh water and siblings with a period of seawater residence. Egg viability averaged 87% for fish reared entirely in freshwater and 83% for the seawater-reared groups. Peterschmidt (1991) also found no significant difference in viability of co110 salmon eggs incubated on a constant versus declining temperature regime. Egg viability averaged 84% for eggs incubated in constant-temperature water and 91% for eggs incubated in ambient-temperature water.

The coho salmon reared for Peterschmidt's (1991) experiments were 1986-brood and were maintained in culture by Domsea for experimental purposes. Infonnation on overall survival during rearing was not presented (Peterschmidt 1991). In spring 1988, fish were culled and groups selected for "family performance" (good growth and survival in culture) and monitored through maturity in fall 1988. Peterschmidt (1991) documented survival during the last 9 months of the experiment: during this period, survival of freshwater reared fish was 38% and survival of seawater reared fish was only 7%.

Peterschmidt (1991) attributed much of the mortality in seawater to toxic phytoplsnkton blooms. Causes of mortality during freshwater rearing were not reported, though he noted:

> Mortality during the freshwater maturation phase for both groups was primarily a result of opportunistic fungal infections (Saprolegnia). Fish in both groups were also lost to poachers during the course of the study.

No explanation was presented in Peterschmidt's (1991) report to help determine why survival for fish held full-term in freshwater was so much lower than the average 50 to 60% survival for Domsea coho salmon reported by McAuley (1981a). However, freshwater rearing conditions for the Peterschmidt (1991) fish were compromised (by holding tank effects) compared to normal Domsea production groups (C. McAuley, NMFS. P.O. Box 130, Manchester, WA. 98353. Pers. commun., June 1994).

Most of the information we reviewed pertained to coho salmon broodstocks selected for commercial culture. However, Sayre (in press) indicated that a captive broodstock has recently been established for a depleted stock of Hood Canal (Washington) coho salmon. In May 1993, a total of about 2,000 juvenile wild coho salmon were captured from 12 northern Hood Canal streams. The fiih are being reared at low density in circular tanks on spring water. Survival of these fish during the 12 months of rearing has been over 95% (J. Sayre, Long Live The Kings, 7981 168th Ave N. E., Redmond, WA. 98052. Pers. commun., June 1994). These fish are expected to mature in the next 2 years and should provide a valuable gene bank resource for aiding restoration of this depleted run (Sayre, in press).

**Chinook** salmon--Noble and Ellis (1960) reviewed efforts by the Washington State Department of Fisheries [now WDFW] to rear chinook salmon to maturity in seawater during the mid-1950s. A total of 63 fish matured. The authors presented no information on starting numbers of fish in culture, so survival cannot be determined. However, they reported that age-4 fish were under 1.5 kg at spawning. Chinook salmon normally range from 5 to 15 kg or larger at spawning. Therefore, it can be assumed that these fish did not thrive in culture. Noble and Ellis (1960) noted that many eggs from spawners were hard, non-fertilizing, and glass-like in appearance. Egg loss during hatching was about 18%. They stated that fry from these captive-reared fish developed normally in all respects.

Mahnken and Waknitz (1979) mentioned early experiments conducted at the NMFS Manchester Marine Experimental Station on rearing chinook salmon during the early portion of their seawater life phase. They indicated that failure of the fish to adapt to salt water was a major cause of mortality and reduced growth in seawater net-pens. Seven grams was given as a minimum size that accelerated chinook salmon must attain before successful adaptation to seawater. Mahnken and Waknitz (1979) caution that poorly smolted chinook salmon often die within the first week after seawater transfer due to osmoregulatory dysfunction. They reported that 5- to 'I-month survival for stocks held in seawater net-pens ranged from 30 to 80%. Vibriosis, caused by ***Vibrio*** anguillurum, and bacterial kidney disease (BKD), caused by ***Renibacterium*** salmoninarum were indicated as major causes of mortality after osmoregulatory dysfunction subsided (Mahnken and Waknitz 1979).

During the 1970s to mid-1980s, a number of groups of chinook salmon were reared to adulthood at the NMFS Manchester Marine Experimental Station (Fig. 2). Fish were reared in floating net-pens in seawater (28 ppt). Net-pens ranged in size from about 5 by 5 by 5 ***in*** deep to 5 by 10 by 5 m deep (about 125 to 250 $m^3$ total rearing volume). Fish were typically reared at densities up to 10 $kg/m^3$. However, excessive mortality was noted at densities greater than 4 $kg/m^3$ (B. Waknitz, NMFS, unpublished data). Cumulative mortality of fish during seawater residence ranged from about 35% to almost 100% (Fig. 2).

Initial mortality during the first few months after seawater entry of chinook salmon reared at the NMFS Manchester facility was usually attributed to osmoregulatory dysfunction. possibly exacerbated by handling stress during transfer to seawater (Harrell et al. 1985, 1987). The

majority of mortality usually occurred after the fish had been in seawater about 1 year, with mortality from the first to second winters in seawater attributed primarily to BKD (Fig. 2). Total losses from BKD rarely exceeded 30% during seawater rearing. Losses to male precocity generally ranged around 30% of the population (Fig. 2), while adult-phase diseases resulted in the loss of most remaining fish prior to adulthood (Fig. 2). Adult-phase diseases affecting chinook salmon included marine infectious anemia, caused by an (unnamed) intranuclear microspordium, and rosette disease, caused by an (unnamed) obligate intercellular eukaryotic pathogen (Harrell et al. 1986, 1987; Elston et al. 1986, 1987).

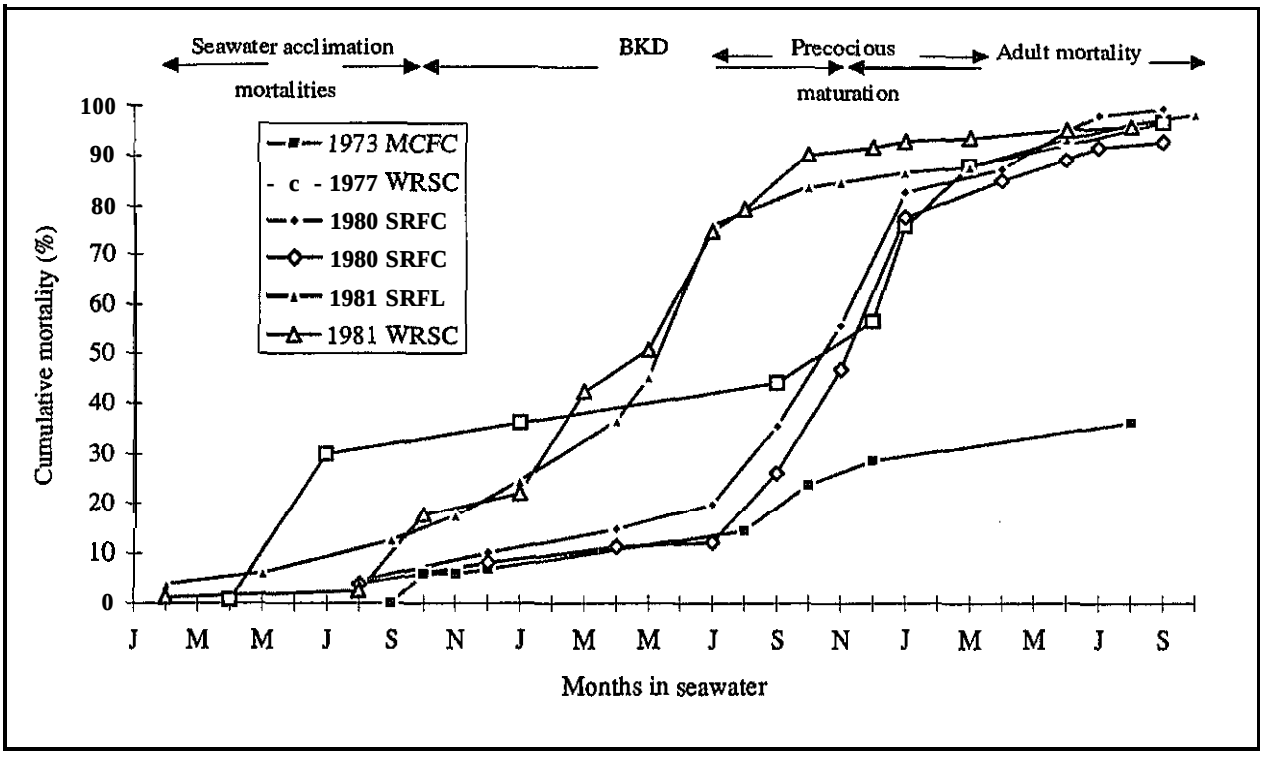


Figure 2. Cumulative mortality during seawater net-pen rearing of Snake River fall chinook salmon (SRFC), Minter Creek fall chinook salmon (MCFC), and White River Spring chinook salmon (WRSC).

Most of the early chinook salmon broodstocks cultured at Manchester were transferred to seawater after freshwater rearing at state and federal fish hatcheries. Therefore, overall (combined freshwater and seawater) survival cannot be estimated for these groups. NMFS also reared broodstock groups of Snake River fall chinook salmon from egg-to-adult in 1980, 1981, 1982, and 1983 (Harrell et al. 1987). Mortality during freshwater rearing ranged from about 5 to 60% for these broods. Mortality in seawater approached 100% for the 1980-, 198 I-, and 1982-broods (as noted below, rearing was terminated before the 1983-brood could be fully evaluated) (Fig. 3).

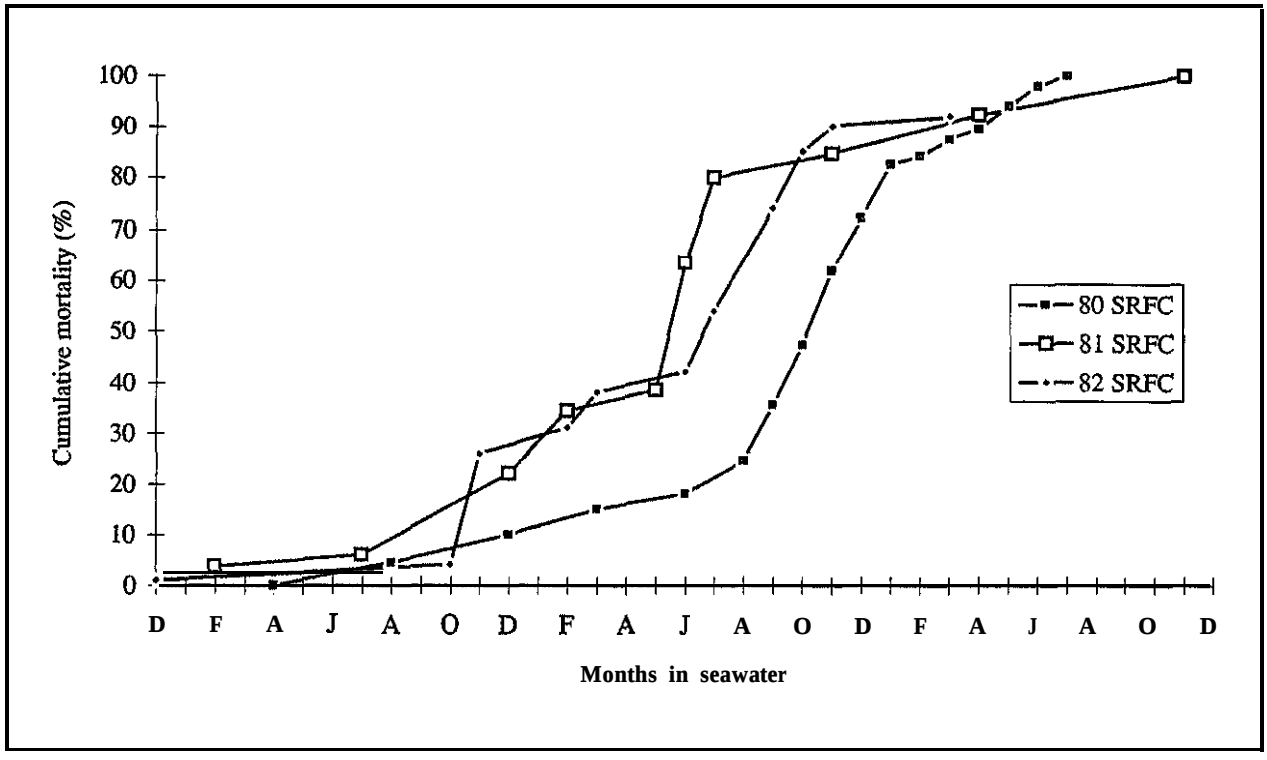


Figure 3. Cumulative mortality during seawater net-pen rearing for 1980- to 1982-brood Snake River fall chinook salmon.

Although a few fish survived and matured, the spawners were heavily infected with rosette disease, and no eggs survived. Average fish size in these broodstocks was about 1.5 to 2.0 kg (Fig. 4) compared to wild chinook salmon, which range 5 to 15 kg or larger at spawning. This suggested that health problems during this time may have compromised growth. Rearing of Snake River fall chinook salmon at Manchester was discontinued in 1986 due to poor survival of the stocks (Harrell et al. 1987).

The 1983-brood Snake River chinook salmon in the NMFS broodstock program had not yet reached spawning age when the study was discontinued in 1986. About 300 of these 1983-brood were retained at the NMFS hatchery for full-tenn rearing to maturity in constant 10°C, fresh well water. Although survival of this stock in seawater was uniformly poor, survival of fish held full-tenn in freshwater was good (Fig. 5). Most freshwater mortality was associated with normal ponding attrition. Growth of fish in freshwater was comparable to fish in seawater (Fig. 6). Survival to 2.5 years of age (mid-1986) was almost 90% for 1983-brood Snake River fall chinook salmon retained in fresh water. However, less than 2% of the age-0 and only about SO% of the age-l fish remained at tennination of the program in 1986 (Fig. 5) (Harrell et al. 1987). It is worth noting that none of the fish in freshwater had been diagnosed with BKD, while cohort groups in seawater had been severely affected with this disease (L. Harrell and T. Flagg, NMFS. unpublished data).

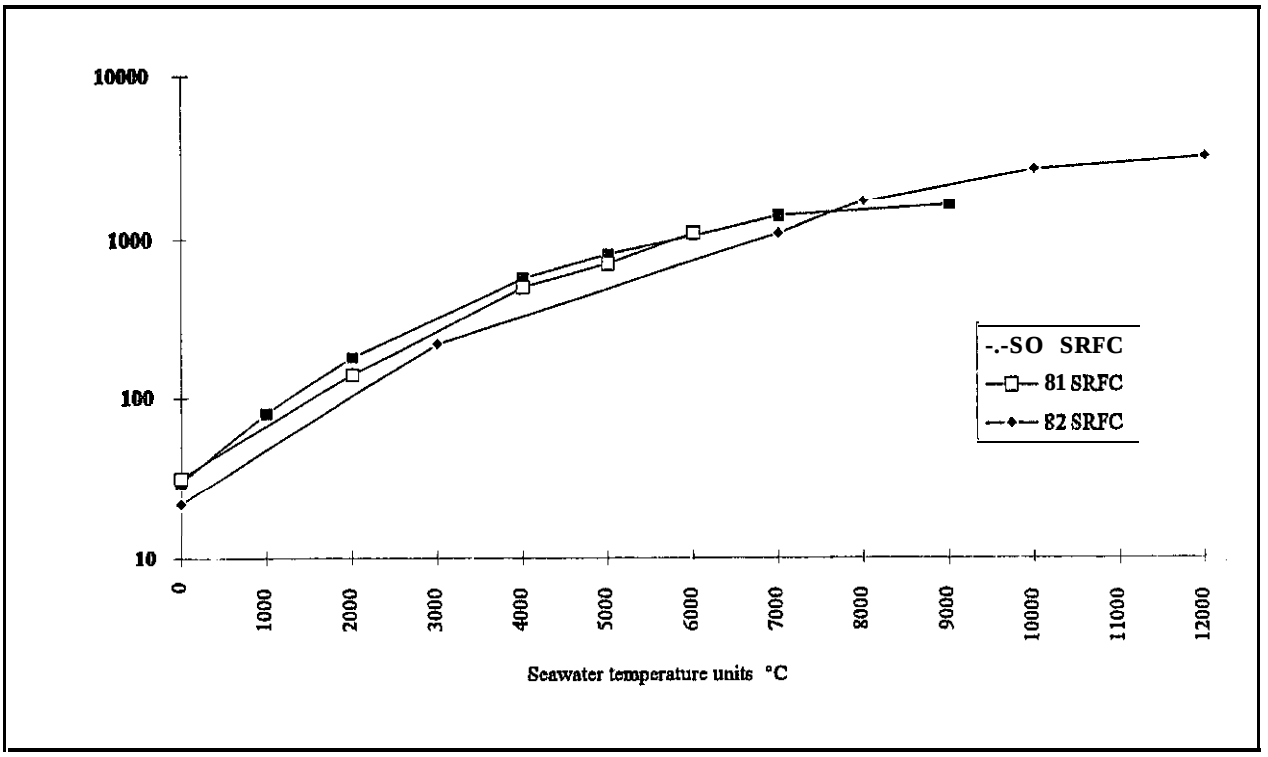


Figure 4. Growth during seawater net-pen rearing for 1980- to 1982-brood Snake River fall chinook salmon (SRFC).

A few 1983-brood Snake River fall chinook salmon were retained in freshwater until spawning in 1986 and 1987. Unfortunately, since the formal Snake River fall chinook rearing program was terminated, detailed records were not kept after fall 1986. One age-3 female (a jill) held full-term in freshwater spawned in 1986: this fish weighed about 0.9 kg, with egg viability of about 90%. This female spawner was certified BKD-free. About 31% of the male fish held full-term in freshwater matured (as jacks) in 1986. Most remaining fish were culled in 1987 to reduce rearing cost.

However, about 10 females and males were retained and spawned in fall 1987. These fish averaged 1 to 1.5 kg at spawning. Exact egg viability information was not available; however, it is thought to have been high. Survival of the 1983-brood Snake River fall chinook salmon in freshwater remained high during rearing, while all cohorts reared in seawater died (Fig. 5). This suggests there may be good potential for full-term captive rearing of chinook salmon broodstocks in freshwater. To our knowledge, no further studies have been conducted to compare survival between chinook salmon grown to maturity in fresh water and seawater.

Cheng et al. (1987) reported survival of two different strains of British Columbia (Canada) chinook salmon and their crosses reared in confinement. These fish were reared to assess aquaculture potential, and rearing was terminated prior to maturity. Juveniles were reared in

freshwater and transferred to seawater net-pens. Survival during the 9 weeks of freshwater rearing ranged from 92 to almost 98% for the groups. Survival of the groups in seawater ranged from 20 to 26%. Gutted body weight of 120-week-old fish averaged about 1 kg.

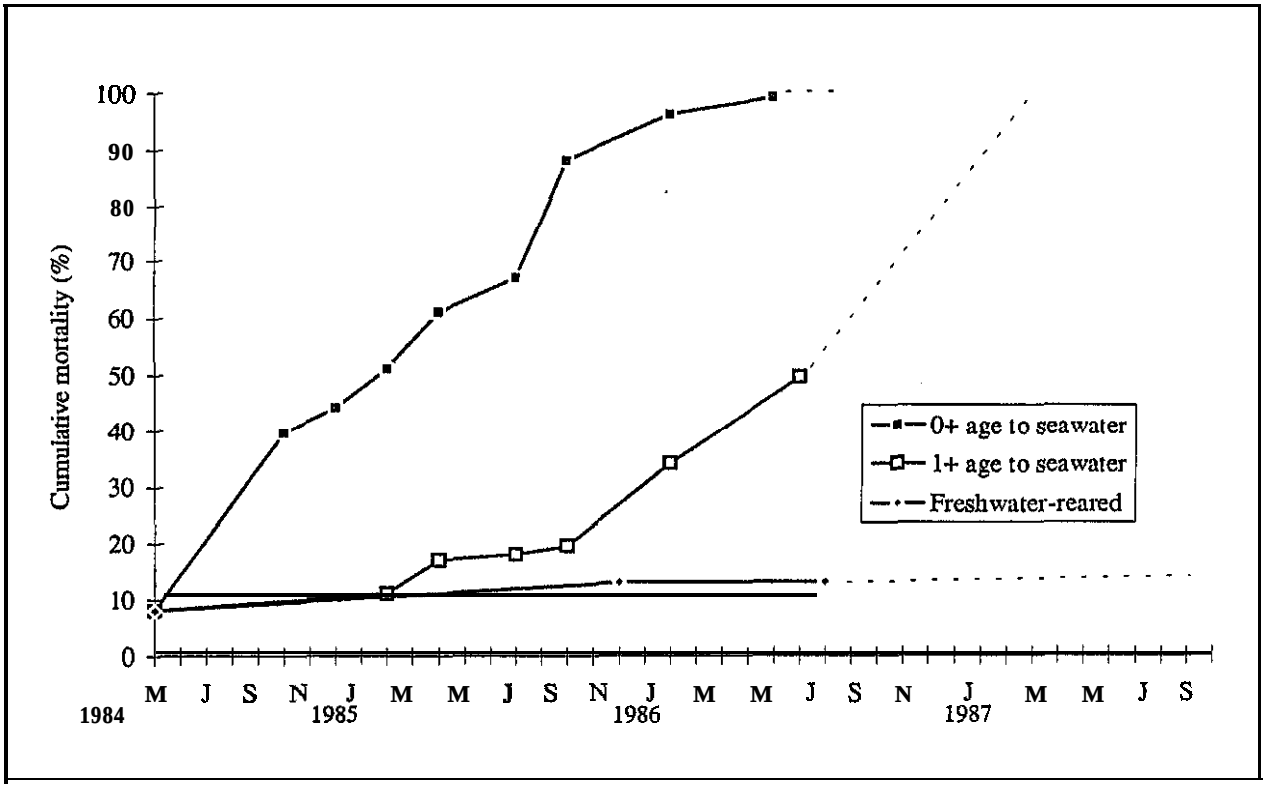


Figure 5. Comparison of mortality for 1983-brood Snake River fall chinook salmon reared to maturity in tanks supplied with 10°C fresh well water and cohorts transferred to seawater net-pens as smolts. Data after mid-1986 are approximate.

Cheng et al. (1987) reported that "The low survival rates observed in these pens were due to predation by sea otters." To our knowledge, predation of pen-reared fish by sea otters (*Enhydra lutris*) has never been documented prior to their report. It is likely that predation was attributable to river otters: a known predator of net-pen reared fish (C. Mahnken, W. Waknitz, and T. Flagg, NMFS, personal observation). It is our experience that river otters will destroy and consume most fish in a net-pen rather than simply cropping the population to the 20% level as indicated by Cheng et al. (1987). We know of no cases where disease has not been a contributor to mortality of fish during rearing. Nonetheless, no causes of death other than sea otters were mentioned by Cheng et al. (1987).

Fedorenko and Cross (1991) reported attempts to rear Squamish River (British Columbia. Canada) chinook salmon to maturity in seapens. Smolt-to-adult survival was less than 10% for the 2 brood years tested. Mortality was attributed to myxobacterial infections (caused by

*Sporocytophaga* sp.), vibriosis, BKD, noxious algal blooms, and poaching and vandalism; however, percent mortality attributed to each of the listed causes was not presented. The fish that survived and matured were comparable in size and fecundity to natural spawners, and averaged about 5 kg and 4,200 eggs per female for age-3 spawners and 8 kg and 6,200 eggs per female for age-4 spawners. Eyed egg survival averaged about 65% and 87% for the 1984 and 1985 brood, respectively.

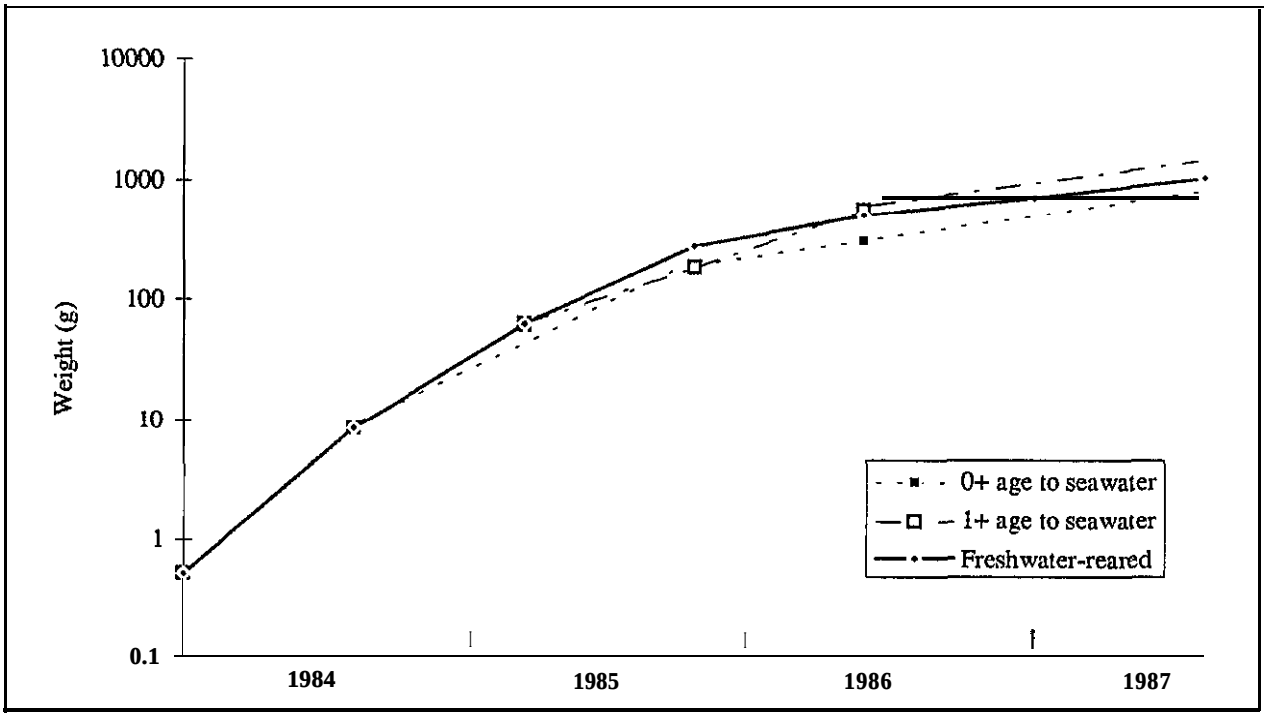


Figure 6. Comparison of growth for 1983-brood Snake River fall chinook salmon reared to maturity in tanks supplied with 10°C fresh well water and cohorts transferred to seawater net-pens as smolts. Data for seawater groups after early 1986 are approximate.

Joyce et al. (1993) reared chinook salmon to maturity in net-pens in Alaska. The captive-reared fish were smaller and younger at maturity than anadromous adults of the same stock. Survival rates for the captive-reared fish were not mentioned, nor were causes of mortality during rearing. Captive-reared spawners averaged 6.7 kg compared to 12.8 kg for anadromous fish. Most captive-reared female fish matured at age-4, whereas anadromous females matured at age-5 and age-6. Captive-reared females produced gametes that averaged 72% survival to the eyed egg stage, whereas average eyed egg survival for anadromous female fish was 65%. Offspring of captive-reared and anadromous fish were the same size (10 g) after 6 months of rearing.

The White River spring chinook salmon captive broodstock has a longer history than other formal chinook salmon captive rearing programs we reviewed. From 1977 to 1985, seven brood

years of White River spring chinook salmon were reared from smolt to maturity in seawater net-pens at the NMFS Manchester facility. Net-pens ranged in size from about 125 to 500 $m^3$ total rearing volume (5 x 5 x 5 m to 10 x 10 x 5 m) . Excessive mortality was noted at densities greater than 4 $kg/m^3$ (W. Waknitz, NMFS, unpublished data). Smelt-to-adult survival to spawning ranged from 1 to 10%. At spawning, captive-reared adults weighed about 4 kg, and eyed egg viability was typically 30 to 60% compared to 80 to 90% for wild fish (C. Mahnken, W. Waknitz, and T. Flagg, unpubl. data., NMFS).

Since 1987, WDFW has been rearing White River spring chinook salmon captive broodstocks in net-pens in south Puget Sound. Results of this program are detailed by Appleby and Keown (1995), this report. Net-pens used by WDFW for this program are about 8 by 10 by 4 m deep (about 300 $m^3$ total rearing volume). Fish are reared at densities ranging from about 2 to 6 kg/m'. Between 1991 and 1993, WDFW spawned between 800 to 1,000 adults (23 to 29%) from each age-class of 3,500 smolts transferred to seawater. At spawning, adult fish typically averaged 65 to 75 cm and weighed 3.0 to 7.0 kg. Fecundity of captive-reared spawners from 1991 to 1993 ranged from 2,200 to 2,700 eggs per female, and egg viability ranged from 63 to 70%. However, Appleby and Keown (1995, this report) indicate that these numbers do not include eggs discarded during spawning, so actual viability might be slightly less than the numbers presented.

Commercial culture of chinook salmon has been expanding on the West Coast of the United States and Canada since the late 1980s. The greatest concentration of chinook salmon farms is currently along the east coast of Vancouver Island. Initially, chinook salmon fanners reared fish in small pens at sheltered seawater sites and fish performance was poor. Today, successful chinook salmon farmers in Canada typically rear fish in seawater in very large, 30 by 30 by 20-m-deep net-pens (18,000 $m^3$ total rearing volume) anchored in areas with extremely high flushing rates and near-oceanic temperatures and salinity (D. Groves, Sea Springs AquaFarms, P.O. Box 870, Chemanus, B.C., Canada VOR1KO. Pers. commun., June 1994).

In Canada, commercial farmers often maintain a small number of chinook salmon in seawater net-pens for broodstock. The best sites for broodstock maturation have seawater temperatures that do not exceed 11°C during the fall maturation period. Smolt-to-adult survival of broodstock typically averages 70%. Fish are often spawned directly from seawater, and egg viability normally averages over 90% (D. Groves, Sea Springs AquaFarms, P.O. Box 870, Chemanus, B.C., Canada VOR1KO. Pers. commun., June 1994).

The recent success of chinook salmon farming in Canada is encouraging. These commercial growers are apparently achieving 3 to 10 times the survival rates to maturity documented by NMFS and WDFW (see above). It is possible that the added success the commercial farmers are enjoying is due in part to their culturing domestic hatchery strains of chinook salmon in very large net-pens, whereas attempts by NMFS and WDFW to culture chinook salmon to maturity have focused on wild strains reared in relatively small net-pens.

In our experience with rearing fish in seawater net-pens at the NMFS Manchester facility, chinook salmon preferentially inhabited only the bottom-most portion of the pen and never appeared to be "at home" in the confines of the pen environment (C. Mahnken, L. Harrell, W. Waknitz, and T. Flagg, NMFS, personal observation). Appleby and Keown (1995, this report) also mention that chinook salmon reared in their net-pen system favored the bottom. The net-pens used to rear chinook salmon at NMFS Manchester and by WDFW in South Puget Sound have less than 3% of the volume of net-pens currently used by successful Canadian chinook salmon fanners.

In addition, the better-performing Canadian net-pen sites are in areas supplied with high-quality oceanic water, while NMFS and WDFW net-pen operations are sited in sheltered bays. Canadian success is undoubtedly bolstered by an advantageous culture environment with good flows, low water temperatures, and seawater low in toxic algae concentrations. Therefore, we suggest that future new attempts at rearing chinook salmon to maturity as captive broodstocks for restoration be conducted in large-volume rearing containers supplied with oceanic-quality water.

Sockeye salmon--Very little information has been published concerning captive broodstock rearing of sockeye salmon. However, some information is available.

Between 1987 and 1991, NMFS collected up to 520 wild sockeye salmon adults annually from the Wenatchee River in Washington State as donors to provide juveniles for experiments iu the Columbia River Basin. During these years, average prespawning survival ranged from 72-95%, egg viability ranged from 40-80%, and survival to yearling smolt generally approached 80% (Flagg et al. 1991).

Early attempts to rear two stocks of sockeye salmon to maturity in seawater net-pens at the NMFS Manchester facility ended in failure. A few 1973-brood Cedar River sockeye salmon survived to spawn; however, all eggs were non-viable. All 1988-brood Lake Wenatchee sockeye salmon died during culture (T. Flagg and W. Waknitz, unpubl. data, NMFS).

Researchers at the NMFS Auke Bay Laboratory transferred about 300 1987-brood sockeye salmon from wild parents to seawater net-pens in May 1988. Survival of these fish to August 1991 was 58%, and average fish length was about 45 cm. On 19 August 1991, 20 females reared in the net-pens were spawned directly out of seawater and their eggs were incubated at the Auke Creek Hatchery. Egg viability was about 10%, but researchers presumed it would have been higher if fish had been returned to freshwater for acclimation prior to spawning (W. Heard, NMFS, Auke Bay Laboratory, Juneau, AK. pers. commun. December 1991).

The Canadian Department of Fish and Ocean's (DFO) Nanaimo Laboratory has reared six stocks and year-classes of sockeye salmon to maturity. Most were reared full-term in freshwater. though a portion of one group was also reared in seawater net-pens. The researchers indicated that portions of all groups held in freshwater survived to maturity and produced viable eggs and progeny, whereas most fish held in seawater died. They were able to accelerate growth rate and

achieve some early maturing 3-year-old females. They indicated that BKD was a problem during rearing, particularly in 3- to 4-year-old fish (C. Wood, DFO, Nanaimo, B.C., pers. commun. December 1991).

Based on the results of sockeye salmon research at DFO, and other freshwater-rearing experiments for chinook and coho salmon (described above), freshwater culture was selected as the method with the highest likelihood of ensuring survival during full-tenn rearing of endangered Redfish Lake sockeye salmon (see discussion below). However, there are numerous unanswered questions regarding the role of seawater residence in overall fitness of both captive-reared fish and their offspring.

To help answer these questions, Flagg and McAuley (in press) compared survival between progeny of 1991-brood Lake Wenatchee sockeye salmon cultured full-term in freshwater and progeny of anadromous parents. In these experiments, about 300 1987-brood Lake Wenatchee sockeye salmon were held full-term in fresh water at a NMFS facility. Survival to first spawning was about 20%. The first female from this group matured in fall 1991, and egg viability was 67% (J. Mighell, NMFS, 2725 Montlake Blvd E., Seattle, WA. pers. commun. January 1991).

Performance of offspring (1991-brood) of these 1987-brood fish was closely monitored and compared to offspring of ocean-return fish of the same brood year. Survival for 1991-brood freshwater offspring from hatch to smolting was about 90%, compared to about 85% for progeny of parents that had migrated to the ocean (Flagg and McAuley in press). There was no difference in smoltification as measured by plasma thyroxine levels between the two treatments (W. Dickhoff and T. Flagg, unpubl. data, NMFS).

At smolting, fish were transferred to seawater net-pens at the NMFS Manchester facility. Survival during the first 4 months of seawater residence averaged about 83% for the 1991-brood freshwater offspring replicates and 79% for the 1991-brood anadromous replicates. There was no significant difference in survival between the two treatments (Flagg and McAuley in press). Results of this experiment suggested that full-term freshwater rearing of sockeye salmon captive broodstocks does not compromise seawater adaptability of offspring. However, it should be noted that these data are somewhat inconclusive because progeny of only one female held full-tenn to maturity in freshwater were available for testing.

In an ongoing sockeye salmon captive-rearing experiment conducted at the NMFS Manchester facility, replicated groups of two year-classes of Lake Wenatchee stock sockeye salmon are being held under the following conditions: 1) circular tanks supplied with pathogen-free freshwater; 2) seawater net-pens; and 3) circular tanks supplied with pumped, filtered, and W-sterilized seawater (Flagg and McAuley in Press, Flagg et al. in prep.). Survival has been documented for experimental groups of 1990-brood Lake Wenatchee sockeye salmon during 29 months of rearing, from the beginning of the experiment in May 1992 through spawning as 4-year-old fish in fall 1994. Survival averaged about 25% in seawater net-pens, 35% in seawater tanks, and 32% in freshwater tanks.

In a second experiment, groups of 1991-brood Lake Wenatchee sockeye salmon reared 20 months, from the beginning of the experiment in 1993 through the end of January 1995, averaged about 26% survival in seawater net-pens, 71% in seawater tanks, and 93% in freshwater tanks. Most mortality was attributed to BKD (Flagg and McAuley in press, Flagg et al. in prep.). This data suggested that captive broodstocks cultured in filtered and UV-sterilized seawater or fresh spring water will survive at a higher rate compared to sockeye salmon cultured in seawater net-pens.

Growth was significantly greater for fish reared in freshwater than for those reared in either of the two seawater treatments (Flagg and McAuley in press, Flagg et al. in prep.). Approximately 15% of 1990-brood fish in the freshwater replicates matured at 3 years of age in late October 1993, but no fish matured in the seawater treatments in 1993. Female 1990-brood sockeye salmon, spawned from freshwater tank-reared replicates in 1993, averaged 41.5 cm in length and 0.87 kg in weight, and 1,359 eggs/female (about 1,560 eggs/kg of female weight) in fecundity. Eyed-egg survival for these eggs averaged only about 36% (Flagg and McAuley in press).

Most remaining 1990-brood in both freshwater and seawater matured as 4-year-old fish in fall 1994 (Flagg et al. in prep.). In 1994, mature female 1990-brood sockeye salmon from the seawater net-pen replicates averaged about 1.0 kg in weight and 1,852 eggs/female (about 1,869 eggs/kg of female weight) in fecundity. Females from the seawater net-pen replicates averaged about 1.6 kg in weight and 1,876 eggs/female (about 1,189 eggs/kg of female weight) in fecundity. Females from the freshwater tank replicates averaged about 2.2 kg in weight and 2,496 eggs/female (about 1 ,I 16 eggs/kg of female weight) in fecundity. Eyed-egg survival from 1990-brood Lake Wenatchee sockeye sahnon spawned in 1994 averaged about 50% from the seawater net-pen groups, 45% from the seawater tank groups, and 50% from the freshwater tank groups spawned in 1994 (Flagg et al. in prep.).

The 35 to 50% eyed-egg survival rates documented by Flagg and McAuley (in press) and Flagg et al. (in prep.) were much lower than the 70 to 90% often seen from sockeye salmon adults collected from the wild (Mullan 1986, Flagg et al. 1991). However, this rate was similar to the 30-60% eyed egg survival (described below) observed by NMFS and Idaho Department of Fish and Game (IDFG) for Redfish Lake sockeye salmon captive broodstock spawned in 1993 and 1994 (Flagg et al. in prep.; K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., December 1994). We are unsure of causes of these low egg-viability rates from captive-reared fish, however, spawning techniques were ruled out. The eggs from these fish were often pale in color (Flagg and McAuley in press; K. Johnson, IDFG, 1800 Trout Road, Eagle. ID. 83616. Pers. commun., December 1993), whereas wild sockeye salmon eggs are normally bright red. Diets may have influenced egg viability in these studies since sockeye salmon are planktivorous, whereas commercial brood diets are fonnulated for piscivorous fish.

Currently there is only one set of captive broodstock to maintain threatened or endangered species of sockeye salmon. Snake River sockeye salmon were listed as endangered under the

ESA in 1991 (Waples et al. 1991a). Interim recovery measures focus on protecting the last known remnants of this species: sockeye salmon that return to Redfish Lake in the Sawtooth Basin of Idaho. Because of the critically low population size of Redfish Lake sockeye salmon, the interim recovery measures are centered around a series of captive broodstocks to maintain the species while habitat improvements are underway.

Between 1991 and 1994, captive broodstocks were initiated to preserve the Redflsh Lake sockeye salmon (Flagg 1993, Johnson 1993, Flagg et al. 1994, Flagg et al. in press, Flagg and McAuley in press, Flagg et al. in prep.). Sources for these broodstocks were outmigrating juveniles and eggs from adults returning to Redfish Lake. Eggs from returning adults were divided between IDFG and NMFS hatcheries to reduce the risk of catastrophic loss. Full-term freshwater rearing was chosen at both hatcheries, based on evidence which suggested that culture in freshwater would ensure the highest survival (see above).

By the end of 1994, fish in four groups had successfully matured in captivity and survival (including successfully spawned fish) rates averaged as follows: about 15% after 44 months of rearing for fish captured as juveniles in spring 1991, 65% after 32 months of rearing for fish captured as juveniles in spring 1992, 38% after 20 months of rearing for fish captured as juveniles in spring 1993, 45% (15% for NMFS fish and 75% for IDFG fish) after 36 months of rearing the progeny of fish that returned and were spawned in fall 1991, and 98% after 12 months of rearing the progeny of fish that returned and were spawned in fall 1993. Most mortality was ascribed to normal attrition, standpipe failures, and BKD; however, other pathogens (e.g., *Aeromonas* sp.) were also noted (Flagg 1993; Johnson 1993; Flagg et al. 1994; Flagg and McAuley in press; Flagg et al. in prep.; K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., February 1995).

Fish captured as juvenile outmigrants from Redfish Lake in spring 1991 were mostly 1989-brood yearlings and a few (about 15% of the population remaining at that time) matured as 4-year-old fish in fall 1993. Twenty-four maturing adults (12 males and 12 females) from this group were released into Redfish Lake in fall 1993 to spawn naturally. An additional 13 females were spawned by IDFG. Fecundity averaged about 2,100 eggs/female, but egg viability was less than 40%.

This first spawning from the captive broodstock produced about 10,000 sockeye salmon juveniles that were released into Redfish Lake in fall 1994. These fish were marked for evaluation and are expected to outmigrate from Redfish Lake in spring 1995. An additional eight females from this group survived to 5 years of age and were spawned in fall 1994; egg viability averaged about 30% for these fish. Progeny from the 1994 spawning of these fish will be released in Redfish Lake in 1995 (K. Johnson. IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun. February 1995).

Progeny of the one female and three male sockeye salmon that returned to Redfish Lake in 1991 were expected to mature as 4-year-old fish in fall 1995. However, most matured as 3-year-

old fish in fall 1994. Survival of these fish was much higher at the IDFG hatchery than at the NMFS hatchery (about 80 vs. 25%), where the primary cause of death was BKD. Female 1991-brood Redfish Lake sockeye salmon spawners at NMFS averaged 43.6 cm and 1.23 kg, while male spawners averaged 45.7 cm and 1.44 kg. Fecundity averaged 1,644 eggs/female (about 1,142 eggs/kg of female weight) and egg viability averaged about 60% for the 1991-brood females spawned by NMFS in 1994 (Flagg et al. in prep.). Fecundity averaged about 1,800 eggs/female and egg viability averaged about 45% for the 1991-brood females spawned by IDFG in 1994 (K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., January 199.5).

Spawning of 1991-brood Redfish Lake sockeye salmon captive broodstock by NMFS and IDFG resulted in approximately 115,000 healthy juveniles; these will be released in Redfish Lake in 1995 (K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. comrnun., February 1995). We anticipate that thousands of fish from the Redfish Lake captive broodstocks will mature over the next few years and hundreds of thousands of progeny will be available for use in recovery efforts to increase the odds of persistence for this endangered stock of fish.

Although Redfish Lake sockeye salmon captive broodstocks are apparently "on the road to success" at this time, it should be cautioned that long-term success is uncertain. In addition to the fish health and egg viability problems noted above, early maturity (male precocity) and asynchronous maturation have been noted in the broodstocks (K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., February 1995). Research on physiological, nutritional, and pathological components of each of these "trouble areas" are required to refine technology for captive broodstock production of sockeye salmon.

**Pink** Salmon--Pink salmon are distinguished from other Pacific salmon by several unique life-history traits: 1) a fixed, 2-year life cycle from egg fertilization to adult maturation, the shortest life cycle of all Pacific salmon; 2) average weight of only 1 to 2.5 kg at maturity, the smallest of all Pacific salmon; and 3) juveniles that migrate to sea within weeks after emergence (Heard 1991). Pink salmon also are the least fecund of all Pacific salmon, nonnally averaging 1,200 to 1,900 eggs/female for wild fish, (Heard 1991). The inflexible life cycle characteristics of this species may limit its captive broodstock rearing options. For instance, the fixed 2-year life cycle limits cross-generational matings except through the use of cryopreservation techniques.

ln the 1970s, several attempts were made to rear pink salmon to maturity in seawater net-pens at the NMFS Manchester facility. However, none of these trials were successful. One hundred percent mortality (primarily due to vibriosis) occurred in both local and Alaskan pink salmon stocks transferred to seawater in accordance with natural life history requirements for this species (i.e., soon after the swim-up stage). When pink salmon were retained in fresh water for up to 9 months prior to introduction to seawater, a few fish (3%) survived nearly to maturity, but all fish eventually succumbed to BKD before gametes were produced (W. Waknitz, unpubl. data, NMFS). Mortalities noted in these early rearing experiments were likely exacerbated by stress associated with confinement in net-pens during spring/summer periods. The extremely small

mesh-size necessary to retain pink salmon fry produced high sea temperature, restricted flow, and low dissolved oxygen levels.

MacQuarrie et al. (1979) reported on rearing trials for pink salmon conducted at the DFO West Vancouver Laboratory. Photoperiod was manipulated during fish rearing in an attempt to alter maturation timing. In an initial experiment, MacQuarrie et al. (1979) reported that very little mortality occurred during the 2 years of fish rearing. Most males developed sexually, but fewer than 50% of females in the normal photoperiod group, and much smaller percentages of females in the accelerated or delayed photoperiod groups, matured. Unfortunately, MacQuarrie et al. (1979) did not provide details on survival or maturation of fish in this first experiment, except to note that ova from all groups exhibited very high mortalities prior to hatching.

In a subsequent series of experiments MacQuarrie et al. (1979) reared groups of pink salmon in seawater tanks. Several year classes of fish were reared under varying photoperiod regimes and reacclimated to fresh (well) water before spawning. MacQuarrie et al. (1979) reported that vibriosis was a major cause of mortality during early rearing for some groups of 1973-brood. MacQuarrie et al. (1979) did not provide details on juvenile-to-adult survival for the 1973-brood experimental groups except to note that survival rates were high.

Spawners from these groups were only about one-half the weight and produced about one-half the eggs of wild fish. Survival to about 3 weeks post swim-up ranged from 5 to 74% and averaged 32% for progeny of 1973-brood spawners in these experiments. MacQuarrie et al. (1979) indicated that both vibrio and BKD were major causes of mortality for the 1974-brood experimental groups, and no fish were successfully spawned.

Beacham and Murray (1990, 1993) reported on two experiments to rear pink salmon to maturity at the DFO Nanaimo Laboratory. In both the (1990 and 1993) studies, photoperiod was manipulated during fish rearing. Beacham and Murray (1990) indicated that in a treatment group with two periods of declining day length, about 50% of males matured within the 15-month study period. However, no males matured in treatments having only one photoperiod of declining day length. Importantly, no females matured in the first study in any photoperiod treatment (Beacham and Murray 1990). In the second study, Beacham and Murray (1993) indicated that an accelerated photoperiod resulted in maturation of both males and females 6 months ahead of naturally-spawning populations. Jn this study, almost all surviving males matured, however, only about 30% of the females matured. Egg fertility ranged from 1 to 93%; however, egg size was only about 50% of the weight of naturally spawned pink salmon eggs. Survival to fry for eggs from captive-reared fish was about 75% (Beacham and Murray 1993). Complete information on fish survival during rearing was not presented for either study (Beacham and Murray 1990. 1993 ).

Recently, researchers at the NMFS Auke Bay Laboratory have been conducting studies regarding captive rearing of pink salmon. In one study. both 1989- and 1990-brood were reared to IO-15 g in freshwater and transferred to seawater net-pens for rearing to maturity. Survival from seawater transfer to maturation was about 50% for both year classes of fish. Fish density

was kept at less than 8 $kg/m^3$, and vibriosis was described as the major health problem during growout in seawater. Fish that matured were not spawned, therefore no information is available concerning reproductive performance for these groups (J. Joyce, NMFS, Auke Bay Laboratory, Juneau, AK. pers. commun. March 1995). In a subsequent experiment to rear 1989- and 1990-brood pink salmon to maturity in the seapens, all fish were lost to outbreaks of BKD (W. Heard, NMFS, Auke Bay Laboratory, Juneau, AK. pers. commun. March 1995).

## Husbandry Techniques

Captive broodstock culture programs involving threatened and endangered nonsalmonid fish species have had to overcome a basic lack of life-history information concerning rearing preferences such as temperature, habitat, feeding requirements, growth rates, and spawning and incubation needs (Rinne et al. 1986, Johnson and Jensen 1991, Brandt et al. 1993). Fortunately, life-history parameters and requirements for culture of most species of salmonids are. known and are applicable to captive broodstock programs. The basic fish husbandry techniques employed in traditional salmonid hatchery fish management can be used for maintaining captive broodstocks, at least during the early life-history stages (Davis 1967, McNeil and Bailey 1975, Leitritz and Lewis 1980, Piper et al. 1982, Stickney 1991).

Traditional hatcheries for anadromous Pacific salmon rear fish to smolt size, which requires a maximum rearing period of about 1.5 years (Piper et al. 1982). Since most basic juvenile rearing constraints have already been addressed over the course of salmon culture development (Moring 1986), they can theoretically be avoided in captive broodstock programs. However, full-term captive broodstock culture may extend the rearing period to maturity at 4-6 years, which encompasses a period in salmonid life history for which cultural requirements are relatively unknown (Mighell 1981, McAuley 1983, Rinne et al. 1986).

Many previous attempts to culture Pacific salmon to maturity have been unsuccessful or have resulted in low survival and poor gamete quality (Waknitz 1981; McAuley 1983; Harrell et al. 1984, 1985, 1987; Peterschmidt 1991). Likewise, attempts to use artificial propagation to supplement naturally-spawning populations of Pacific salmon have often yielded mixed results (Moring 1986, Miller 1990, Cuenco et al. 1993). Accordingly, some modifications in standard fish-culture systems and rearing techniques appear necessary to ensure reasonable survival in full-term protective culture. In the following sections, we describe some husbandry strategies that deserve special consideration for use in captive broodstock programs.

Optimally, captive broodstock culture methods should emulate natural life-cycle events of the wild fish. In addition, they should produce offspring that are indistinguishable from wild fish with respect to appearance, physiology, behavior, and genetic diversity. Therefore, the potential detrimental effects of standard hatchery practices on captive broodstock and their offspring must be minimized. To mitigate these effects, Kincaid (1993) suggested the following precautions:

> (I) plant fish at the earliest possible life stage, (2) maintain fish at low rearing densities during culture, (3) maintain high numbers of brood fish (effective population numbers), (4) equalize the genetic contribution of all parental fish to the next generation. (5) capture brood fish from throughout the fishery and spawning season, (6) spawn all mature adults available, and (7) avoid selection of brood fish and progeny based on physical appearance and captive performance.

To this list, we would add that juveniles from captive-bred fish should be reared under conditions that mimic the natural rearing habitat. In one study, a substantial increase in survival to maturity was gained by simulating the natural life history of a stock of fall chinook salmon in culture, rather than applying standard roaring procedures for the species (Reimers 1979).

Culture techniques that provide cover, substrate, and structure in the rearing container can increase the post-release survival of fall chinook salmon through the freshwater migratory corridor by 40% or more (Maynard et al. in press). Natural-type rearing techniques may reduce domestication effects and provide hatchery-reared juveniles that are physiologically and behaviorally equivalent to naturally produced salrnonicls.

## Hatchery Water Supply

It is essential that the water supplied to a captive broodstock facility be of the highest quality. Restoration programs have often been aided by improvements in water quality (Moring 1986). Beside being free of pollutants and toxins, water for captive broodstock facilities must be highly oxygenated, at or below nitrogen saturation, and within the proper temperature range for salmonids. Pathogen-free water may be required at different life stages, especially during egg incubation.

According to state (California, Idaho, Oregon, Washington, and Montana) and federal disease policies, eggs infected with pathogens, such as infectious hematopoietic necrosis and infectious pancreatic necrosis, cannot be moved from the spawning/incubation facility (PNWFHPC 1993). Therefore, captive broodstock facilities must have the capability to treat at least a portion of the incoming water supply to prevent exposure to pathogens which might prohibit the release of the progeny. For further details on diseases affecting captive broodstocks, see Harrell's (1995) discussion in this report.

Water for captive broodstock facilities should be at or near saturation with dissolved oxygen (DO). Supplemental oxygen improves both growth and survival and is beneficial to overall fish health (Colt et al. 1991). Well water is often low in DO, requiring the addition of supplemental oxygen. If the incoming water is below saturation, oxygen can be injected into the water system prior to delivery to the fish. It has been shown that in hatchery applications, supplementation with commercial oxygen is more effective at raising DO than with conventional aerators, although it is also more expensive (Speece 1980). The ability to add oxygen, for both beneficial and emergency purposes, must be part of the captive broodstock hatchery design.

Most available water supplies in the Pacific Northwest fall within the range of suitable temperatures for salmonids. However, the ability to heat or chill the water supply adds a great deal of flexibility to the captive broodstock facility and allows the operator some control over growth and reproduction. For further details on factors affecting growth and reproductive development, see reviews by Swanson (1995) and Forster and Hardy (1995) in this report.

Water supersaturated with atmospheric gases is not suitable for rearing salmonids (Stickney 1991). Levels of total dissolved gas (TDG) should be kept below 102% for eggs and fry, and below 105% for fingerlings (Chvsley 1980). Potential sources of high TDG in the water supply include dams, air leaks in the pumping system, elevated temperatures, welt-water, and gravity-water intakes (Owsley 1980). Removal of excess TDG with the use of packed-column degassers is simple and economical (Owsley 1980). Although production hatcheries do not often test for TDG until a problem is suspected, it is essential that water-quality monitoring equipment be installed at captive broodstock facilities to reduce the risk of loss (Hendrix 1990). This equipment should continuously monitor water quality parameters such as flow and temperature and alert fish culturists at work and at home if parameters deviate from specified limits.

Filtration and disinfection of incoming water should also be considered in the design of a captive broodstock facility. The primary rationale for disinfection is to provide a measure of disease control (Dupree 1980). Sterilization of all incoming water is probably more amenable to conservation facilities such as captive broodstock hatcheries, which tend to be small, than to enhancement facilities, which tend to be large (Dupree 1980). Filtration of the incoming water will remove multicellular pathogens, after which the water can be subjected to ultraviolet (UV) radiation, chlorination, or ozonization to kill single-cell organisms. Control of water-quality parameters can also be increased with partial or complete recycling systems (Losordo 1991, Wheaton 1991).

Ultraviolet radiation treatment of the water supply is recommended for a captive broodstock facility. Chlorine and ozone, although effective sterilizing agents, must be removed before fish can be exposed to water, as they are both highly toxic even at small concentrations (Dupree 1980, Huguenin and Colt 1989). Ultraviolet radiation treatment, although somewhat ineffective in turbid water, is nontoxic and therefore presents lower risk to the fish (Dupree 1980). Additional information regarding water-quality criteria that can be applied to captive broodstock facilities has been published by Laio (1971), Laio and Mayo (1972), Wheaton (1977), Piper et al. (1982), Colt and Watten (1988), and Petit (1990).

## Broodstock Collection

**A** fundamental difference between traditional hatchery operations and captive broodstock programs involves the acquisition of adults. Hatcheries that release fish to enhance sport and commercial fisheries rely primarily on adults returning to the hatchery, or on eggs transferred from other facilities, to fulfill their production needs. Hatchery fish may be the product of many generations of artificial selection, Captive broodstock facilities, whose goal it is to recover wild fish populations, will ideally use fish that have not been domesticated by hatchery operations. The potential consequences of such selection are discussed in detail by Hard and Hershberger (1995) in this report.

Captive broodstocks may be established by collecting sperm and eggs from migrating adults on or near the spawning grounds, or eggs from redds, fry, or migrating juveniles. In any event, broodstock collections must be made at or near the natal habitat to ensure that the target stock is being conserved (Cuenco et al. 1993). Genetic advantages and disadvantages of various broodstock collection methods are also discussed by Hard and Hershberger (1995) in this report.

In some cases, gametes can be taken without killing wild adults. For instance, cryopreservation techniques allow the stream-side collection of sperm from wild male fish, which can be crossed with wild- or hatchery-reared females in the same or subsequent years (Cloud et al. 1990). Likewise, some eggs can be expressed from wild female fish captured on their spawning grounds. The fish can then be released to contribute to the naturally-spawning population.

Cryopreserved sperm can also be stored for use until assays for pathogens have been completed. This reduces the risk of vertical disease transmission by avoiding the use of pathogen positive males. However, in recent experiments, the viability of eggs fertilized with cryopreserved sperm was only 23% for steelhead (Cloud et al. 1990) and less than 30% for sockeye salmon (K. Johnson, IDFG, 1800 Trout Road, Eagle, ID. 83616. Pers. commun., June 1994). Therefore, use of cryopreserved sperm might not be advantageous when the number of eggs available is critically low.

Regardless of the method chosen to source captive broodstock, it is essential that physical handling of adult and juvenile fish be minimized. Long-term holding of captured wild adult salmon can often lead to significant mortality, especially when fish are held to maturity in seawater (Johnston and Mercer 1977, Allee 1981, McAuley 1981, Wertheimer 1984, Peterschmidt 1991). Wild fish are also more susceptible to stress than hatchery fish. For instance, in one study, wild coho salmon and chinook salmon experienced greater stress during handling (as measured by increased blood cortisol levels) than their hatchery-reared cohorts (Salonius and Iwama 1993). In addition, fish may experience increased susceptibility to disease after handling (Stickney 1983, Pickering 1988).

Capture methods that bind or encumber the fish should not be used. Preferred capture methods are those that guide fish into watered boxes or fine-mesh pockets in seines, offering fish little opportunity to become overly excited or harm themselves. For instance, black vinyl tubes have been used for water-to-water transport of individual adult Atlantic salmon from an upstream migrant trap to a hatchery in order to reduce the amount of handling-induced fungal infection Hendrix 1990). In addition, the holding facility should be designed to prevent adult fish from jumping out or otherwise sustaining injury. For example. facilities used to hold Atlantic salmon prior to spawning are designed to have at least 180 cm of freeboard Hendrix 1990).

These types of extra precautions are increasingly important when handling groups of endangered fish, even though the cost per unit capture may be relatively high. For example, a downstream migrant trap with individual, protected fish-holding areas below each of five incline-plane traps was used to collect endangered sockeye salmon smolts exiting Redfish Lake. Idaho

(Johnson 1993). Mortality of wild sockeye salmon smolts due to collection procedure was less than 7%.

Careful handling after capture, transport to the captive broodstock facility, and subsequent individual PIT tagging resulted in an additional 1.5% mortality of the approximately 1,000 fish captured during 2 years of trapping. Similar methods were used to collect upstream-migrating adult sockeye salmon returning to Redfish Lake. Adults voluntarily entered a trap and were then netted and transported to the captive broodstock facility, held, and spawned with no mortality (Johnson 1993).

In another restoration project, wild sockeye salmon adults were collected at a fish ladder and transferred, using a water-to-water method, to net-pens in a mountain lake, where they were held for several months until maturity (Flagg et al. 1990). Survival from capture to spawning in the net-pen enclosure ranged from about 72 to 95% during the 5-year study, compared to survivals of 25% and less for sockeye salmon held to maturity in more traditional hatchery raceways and tanks (Mullan 1986, Amos et al. 1989).

## Gamete Collection and Fertilization

Gamete collection and fertilization methods for adult fish sourced for and produced from captive broodstock programs are similar to those used in production salmon hatcheries, with the exception that every egg is valuable and wastage must be kept to a minimum. Prespawning adults should be maintained in freshwater, if possible. Attempts to spawn salmonids directly from seawater have generally resulted in lower egg viability than attempts to spawn salmon matured in freshwater (Allee 1981, Stoss and Fagerlund 1982, Lam et al. 1982, Wertheimer 1984, Peterschmidt 1991).

In production facilities, ripe females are usually killed and eggs excised. However, sometimes fish spawned in this manner are not fully mature and immature eggs are wasted. In captive broodstock programs, which seek to produce as many progeny as possible, adult fish should be live-spawned until all eggs have been removed (Leitritz and Lewis 1980, Hendrix 1990). Since it may take several spawnings to retrieve all eggs, it is essential that handling stress during spawning not result in mortality. Prior to spawning, fish should'be anesthetized with an approved chemical to reduce stress during spawning (Schnick et al. 1989). During spawning, fish should not be held by the gills or other areas where injury and bleeding may occur. Vessel designs that reduce or soften collisions with the sides of the container (e.g., circular or semi-square tanks or net-pens) are recommended for holding valuable broodstocks.

Temperature and quality of holding water for adults can affect egg quality and survival of progeny after spawning. For example, cutthroat trout adults reared in stream water with fluctuating temperatures (2-10°C) produced significantly more eyed eggs than adults reared in constant temperature spring water (10°C). even though water quality was essentially the same.

Eggs from adults reared in spring-water were of poor quality and easily broken, while eggs of adults reared in creek-water were of good quality (Smith et al. 1983). Similar results have been observed for Atlantic salmon, where eggs from prespawning adults held in fluctuating temperature water (8 to 12°C) had higher viability than eggs from adults held in constant 10°C water (T. Flagg, unpubl. data, NMFS).

Nevertheless, it may be desirable to use spring water to prevent exposure of maturing fish to natural pathogens. In this case, the constant temperature of spring water may be varied by using a heat exchanger placed in a natural creek to impart a fluctuating temperature profile to the spring water. In the absence of creek water, heating or cooling by standard mechanical methods can provide suitable varied-temperature incubation and rearing water for the captive broodstock.

Salmonids can be induced to spawn a year or more before normal by rearing them in water warmer then they naturally experience. If some fish from any given brood year are reared on an accelerated growth profile, and others are not, gametes will be available in several seasons, allowing for extensive inter-year crosses with succeeding or previous brood years (Fowler and Banks 1980, Banks and Fowler 1988). For example, after accelerated rearing in 12°C well water, chinook salmon matured and spawned a year earlier than normal. Many males matured as precocious parr or jacks. However, there was no difference in the size of mature adults between accelerated and nonaccelerated groups (Banks and Fowler 1988). Chinook salmon reared in warmer, recycled well water grew faster and produced more adults than fish grown in cooler recycled creek water (Fowler et al. 1980).

Spawning techniques to maximize genetic variability of the captive brood and minimize the incidence of disease are presented elsewhere in this report; see Hard and Hershberger (1995) and Harrell (1995). Additional information regarding spawning methods that can be applied to captive broodstock programs has been published by Piper et al. (1982), Ingram (1988), Laird and Needham (1988), and Flagg et al. (1990).

## Egg and Juvenile Culture

Incubation systems presently used in production hatcheries have been used for captive broodstocks, although some modifications may be beneficial. For instance, incubators with a matrix substrate produced Atlantic salmon fry up to 45% larger than fry incubated in smooth-bottomed units (Leon and Bonney 1979). These incubators were recommended for use in programs to rehabilitate depleted stocks of Atlantic salmon. Incubation of eggs at low density with substrate resulted in better survival, reduced yolk sac problems, and larger fry (Murray and Beacham 1986). In addition, darkened incubators produced heavier Atlantic salmon fry than undarkened incubators (Mighell 1981). A combination of cover and substrate resulted in higher mean weight of coho salmon alevins than with substrate alone (Fuss and Johnson 1988).

In recent years, a low volume, down-welling incubation system has been used for restoration efforts with Atlantic salmon and sockeye salmon (Harrell et al. 1984, Novotny et al.

1985, Flagg et al. 1991). In this system, the eggs of a single female are incubated in isolated containers, which prevent horizontal transmission of disease to other families as well as providing a convenient means of maintaining distinct groups. Although egg densities of up to 10,000 eggs per Heath-type vertical incubator tray have been used in production salmon hatcheries (Fuss and Seidel 1987), the objective in captive broodstock programs is not to maximize biomass per unit space, but to maximize overall survival at all life stages. Therefore, although no list of species-specific maximum egg loading densities were found in the literature, it would seem prudent to incubate valuable broodstocks at no more than half the density currently used in production facilities.

Care must be taken not to exceed the proper incubation temperature. At IO to 11.5°C, chinook salmon alevins experienced about 98% survival to swim-up, whereas at 15°C, only 73% survived to the swim-up stage (Garling and Masterson 198.5). Although preferred incubation temperature varies with species (Brett 1952), keeping incubation temperature below 12°C should prevent thermal problems (Heming 1982). Additional information regarding various incubation techniques that can be applied to captive broodstock facilities has been published by Leitritz and Lewis (1980), Fuss and Seidel (1987), and Brannon (1991).

Standard salmonid rearing techniques and diets (Leitritz and Lewis 1980, Piper et al. 1982, Stickney 1991) can be used for juvenile culture of salmonids. However, captive broodstock facilities should use rearing densities substantially lower than those used in production hatcheries (Stickney 1991). Lower rearing density will reduce crowding, stress and alterations in natural behavior (Cuenco et al. 1993). Rearing densities in captive broodstock programs should never be allowed to become so high that constant attention must be exercised to prevent adverse density-related effects. To ensure that rearing conditions will not become a determining factor in the success of the captive brood effort, concentrations in the rearing vessels should be much less than the Density Index values presented by Piper et al. (1982).

Additional information regarding various juvenile fish rearing techniques that can be applied to captive broodstocks has been published by Leitritz and Lewis (1980), Piper et al. (1982), and Stickney (1991). If a supply of seawater is available to the captive broodstock program, the decision will have to be made whether to continue rearing juveniles full-tenn to maturation in fresh water, or to introduce the fish to seawater when they undergo smoltification. There are risks and benefits to each mode of rearing and each may require different husbandry techniques (Waknitz 1981).

## Full-term Freshwater Rearing

Freshwater sites selected for full-tenn rearing of captive broodstocks should have access to a pathogen-free water source. Water source considerations described under the hatchery water section (above) can be applied to facilities dedicated to full-tenn freshwater rearing of salmonids. However. artesian well water is the preferred source for captive broodstock facilities. Well water is not only less likely to contain pathogens than surface water, but also normally has fewer

suspended solids and debris than surface water, and provides better flexibility for temperature control (Brannon 1991). Full-term rearing to maturity in fresh well water was selected as having the highest likelihood of ensuring survival during culture for endaugered Redfish Lake sockeye salmon (Flagg 1993, Johnson 1993).

Several species of anadromous salmonids have been reared to maturity entirely in freshwater with varying degrees of success. It has been shown that anadromous stocks of Atlantic salmon and brown trout will mature when reared full-term in freshwater (Jarrams 1979). However, egg fertilization was not as successful with freshwater-reared fish as it was in those held to maturity in seawater (47% vs. 85% for Atlantic salmon and 62% vs. 70% for brown trout).

Coho salmon, sockeye salmon, and chinook salmon have also been successfully reared to maturity in freshwater; these are described (above) in the sections on captive broodstock performance. There appeared to be no difference in health of Atlantic salmon fiy from parents reared in freshwater or seawater (Jarrams 1979). In addition, in a pilot study, there was no difference in smoltification of sockeye sahnon juveniles from parents of anadromous fish compared to those reared full-term to maturity in freshwater (W. Dickhoff and T. Flagg, unpubl. data, NMFS). Most data on performance of offspring from salmonids reared full-term to maturity in freshwater is anecdotal. However, the presence of large populations of land-locked Pacific salmonids in the Great Lakes over the last three decades has clearly shown that saline waters are not necessary for successful completion of the life cycle for hatchery-reared or naturally-spawning anadromous salmonids (Stewart and Ibarra 1991).

The potential problems associated with full-term fresh water rearing of salmonids seem to be less numerous than those related to seawater culture (see discussion of seawater rearing, below). In general, procedures established for rearing juvenile salmonids in freshwater can be applied to the smelt-to-adult phases and a well-developed technology exists for freshwater salmonid culture. Large culture vessels should be used for rearing fish to adult size. Stickney's (1991) criteria of maximum densities of 30 $kg/m^3$ for conservation hatcheries seems too high, especially for larger fish: captive-brood chinook salmon held from smolt-to-adult in net-pens in Puget Sound experienced an increase in mortality whenever rearing density exceeded 5 kg/m" (W. Waknitz, unpubl. data, NMFS).

We have recommended that densities for full-term rearing of endangered Redfish Lake sockeye salmon in freshwater be maintained between 2 to 8 kg/m" (Flagg 1993, Johnson 1993). Additional information regarding freshwater rearing techniques that can be applied to captive, broodstock programs has been contributed by. Davis (1970), Laio (1971), Laird and Needham (1988), Leitritz and Lewis (1980), Piper et al. (1982), Brannon (1991), and Stickney (1991).

## Seawater Rearing

Several recent programs to restore depressed stocks of anadromous salmonids (e.g., Atlantic salmon, Snake River fall chinook salmon, and White River spring chinook salmon) have reared fish to maturity in seawater net-pens in Puget Sound (Harrell et al. 1984, 1985, 1986; Appleby and Keown 1995, this report). In some years, growth and survival of these pen-reared captive broodstocks was sufficient to produce more eggs than were available from wild or ocean-ranched cohorts. However, in other years a variety of factors reduced the total number of eggs produced by captive broodstock to below what would have been available if the fish had been left in the wild (W. Waknitz, unpubl. data, NMFS). Factors limiting captive broodstock survival in seawater included osmoregulatory dysfunction after transfer to seawater, stonns, harmful encounters with phytoplankton, predation from birds and marine mammals, poaching, and disease.

Exposing non- or partially-smoked salmonids to full-strength seawater can result in severe mortality (Mahnken and Waknitz 1979, Folmar and Dickhoff 1980, Folmar et al. 1982, Mahnken et al. 1982). Maintenance of juveniles (e.g., Atlantic salmon) at constant elevated temperatures can be detrimental to growth and survival after transfer to seawater (Dickhoff et al. 1989). Procedures that alter the physiology of fish should be used with caution (Wedemeyer et al. 1980). For example, it has been shown that rearing steelhead smolts in 13°C fresh water inhibited the production of gill sodium-potassium adenosine triphosphatase (ATPase), which presumably compromises the ability of the fish to successfully osmoregulate after exposure to seawater (Zaugg et al. 1972). Therefore, if the decision is made to transfer or release captive broodstock in the marine environment, it will be essential to employ rearing procedures which do not compromise the ability of the fish to adapt to a saline environment.

Care must be taken to reduce stress when handling fish, especially during transfer into seawater. Handling of smolts prior to transfer to seawater can cause descaling, which may promote loss of electrolytes and increase mortality after transfer (Bouck and Smith 1979). Handling can also inhibit the production of gill ATPase, reducing seawater adaptability (Zaugg 1982).

In one study, fish dip-netted from a hatchery truck into full strength seawater using standard hatchery methods of dewatering fish in the nets experienced a 3.5-times higher mortality than fish transferred using a more time consuming, but less stressful water-to-water transfer method (Flagg and Harrell 1990). Although stress-reducing procedures are usually not as efficient as standard practices in terms of fish moved per unit effort, the health of fish in a captive broodstock program must be weighed against temporary convenience to the personnel.

The time of year when salmonids are introduced to seawater can also affect subsequent survival. Atlantic salmon moved to seawater after the summer solstice had difficulty adapting to the saline environment (Mighell 1981). A similar situation was observed in chinook and coho salmon (Mahnken and Waknitz *1979,* Mahnken et al. 1982, Martin and Wertheimer 1987). Monitoring increases in both gill ATPase and plasma thyroid hormones, as well as seawater

challenge tests to ascertain blood osmolality, are effective means of determining when sahnonids are best able to adapt to the seawater environment (Wedemeyer et al. 1980, Clarke 1982, Dickhoff and Fohnar 1982, Zaugg et al. 1983). However, some of these methods may require the sacrifice of up to 40 fish per month for 3 or 4 months to establish base-line profiles necessary to detect the rises in physiological parameters associated with smoltification.

Pre-seawater feeding of salt-enhanced diets is a practical, non-intrusive method to induce an increase in gill ATPase activity, which will increase survival after introduction to the marine environment. Addition of about 7% dietary salt to the feed of Columbia River hatchery chinook salmon several months prior to release resulted in a 65% greater adult return to the hatchery compared to non-salt fed cohorts (Zaugg et al. 1983). A similar diet increased survival of chinook salmon (Zaugg et al. 1983) and Atlantic sahnon (T. Flagg and W. Waknitz, unpubl. data, NMFS) transferred directly to seawater net-pens. Other studies have shown that addition of dietary salt increased survival after seawater introduction for coho salmon (Zaugg and McLain 1969), Atlantic salmon (Basulto 1979), and rainbow trout (Jackson 1977, Salman and Eddy 1990).

State-of-the-art, captive broodstock facilities should be designed to supply both high-quality seawater and fresh water to the site (Huguenin and Colt 1989). This arrangement will allow juvenile captive broodstock to be gradually introduced to full strength seawater over a period of days; a strategy that has been shown to increase survival. Progressive increases in salinity over a 6-day period improved survival rate of brook trout over 3.5-times the rates of fish introduced less gradually (Pelletier and Besner 1992).

In addition, post-seawater transfer mortality of Atlantic sahnon smolts was markedly reduced by pumping fresh water into a I-m-deep, vinyl-skirted net-pen. This formed a salinity gradient of 4 to 25 ppt, and allowed fish to seek their preferred salinity level (Harrell et al. 1984). In another study, yearling chinook sahnon introduced to the same lens system in the fall of the year experienced 2.4% mortality after 7 days, while a control group placed directly into full-strength seawater sustained 63.2% mortality during the same period (W. Waknitz, unpubl. data, NMFS).

Potential sites for seawater net-pen captive broodstock facilities are restricted by several factors, including susceptibility to damage from waves and debris, which can tear nets and lead to the escape of fish (Edwards 1978, Kennedy 1978, Sedgwick 1982, Laird and Needham 1989). However, turbulent waters will not usually preclude the establishment of a land-based seawater facility unless tidal fluctuations are so extreme as to prevent pumps from delivering water continuously (Huguenin and Colt 1989). Land-based facilities supplied with processed (pumped, filtered, and sterilized) seawater are preferred over open-environment net-pens for rearing valuable broodstocks because of the control of water quality and security inherent to the land-based operation. Growth and survival of salmonids are improved when grown on environmentally controlled. processed seawater (Flagg and McAuley in press, Flagg et al. in prep.).

Exposure to harmful phytoplankton can cause uncontrollable losses in salmonids maintained in seawater, although the risk is substantially reduced when fiih are held in land-based tank facilities supplied with filtered seawater. Net-pens, while far less costly than land-based structures, offer virtually no protection from plankton blooms. For example, salmon facilities in Europe, North America, and Japan have occasionally sustained severe losses as a result of plankton blooms (Saunders 1988, Rensel 1992).

In addition, a recent bloom of a toxic dinoflagellate in Puget Sound caused extensive loss in a chinook salmon captive broodstock (Prentice 1990). In this case, over 500 4- and 5-year-old maturing White River spring chinook salmon were killed only weeks prior to their transfer to a freshwater hatchery for final maturation and spawning (W. Waknitz, unpubl. data, NMFS). To avoid fish kills due to noxious phytoplsnkton, it is recommended that valuable stocks be held in land-based tanks supplied with filtered sterilized seawater. Filters should be capable of removing particles as small as 2 microns to prevent phytoplankton from entering the fish tanks (Huguenin and Colt 1989).

An additional consideration in favor of land-based seawater captive broodstock facilities is better protection from predators. Although net-pens can be covered to prevent damage from predatory birds, they are especially vulnerable to attack from marine mammals. For example, an entire brood year of a depressed stock of chinook salmon was eliminated from marine net-pens by river otters in a single night (L. Harrell, NMFS, P.O. Box 130, Manchester, WA. 98353. pers. commun. May 1994). In addition, large pinnipeds can rip holes in nets, allowing the escape of fish that are not killed outright (W. Waknitz, NMFS, pers. observation).

Human poachers can also harm captive broodstocks. For example, in 1976, thieves arrived by boat and stole or killed several hundred maturing Atlantic and chinook salmon broodstock held in seawater net-pens at the NMFS Manchester facility (L. Harrell, NMFS, P.O. Box 130, Manchester, WA. 98353. pers. commun. May 1994). As a result, the program was set back several years until the next available year classes were ready to spawn.

Land-based fresh and seawater captive broodstock facilities can be made almost impervious to animal predators, although no affordable design will be 100% secure. Enclosing the entire facility in a building will deter most predators (animal and human) while providing an environment that is convenient for control of water quality. Cover will also significantly reduce bio-fouling in the captive broodstock facility which decreases the amount of cleaning necessary, thereby reducing stress to the stock.

Additional information regarding seawater rearing methods for anadromous salmonids in captive broodstock programs has been contributed by Edwards (1978), Kennedy (1978). Sedgwick (1982), Laird and Needham (1988), and Spotte (1992).

## Rearing and Release of Offspring from Captive Broodstocks

A primary objective of a captive broodstock program should be to produce wild-like fish capable of establishing self-perpetuating populations in natural habitats, instead of providing large numbers of fish to meet short-term harvest goals. The basic approach during rearing of juveniles should be to provide natural rearing conditions to ensure that fish for release are suited to the natural environment (Cuenco 1993).

Rearing environment may affect startle response and aggressive behavior in hatchery-reared salmonids (Roadhouse et al. 1986, Olla et al. 1992). Therefore, changes in behavior associated with artificial rearing of juvenile salmonids, which are generally nongregarious, must be minimized or avoided when the progeny of captive brood fish are destined for release. Salmmonids are known to seek concealment (Woodhead 1957, McCrimmon and Kwain 1966). However, standard production-rearing techniques do not allow fish to choose between open and exposed areas. Therefore, released fish may not immediately recognize that open areas in natural habitat present more dangers than covered areas or those containing structure.

Culture techniques that provide cover, substrate, and structure in the rearing container produce fish with natural cryptic coloration and fright responses, and higher post-release survival than standard hatchery-reared fish (Maynard et al. in press). These natural-type rearing techniques may reduce domestication effects and produce hatchery-reared juveniles that are physiologically and behaviorally equivalent to their wild counterparts. Fish destined for release should be reared at low density; low rearing densities combined with high water-flow in the hatchery, during rearing and in the receiving stream after release, were the only factors that correlated with increased survival of Atlantic salmon in a restoration project in the Eastern U.S. (Hosmer et al. 1979). Releases of juveniles or adults from captive broodstocks must be scaled to the carrying capacity of the area to be enhanced in order to reduce ecological risks to the population as a whole.

## Conclusions

At present, we regard the full-term culture to maturity of all species of Pacific salmon as problematic: high fish mortality and low egg viability were noted for most species. Based on our review, we rate the ease of captive culture of Pacific salmon by species as follows: 1) coho salmon and steelhead, 2) chinook salmon and sockeye salmon, and 3) pink salmon and chum salmon. Ideally, fish should be reared at extremely low densities in the largest rearing vessels possible. Captive broodstock facilities should be equipped with state-of-the-art monitoring, alarm, and response systems to help ensure fish survival during emergencies. Multiple facilities are recommended for each broodstock to reduce the risk of catastrophic loss.

Overall egg-to-adult survival for captive broodstocks documented in this report was generally lower than expected, normally never ranging upwards of 30%. viability of eggs from captive-reared spawners generally ranged 30-60%. In addition, size of captive-reared fish was generally slightly smaller than wild cohorts. Nevertheless, existing captive broodstocks for salmon and trout have often contributed the majority of gametes available for recovery efforts. It appears that agencies involved in recovery of other fish species (e.g., trout and desert fish) often apply the pragmatic view that survival of fish in nature is often less than a few tenths of one percent.

Even a 30% survival in culture provides survival rates several orders-of-magnitude higher than found in nature. However, for captive broodstocks to reach their full potential for recovery efforts, exacting fish culture methods must be developed that ensure that their offspring have the same genetic, physiological, and behavioral makeup as their wild grandparents. It would seem prudent for captive culture to mirror the natural life-cycle of the fish; whenever this is not possible (e.g., full-tenn freshwater rearing, accelerated growth and maturation, etc.), potential effects to the broodstock and their offspring should be evaluated.

Captive broodstocks can provide an egg-base to help “jump-start” a population and will often be a preferred alternative for responsible resource managers, especially when populations have reached critically low numbers and extinction is eminent. However, captive broodstocks should be always viewed as a short-term measure to aid in recovery: never as a substitute for returning naturally spawning fish to the ecosystem.

There is limited data regarding the effects of broodstock manipulations (e.g., sourcing, mating, rearing, feeding, and release strategies) on the health, physiology, or genetic stability of the population, or, most importantly, on reproductive performance. Each of these areas of concern will be discussed in depth in succeeding sections of this report. We caution that the lack of basic knowledge of captive broodstock performance makes success uncertain.

As we have pointed out above, primary consideration should be to the fish themselves. Other avenues of restoration should be exhausted. and the fish facing eminent threat of extinction. before experimental measures such as captive broodstocks are employed. Nonetheless, in some

cases captive broodstocks may provide the only mechanism to prevent extinction of a stock and may be undertaken regardless of prospects for immediate habitat improvement.

We conclude that captive broodstock technology for salmonids, although in its initial development stages, is sufficiently advanced to allow careful captive rearing to proceed. However, the husbandry methodology for full-term culture of Pacific salmon in captivity is poorly developed and has not yet been thoroughly evaluated and success of supplementation using offspring from captive broodstock is uncertain. Because the benefits and risks have not been determined through appropriate monitoring and evaluation, captive broodstock development should be considered an experimental approach and used with caution. Releases from captive broodstocks must be scaled to a level appropriate to reduce ecological risks to the population as a whole.

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# QUANTITATIVE GENETIC CONSEQUENCES OF CAPTIVE BROODSTOCK PROGRAMS FOR ANADROMOUS PACIFIC SALMON (*ONCORHYNCHUS* SPP.)[1]

by

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Although many of these programs have involved salmonids, the focus of these reviews has been primarily on the use of quantitative genetic methods to increase aquacultural production or contribution to fisheries, both traditional purposes of artificial propagation.

The purpose of this review is to summarize current knowledge in the area of quantitative genetics related to the genetic consequences of captive culture programs for Pacific salmon (*Oncorhynchus* spp.), especially captive broodstock programs, and to identify issues in this area in need of further research.

Although the focus of this review is on the quantitative genetic management of Pacific salmon populations, most of the empirical work in quantitative genetics has involved plants and animals with short generation times or of widespread economic importance, primarily in agriculture. Consequently, the literature reviewed here encompasses a wide range of organisms. Nevertheless, these studies--as well as the relatively few studies involving salmonids that do exist--provide a foundation for developing future quantitative genetic research on Pacific salmon.

For this report, we outline prominent quantitative genetic risks associated with captive broodstock programs, discuss the importance of genetic monitoring for quantitative characters, and introduce some basic approaches to address these issues. The quantitative genetic consequences of these programs can be grouped into three main categories: 1) loss of genetic variability within a population resulting from the establishment of a captive broodstock, and the inbreeding depression that may result; 2) genetic change that may result from natural selection (domestication) on captive fish in protective culture; and 3) genetic divergence of the captive fish from their natural source population, and the consequences of genetic interactions between these groups.

## Quantitative Genetic Approaches to Genetic Inference

The approach to genetic analysis of variation in quantitative characters is affected by three common aspects of these characters: 1) their phenotypic expression is typically sensitive to environmental variation (Falconer 1989), 2) in general, they do not fall into discrete phenotypic classes but instead exhibit continuous phenotypic distributions (for "threshold" characters, such as disease resistance or migratory tendency, they are thought to have an underlying distribution of genetic effects that is approximately continuous; Falconer 1965, 1989), and 3) they usually appear to be under the control of several genes of generally unknown effect on the character (Wright 1968, Lande 1981).

Quantitative genetic investigations use statistical analyses of these characters to describe the composite behavior of the underlying genes. These analyses generally require an assumption of Mendelian inheritance at constituent loci and small, independent effects of many genes on the characters of interest (Bulmer 1985). With this assumption, these analyses use the phenotypic resemblance of individuals of known average relationship to permit inferences about the inheritance and evolution of quantitative characters, processes that are controlled by hidden variation in gene frequencies and effects (Falconer 1989). Through often elaborate statistical analyses, observed patterns of means, variances, and covariances in a population, when combined with appropriate breeding designs and statistical techniques, can be used to estimate the genetic parameters that determine the population's response to selection.

Unlike most molecular genetic analyses, which focus on traits controlled by a single gene or a few major genes, quantitative genetic analyses are generally incapable of detecting the effects of individual genes on the expression of quantitative traits. Despite this limitation, it is significant that genetic variation not detectable with molecular genetic analyses may be revealed with the appropriate quantitative genetic tool. Thus, molecular and quantitative genetic techniques detect different facets of the genome, a point worth remembering when considering different techniques for applications to problems in conservation biology.

The quantitative genetic approach is fundamentally one of partitioning observed variation into its genetic and environmental components. Because it does not focus on genes themselves, but rather on composite genetic and environmental effects on the phenotype, the power of this approach to resolve the details of genetic architecture is limited. Quantitative genetic approaches are therefore more synoptic and less mechanistic than molecular genetic approaches. Nevertheless, this quality of quantitative genetic analysis contributes to its suitability for investigating patterns of adaptive evolution and comparing them in populations that occur over broad environmental or geographic gradients. Such analyses are especially appropriate for investigating variation in life-history traits, which are known to be influenced strongly by both genetic and environmental variation.

The rapid development of molecular genetic techniques such as the analysis of restriction fragment length polymorphisms (RFLPs) might appear to offer an alternative to conventional

quantitative genetics for assessing genetic variation, but these methods can at best provide only part of the picture. For example, molecular analyses like these are not designed to detect the effects of environmental variation, or its interaction with genotypic variation, on the phenotype. Interpreting molecular genetic variation in terms of adaptive evolution is always problematic because the direct relationship between molecular variants and fitness differences is obscure (Eanes 1987, Houle 1989, Lewontin 1991, Avise 1994).

Quantitative genetic techniques also differ from molecular ones in that they are prospective rather than retrospective (Ewens 1979): quantitative genetic approaches generally focus on the potential evolutionary consequences of particular genetic states rather than on describing the genetic states that have resulted from past evolution. Thus, quantitative genetics can be useful for generating hypotheses for what the consequences of genetic change will be, at least in the short term.

Quantitative genetic approaches are not without potential problems. Although it is relatively straightforward simply to determine whether or not a character has a genetic basis (Lawrence 1984, Crow 1986, Falconer 1989), the estimation and interpretation of genetic parameters can be difficult and have some limitations. Most parameters that are estimated are specific to the population and environment in which they are measured. They are also sensitive to the influence of migration, mutation, selection, non-random mating, level of inbreeding, genotype hy environment correlation or interaction, and ecological or social factors (Barker and Thomas 1987). In addition, quantitative genetic estimates are based on an assumption that the underlying genes are unlinked structural genes, not modifiers.

Despite these limitations, quantitative genetic methods are the tools of choice to analyze the mechanisms of adaptive evolution. These methods are designed to measure the inheritance of life-history characters, to estimate their responses to evolutionaty forces (mutation, gene flow, genetic drift, and selection), and to identify the consequences of these responses for the adaptation and divergence of populations. There are several of these methods available to the experimental quantitative geneticist, and they can be organized into a few general categories defined by their respective objectives: 1) determine the inheritance of quantitative characters and the genetic basis of their phenotypic variation, 2) identify the mode of gene action affecting phenotypic expression within and among populations, 3) estimate the “effective” number of underlying genes, and 4) assess and predict the response to selection (and limits to this response). All of these objectives and their corresponding techniques have well-developed theoretical foundations and are based on analyzing patterns of observed variation within or among groups of individuals of known relatedness (Barton and Turelli 1989).

Thus, quantitative genetics has applications to a wide variety of genetic problems. The primary objectives of animal and plant breeders are to maximize a population’s response to selection in particular environments and to estimate “breeding values” (Falconer 1989) to predict and enhance this response. Evolutionary quantitative geneticists analyze pbenotypic variation within and among natural populations, both to understand its genetic basis and describe the

evolutionary mechanisms that produced its observed variation. Quantitative genetic methods have not yet been widely applied to the supplementation and conservation of natural populations, but much of what is known from evolutionary genetics and from applied breeding may be useful in increasing the prospects for success in these efforts.

## The Significance of Quantitative Genetic Variation

Evolutionary geneticists recognize four primary agents that affect gene frequencies and, hence, genetic variation: mutation, genetic drift, gene flow, and selection (Futuyma 1986, Hart1 and Clark 1989). Because the distribution and maintenance of genetic variation within and among natural populations is a fundamental problem in evolutionary genetics, the effects of these agents on patterns of genetic variation have received a great deal of theoretical and empirical attention (reviewed by Barton and Turelli 1989, Falconer 1989, Avise 1994). Traditional approaches to characterizing these patterns of variation have concentrated on single-locus polymorphism (Lewontin 1974, 1991) and have often neglected quantitative genetic variation. Quantitative genetic variation differs from single-locus polymorphism in that a distribution of genotypic values potentially exists at each locus; these values are usually assumed to be normally distributed (Kimura 1965, Lande 1976). Major changes in observed variation that affect adaptation are thought to result largely from polygenic variation rather than variation at single loci (Wright 1968, Lande 1981). For this reason, Lande and Barrowclough (1987) argued that quantitative characters and their inheritance should be considered distinct from single-locus characters (but see Orr and Coyne 1992 for a counterargument).

A common explanation for the observed maintenance of quantitative genetic variation in populations is a balance between the "forces" of mutation and selection. A variety of models have been advanced to describe the mechanism for this maintenance. Although these models take into account the incidence of mutation, strength of selection, and distribution of alleles at each locus, and assume polygenic inheritance (e.g., Lande 1976, Lynch 1984, Turelli 1984, Houle 1989), the true situation is probably more complicated. For example, other factors affecting genetic variation include phenotypic plasticity (Via and Lande 1985), frequency-dependent selection, spatial variation, and gene effects expressed through multiple characters (i.e., pleiotropy; Rose 1982). In addition, theoretical (Griffing 1960; Goodnight 1987, 1988; Lynch 1988; Gimelfarb 1989) and empirical (Wade and McCauley 1984, Bryant et al. 1986, Carson and Wisotzkey 1989, Cohan et al. 1989, Bryant and Meffert 1992, Hard et al. 1993) studies suggest that genetic variation may be redistributed into additive genetic variation under certain conditions (e.g.., sharp population bottlenecks).

The dynamics of genetic variation are potentially much more complex for quantitative characters under selection because quantitative characters are affected by many more genes than single-locus characters, whose dynamics are thought to be affected primarily by genetic drift. The consequences of neglecting quantitative genetics in the management or conservation of natural populations are not clear, but Bentsen (1991) has claimed that one possibility is that monitoring

only the "qualitative" genetics of a population may cause undesirable genetic change. For example, if populations are differentiated only by allele frequencies and not by the distribution of unique alleles, as is often the case among anadromous salmonid populations, the use of a low nutnber of breeders thought to represent a population's 'unique" genetic architecture may lead to a reduction in quantitative genetic variability, potentially eroding local adaptation.

Bentsen (1991) believed that the erosion of local adaptation in several Atlantic salmon (*Salmo salar*) populations could have resulted from relying solely on electrophoretic surveys for monitoring genetic variability in these populations. Other evidence suggests mat it may be easier to maintain quantitative genetic variation than single-locus variation (Lande and Barrowclough 1987, Bryant and Meffert 1992). Unfortunately, little explicit guidance is available for monitoring salmon populations to reduce genetic problems associated with interaction between hatchery and wild fish (Lande and Barrowclough 1987, Hindar et al. 1991; but see Hard, in press).

On the whole, fishery genetic researchers are not taking advantage of approaches being developed by evolutionary quantitative geneticists. Most fishery genetic work published to date has dealt with estimating levels of genetic variation within and among populations. While this objective is important, it ultimately prompts the question, What maintains or erodes this variation? Attempts to answer this question are not only of basic evolutionary interest, but impinge on issues essential to informed management and conservation.

## A Review of Applications of Quantitative Genetics to Problems in Salmon Biology

### The Components of Quantitative Variation

As explained above, quantitative genetics is used to address a variety of issues associated with inheritance and evolution. These include the distribution of genetic variation within and among populations and the consequences of mutation, gene flow, genetic drift, and selection for this distribution. In order to familiarize the reader with concepts and terms used in our review of empirical quantitative genetics, this section outlines a fundamental objective of quantitative genetics: characterizing the elements of phenotypic variation for quantitative traits. At its simplest level, this objective requires partitioning phenotypic variation into genetic and environmental components (Falconer 1989). For any quantitative trait, the relationship among these components can be expressed in terms of variances as follows:

$$V_P = V_G + V_E \tag{1}$$

where $V_P$ is the trait's phenotypic variance, $V_G$ is its genotypic variance, and $V_E$ is its environmental variance. Thus, this relationship can be interpreted as the phenotypic variation (i.e., measured or observed variation) in a trait that results from variation in both genotypes and environmental factors.

Based on what is known about each of these components, they can be further subdivided to yield a more realistic equation. The genotypic variance can be dissected into three parts that reflect three general types of gene expression:

$$V_G = V_A + V_D + V_I \tag{2}$$

where $V_A$ is the additive genetic variance, or variance due solely to the composite independent effects of genes exclusive of net directional dominance and other effects; $V_D$ is the dominance genetic variance, or that due to dominance effects within loci; and $V_I$ is the interaction (or epistatic) genetic variance, or that due to interactions among loci. This partitioning of genotypic variance reflects the influences that these different categories of allelic or genic variation may have on phenotypic expression.

Another term is usually added to Equation 1 to indicate the fact that genotypic and environmental effects on the phenotype are often not independent. This term, $V_{GE}$, reflects the genotype-environment interaction that can contribute to local adaptation, and renders a more realistic equation:

$$V_P = V_A + V_D + V_I + V_E + V_{GE} \tag{3}$$

This equation is widely used in quantitative genetics and is applicable as long as genotypic and environmental differences are uncorrelated (Falconer 1989).

Because most quantitative characters may be under the control of many genes with small phenotypic effects, it is typically impractical to identify either the location or the effect of individual genes. Quantitative genetic techniques attempt to characterize the composite behavior of a quantitative trait's underlying genes by analyzing two pieces of information: the statistical behavior of the observed trait, and the relationship between the individuals examined. The elegance of quantitative genetics is that the inheritance and evolution (in the very short term, at least) of such traits can be understood and, in principle, predicted with this information alone.

The basic metric used in quantitative genetics to depict the relative contribution of genetic and environmental variation to a trait's phenotype in a population is heritability. The origin of this term is unknown, but probably arose before Mendel (Bell 1977). It is currently used in one of two ways: broad-sense or narrow-sense. Broad-sense heritability (designated $H^2$) is used to estimate the ratio of all "genetic" sources of variance ($V_G$) to $V_P$. These sources include dominance, epistasis, and some specific environmental effects such as maternal and cytoplasmic effects (see Falconer 1989). Narrow-sense heritability (designated $h^2$) is used to estimate the ratio of $V_A$ to $V_P$. Both are ratios with scales from 0 (no "genetic" variance) to 1 (no "environmental" variance) (Figure 1). Narrow-sense heritability is a more reliable predictor of a trait's short-term evolutionary response than broad-sense heritability and, consequently, is more widely estimated in breeding programs. However, the broad-sense heritability is more appropriate when dealing with asexually reproducing organisms, in which additive and non-additive sources of genetic variance are not separable, or in circumstances where it is not feasible to implement breeding designs to estimate $h^2$.

A variety of methods exist to estimate $h^2$ and its analog for determining the genetic relationship between two traits, the additive genetic correlation, $r_A$ (Falconer 1989). Three basic techniques are commonly used: sib analysis, offspring-parent regression, and response to selection. The first two techniques use the relationship among relatives. When estimated from this relationship, the $h^2$ of a trait and the $r_A$ between two traits are ratios of covariances and variances. For example, using a conventional half-sib analysis (see Falconer 1989), $h^2$ of trait x is estimated as four times the ratio of the phenotypic covariance (cov) among half sibs (HS) to the phenotypic variance (var):

$$h^2 = \frac{4\ cov_{HS}(x)}{var(x)} \qquad (4)$$

The coefficient of 4 indicates the fact that half sibs have an average coefficient of relationship of 1/4 (i.e., share 1/4 of their genes).

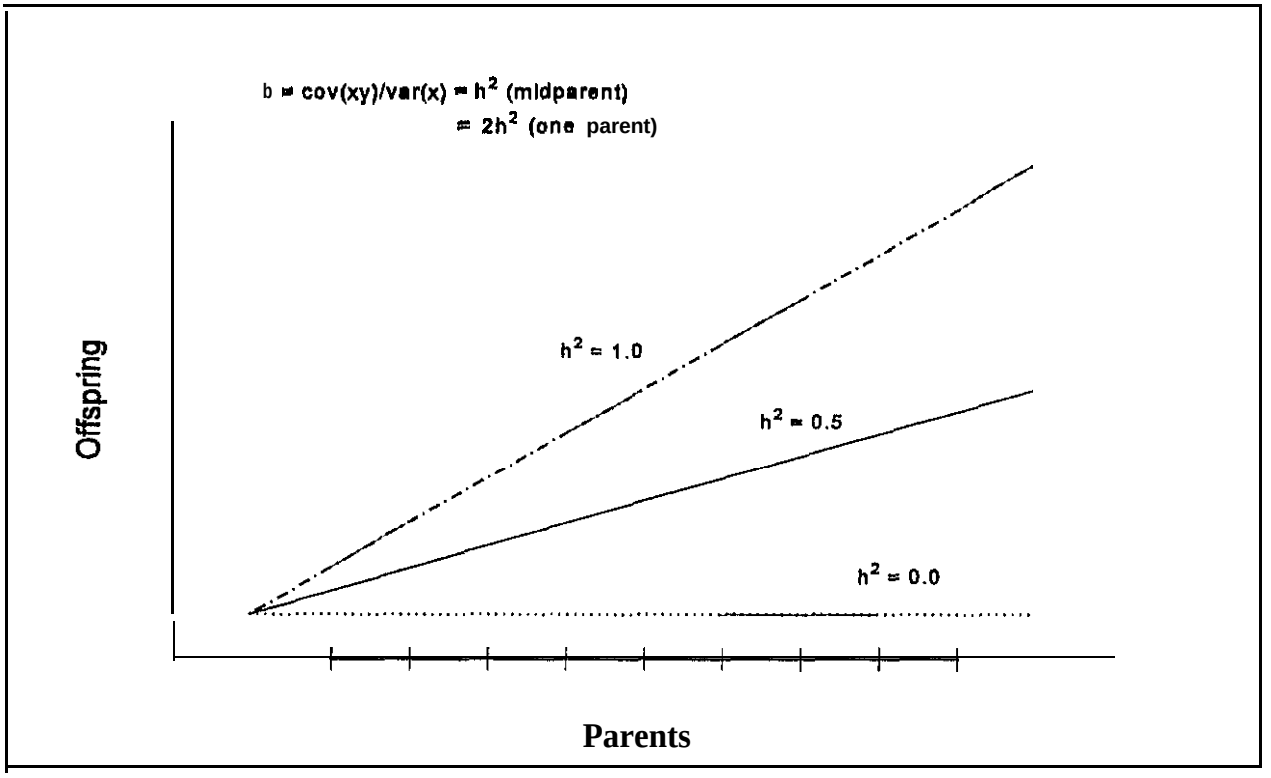


Figure 1. Graphical representation of estimating the heritability ($h^2$) of a quantitative trait by regressing offspring phenotypes on those of their parents. The relationship of the least-squares linear regression coefficient b to $h^2$ depends on which parents are used in the regression (Falconer 1989). The estimate of $h^2$ ranges from 0 (no genetic variance) to 1 (no environmental variance).

The $r_A$ between traits x and y is the ratio of their covariance to the square root of the product of their variances:

$$r_A = \frac{cov_{HS}(xy)}{\sqrt{var_{HS}(x)\ var_{HS}(y)}} \tag{5}$$

With offspring-parent regression, $h^2$ can be estimated directly from the slope of the regression line relating the trait in parents and offspring. If the regression is of offspring on midparent (parental mean), the slope estimates $h^2$; if the regression is of offspring on one parent, the slope estimates $1/2(h^2)$.

In terms of variances and covariances, the equations for $h^2$ and $r_A$ using offspring-parent regression are

$$h^2 = \frac{cov_{OP}(x)}{var_P(x)} \tag{6}$$

and

$$r_A = \frac{cov_{OP}(xy)}{\sqrt{cov_P(xy)\ cov_O(xy)}} \tag{7}$$

It should be noted that if the phenotypic variances in the two sexes are unequal, some adjustments to these equations are necessary (Falconer 1989).

The other primary method of estimating $h^2$ and $r_A$ relies on phenotypic responses to an applied amount of selection on a trait. This method is derived from the fact that the response of a trait to a precise amount of selection, when expressed as a ratio, estimates its heritability. The "realized" $h^2$ of trait x under selection is estimated from the breeder's equation (Falconer 1989):

$$h^2 = \frac{R}{S} \tag{8}$$

where R is the change in the phenotypic mean of x after selection (the response) and S is the difference in the mean of x between the unselected and selected groups (the selection differential). Figure 2 depicts graphically how realized $h^2$ is related to R, S, and trait means.

The genetic correlation between traits x and y, when selection is imposed directly on trait x, can be estimated from the equation

$$r_A = \frac{CR(y)\ var(x)}{S(x)\ var(y)\ \sqrt{h^2(x)h^2(y)}} \tag{9}$$

where CR(y) is the correlated phenotypic response in trait y to selection on trait x, and var (x) and var (y) are the phenotypic variances of the two traits (Falconer 1989).

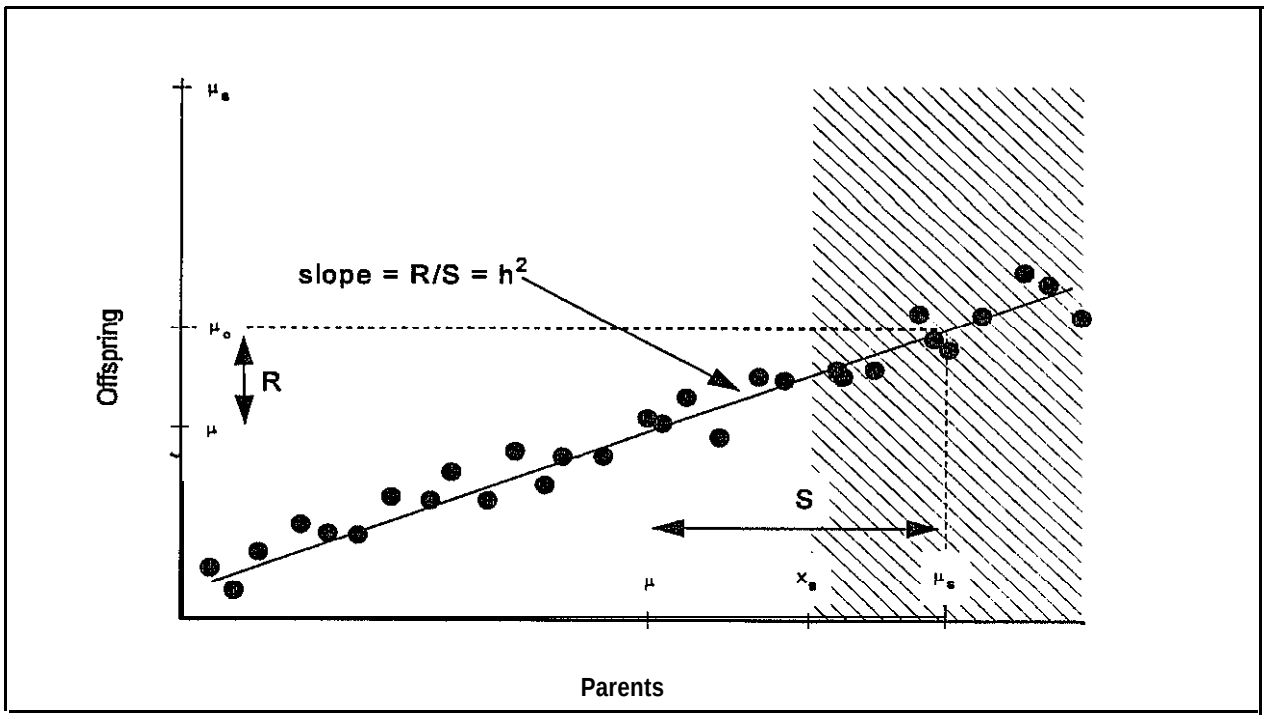


Figure 2. Graphical representation of truncation selection on a quantitative character and its phenotypic response. $\mu$ and $\mu_o$ are the respective trait means in parents and offspring before selection, $x_s$ is the threshold for selection (the cross-hatched area represents parental phenotypes allowed to contribute to the next generation, and $\mu_s$ is the phenotypic mean of the selected parents). The selection differential is $S = \mu_s - \mu$. $\mu_o$ is the phenotypic mean of the offspring of the selected parents, and the response to selection (R) is $\mu_o - \mu$. The realized $h^2$ of the trait is estimated from the slope of the regression line, which for this one-generation case is R/S. Note that as $h^2$ increases, $\mu$ approaches $\mu_s$.

The choice of technique depends on several factors, including the types of relatives that can be analyzed, the number of individuals available for analysis, and practical limitations on the design of the analysis. Each technique has peculiar strengths and limitations. More comprehensive discussions of these and other methods are presented by Becker (1984) and Falconer (1989). An introduction to some of the multivariate forms of these equations is presented by Lande (1982b, 1988b).

Heritabilities and genetic correlations are in principle simple to estimate, but they generally have such large sampling errors that the experimental requirements necessary to quantify them with reasonable precision can exceed the capacity of many facilities. Readers interested in experimental designs necessary to estimate these genetic parameters and their precision should refer to Kempthome (1957), Klein et al. (1973), Klein (1974), Becker (1984), and Falconer (1989).

Estimates of genetic parameters depend on gene frequencies as well as the environment in which they are measured, and, therefore, any particular estimate **is** accurate only for the population and environment from which it is estimated. Additionally, selection over even just a few generations can alter heritabilities (Hill 1972, Sheridan 1988, Falconer 1989). Nevertheless, despite the difficulties associated with estimating these genetic parameters and the limitations of inference based upon them, such estimates are the only means to quantify with reasonable precision genetic and environmental sources of variation and covariation in traits under selection in captive broodstock programs.

## Breeding Programs

**Specific phenotypic and** genetic objectives--Given the importance of quantitative traits and their analyses to plant and animal breeding programs, it is not surprising that most genetic research applied to fish has been directed toward aquacultural production. The major objective in conducting aquaculturally oriented investigations has been to develop populations of fish that express phenotypes or phenotypic traits leading to increased production efficiency and marketability. To accomplish this, 1) one to several traits are chosen such that their improvement will yield the maximum increase in production or marketability, 2) genetic parameters for these traits are estimated, and, based on the results, and 3) selection and breeding designs are implemented. While the details of such programs are often complex (Shultz 1985), the overall aim is to utilize available genetic variability to develop a population of fish with traits that enhance aquaculturalproduction.

**Genetic and environmental components of trait variation--*Growth and*** size. With increased production as the basis for the research conducted, several salmonid traits that contribute to aquacultural production have received major attention. Among the most prominent of these are growth and size. Growth and body size are important to successful commercial aquacuiture and are relatively easy to measure; consequently, the emphasis on these traits is not surprising. In general, analyses have shown that the genetic component defining size characteristics (typically, length or weight) is of a magnitude that a reasonable response to selection can be anticipated.

Many of these analyses have been summarized by Gjedrem (1983, Table III) and Tave (1993, Tables 4.1 and 4.5). Estimated heritability estimates ($h^2$) for weight or length range from about 0.10 to about 0.90, depending on the species and population from which the fish were derived. In most cases, $h^2$ for size measured early in the life cycle is fairly large (0.50-0.70) and decreases as the fish ages and approaches maturity. This could suggest that the genetic determination of size during the early pan of the life cycle is different than that for later in the life cycle. On the other hand, some results may be due to breeding designs that do not permit separation of maternal or common environmental effects from additive genetic effects. Research has shown that maternal effects on size are diminished at about 150 days after hatching in salmonids (Kincaid 1972), although in some species. environmentally induced size differences

expressed early in life can be maintained throughout the life cycle, at least in captive culture (Wohlfarth and Moav 1972).

***Reproduction.*** The next area of quantitative genetic research that has received major emphasis in salmonids is reproduction. Research on sahnonid reproduction can be divided into two groups of traits: maturation and egg production.

A phenomenon that is relatively common among fish species is precocious maturation. In Pacific salmon (*Oncorhynchus* spp.), precocious maturation is usually expressed in males, often referred to as "jacks." With the onset of maturation, growth is halted, and consequently, precocious maturation leads to the production of adult fish that are smaller (often dramatically) than those maturing at older ages. Because of the small sire and the deterioration in flesh quality (i.e., decrease in red coloration and softening of texture) brought on by maturation, precociously maturing fish are generally unmarketable and, from an aquaculture-production standpoint, result in a loss of investment and resources. On the other hand, precocious male maturation may be a valuable life-history trait for the viability of fish populations in the natural environment (Gross 1991). Thus, numerous studies have been conducted to attempt to identify the genetic and environmental components responsible for this phenomenon, especially in salmonids.

Much of the research on sexual precocity has been focused on captive populations, especially of Atlantic salmon, and the results have implicated both genetic and environmental influences (Piggins 1974, Bailey et al. 1980, Glebe et al. 1980, Saunders 1986, Rowe and Thorpe 1990, Herbinger and Friars 1992). Although infonnation for Pacific salmon is less complete, results from captive populations have also shown that genetic or environmental factors or both may be involved in determing the incidence of precocious male maturation (Garrison 197 1, Childs and Law 1972, Hager and Noble 1976, Bilton 1978, Hard et al. 1985, Heath et al. 1994). In both Atlantic salmon (Thorpe et al. 1983) and coho salmon, (0. *kisutch*) (Iwamoto et al. 1984), the incidence of male precocity in progeny sired by precocious males was 4 to 5 times higher than that shown in progeny sired by non-precocious males. Yet other studies to estimate the heritability of this trait have yielded low values for rainbow trout, (0. *mykiss*) ($h^2$ = 0.04-0.06), coho salmon ($h^2$ = 0.05), and Atlantic salmon ($h^2$ = 0.07-0.25) (Gjerde 1986, Silverstein and Hershberger 1992). Consequently, lowering the incidence of precocious males in these species will require exacting selection and breeding procedures, which may be slow to yield results. On the other hand, recent work with chinook salmon (0. *tshawytscha*), which generally mature at any of a wider range of ages, has yielded $h^2$ estimates of 0.30 to 0.50 for the same trait (Heath et al. 1994). Response to selection in this species should be somewhat more rapid, at least in some populations.

ln addition to genetic factors, some environmental factors have been shown to produce precocious maturation in such diverse species as brook trout (*Salvelinus fontinalis*), Arctic charr (S. *alpinus*), brown trout (*Salmo trutta*), rainbow trout (0. *mykiss*), and kokanee (0. *nerka*). These factors include high feeding rate (Bagenal 1969, Kato 1975, McCormick and Naiman 1984) and high temperature (Saunders et al. 1983, Papst and Hopky 1984, Crandell and Gall 1993a).

However, results from other studies are not in agreement with these conclusions. Iwamoto et al. (1984) showed that high temperature, while increasing the growth rate of coho salmon, did not produce a higher percentage of early maturing fish. Glebe et al. (1980) reported little difference in the proportions of precocious male Atlantic salmon raised at ambient and high temperatures. Nævdal (1983) reported faster growth of rainbow in full-strength seawater than in brackish water, but noted little difference in the maturity schedule of male fish. Similarly, Siitonen (1986) found more rapid growth of rainbow trout in brackish water than in fresh water, but a lower proportion of mature fish in brackish water. It would appear that fish of different strains have different capabilities for early maturation (Nævdal 1983) and that environmental factors will influence those fish in which sensitivity to external factors is high.

In addition to precocious maturation, the genetic component for "normal" age at maturity has been estimated for rainbow trout, Atlantic salmon, and chinook salmon. In rainbow trout and Atlantic salmon, Gjerde (1986) reported relatively high $h^2$ estimates for age at maturity (0.20 to 0.37), based on data transformed to a continuous scale. The estimates reported for chinook salmon were somewhat higher (0.4 to 0.6), although these results were obtained from selection response calculations (Hankin et al. 1993), which can differ substantially from estimates obtained from sib analyses or parent-offspring regression.

Within-season variation in spawning time has also been analyzed genetically in several species of fish, and this trait has been found to be highly heritable ($h^2 > 0.50$) (Campton and Gall 1988, Gall et al. 1988, Siitonen and Gall 1989, Crandell and Gall 1993a, Silverstein 1993). Thus, it would appear that the age at spawning and the timing of spawning within a season could both change rather rapidly with selection.

Obtaining large numbers of high-quality eggs at the right time of year is important for programming aquaculture production In addition, egg size has been correlated with hatching percentage in rainbow trout (Gall 1974), with growth between 1-4 months of age in that species (Chevassus and Blanc 1979, Springate and Bromage 1985), and with survival and growth in chinook salmon (Fowler 1972). Heritabilities for egg weight, egg volume and egg number have been estimated for rainbow trout and Atlantic sahnon (Gall 1975, Gall and Gross 1978, Haus 1984, Halseth 1984). The values are all fairly high ($h^2$ = 0.19-0.52) and suggest these traits could be altered relatively easily by selection. In fact, Gall and Huang (1988) developed a selection approach utilizing these traits for enhancing reproductive performance in rainbow trout.

Disease resistance. Another suite of traits important to aquaculture, although it has received relatively little attention, represents those involved in the inheritance of disease resistance. One of the earliest reports of successful selection with salmonid species was that of Embody and Hayford (1925), in which they demonstrated increased resistance to furunculosis (*Aeromonas salmonicida*) in brook trout. 'Subsequent genetic work on disease resistance has involved a variety of approaches (Chevassus and Dorson 1990). although relatively little research has emphasized the estimation of $h^2$ and the potential for response to selection. In fact, the only

other reported selection program to improve disease resistance in fish has involved infectious dropsy *(A. hydrophila) in* common *carp, (Cyprinus carpio)* (Schaperclaus 1962, Byassov 1987).

Considerable data on salmonids have demonstrated differences between populations in susceptibility to bacterial (Cipriano 1983; Wolf 1953; Ehlinger 1964, 1977; Snieszko et al. 1959; Suzumoto et al. 1977; Gjedrem and Aulstad 1974; Refstie 1982; Beacham and Evelyn 1992a), viral (Amend and Nelson 1977, McIntyre and Amend 1978, Silim et al. 1982, Okamoto et al. 1987) and parasitic (Zinn et al. 1977) infections. Published estimates of genetic influence on disease resistance range widely in magnitude. The heritability of resistance to bacterial kidney disease, caused by *Renibacterium salmoninarum* infection, was estimated at 0.00-0.38 in chinook salmon by Beacham and Evelyn (1992a).

However, Beacham and Evelyn (1992b) found that the heritability of mortality and time to death in chinook salmon challenged with *R. salmoninarum* was not different from zero. Heritabilities of these traits in coho and chum salmon (0. *keta*) were also low (Beacham and Evelyn 199213). The heritability of resistance to *Vibrio anguillarum* infection (vibriosis) was estimated at 0.11 in Atlantic salmon (Gjedrem and Aulstad 1974) and 0.00-0.13 in chinook salmon (Beacham and Evelyn 1992a,b), whereas the heritability of the same trait in chum salmon was estimated at 0.5 (Smoker 1981).

On the other hand, the heritability of susceptibility to the causative agent for furunculosis in Atlantic salmon was estimated at 0.48 (Gjedrem et al. 1991) and from 0.00 to 0.34 in chinook salmon (Beacham and Evelyn 1992a,b). Measurement of resistance to viral hemorrhagic septicemia (VHS) in rainbow trout yielded $h^2$ estimates of -0.10 to 0.30 (Kaastrup et al. 1991). Assessment of tolerance to infectious hematopoietic necrosis (IHN) virus in sockeye salmon (0. *nerka*) produced $h^2$ estimates of 0.27 to 0.38 (McIntyre and Amend 1978).

There are a number of reasons why the genetic basis of disease resistance and susceptibility has not received more attention (Chevassus and Dorson 1990). A major reason is lack of understanding of the mechanisms fish employ to resist infection, the interactions among these mechanisms, and their relationship to fish mortality. For example, a pathogen may not invade the fish because of a barrier or destructive effect presented by mucous, gastric, or intestinal secretions. Alternatively, a pathogen may penetrate but be inactivated by spontaneous mechanisms such as bactericidal serum effect; macrophage, complement, or killer cells; or induced mechanisms such as interferon or antibody production.

These agents represent numerous means through which genetic variability could impact disease resistance in fish. Refstie (1982) studied the development of serum antibodies to *V. anguillarum* in rainbow trout families after immunization and calculated heritabilities of 0.15 to 0.25. However, low antibody titers and the consequent inability to identify large variations make these estimates suspect. Genetic analysis of serum hemolytic activity in rainbow trout as a measure of natural immunological defense revealed high $h^2$ estimates of 0.34-0.96 (Røed et al. 1990). However. the relationship of this trait to disease resistance has not been clearly defined.

Recent quantitative genetic work on susceptibility of rainbow trout to the myxosporeau parasite *Ceratomyxa shasta* revealed several significant genetic components involved in the expression of susceptibility (Ibarra et al. 1994). Further, it appeared that the nature of the genetic causal components differed with time of exposure to the parasite.

As a consequence of this lack of basic information, there has been little progress on the use of genetics for improving disease resistance in fish. Instead, reliance on the use of antibiotic treatment and immunization to address disease problems continues.

***Body composition and flesh quality.*** A final set of traits that has direct application to salmonid aquaculture is that dealing with body composition and flesh quality. A number of studies have been conducted to estimate the genetic determination of the quantity of various biochemical compounds (e.g., proteins and lipids) in the flesh. Estimates based on proximate analyses of proteins, lipids, and moisture in the flesh of rainbow trout and coho salmon have yielded relatively low heritabilities (< 0.10) for percent protein and somewhat higher values (0.14-0.47) for percent lipid and percent moisture (Gjerde and Schaeffer 1989, Iwamoto et al. 1990).

Another quality that is important to the marketability of cultured salmon and trout is flesh color. Genetic analyses of flesh color in rainbow trout and coho sahnon have yielded $h^2$ estimates that would suggest a relatively rapid response to selection (0.27 and 0.30, respectively; Gjerde and Schaeffer 1989, Iwamoto et al. 1990). Work with chinook sahnon suggested the possibility of a rather simple genetic system defining whether or not the flesh contained red coloration (Withler 1986). Hard (1986) observed both strong genetic and environmental effects on flesh pigment coloration in chinook salmon reared in seawater netpens. The genetic basis of pigmentation intensity in this species is unknown but is probably polygenic. Research on Atlantic sahnon yielded low $h^2$ estimates (0.01) for meat color scores (Gjerde and Gjedrem 1984), suggesting that little change in the flesh coloration could be achieved in this species through selection.

Life ***history.*** In addition to work on performance traits that are of direct consequence to aquaculture production, a number of studies have been conducted to estimate genetic parameters on traits that could be termed “adaptive” (Table 4.1, Tave 1993). These include such characteristics as incubation performance, early development traits, survival, and behavior. Without going into detail on each of these traits, there are two generalizations that can be made from the data obtained from studies on sahnonids.

First, traits associated with incubation, hatching, and early development exhibit a wide range of genetic underpinnings. Analyses of hatching rate (McIntyre and Blanc 1973. Beacham 1988. Sato and Morikawa 1982) and hatching time (Sato 1980, Sato and Morikawa 1982) have yielded relatively high heritabilities. On the other hand. most traits associated with survival during early development exhibit rather low heritabilities (< 0.20) (Kanis et al. 1976, Robison and Luempert 1984, Withler et al. 1987, Sato and Morikawa 1982, Beacham 1988). In addition, most

alevin and fry body traits (e.g., yolk weight, alevin weight and length, and fry weight and length) exhibit relatively low heritabilities (Withler et al. 1987, Beacham 1988). Since the early survival and physical characteristics of alevins and fry have been shown to be strongly influenced by the physical characteristics of the eggs (Fowler 1972, Gall 1974, Chevassus and Blanc 1979, Springate and Bromage 1985), a large amount of additive genetic variability (expressed by a high $h^2$) should not be expected for traits measured during early development.

In fact, Beacham (1988) concluded from his results that increased survival rates for the embryos and alevins would be more readily obtained by providing suitable environments than by selection or crossbreeding. By contrast, Withler et al. (1987) detected genetic variability in early developmental traits among three strains of chinook salmon; for example, $h^2$ estimates for survival of uneyed eggs varied from 0.0 to 0.21 among the strains. Additional investigations are needed to better define the contribution of genetics to phenotypic expression during early development.

Second, where genetic analyses have been conducted over different environmental conditions there appeared to be a fairly strong interaction between genotype and environment. Beacham (1988) identified the genetic determinants of early developmental traits in pink (0. *gorbuscha*) and chum salmon under three different temperature regimes (3°, 8°, and 16°C) and found that heritabilities varied in different ways with temperature. For example, the $h^2$ estimate for alevin tissue weight varied from 0.06 at 3°C to 0.45 at 16°C, while the $h^2$ estimate for hatching time was highest at 3°C (0.52) and lowest at 8°C (0.30). Studies of hatching time in steelhead (0. *mykiss*) demonstrated that the $h^2$ estimate differed depending on the type of container in which eggs were incubated (McIntyre and Blanc 1973). In addition, although the results vary, there may be greater additive genetic variance (i.e., higher $h^2$) for traits associated with survival when salmonids are raised under adverse conditions. Assessment of brown trout egg survival in acidic water showed $h^2$ estimates of 0.27 to 0.33 (Edwards and Gjedrem 1979). Estimates of $h^2$ for survival of Atlantic sahnon in acidic water varied with the length of exposure, but were relatively high (0.29 to 0.72; Schom 1986).

***Inbreeding and inbreeding depression.*** Inbreeding and inbreeding depression have been topics of considerable concern to fishery geneticists. Inbreeding can be defined as the mating of individuals more closely related to each other than to individuals chosen at random from the population (Wright 1978, Gall 1987). Related individuals have one or more common ancestors and, therefore, may have received one or more identical genes. The measure of inbreeding, designated the inbreeding coefficient (F), is the probability that two alleles at a common locus are identical copies of an ancestral allele (Falconer 1989). Inbreeding can occur in populations either as a directed or a random process, and it can occur in large or small groups of animals. The basic impact of inbreeding on the genetic constitution of a population is an altered distribution of Genotypes toward fewer heterozygotes and more homozygotes. This distribution leads to the "unmasking" of deleterious recessive alleles and reduces the frequency of genotypes expressing codominance and overdominance.

Often, the result of increased inbreeding is a decrease in mean phenotypic value of one or more traits with respect to fitness, a phenomenon known as inbreeding depression. Inbreeding depression is commonly measured by comparing the average phenotypic values between an inbred population and the base population from which it was derived (Gall 1987). The traits often most strongly affected by inbreeding are those connected with reproductive capacity (e.g., fecundity, egg size, hatchability) or physiological efficiency (e.g., growth rate, feed conversion efficiency, survival) (Fakoner 1989).

In general, inbreeding depression tends to increase in proportion to the inbreeding coeffkient during the early stages of inbreeding. However, as Kincaid (1983) pointed out, when the impacts of inbreeding on viability and survival traits become severe enough to result in the actual loss of inbred lines, the relationship between inbreeding depression and the inbreeding coefficient is often unpredictable.

Because the exact level of inbreeding in a population is usually impossible to measure, inbreeding in practice is measured relative to the initial level in the source population (or assumed to be initially zero; Lande and Barrowclough 1987). A major problem when investigating inbreeding and its effects in populations of fish is that information on the breeding history is generally inadequate to determine even the initial level of inbreeding. Genealogies are rarely known for fish populations. This precludes the use of pedigrees for calculation of inbreeding coefficients (Gall 1987), which requires definition of the relationship of individuals over generations and the ability to follow and analyze a pathway of inheritance (Wright 1969). Consequently, estimates of inbreeding are most often based on the number of breeding individuals in the population. The generalized formula for making this calculation with a random mating population is

$$\Delta F = \frac{1}{2N} \qquad (10)$$

where AF is the expected increase in the inbreeding coefficient per generation and N is the number of individuals that mate to produce the next generation.

Under non-ideal conditions (e.g., non-random mating, different numbers of each sex, or greater than binomial variation in family size) the "effective" number of individuals contributing to subsequent generations can be substantially lower than the census number. This effective number of breeding individuals ($N_e$) can be estimated by several methods (Fakoner 1989) and is substituted for N in the above equation to determine the rate of increase in inbreeding. The major problem with this approach to calculation of the inbreeding coefficient is that the estimates of inbreeding are highly sensitive to departure from non-ideal conditions, especially variation in family size. Consequently, values estimated by this procedure yield overestimates of the actual inbreeding rate after the first generation, and the overestimation increases as the effective population size decreases.

Additional factors that complicate accurate estimation of inbreeding include overlapping generations and nonrandom mating (as well as mutation, gene flow, and selection). Also, estimates using population size (N or $N_e$) yield an average rate of inbreeding over the population and do not provide information on individual fish. Finally, these estimates depend on the assumptions that population size is stable between generations and that mating is random.

Nevertheless, in the absence of records on breeding practices and pedigrees, this method of approximation provides a valuable estimate of the rate that inbreeding accumulates in a population. The calculated AF value for each generation can be added to the inbreeding coefficient of the previous generation to obtain an estimate of the current level of inbreeding (Falconer 1989). Falconer (1989) discusses this topic in detail and provides alternative formulae for estimating levels of inbreeding when population size or family size vary or when generations are not discrete.

Results from studies on the effects of inbreeding in fish have shown that increasing the level of inbreeding in a population yields reduced performance in a variety of traits. The equivalent of one generation of brother-sister mating (i.e., full-sib mating; AF = 0.25) led to an increase in the occurrence of fry deformities in rainbow trout (Aulstad and Kittelson 1971). Results from this same level of inbreeding in brook trout demonstrated a decrease in body weight of 27.7% after 7 months and 34.4% after 19 months (Cooper 1961).

Longer-term studies have permitted estimates of changes in traits with increased inbreeding. Bridges (1973) reported depression estimates of 5.1% in fish weight and 0.4% in formalin tolerance at 150 days of age in rainbow trout with each 10% increase in inbreeding. Gjerde et al. (1983) reported that three generations of inbreeding in rainbow trout led to increases of 2.5% in eyed egg mortality, 1.9% in alevin mortality, and 3.2% in fry mortality. They also found decreases of 3.0% in fingerling growth and 5.1% in growth to 18 months in seawater per 10% increase in inbreeding. One problem with these results is that increases in inbreeding were achieved by use of sequential generations, which necessitates comparing data on trait performance over environmental conditions that are not exactly comparable. This method interjects error in the estimates of inbreeding depression that is difficult to quantify.

To address this problem, Kincaid (1976a,b) designed an experiment that allowed comparisons between rainbow trout families with different levels of inbreeding, as well as comparisons between related but outbred families, in a single year. Analyses of the phenotypic values for a number of traits expressed with the equivalent of one and two generations of full-sib matings (AF = 0.25 and 0.375, respectively) revealed that inbreeding depression increased with the level of inbreeding. At inbreeding coefficients of 0.25 and 0.375, respectively, fry defonnities increased 37.6% and 191% over outbred levels. Also, feed conversion efficiency decreased by 5.6% and 14.9%, fry survival declined by 19.0% and 29.7%. and fish weight at 147 days and 364 days of age decreased by 11.0% and 13.4% (AF = 0.25) and 23.2% and 33.5% ($\Delta F$ = 0.375).

Subsequent work using this same design to investigate field performance and later growth and reproductive traits revealed lower fishery recovery with inbred groups, resulting from lower rates of survival and growth (Kincaid 1983). Additionally, the total weight of eggs produced decreased with increased levels of inbreeding, probably resulting from the smaller size of inbred females. Lower performance in the natural environment was also shown in Atlantic salmon by lower recapture frequencies for members of inbred families (Ryman 1970). Thus, inbreeding can impact all phases of the life cycle and the deleterious effects appear to be, to a large degree, linear with increases in the level of inbreeding.

There are several precautions that need to be considered when dealing with inbreeding and its phenotypic effect, especially on animals with little or no history of domestication. First, accurate prediction of the response of a particular trait to increased levels of inbreeding may not be possible. For most quantitative traits, little is known about the number of genes that define the traits, the frequencies of these genes in the population, or the relationships between the structure (e.g., linked vs. independently segregating) and expression (e.g., additivity vs. dominance) of these genes. Since inbreeding affects the genotypic distribution within the population, the frequency, arrangement, association, and interaction of genes determining a trait are all critical to any change in phenotypic expression. In addition, environmental factors play a major role in expression of quantitative traits and may act to "buffer" otherwise negative changes in genotypic distribution.

Second, fish populations with little history of domestication could exhibit stronger responses to increased levels of inbreeding (i.e., inbreeding depression) than those with some history of controlled breeding. One predictable result from increased levels of inbreeding is the unmasking of deleterious recessive alleles via increased homozygosity and the potential for elimination of these alleles from the population. The unmasking and elimination of these alleles may have fewer opportunities to occur under natural circumstances and, thus, inbreeding depression levels may be somewhat higher as an initial response to an increase in inbreeding.

Finally, changes in the phenotypic expression of a trait with increased levels of inbreeding are a consequence of a number of genetic factors. Perhaps the most important to consider is selection, although other elements (e.g., migration, mutation, drift) may play a role. Although natural selection acts to eliminate the less fit genotypes produced by inbreeding, it may not be sufficient to offset inbreeding depression. However, a change in selection pressures, such as might be expected in the initial phases of domestication, can counteract the effects of inbreeding and result in weaker inbreeding depression. There is some evidence for this effect in domesticated animals (Falconer 1989). These considerations lead to the conclusion that assessment of the effects of inbreeding must be approached very carefully and with an appropriate experimental design (Gall 1987).

## Utility and Reliability of Genetic Estimates

In attempting to interpret these data, it must be remembered that heritability is calculated as a proportion of the total phenotypic variance and that its magnitude for a specific trait in a specific population wilI be strongly influenced by nongenetic variation. Allendorfet al. (1987) pointed out that while fish species demonstrate a large amount of phenotypic variation (as measured by coefficient of variation), comparison of heritability estimates for similar traits with other animal species reveals smaller values in general. This could be explained by greater fish susceptibility or responsiveness to environmental factors mediated by 1) poikilothenny, 2) indeterminate growth, and 3) flexibility of age and size at sexual maturation.

Estimation of environmental influence on quantitative traits can be achieved from results of studies analyzing the magnitude of genotype-environment interactions. These interactions can be defined as the consistent variation in phenotypic expression of specific genotypes under different environments. These interactions are usually estimated by analyzing the phenotypic response of organisms with the same or similar genotype(s) under different environmental conditions.

For example, Donald and Anderson (1982) found that 72% of the total variation in weight of 2-year-old rainbow trout from a strain stocked in mountainlakes could be attributed to stocking density and overall productivity of food organisms, i.e., environmental variation. In another study, Ayles and Baker (1983) determined that growth differences among rainbow trout strains stocked in central Canadian lakes were primarily due to lake-to-lake variability. Under more controlled culture conditions, Atlantic salmon strain (genetic) differences accounted for only 6.4% and 7.0% of the total phenotypic variance in body weight and body length, respectively (Gjerde and Gjedrem 1984).

In addition, Gjerde (1986) found large variation in percent immature fish in comparisons between fish from the same full-sib families (families of fish sharing the same parents) reared on different fish farms, fish from different year classes reared on the same farm, and full-sib fish reared in different cages on the same fann. Further, in analyses of results from rainbow trout grown under various combinations of density and feeding regimes, environmental factors accounted for more than 40% of the total variance in weight (lwamoto et al. 1986). Consequently, it is critical that environmental factors be estimated when attempting to interpret quantitative genetic influence on phenotypic variation.

How satisfactorily $h^2$ estimates explain the genetic component of phenotypic variation for-a trait can be determined only through a directed selection program that assesses the magnitude of actual response. There have been few reports on directed selection progrsms of sufficient length to test the predictions of quantitative genetic analyses with fish (Hershberger 1993). Those that have been conducted beyond one or two generations have demonstrated the efficacy of selection programs to develop strains of fish with improved aquaculture characteristics.

Probably the most extensive program with this purpose is in Norway, where stocks of Atlantic salmon are being developed for aquaculture in marine net pens (Gjedrem et al. 1987). There are also programs in Israel and Hungary for the development of carp stocks for pond rearing (Moav and Wohlfarth 1966, Bakos 1976). In the United States, several programs have been conducted with sahnonids that have applications to various sectors of the aquaculture industry (Donaldson and Olson 1957, Gall and Gross 1978, Hershberger et al. 1990). Results in all of these studies have demonstrated that selection can have major effects on phenotypic traits in fish, although not all studies were based on prior quantitative genetic analyses or had appropriate experimental designs for estimation of genetic influence.

Where selection programs with fish have been conducted based on a priori genetic heritability estimates, the realized estimates have generally corroborated estimates made before initiating selection (see Sheridan 1988 for counterexamples from other domesticated organisms). For example, programs conducted to increase size-related traits in salmonids have shown that $h^2$ estimates are adequate predictors of the gains that can be realized (Gjedrem et al. 1987, Hershberger et al. 1990). Not surprisingly, however, work with other fish has demonstrated that the results are dependent on the species and strain being analyzed and tested.

Study of the common carp has demonstrated an asymmetrical response to selection (Moav and Wohlfarth 1976): no response was realized in selection for larger fish, but a response was apparent when selecting for smaller fish. Also, a positive response was reported for divergent selection on body weight in the Tifton strain of blue tilapia, *Tilapia aurea* (Bondari et al. 1983), whereas little response to selection on this trait was realized in either the Ivory Coast strain of *T. aurea* (Tave and Smitbennan 1980) or the Ghana strain of Nile tilapia, *Oreochromis niloticus* (Hulata et al. 1986).

These differences have been attributed to a lack of genetic variability in these latter strains, due to either a long history of selection or a small founding population size or both. Although many other explanations can be cited for either an asymmetrical response to selection or a lack of response (Falconer 1989), the exact mechanism will be a function of the genetic composition of the population of fish being investigated.

A final area where breeding programs have been valuable in understanding quantitative genetic variation is in characterizing genetic relationships among traits. These relationships are estimated by analyses of correlations between traits and, depending on the experimental design, can be defined on a phenotypic or genetic basis (Falconer 1989). A correlation between traits. whether phenotypic or genetic, is a measure of rhe proportion of observed covariance between them, scaled by the square root of the product of the trait variances. It is typically calculated from variance and covariance components in an analysis of variance.

Estimation of the genetic correlation differs from that of the phenotypic correlation in that its computation requires knowledge of the genetic relationship of the individuals that the variance and covariance components are estimated from (see Equation 5). Clearly, given the number of

traits that have been analyzed in fish, delving into the variety of combinations of traits that may exhibit genetic or phenotypic correlations is a complex undertaking (see Table 4.5, Tave 1993). Nevertheless, there are several generalizations apparent from the results that have been obtained.

First, in most investigations with fish it has been difficult to obtain accurate estimates of relationships between traits other than phenotypic correlations between measurements of the same trait made at different points in the life cycle. Until recently (with the development of the Passive Integrated Transponder (PIT) tag) it has not been possible to ensure that measurements at different stages of the life cycle were conducted on the same animal. Without this capability there is an unknown quantity of environmental influence on the results, and thus potential bias in genetic correlation estimates.

Second, correlations between measurements made on the same body-size trait at different times decrease with age. For example, in rainbow trout the phenotypic correlation between length at 6 months and length at 12 months was 0.81, whereas length at 6 months and length at 30 months exhibited a correlation of 0.06 (Møller et al. 1979). Estimations of correlations between lengths at intermediate points in the life cycle were between these two values. Consequently, it would seem that either different genes affecting size are expressed at particular ages or environmental factors are exerting cumulative influence over time.

A third general observation is that genetic correlations between traits associated with body size (e.g., weight and length) and between these traits and other morphological measurements at a particular life cycle stage can be rather high (> 0.80). For example, the genetic correlation between length and weight in coho sahnon at 84 days post swim-up was reported to be 0.95 ± 0.04 (Iwamoto et al. 1982). In Atlantic salmon, the genetic correlation between these same two traits at two years of age was reported to be 0.99 ± 0.01, and the genetic correlation between body length and pectoral fin length at 1 year was 0.95 (Riddell et al. 1981). Thus, it appears that there is a strong pleiotropic effect of genes associated with morphological size.

Finally, it appears that body-size traits are fairly strongly correlated with some prominent life-history traits. Gall (1975) and Huang and Gall (1990) obtained some relatively high genetic correlations between female body weight and egg volume (0.37-0.47); these and other data suggested that body size has evolutionary implications for reproductive performance. For pink salmon, Beacham and Murray (1988) obtained genetic correlations in excess of 0.90 for the relation between 315- and 500-day body weight and the gonadosomatic index in males and females. In coho salmon, Saxton et al. (1984) found that the best predictor of successful transfer to saltwater was body weight at transfer. For rapidly growing coho sahnon raised in freshwater. moderate phenotypic correlations were estimated between precocious maturation and body length (0.45) and weight (0.47) (Silverstein and Hershberger 1992). Consequently, sahnonids appear to exhibit a fairly strong genetic and phenotypic relationship between body size and life-history traits. at least in an aquacultural setting.

This brief consideration of results from breeding programs indicates that information on genetic variation in quantitative traits in salmonids has been directed primarily at traits affecting aquaculturalproduction.For example, our understanding of the quantitative genetic basis of life-history variation is meager. However, new developments in technology, such as molecular genetic markers and PIT tags or other nonlethal tags that identify individuals, will undoubtedly allow increases in the type and number of traits analyzed and in the breadth of experimental applications.

### Mixed-Stock Management

Quantitative genetic analyses as a source of information for assisting stock identification of salmon in mixed-stock fisheries are problematic because unequivocal identification of component populations is a management requirement. Given the synoptic nature of the statistical analyses used for quantitative traits and the interplay of environmental influences and genetics in the expression of phenotypic differences, the results are usually not definitive enough for effective mixed-stock management. The integration of molecular and quantitative genetic methods may be useful in providing a means to identify the genes responsible for the determination of quantitative traits affecting performance or fitness (Paterson et al. 1988, Lander and Botstein 1989, Andersson et al. 1994). However, this approach has limited ability to explain the genetic basis of quantitative traits because it relies heavily on the analysis of single loci with large effects on the character.

Nevertheless, there are at least two important applications of quantitative genetic analyses to management of mixed-stock fisheries. First, identification of quantitative genetic differences between groups of animals can provide a basis for separate management of groups that had been considered genetically homogeneous. For example, Gharrett and Smoker (1993) identified pink salmon subpopulations within a stream by their temporal segregation in adult return timing. Other measures of genetic differentiation (e.g., allozyme electrophoresis) did not separate these two groups, and genetic variability in return timing apparently was the factor responsible for their divergence. These results are consistent with a hypothesis of finer population differentiation at the level of life history (quantitative trait loci) than at neutral genetic markers (single loci) (Utter et al. 1993).

The second application is the identification of changes that may result from the selective effects of management activities such as fishing regulations. Many of the restrictions intended to control harvest are selective on fish populations by the way they are practiced. For example, Burgner (1963) demonstrated that the selective effects of the gillnet fishery on Bristol Bay sockeye salmon could decrease the size of a population and skew the sex ratio. Alexandersdottir (1987) suggested that the run timing of southeastern Alaskan pink salmon was altered as a result of directed fishing.

There have been few analyses of such effects on anadromous salmon that utilize a quantitative genetic approach. Ricker (1981) analyzed a large data set on body size collected

over a number of years from different typos of fisheries to estimate a realized heritability for this trait on the basis of realized response to selection. Results from analysis of body size (weight) data on pink salmon yielded a heritability estimate that was very close to similar estimates for other fish species ($h^2$ = 0.22-0.33). His interpretation of this result was that selection imposed by gillnet, troll and seine fisheries was a major cause for the observed decrease in body size of pink salmon in British Columbia. Hankin et al. (1993) used a similar approach to estimate, after a single generation, the realized $h^2$ of age at maturity ($h^2 \approx$ 0.4-0.6) in hatchery chinook salmon harvested in troll fisheries. Their result indicates a strong potential for this character to decline under exploitation by size-selective (and, hence, age-selective) fisheries. Since many life-history traits in fish populations are quantitative in nature, it seems prudent to emphasize the quantitative genetic analysis of these traits.

## Genetic Conservation

Quantitative traits are the major genetic determinants of the adaptive capability of a population, and their genetic architecture constitutes a fundamental constraint on the population's evolutionary pathway. Consequently, the monitoring of these traits should be an integral part of a genetic conservation program. However, while much evidence suggests substantial genetic determination of adaptive traits, there have been few attempts to estimate the magnitude and type of genetic determinants underlying these traits. The value of these estimates would reside in 1) better understanding of the genetic and environmental basis of variation in adaptive traits and 2) prediction of the evolutionary response of these traits to selection or other factors. One problem of growing interest to salmon geneticists and managers that such estimates could provide a better understanding of is the genetic consequences of artificial propagation for hatchery and natural populations.

**Genetic concerns in artificial propagation--There** is evidence that quantitative traits affecting adaptation in salmonids can change under human influences, including harvest practices, habitat alteration, and artificial propagation (as in conventional hatcheries or in captive broodstock programs Until recently, the effects of artificial propagation on adaptation of salmonids in the wild were not widely appreciated. In the last 10-15 years, however, many authors have pointed to genetic problems that can arise during salmon artificial propagatiou (Krueger et al. 1981, Reisenbichler and McIntyre 1986, Allendorf et al. 1987, Lichatowich and McIntyre 1987, Nelson and Soulé 1987, Lannan et al. 1989, Hindar et al. 1991, Waples 1991a). Much of this attention has been sparked by declines in productivity (returns per spawner) of hatchery populations.

To address some of these problems, a number of authors have drafted documents in an attempt to guide hatchery managers in certain areas of genetic management; these authors include Hershberger and Iwamoto (1981), Hynes et al. (1981). Krueger et al. (1981), Kincaid (1983), Davis et al. (1985), Allendorf and Ryman (1987), Kapuscinski and Jacobsen (1987), and Simon (1991). Almost without exception, the detailed guidelines developed and recorded by these

workers focus heavily on the maintenance of a large effective breeding population size (NJ, the monitoring and control of sex ratio, and broodstock sampling.

The immediate genetic concerns of artificial propagation for conservation may differ substantially from those of artificial propagation for enhancement and mitigation. For example, minimizing the genetic differentiation of hatchery fish and the natural fish they are intended to supplement can be of equal concern to maximizing genetic variability in the hatchery population in a conservation program. However, few of the publications that address the genetic management of threatened or endangered populations of fishes, including papers by Meffe (1986), Nelson and Soul6 (1987). Kapuscinski and Phillip (1988), Johnson and Jensen (1991), and Ryman (1991), have explicitly made this point. Much of this work has also failed to stress the importance of minimizing unintentional selection (Tave 1993) during captive culture.

Lichatowich and Cramer (1979) surveyed natural variation in a number of life-history characters in several populations of Pacific salmon They argued that investigators interested in tracking natural variation should monitor traits that have a strong influence on survival rather than survival or abundance directly, because natural variation in survival and abundance may be large enough to preclude detecting effects of hatchery practices on these traits in natural populations, at least over the short tenn. Lichatowich and Cramer (1979) based their recommendation on the greater statistical power to detect changes in traits such as growth rate, age and size at outmigration and return, migration timing, and spawn timing. These are precisely the kinds of traits that are amenable to quantitative genetic analysis.

Interest in the use of artificial propagation as a tool to assist in the recovery of threatened or endangered populations has grown dramatically in the past few years. Reviews of the salmon supplementation literature (Bakke 1987, Miller et al. 1990, Steward and Bjomn 1990, Cuenco 1991, Cuenco et al. 1993) have identified several areas where deficiencies make it difficult to determine the genetic consequences of supplementation for natural populations. These deficiencies generally fall into two categories: infomration on levels of genetic variation for traits important to reproductive success in the wild, and knowledge of the effects of supplementation on this genetic variation.

Concern about the uncertainties associated with supplementation of natural salmon populations with artificially propagated fish has produced a flurry of recent research intended to guide the use of this technique (e.g., Busack 1990, Kapuscinski et al. 1991, Hard et al. 1992a, RASP 1992, Kapuscinski and Miller 1993, Lichatowich and Watson 1993). Recommendations of this research have focused on ways to reduce the genetic risks associated with supplementation.

Unfortunately, while these guidelines have often provided specific information on methods to minimize the random loss of genetic variation, they have generally failed to recommend specific ways to minimize genetic change that may arise during captive culture through the directional process of selection. Such guidelines must often be constructed from infonnation in the quantitative genetics and animal breeding literature, which may not be easily accessible to fisheries

biologists with little or no genetics training. The texts by Kempthorne (1957), Turner and Young (1969), Mather and Jinks (1982), Becker (1984), and Falconer (1989) are prominent storehouses of this information.

**Inbreeding and loss of genetic variability during supplementation**--Recently, Ryman and Laikre (1991) pointed out that, in a supplementation program, the effective size of the hatchery-wild system as a whole is a more important consideration than the effective size of either component separately. They showed that differentially enhancing only part of the gene pool of a population through artificial culture (as might easily occur in a captive broodstock program) can lead to higher levels of inbreeding and loss of genetic diversity in the overall population. However, Ryman and Laikre's study assumed discrete generations and considered only a single generation of enhancement.

In a study conducted as part of a genetic monitoring and evaluation program for Snake River chinook salmon and steelhead (BPA Project 89-096), Waples and Do (in press) evaluated more fully the Ryman and Laikre effect in age-structured Pacific salmon populations. Waples did Do used computer simulations to model the level of inbreeding in the hatchery-wild system as a whole and how this level is affected by various types of captive broodstock programs. Three scenarios were considered in the simulations: control (no supplementation), increase (population increases through supplementation and remains large), and crash (population temporarily increases but declines after supplementation ends).

Results were summarized in terms of the parameter ΔIBD, which represents the change in level of inbreeding in the postsupplementation population compared to the control. Waples and Do (in press) found that: 1) The single most important factor affecting ΔIBD was whether the increase in population size was sustained. Compared to the control, higher levels of inbreeding were found under the crash scenario and lower levels were found under the increase scenario. 2) The absolute number of wild adults taken for broodstock had a stronger influence on ΔIBD than did the proportion of the population sampled. 3) In both the crash and increase scenarios, over 99% of genes in the postsupplementation population could be traced to hatchery fish. In the crash (but not the increase) scenario, broodstock taken in later years of the program dominated the final genetic makeup of the population. 4) ΔIBD was higher if broodstock collection lasted longer than l-2 generations, but the increase was not linear and there were some exceptions to the trend. 5) Marking hatchery fish so that they could be avoided in subsequent broodstock collections postponed further increases in ΔIBD but did not prevent them altogether. Furthermore, appreciable reductions in ΔIBD occurred only when the proportion marked was nearly 100%. 6) Captive breeding and rearing strategies such as sib-avoidance mating and equalizing progeny number generally had little effect on ΔIBD.

These results should be useful in evaluating genetic risks of captive broodstock programs and in developing programs to minimize these risks. However, although Waples and Do (in press) attempted to provide a comprehensive evaluation of the Ryman-Laikre effect on captive broodstock programs for Pacific salmon, it was not possible to consider every possible

combination of parameter values. Similarly, there were a number of factors not considered in their study (e.g., selective changes due to the culture environment and fitness consequences of particular values of ΔlBD) that should also be evaluated in deciding whether or how to implement captive broodstock programs.

**Quantitative genetic issues in supplementation**--Research to guide the use of artificial propagation for supplementation of natural salmon populations has generally provided few details about how supplementation should be monitored and about which traits should be monitored closely. The lack of guidance on how to detect, monitor, and respond to the effects of selection in hatchery fish undoubtedly has resulted largely from uncertainties about how adaptation operates in novel environments. This process ***is a*** topic of active research in evolutionary genetics (Bryant et al. 1990, Holloway et al. 1990, Allendorf 1993, Frankham et al. 1993).

As the previous review of quantitative genetic studies indicates, most quantitative genetic research on salmonids has been applied primarily to estimate levels of genetic variation and covariation in traits important to hatchery production or market demand. Genetic variation and covariation in life-history traits important to adaptation in the wild are, with few exceptions, unknown for most Pacific salmon populations. These traits include age at juvenile ouhnigration and adult maturity, juvenile outmigration and adult run timing, fecundity, and habitat preference (Riggs 1990, Kapuscinski and Miller 1993). To this list could be added stage-specific survival rates, sex ratio, egg size, development and growth rates, food conversion, temperature and pH tolerance, body morphometry and composition, migration tendency, stamina and burst swimming speed, stress and disease resistance, seawater tolerance, agonistic behavior and competitive ability, and homing ability (Steward and Bjomn 1990, RASP 1992).

Estimation of genetic variation and covariation is essential to the implementation of quantitative genetics in the management of natural populations, but other genetic problems also warrant investigation. There are a number of quantitative genetic issues relevant to salmon supplementation efforts, including captive broodstock programs, that remain largely unexplored. One of these issues is inbreeding depression expressed during short-term captive propagation of small populations composed of close relatives. Another is domestication selection, or directional genetic change during captive propagation that results from adaptation to the protective culture environment. A third issue is outbreeding depression resulting from interbreeding between cultured and wild individuals.

***Inbreeding depression.*** Geneticists generally consider inbreeding depression to be the one of the most serious tlueats to the viability of small captive populations Ralls and Ballou 1983, Lande and Barrowclough 1987. Ralls et al. 1988, Simberloff 1988, Hedrick 1992, Hedrick and Miller 1992). As described above, inbreeding depression is the reduction in fitness resulting from mating between close relatives that occurs by chance in small populations, or from assortative mating in large populations. Inbreeding depression is a consequence of the expression of deleterious recessive alleles as homozygosity increases; therefore, it depends largely on dominance, or interactions between alleles within loci (Falconer 1989, Lynch 1991). Inbreeding

depression is an important concern in captive broodstock programs for threatened or endangered species because it addresses the additional risk of extinction that results when related individuals are mated. In populations that have existed at low numbers for any appreciable length of time, this risk can be high (e.g., Templeton and Read 1984).

Inbreeding depression has been documented repeatedly in many plant and animal taxa (Hedrick et al. 1986, Charlesworth and Charlesworth 1987, Hedrick 1992). To our knowledge, however, in Pacific salmon inbreeding depression has not been documented quantitatively in hatchery populations, and its prevalence in wild salmon populations is unknown (Allendorf and Ryman 1987, Gall 1987). Inbreeding depression is often a laborious quantitative genetic problem to investigate because, unlike estimation of simple inbreeding, estimation of inbreeding depression requires the assessment of some measure of fitness as well as knowledge of the parentage of the individuals under consideration. Such assessment requires a level of manipulation that is not typically practiced (and, indeed, would be difficult to practice) in most production situations. Nevertheless, further research is needed to characterize the relationship between inbreeding and fitness in salmon populations.

In addition to examining the extent of inbreeding and inbreeding depression in both hatchery and natural populations, three research topics are particularly worthy of attention: 1) effective mating schemes to minimize further inbreeding, 2) estimation of critical levels of inbreeding that lead to substantial reductions in fitness, and 3) conditions necessary to avoid or recover from inbreeding depression (Le., amount and type of outbreeding) (Waples, in press).

Some experimental studies have shown that even though population bottlenecks increase the opportunity for inbreeding depression to occur, these events sometimes increase the genetic variance in quantitative traits (Bryant et al. 1986, Carson and Wisotzkey 1989, Cohan et al. 1989, see also Hard et al. 1993). This expression of "hidden" variance is thought to result from a redistribution of genetic variance under strong genetic drift (Robertson 1952; Griffing 1960; Cockerham 1984; Goodnight 1987, 1988; Gimmelfarb 1989; Willis and Orr 1993). Nevertheless, strong bottlenecks pose considerable risk to populations. Bryant et al. (1990) found that fitness dropped sharply after a single bottleneck (of 1, 4, or 16 mating pairs) in *Musca domestica,* although fitness in surviving lines began to rise by the third successive bottleneck. In *Drosophila melanogaster*, Frankham et al. (1993) found that selection for high fitness in inbred lines substantially reduced the loss in fitness due to inbreeding depression; however, fitness was still significantly lower than in an outbred, unselected control line. Thus, while bottlenecks may provide some adaptive opportunities for populations in unstable environments, the additional risk of extinction posed by bottlenecks should be avoided if possible (Willis and Orr 1993).

Domestication selection. In captive broodstock programs intended to supplement natural populations. the genetic objective must be to minimize genetic and phenotypic divergence of cultured fish from the natural fish they are intended to supplement. The opportunity for selection to produce this divergence in a captive broodstock program is large because fish are cultured entirely in captivity for one or more generations. The greater potential control of mortality in

captive broodstocks may limit the effects of natural selection during captivity--but only if the genetic divergence that results is sufficiently low that it entails only nominal fitness costs once the fish are released to the wild.

Genetic change in a quantitative character depends on three basic parameters: the amount of genetic variation for that character, the amount of selection exacted by factors in the environment, and me number of generations exposed to the environment. Only recently has it been explicitly recognized that the hatcheryenvironment may be sufficiently different from the natural environment that, even if fish are held for a few generations for only a small portion of their lives in captivity, substantial quantitative genetic divergence of hatchery and wild fish might occur.

Genetic divergence can result from selection or genetic drift (as well as mutation and migration), and it can be difficult to distinguish between the effects of these agents if populations are at low effective population sizes (Lynch 1988). Yet this is precisely the condition that exists when establishing most captive broodstock programs. The effects of drift, because they are determined by the effective population size, are to a large degree determined by the time a captive broodstock is established and are typically random in nature. By contrast, genetic change through selection is directional (i.e., through adaptation to the protective culture environment) and can be more difficult to control.

Artificial selection is not required in order to domesticate animals, a process that can occur through “inadvertent” selection (Doyle 1983, Kohane and Parsons 1988). In supplementation schemes such as captive broodstock programs, which are designed for reintroduction of animals to the wild, domestication is not desirable. Domestication is a process of adaptation to a novel, usually controlled environment, and this process is only partially independent of experimental design. The environment may be artificial, but the adaptive process is a natural one. The bulk of evidence for domestication in wild animals comes from animal breeding experience (Spurway 1952, Hale 1969, Price and King 1969, Lande 1983, Price 1984, Fredeen 1986, Kohane and Parsons 1988) and from experimental work on other organisms, primarily invertebrates (Doyle and Hunte 1981a,b; Frankham et al. 1986; Parsons 1986; Holloway et al. 1990; Briscoe et al. 1992).

Because domestication is a form of adaptation, it is thought to involve primarily quantitative characters that affect fitness. Evolutionary theory predicts that the number of genes affecting trait expression should increase with the trait’s correlation with fitness. Indeed, many quantitative characters (especially life-history traits) which are known to affect fitness show evidence ofpolygenic control (Wright 1968, Laude 1981). Williams (1966), Gadgil and Bossert (1970). Rose (1982), and Clark (1987) have argued on theoretical grounds that many of these constituent genes have pleiotropic effects on different characters, which are manifested as genetic correlations between these traits.

Adaptation should drive allelic combinations with positive genetic correlations with respect to fimess toward higher frequencies, with the result that, when near selective equilibrium, the correlations that remain will be predominantly negative. These negative correlations mean that selection to increase one character with respect to fimess will tend to decrease correlated characters with respect to fitness. As a result, genetic variation for fitness may be low, but genetic variation for its components can remain high. The major prediction from this theory is that populations that have adapted to a particular range of environments should exhibit negative correlations among characters affecting fimess, a result supported by empirical evidence (Rose 1984).

A population apparently adapted to one environment, upon exposure to a novel environment with a different selective regime, can face a formidable evolutionary challenge. Different selective pressures will favor different allelic combinations; the process of adaptation to new environmental characteristics should result in rapid changes in allele frequencies corresponding to the newly favored alleles. During this process, prior to selective equilibrium, genetic correlations between fitness characters should become more positive, and the additive genetic variance for these characters should increase (Service and Rose 1985, Holloway et al. 1990). The expected result is a rapid reorganization in the genetic architecture of life history, including a phase characterized by positive genetic correlations among life-history traits.

Empirical results generally appear to support the notion of rapid adaptation to novel environments. For example, Doyle and Hunte (1981a,b) found a substantially higher population growth rate in a laboratory environment in a domesticated population (25 generations in the laboratory) of the estuarine amphipod *Gammarus lawrencianus*, relative to a wild population. Lande (1983) concluded from a survey of the literature that major adaptive changes occur in domesticated and artificially disturbed populations due to mutations with large phenotypic effects.

The evidence for such effects comes primarily from resistance to toxins, pathogens, and predation. Service and Rose (1985) showed that rapid adaptation to a novel environment (as might result if wild fish are cultured in a hatchery) altered the genetic covariance stmcture of life history ***in Drosophila melanogaster*** transferred after 80 generations from an environment of banana-agar-corn syrup medium, 25°C, and constant light to one of Instant Drosophila Medium Blue medium, 15.5°C, and constant darkness. Holloway et al. (1990) showed similar results when they introduced rice ***weevil*** (*Sitophilus oryzae*) that had been cultured for over 50 generations on wheat (*Triticum aestivum*) and transferred to yellow split-pea (*Pisum sativum*). These results were consistent with the above predictions.

Several studies have failed to detect negative genetic correlations among life-history traits (e.g., Giesel and Zettler 1980; Giesel et al. 1982: Murphy et al. 1983; Bell 1984a,b). However, Rose (1984) and Service and Rose (1985) argued that these correlations were measured in populations that either I) were partially inbred--i.e.. exhibited some degree of inbreeding depression--which can bias patterns of genetic correlation (as in the case of the studies led by Giesel) or 2) had been subjected to the laboratory environment for only a few generations--i.e.. were not near selective equilibrium (as in the case of the other studies).

On balance, experimental evidence supports a prominent role for antagonistic pleiotropy in life-history structure and the ability of rapid adaptation in novel environments to disrupt this stmcture. However, more work is needed on fishes and other organisms to confirm the generality of these results. Research on the genetic consequences of hatchery culture for sahnonid life history is required to fully evaluate the genetic risks of captive broodstock programs for these fish.

The large apparent differences in rearing environment between traditional salmon hatcheries and natural rearing habitats suggest that the types of selection experienced by fish developing in these environments may differ substantially (Hynes et al. 1981, Doyle 1983). Waples (1991a) argued that sharp differences exist in mortality profiles between hatchery and natural salmon, and that these differences transcend metamorphosis (smoltification) and preclude almost any chance that genetic differentiation between these two groups can be avoided.

The empirical evidence for domestication in artificially propagated salmonids that are not subjected to artificial selection is equivocal; most of it is based on reduced performance of hatchery fish in the wild, including reduced survival (Schuck 1948, Reisenbichler and McIntyre 1977, Leider et al. 1990), reduced stamina (Green 1964, Leon 1986), altered behavior (Vincent 1960, Moyle 1969, Swam and Riddell 1990, Riddell and Swam 1991, Fleming and Gross 1992), and reduced reproductive success (Fleming and Gross 1993). Skaala et al. (1990, Table 1) summarized genetic changes connected with salmon culture, although their survey clearly included other genetic problems such as inbreeding and inbreeding depression.

Indirect evidence for domestication selection comes from changes in quantitative genetic parameters in artificial selection experiments that are difficult to account for on the basis of the applied selection differential alone. Gjedrem (1979) observed a larger response to selection for increased growth rate in Atlantic salmon than he could ascribe to the amount of artificial selection that he applied. Kinghom (1988) presented evidence from farmed Norwegian Atlantic salmon compared to wild fish that was consistent with a response within four generations to domestication selection for growth. In an experiment to determine response to selection for high 8-month weight in coho salmon, Hershberger et al. (1990) detected a weight increase in unselected (control) populations reared initially in a hatchery and then transferred to marine netpens.

It should be recognized, however, that the first three conditions are not very restrictive and that the last condition is superfluous if no artificial selection is being practiced. The evolutionary genetic literature contains several examples of genetic change occurring over a few generations in quantitative traits with relatively low heritabilities (but potentially high additive genetic variances) that are difficult to attribute to genetic drift. The point is not that genetic change is difficult to produce, but rather that its direction and magnitude are difficult to predict (e.g., Sheridan 1988).

Table 1. Fitness consequences of crossbreeding between conspecific populations, exclusive of salmonids (updated from Endler 1977, Table 4.6). The symbols +, –, and = refer to the character's value in the crossbred offspring relative to the parental mean; a period (.) indicates no data. "Overall" refers to me general result of crossbreeding for the character across studies. See Endler (1977) for further notes and qualifications.

| | | Mean | | Variance | | |
|---|---|---|---|---|---|---|
| Character | Species | $F_1$ | $F_2$ | $F_1$ | $F_2$ | References |
| Viability | ***Drosophila melanogaster*** | + | – | | + = | Wailnce (1955) |
| | | = | = | – | + | King (1955) |
| | ***Drosophila pseudoobscura*** | + | – | += | = | Vetukhiv (1953, 1955) |
| | | + | –= | | | Brncic (1954) |
| | | + | – | | | Wallace and Vetukhiv (1955) |
| | | | | | + | Spassky et al. (1958) |
| | | += | –= | = | + = | Vetukhiv and Beardmore (1959) |
| | ***Drosophila willistoni*** | + | – | + | + | Vehkhiv (1954) |
| | | + | – | | | Wallace tend Vetukhiv (1955) |
| | ***Drosophila paulistorum*** | + | – | += | + | Vetukhiv (1954) |
| | | + | – | | | Wallace and Vetukhiv (1955) |
| | *Drosophila pavani* | – | | | | Brncic (1961) |
| | *Drosophila persimilis* | . | – | | | Spiess (1959) |
| | *Drosophila prosaltans* | – | | | | Dobzhansky et al. (1959) |
| | *Phyciodes tharos* | = | – | += | + | Oliver (1972) |
| | *Boloria toddi* | += | – | | | Oliver (1972) |
| | *Cisseps fulvicollis* | – | – | + | + | Oliver (1972) |
| | *Hyperia postica* | – | – | | | Blickenstaff (1965) |
| | *Rana pipiens* | – | | | | Ruibal(l955), Fowler (1964) |
| | | –= | | | | Moore (1950, 1967), Volpe (1957). Cuellar (1971) |
| Viability | *Triturus critatus* | + | – | | | Callan and Spurway (1951), Spurway (1953, 1954) |
| | *Streptanthus glandulosus* | + | | . | | Kruckeberg (1957) |
| | ***Mimulus luteus*** | –= | | | | Hughes and Vickery (1974) |
| | ***Mimulus tigrinus*** | = | . | . | | Hughes and Vickery (1974) |
| | ***Mimulus cupreus*** | – | | . | | Hughes and Vickery (1974) |
| | ***Drosophila mojavensis*** | = | = | . | | Etges ( 1989) |
| | ***Delphinium nelsoni*** | + | = | | | Price and Waser (1979) |
| | ***Chamaecrista fasciculata*** | += | | | | Fenster (1991) |
| | *Amphicarpaea bracteata* | – | | | | Parker (1992) |
| | *Tigriopus californicus* | – | | | | Burton (1986, 1990) |
| | *Capra* ***ibex*** subspp. | – | | | | Grieg (1979) |
| | *Micropterus salmoides* | = | | | | Philipp and Whitt (199 1) |
| | *Ipomopsis aggregata* | –= | | | | Waser and Price (1989) |
| | OVERALL | += | – | += | + | |

the resulting genic interactions may be so strong that outbreeding depression is expressed in the F,. However, outbreeding depression may also be expressed in the F, if hybrid offspring are poorly adapted to the habitat they occupy, regardless of the mode of gene interaction.

Although current interest in outbreeding depression is high, its extent and consequences in natural salmon populations or between hatchery and wild populations is unknown. Evidence exists for outbreeding depression in other organisms, but the quality of this evidence varies and it relies largely on a few, primarily invertebrate, species. The contentious nature of this issue for salmon supplementation warrants a detailed examination of the evidence. This evidence is summarized in Tables 1 and 2.

The results surveyed in Table 1 indicate a frequent tendency for outbreeding to yield heterosis with respect to correlates of fitness in the $F_1$, followed by reduced fitness in the $F_2$. Where variances in these hybrids have been examined, the trend is for increased variance in both generations. However, phenotypic variance is expected to increase in the $F_2$ due to segregation. Thus, it is not clear from these results to what extent the observed increases in $F_2$ variance exceed the amount resulting from segregation (potentially by disruption of coadapted gene complexes).

One way to test observed versus expected increases in trait variances in second-generation hybrids ($F_2$ and first backcrosses) is by comparing observed means and variances with the expectations of an additive genetic model (Cockerham 1986). Hard et al. (1992b, 1993) tested the means and variances of six geographic populations of the pitcher-plant mosquito (*Wyeomyia smithii*) and their F,, $F_2$, and first-generation backcrosses against the additive genetic expectation to show that the lower $F_1$ variances and higher $F_2$ variances they observed cannot be attributed to additive effects alone. Large increases in $F_1$ or $F_2$ variance with respect to fitness may be consistent with outbreeding depression, but they do not necessarily reflect it.

The evolutionary consequences of outbreeding among salmonid populations are not clear. Virtually all the studies surveyed in Table 2 have examined only first-generation hybrids, and, consequently, most of these studies were not designed to detect outbreeding depression. Nevertheless, relatively few studies have found evidence for heterosis in first-generation hybrids, suggesting little directional dominance (or epistasis involving directional dominance) for fitness among populations. Some crosses have shown reductions in fitness in the F,, which could portend severe outbreeding depression in subsequent generations through the breakup of coadapted gene complexes if epistasis has contributed to population divergence.

In the only empirical study designed specifically to detect outbreeding depression in salmonids beyond Fist-generation hybrids, Gharrett and Smoker (1991) examined marine survival. return date. body size. and bilateral assymetry in two generations of crosses between even- and odd-year populations of Auke Creek (Alaska) pink salmon. These workers observed substantially lower survival and increased assymetry in the $F_2$ but not the $F_1$ hybrids, a result consistent with outbreeding depression by breakdown of coadapted genes. However, it is important to recognize that even- and odd-year populations of pink salmon from the same stream may have been

reproductively isolated for potentially thousands of generations. Indeed, such populations are genetically more distinct from each other than from populations spawning in the same year in different streams (Aspinwall 1974, Beacham et al. 1988, Shaklee et al. 1991). Consequently, the results found by Gharrett and Smoker (1991) may not be representative of those expected between hatchery and natural populations with a greater natural opportunity for gene flow.

Table 2. Fitness consequences of crossbreeding between conspecific salmonid populations. The symbols are as in Table 1.

| | | Mean | | Variance | | |
|---|---|---|---|---|---|---|
| Character | Species | $F_1$ | $F_2$ | $F_1$ | $F_2$ | References |
| Viability | *Oncorhynchus gorbuscha* | = | – | | | Gharrett and Smoker (199 I) |
| | *Oncorhynchus mykiss* | – | | | | Reisenbichler and McIntyre (1977) |
| | | = | + | | | Ayles and Baker (1983) |
| | | + | = | | | Hörstgen–Schwark et al. (1986) |
| | *Oncorhynchus nerka* | = | | | | Wood and Foote (1990) |
| | Oncorhynchus *tshawytscha* | = | | | | Cheng et al. (1987) |
| | *Salvelinus fontinalis* | –= | . | | | Mason et al. (1967) |
| | | + | | | | Webster and Flick (1981) |
| | | = | | | | Fraser (1989) |
| | | = | + | | | Lachance and Magnan (1990a) |
| | *Salmo salar* | = | | | | Gjerde and Refstie ( 1984) |
| | *Salmo* spp. | –=+ | | | | McGowan and Davidson (1992) |
| | OVERALL | = | . | | | |
| Seawater adaptability | *Oncorhynchus nerka* | = | . | | | Foote et al. (1992) |
| | *Oncorhynchus tshawytscha* | – | . | = | | Clarke et al. (1992) |
| | OVERALL | –= | . | = | | |
| Growth rate | Oncorhynchus mykiss | = | | | | Reisenbichler and McIntyre (1977) |
| | *Oncorhynchus gorbuscha* | += | = | + | | Gharrett and Smoker (199 1) |
| | | = | + | | | Ayles and Baker (1983) |
| | | –=+ | | | | Ferguson et al. (1985) |
| | | = | | | | Hörstgen–Schwark et al. (1986) |
| Growth rate | Oncorhynchus *mykiss* | = | . | | | Wangila and Dick (1987) |
| | | –= | | | | Johnsson et al. (1993) |
| | Oncorhynchus *tshawytscha* | –=+ | . | | | Cheng et al. (1987) |
| | | + | = | = | | Clarke et al. (1992) |
| | *Salvelinus fontinalis* | = | + | | | Mason et al. (1967) |
| | | = | + | | | Keller and Plosila (1981) |
| | | + | | | | Webster and Flick (1981) |
| | | = | | | | Fraser (1989) |
| | | =+ | | | | Lachance and Magnan (1990a) |
| | *Oncorhynchus nerka* | = | | + | | Wood and Foote (1990) |
| | *Salmo trutta* | | | | | Maisse et al. (1983) |
| | OVERALL | = | | +=: | | |

Table 2. Continued.

| character | Species | Mean F, | Mean $F_2$ | Variance F, | Variance $F_2$ | References |
|---|---|---|---|---|---|---|
| Catchability | *Oncorhynchus mykiss* | = | + | | | Pawson and Purdom (1987) |
| | *Salvelinus fontinalis* | + | | | | Keller and PIosila (198 1) |
| Hatching the | *Salmo clarki* | –=+ | | . | | Ferguson et al. (1988) |
| Parasite resistance | *Oncorhynchus kisutch* | –=+ | | . | | Hemmingsen et al. (1986) |
| Homing rate | *Oncorhynchus gorbuscha* | + | | . | | Barns (1976) |
| *Maturation/ spawn* timing/ GST[a] | *Oncorhynchus mykiss* | = | | . | . | Marrocco (1982) |
| | *Salmo salar* | = | | . | . | Sutterlin and MacLean (1984) |
| | *Salvelinus fontinalis* | = | + | . | . | Lachance and Magnan (1990b) |
| Rheotaxis | *Oncorhynchusmykiss* | = | | . | | Kelso and Northcote (1981) |
| Meristics/ fluctuating assymetry | *Oncorhynchus gorbuscha* | = | – | . | . | Gharrett and Smoker (199 1) |
| | *Oncorhynchus mykiss* | + | = | . | . | Ferguson and Danzmann (1987) |
| | *Salvelinus* spp.[b] | + | | . | . | Leary et al. (1985) |
| | *Oncorhynchus* spp.[b] | + | | . | . | Leary et al. (1985) |
| | *Oncorhynchus mykiss* | – | | . | . | Ferguson (1986) |
| | *Salmo clarki* | + | | . | . | Ferguson et al. (1988) |
| | | = | | . | . | Forbes and Allendorf ( 199 1) |
| | *Salmo trutta* | = | | . | | Ielli and Ducbi (1990) |
| | OVERALL | + | = | . | | |

[a] GST = Gonadosomatic index
[b] Narurally occurring interspecific hybrids

In the following section, we outline prominent quantitative genetic risks associated with captive broodstock programs, discuss the importance of genetic monitoring for quantitative characters, and suggest some basic approaches to address these issues.

Table 3. Major control points and possible genetic consequences of activities associated with salmonid captive broodstock programs.

| Control point | Genetic consequences |
|---|---|
| Selection of broodstock | Founder event (genetic change) |
| Collection of broodstock | Founder event (genetic change)<br>Bottleneck (reduced genetic variation) |
| Mating of broodstock | Genetic drift (reduced genetic variation)<br>Inbreeding depression (reduced fitness)<br>Domestication selection (genetic change) |
| Rearing of first-generation descendants to maturity in captivity | Bottleneck/genetic drift (reduced genetic variation)<br>Domestication selection (genetic change) |
| Sampling of descendants for broodstock | Bottleneck/genetic drift (reduced genetic variation)<br>Inbreeding depression (reduced fitness)<br>Founder event/domestication selection (genetic change) |
| Rearing of second-generation descendants in captivity | Bottleneck/genetic drift (reduced genetic variation)<br>Domestication selection (genetic change) |
| Release of second-generation descendants to the wild | Bottleneck (reduced genetic variation)<br>Domestication selection (genetic change)<br>Genetic introgression (genetic change) |

Pacific salmon (e.g., Waples et al. 1993) and, to our knowledge, none of these monitor quantitative characters. It is important to consider monitoring quantitative characters to fully evaluate the effects of captive propagation on adaptation in the wild.

Monitoring quantitative characters involves one consideration that is not at issue when monitoring single-locus characters: discrimination between environmental and genetic sources of observed variation. Determining the genetic basis of phenotypic variation is the raison d'être of quantitative genetics. Two main issues should be considered in monitoring quantitative genetic variation: the first is genetic, the second statistical (Hard. in press).

The genetic problem arises from the fact that observed variation in quantitative characters has an environmental as well as a genetic component. A portion of this variation often reflects

interaction between these components, so that the phenotypic variation expressed by particular genotypes depends on the environment in which they exist. For captive broodstock programs, the primary significance of environmental influence on phenotypic expression is that phenotypic change during the course of the program is likely to reflect both genetic and environmental change. Determining the amount of genetic change associated with an observed phenotypic change is essential to evaluating the genetic consequences of captive propagation for the population, and this determination requires quantitative genetic methods.

However, to achieve this objective it is probably not necessary to estimate genetic parameters such as heritability with the precision usually employed in most quantitative genetic research or in selective breeding programs. Because these estimates depend on the population's environment as well as its gene frequencies, estimates for captive populations are often higher than corresponding estimates for the same traits in populations developing under naturally more variable conditions (Prout 1958, Coyne and Beacham 1987). Furthermore, the reliability of such estimates in predicting phenotypic response to selection has been called into question (Sheridan 1988).

Thus, it may be sufficient to determine only whether additive genetic variance exists for a character, as this parameter is what determines a population's potential for genetic change (Hard, in press). If significant additive genetic variance exists for a quantitative character in a captively propagated population, and if appreciable phenotypic change has occurred during propagation, concern should arise that genetic change has resulted during the captive broodstock program.

The statistical problem arises directly from the objective to minimize the genetic and phenotypic differentiation of captive and natural fish during supplementation. This objective generates a different approach to hypothesis testing than other goals might. For a captive broodstock program with this objective, an appropriate null hypothesis is that captive and natural fish do not differ for the trait in question.

There are two statistical errors that can result in testing this hypothesis, and only one of these has been widely appreciated by fisheries biologists. This error, known as type I error (Winer 1971, Sokal and Rohlf 1981), is in the present context the probability of concluding that these two groups differ when in fact they do not. Fortunately, the level of type I error (designated a), can be controlled a priori by the investigator by setting the significance level for the test.

The other error, known as type II error (Dixon and Massey 1957, Winer 1971), is the probability of concluding that these groups do not differ when in fact they do. Unfortunately. type II error cannot be controlled by the investigator except indirectly through sample size and "effect size" (the direction and magnitude of the effect the investigator wishes to detect), and it tends to rise with more stringent control of type I error (Cohen 1988).

The consequences of type I error have received a great deal of attention by empirical biologists because it has generally been presumed that this error is the most serious of the two

types (Cohen 1988; Peterman 1990). However, in many studies in environmental toxicology, industrial and chemical safety, and conservation hiology, type II error may be more serious than type I error. In general, the risks of these errors should be weighed in any evaluation to minimize unanticipated problems and guide experimental design.

A comprehensive treatment of the statistical risks in hypothesis testing and an explanation of experimental power is beyond the scope of this report. Peterman (1990) gives an exemplary introduction to this issue in fisheries applications; statistical texts by Dixon and Massey (1957) and Sokal and Rohlf (1981) provide additional detail for the interested reader.

For the purposes of captive broodstock programs the following point should be remembered: The genetic consequences of concluding that appreciable genetic change has not occurred during protective culture, when such change has actually occurred (type II error), are arguably more serious than the consequences of falsely concluding that such genetic change has occurred (type I error).

The former conclusion has irreversible biological consequences; the second has serious (but reversible), primarily economic, consequences. The risk of type II error is inversely related to the intensity of genetic monitoring (i.e., sample size) and the direction and magnitude of genetic divergence considered acceptable (the effect size). These risks, in turn, depend on character variability and bow divergence is measured. Hard (in press) discusses this issue in further detail and illustrates difficulties in avoiding type II error that could arise during genetic monitoring of quantitative characters.

The success of supplementation efforts that involve captive broodstock programs can be enhanced if quantitative characters are monitored because these characters are sensitive to changes in environment, and consequently, the potential for domestication. The desirable features of such programs include 1) quantitative genetic monitoring as an integral part of the supplementation process, involving differential marking of released individuals and adequate sampling of life-history characters on captive and natural individuals; 2) identifying the amount of genetic differentiation allowed to occur in the characters that are assessed, this amount to be determined by consideration of information on character variation; and 3) determining appropriate responses to genetic problems that surface in the program.

### Future Research Priorities

As the discussion above indicates, there are several potential genetic consequences of salmon captive broodstock programs, and little research has been done on any of them. A considerable amount of work, firmly grounded in quantitative genetics, will be required to determine the likelihood and extent of these consequences, which can be grouped into three main categories: I) loss of genetic variability within a salmon population resulting from the establishment of a captive broodstock program, and the inbreeding depression that may result

from small population size and patterns of mating; 2) genetic change that may result from adaptation (domestication) in captive fish to the protective culture environment; and 3) genetic divergence of the captive fish from their natural source population and the subsequent consequences of genetic interactions between these groups.

The experimental requirements of studies designed to address these issues are formidable. These studies require large numbers of available spawners and at least one, and generally two or more, fish generations to address the experimental objectives. Previous studies have attempted to address three main categories of quantitative genetic risk in captive broodstock programs:

1) To relate the degree of inbreeding to the degree of inbreeding depression by determining the extent of inbreeding depression that results from various levels of inbreeding, and compare the levels of inbreeding incurred by different mating schemes. The simplest approach is to subject a population to several generations of full-sib mating (Kincaid 1976, 1983) and compare its response to that of other mating techniques.

2) To determine whether natural selection ("domestication") that acts on captive populations during protective culture differs qualitatively from selection that acts on salmon in nature. The approach used could examine variation in family size as an indicator of domestication selection, or could use the relative inter-generational variation in a trait(s) between a selected and control (i.e., unselected) captive population to characterize the direction and general magnitude of natural selection acting on that trait during protective culture (Kinghorn 1988, Hershberger et al. 1990).

3) To determine the genetic consequences of interbreeding between captive broodstock and natural fish. The approach involves making crosses between cultured and natural fish, establishing their first- and second-generation hybrids, measuring the means and sampling variances of life-history traits in each derivative line, and testing the goodness of fit of the variation in these traits among cross derivatives to various simple models of gene expression.

Such an approach can allow one to determine not only whether adaptive divergence between captive and natural fish has occurred during a captive broodstock program, but also whether the most commonly accepted mechanism for this "outbreeding" depression (i.e., the breakup of "coadapted gene complexes"; Dobzhansky 1948) is likely to be responsible for the depression. At a 1994 American Fisheries Society Symposium on the "Uses and Effects of Cultured Fishes in Aquatic Ecosystems" in Albuquerque, New Mexico, outbreeding depression was identified as a primary concern among fishery geneticists.

## Conclusions

Quantitative genetics has applications to a wide variety of problems. However, most fishery work published to date has dealt with estimating levels of genetic variation within and among populations. This objective is important, but it raises questions about what maintains or erodes this variation. Attempts to address this issue are not only of basic evolutionary interest, but can contribute to informed management and conservation.

The majority of research in quantitative genetics has emphasized determining the genetic and environmental components of variation in traits important to aquacultural production. These traits include growth and size, reproduction (especially maturation and egg production), disease resistance, and body composition and flesh quality. In general, the genetic basis for most of these characters is often large enough that a reasonable response to selection can be realized, but individual responses depend strongly on the stock aod environment involved.

A number of studies have examined the quantitative genetic basis of life-history characters and their covariation with morphological and other characters. Life-history characters examined include incubation performance, early development traits, survival, age at maturity, and behavior. Four general observations emerge from these studies.

First, white life-history characters tend to exhibit a wide range of genetic underpinnings, the reliability of genetic parameter estimates such as heritabities and genetic correlations is questionable because of inadequate experimental designs or sampling techniques. Second, where analyses have been conducted over different environmental conditions, there often appears to be substantial interaction between genetic and environmental effects on the phenotype. Third, some traits appear to evolve in close association with ether traits, often precluding selection on them independently. Finally, genetic relationships among traits are often (but not always) qualitatively similar to their phenotypic relationships.

The genetic basis of adaptation has received little attention in fishery quantitative genetics. Topics in particular need of attention include inbreeding depression, selection, and population differentiation and outbreeding depression. Until these topics receive empirical attention, fishery geneticists will find it difficult to predict the evolutionary consequences of small population size, intensive hatchery culture, or interbreeding between hatchery and wild fish. Consequently, we believe it is crucial to integrate investigation of these topics into genetic conservation research.

Evidence is growing that quantitative traits that contribute to adaptation in salmonids can change under human influences, such as harvest, habitat alteration, and artificial propagation (as in conventional hatcheries or in captive broodstock programs). Many authors have recently pointed out potential genetic problems that can arise during artificial propagation as a result of these influences. since the immediate genetic concerns for conservation may differ substantively from those for enhancement and mitigation. For example. minimizing genetic differentiation between hatchery fish and the natural fish they are intended to supplement may be as important a concern

as maintaining genetic variability within the hatchery population. Lack of guidance on how to detect, monitor, and respond to the effects of selection in hatchery fish undoubtedly has resulted from uncertainties about how adaptation operates in novel environments. Although these and other quantitative genetic issues should be addressed empirically before artificial propagation is applied to salmon conservation on a large scale, there are several reasons why they have not yet been adequately addressed. Two of these issues stand out as particularly significant.

First, it is only recently that possible adverse genetic effects of hatchery culture have been widely appreciated. Supplementation of wild salmon populations and the concept of captive broodstocks are recent developments associated with acknowledged declines in wild populations. Traditional hatchery programs were developed primarily to enhance fisheries or to mitigate for lost abundance of naturally reproducing fish due to harvest or habitat loss or degradation. In such programs, issues such as inbreeding depression, selection, and the consequences of hatchery-wild stock interactions have not received the attention they deserve because hatchery production has generally been considered to be independent of wild production (Larkin 1974, Riddell 1993, Lichatowich and Watson 1993) or even a replacement for it (e.g., Netboy 1974).

Assessment of stock performance has been gauged in terms of fishery contributions and adult returns to the hatchery, not in terms of reproductive success and genetic and phenotypic change. In addition, transfers of fish within and among major river basins removed many constraints on broodstock development. Growing evidence that hatchery production may have adverse effects on wild production has forced a reevaluation of these attitudes (Hindar et al. 1991, Riddell 1991, Waples 1991a).

Second, most quantitative genetic issues are difficult to address experimentally with sahnon. Most empirical work done on inbreeding depression, selection, and population differentiation has involved invertebrates and plants that have short generation times and can be cultured in large numbers of closely related groups. Pacific sahnon satisfy neither of these criteria very well. Few salmon biologists have adequate trainiig in quantitative genetics, and this limits their ability to design breeding programs that evaluate the evolutionary basis of phenotypic variation. These factors have proven to be a major obstacle to research on the quantitative genetics of Pacific salmon.

The use of artificial propagation techniques such as captive broodstock programs to help reverse declines in wild salmon production should be. accompanied by aggressive research to understand the genetic basis of population differentiation and adaptation. This research must entail quantitative genetic approaches. These approaches are often costly, logistically difficult to implement, and protracted, but they are necessary to directly address genetic issues of primary importance to fishery managers and salmon producers.

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# ENVIRONMENTAL AND ENDOCRINE CONTROL OF REPRODUCTION IN CULTURED SALMONIDS

by

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## Introduction

Reproduction in salmonid fish involves the growth, development, and release of mature gametes (eggs and sperm), as well as the development of appropriate secondary sex characteristics and spawning behavior. Cultured salmon are artificially spawned; therefore, spawning behavior is not required for successful reproduction of a captive broodstock. The major problems with reproduction of salmonids in captivity involve timely maturation of broodstock, losses due to prespawning mortality, reliable production of high quality gametes, and precocious maturation of male fish. There is an obvious need to develop methods to monitor and control sexual maturation in captive broodstock, to ensure production of high quality gametes and high survival of offspring, and to minimize asynchronous maturation of male and female fish. In this report, literature relevant to these problems will be reviewed as follows: 1) endocrine control of reproduction in salmonids; 2) environmental regulation of reproduction in salmonids; 3) hormonal induction of final oocyte maturation, ovulation, and spermiation salmonids; 4) precocious or early male maturation in salmonids; and 5) factors affecting gamete quality.

## Endocrine Control of Reproduction in Salmonids

In temperate-zone fishes like salmonids, the seasonal timing of reproduction is strongly influenced by photoperiod, with temperature having a supplementary role. This environmental information is perceived and processed by the brain, which in mm regulates internal processes through the endocrine system. Major components of the reproductive endocrine system are the brain (hypothalamus), pituitary, and gonads (Fig. 1). The pituitary gland plays a central role in initiating reproductive maturation (puberty), maintaining production of sperm and eggs by the gonads, and inducing final maturation and gamete release (spawning).

In fish gonadotropins are the major pituitary hormones responsible for regulating production of gametes (gametogenesis). Other pituitary hormones such as prolactin, growth hormone, and somatolactin have been shown to stimulate gonadal steroidogenesis in fish or to potentiate the response to gonadotropins, but their potencies are far less than gonadotropins and their specific roles in regulation of gametogenesis are not fully understood. Synthesized by gonadotropes of the pituitary, gonadotropins are secreted into the peripheral circulation and bind to receptors in the gonad with subsequent effects on gametogenesis. In most cases, gonadotropins act through the biosynthesis of steroids, which in turn mediate various stages of gametogenesis: oocyte growth, oocyte maturation, spermatogenesis, and spermiation. The ability of the gonadotropins to modulate gametogenesis depends not only on circulating levels of gonadotropins, but also on expression of the appropriate receptor proteins by potential target cells in the gonad. In this section, the biochemistry and physiology of gonadotropins and how they regulate reproduction in salmon will be briefly reviewed.

### Biochemistry of Gonadotropins

Pituitary gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH), along with placenta-derived chorionic gonadotropin (CG) and a third pituitary hormone, thyroid stimulating hormone (TSH) constitute a family of chemically related hormones (Pierce and Parsons 1981). Each member of this family is a heterodimeric glycoprotein, consisting of the noncovalent association of a common a subunit and a unique β subunit, which confers biological specificity to the hormone. For full expression of biological activity, carbohydrate moieties and association between subunits are necessary (Ryan et al. 1987).

Historically there has been controversy over whether the fish pituitary gland produces one or two types of gonadotropins (Burzawa-Gerard 1982, Idler and Ng 1983, Van Oordt and Peute 1983, Fontaine and Dufour 1987).

Initially it was thought that all phases of gametogenesis in fish were regulated by a single "LH-type" gonadotropin, designated "maturational" gonadotropin (Burzawa-Gerard 1982). Later, two types of gonadotropins were isolated by Idler and colleagues, and were designated carbohydrate rich "maturational" gonadotropin, and carbohydrate poor "vitellogenic" gonadotropin, which does not belong to the LH/FSH hormone family (Idler and Ng 1983). Unfortunately, the biochemical nature of the vitellogenic gonadotropin identified by Idler and colleagues has not been fully characterized, and the original physiological work done with this protein has not been verified in other laboratories. Thus, the controversy of one versus two types of gonadotropins in fish persisted until the late 1980s.

The debate was resolved primarily through the efforts of Kawauchi and colleagues (Suzuki et al. 1988a, b; Itoh et al. 1988, 1990; Sekine et al. 1989; Kawauchi et al. 1989; Swanson et al. 1991) who initially characterized two types of gonadotropins, GTH I and GTH II, in chum salmon (*Oncorhynchus keta*) and coho salmon (0. kisutch). Like tetrapod LH and FSH, both GTH I and GTH II consist of an $\alpha$ and $\beta$ subunit. The $\beta$ subunits of salmon GTH I and GTH II have only 31% ammo acid sequence identity to each other, and 30-40% sequence identity to mammalian LH and FSH $\beta$ subunits (Itoh et al. 1988). In salmon, unlike tetrapods, two types of a subunits, a-l and a-2, have been identified (Itoh et al. 1990). Both of the salmon a subunits are highly conserved, showing approximately 65% sequence identity to mammalian a subunits. The salmon GTH I $\beta$ subunit associates with either of two a subunits, a-l or a-2. On the other hand, the $\beta$ subunit of GTH II associates only with the a-2 subunit. GTH II is identical to the previously described chinook salmon (0. *tshawytscha*) "maturational" gonadotropin and is most chemically similar to mammalian LH. Neither GTH I nor GTH II show any biochemical similarity to the vitellogenic gonadotropin identified by Idler and colleagues. Since the identification of the two types of gonadotropins in salmon, considerable progress has been made in understanding their physiological roles.

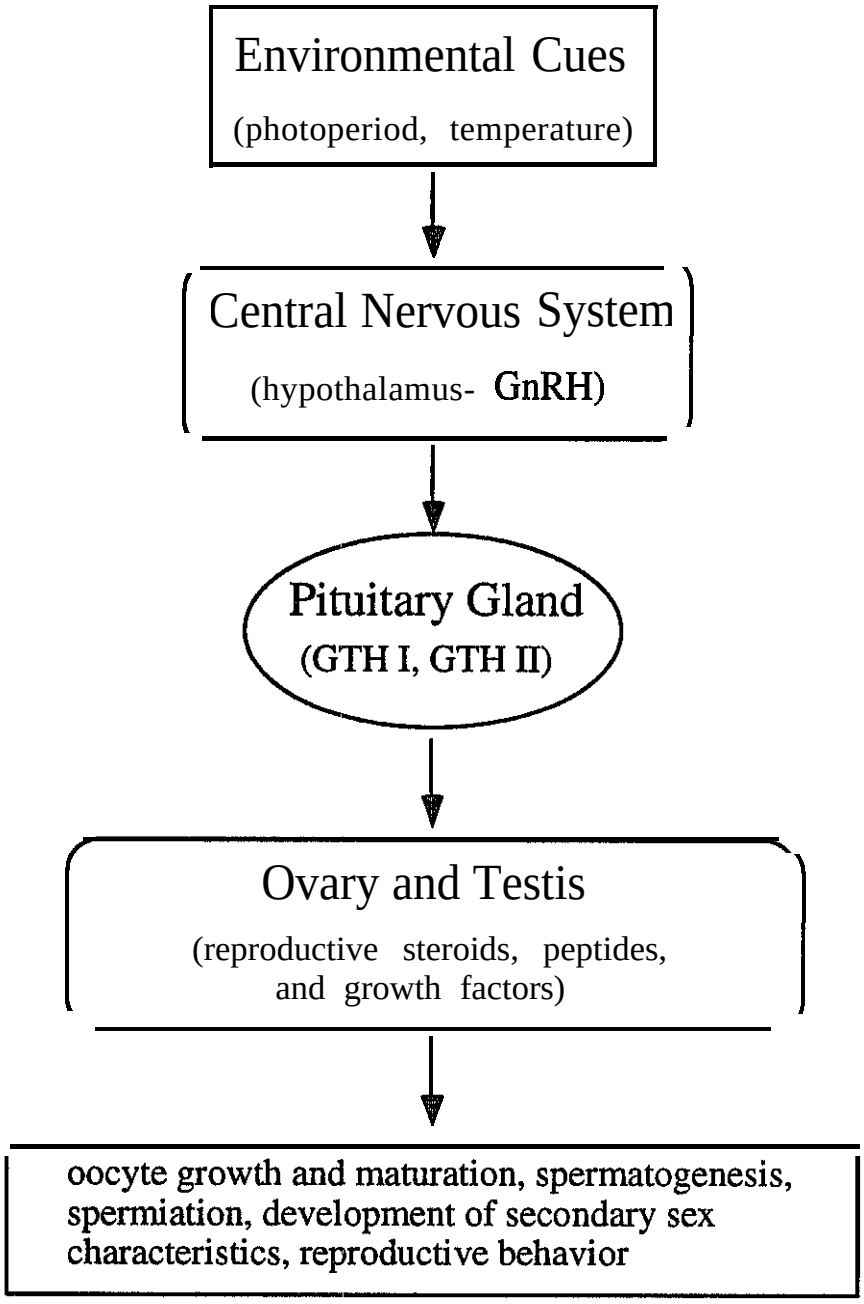


Figure 1. Reproductive endocrine axis in salmonids. Environmental cues are perceived and processed by the central nervous system, which regulates production of hypothalamic peptides such as gonadotropin-releasing hormone (GnRH). GnRH acts on the pituitary gland to regulate synthesis and secretion of gonadotropins (GTH I and GTH II). The gonadotropins are secreted into the peripheral circulation and regulate gametogenesis primarily through effects on the production of reproductive steroids, which also affect the development of secondary sex characteristics and reproductive behavior.

### Plasma and Pituitary Levels of Gonadotropins

Plasma levels of GTH II show a similar pattern during the reproductive cycle in a variety of salmonids: levels remain relatively low or nondetectable until the period of final oocyte maturation and ovulation or spermiation, when levels increase in a pattern similar to that of the preovulatory LH surge in tetrapods (Fitzpatrick et al. 1986; Sumpter and Scott 1989; Suzuki et al. 1988c; Swanson 1991; Amano et al. 1992, 1993, 1994; Oppen-Bemsten et al. 1994; Slater et al. 1994). In contrast, GTH I levels increase and remain elevated during the period of vitellogenesis or spermatogenesis and then decline during final maturation (Suzuki et al. 1988c, Swanson et al. 1989, Swanson 1991, Oppen-Bemtsen et al. 1994, Slater et al. 1994). In coho salmon, plasma GTH I levels increase only in maturing fish 6 to 9 months prior to spawning. The lack of GTH II in the peripheral circulation during periods of gonadal growth suggests that GTH II does not play a role in regulating this phase of reproductive development (Pig. 2).

Immunocytochemical studies in salmonids indicate that GTH I and GTH II are produced in distinctly different cell-types (Nozaki et al. 1990a, Naito et al. 1993) and are differentially synthesized during gonadal development (Nozaki et al. 1990b, Naito et al. 1991, Saga et al. 1993). Immunoreactive (ir) GTH I β subunit is first detected about 56 days post-fertilization in the pituitary of the developing embryo (Mal et al. 1988, Mal 1991, Saga et al. 1993), and levels increase during vitellogenesis and spermatogenesis (Nozaki et al. 1990b, Naito et al. 1991). On the other hand, the ir-GTH II β subunit is not detected until late stages of vitellogenesis or spermatogenesis, with the highest pituitary levels at spawning development (Nozaki et al. 1990b, Naito et al. 1991). The a subunits are detected throughout gametogenesis. However, levels of messenger RNA for the a subunit decline in GTH I-producing cells at the time of spawning (Naito et al. 1991).

### Regulation of gonadotropin secretion

Secretion of GTH II in fish is regulated by hypothalamic peptides such as gonadotropin-releasing hormone (GnRH) and neuropeptide Y (NPY) as well as by other neuromodulators such as dopamine (DA), serotonin (5-HT), norepinephrine (NE) and gamma-amino butyric acid (GABA) (Peter 1983, Peter et al. 1991). Like LH in other vertebrates, secretion of GTH II in fish is primarily regulated by GnRH (Peter 1983). The endocrine mechanisms involved in regulating GTH I secretion have not been extensively studied; however, GnRH has been shown to stimulate in *vitro* release of GTH I in salmon (Swanson et al. 1987, 1989; Swanson 1992).

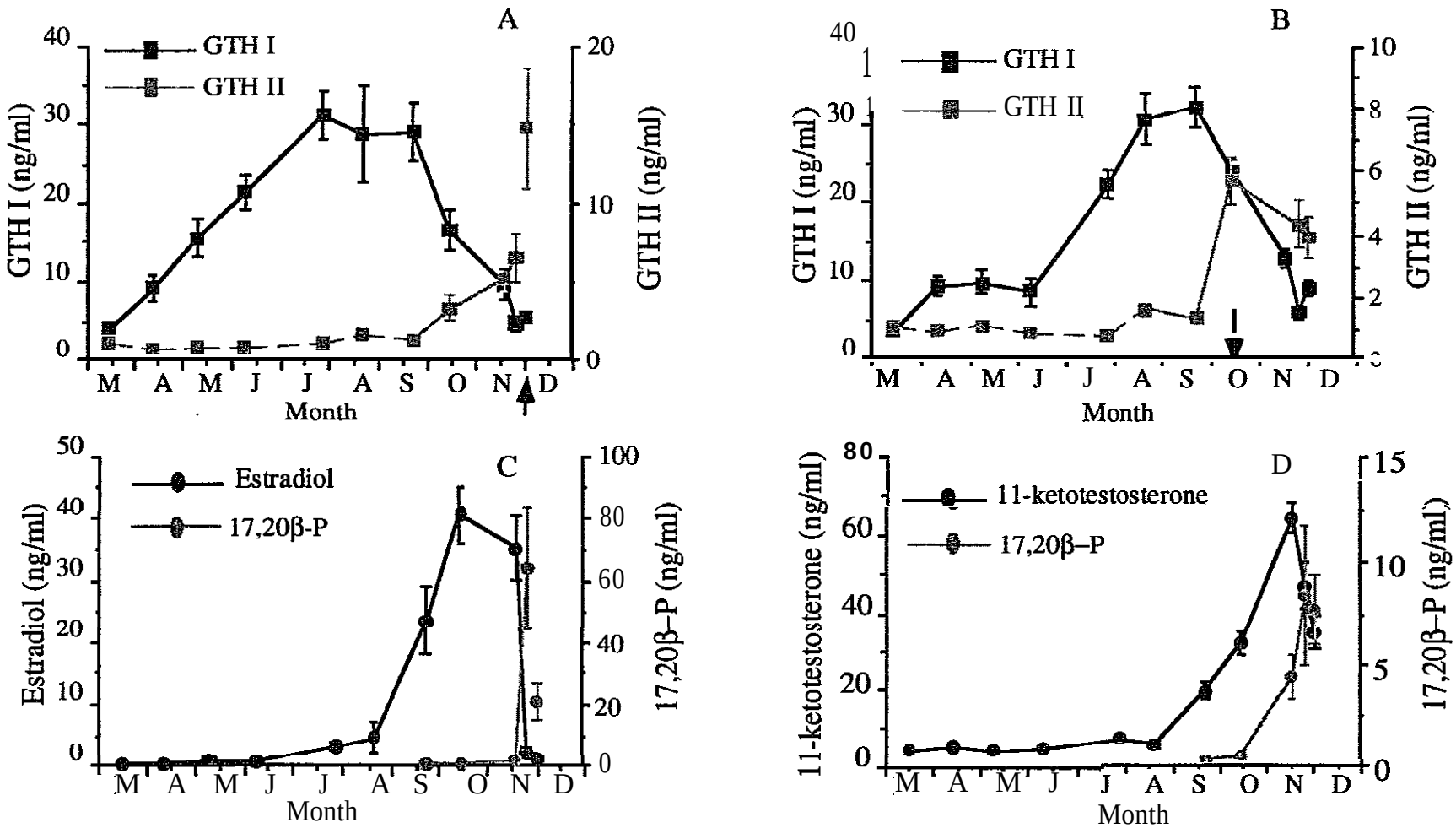


Figure 2. Plasma levels of gonadotropins, GTH I and GTH II, (A,B) and reproductive steroids (C,D) in male (B,D) and female (A,C) coho salmon during sexual maturation. In females, secondary oocyte growth occurred between March and November, and final oocyte maturation and ovulation occurred during late November and December. The last two data points in A and C are from two different groups of ovulated females. The arrow in A indicates when the first ovulated female was observed. In males, spermatogenesis occurred from March through mid-October, and spermiation occurred from October through December. The arrow in B indicates when milt could first be manually stripped from males. Each data point represents the mean ± standard error of 4-8 samples from individual fish. Dashed portion of line indicates levels of GTH II were nondetectable (< 1 ng/ml). 17,20β-P refers to 17α,20β-dihydroxy-4-pregnen-3-one. (from Swanson 1991 and upubl. data, NMFS).

Although the terms GnRH and luteinizing hormone-releasing hormone (LHRH) are frequently used interchangeably, LHRH has been generally used to describe the mammalian form of GnRH. The current and most widely used terminology to describe all members of this hormone family is GnRH. The specific type of GnRH is named according to the species in which it was first discovered.

In salmonids, at least two forms of GnRH have been identified. Salmon (s)GnRH has been purified and sequenced (Sherwood et al. 1983). Like mammalian GnRH, sGnRH is a decapeptide, but it differs from the mammaliam form in amino-acid postions seven and eight. A second form of brain GnRH (chicken (c) GnRH-II) has been found in salmonids (Sherwood et al. 1983, Okuzawa et al. 1990, Amano et al. 1991), which differs from sGnRH in amino-acid positions five and eight. The second form, cGnRH II, is probably not involved in the regulation of gonadotropin secretion in salmonids because cGnRH II was found throughout the brain, but could not be detected in the pituitary (Okuzawa et al. 1990, Amano et al. 1991). Interestingly, both sGnRH and cGnRH II stimulate *in vitro* secretion of GTH I and GTH II in a similar manner (Swanson 1992). However, the response of the pituitary varies according to stage of gametogenesis and the pituitary content of GTH I and GTH II. During vitellogenesis and spermatogenesis when ir-GTH I is elevated and ir-GTH II is low or nondetectable, GnRH stimulates release of GTH I. At the stage of spawning, when ir-GTH II is highest, GnRH stimulates release of GTH II and has either a weak or no effect on GTH I secretion.

In many but not all teleosts, G&I-I-stimulated GTH II release can be blocked by DA through direct effects on gonadotropes and by inhibiting release of GnRH (Peter 1983, Peter et al. 1991). The degree of this inhibition is strong in cyprinids, but either weak or absent in salmonids (Billard et al. 1984).

### Gonadotropin Receptors

It is well established that gonadotropins act via binding to specific membrane receptors. Initial studies in fish suggested that there was a single type of GTH receptor (Breton et al. 1986, Kanamori et al. 1987, Kanamori and Nagahama 1988, LeGac et al. 1988). However, recent studies using purified GTH I and GTH II in salmon demonstrated two types of GTH receptors: a type I receptor (GTH-RI), which binds both GTH I and GTH II, and a type II receptor (GTH-RII), which binds GTH II but not GTH I (Yan et al. 1992, Miwa et al. 1994). The GTH-RI was localized in three cell-types by in vitro ligand autoradiography: in the thecal cell-layer and granulosa cells of the vitellogenic follicle, in the thecal cell-layer of the preovulatory follicle, and in presumptive Sertoli cells of the testis at all stages of spermatogenesis (Miwa et al. 1994). In contrast, the GTH-RII was localized in only the granulosa cells of the preovulatory follicle and Leydig cells of the fully mature (sperrniating) testis.

## Gonadotropin Regulation of Oocyte Growth

The salmon ovary consists of oogonia, oocytes and surrounding follicle cells, supporting stromal tissue, vascular, and nerve tissue. Oocytes grow while arrested in meiotic prophase. Enormous growth of the salmon oocyte occurs during the phase termed vitellogenesis, which involves the sequestration and packaging of the hepatically derived yolk precursor protein, vitellogenin. The selective uptake of vitellogenin into the oocyte occurs through a receptor-mediated process (Tyler et al. 1987.1988; Kanungo et al. 1990). The process of vitellogenesis is regulated by a two-step mechanism whereby gonadotropin stimulates ovarian synthesis of estradiol-17β (E), which in turn stimulates hepatic vitellogenin synthesis. The specific uptake of vitellogenin by the oocyte is stimulated by GTH I (Tyler et al. 1991) and possibly other growth factors.

During oocyte growth, the eggshell or vitelline envelope between the granulosa cells and oocyte is also formed (Yamagami et al. 1992). The eggshell consists of a thin outer zona pellucida and thick inner zona radiata. The zona radiata (zr) proteins are glycoproteins produced in the liver in response to E (Hamazaki et al. 1989, Oppen-Bemtsen et al. 1992). The mechanism whereby zr-proteins are deposited on the oocyte surface is not understood.

In salmonid fiih, it has been demonstrated that during oocyte growth, plasma levels of E increase and subsequently decline prior to fmal oocyte maturation (Fostier et al. 1978). The production of E by the follicular cells of the salmon ovary requires the involvement of both the special thecal cells and granulosa cells (Kagawa et al. 1982a; Nagahama et al. 1982b; Nagahama 1983, 1987) and is stimulated by gonadotropin (Kagawa et al. 1982b). Testosterone, produced by the thecal cell layer, is converted to E by aromatase in granulosa cells. Both GTH I and GTH II stimulate in vitro production of testosterone and E by intact ovarian follicles (Suzuki et al. 1998d, Swanson et al. 1989, Planas 1993), and production of testosterone by isolated thecal cell-layers (Kanamori et al. 1988, Suzuki et al. 1988d, Planas 1993). The effect of GTH I and GTH II on E production may occur through stimulatory effects on T production because a direct stimulatory effect of gonadotropin on aromatase activity has not been reported in salmon.

The similar ability of GTH I and GTH II to stimulate steroid production at this stage is most likely due to a single type of gonadotropin receptor present in both the thecal and granulosa cells, which binds both gonadotropins (Yan et al. 1992, Miwa et al. 1994). Although both GTH I and GTH II show similar steroidogenic activity in the vitellogenic follicle, it is unlikely that GTH II plays a physiological role at this stage in salmonids because plasma levels are low or nondetectable. Furthermore, levels of GTH I, but not GTH II, are significantly correlated with plasma E and zr-proteins in salmon (Oppen-Bemsten et al. 1994).

Thus, the production of both vitellogenin and eggshell proteins in salmonids is probably regulated indirectly by GTH I through its effects on E production (Fig. 3). Non-steroid mediated effects of GTH I on oogenesis may also exist, but have not yet been reported.

### Gonadotropin Regulation of Final Oocyte Maturation

Oocyte growth is followed by a process called final oocyte maturation (the resumption of meiosis), which must precede ovulation and is required for successful fertilization. It is well established that gonadotropin initiates final oocyte maturation by stimulating production of maturation-inducing hormones (MIH) by ovarian folllcular cells (Nagahama *et* al. 1982b; Nagahama 1983, 1987). A number of C21-steroids induce oocyte maturation *in* vitro, including 17α-hydroxyprogesterone (17-OHP); 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P), 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), cortisol, and deoxycorticosterone. In salmonids 17α,20β-P is the most potent MIH (Nagahama 1983, 1987).

Several studies have shown that gonadotropin stimulates production of 17-OHP in the thecal cells; the 17-OHP is then converted to 17α,20β-P in granulosa cells by the enzyme, 20β-hydroxysteroid dehydrogenase (20β-HSD). In the preovulatory salmon follicle, both GTH I and GTH II stimulate production of 17-OHP by thecal cell layers; however, GTH II is far more potent than GTH I in stimulating conversion of 17-OHP to 17α,20β-P (Suzuki et al. 1988d, Planas 1993). The enhanced potency of GTH II during this period is associated with the appearance of a GTH II-specific receptor in the granulosa cells (Miwa et al. 1994). Additionally, the gonadotropin receptor, which binds both GTH I and GTH II (GTH-RI), is not present in granulosa cells of the preovulatory follicle. The inability of GTH I to stimulate activity is probably due to the loss of GTH-RI in the granulosa cells at this stage.

During the post-vitellogenic period, the capacity of the follicles to produce E declines and is associated with a decline in aromatase activity in granulosa cells, while 20β-HSD activity increases (Kanamori et al. 1988). The capacity of the thecal cells to produce testosterone in response to GTH increases at this time. Just prior to final oocyte maturation, the production of 17-OHP by thecal layers increases. Thus, there is a shift in the steroidogenic pathway in granulosa cells from production of E to 17α,20β-P, and there is enhanced production of 17-OHP by theca cells.

The induction of 20β-HSD and production of 17α,20β-P during final oocyte maturation are most likely controlled by GTH II, because plasma levels of GTH II increase during this period, and a receptor that binds GTH II but not GTH I is present in granulosa cells of the preovulatory follicle (Miwa et al. 1994). The appearance of the GTH II-specific receptor is coordinated with the increase in plasma levels of GTH II that precedes ovulation. Thus, key steps in the process of final oocyte maturation are regulated by GTH II (Fig. 3).

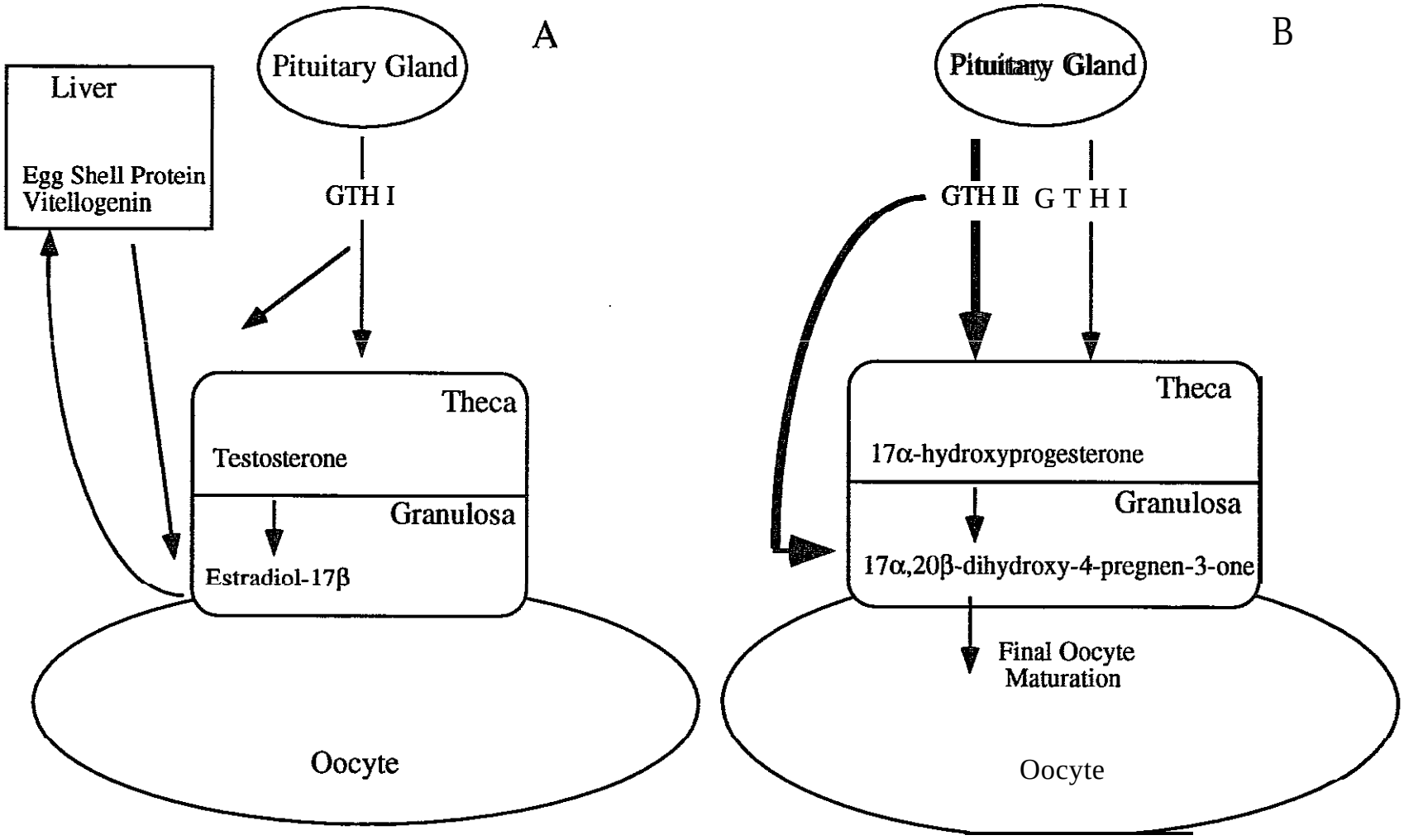


Figure 3. Diagramatic representation of the actions of gonadotropins (GTH I and GTH II) in the salmon ovary during secondary oocyte growth (A) and final oocyte maturation (B). During oocyte growth, plasma levels of GTH I increase. GTH I stimulates production of estradiol-17β, which is secreted into the peripheral ciruculation and stimulates the liver to produce the egg yolk precursor protein (vitellogenin) and the egg shell protein. GTH I also stimulates the uptake of vitellogenin by the oocyte. During final oocyte maturation, plasma levels of GTH I decline, whereas levels of GTH II increase. At this stage, GTH II stimulates production of the maturation-inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one, which stimulates fmal oocyte maturation. GTH II stimulates production 17α-hydroxyprogesterone by thecal cells and the conversion of this steroid to 17α,20β-dihydroxy-4-pregnen-3-one. GTH I also stimulates production of 17α-hydroxyprogesterone by thecal cells, but is less potent that GTH II. The thickness of the arrows in B reflects the relative difference in plasma levels of GTH I and GTH II.

## Gonadotropin Regulation of Spermatogenesis and Spermiation

The salmon testis is composed of interstitial and lobular (tubular) compartments (Billard 1983, 1992; Billard et al. 1986). In the interstitial compartment, Leydig cells are present and are involved in steroid biosynthesis. Within the lobule, two cell types are present: germ cells and Sertoli cells, which line the periphery of the lobule. Sertoli cells in fish have both histcchemical and ultrastructural features, which suggest that they are steroidogenic. However, the role of Sertoli cells in testicular steroidogenesis is unclear (Nagahama 1983). In male salmonids, as well as in other teleost species, plasma levels of testosterone and 11-ketotestosterone (11-KT) increase during later stages of spermatogenesis and decline slightly at the time of spermiation, when plasma levels of 17α,20β-P increase (Hunt et al. 1982, Fostier et al. 1983, Ueda et al. 1984, Baynes and Scott 1985, Billard et al. 1990, Billard 1992).

Spermatogenesis is regulated by gonadotropin indirectly through stimulation of steroid biosynthesis (Billard et al. 1986, 1990; Billard 1992, 1993). Miura and colleagues (Miura et al. 1991) have shown that the entire process of spermatogenesis could be induced ***in vitro*** by 11-KT. However, spermiation and the acquisition of sperm motility are mediated by 17α,20β-P (Miura et al. 1992). Gonadotropin stimulates production of testosterone and 11-KT by somatic cells of the testis (Schulz 1986, Sakai et al. 1989, Schulz and Blum 1990, Planas 1993, Planas et al. 1993, Planas and Swanson 1994), and the capacity of the testis to produce these steroids in response to gonadotropin increases during spermatogenesis. The production of 17OH-P 17α,20β-P increases during late stages of spermatogenesis and spermiation (Depeche and Sire 1982, Sakai et al. 1989, Planas et al. 1993, Planas and Swanson 1994). Planas and Swanson (1994) demonstrated that during early to mid-stages of spermatogenesis, both GTH I and GTH II stimulate production of testosterone, 17α,20β-P, and 11-KT with similar potencies. However, during late stages of spermatogenesis and at spermiation, the steroidogenic potency of GTH II exceeds that of GTH I.

Similar potencies of GTH I and GTH II during the early stages of spermatogenesis could be correlated with the presence of a single type of gonadotropin receptor (GTH-RI) that binds both GTH I and GTH II (Miwa et al. 1994). This receptor was localized in presumptive Sertoli cells of the testis throughout spermatogenesis, but may also be present in Leydig cells. In the spermiating testis, when GTH II has enhanced steroidogenic potency, a second gonadotropin receptor (GTH-RII), which binds GTH II specifically appears in Leydig cells.

It is likely that spenniation and the acquisition of sperm motility in salmonids are regulated by GTH II, not GTH I, because during this period plasma levels of GTH II increase, a GTH II-specific receptor appears in Leydig cells, and the capacity of the testis to produce 17α,20β-P in response to GTH II increases.

In contrast, spermatogenesis is probably regulated by GTH I via stimulation of 11-KT production because plasma levels of GTH I and 11-KT increase during early phases of spermatogenesis and remain elevated throughout spermatogenesis when GTH II levels are non-detectable (Swanson 1991).

### Applications of Reproductive Endocrinology to Captive Broodstock Programs for Salmonids and Future Research Needs

Reproductive endocrinology may be applied to captive broodstock programs by using hormones to control and monitor reproduction in broodfish. Later in this report, the use of exogenous hormones to control reproduction in salmonids is discussed. Plasma levels of hormones may be used to determine state of maturity and sex; gonadotropins to distinguish maturing from non-maturing broodfish, and steroids (E and 1 l-KT) to distinguish male from female fish. In both male and female salmonids, the period of gonadal growth appears to be regulated primarily by GTH I, whereas the period of final oocyte maturation and spermiation is regulated primarily by GTH II. Because plasma levels of GTH I increase 6 to 9 months in advance of the spawning period in maturing coho salmon (Swanson 1991, Fig. 2), it may be possible to use GTH I levels as a nondestructive method to distinguish maturing from non-maturing broodstock well in advance of the spawning period. Plasma GTH I and GTH II levels may also be used as a research tool to monitor the progress of maturation of experimental fish in response to various rearing conditions. Further research on the feasibility and reliability of using GTH I levels as a non-destructive method to distinguish maturing from non-maturing in a variety of salmonids will be required.

## Environmental Regulation of Reproduction in Salmonids

Fish of the salmon family Salmonidae am native to the oceans, rivers and lakes of the northern temperate zone and are seasonally breeding fish. The majority of sahnonids in the temperate zone spawn during autumn (September to December). However, some stocks of rainbow trout (0. *mykiss*) and steelhead (0. *mykiss* ) spawn during winter months (January to March) and a few spawn during the spring and summer. Spawning time has been shown to be a heritable trait in salmonids (Gall 1975, Gardner 1976, Gall et al. 1988). For each strain or stock, spawning occurs at a time which will ensure that the young fish or fry emerge when local climatic conditions am favorable and natural supplies of food are abundant (Brannon 1987, Heggeberget 1988). In addition to genetic factors, the seasonal timing of spawning in salmonids is controlled by photoperiod, temperature, and an endogenous circannual rhythm (reviewed by deVlaming 1972, Lam 1983, Scott 1990, Bromage et al. 1993).

## Regulation of Reproduction in Salmonids by Photoperiod

There is substantial evidence that the reproductive cycle in salmonids is driven by an endogenous rhythm (Whitehead et al. 1978a, b; Scott 1979; Duston and Bromage 1986, 1987, 1991; Bromage and Duston 1986). Spawning occurs at approximately annual intervals in fish experimentally maintained on constant photoperiods (LD 12:12). The endogenous rhythm is self-sustaining, has an approximate circannual rhythmicity, and is synchronized or entrained to the annual cycles by environmental cues. Photoperiod is the most important of these environmental cues in sahnonids.

The majority of studies on photoperiod control of reproduction in sahnonids have been conducted in domesticated strains of rainbow trout (Hoover 1937, Bromage et al. 1993) and brook trout (*Salvelinus fontinalis)* (Hazard and Eddy 1951, Henderson 1963, Carlson and Hale 1973), while relatively few studies have been conducted on Pacific salmon (Combs et al. 1959; MacQuarrie et al. 1978, 1979; Johnson 1984) and Atlantic salmon (*Salmo salar*) (Johnston et al. 1987, 1990, 1992; Taranger et al. 1991; Hansen et al. 1992). These studies have demonstrated that advanced or compressed seasonal light cycles induce precocious gonadal development; whereas delayed or extended photoperiods result in later spawnings.

From the early 1960s to the mid-1980s there was considerable debate about whether gonadal development in salmonids is cued by “long” or “short” day lengths. This debate was fueled in part by conflicting definitions of what constituted a “long” or “short” day. In numerous studies, the terms “long” and “short” day were used rather loosely to describe day lengths mom than 16 and less than 8 hours of light per day, respectively.

It was thought that a specific quantity of light (in terms of day length) was required to exert an effect on reproduction.

Now it is clear that the direction of change of photoperiod, rather than the absolute number of hours of light, is the critical factor for entrainment of reproduction by photoperiod (Duston and Bromage 1987, Randall et al. 1987). Therefore, "long" days can be defined as those which are preceded or followed by a photoperiod which is shorter, and "short" days are those which are preceded or followed by a photopetiod which is longer. Based on this definition, Scott (1990) concludes that in all salmonids the reproductive cycle is initiated in springtime, under conditions of increasing or "long-day" photoperiods. Decreasing or "short-day" photoperiods only advance the reproductive cycle in autumn-spawning salmonids.

One of the questions that arose early in the studies on photoperiod control of reproduction was whether seasonally changing light was critical for controlling spawning time. Studies in rainbow trout have demonstrated that the timing of reproduction can be modified by an altered seasonal light cycle; however, seasonally changing light is not essential for maturation (Whitehead et al. 1978a, b; Bromage et al. 1982, 1984; Elliot et al. 1984; Bromage and Duston 1986, 1987; Duston and Bromage 1986, 1987). For example, the rise and decline in day length, which occurs seasonally, can be replaced by constant "long" and "short" photoperlods, respectively (Whitehead and Bromage 1980, Bromage et al. 1982, Bromage and Duston 1986). Because of the endogenous rhythm controlling maturation, the timing of exposure to changes in day length relative to the phase of endogenous rhythm determines whether spawning is advanced or delayed and the degree to which spawning time is altered. This phenomena has been studied extensively in rainbow trout and is reviewed below. Studies conducted on other salmonids are cited when relevant

Spawning time can be advanced if rainbow trout are exposed to "long" days or continuous light during the early part of the reproductive cycle, or from the winter solstice throughout the spring (Whitehead and Bromage 1980; Bromage et al. 1982.1984; Scott et al. 1984; Bromage and Duston 1986, 1987; Duston and Bromage 1987, 1988). The degree of advancement of spawning time appears to be greatest if trout are exposed to "long" days or continuous light just after spawning at the winter solstice (Bromage et al. 1984, Scott et al. 1984). In contrast, the reproductive cycle of salmonids can be extended and spawning time can be delayed by exposing fish to "long" days or continuous light after the summer solstice (Allison 1951; Combs et al. 1959; Henderson 1963; Shirashi and Fukuda 1966; MacQuarrie et al. 1978, 1979; Lundqvist 1980; Whitehead and Bromage 1980; Bromage et al. 1982; Johnson 1984; Bourlier and Billard 1984; Takashima and Yamada 1984). Fish exposed to continuous light after the summer solstice not only show a delay in spawning, but their period of spawning can be extended two- to three-fold (Bourlier and Billard 1984).

However, exposure to continuous light will also cause spawning to be asynchronous within a group of fish and a significant amount of atresia of eggs can occur (Bourlier and Billard 1984).

In general, exposure of trout to constant "short" days during the very early stages of maturation results in a delay in spawning (Whitehead and Bromage 1980, Bromage et al. 1984, Duston and Bromage 1987). The extent of the delay depends on the point at which the photoperiod is applied relative to the stage of reproductive development and natural spawning time of the particular stock of fish. Once maturation has proceeded for approximately 3 to 4 months in rainbow trout, exposure of fish to "short" days advances spawning time (Bromage et al. 1984, Duston and Bromage 1987).

**Regulation of Reproduction in Salmonids by Temperature**

Although photoperiod is regarded as the most important environmental factor controlling gonadal development and spawning time in sahnonids, it does not act alone in controlling reproductive function.
The extent to which temperature acts in concert with photoperiod to regulate the timing and rate of gametogenesis is poorly understood. Most studies on photoperiod manipulation of reproduction have maintained fish on either constant or ambient temperatures averaging 10 °C Few experimental studies have been conducted on the effects of rearing temperature on reproduction in sabnonids (Henderson 1963; Breton and Billard 1977; Morrison and Smith 1986; Beacham and Murray 1988; Bromage and Cumaranatunga 1988; Nakari et al. 1987, 1988; Johnston et al. 1987, 1990, 1992; Taranger and Hansen 1993).

In rainbow trout, studies have shown that temperatures ranging from 8 to 16 °C have little effect on timing of the reproductive cycle, but higher temperatures adversely affect the quality and quantity of gametes (Billard 1985). The approximate upper limit for successful reproduction of salmonids in the wild is considered to be 13 °C (MacCrimmon 1971, Scott 1990). However, this upper limit may be lower for salmonids which naturally spawn in the northernmost latitudes or high altitudes (Tarauger and Hansen 1993).

While the range of water temperatures for survival and successful reproduction has been studied for a number of salmonids, the question of whether seasonally fluctuating temperatures are important for reproductive performance of broodstock has not been thoroughly investigated. In many hatchery conditions, particularly those using wellwater, fish am reared on relatively constant water temperature. Only one study has compared the effects of constant versus seasonally fluctuating rearing temperature on spawning time. Davies and Bromage (1991) maintained rainbow trout on a river-water supply with seasonal variations in water temperature ranging from 4°C in February to 16.5°C in July. They found that these fish responded similarly to a parallel group of fish maintained on well-water with constant temperature of 8.5°C and exposed to the same stimulatory photoperiod.

Spawning time of both groups was advanced. However, Davies and Bromage did not describe the effects of temperature on the quality of gametes produced by fish reared on the two thermal regimes. Other important factors to consider are that the response of domesticated stocks to rearing temperatures may be very different than that of wild stocks and may vary considerably among species and stocks.

Because fish are poikilothermic animals, temperature directly affects their gonadal physiology by changing the rate of yolk sequestration, steroid biosynthesis, and other general metabolic reactions. In salmonids, low temperatures (< 8°C) reduce the rate of maturation; causing vitellogenesis (Johnston et al. 1987, Korsgaard et al. 1986) and oocyte growth (Crim et al. 1983b) to proceed slowly. At very low temperatures (< 3°C) the final stages of oocyte maturation and ovulation are completely inhibited (Billard 1985). Testicular steroid production is also reduced at low temperatures (Manning and Kime 1985). Spawning time in autumn-spawning trout can be delayed until spring by adversely cold weather conditions, and conversely, spring-spawning trout can be made to spawn in winter or autumn by movement into temperate water conditions (Morrison and Smith 1986, Nakari et al. 1987, Titarev 1975). In Atlantic salmon, high water temperatures (increases from 10 to 13-14°C) during the spawning season inhibit ovulation and have a detrimental effect on gamete quality (Taranger and Hansen 1993). Heggeberget (1988) found that in this species, peak spawning in Norwegian rivers occured when water temperatures were decreasing. This suggests that among sahnonid species and/or strains temperature may affect reproduction differently.

### Applications of Environmental Control of Reproduction to Captive Broodstock Programs for Salmonids and Future Research Needs

In commercial farming of salmonids, manipulation of spawning time has been used to spread the availability of juvenile fish over the year. This regulation provides the market with continuous production, and synchronizes or compresses the spawning period. In captive broodstock programs for depleted fish stocks, off-season spawning of fish may not be an appropriate goal, since production of progeny for release into the natural habitat should be timed appropriately for the requirements of the stock. However, photoperiod in captive rearing facilities must be controlled, and inadvertent exposure of fish to light during the dark phases should be avoided. Uncontrolled lighting could have catastrophic consequences if, for example, it artificially extended spawning periods into periods when ambient water temperatures are sufficiently high to impair gamete quality and offspring survival. Furthermore, exposure to continuous light can induce asynchronous maturation and atresia of oocytes (Bourlier and Billard 1984).

MacQuarrie et al. (1978) observed abnormalities in the process of oogenesis and poor fertilization of eggs when coho salmon were exposed to advanced photoperiod and natural thermoperiod.

In addition, the photoperiod history, stage of reproductive development, and natural spawning time of the fish must be considered if alterations in light-cycles are made because the response of the fish to changes in photoperiod depends on all three factors.

The effects of temperature on reproductive performance in salmonids have not been extensively examined. Further studies are necessary to develop better guidelines for rearing temperatures for Pacific salmon broodstock, and to determine whether constant or seasonally fluctuating water temperatures affect reproductive performance. This is particularly important because facilities for captive rearing of broodstock may be practically limited by the ability to control water temperature and the availability of water of constant versus fluctuating temperatures.

## Hormonal Induction of Final Oocyte Maturation, Ovulation, and Spermiation in Salmonids

Reproduction in captive broodfish can artificially controlled at two levels: through manipulation of environmental cues and through alterations in the reproductive endocrine system. As mentioned in the previous section on environmental control of reproduction in sahnonids, spawning time can be delayed or advanced through alterations in environmental factors such as photoperiod. Photoperiodic influences on reproduction, after perception and integration by the central nervous system, alter release of hormones by the hypothalamus (primarily GnRH), which in turn regulate secretion of pituitary gonadotropins (Fig. 1). Techniques for the control of reproduction through exogenous administration of hormones have been developed which intervene at each level of the brain (GnRH)-pituitary (gonadotropin)-gonad (steroid) axis.

Although manipulation of gametogenesis (gonadal growth) has been achieved in some species through chronic treatment of fish with various preparations of gonadotropins and/or steroids (Billard et al. 1986, 1990; Billard 1992), this technology is not presently used on cultured salmonids. However, hormonal induction of final oocyte maturation, ovulation, and spermiation is widely used on salmon broodstock to prevent losses due to prespawning mortality and to advance or synchronize spawning time (Donaldson and Hunter 1983; Donaldson 1986; Zohar 1988.1989).

### Hormones Used to Induce Final Oocyte Maturation, Ovulation, and Spermiation

The hormonal induction of spawning is achieved by producing an increase in plasma levels of gonadotropin (GTH II or homologous proteins) in fish that have completed vitellogenesis and spermatogenesis. The gonadotropins act on the ovary to induce final oocyte maturation and ovulation or on the testis to induce spermiation (as previously described). Plasma levels of gonadotropins can be elevated either by stimulating secretion of endogenous gonadotropins, or by administration of pituitary extracts, human chorionic gonadotropin (a human hormone which is chemically similar to fish GTH II), or purified preparations of fish gonadotropins.

Induction of spawning with pituitary extracts or partially purified salmon gonadotropin has been demonstrated widely in salmonids (Jalabert et al. 1978; Hunter et al. 1978, 1979, 1981; Sower et al. 1982; Donaldson et al. 1985; Van Der Kraak et al. 1985). However, this technique has some major limitations. There is a high degree of variability in the purity and quality of the gonadotropin preparations, and highly purified gonadotropins are expensive and difficult to obtain.

Furthermore, there is a high degree of species specificity in fish gonadotropin: a preparation from one species may not be effective in another. Finally, the content of biologically active gonadotropin in pituitary extracts is variable, and the presence of other hormones in the extracts can lead to undesirable effects.

A more reliable and economical alternative to administration of gonadotropin preparations has been the use of GnRH or superactive analogues of GnRH (GnRHa) for induction of spawning. This appears to be the most efficient therapy because GnRH stimulates release of endogenous gonadotropin, thus avoiding problems with species specificity and quality of the gonadotropin preparations. GnRH and GnRHa are non-immunogenic decapeptides, which can be synthesized and obtained in pure form and are readily available from a variety of chemical companies. They also can be administered in very low doses (few micrograms/kilogram body weight), which is more economical than gonadotropin preparations.

### Induction of Spawning with Analogues of GnRH in Salmonids

Induction of ovulation and spermiation in fully mature fish using GnRH or GnRHa has been achieved in a wide variety of salmonids (Donaldson et al. 1981, 1984; Sower et al. 1982, 1984; Crim et al. 1983a, b, 1986, 1987, 1988a, b; Donaldson and Hunter 1983; Weil and Crim 1983; Crim and Glebe 1984; Fitzpatrick et al. 1984, 1987; Van Der Kraak et al. 1985; Zohar et al. 1990a, b; Breton et al., 1990; Taranger et al. 1992; Mylonas et al. 1993; Haraldsson et al. 1993; Slater et al. 1995). In initial studies of GnRH or GnRHa-induced spawning in salmonids, GnRH or GnRHa were tested in conjunction with gonadotropin preparations (Donaldson et al. 1981, 1984, 1985; Sower et al. 1982, 1984; Van Der Kraak et al. 1983, 1984, 1985). However, treatment with GnRHa alone has been shown to be equally effective to that of GnRHa combined with gonadotropin as long as two injections of GnRHa were given.

Superactive analogues of GnRH are generally more potent *in* vivo than native forms of GnRH (Crim et al. 1987, 1988b; Donaldson et al, 1981) because of their prolonged biological half lives (Goren et al. 1987, Zohar et al. 1990a) and higher affinity to GnRH receptors (Habibi et al. 1987). The analogues of GnRH most commonly used for induction of spawning are as follows: [D-Arg$^{6}$-Pro$^{9}$NET] salmon GnRH, [D-Ala$^{6}$-Pro$^{9}$-NET] mammalian GnRH, and des-Gly$^{10}$[D-Ala$^{6}$]-mammalian GnRH ethylamide. These analogues differ somewhat in their ability to induce spawning in goldfish and in their affinity to goldfish GnRH receptors (Habibi et al. 1987, Habibi and Peter 1991); however, no significant differences have been observed among these analogues when used in salmonid fish or seabream (*Sparus aurata*) for spawning induction (Zohar et al. 1989, 1990a; Haraldsson et al. 1993).

For successful induction of ovulation in salmonid fish, GnRHa dissolved in saline has been administered by two intramuscular or intraperiotoneal injections spaced at an interval of 3 days (Donaldson 1986; Crim et al. 1987; Van Der Kraak et al. 1984, 1985; Fitzpatrick et al. 1984, 1987; Zohar et al. 1989, 1990a; Slater et al. 1995). In general, G&Ha-induced ovulation was observed within 10- 14 days after the initial injection. However, the rate of response to the GnRHa depended on the timing of treatment relative to the natural period of ovulation and the dosage of GnRHa. If GnRHa was administered within 3 to 4 weeks prior to the normal spawning time, ovulation was synchronized and advanced by approximately 2 weeks (Taranger et al. 1992). Ovulation was synchronized, but not significantly advanced when fish were. treated within 2 weeks of the normal spawning time. Dosages ranging from 10 to 150 µg GnRHa per kg body weight have been used to advance or synchronize ovulation in female salmonids. However, several studies (Crhn and Glebe 1984, Taranger et al. 1992) have reported reduced fertilization rates and survival of offspring to the eyed-stage when females were treated with high dosages of GnRHa (100-150 µg/kg body weight). Dosages of 10-20 µg GnRHa/kg body weight have been shown adequate for advancing or synchronizing ovulation without impairing offspring survival (VanDerKraak et al. 1985, Taranger et al. 1992).

Technology for controlled-release GnRHa delivery systems has been developed for induction of ovulation and spermiation in sahnonids (Crim et al. 1983b, 1988a; Crim and Glebe 1984; Zohar et al. 1990b; Breton et al., 1990). The advantages of this technique are that 1) the quantity of hormone administered and labor required for the treatment can be reduced, making the treatment more cost-effective, and 2) stress to the broodfish associated with protocols requiring mutiple injections can be reduced. Several types of delivery systems for GnRHa have been developed and tested in salmonids.

Crim and colleagues (Crim et al. 1983b, 1987, 1988a; Crim and Glebe 1984) advanced and synchronized ovulation with a pelleted form of GnRHa in a cholesterol matrix. The minimum dose of GnRHa has not been determined for this technique; however, a single treatment with 20-25 µg GnRHa/kg body weight was effective in advancing ovulation (Crim and Glebe 1984). Zohar and colleagues (Zohar et al. 1990b) have developed two types of GnRHa delivery systems: nonbiodegradable and biodegradable. The nonbiodegradable delivery system is a pellet (approximately 0.5 mm diameter) composed of an ethylene vinyl acetate copolymer (EVAC). Implants containing dosages of GnRHa as low as 25 µg per fish (body weight ranging from approximately 0.8 to 2.5 kg) effectively induced ovulation in trout (Breton et al. 1990) and coho salmon (Fig. 4) without impairing offspring survival. These implants were administered by intramuscular injection and induced ovulation or spermiation approximately 8-12 days after treatment.

A second type of delivery system developed by Zohar and colleages (Zohar et al. 1990b) is a preparation of biodegradable microspheres consisting of a polyglycolic acid copolymer.

The microspheres containing GnRHa (75 µg GnRHa/kg body weight) and administered by intramuscular injection have been used to induce ovulation and spermiation 2 to 4 weeks in advance of the normal spawning time in coho salmon (Pig. 5). In farmed coho salmon, a combination of compressed photoperiod and treatment with GnRHa-microspheres was used to advance spawning by 4 to 8 weeks (Greg Hudson, Domsea Broodstock, Inc., Rochester, Washington, Pers. commun. June, 1991). Similar results have been obtained for advancement of maturation in sockeye (0. *nerka)* salmon without impairing gamete quality using microspheres containing GnRHa (Fig. 6). Presently, the minimum dosage of GnRHa-microspheres required for induction of ovulation and spermiation has not been established in salmonids.

### Timing of Hormone Treatments for Spawning Induction

One of the major considerations in developing technology for induction of ovulation and spermiation is the timing of hormone injection. Presently, females are selected for treatment on the basis of overall appearance (coloration, shape of abdomen, etc.) or stage of oocyte development, which is determined after ovarian biopsy in fish with sufficiently small eggs. Unfortunately, morphological indicators are not always reliable indicators of responsiveness, and fish with large eggs like sahnonids am not easily biopsied. Determination of the stage of maturity can be particularly difficult in some wild salmon stocks that have extended spawning periods.

Fitzpatrick et al. (1987) have suggested that plasma testosterone levels, which are elevated preceding ovulation in sahnonids, may be used as a hormonal indicator to predict the sensitivity of the female to GnRHa. This technique is somewhat limited because it is most reliable if two plasma samples can be taken at an interval of several days, so that a relative change in testosterone levels can be observed to gauge more precisely the stage of maturation. In addition, it currently requires sophisticated laboratory facilities to analyze the testosterone levels by radioimmunoassay, and several days or weeks before the information is available to hatchery managers. The reliability of testosterone levels as indicators of responsiveness to GnRHa will require further testing to reach a practical level of use in management of captive broodstock.

Another method to determine the timing of hormone-induced spawning is simply use of the calendar. Hormone-induced spawning has been successful when treatments were administered within 2 to 4 weeks in advance of the normal spawning period, depending on the mode of administration of GnRHa. This technique is practical when the normal spawning period for the broodstock is well characterized and occurs within a 4- to 6-week period, as in the case of domesticated stocks of farmed salmon. Obviously, when secondary sex characteristics develop near the time of spawning, GnRHa can be used reliably to synchronize spawning among broodstock.

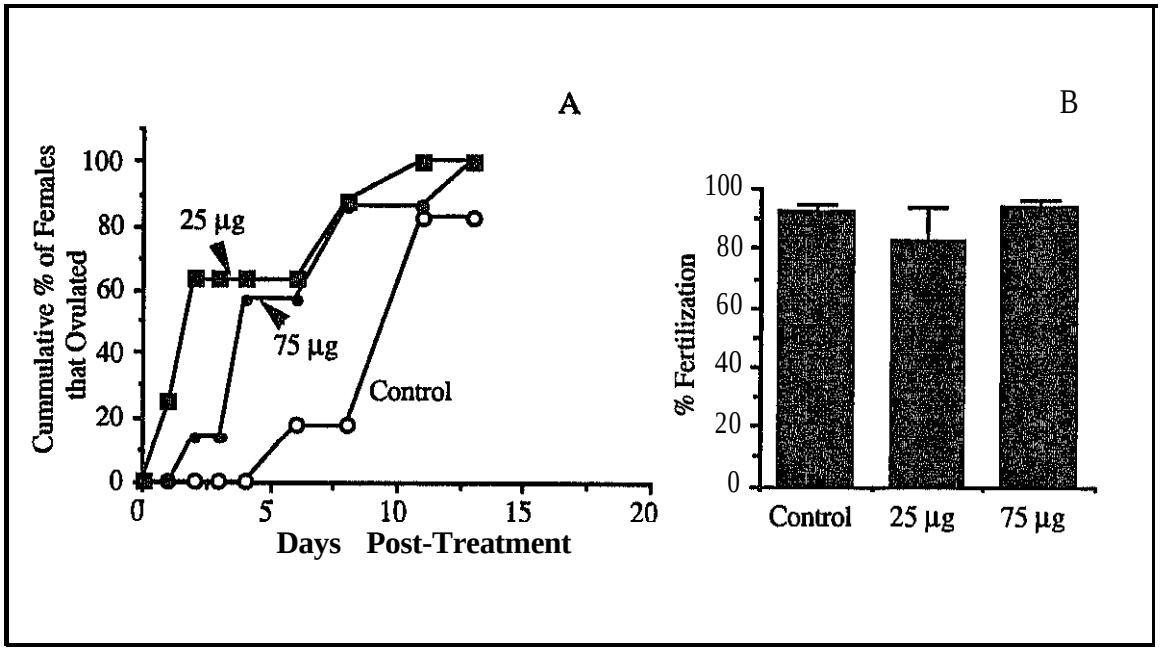


Figure 4. Induction of ovulation in female coho salmon using gonadotropin-releasing hormone analogue (GnRHa) administered via ethylene vinyl acetate copolymer (EVAC) implants. Fish (N = 10 per treatment) were injected intramuscularly with EVAC implants containing either 25 or 75 micrograms GnRHa. Control fish received blank implants. Data on ovulation are expressed as cummulative percent of females that ovulated during the course of study. Both doses of GnRHa effectively advanced ovulation (A) without impairing egg quality (B). Data on fertilization are mean ± standard error. (Swanson, Dickhoff, Larsen, and Zohar, unpubl. data, NMFS).

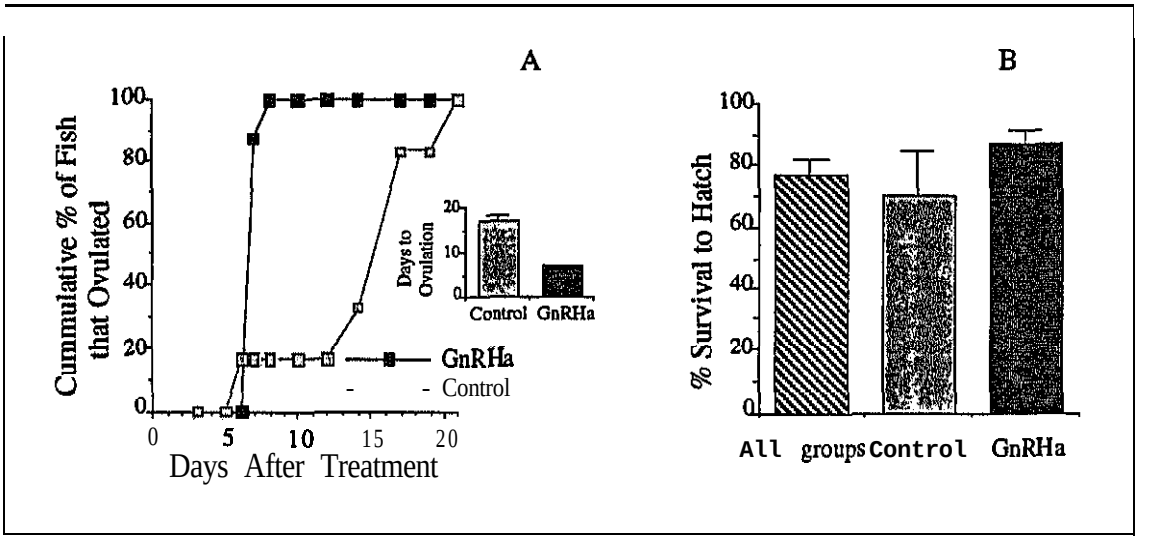


Figure 5. Induction of ovulation in adult female coho salmon using microspheres containing GnRHa (75 micrograms& body weight). Fish (N = 6 per treatment) were treated approximately 3 weeks in advance of the historical spawning date for the stock. Treatment with GnRHa-microspheres advanced ovulation by about 2 weeks without affecting survival of embryos to hatching. (Swanson, Dickhoff, Larsen, Mylonas and Zohar, unpubl. data, NMFS)

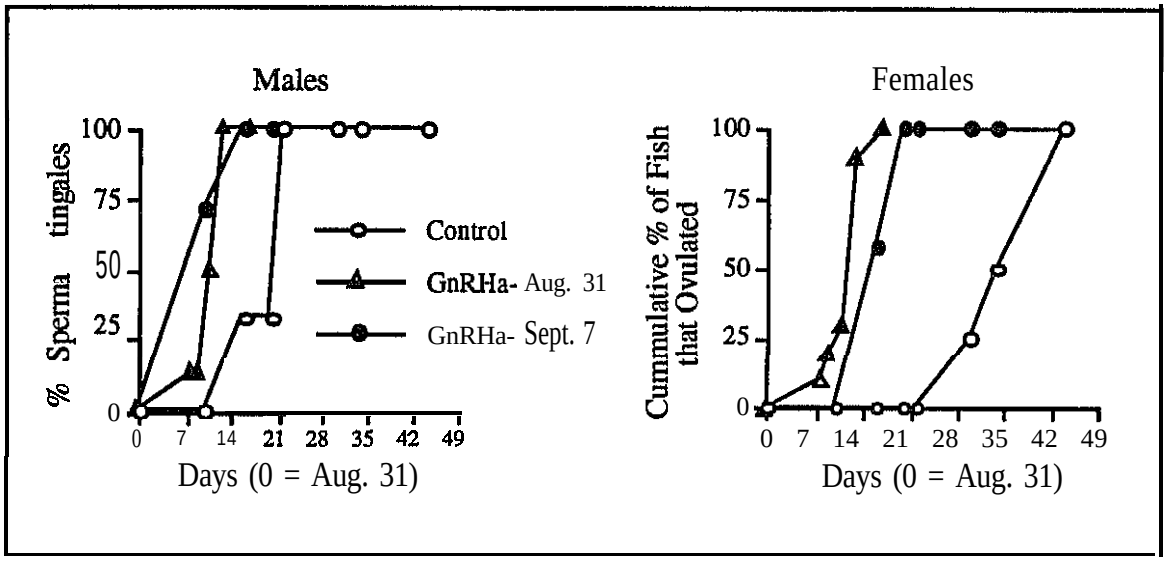


Figure 6. Induction of spermiation and ovulation in Lake Wenatchee sockeye salmon. Fish were injected intramuscularly with microspheres containing GnRHa (75 micrograms/kg body weight) either on day 0 (Aug. 31) or day 7 (Sept. 7). Control fish received blank microspheres on day 0. N = 10 fish of each sex per group. Data are expressed as cummulative percent of either spermiating males or ovulated females. The GnRHa treatment successfully advanced and synchronized maturation in both male and female fish without affecting the rate of fertilization or survival of fertilized eggs to the eyed stage (Swanson, Yan, Dickey, and Zohar unpubl. data, NMFS).

**Applications of Hormone-Induced Maturation to Captive Broodstock Programs for Salmonids and Future Research**

Technology for hormonal induction of ovulation and spermiation has several important applications to captive broodstock programs for endangered or threatened salmonid fish. This technology can be used to 1) prevent loss of gametes due to prespawning mortality, 2) synchronize spawning in wild and captive fish, 3) extend the period of spermiation and yield of sperm, and 4) to synchronize and/or advance spawning in male and female broodfish. Hatchery managers may not need to routinely induce spawning in broodstock, but this technology is a tool that should be available to managers who may need to produce a predictable spawning period or advance spawning if there is a risk of prespawning mortality. However, artificial induction of spawning using GnRHa should be further refined for general use in control of reproduction in Pacific salmon broodstock.

Further research is necessary to develop a reliable index of when GnRHa can be administered to both male and female fiih for successful induction of spawning. This will be particularly important for species with extended spawning seasons. The minimum effective dosages and optimal modes of administration of GnRHa must be established in several species and stocks for both males and females.

## Precocious or Early Maturation in Male Salmonids

In both captive and wild populations of anadromous salmonids, sexual maturation may occur at an earlier age for males than for females in the breeding population. Early, or precocious maturation in male salmonids may occur in fresh water prior to smoltification and seawater migration or after a period of seawater residence. The term "precocious parr" has been used to describe male salmonids that mature without smolting and migrating to sea, whereas the term "jack" has been used to describe males that mature after a period of seawater residence, usually at least 1 year prior to the first maturation of females. The period of seawater residence for jacks depends on the species, and is less than 1 year for coho salmon and up to a year for chinook salmon.

Precocious maturation of male parr is common in Atlantic salmon (Thorpe 1986), but also has been documented in a wide variety of anadromous ***Oncorhynchus*** sp. including chinook salmon (Robertson 1957, Gebhards 1960, Taylor 1989, Foote et al. 1991), sockeye salmon (Ricker 1938, 1959; Burgner 1991), coho salmon (Silverstein and Hershberger 1992), steelhead (0. *mykiss*) (Schmidt and House 1979), amago (0. ***rhodurus)*** (Nagahama et al. 1982a), and masu salmon (0. *masu*) (Aida et al. 1984, Kato 1991). Early maturation of males after seawater residence has been documented for chinook salmon (Hard et al. 1985, Healy 1991, Bocking and Nass 1992), coho salmon (Bilton 1980, Bilton et al. 1981, Sandercock 1991) and sockeye salmon (Burgner 1991).

The phenomena of precocious sexual maturation of male salmonids is an evolutionarily viable, alternative life-history strategy. Smaller, younger males gain access to females for reproduction by utilizing "sneaking" behavior, while the larger, older males guard females and fight each other to defend their territory (Gross 1985). Thus in nature, precocious male maturation is not necessarily an undesirable characteristic. However, in captive breeding programs for depleted salmon stocks, maturation of males in the absence of matnre females could be catastrophic.

In commercial aquaculture, early maturing male fish have reduced market value and poor survival, thus representing serious financial loss to the farmer. In addition, precocious male maturation in hatchery-reared Atlantic salmon causes a significant decrease in the rate of adult recaptures from hatchery releases (Lundqvist et al. 1988) and a loss of adult chinook salmon to the fishery (Foote et al. 1991, Mullan et al. 1992). Precocious male parr may reabsorb gonadal tissue and smolt in the year following maturation (Robertson 1957, Gebhard 1960, Lundqvist and Fridberg 1982, Bemier et al. 1992).

However, the maturation process impairs seawater adaptability and smolting during the subsequent spring in precocious Atlantic salmon (Saunders et al. 1982, Langdon and Thorpe 1985, Lundqvist et al. 1988, Thorpe 1987), sea trout (S. *trutta*) (Dellefors and Faremo 1988), masu salmon (Aida et al. 1984), amago salmon (Nagahama et al. 1982a) and chinook salmon (Foote et al. 1991). It has also been shown that the mortality rate of mature male parr is higher than that of immature parr (Myers 1984, Dempson et al. 1986). Therefore, a better understanding of the control of early male maturation is needed to develop rearing methods that will maximize the synchronous maturation of both sexes and reduce the proportion of precocious males while not adversely affecting the survival of sexually immature smolts.

There is strong evidence that the age of sexual maturity in salmonids is regulated by both genetic and environmental factors (Purdom 1979, Randall et al. 1986, Gjerde 1984a). Environmental factors include both abiotic factors such as photoperiod and temperature, which affect the seasonal timing of maturation, and biotic factors such as food availability and diet composition, which affect growth and energy status. Although effects of both genotype and environment on precocious male maturation have been found, clarifying the relative roles of these factors has been difficult because of their interrelatedness. In the following sections, biological factors that affect the rate of early maturation in male sahnonids are discussed.

### Genetic Basis of Precocious Maturation

There is strong evidence that genetic factors play a role in determing the age of sexual maturation in several species of salmonids (Purdom 1979, Gjedrem 1985, Naevdal 1983). Most genetic evidence for age of sexual maturity has come from work on Altantic salmon (Gardner 1976; Naevdal et al. 1978a; Thorpe and Morgan 1978.1980; Thorpe et al. 1983; Gjerde 1984a, b; Myers et al. 1986; Glebe and Saunders 1986) and rainbow trout (Gall 1975; Moller et al. 1976; Naevdal et al. 1979; Gjedrem 1985; Gall et al. 1988; Crandell and Gall 1993a, b). However, there is also evidence in coho salmon (Iwamoto et al. 1984, Silverstein and Hershberger 1992), chinook salmon (Hard et al. 1985, Heath 1992), and ***Arctic*** charr, ***Salvelinus alpinus*** (Nilsson 1992).

One difficulty in genetic analyses of precocious maturation in salmonids is sorting out clear genetic from non-genetic maternal effects. For example, egg sire can affect early growth of offspring, which in turn may play a role in precocious maturation (Silverstein and Hershberger 1992). A maternal effect on early growth could have an impact on estimates of the dam component of observed variance in maturation timing and must be considered in the analyses (Bradford and Peterman 1987).

Breeding experiments have identified both a maternal and paternal genetic component to precocious male maturation. An effect of sire age has been found in chinook salmon (Heath 1992), coho salmon (Iwamoto et al. 1984, Silverstein and Hershberger 1992), and Atlantic salmon (Thorpe and Morgan 1980; Glebe and Saunders 1986; Thorpe et al. 1983; Gjerde 1984a, b). Maternal genetic effects on precocious maturation have also been found (Heath 1992, Silverstein and Hershberger 1992).
In addition, significant genotype-by-environment interactions for temperature and growth have been found in a number of salmonids (McKay et al. 1984, Iwamoto et al. 1984, Heath 1992, Nilsson 1992). In other words, the maturation response to growth acceleration in early life is not equivalent in all genetic groups or genotypes.

Clearly, genetic effects on maturation may be linked to genetic effects on growth (Thorpeand Morgan 1978; Purdom 1979; Thorpe et al. 1983, 1984; Crandell and Gall 1993a, b). Thorpe et al. (1983, 1984) have shown that fast growth rate and early maturation were genetically linked to developmental rates. In a later study, Thorpe (1986, 1994) proposed that maturation depended on a genetically determined rate of development that must be exceeded during a specific time of year.

**Growth and Age at Maturity**

In fish, different populations of the same species may show a wide range in mean age of maturity. There is also variation within the same population that suggests that age at first maturation in fish is probably linked to environmental effects on individual growth rates. Each species may need to reach a certain size or body condition before successful sexual maturation and breeding can take place (Policansky 1983), and these thresholds may be partially determined by environmental conditions.

Within populations of salmonids, there is a high degree of variation in age of maturity, ranging from 1 to 7 years. It is generally thought that males are able to mature at an earlier age than females because the energetic "cost" of producing sperm is very low compared with that of producing viable eggs (Wootton 1985). Thorpe (1994) suggested that the high degree of phenotypic plasticity in age of maturity in salmonids is most likely an adaptation to the variable productivity of the freshwater environment, which the fish occupy during early life-history stages. Furthermore, he has proposed that timing of maturation is influenced by growth opportunity at critical life-history stages.

It is well established that age at fist maturity in salmonids is strongly dependent on size and growth. Ahn (1959) was the first to show that in a population of brown trout (*Salmo trutta*), fish which grew fastest matured earliest. Similarly, Lamont (1990) used individually tagged rainbow trout and coho salmon to show that the fastest growing individuals were early maturing males. Several studies have shown that maturing male parr are usually larger than nonmaturing siblings (Naevdal et al. 1978b, Bailey et al. 1980, Thorpe et al. 1983, Rowe and Thorpe, 1990a, Heath 1992).

Furthermore, age at first maturity in salmonids is negatively correlated with growth rate, or in other words, early maturation is positively correlated with growth rate ( Kato 1975, 1978; Gardner 1976; Hagar and Noble 1976; Glebe et al. 1978; Naevdal et al. 1978b, 1979; Bilton et al. 1981; Lundqvist 1980; Hunt et al. 1982; Thorpe et al. 1983; McCormick and Naiman 1984; Gjedrem 1985; Thorpe 1986; Rowe and Thorpe 1990a; Bergland 1992; Clarke and Blackburn 1994).

However, the correlations of early male maturation to growth rate are not maintained throughout gonadal development. Several studies have shown that during later stages of gonadal growth the specific growth of maturing males decreases (Thorpe and Morgan 1980; Saunders et al. 1982; Thorpe et al. 1983; Glebe and Saunders 1986; Rowe and Thorpe 1990a, b, Foote et al. 1991). This is consistent with the generally accepted idea that as fish mature, a reduction in somatic growth occurs due to allocation of energy to gonadal development (Ware 1980, Roff 1983). In contrast to this model, a reduction in somatic growth was not observed in in one study of early maturing male chinook salmon (Heath 1992).

A number of studies have indicated that the rate of early male maturation can be modified by the hatchery rearing environment, primarily by factors that affect growth opportunity (Saunders and Henderson 1965, Schmidt and House 1979, Bilton et al. 1981, Saunders 1986, Saunders et al. 1982, Taylor 1990, Rowe and Thorpe 1990b, Thorpe et al. 1990, Bergland 1991, Herbinger and Friars 1992, Clark and Blackburn 1994). Bilton et al. (1981) demonstrated that acceleration of growth in chinook salmon fry with elevated water temperature also increased the incidence of early male maturity. In Atlantic salmon, Bailey et al. (1980). Saunders et al. (1982) increased the number of mates maturing at 0+ age by increasing growth during the winter months with elevated water temperatures. Similar results of elevated termperamre on precocious maturation in Atlantic salmon were found by Bergland et al. (1991) and Herbinger and Friars (1992).

Rowe and Thorpe (1990b) found that in Atlantic salmon, reduced feeding opportunity during the spring prior to maturation suppressed early male maturation, whereas increased feeding at this time increased the incidence of male maturation. A similar effect of spring feeding opportunity on grilsing in Atlantic salmon held in seawater cages has been reported (Thorpe et al. 1990). In chinook salmon, Clarke and Blackbum (1994) increased the proportion of sexually maturing yearling fish by increasing ration from November through June. They also noted that males destined to mature as yearlings grew more rapidly as underyearlings than their immature cohorts, and thus concluded that sexual maturation is conditional to and facilitated by rapid growth.

These relationships between maturation and size have led to several hypotheses to explain the role of growth in the initiation of sexual maturation. Early work led to the proposal that there was a minimum threshold of size for maturation of male parr (Elson 1957, Refstie et al. 1977, Bailey et al. 1980, Myers et al. 1986) and that this size threshold' was higher for maturation than for smoltification (Thorpe et al. 1980, Saunders et al. 1982, Thorpe et al. 1987). Myers (1984) and Myers et al. (1986) found that the proportion of mature male parr over a 5-year period was correlated with growth to a size threshold of 70-72 mm in Atlantic salmon.

Later, it was recognized that growth opportunity at critical seasonal periods was important for initiation of maturation. Thorpe (1986) proposed that maturation is initiated if a set-point in growth rate is exceeded at a particular time of year. Because maturation in salmon is initiated under increasing day lengths (Scott 1990, Adams and Thorpe 1989), Thorpe proposed that the initiation of maturation depended on growth performance during the winter or spring. Rowe and Thorpe (1990b) could reduce the proportion of maturing male parr by reducing feeding and growth during the spring months. Although maturing male parr tended to be larger than nonmaturing siblings in the winter, no relationship between monthly specific growth rates and maturation could be established in this study. However, maturing parr showed greater increases in condition factor than nonmaturing fish during the spring.

This led to another hypothesis: that levels of stored energy reserves in the spring, rather than body size, were involved in the physiological initiation of maturation (Herbinger and Newkirk 1990, Rowe and Thorpe 1990b, Rowe et al. 1991, Simpson 1992). In salmonids, mesenteric fat is the major energy store (Henderson and Sargent 1981). A study by Rowe et al. (1991) demonstrated that levels of mesenteric fat in May, prior to maturation, are higher in precocious Atlantic salmon male parr than in their nonmaturing siblings. These investigators proposed a model describing a potential mechanism for the effect of fat on maturation.

In this model, mesenteric fat stores act as sites for aromatization of androgens to estrogens, which in turn trigger maturation through stimulation of pituitary gonadotropin synthesis. However, no further endocrine studies have been reported to support this model. It is likely that the endocrine mechanism involved in initiation of maturation is far more complex than proposed in this model and probably involves metabolic hormones such as insulin.

More recently, Thorpe (1994) has suggested that during the life-cycle of salmon, there is an annual opportunity for sexual maturity and that during critical periods of this annual cycle, growth opportunity acts as a gate permitting maturation to be initiated. In Thorpe's model, the maturation process is initiated by the time of fist feeding, and takes priority over somatic growth: completion of maturation is environmentally dependent and can be arrested annually.

Whether or not maturation is arrested depends on the status of energy stores of the individual at particular critical times of year. Thii model and data on the relationship between growth and maturation has several implications for the impact of feeding/growth regimes in captive culture on maturation timing.

In captive culture, the feeding opportunity for fish far exceeds that normally experienced in the wild. Food availablity is constant, not seasonally variable as in nature. Frequently hatchery managers rear juvenile fish to a specific size for release, but when during the season that size is achieved varies according to management practices. The ration is relatively constant and is generally adjusted according to rearing temperature and desired growth pattern. In fish, the rate of growth also affects the rate of development. Because growth in culture conditions frequently exceeds that in the wild, the developmental cycle is also accelerated. As a result, life-history transitions during seasonal periods that are abnormal for the stocks often occur. Thus, it is not surprising to find that hatchery-reared fish are substantially fatter than wild fish (Ludwig 1977, Shearer and Swanson, 1994) and that precocious male maturation rates as high as 80% occur in captive culture.

**Future Research Needs on Early Male Maturation**

In a captive broodstock program for depleted stocks of salmon, it is undesirable to produce mature males at a time when females of the same stock are not mature. In addition, selective mortality of precocious males could reduce the effective breeding population size of a captive broodstock. Thus, there is a critical need to develop methods to minimize precocious male maturation in captive broodstock programs for depleted salmon stocks. From the above review of literature, it is clear that the time of sexual maturation is controlled by genetic, abiotic (e.g., photoperiod, temperature, salinity) and biotic (e.g., diet, growth rate, energy stores) factors. The relative importance of these factors and how they interact are poorly understood. Because artificial genetic selection should be minimized in a captive broodstock program for depleted stocks, rearing strategies that minimize expression of precocious male maturation should be developed.

Research to date, primarily from work on Atlantic salmon, indicates that growth rate, size, and levels of stored energy at specific times of year or critical periods of the life cycle, are important factors affecting the incidence of precocious maturation. It may be possible to reduce levels of precocious male maturation through alterations in rearing conditions, growth rates, and diet. However, it is not known whether all of the results from studies of Atlantic salmon are applicable to Pacific salmon species such as chinook salmon.

Before methods that minimize the rate of precocious male maturation can be developed, research is necessary to determine how stored energy levels (body fat content), growth rates, or rates of energy deposition at critical developmental stages either permit or prevent the onset of maturation.

It will be necessary to determine the relative roles of growth rate and stored energy levels, as well as interactive effects of these factors, on precocious maturation. Diets and growth regimes that sustain somatic growth and provide sufficient stored energy for appropriate life-cycle transitions must be developed: in other words, rearing methods that minimize precocious maturation in a population but do not affect the quality of smolts are needed.

## Factors Affecting Gamete Quality

Successful fertilization of eggs and subsequent development of offspring depend greatly on the quality of gametes produced by the parent fish.Various biological and nonbiological factors have been implicated as determinants of gamete quality and subsequent survival of progeny. These include: composition and size of the egg, quality of the sperm, genetic makeup and nutritional status of the parents, husbandry procedures, and quality of the water supply (Bromage and Cumaranatunga 1988; Bromage et al. 1992; Springate and Bromage 1983, 1984a, b, c, 1985a, b; Springate et al. 1984; Springate 1985).

In many instances it is difficult to separate the relative effects of these parameters because they are frequently interrelated. For example, the chemical composition of the egg is affected by nutrition of the female (diet composition and quantity, Forster and Hardy 1995), genetics of the fish, and water quality. Environmental factors and husbandry practices that affect egg size, fecundity, and egg composition frequently also affect intake of food (see discussion below on stress), therefore is it difficult to determine cause-effect relationships among these variables.

### Composition and Size of Eggs

The composition, size, and number of eggs produced by an individual female are influenced strongly by nutrition and are reviewed by Forster and Hardy (1995). There have been a considerable number of studies conducted on the effects of dietary constituents on egg quality, and the relationship between chemical composition of the egg and egg qualtiy in rainbow trout. However, Bromage and Cumaranatunga (1988) concluded that 1) there is no clear relationship between any single component of the egg and egg quality, and 2) profound effects of dietary constituents on egg quality are observed only when specific vitamins or minerals are absent or present at very low levels. Much of the data suggesting that egg size affects egg quality are inconclusive because experiments were not controlled for ripeness of the egg. For example, when eggs were collected within 1 week of ovulation and fry were given proper nutrition and water quality, no relationship between egg quality and egg size was observed (Springate and Bromage 1985a).

### Timing of Egg Collection and Husbandry Practices

Fish husbandry practices can have dramatic effects on egg quality. Probably the most profound effects are those that result from the timing of egg collection (stripping) and handling of the gametes (Craik and Harvey 1984, Springate et al. 1984, Springate 1985, Springate and Bromage 1985a, Bromage and Cumaranatunga 1988, Bromage et al. 1992).

The time of stripping relative to ovulation can have marked effects on fertilization rates and survival of developing embryos (Nomura et al. 1974, Sakai et al. 1975, Escaffre and Billard 1979, Springate et al 1984). In eggs stripped too soon after ovulation, a modest reduction in fertilization has been observed (Sakai et al. 1975, Springate et al. 1984, Springate and Bromage 1984b), and this may be due to excessive force used during stripping or incomplete ripening of the eggs. In addition, overripe eggs have very low rates of fertilization and subsequent survival (Sakai et al. 1975, Springate et al. 1984). Eggs collected more than 10 days after ovulation in rainbow trout exhibited reduced viability. Viabilities of over 70% were observed when eggs were stripped within 10 days of ovulation, whereas when eggs were collected 30 days after ovulation 0% viability was observed (Nomura et al. 1974, Sakai et al. 1975).

Overripeness of the egg is characterized by the aggregation and fusion of oil droplets and migration of the cortical alveoli (Nomura et al. 1974). It is not known exactly how these morphological changes are related to specific chemical changes in the egg and reduction in viability of the egg and fry. Whatever the cause of reduced viability when eggs remain in the body cavity for an extended period after ovulation, overripeness of the egg remains a major cause of egg loss in fiih culture (Lam et al. 1978, Bromage et al. 1992).

The timing of egg collection has been most precisely determined for rainbow trout reared at 10 °C (Springate and Bromage 1984a, b; Springate et al., 1984; Bromage and Cumaranatunga 1988; Bromage et al. 1992). In studies conducted under these conditions, eggs that were collected between 4 and 10 days after ovulation exhibited high rates of fertilization. Thus, checking female rainbow trout for “ripeness” every 7 to 10 days was recommended by Bromage et al. (1992). They also recommended that sorting and stripping of ripe fish should be carried out under anesthesia to avoid stressing broodstock and damaging eggs, and that recovery of fish that are not spawned should be done with good water flow or auxilliary aeration. When administered properly, anesthetics have no effect on the quality of gametes (Billard 1981). Unfortunately the rate of ripening may vary with rearing temperature, and differences among salmonid species as well as stocks within a species may exist.

Methods of collection and handling of gametes also affect gamete quality. General handling procedures have been described in several texts on fish farming (Leitritz and Lewis 1976, Piper et al. 1982, Springate and Bromage, 1985b). Batches of broken or overripe eggs, as well as eggs that have poor fertilization or survival 24 hours post-fertilization, should be discarded so that contamination of good eggs is avoided. Wilcox et al. (1984) demonstrated that broken eggs caused poor survival of embryos in coho salmon. Generally, fertilization rate and survival during the first few days of incubation is a good predictor of subsequent survival to hatch (Springate and Bromage 1983, 1984 a, b, c; Craik and Harvey 1984).

**Effects of Stress on the Quality of Gametes**

Studies in rainbow trout have shown that acute and chronic stress have suppressive effects on the reproductive endocrine system. Both types of stress reduced plasma testosterone and gonadotropin levels in males, and reduced plasma sex steroids, vitellogenin, and gonadotropin in females (Billard and Gillet 1981, Pickering et al. 1987). During both acute and chronic stress, plasma levels of cortisol increased to a point that impaired reproductive function in trout (Carragher et al. 1989, Carragher and Sumpter 1990). A thorough study by Campbell et al. (1992) evaluated the effects of repeated acute stress on gamete quality and quantity in rainbow trout. The stress consisted of exposure to a brief period of emersion approximately once per week at random intervals during a 9-month period. This stress delayed ovulation, reduced egg size and sperm count, and lowered survival rates of progeny of stressed fish. However, it had no effect on fertilization rates or somatic growth in the adults. Mortality of offspring was highest from fertilization up to hatch, but also persisted through 28 days post-hatch.

Other studies have examined the effects of stress on oogenesis and spermatogenesis using fish exposed to sublethal levels of pollutants or low pH. Exposure of female fiih to acid stress has been shown to delay ovulation and reduce fecundity, egg quality, and survival of progeny (Tam and Payson 1986, Mount et al. 1988, Weiner et al. 1986). Negative effects of low environmental pH on the reproductive system of male fish have also been observed @aye and Glebe 1984). However, one of the problems in interpreting results of these studies is that acid stress also reduces food intake and growth (Tam et al. 1987, 1990). This would explain the reduction in egg and body size in acid-stressed fish, since reduction in food intake suppresses gametogenesis and reduces fecundity (Love 1980, Billard and Gillet 1981). Thus when stress impairs growth, it is not possible to distinguish between the direct effects of stress on reproduction and the indirect effects, which are due to nutritional factors.

The effects of stress on reproduction, which have been described primarily in trout, may not be generalized to all sahnonids. Several investigators have demonstrated that there is considerable variation in the sensitivity of different strains and species of trout to stress (Pickering et al. 1982, Refsie 1982). In addition, the stage of development and history of exposure to stress also affect the response. Mature or maturing trout show a substantially reduced response to stress compared to juvenile fish (Sumpter et al. 1987). It is also possible to acclimate trout to regular periods of mild stress (Pickering and Pottinger 1985, Barton et al. 1987).

Most work on stress and reproduction in fish has been conducted on females, with little work carried out on the effects of stress on sperm quality. It has been shown that frequent stripping decreases sperm density and total sperm number, and reduces sperm motility (Billard 1992, Buyukhatipolglu and Holtz, 1984).

Unfortunately, it was not possible to determine whether the effects of stripping on sperm quality were due to the frequent handling stress or to a change in quality of the sperm released by the testis during the period of spermiation. One of the problems encountered in these studies has been the difficulty in assessing the quality of sperm.

### Assessing the Quality of Sperm

At present there is no truly dependable criteria for estimating sperm quality. In fish, the length of time and intensity of spermatozoon motility (Temer 1986, Moccia and Munkittrick 1987, Billard and Cosson 1992), the percentage of motile spermatozoa (Levanduski and Cloud 1988), sperm density (Moccacia and Munkittrick 1987, Scott and Baynes 1987), and the chemical composition of seminal plasma (Hwang and Idler 1969, Morisawa 1988) are all factors that have been measured in an attempt to assess sperm quality. However, there is little strong evidence directly linking any of these factors with fertility.

In salmonids, greater length of time and intensity of motility are not consistently correlated with higher fertilizing ability (Billard 1988). Moccia and Munkittrick (1987) concluded that the critical factor for fertilization was the number of motile sperm, not the motility per se. It is widely agreed that functional tests of sperm quality, such as fertilizability, probably provide a more valuable means of assessing quality of the sperm. However, until more techniques are developed that allow evaluation of sperm quality in the field, hatchery managers will probably have to rely on counts of motile sperm.

### Future Research Needs on Gamete Quality

Most studies on gamete quality in sahnonids have been conducted on domesticated stocks of rainbow trout. Little is known about the factors affecting egg and sperm quality in wild stocks of Pacific salmon captive broodfish. It is likely that wild stocks of fish may be more stressed by the rearing environment and handling, thus the impact of stress on the quality of gametes may be more dramatic. Procedures which have been established for domesticated stocks of salmonids may not be applicable to wild stocks of fish. Thus, research is needed on the effects of rearing environment (water quality and rearing density) and on developing handling procedures that minimize stress in wild stocks of Pacific salmon. The effects of rearing temperature on maturation timing and gamete quality in captive broodstocks needs to be evaluated in a variety of Pacific salmon species, as does the impact of diet on the quality of gametes (Forster and Hardy 1995). In addition, better methods to accurately determine the quality of sperm in the field are needed.

## Conclusion

The major problems with reproduction of Pacific salmon species in captivity include: inappropriate tuning of sexual maturation of broodstock, losses due to prespawning mortality, unreliable production of high quality gametes, and precocious maturation of male fish. Most studies to date on factors affecting gamete quality and the age and seasonal timing of sexual maturation have been conducted on domesticated stocks of rainbow trout and Atlantic salmon. Although some of the information generated from these species is applicable to Pacific salmon, further research is necessary to solve problems with poor reproductive performance of wild Pacific salmon stocks reared in captivity. There is an obvious need to develop methods to monitor and control sexual maturation in captive broodstock, to ensure production of high quality gametes and high survival of offspring, and to minimize asynchronous maturation of male and female fish.

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# CAPTIVE SALMON BROODSTOCK NUTRITION LITERATURE REVIEW

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## Introduction

One of the limitations of modem aquaculture is the availability of reliable sources of high-quality juvenile fish for stocking into grow-out farms. Juveniles can be obtained from the wild or from hatching and rearing offspring of artificially spawned, wild, maturing fish or captive broodstock. As is the case for terrestrial agriculture species, procurement of juveniles from captive broodstock is preferable because it allows the use of breeding programs and affords control of the rearing environment to the culturist. When the objective of fish culture is the restoration of depleted stocks, as is the case for many salmonid stocks in the Columbia River Basin, captive broodstock use is essential.

Each female salmon is capable of producing thousands of viable eggs, meaning that even severely depleted stocks with only a few adults remaining can, in theory, be restored in a few generations or even in a single generation. Similarly, a partially depleted stock can be enhanced to historic levels by artificial spawning of a relatively small number of parents. Concerns about reduction in genetic diversity resulting from using a small number of parents, however, usually dictate that greater numbers of adults be spawned than would be required for production of a given number of offspring.

Maintaining fish for use as broodstock involves different rearing methods than those used to rear salmon for food. When fish are grown for use as food, characteristics such as fast growth and high efficiency of feed utiliiation are desirable. Early sexual maturation in food fish lowers their value. In contrast, the desired characteristics of broodstock fish are high egg production, high quality (survival and growth) of rhe offspring, and high rates of maturity. Egg production is generally measured either as the number of eggs per female, or as the number of eggs per unit of body weight. These parameters are referred to as fecundity and relative fecundity, respectively.

Each of the desirable characteristics listed above for broodstock have been shown to be affected by environmental rearing conditions, including the diet. Conditions which enhance one characteristic may diminish others. Deciding which of these criteria to maximize will depend on the objectives and capacity of the culture facility. When the rearing facility is large enough to handle the entire available broodstock, as may be the case for severely depleted salmon stocks, fecundity will be the criteria to maximize Since larger fish produce more eggs, a policy of maximizing fecundity entails feeding broodstock in such a way as to maximize broodfish size at maturation. Alternatively, if the facility is limited in the biomass of broodfish that it can accommodate, as is normally the case, then maximizing relative fecundity will increase the number of juveniles that can be produced. Achieving these objectives by manipulating diet and feed practices will be discussed later in this report.

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[1] In practice, however, to achieve and maintain historical levels. the factors contributing to the original decline (i.e., fisheries management and environmental alteration) must also be addressed.

Efficient development and implementation of a successful fiih culture program requires an understanding of the principals of management, genetics and nutrition, and how these are integrated with each other. Many studies have indicated that the diet and feed level of fish can affect egg production and fry quality (Luquet and Watanabe 1986, Hardy 1985, Bromage et al. 1992), but gaps in our knowledge remain. Filling in these gaps will improve the efficiency with which salmonid broodstock can be utilized for the production of juveniles for commercial concerns and for the restoration of depleted stocks.

This report provides a critical review of what is known about the manner in which diet and feeding of salmonids can affect their reproductive performance, and is presented in four sections. The first section describes the natural diet of Pacific salmon and steelhead in the sea and in freshwater. The second section reviews experimental work conducted to relate nutrition and feeding of salmonids to their reproductive performance. The third section summarizes the current state of knowledge, indicates the gaps in our knowledge, and provides recommendations for further experimental study. Finally, the composition of some commercial and experimental diets designed for salmonid broodstock are given in the fourth section.

## Conditions of Wild Salmon

Wild adult Pacific salmon are highly variable in their feeding habits, spawn timing, and fecundity along their geographic range and from year to year. There is great variability in these factors even within stocks of fish. In their momentous work, Groot and Margolis (1991) have synthesized a great deal of information on these factors which allows some perception of patterns of these factors among the species of Pacific salmon. The great variability, however, makes application of generalizations to conditions of cultured broodstock difficult and risky.

### Body Size and Fecundity

Size, as measured by length of the maturing females of all Pacific salmon species, is positively correlated to the number of eggs produced (Forster and Pritchard 1941, Healey 1987, Godfrey 19.59, West and Mason 1987, Major and Craddock 1962, Healey and Heard 1984, Nicholas and Hankin 1988, Drucker 1972, Salo and Bayliff 1958), although the numbers of eggs per female between and within a stock may be highly variable. Sockeye sahnon maturing at a given age are larger (weight and length, both sexes combined) than their immature cohorts (Lander et al. 1966, French et al. 1976). Average size at maturity varies, however, over the geographic range, with Chignik River sockeye salmon being the largest (3.2 kg, sexes combined), and Columbia River sockeye salmon the smallest (1.6 kg, sexes combined) (Groot and Margolis 1991). Healey (1987) examined 51 sockeye salmon populations in Alaska and found a great degree of interpopulation variation in size within age classes.

Fecundity in chinook salmon appears to be less related to body size than in other species. Healey and Heard (1984) reported high inter- and intra-population variability of egg production in chinook salmon. These researchers also found that although female body length was significantly correlated with fecundity, size explained less than half of the individual variation in fecundity within a population. Further, they found that the influence of female body length was less for chinook salmon than for other salmon species.

Crone and Bond (1976), in summarizing data for coho salmon, found a positive tendency for fecundity to increase over the range from California to Alaska. However, these researchers acknowledge that the numbers for each stock are not strictly comparable because of differences in methods used and large variations in sample size (chinook salmon fecundity was also reported to increase from south to north (Groot and Margolis 1991)).

The number of oocytes has been found highest in young salmon, declining over the course of ocean life. The rate of oocyte degeneration in pink salmon was found to increase during the fall and early winter periods (Grachev 1971). Pink salmon maturing early in the spawning season had smaller ovaries and were less fecund than fish that matured later in the spawning season.

Natural Diet of Pacific Salmon and Steelhead Trout

The Pacific salmon and rainbow trout are carnivorous, and as such have natural diets that are high in protein content. The wide variety in the prey of adult sahnonids is consistent with that of opportunistic carnivores.

Freshwater Juvenile Stage--In freshwater, newly emerged sockeye salmon (0. *nerka)* fry consume principally copepods, chironomids, dipterean larvae, and cladocerans (McCart 1967, Siminova 1972). Larger pre-smolt sockeye salmon eat cladocerans, copepods, and insects (Rogers 1968, Hoag 1972, Parr 1972).

Chinook salmon in fresh water principally consume larval and adult insects (Becker 1973) and plankton. Cladocera, diptera, copepoda, and homoptera predominated in the diet of chinook fry in the freshwater regions of the Sacramento-San Joaquin River delta (Kjelson 1982). Hermann (1970) found Chehalis River chinook salmon feeding primarily on crustacea and immature and adult insects. Insects were found to comprise more than 95% of the diet of Columbia River chinook fry salmon (Becker 1973), with adult Chironomidae accounting for 58-63% and larval chironomids 17-18%.

The diet of chinook salmon residing in estuaries varies with body size. Smaller fish, residing in tidal channels, consume primarily insect larvae and pupae (Levy et al. 1979, Levy and Northcote 1981). Larger fish, residing near the delta front, feed on small fish such as juvenile herring and stickleback, as well as insects (Levy and Northcote 1981, Levings 1982).

In freshwater, the diet of juvenile chum salmon includes the larvae and chrysalis of chironomid, mayfly larvae, tricoptera, and other insects (Frolenko 1970, Kostarev 1970). Supplementation of the natural diets of juvenile chum salmon with commercial feeds has improved their rate of return in Japan. Japan has a highly successful hatchery program for chum salmon and released more than 2 billion chum fry annually from 1982 to 1985. Before 1966, fry were released with no supplemental feeding, and the average rate of return was about 1%. Since artificial feed supplementation was established in 1966, the return rate has increased to about 2%, and on occasion, 3% (Shirahata 1985).

The diet of coho salmon juveniles is comprised primarily of insects and secondarily of zooplankton (Mason 1974, Zorbidi 1977). In Cultus Lake, British Columbia, young sockeye salmon fry were the principal food item of coho salmon yearlings (Foester and Ricker 1953).

Saltwater Stage--The prey items of adult sockeye sahnon have been summarized by many authors. The composition of food is clearly dependent on the availability and relative abundance of prey items, which vary with season and location. In saltwater, sockeye salmon juveniles were found by McAllister et al. (1969) to seek areas of high concentrations of macrozooplankton south of the central Aleutian Islands. Favorite (1970) reported that food-item composition was different among 82 geographic locations in the central North Pacific Ocean and Bering Sea from May to

August, 1960. On average, the fish consumed amphipods (43%), fish (18%), squid (16%), euphausiids (12%), copepods (7%), pteropods (2%), and other items (2%).

Food items of maturing sockeye salmon were also found to vary according to location. Nishiyama (1974, 1984) found that in the basin area of central Bering Sea, food items included squid, fish larvae, amphipods, and euphausiids, while fish in the shelf area to the east consumed almost exclusively euphausiids, with a small amount of fish. The differences in stomach items were coincidental with the relative abundance of food items. Both Dell (1963) and LeBrasseur (1966) found that young, more so than maturing sockeye salmon, favored euphausiids.

Of the Pacific salmon, chinook salmon in seawater appear to be the most dependent on fish as food (Healey 1976). In general, herring and sand lance are the most important prey items, although euphausiids have been found in significant quantities (43%) in the stomachs of chinook salmon off the coast of Washington State (Silliman 1941).

Chum salmon are planktivores during early marine life. The most important items in their diets are calanoid copepods, hyperiid amphipods, euphausiids, chaetognaths, decapod larvae and fish larvae (Okada and Taniguchi 1971, Healey 1976, Simenstad et al. 1982).

When coho salmon enter salt water, they feed mostly on marine invertebrates, but as they grow larger they become more piscivorous (Shapovalov and Taft 1984). Herring and other fish predominate in the diet of adult coho salmon (Pritchare and Tester 1943, 1944, Foerster 1955), although there is considerable plasticity in prey items (Prakash 1962).

Once salmon enter freshwater on their spawning migration, they no longer eat. Therefore, nutrient stores must supply all of the metabolic and reproductive needs of the maturing fish. This phase can last for several days or for weeks depending on the distance fish migrate to the spawning area.

De la Noue and Choubert (1985) determined the amino acid composition and apparent digestibility of daphnids, chironomids, and gammarids to rainbow trout. These researchers found that the dry matter, crude protein, and energy-apparent digestibility was high for each of these invertebrate groups, although digestibility coefficients for gammarids were found to be slightly lower than for the other two. De la Noue and Choubert (1985) also compared the amino acid profile of these invertebrate groups with the known amino acid requirements of salmonids and confirmed that the amino acid requirements can be met by diets comprised of these invertebrate groups. The values for crude protein digestibility found by De la Noue and Choubea (1985) are similar to what is found for fish meals (R. W. Hardy, unpubl. data, NMFS).

In conclusion, the dietary composition of salmonids in wild conditions is somewhat dependent on food availability, but each species will target specific groups of organisms. Though there is great variety in the composition of prey items between and within salmonid species, all diets studied appear to easily fulfill the requirements for growth, health, and reproduction. A

report will be published soon that summarizes a great deal of information on the diet of wild salmonids at various life-history stages and details the chemical composition and nutrient bioavailability of wild sahnonid diets (D. Higgs, West Vancouver Laboratory, Department of Fisheries and Oceans, Canada). Higgs concludes that as long as sufficient prey are available, the nutrient composition and feed intake levels of wild salmonids will be consistent with known requirements for growth and health.

## Comparison of Chemical Composition of Wild and Farmed Salmonids

Few studies have found substantial differences in the chemical composition of wild and cultured salmonids, except in the lipid fraction. Diet has a strong influence on the lipid level, fatty acid (FA) profile, and lipid-soluble nutrient levels (i.e., some vitamins and carotenoids) of sahnonid tissue and organs.

Ludwig (1980) examined wild and cultured juvenile coho salmon in fresh water for chemical differences in carcass composition. He found that the protein and ash portions of the fish were similar, but that there were differences both in content and composition of the lipid fraction. Omega-3 FA levels were lower in the neutral lipid of artificially reared fish than wild fish (28.3% vs 58.2%), whereas there were no differences in the omega-3 FA level of the polar fraction of the lipid. The cultured fish had been reared on Oregon moist pellet, which was found to have a lower level of omega-3 FA relative to some of the prey items consumed by the wild fish (Table 1).

Table 1. Fatty acid composition of some prey species of salmon in freshwater and of Oregon moist pellets. Data from Ludwig (1980).

| Lipid group [a] | Ephemeroptera | Plecoptera | Chiromonidae | Trichoptera | OMP |
|---|---|---|---|---|---|
| Saturated | 32.4 | 22.8 | 29.6 | 30.9 | 20.0 |
| MUFA | 36.8 | 43.9 | 42.6 | 39.4 | |
| 58.2 | | | | | |
| PUFA | 29.9 | 32.3 | 26.5 | 26.9 | 21.1 |
| n6 | 4.5 | 3.6 | 13.6 | 5.7 | 5.1 |
| n3 | 25.4 | 28.7 | 12.9 | 21.2 | 16.0 |

[a]MUFA--mono-unsaturated fatty acid; PUFA--poly-unsaturated fatty acid; n6, n3--number of carbon atoms from terminal methyl group of fatty acid of first doube-bond.

Ashton et al. (1993) compared the FA composition of wild and cultivated chinook salmon eggs and alevins. They obtained eggs from farm-reared fish of two stocks, each fed diets containing low or moderate levels of omega-3 FA lipids (15 or 24% of dietary lipid), and from returning wild fish of both stocks. The omega-3 FA content of the eggs from cultured fish

reflected the dietary levels of this lipid family and the eggs from all cultured fish fed either diet were lower in omega-3 FA than those obtained from wild fish.

Survival of fertilized eggs from cultured fish to the eyed stage was generally lower than that of eggs from wild fish, hut among the eggs of cultured fiih, levels of dietary omega-3 FA had no effect. Ashton et al. (19[illegible]) also found that although the 22:6 omega-3 FA[2] content of eggs correlated with dietary levels, and was generally lower in the eggs of cultured fish, the level of this FA was invariant in the polar lipid fraction, and was the same as that found in wild fish. This FA (22:6 omega-3 FA) is required specifically during reproduction, first in the synthesis of polar lipids required for vitellogen production by the broodfish, and then in the synthesis of the polar lipid component of membranes during embryonic development (Ashton et al. 1993).

In the second study by Ashton et al. (1993), alevins were obtained from wild and cultured chinook salmon from the same stocks as before, but all cultured fish were fed the same commercially available diet. As in the first experiment, differences in fatty acid composition between alevins from wild and cultured fish were greater in neutral than in polar lipid levels.

Poppe et al. (1985) compared wild and farmed Atlantic salmon for liver content of three minerals and serum levels of vitamin E. They found that wild salmon had much higher levels of copper and selenium and serum vitamin E. Fish used in this study ranged in size from 1.5 to 9.0 kg and were kept in seawater.

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[2] An omega-3 FA containing 22 carbon atoms arranged in series. with 6 double-bon& between them, one of which is between the third and fourth carbons from one end.

## Dietary Factors Affecting Captive Broodstock Performance

The ability of feeding regime to affect egg production and the maturation rate of rainbow trout has been well documented (Bromage et al. 1992). Studies have generally been concerned with daily feed rate of maturing fiih or alterations in feeding rate, including periods of starvation during the year prior to spawning.

Springate et al. (1985) examined the effect of different ration sizes on fecundity and egg quality of rainbow trout. These researchers fed previously spawned fish either at the rate of 0.7% or 0.35% body weight per day for 1 year. They found that fish fed at the higher rate were larger than those fed at the lower rate (1.3 kg vs 0.8 kg) and had higher fecundity (2,800 eggs/female vs 2,100 eggs/female), and egg size (4.7 mm OD vs 4.5 mm OD). The survival of the eggs to hatch was not significantly different between the treatments.

Progeny of fish fed at the high rate of intake were larger than those of fish fed at the lower rate at hatch. This size difference persisted for 4 months post-hatch. Perhaps the most significant finding was that although all of the fish fed at the high rate of intake spawned, only 89% of the half-ration group matured.

Scott (1962) and Bagenal(1969) worked with rainbow trout and brook trout, respectively, and found that the proportion of fish reaching maturity and the fecundity were reduced for fish fed restricted rations. However, the studies were seriously compromised by their experitnental designs and by the high rate of mortality (Bagenal 1969).

Roley (1983) fed rainbow trout either to satiation or one-half satiation under two temperature regimes for 8 months prior to spawning (diet contained 60% protein and 17% lipid on a dry weight basis). Size at spawning, fecundity, relative fecundity, and egg survival of fish in each treatment were analyzed using analysis of variance and analysis of covariance (with female spawning weight as the covariate). He found that feeding at a higher rate had a positive effect on growth and fecundity, but a negative effect on relative fecundity and hatchability (Table 2). Broodfish that were reared in the warmer water exhibited higher fecundity, but lower relative fecundity and hatchability, than those fish reared in the cooler water. The maturation rate of the broodfish was not affected by treatment.

Thorpe (1986) suggested that maturation of Atlantic salmon is determined by the rate of storage or turnover of surplus energy (ultimately dependent of the rate of feed intake). This rate must exceed a genetically fixed threshold value during a critical, limited season, defined by rate of increase of day length (Thorpe et al. 1990).

Recent work by Thorpe et al. (1990) with Atlantic salmon indicated that restricting feed intake during any month from December to April reduces the proportion of females maturing in the subsequent summer and autumn. The researchers fed six groups of fist-sea winter Atlantic salmon (approximately initial weight of 450g/fish) a commercial feed either to apparent satiation or in a restricted fashion from December to May. One tank of fish was fed to apparent satiation throughout this time.

Table 2. Summary of fecundity, relative fecundity, and egg survival of rainbow trout broodfish fed at two rates of feed intake and reared in two water temperatures (means ± sd). Data from Roley (1983).

| | Treatment | | | |
|---|---|---|---|---|
| | Cool, low feed | Cool, high feed | Warm, low feed | Warm, high feed |
| Fecundity (eggs/fish) | 5,546 ± 1,248 | 6,017 ± 1,772 | 6,039 ± 1,779 | 6,694 ± 2,103 |
| Relative fecundity (eggs/kg fish) | 3,008 ± 630 | 2,254 ± 526 | 3,562 ± 967 | 2,287 ± 699 |
| Hatchability (%) | 45.4 ± 33.0 | 25.3 ± 23.8 | 19.9 ± 27.1 | 5.9 ± 12.6 |
| Number of fish | 32 | 35 | 24 | 12 |

Fish in the other tanks were fed in the same manner except that for some period of time feed intake was restricted by withholding feed on alternate weeks (Table 3). The maturation status of each fish was determined by dissection. It was found that the average female maturation rate in all the groups that had been fed in restricted fashion was lower than for the control group, and that this reduction was most pronounced in those fish receiving restricted rations during February/March and March/April. Fecundity was not determined in this experiment.

Reimers et al. (1993), working with second sea winter Atlantic salmon (approximate initial weight of 5.5 kg/fish), also found that restricting feed intake during February and March substantially reduced the maturation rate. In this experiment, feed was withheld entirely for these 2 months. The maturation rate of females that had been starved was only 52% of that found for the satiation-fed fish (21.2% for starved fish; 40.4% for fed fish). The maturation rate of males followed a similar pattern (35% for starved fish; 52% for fed fish).

Jones and Bromage (unpublished data, cited by Bromage et al. (1992)), investigated the effect of seasonal alterations in relation to the reproductive performance of rainbow trout. One-year-old rainbow trout were fed at variable rates over their second year until first spawning as 2-year-olds. Their subsequent spawning rates and fecundity were measured (Table 4). Details of this experiment, such as nutrient composition of the diet, number of replicates per treatment, and hatchability of eggs were not presented, but some interesting patterns were evident in the results. The proportion of fish spawning in their second year was most influenced by the ration size during the period between 12 and 9 months prior to spawning season. All groups of fish fed

Table 3. Feedmg regime and subsequent maturity rate of Atlantic salmon. Data from Thorpe et al. (1990).

| Month | Control Diet | Test Diets | | | | |
|---|---|---|---|---|---|---|
| Dec | Standard[a] | EOW[b] | EOW | Standard | Standard | Standard |
| Jan | Standard | Standard | EOW | EOW | Standard | Standard |
| Feb | Standard | Standard | Standard | EOW | EOW | Standard |
| Mar | Standard | Standard | Standard | Standard | EOW | EOW |
| Apr | Standard | Standard | Standard | Standard | Standard | EOW |
| May | Standard | Standard | Standard | Standard | Standard | Standard |
| male' (%) | 73.7 | 81.3 | 75.0 | 82.6 | 48.1 | 68.0 |
| female' (%) | 42.3 | 30.0 | 28.0 | 26.1 | 14.3 | 15.0 |

[a] Standard = normal daily feed schedule (three times daily).
[b] EOW = every other week, i.e., alternate 7-day periods of no feeding and feeding.
[c] Maturation rate of fii in each treatment.

Table 4. Reproductive performance of rainbow trout on varying rations. Data from Bromage et al. (1992").

| MR[b] | Fec[c] | Wt[d] | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | 3060 | 543 | + | + | + | + | + | + | + | + | - | - | - | - |
| 68 | 3036 | 1205 | + | + | + | + | + | + | + | + | + | + | + | + |
| 68 + | 2268 | 843 | + | + | + | + | - | - | - | - | + | + | + | |
| 64 | 2355 | 603 | + | + | + | + | - | - | - | - | - | - | - | - |
| 48 | 2562 | 1070 | - | - | - | - | + | + | + | + | + | + | + | + |
| 47 | 2693 | 822 | - | - | - | - | + | + | + | + | - | - | - | - |
| 41 | 1865 | 1417 | - | - | - | - | - | - | - | - | + | + | + | + |
| 35 | 1864 | 1205 | - | - | - | - | - | - | - | - | - | - | - | - |

[a] + denotes periods of feeding at 1% body weight per day; - denotes periods of feeding at 0.4% body weight per day.
[b] Maturity rate (%) defined as percentage of females in a treatment that matured.
[c] Number of eggs per spawning female.
[d] Biomass (kg) of females required to produce one million eggs (includes non-maturing females).

at the low rate of intake during this period (0.4% body weight per day) had the lowest rates of maturation. Their spawning rates, ranging from. 35-48%. were compared with those of groups fed at the high rate (1 .O% body weight per day). which ranged from 64-70%. In general, the fecundity of the fish followed a similar pattern, although fish fed at the high rate during the period

between 9 and 6 months prior to spawning exhibited the greatest fecundity. Jones and Bromage suggested that a finite range of potential fecundity is established prior to the final 3 months of the maturation cycle. In addition, these researchers expressed the reproductive success of the fish in terms of the number and weight of fish required to produce a specified number of eggs (one million, in this instance). This measure combines fecundity and maturity rate data, and can be used to calculate the maximum production capacity of a facility based on the biomass it can sustain. The data indicate that feeding broodfish at less than maximum rate for patt of the maturation cycle can improve the efficiency of egg production and increase the potential yield of spawning facilities.

Kato (1975) and Ridehnan et al. (1984) reported that short-term diet restriction immediately prior to spawning produced no changes in either fecundity or egg size of rainbow trout. Ridehnan et al. (1984) examined the fecundity and egg hatchability of maturing rainbow trout starved for 40 days prior to spawning. They found that fecundity and proximate composition and hatchability of the eggs were unaffected by this period of starvation, indicating that sufficient material was present in the maternal tissue of maturing fish to allow vitellogenesis to proceed to completion. Ridehnan et al. (1984) concluded that the expense of feeding rainbow trout during the period immediately prior to spawning could be saved, without any loss of egg production, by withholding feed at this time. However, these authors also recommend further research on the effect of such starvation on post-spawning survival of mature fish.

Recently, Erdall and Barrows have examined the effect of feed rate on rainbow trout reproduction (Dr. Dave Erdall, US Fish and Wildlife, Bozeman, MT Pers. commun. January, 1994). These researchers fed rainbow trout, which had previously spawned, at the rate of 0.4, 0.8, or 1.2% of body weight daily for 10 months prior to second spawning. No difference was found in egg size or survival among the treatments. Fecundity was found to increase with ration size, while relative fecundity was inversely related to ration size. This finding agrees with the findings of Jones and Bromage previously mentioned.

In summary, the rate of feeding and the alteration of feed rate during maturation influences the reproductive output of previously spawned rainbow trout. Fecundity is positively related and relative fecundity is negatively related to fish size. Maturation rate is also related to the feed regime and is highest in fish that have been fed 9-12 months prior to spawning. Similar work has not been reported with Pacific salmon captive broodfish. Results of the work reported here suggest that when properly applied, modification of the feeding regime of broodfish is a potent tool for hatcheries to meet their production goals.

## Nutrient Requirements

Dietary Protein--Various studies have shown that the dietary protein requirement of maturing rainbow trout broodstock for maximum reproductive output is less than that required for optimum growth rate.

Takeuchi et al. (1981) examined the ability of low protein, high-energy diets to support growth and reproduction of rainbow trout. These researchers fed three groups of juvenile rainbow trout either a commercial diet (43-47% protein) or one of two fishmeal-based diets containing reduced protein (33-35%) for 3 years. At the second spawning of these fish there was no significant difference among the treatments for body weight, fecundity, relative fecundity, and egg hatchability.

Roley (1983) examined the growth and reproductive performance of rainbow trout broodfish in response to feeding diets containing 27, 37, 47 or 56% protein for 8 months prior to spawning. The weight of females immediately prior to spawning was positively correlated with dietary protein, but maturation rate, fecundity, relative fecundity and hatchability were not related to diet.

Watanabe et al. (1984) fed three fishmeal-based diets containing different levels of protein and lipid to rainbow trout for 3 months prior to spawning (Table 5). These researchers found that fecundity, relative fecundity, and egg survival of fish fed the diet containing the lowest level of fishmeal (diet #1; 28% protein, 21% lipid) was higher than that of fish fed the diet containing the highest level of fishmeal (diet #3; 46% protein, 15% lipid). Because these diets varied in lipid and protein content simultaneously, it is not possible to differentiate the effect of each nutrient separately on reproductive performance. Furthermore, the significance of the findings could not be determined due to lack of treatment replication in this study.

Washburn et al. (1990) investigated the relationship between dietary protein and reproductive performance of rainbow trout by feeding diets containing two levels of protein to 2-year old maturing fish. The two experimental diets contained either 57.5% or 30.3% protein, and both contained 10% lipid. The principal sources of protein in these diets were fish meal and soybean meal, with wheat flour replacing these ingredients in the low protein diet. In addition, a commercial diet (source unknown) known to support good growth and reproduction of trout was fed.

All fish were fed at 1% of body weight daily for 9 months until 4-6 weeks prior to spawning. These researchers found that the high protein diet promoted the greatest growth in fish. There was no difference in weight among fish fed the different diets when eggs were removed. Dietary treatment had no effect on fecundity. and relative fecundity (# eggs/ body weight) was significantly lower in fish fed the high protein diet.

Survival of eggs to hatch was also markedly lower in fish fed the high protein diet relative to those fed the low protein diet (36% and 65%. respectively). The number of hatched eggs produced per kilogram of adult body weight was about 1,000 for fish fed the low protein diet and the commercial diet, and about 600 for those fed the high protein diet. This level of productivity is quite low compared to that found by other researchers (e.g., Bromage et al., (1992).

Table 5. Protein and lipid content of diets fed to rainbow trout broodstock and subsequent fecundity and egg survival. Data from Watanabe et al. (1984).

| | 1* | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| dietary protein | 28.2 | 36.3 | 45.7 | 40.1 | 42.9 | 42.9 | 43.1 |
| dietary lipid | 20.8 | 17.5 | 14.6 | 19.4 | 14.0 | 14.0 | 12.7 |
| eggs/kg fish | 2821 | 1817 | 2355 | 2573 | 2485 | 2632 | 2295 |
| eggs/fish | 2315 | 1699 | 2197 | 2015 | 1429 | 1937 | 1737 |
| % total hatch | 70.0 | 85.8 | 57.1 | 68.5 | 46.4 | 82.4 | 70.7 |

* Diets 1.2, 3 and 4 contained fish meal. Diet 4 contained 7% beef tallow. Diets 5 and 6 contained casein. Diet 5 contained 15% methyl laurate. Diet 6 contained 10% methyl laurate and 5% ethyl linoleate. Diet 7 was a commercial diet.

Recently, Erdall and Barrows (unpublished data) investigated the effect of varying dietary lipid and protein levels on growth and reproductive performance of rainbow trout. These researchers fed six diets containing one of two levels of protein (32 or 43% of diet) and one of three levels of lipid (9, 12 or 15% of diet) for 2 years. After 1 year of feeding, no differences in growth or fecundity were found to be statistically significant. After 2 years, however, fish size was positively related to both dietary protein and lipid level. There was no influence of dietary treatment on fecundity or egg survival, but relative fecundity was higher in the low-protein diet.

In summary, evidence from several experiments indicates that the level of protein in rainbow trout diets required for optimum reproductive performance is lower than that needed for maximum growth. This is especially true if relative fecundity is considered. The level of dietary protein required for optimum reproductive performance has not been quantified for salmonids. No studies investigating the dietary protein requirement for optimum reproductive output of Pacific salmon have been published.

The studies described above suffer from lack of treatment replication, which reduces their scientific merit. Experimental diets were fed to only one group of fish, making it impossible to ascribe differences in results to treatment effects. Even so, the near uniformity of the findings indicates that efficiency of egg production may be increased if maturing female trout are maintained on diets that are relatively low in protein. Further experimentation, incorporating replication and multiple levels of dietary protein, will greatly enhance the design of feeds for optimum reproductive performance.

Dietary Lipids--Dietary lipids are used by fish both for energy and as a source of fatty acids. Fatty acids are used in the structure of cell membranes and in the production of bioactive molecules such as prostaglandins. For growth and health, salmonids require specific types of fatty acids. Some of these cannot be produced by the animal and must be acquired from the diet. These fatty acids are referred to as the essential fatty acids (EFAs).

Fatty acids (FAs) of the linolenic acid series, designated as omega-3 FAs, have been known to be essential in the diets of salmon and trout for growth and health since the pioneering work of Yu and Sinnhuber in the early 1970s (Caste11 et al., 1972a, b, c; Yu and Sinnhuber 1975, 1976; Yu et al. 1979). Subsequent work has established the requirements of these fish species for omega-3 FA for reproduction (Yu et al. 1979, Hardy et al. 1989, LeRay et al. 1985, Watanabe et al. 1984).

There is no published data of the effects of differing fat levels on egg quality in salmonids. Jones and Bromage (unpublished data, cited in Bromage et al. 1992) report that fecundity and the cost of egg production in rainbow trout are optimized by using diets containing 7-12% gross fat, although egg survival was the same among fish fed diets containing 7, 12, 18, or 25% lipid.

Yu et al. (1979) investigated the ability of omega-3 FA to meet the requirements of maturing rainbow trout for EFAs. These researchers fed semi-purified casein-gelatin diets containing either 1% ethyl linolenate (omega-3 FA ethyl ester) either by itself or in combination with 1.5% ethyl linoleate (omega-3 FA ethyl ester). In both diets the total lipid content was increased to 6% by addition of ethyl laurate (saturated FA ethyl ester).

Fish in this experiment were initially less than 0.5 g and were raised to maturity (from 22 to 34 months). The investigators concluded, based on growth, reproductive success and early growth of progeny, that omega-3 FAs are essential in the diets of rainbow trout for adequate reproduction and that omega-3 FAs are not required by trout. Fish that were fed experimental diets, however, had considerably fecundity and hatchability than did comparable fish fed on Oregon moist diet (Table 6). Of fish on experimental diets, those fed the diet containing omega-3 FA ethyl esters had higher fecundity and hatchability than those fed the experimental diet containing no omega-3 FA ethyl esters (Table 6). It is not possible to assess the statistical significance of these treatment differences because of the lack of replication.

Hardy et al. (1989) assessed the effect of dietary lipid source on the reproductive performance of maturing coho salmon. These researchers fed diets containing lipids from three sources: herring oil, soybean oil or beef tallow, either singly or in combination, for 5 months prior to spawning. These lipid sources are highly variable in omega-3 FA content, with herring oil being the highest and beef tallow containing none. Although significant differences were found in fatty acid composition of the lipid fraction of coho salmon muscle and eggs, especially among the neutral lipids, no differences were detected in fecundity, egg viability, or egg size among the various treatments. All the diets contained omega-3 FA, at or above 1% of the diet, which is the reported requirement for optimum growth for salmonids (Yu et al. 1979). Hardy et al. (1989)

concluded that reproductive performance of coho salmon was not impaired by dietary lipid source, providing the diets contained at least 1% omega-3 FA.

Table 6. Composition of diets fed to rainbow trout and the resulting fecundity and egg survival. Data from Yu et al. (1979).

| Ingredient | A | B | OMP[a] |
|---|---|---|---|
| Casein | 51 | 51 | |
| Gelatin | 9 | 9 | |
| Ethyl linolenate (n3FA) | 16.8 | 16.8 | |
| Ethyl linoleate (n6FA) | 1 | 1 | |
| Ethyl laurate (saturated FA) | 0 | 1.5 | |
| Carboxymethylcellulose | 5 | 3.5 | |
| Alpha-cellulose | 1 | 1 | |
| Mineral and vitamin premix[b] | 7.2 | 7.2 | |
| Fecundity (eggs/female) | 2030 | 2235 | 4780 |
| Hatch (%) | 60.9 | 80.6 | 75.1 |

[a] OMP = Oregon moist pellets.
[b] Percent in diet: mineral mix 4: vitamin mix 2.0; choline chloride (70%) 1: vitamin E 0.2 (660 IU/kg); total 7.2; Mineral mix: Bernhardt - Tomarelli salt mix. Vitamin mix supplies (mg/kg of diet): thiamin 64: riboflavin 144; niacin&de 512; biotin 1.6: pantothenate 288; pyridoxine 48; folic acid 19.2; menadione 16: cobalamine0.16; inositol 2500; ascorbic acid 1200; PABA 400; vitamin $D_2$ 4000 IU/kg; vitamin A 25,000 IU/kg.

LeRay et al. (1985) investigated the effect of EFA deficiency on reproductive success of rainbow trout. These researchers fed fish diets containing omega-3 FAs either at the level of 12% or 0% of the diet. Fish were fed the experimental diets once daily for 1 year at the rate of 1% of body weight per day. The authors found that omega-3 FA levels in both the neutral-lipid and polar-lipid fraction of maturing fish and the eggs produced by these fish were drastically lower in the fish fed the diet devoid of omega-3 FA. Fecundity and egg survival were impaired in fish fed the omega-3 FA deficient diet. LeRay et al. (1985) concluded that omega-3 FA is required by rainbow trout for optimal reproductive success and that reproductive processes are impaired in fish fed omega-3 FA deficient diets for extended periods,

Watanabe et al. (1984) examined the effect on reproduction of feeding omega-3 FA deficient diets to rainbow trout. Semipurified casein-based diets containing methyl laurate (saturated) or a combination of methyl laurate and ethyl linoleate (omega-6 FA) as the sole sources of lipid were fed to groups of fish. Fecundity and egg survival of fish fed these diets were compared to those of fish fed fish meal-based diets containing adequate levels omega-3 FA (supplied from marine oil). Fecundity and egg survival of fish fed diets containing methyl laurate as the sole source of fatty acids were considerably lower than for fish fed diets containing marine oil. Watanabe et al. also found that partially replacing methyl laurate with omega-6 FA improved the fecundity and egg survival of fish to levels comparable to that found for fish fed diets

containing marine oil. These authors concluded that dietary omega-6 FA may satisfy the EFA requirement of rainbow trout for reproduction. From the data presented by these authors, it seems that omega-6 FA may reduce the requirement of rainbow trout for omega-3 FA, or at least may prolong the length of time that broodstock can be fed diets devoid of omega-3 FA without reduction of reproductive success.

To summarize, results of the above experiments provide evidence that trout have a dietary requirement for omega-3 FA for reproduction. The level of this requirement is not known, but appears to be no greater than the requirement for optimum growth and health. No work has been done with Pacific salmon species to determine the effect of dietary lipid level on reproductive performance. It has been shown in coho salmon that diets containing omega-3 FA at minimal levels required for growth and health also contain sufficient levels for reproduction.

Dietary Minerals--There is very little published work on the effect of dietary mineral levels on reproduction of salmonids. Most of the work that has been done with mature fish has examined the effect of either deletion of trace elements (Takeuchi et al. 1981) or fortification of a standard formulation (Hardy et al. 1984) on salmonid reproductive success.

Early work by Hirao et al. (1954, 1955) found that egg iron level correlated with survival to eyed stage in rainbow trout. Craik and Harvey (1984), however, were unable to find any significant correlation between iron content and survival to hatch. The techniques used by Craik and Harvey (1984) appeared to be suspect, as nearly half of the batches of eggs collected were blanks (i.e., 100% mortality). Takeuchi et al. (1981) found that rainbow trout fed f&meal-based diets not supplemented with trace minerals had poorer reproductive success than those fed a similar diet containing supplemental minerals.

Hardy et al. (1984) investigated the effect of fortifying an experimental salmonid fingerling diet (Abernathy 19-1 diet, Table 7) with five essential minerals on elemental composition of eggs and maternal soma of coho salmon. Coho salmon were reared in saltwater net pens and fed with demand feeders for 4 months (June-Sept.) prior to freshwater transfer and spawning, during which time feed was withheld. Two groups of fish were fed either Abernathy 19-1 diet with or without additional supplementation of cobalt, copper, iron, manganese, and zinc (Table 7). The authors found that fortification of these trace elements to the Abernathy 19-1 diet resulted in no detectable differences in elemental composition of maternal soma or eggs. They suggested that dietary requirements for these elements for coho salmon broodstock were met by levels found in the Abernathy 19-1 diet.

Table 7. Formulation of experimental diets fed to maturing coho salmon. (Abernathy diets 19-1, 82 and 1%2). Data from Hardy et al. (1984) and Hardy (1991).

| Ingredient | 19-1 | 19-2 |
|---|---|---|
| Herring meal | 50 | 50 |
| Dried whey | 10 | 5 |
| Dried shrimp meal | 5 | |
| Condensed milk solids | | 3 |
| or Poultry byproduct meal | | 1.5 |
| Wheat germ meal | | 5 |
| Wheat middlings | 13.9 | 12.22 |
| Dried blood flour | 5 | 10 |
| Dried brewer's yeast | 5 | |
| Lignin sulfonate (binder) | | 2 |
| Vitamin premix | 1.5[a] | 1.5[b] |
| Trace mineral premix | 0.1[c] | 0.1[d] |
| Choline chloride (50%) | 0.5 | 0.58 |
| Ascorbic acid | | 0.1 |
| Fish oil | 9 | 9 |

[a] Vitamin premix supplied the following per kg of diet: 105 mg D-calcium pantothenate; 31 mg pyridoxine-HCl; 53 mg riboflavin: 220 mg niacinamide; 13 mg folic acid; 43 mg thiamin mononitrate; 0.59 mg biotin; 59 pg $B_{12}$; 11 mg meadione sodium bisulfite; 501 mg alpha-tocopheryl acetae; 6600 IU vitamin A palmitate or acetate; 264 mg myo-inositol: 891 mg ascorbic acid, 440 IU vitamin $D_3$.

[b] Vitamin premix supplied the following per kg of diet: 6600 IU vitamin A (palmitate or acetate); 441 IU vitamin $D_3$; 502 IU alpha-tocopheryl acetate; 28 mtg menadione sodium bisulfite; 47 mg thiamine mononitrate; 53 mg riboflavin; 30.8 mg pyridoxine-HCl; 115 mg calcium pantothenate; 220 mg niacin; 0.6 mg biotin; 0.06 mg viamin $B_{12}$; 12.7 mg folic acid; 132 mg myo-inositol.

'Trace mineral premix supplied the following per kg of diet: 184.8 mg $ZnSO_4$; 206.8 mg $MnSO_4$; 49.5 $FeSO_4 \bullet 7H_2O$; 3.85 mg $CuSO_4$; and 0.836 mg $KIO_3$.

[d] Trace mineral premix supplies the following per kg diet: Zn as $ZnSO_4$ 75 mg; Mn as $MnSO_4$ 20 mg; Cu as $CuSO_4$ 1.54 mg; and I as $KIO_3 \bullet C_2H_8N_2 \bullet 2HI$ 10 mg.

Recently, chinook salmon eggs of fish taken from the same stock, but reared either in the wild or in captivity, were analyzed for mineral content (D. Groves, Sea Spring Salmon Farms, Chemainus, BC, Canada, Pers. cornmun. December, 1993). The eggs of wild fish were found to contain lower levels of selenium (Se) relative to the wild fish. Survival of eggs from the captive broodstock was also much lower than eggs obtained from the wild fish (20% vs. > 90%).

The commercial diet that had been fed to captive fish was found to contain 1.5 ppm Se This level was increased to 2.0% by supplementation of sodium selenite and fed to the

broodstock. Survival of the eggs from cultured fish fed the Se-supplemented diet was 85%. This study was conducted at a commercial hatchery and was not rigorously designed for statistical analysis with controls and replication. Such observations, however, suggest that mineral levels in broodstock diets can influence egg quality.

In summary, very little work has been published on the differences in mineral requirements for salmonid growth and reproduction. Although the above-described studies are not conclusive, it appears that diets containing at minimal levels required for growth and health also contain sufficient minerals for reproduction. Because the dietary requirements for most elements are reasonably well known, and since supplemental minerals add very little to cost for feed manufacturers, there is very little incentive to commit research resources to establish the actual dietary requirement levels for specific minerals for reproduction.

Dietary Vitamins--Except for ascorbic acid, very little work has been published concerning the vitamin requirements for salmonid reproduction. Requirements for growth and health have been reasonably well established, and commercial feeds that contain these levels of vitamins appear to produce good results with broodfish. Ascorbic acid is important in the formation of connective tissue and has been found by Sandnes and Braekkan (198 1) to increase during ovarian development in cod *(Gadus morrhua).*

Comparison of the ascorbic acid content of wild and cultured Atlantic sahnon eggs has shown that wild sahnon have markedly higher levels of this nutrient than do cultivated salmon (SO -100 mg/kg fish weight vs 15 - 31 mg/kg; unpublished data presented by Sandnes et al. (1984)). Sandnes et al. (1984) examined the effect of dietary ascorbic acid on the reproductive success of rainbow trout. Three diets were fed for 4 months to groups of 2-year-old trout held in seawater. Two of the diets were experimental (50% fish meal, 25% fat extracted soybean meal, 10% fish oil). One contained no ascorbic acid, while the other was supplemented with 1,000 mg ascorbic acid per kg of dry feed. In addition, a commercial feed was used as a control. All diets were fed to the fish twice daily to apparent satiation.

Ascorbic acid levels of the eggs from each female were monitored and related to fecundity and egg hatching percentage of individual fish No significant reduction in fecundity was found in fish fed diets containing no supplemental ascorbic acid, but egg survival was significantly higher in fish fed the control diet and ascorbic acid-supplemented diet. Sandnes et al. (1984) found a significant positive relationship of egg ascorbic acid content and hatchability, although there was a great deal of variability. Indeed, 50% of the egg lots of fish fed the unsupplemented diet had hatchability rates of 60% or greater.

The authors determined that ascorbic acid is required in diets of fish for reproduction, and that the effects of ascorbic acid deficiency can be noticed in fish fed diets containing no ascorbic acid for 4 months prior to spawning. The requirement for optimum reproductive performance was not detennined, nor was there an assessment of the difference between requirements for reproduction and growth. Sandnes et al. (1984) recommended, however, that salmonid

broodstock feeds should be fortified with sufficient available ascorbic acid to at least 100 mg/kg dry diet at the time of feeding. The researchers suggested that this dietary level should ensure ascorbic acid levels of at least 20 ppm (wet weight) in the eggs. The technique of relating dietary nutrient level to subsequent egg concentrations and survival appears to be a useful one in estimating requirements, but as the authors stated, experiments with graded levels of ascorbic acid are needed.

The effect of dietary ascorbic level on reproductive performance of coho salmon was investigated by Hardy and King (unpubl. data, NMFS), as quoted by Hardy (1991). These researchers fed diets containing either 430 or 2200 mg/kg of ascorbic acid (the diets contained 1,045 and 10,450 mg/kg, respectively, prior to pelleting and storage). The ascorbic acid content in the eggs was 310 and 513 µg/g, respectively, but survival of eggs to hatch between the two groups was identical at 92%. Increasing the dietary level of alpha-tocopheryl acetate (Vitamin E) by 10 tunes over normal levels resulted in a decrease in percent survival to hatch of coho sahnon eggs, in a study cited by Hardy (1991) (Hardy and King unpubl. data, NMFS).

Hardy (1991) cited the results of a study by Hardy and Masumoto (unpubl. data, NMFS) wherein doubling the level of vitamin premix in diets for coho salmon yielded promising, but nonsignificant, increases in fecundity and egg hatchability. However, the difficulties of conducting scientific feeding trials at commercial facilities compromised the value of these data (Hardy 1991).

ln summary, as with minerals, there has been no work on sahnonids to demonstrate that any vitamin requirement for reproduction, aside from ascorbic acid, is greater than for growth and health. There is some evidence that the dierary ascorbic acid requirement of Atlantic broodstock for reproduction is at least 100 mg/kg of diet. However, this estimate is not based on standard nutritional methodology.

Dietary Carotenoids--Carotenoids are pigments that occur naturally in many animal species, including salmonids. These compounds are related to vitamin A and are largely responsible for the vivid red color of salmon flesh and eggs. The two principle carotenoids of interest to salmon producers are canthaxanthin and astaxanthin. Currently only canthaxanthin is permuted in commercial fiih feeds in the US, although in other countries astaxanthin is also permitted, and astaxanthin is present in considerable amounts in common feed ingredients.

Early work demonstrated that fish fed diets supplemented with synthetic csnthaxanthin exhibited improved reproductive performance (Deufel 1965, Harris 1984). More recently, however, Quantz (1980) was unable to find a significant increase of egg fertility in rainbow trout fed diets supplemented with up to 20 mg astaxanthin per kilogram of diet for up to 15 weeks prior to spawning. Similarly, Morrison and Smith (1981), working with brown trout, were unable to find any difference in fecundity between fish fed diets supplemented with csnthaxanthin and red crab processing wastes and unsupplemented controls. Harris (1984) fed rainbow trout diets supplemented with canthaxanthin at levels of either 20 or 40 mg per kilogram diet for either 3 or 6 months prior to spawning. He found that the addition of synthetic canthaxanthin to the diets

caused no increase in fecundity. The proportion of infertile females was much higher in the control group, but the lack of replication in this experiment precluded assessment of the statistical significance of this finding.

Torrissen (1984) examined the relation between egg carotenoid level and subsequent survival of Atlantic salmon. He examined the eggs of fish from three broodyears. All the parental fish had been reared at the same facility and presumably had the same access to feed. He found no correlation between levels of either canthaxanthin or astaxanthin in fertilized eggs and egg or alevin survival rates. In a related experiment, Torrissen (1984) found that supplementation of 30 mg of either astaxanthin or canthaxanthin in starter diets improved the growth rate of fry.

Murayama and Yanase (1961) reported the hatching rate and carotenoid level of rainbow trout eggs obtained from nine farms in Japan. The two researchers concluded from their data that there was “no definite correlation between the hatching (percentage) and the amount of any chemical constituent examined.” However, Craik (1985) noted that this statement only applied to results obtained from each particular farm and concluded from the same data that there is “strong suggestion that eggs with lower carotenoid content (under l-3 µg/g) tend to have low hatching percentages, and that above this value hatching tended to approach 100%.” Yet Craik (1985) cautions that this conclusion is tentative because of the differing conditions of broodstock nutrition and egg incubation on the various farms.

## Replication and Controls

Several elements of experimental design that are considered essential for scientific research are missing in almost all published studies that examine effects of feeding rates or nutrient levels on reproductive performance of salmonid broodstock. These include proper replication of experimental units, use of proper controls, and use of graded levels of nutrients to establish requirement levels. Incorporation of these elements into nutritional studies would increase considerably the confidence in results of such experiments. However, the high expense involved in incorporating these elements of experimental design has limited their use.

Almost all published nutritional studies of salmonid broodstock assign each treatment to only one group of animals. As a result, it is not possible to separate the effects of random, non-treatment effects from the treatment effects on the results. Factors contributing to non-treatment effects include differences in environmental conditions between experimental units, such as water temperature and flow rate, and lighting. Other non-treatment effects include differences in initial body size and health of the animals in each experimental unit.

Replication of experimental units and the random assignment of animals and treatments allows application of standard methods of statistical analysis to remove non-treatment effects from the results of treatments. The greater the number of replications, the more confidence can be had in assigning differences in experimental results to treatment effects, but the more expensive the experiment will be. Salmonid hatcheries rarely have sufficient resources available to permit the level of replication that is generally considered adequate for nutritional research. Repeating experiments over multiple broodyears, although not as statistically powerful as replication of experimental units, may nevertheless increase the confidence in a result if it is consistently observed.

The second element of experimental design that is frequently left out of broodstock nutrition research is the utilization of suitable controls, which provide a means by which results of experiments may be compared to each other. In nutritional studies, a control treatment consists of the feeding of a reference diet under environmental conditions and feeding regimen that are known to produce optimal results. When a series of connected experiments over time is conducted, the consistent application of a standard control permits unification of the findings and strengthens the overall conclusions of the study. There is no standard reference diet that has been uniformly applied as a control by researchers. Rather, one of a variety of commercially available feeds, or a diet based on standard fonnulations, is commonly used for this purpose. Since these feeds generally promote reasonable reproductive performance, there is no need to standardize between facilities.

Almost all published work on dietary nutrient requirements of salmonid broodstock has been concerned with the qualitative aspects of requirement, or whether the nutrient is required or not. Very few studies have investigated levels of nutrients required for optimal reproductive performance. As a result, little is known about differences in nutrient requirements for reproduction and growth.

The standard technique for investigating nutritional requirements, the dose-response method, involves feeding graded levels of the nutrient to the fish and measuring the response. This method permits estimation of the minimum level of a particular nutrient that will induce the maximum response and can be adapted to investigate the effects of other nutritional aspects as well. For example, to examine the relationship of feeding rate on reproductive performance, it is much more informative to feed at several rates of intake, ranging from satiation to near starvation, than to feed at only two levels of intake, as has been most commonly done.

### Nutrient Requirement Levels and Optimum Feeding Regime

As described above, the nutrients that are known to be required by salmonids for growth and health are also required for reproduction. It is not known if the dietary nutrient requirements for these process are the same, although some work has been done with protein in rainbow trout.

Prioritizing investigations of quantitative requirements for various nutrients for optimal reproductive performance will permit the greatest utilization of limited resources. In establishing list of priorities, the potential for feed cost reduction and the potential for improving reproductive performance must be considered.

The protein component is the single greatest contributor to the cost of salmonid feeds. Studies conducted with rainbow trout have demonstrated that broodfish fed diets containing less protein than production diets have equal fecundity and higher relative fecundity than those fed diets containing protein at levels that maximize growth. However, there is no published work identifying the minimum dietary protein level that will optimize reproductive output in rainbow trout. Furthennore, there are no published studies on the relationship between dietary protein and reproductive output in Pacific salmon.

After protein, the lipid component contributes most to the cost of salmonid feeds. The effect of dietary lipid level on reproductive performance of Pacific salmon has not been investigated, except with regard to the level of specific fatty acids. Unpublished work (Jones and Bromage, cited in Bromage et al, 1992) with rainbow trout has indicated that lowering the lipid level to 7% of the diet may reduce the cost of egg production in rainbow trout with no adverse effect on quality. This work did not examine potential cost savings from reducing levels of dietary lipid below 7%. Further work should be undertaken with other species of *Oncorhynchus* to determine the optimum level of dietary lipid for reproductive output.

There is little justification to examine the effect of vitamins, other than ascorbic acid, on reproductive performance at this time. With the exception of ascorbic acid, supplemental vitamins add very little to the cost of feeds, and there is no evidence indicating that their inclusion above the level required for optimum growth and health has any effect on reproductive output.

Minerals, like vitamins, do not contribute appreciably to the cost of producing salmonids, and have also not been found to be required in rainbow trout diets for reproduction at levels above those required for maximum growth. Low priority should therefore be given to establishing the mineral requirements of salmonids for reproduction.

## Current Diet Formulation for Captive Salmonid Broodstock Culture

### Commercial Feeds

The development and manufacture of feeds for broodstock seem to be generally of low priority to feed companies. There is not enough demand for the product to warrant the diversion of large amounts of resources. Broodstocks comprise a small percentage of the total fish biomass on most farms, reducing the demand for feed. The formulation of feeds that are currently promoted by private industry for use with captive salmonid species are naturally confidential. The ingredient content and proximate composition of these feeds is available, however, as required by U.S. law (Table 8).

Many feed companies produce broodstock feeds that are lower in energy and that are more fortified with vitamins (especially ascorbic acid and vitamin E) and minerals (especially calcium and phosphorous) relative to feeds formulated for growing salmonids. Each of the larger feed manufacturers has its own research program to formulate and test feeds. It is not publicly known how extensive these programs are, and the specifics of results of tests are generally confidential

Table 8. The proximate composition of broodstock and growout diets manufactured by Moore-Clark. Oncor Brood and Oncor Marine are fonnulated for use with chinook and coho salmon, whereas Royal Brood and Royal Superfeed are formulated for use with Atlantic salmon. Data supplied by Moore-Clark Co. Inc.

| | Feed* | | | |
|---|---|---|---|---|
| | Royal Brood | Oncor Brood | Royal Superfeed | Oncor Marine |
| Protein (%) | 46 | 48 | 46 | 45 |
| Lipid (%) | 22 | 18 | 30 | 18 |
| Carbohydrate (%) | 13 | 15 | 11 | 17 |
| Ash (%) | 10 | 10 | 10 | 10 |
| Moisture (%) | 9 | 9 | 6 | 10 |
| Calcium (%) | 2.3 | 2.3 | 2 | 2.1 |
| Phosphorous (%) | 1.6 | 1 . 6 | 1.5 | 1.5 |
| Vitamin A (IU/kg) | 5,000 | 5,000 | 10,000 | 2,500 |
| Vitamin $D_3$ (IU/kg) | 3,000 | 3,000 | 3,000 | 2,400 |
| Vitamin E (IU/kg) | 200 | 200 | 200 | 100 |

* Diets contain the following ingredients (not necessarily in order of proportion): Fish meal, fish oil, whole wheat. cane molasses. vitamin and mineral premixes. ethoxyquin, betaine, pigment (canthaxanthin). The broodstock diets are differentiated from the growout diets by the addition of krill meal and brewers yeast, and by higher levels of betaine pigment. and some vitamins and minerals.

## Experimental Diets

Experimental diets are formulated to meet criteria specific to the objective of a particular study. These diets can be either purified, semi-purified, or commercial type (Tables 8, 9, 10, 11, 11, 12, and 13). Purified diets are those in which each nutrient is added individually. Halver's diet, called H440, is an example of such a diet (Table 12). A semi-purified diet is one in which the nutrients are added in combination with each other, but from highly purified ingredients. The WV diet is an example of this type (Table 12). Commercial type diets contain ingredients that are typically found in commercial feeds. An example of this type of diets is OMP (Table 13).

Table 9. The composition of broodstock and growout diets manufactured for use with chinook and coho salmon by White Crest Mills and EWOS Canada.

| | White Crest Salmon Brood* | EWOS Salmon Grower | Vextra Brood | Vextra Chinook |
|---|---|---|---|---|
| Protein (%) | 47 | 45 | 50 | 45 |
| Lipid (%) | 14 | 16 | 12 | 18 |
| Fiber (%) | 3 | 3 | 1 | 1 |
| Ash (%) | 8-10 | 8-10 | 8 | 8 |
| Moisture (%) | | | 10 | 10 |
| Calcium (%) | 2.2 | 2.2 | | 2.1 |
| Phosphorous (%) | 1.5 | 1.5 | | 1.1 |
| Vitamin A (IU/kg) | 10,000 | 3,000 | 3,000 | |
| Vitamin D (IU/kg) | 2,400 | 3,000 | 3,000 | |
| Vitamin E (IU/kg) | 500 | 150 | 150 | |
| Vitamin C (mg/kg) | 12,00 | | | |

* Feeds contain the following ingredients (not necessarily in order of proportion): EWOS diets contain: Fish meal, fish oil. blood meal. wheat, vitamin and mineral premixes, ethoxyquin, pigment: WhiteCrest feeds contain: Fish meal, soybean meal, canola meal. wheat middlings. wheat germ, poultry byproduct meal, herring oil, whey powder, vitamin and mineral premixes. pigment. The White Crest broodstock diet contains krill meal and is supplemented with extra levels of vitamins E and C.

Table 10. The composition of broodstock and growout diets manufactured by Rangen Inc. for use with chinook and coho salmon and trout.

| | Salmon Brood* | Salmon Extruded | Trout Brood | Trout Production |
|---|---|---|---|---|
| Protein (%) | 44 | 45 | 40 | 40 |
| Lipid (%) | 14 | 20 | 10 | 12 |
| Fiber (%) | 5 | 5 | 5 | 5 |
| Ash (%) | 12 | 15 | 12 | 15 |

* Salmon feeds contain the following ingredients (not necessarily in order of proportion): fish meal, wheat meal, blood meal. fish oil. soy lecithin, vitamins and minerals. The trout diet contains the same ingredients, but in addition contains soybean meal and brewers yeast.

Table 11. U.S. Fish and Wildlife brood diet for trout (open formula).

| Ingredient | % |
|---|---|
| Fish meal: Herring, anchovy, menhaden (or combination) stabilized, maximum moisture 10%. stored at manufacturers plant no longer than 6 months. Pepsin digestibility ≥ 92.5% | 30 |
| Wheat middlings: protein ≥ 15%; fiber ≤ 1.5% | 17.5[a] |
| Wheat flour: protein ≥ 14%; fiber ≤ 1.5% | 5 |
| Soybean meal: solvent extracted and dehulled: protein ≥ 45.5%. Cottonseed meal: solvent extracted; protein ≥ 48%. free gossypol ≤ 0.04% may replace soybean meal for not more than 15% of the total diet. | 2.5 |
| Blood meal: ring-dried; protein ≥ 80% | 10 |
| Trace minerals premix | 0.1 |
| Vitamin premix | 0.4 |
| Choline chloride (50%) | 0.175 |
| Ascorbic acid | 0.075 |
| Fish oil: stabilized with 0.04% BHA:BHT (1:1) or 0.01% ethoxyquin and less than 3% free fatty acids. | 10[a,b] |
| Lignansulphonate pellet binder | 2 |

[a] Fish meal may be increased (not decreased) depending upon protein content, but must provide not less than 20% fish protein. Quantity of added oil may be adjusted upwardly only so that finised feed shall contain not less than 10% crude lipid. Wheat middlings to be adjusted to compensate for the above variations.

[b] Up to 4% soybean oil. once refined (NSPA standard) with 0.04% BHA:BHT (1: 1) or 0.01% ethoxyquin may be substituted for an equal amount of fish oil in the feed mix. Not less than 3% of the total fat shall be sprayed on the pellets as a top dressing. the rest to be included in the feed mix Only fish oil may be used to top-dress feeds.

Table 12. Percent composition of test diets used for Pacific salmon (%). Data from Hardy (1991).

| Ingredient | West Van 33 Diet | H440 modified |
|---|---|---|
| Herring meal | 50.42 | |
| Poultry by-product meal | 7.35 | |
| Dried whey | 7.69 | |
| Blood flour | 5.02 | |
| Shrimp meal | 3.15 | |
| Euphusiids | 2.06 | |
| Wheat middlings | 6.97 | |
| Casein, vitamin free | | 40.8 |
| Gelatin | | 8.0 |
| Dextrin | | 16.0 |
| Alpha-cellulose | | 4.7 |
| Vitamin premix | 4.30[a] | 2.0[b] |
| Mineral premix | 2.04[c] | 8.0[d] |
| Herring or salmon oil | 8.45 | 15.0 |
| Lignin sulfonate | 1.89 | |
| Ascorbic acid | 0.19 | 0.1 |
| Choline chloride | 0.47[e] | 1.0[f] |
| Amino acid mixture | | 4.4 |

[a] Tbe vitamin supplement supplied the following per kg diet: vitamin A acetate, 9455 IU; cholecalciferol, 2269 IU; DL-alpha-tocopheryl acetate, 567 IU; menadione, 24.8 mg; D-calcium pantothenate, 182.9 mg; pyridoxine•HCl, 42.2 mg; riboflavin, 56.7 mg; niacin, 284 mg; folic acid, 18.9 mg; thiamine mononitrate, 3x.4 mg; biotin. 2.84 mg; cyanocobalamin, 0.057 mg; and inositol, 378 mg.

[b] The vitamin supplement supplied the following per kg diet: D-calcium pantothenate, 564 mg; pyridoxine•HCl, 41 mg; riboflavin, 222 mg; niacin, 586 mg; folic acid, 34 mg; thiamine mononitrate, 104 mg; biotin, 1.58 mg; cyanocobalamin, 0.057 mg; ascorbic acid, 893 mg; and inositol, 132 mg.

[c] The mineral supplement supplied the following levels of minerals per kg of diet: Mn as $MnSO_4 \bullet H_2O$, 69.2 mg; Zn as $ZnSO_4 \bullet 7H_2O$, 28.4 mg; Co as $CoCl_2 \bullet 6H_2O$, 0.94; Cu as $CuSO_4 \bullet 5H_2O$, 3.29: Fe as $FeSO_4 \bullet 7H_2O$, 47.3 mg; I as KI, 5.1 mg; and Na as NaCl, 1951 mg.

[d] The mineral supplement supplies the following levels of minerals per kg of diet: $CaHPO_4$, 29.3 g; MgO, 1.6 g; $NaHPO_4$, 16 g; $MnSO_4 \bullet H_2O$, 25 mg; $ZnSO_4 \bullet 7H_2O$, 163 mg; $CoCl_3 \bullet 6H_2O$, 4 mg.

[e] 60%.

[f] 70%.

Table 13. Formulation of Oregon moist pellet (formulation OP-2 and as modified by Hardy et al. (1984)).

| Ingredient | OP-2 (g/100 g) | OMP (g/100 g) |
|---|---|---|
| Herring meal | 28.0 | 30 |
| Cottonseed meal | 10.0 | 7 |
| or | | |
| Poultry byproduct meal | 8.0” | 8 |
| Wheat germ | Remainder | 7.85 |
| Wheat flour | | 5 |
| Vitamin premix | 1.5[b] | 1.5[b] |
| Trace mineral premix | 0.1’ | 0.1 |
| Choline chloride (70% active) | 0.5 | 0.5 |
| Roxanthin red (a pigment) | | |
| 0.05 | | |
| Oil | 6.0-6.75 | 10 |
| Wet fish hydrolysate | 30.0 | 30.0 |

[a] Values represent maximums, together cannot exceed 1.5% of diet.

[b] The vitamin supplement supplied the following per kg diet: vitamin A acetate, 1642 IU; cholecalciferol, 2269 IU; DL-alpha-tocopheryl acetate, 503 IU; menadione, 18 mg; D-calcium pantothenate, 115 mg; pyridoxine•HCl, 30.8 mg; riboflavin, 53 mg; niacin, 222 mg; folic acid, 16.5 mg; thiamine mononitrate, 46 mg; biotin, 0.6 mg; cyanocobalamin, 0.057 mg; ascorbic acid. 893 mg: and inositol, 132 mg.

‘The mineral supplement supplies the following levels of minerals per kg of diet: Mn as $MnSO_4 \bullet H_2O$, 20 mg; Zn as $ZnSO_4 \bullet 7H_2O$, 75mg; Cu as $CuSO_4 \bullet 5H_2O$, 1.54: I as $KIO_3 \bullet C_2H_8N_2 \bullet 2HI$, 10 mg.

## Conclusions and Recommendations

### Summary

The reproductive performance of salmonids *(Oncorhynchus* and *Salmo)* is influenced by the nutrient intake of maturing fish. This influence is exerted both through the nutrient composition of the diet and through the feeding regime. For each salmonid species, females that produce the greatest number of viable offspring are those permitted to attain their greatest growth potential; larger fish produce larger numbers of healthy eggs. The number of eggs produced per unit of fish weight, however, is often the converse. If fish are held under a restricted feeding regime, or fed a low protein diet, the relative fecundity can be increased without compromising the quality of the fry. Broodfish held under restricted feeding regimes, however, may have lower rates of maturity. For this reason, it is most important that the relationship between feed regime and egg production be examined more closely.

Reproductive performance is clearly diminished in fish that are fed diets containing one or more essential nutrient, other than protein, at levels below the levels required for optimum growth. The dietary protein requirement of trout may be lower for reproductive performance than it is for growth, but this is far from established. There are no reports in the literature that indicate that the requirement of any nutrient for optimum salmonid reproductive performance is different than for growth and health.

The standard approach for establishing a nutrient requirement is to feed diets containing graded levels of the nutrient of interest to replicate lots of fish and to measure the response under these treatments. Provided that suitable precautions are taken concerning assignment of fish to experimental units and assignment of experimental units to treatments, the statistical significance of differences of mean treatment response can be assessed.

Experiments carried out primarily with rainbow trout have demonstrated the possibility that feed can be reduced for maturing fish up to 4 months prespawning with no adverse effect on reproductive performance. Should this be possible with other salmonids, the savings in feed costs would be immediate.

### Conclusion

There has been sufficient work done with salmonids to demonstrate that diet and feeding regimes can have marked influence on reproductive performance. The work done to date has not been designed to illuminate the dietary requirement of specific nutrients, nor is it known if the required level of any nutrient for reproduction is different from that required for growth and health. There is good evidence that withholding feed from broodstock for short periods of time immediately preceding ovulation has no effect on reproductive success, at least in rainbow trout. Most of the research conducted on sahnonid broodstock nutrition has not employed traditionally accepted standards of experimental design for requirements.

A firm understanding of how the dietary nutrient requirements of salmonids for optimum reproductive success differ from the requirement for growth can be helpful, both in increasing the reproductive performance of captive broodstock and in improving the cost effectiveness of maintaining broodstock. This can be accomplished either by altering the mix of ingredients in broodstock diets to reflect the actual dietary requirements of the fish or by altering the feeding regime of maturing fish.

## Recommendations

The above subsection titled Replication and Controls details some aspects of experimental design that are commonly missing from studies on broodstock nutrition (see page 4-21). Implementation of these elements will probably add considerably to the costs and resource allocation of broodstock nutrition studies. However, given the greater reliability and applicability of the findings, the added expense may well be justifiable.

Establish optimum and most cost-efficient feed regime for reproduction--Improving the state of understanding of the effect of feed regime on reproductive output of salmonids shows great promise of improving the efficiency of hatcheries and should receive highest priority. Examples of such work are studies to determine the optimum feed rate and timing of starvation or reduced intake, relative to freshwater transfer, that will maximize fecundity or relative fecundity. In addition, the effects of feeding rate or protein/energy intake on age at maturity in Pacific salmon species need to be studied. Some of the relevant published reports on this topic are described in the section Dietary Factors Affecting Captive Broodstock Performance (see page 4-S).

Establish nutrient Requirement levels for captive broodstock--The subsection Nutrient Requirement Levels and Optimum Feeding Regime prioritizes the nutrients to investigate (see page 4-22). Establishing the reproductive requirement for protein, of all the nutrients studied, is the most likely to improve the ability of fish hatcheries to produce Pacific salmon juveniles.

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# FISH HEALTH ASPECTS OF BROODSTOCK RESTORATION

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## Introduction

The following information was assembled from communications with fish health personnel and a review of the scientific literature. We gathered information on infectious diseases and parasites potentially affecting at-risk native salmonid broodstocks during culture and determined the best husbandry and medical techniques available for treatment and control.

Fish health management practices in salmonid hatcheries have historically been directed at production of a healthy smolt prepared to enter ocean pastures and survive and grow to maturity. A wealth of literature in textbooks and scientific journals considers the causes, diagnoses, and possible controls for the many infectious, parasitic, and non-infectious diseases affecting salmonids in the freshwater hatchery and in the marine environment (Wood 1974, Kent 1992, Rohovec 1991).

Most attempts to hold salmonid broodstocks to maturity in the Pacific Northwest have not been entirely successful, particularly for chinook salmon (*Oncorhynchus tshawytscha*) and sockeye salmon (0. *nerka*). Harrell et al. (1986) described excessive mortality due to infectious diseases and precocious maturity in chinook salmon broodstocks during the final 2 years of residence in marine net-pens. Sockeye salmon reared to maturity in freshwater tanks and in seawater net-pens have also experienced higher than expected mortalities due to infectious disease (Flagg and McAuley 1994).

## Viral Diseases: Infectious Hematopoietic Necrosis

When diseases of fish are ranked for importance, many biologists emphasize the viral diseases, since no therapeutants are available. Rucker et al. (1953) reported a disease of possible viral origin in sockeye salmon at a federal hatchery in Washington State after he was able to pass the condition to naive fish with filtered tissue homogenate from infected fish. Amend et al. (1969) and McCain et al. (1974) later demonstrated that the disease was identical to Sacramento River chinook salmon disease and Oregon sockeye salmon disease and identified the virus as the pathogen responsible for infectious hematopoietic necrosis (IHN).

Fish affected by II-IN are primarily the alevins and swim-up fry of sockeye salmon, chinook salmon, steelhead trout (0. *mykiss*), rainbow trout (0. *mykiss*), and brook trout (*Salvelinus fontinalis*). There is no known medical treatment for fish with IHN. Mulcahy and Pascho (1985) demonstrated horizontal (fish-to-fish) transmission of IHN and suggested that vertical transmission of the virus (via eggs or coelomic fluid) was also possible. However, Groberg (1988), after extensive laboratory experimentation, failed to show that progeny from parents infected with IHN developed clinical IHN disease or carried the virus in a fonn normally detected by cell culture assays.

LaPatra et al. (1987) presented evidence that adult salmon are infected with IHN virus in the hatchery, and that hatchery designs are often conducive to horizontal transmission between adult salmon. Although II-IN has been implicated as causing minor losses in some feral sahnonid populations, it is primarily a hatchery disease (Busch 1988), particularly when salmonid fry are exposed to the pathogen concomitant with the stress of artificial culture.

Because sockeye salmon are particularly susceptible to IHN, they were not considered for artificial propagation in the Northwestern United States from 1960 through about 1980 (McDaniel et al. 1994). The Alaska Department of Fish and Game began intensive culture of sockeye salmon in the early 1980s, and by 1992 they were taking over 80 million healthy eggs a year for enhancement purposes. Rigorous disinfection with steam and iodophors and the use of virus-free water supplies were responsible for keeping losses due to the disease and prophylactic destruction of eggs at less than 5% for about 10 years.

However, in 1993 a combination of higher than normal environmental temperatures, failure to use virus-free water sources, and several management changes (e.g., fry transfers between different facilities) resulted in losses to IHN of over 13.6% (Follet 1993). Currently IHN is classified as a Class B disease (quarantine and controlled transfer or release) by the Pacific Northwest Fish Health Protection Committee (PNFHPC). Nevertheless, a review of pathologists' reports and fish disease status reports from six northwest states and British Columbia, submitted to the Committee over the past 14 years, suggests that losses of fish directly due to IHN disease are not as severe as losses due to destruction of fish and eggs because of virus detection.

Salmonid culture programs for broodstock restoration should include routine testing of mature females for IHN virus and infectious pancreatic necrosis virus (IPNV), another viral disease that may affect rainbow or steelhead trout. Results of these tests could aid efforts to segregate infected gametes and alert biologists of the potential for disease. The use of virus-free ground water and iodophor disinfection of eggs is essential for healthy fertilization, water hardening, and all stages of egg incubation.

Usually IHN disease is associated with the presence of comparatively large numbers of well-matured spawners, particularly when spawning operations are spread over several weeks time and infected coelomic fluid, blood, and tissues are not adequately contained and disinfected. However, IHN can usually be avoided in juveniles even when the parent stock is positive. For instance, from 1987 through 1990, NMFS obtained gametes from approximately 400 wild adult sockeye salmon from the Wenatchee River each year (Flagg et al. 1991). With careful isolation of egg takes, containment and quarantine of gametes in iso-buckets, and thorough hatchery disinfection and water hardening with iodophors, viral diseases were completely avoided during this project.

## Bacterial Diseases

### Bacterial Kidney Disease

The most pernicious disease limiting culture of sockeye salmon and chinook salmon in the Pacific Northwest is bacterial kidney disease (BKD) (K. Johnson, IDFG, 1800 Trout Rd., Eagle, ID 83616. Pers. commun., July 1994). Renibacterium salmoninarum, the causative bacterium of BKD (Sanders and Fryer 1980), is an obligate pathogen of salmonids and is fastidious and slow growing on laboratory media. These characteristics, combined with generation times of over 24 hours, have impeded experimental work with the disease (Rohovec 1991).

Bacterial kidney disease is a chronic, insidious infection and is manifested as a glomerulonephritis with grey-white necrotic abscesses of the kidney and occasionally the spleen, liver, or heart. Mortality from BKD occurs throughout the life span of salmonids. The disease can be transmitted horizontally (Mitchum and Sherman 1981, Bell et al. 1984, Austin and Rayment 1985) and vertically via the egg (Bullock et al. 1978; Rohovec 1991; Evelyn et al. 1984a, 1986).

Some control of vertical transmission of BKD has been accomplished with injections of erythromycin (at 20 mg/kg fish) in female salmon 28 days before maturation (Lee and Evelyn 1994) and gamete culling or segregation after determination of severity of BKD in maturing female salmon (Pascho et al. 1991). However, current therapeutic measures for prevention or control of horizontal transfer of BKD have been generally inadequate (Austin 1985, Elliot et al. 1989). Although some success at control of morbidity and mortality due to BKD has been achieved using erythromycin as a feed additive at a dose of 100 mg/kg fish for 21 days (Moffitt and Bjomn 1989). it has been difficult or impossible to eliminate the causative organism (Austin 1985, Evelyn 1988).

Mazur et al. (1993), demonstrated that the prevalence of BKD in chinook salmon was directly related to rearing densities (i.e., higher densities with higher infection rates). Other biologists relate unpublished anecdotal evidence that low rearing densities result in less mortality due to BKD than higher densities (J. Morrison, USFWS, Olympia Fish Health Center, 3704 Griffin Lane SE, Olympia, WA 98501, Pers.commun., June 1995). However, sockeye salmon reared at very low densities and treated repeatedly with erythromycin suffered intolerable mortality due to BKD (Flagg and McAuley 1994).

Attempts to develop specific vaccines or to enhance non-specific immunity as prophylaxis against BKD have also been largely unsuccessful (Evelyn et al. 1984b, Kaattari et al. 1989, Baudin Laurencin et al. 1977). The poor control of BKD achieved by vaccination and chemotherapy has been attributed to the intracellular nature of R. salmoninarum (Evelyn 1988) and the fact that the organism can survive and multiply within host phagocytes (Young and Chapman 1978), thereby apparently avoiding stasis or destruction by antibacterial drugs.

Renibacterium salmoninarum survives in seawater and is horizontally transmitted between salmon in marine cages (Evelyn 1988, Banner et al. 1986). During the early 1980s, NMFS was involved in a captive broodstock program for Snake River fall chinook salmon and White River (Puget Sound) spring chinook salmon. Several brood years of each stock were reared in adjacent marine net-pens, and the presumed continual shedding of R. salmoninarum from infected fish and horizontal transmission in seawater resulted in mortality rates of 30 to 40% in each stock after 1 or 2 years of marine residence (Harrell et al. 1987). Attempts to control the ongoing epizootic in marine net-pens with orally administered erythromycin (100 m&g fish for 10 days) were not successful.

The use of erythromycin at various dosages, as a feed additive and as an injectable, as a means of controlling BKD in salmonids in the Pacific Northwest is being investigated under a BPA-supported grant to the University of Idaho for a U. S. Food and Drug Administration Investigational New Animal Drug (INAD) application. R. salmoninarum can develop resistance to erythromycin (Bell et al. 1984), and the continued widespread use of this drug will undoubtedly result in drug-resistant strains of the bacterium. Therefore, the potential effectiveness of other drugs should be investigated. Preliminary investigations of the quinoliie antibiotic enrofloxacin at doses of 5 to 10 mg/kg fish as a means of control for BKD in rainbow trout were encouraging (Hsu et al. 1994). In addition, the potentiated sulfonamide tribrissen has shown some promise as a means of control of BKD in chinook salmon and sockeye salmon broodstocks (D. Groves, Sea Springs Salmon Farms, P.O. Box 870, Chemainus, B. C. VOR 1KO. Pers. commun., July 1994).

Research on nutritional intervention as a means of controlling BKD should be pursued (Evelyn 1988, Bell et al. 1984). Resistance of fish to bacterial infections can be compromised by the amino acid balance of the dietary protein source (Wedemeyer and Ross 1973), improper balance between dietary protein and pyridoxine levels (Hardy et al. 1979), and inadequate dietary ascorbic acid (Durve and Love11 1982). Selective breeding for BKD resistance is another tool that has promise for control of the disease in salmonid populations (Withler and Evelyn 1990, Evelyn 1988).

## Other Bacterial Diseases

There are several infectious bacterial diseases that may compromise salmonid culture during the freshwater phase and be carried to the seawater phase. Furunculosis, caused by Aeromonas salmonicida, is a salmonid disease that is exacerbated by high rearing densities and excessive handling (Nomura et al. 1992). The disease is of particular concern to Atlantic salmon (*Salmo salar*) smolt producers and sea farms. Licensed injectable vaccines for furunculosis are marketed by Biomed Inc. (1730 130th Ave. N. E. Bellevue, WA. 98005-2203). These bacterins require anesthesia and injection of individual fish and appear to provide effective protection to cutthroat trout (0. *clarki*), steelhead, and Atlantic salmon.

However, efficacy of these vaccines has been reported as equivocal in adult spring chinook salmon (R. A. Holt, ODFW, 220 Nash Hall, Corvallis, OR 97331-3804. Pers. commun., July 1994). Alternatively, the antibiotic oxytetracycline is approved by the U.S. Food and Drug Administration as a feed additive at 100 mg/kg fish per day for 10 days for treatment of furunculosis in salmonids. The potentiated sulfonamide Romet-30 was also recently approved by FDA as a feed additive for control of this disease. Furunculosis could be problematic to broodstock restoration programs for salmonids if strains of antibiotic-resistant A. *salmonicida* develop (Aoki et al. 1971).

Vibriosis is an acute bacterial disease of salmon in seawater due to Vibrio *anguillarum* or V. *ordalii* (Harrell et al. 1976, Schiewe et al. 1981). The obvious means of avoiding this disease is total culture in freshwater. If this is not possible or if some seawater residence is necessary for optimum production, an effective vaccine is available from Biomed Inc. Vaccines are preferable to antibacterial feed additives because of the possibility of the development of antibiotic resistant strains of the vibrio bacterium (Austin and Austin 1987). In NMFS's experience with vibriosis in marine net-pens, intraperitoneal injections with commercial vaccines afforded excellent protection against mortality, but only for approximately 90 days (Harrell 1979, Mahnken and Waknitz 1979). Oxytetracycline added in feed at a dose of 100 mg/kg fish for 10 days is also effective therapy if fish are still infected with vibriosis after 90 days (Hodgius et al. 1977).

Bacterial coldwater disease may cause high mortality in alevins or fry in the early spring when water temperatures are between 4 and 10°C. The disease is caused by Flexibacter psychrophilus and is particularly prevalent in coho salmon stocks but has been reported in sockeye salmon culture in the Pacific Northwest (Wood 1974). Most salmon culturists attempt to control coldwater disease with a water-soluble oxytetracycline bath for 1 hour (l-2 ppm), since affected alevins often have not yet begun to feed (Rohovec 1991).

## Other Infectious and Physiological Conditions

There are other bacterial diseases (Austin and Austin 1987) and fungal, helminth, and protozoan parasites that could have an effect on captive broodstock culture in both freshwater and marine facilities. However, relatively low-intensity culture (e.g., low densities and infrequent handling), as compared to culture by standard hatchery practices, should preclude infections with most of these agents. The diagnosis, pathogenesis, and recommended treatments, as well as references to scientific literature, are adequately covered in texts by Hoffman (1967), Wood (1974), Margolis (1982), Roberts (1989), and Kent (1992).

The presence of a pathogenic agent does not necessarily constitute a disease in fish or populations of fish. However, when animals are crowded and subjected to artificial environments and foods, clinical signs of disease and mortality may occur. Restoration programs designed for salmonid stocks should be designed to minimize common stressors that usually accompany intensive artificial culture.

Husbandry techniques for total (gamete-to-mature adult) culture of anadromous salmonids have been under development for only the past 20 years or so. Knowledge of physiological processes, and a proper understanding of the epizootiology and biology of infectious, nutritional, and metabolic diseases that develop during previously inaccessible life stages (e.g., marine phase) is becoming available as research on these husbandry techniques progresses.

For example, during research of total culture of anadromous salmon in marine net-pens at the NMFS Manchester Marine Experimental Station in central Puget Sound, biologists discovered a severe systemic disease of chinook salmon that only affected fish after 18 to 20 months of seawater residence (Harrell et al. 1986, Elston et al. 1986). This “Rosette Disease” was subsequently diagnosed in endangered Sacramento River winter-run chinook salmon held in marine net-pens in Bodega Bay California (J. S. Foote, USFWS, 24411 Coleman Fish Hatchery Rd., Anderson, CA. 96007. Pers. Commun., November 1994). Another previously undescribed anemia caused by an intranuclear protozoan was first detected in chinook salmon broodstocks in seawater at Manchester (Elston et al. 1987) and later reported in chinook salmon pre-smolts and kokanee in freshwater by Morrison et al. (1990). Further epizootiological studies of these two diseases are in progress and may indicate a freshwater origin.

Other infectious and physiological conditions will undoubtedly be detected in future captive broodstock endeavors, particularly when anadromous fish are held full-term in either freshwater or saltwater environments. For instance, NMFS is currently holding sockeye salmon to maturity in marine net-pens, circular tanks supplied with disinfected and filtered seawater, and circular tanks supplied with constant 10.5$^{0}$C pathogen-free groundwater (Flagg and McAuley 1995). Results of these experimental trials should provide data on physiological development, growth, and survival that will be applicable to sockeye salmon as well as other captive broodstock programs.

## Conclusion and Recommendations

In February 1994, the PNFHPC surveyed 187 committee members and non-members involved with fish health in the Pacific Northwest. The survey was designed to 1) rank fish diseases by impact on production and program loss and 2) rank research approaches that might lead to control of the specified diseases. Most respondents were production hatchery biologists and managers and rankings reflected problems usually associated with mass cultivation of salmonids from egg-to-smelt.

For example, the number one ranked disease in terms of impact on programs and production was coldwater disease, a salmonid disease that primarily affects coho sahnon fry held in relatively dense conditions in water containing heavy levels of organic debris at temperatures between 4°C and 10°C (Roberts 1989). Another high ranking disease was bacterial gill disease, another problem associated with overfeeding, excessive fines, and organic particulate (Roberts 1989). Neither of these diseases should impact a well-mn captive-broodstock rearing program, since less intense methods of culture and pathogen-free water supplies during early rearing should minimize the probability of these infections.

Clearly, BKD will be the primary obstacle to successful captive broodstock programs, particularly when culturing chinook salmon and sockeye salmon. As stated earlier, some control of vertical transmission of BKD can be achieved by injection of erythromycin in maturing adults and gamete segregation after quantification of the disease in the spawning females. However, research is needed to develop a method of control of fish-to-fish (horizontal) transfer of R. *salmoninarum*. Development of an antibacterial drug that would eliminate *R. salmoninarum* from infected fish would be the preferred method of control of horizontal transfer of BKD.

Other solutions to the problem of horizontal transmission may be realized with research on treatment of holding waters with chemicals such as ozone or other oxidizing agents (e.g., peroxides). For instance, Bans (1990) was able to inactivate 99.9% of IHN virus in a hatchery water supply using 0.1 ppm active iodine without adverse effect on hatchery fish. There is a need to investigate the efficacy of other chemicals or elements as water-additive therapeutants for BKD.

All aspects of fish culture, including captive broodstock programs, are subject to state, local, and federal statutes and policies that regulate culture and transfers of fish and gametes both intra- and interstate. For example, Washington State legislation (WAC 220-77) requires cultured fish stocks with certain diseases that pose a threat to wild native stocks of fish be quarantined, confiscated, or destroyed. A thorough knowledge of state, county, and federal regulations regarding fish health and necessary disease certifications, as well as hatchery discharge permits and policies. is essential for the success of any captive broodstock program. Continued research in the areas of fish health, physiology, and nutrition, as well as a sound program of integrated fish health management is required for captive broodstock programs to aid restoration of imperiled salmonid stocks.

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# HISTORY OF WHITE RIVER SPRING CHINOOK BROODSTOCK AND CAPTIVE BROOD REARING EFFORTS

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# Forword

The following is a compilation of a number of internal agency documents, memos and notes from Washington Department of Fish and Wildlife files. We extend our thanks to Bill Hopley, Chuck Baranski, Bill Tweit, Rich Eltrich, Tom Langley, Denis Popochock and Carol Smith for their assistance in producing this document. We would also like to acknowledge the efforts of Ross Fuller, Howard Fuss, Mark Kimbel, Stan Hammer, Dan Doty, BilI Hopley and Charmane Ashbrook for their work in reviewing the manuscript.

## Introduction

The White River spring chinook (*Oncorhynchus tshawytscha*) is the last remaining stock of spring chinook in the South Puget Sound Region (A race of spring chinook that formerly inhabited the Nisqually watershed is now thought to be extinct). The White River (a tributary to the Puyallup) stock is genetically distinct from alI remaining Puget Sound stocks as shown by protein electrophoresis (A. Marshall, WDFW, 600 Capitol Way N., Olympia, WA 98501-1091 Personnel Communication, May 1994) and is, therefore, believed to be uniquely adapted to South Puget Sound river systems.

In the mid-1960's, escapement to the White River was reduced to fewer than 600 fish (from an earlier average of 2,000-3,000). By 1977, escapement had declined precipitously to around 50 individuals and the number of wild spawners has remained low ever since (Salo and Jagielo, 1983). At present (1994), the adult population is approximately 1,000 fish and exists, for all practical purposes, entirely under some degree of artificial production, having reached an extremely depressed population size in the White River. These, along with other Puget Sound Basin spring chinook, are among the most depressed stocks in the Pacific Northwest, outside the Columbia River Basin.

The primary goal for White River spring chinook (WRSC) is to restore the native population within the White River watershed. Potential also exists for establishing populations in other South Puget Sound streams such as: Puyallup, Nisqually, Carbon, and Green (not prioritized).

The efforts to culture White River spring chinook are only one part of a larger effort by state, tribal, and federal agencies to rebuild this stock. The White River Technical Committee continues to work toward habitat restoration, solving passage problems at Mud Mountain dam and other water diversions and maintaining adequate minimum stream flows, in order to rebuild the population (Production Recommendations for White River Spring Chinook; B. Graeber, WDFW memo to Muckleshoot Tribe, December 1987, WDFW 600 Capitol Way N., Olympia, WA 98501).

This paper is intended to document the history of artificial production efforts by describing past and present cultural strategies. The Washington Department of Fish and Wildlife (WDFW) and the Squaxin Island Tribe have made significant progress in the White River Spring Chinook captive broodstock program. As with all segments of the restoration effort, this program was undertaken for the purpose of restoring the spring chinook run indigenous to the White River.

## History of Artificial Production

White River spring chinook have been under some degree of artificial production since 1971. The production strategies have ranged from capturing adults returning to the White River to off-site captive broodstock maintenance programs conducted in seawater net-pens. During this period, there has been significant change in the role of artificial culture with respect to White River spring chinook. That role will be examined below, first through a historical review, and then by a description of the current production program.

From a historical perspective, hatchery production of White River spring chinook has evolved through three general stages beginning with the 1971 brood year. The first stage involved capture of wild male spring chinook to be used in an enhancement program. The second stage was an attempt to restore declining runs of spring chinook by direct out-plants of smolts into the White River following off-site rearing. The third, and current stage, is designed to build an egg bank of White River spring chinook in an effort to stem the decline in the stock until certain habitat and passage improvements in the White River can be accomplished. Appendix 6-A provides a summary of the results of each year's efforts.

### Stage 1: 1971-72

The first stage involved the 1971 and 1972 broods of White River spring chinook. Male spring chinook were captured at the Puget Sound Power and Light Company's diversion dam at Buckley, Washington. The males were hybridized with females from several other chinook stocks for use in a Washington Department of Fisheries' plan for restoring the south Puget Sound sport fishery, commonly called the "Thirteen Point Plan" (WDF, 1973). While this stage has been included for historical accuracy, WDFW has long since viewed this hybridization approach as undesirable and does not condone or continue this practice.

### Stage II: 1974-76

The second stage was directed at restoration of the White River spring chinook in their native habitat, in recognition of the severely depressed status of the run. Adults of both sexes were captured at the Buckley trap (near Buckley, WA) and were spawned at one of two Department of Fisheries (currently WDFW) hatcheries (either Garrison Spr. or Puyallup). The progeny were returned to the White River as fingerlings or smolts. This stage affected the 1974, 1975 and 1976 broods. There was no artificial production of the 1973 brood (reason unknown).

**Stage** III: **1977-present**

The third and current stage was designed to build an egg bank of White River spring chinook for eventual return to the White River System. This stage was initially promoted by harvest management and habitat biologists within WDFW, who cited the inadequacy of the then-existing White River enhancement effort. By mid-1979 habitat and passage problems were also gaining notice and provided further impetus to formalize an off-site egg bank program. The newly-constructed Hupp Springs facility (near Purdy, WA) was identified as the only available site providing the cool, high quality water necessary to hold spring chinook adults, and rear high quality smolts. The stage began when the 1977 brood spring chinook, which were being raised at Skagit Hatchery, were released into Minter Creek rather than the White River.

This programming change signalled the beginning of the effort to maintain White River spring chinook through off-site restoration and all subsequent releases (until 1991) were limited to Minter Creek. The third stage went through its own evolutionary process as the current egg bank program emerged. Initially, WDFW and the National Marine Fisheries Service (NMFS) worked to maintain two complimentary programs; (1) an anadromous broodstock program at Hupp Springs Hatchery, and (2) a captive brood program at the NMFS net-pen complex near Manchester, WA. (U.S. Department of Commerce, NOAA, NMFS, 7305 Beach Drive East, Port Orchard, WA 98366).

This stage can be further sub-divided by changes in the captive brood rearing operation. This involved a change from NMFS operations at Manchester, WA (1977-1986 broods) to a WDFW program managed cooperatively with the Squaxin Island tribe at the South Sound Net-pen complex (SSNP), near Olympia, WA (1987-present broods). In addition, the anadromous program expanded when the Muckleshoot Indian Tribe constructed a hatchery on the White River, at the Buckley trapping site. This facility was patterned after the Hupp Springs site and has very similar rearing/incubation capacities.

Currently, all White River spring chinook juveniles produced above the needs of the captive brood program (3,500 smolts) and the Hupp Springs program (about 320,000 smolts) are released into the White River at the Muckleshoot hatchery site. Some fish are reared in acclimation ponds in the upper White River drainage, but are currently returned to the hatchery site for release, until downstream passage problems can be corrected. Upon return as adults, the unmarked portion of these “acclimation” fish will be allowed to return and hopefully, spawn in the White River near the pond sites. A detailed listing of the releases and returns of these programs are presented in Appendices 6-A and 6-B.

## The Captive Broodstock Program

Captive broodstock rearing can differ from fish culture used in agency enhancement programs or aquaculture enterprises. Enhancement programs produce mostly smolted juvenile fish with minimum target sizes and growth uniformity being important objectives. Salmon grown for food are selected for factors such as: fast growth, disease resistance, high fecundity and potential to domesticate. With the captive brood programs these factors are of less importance. The number one priority in this program is to produce viable eggs that meet genetic fitness criteria in order to maintain the health of the stock.

Chinook populations mature at various ages. Each brood has individuals that are in various stages of sexual maturity. This is evident in the net-pen populations where sexual dimorphism can be observed in some of the older (3, 4, 5-year-old) fish as early as March, as they progress toward spawning in September. This variation requires our rearing program to change depending on year class. Our program does not focus just on the number of spawners generated or the resulting fecundity, but also must keep in mind the natural fitness of individual fish throughout the life cycle, in order to produce competitive quality offspring.

Many of the current fish handling practices; such as transfer of juveniles, some rearing strategies, and adult transfer methods have their origins in work conducted at the NMFS, Manchester Net-pen site. At the height of their participation, they produced 66% of the eggs available for the rebuildmg program (1985). The current phase, conducted at the SSNP site in a cooperative effort with the Squaxin Indian Tribe, is described below (Squaxin Is. Tribe S.E. 70th Squaxin Lane, Shelton WA 98584). The current freshwater adult holding and spawning operations at Hupp Springs are similar to those used historically and are representative of many operations rearing spring chinook in Puget Sound.

### The Captive Brood Program at the South Sound Net-pen Complex

The following section describes the seawater captive broodstock program as it is cooperatively managed by the Squaxin Island Tribe and WDFW. Current practices such as low rearing densities and a lack of monthly sampling or inventorying (which minimizes stress) are in response to the primary goal of maximizing the broodstocks' well being and survival. Included is a description of the net-pen site, how the smolts are transferred to saltwater, current fish culture practices and management views on feeding, rearing densities, pathology, and environmental problems at the site. Handling of mature fish and the transfer to freshwater are described in detail.

Site Description--The net-pen complex is composed of 73 pens set in three groups (2 groups of 20 pens and 1 group of 33 pens, a portion of which is used for captive broodstock). These are anchored about 100 m apart, perpendicular to normal current flow in Peale Passage (just east of Squaxin Is. near Olympia WA). Although individual pen size varies, they are approximately 7.7 m x 9.8 m x 3.7 m (see section on pen densities for details).

Since the early 1970's, the site has operated as one of the most successful enhancement facilities in Washington State. It continues to produce substantial numbers of coho salmon *(Oncorhynchus kisutch)* for sport and commercial fisheries. Initial progrsms involved short term rearing and release (from late winter to early June). Before the Squaxin Island Tribe developed a successful steelhead ***(Onchorynchus mykiss)*** broodstock rearing program (1985-1990), it was not known if the site was suitable for year round rearing programs.

Peale Passage is a tidal channel that connects with Dana Passage on the south and Pickering Passage on the north. An early study of hydrographic conditions in the area, including measurements of physical and chemical parameters was conducted by Oclay (1959). Moring, 1973 was the first to document detailed water quality and basic information concerning the pen site. Currently, personnel from the Squaxin Island Tribe and WDFW, Deschutes Hatchery crew, collect pertinent water quality infomration (such as dissolved oxygen, water temperature, saiinity, and presences of certain phytoplankton species). Rensel(l989) characterized the seawater as being well mixed during the late fall to spring months.

The pen site depth is shallow when compared to other sites in Western Washington, with an average depth of about 5.0 m at mean lower low water (Rensel 1989). On the lowest summer tide cycles, (mean lowest low) the bottom of the pen at the west end of the broodstock complex rests on the sea floor. Mean water current velocity readings are 6 to 7 cm/sec. at the northern end of the pen complex (Weston and Gowan 1989). The current diminishes in the vicinity of the third pen complex (unpublished survey data of B. Wood, Squaxin Island Tribe SE. 70th Squat& Lane, Shelton WA 98584). It has been noted that water passing through the net-pen site does not completely exit Peale Passage on moderate tides and the situation can lead to an increased abundance of phytoplankton (Rensel 1989).

During low tide, zero current events, feeding can reduce dissolved oxygen within net-pens compared with water outside the pens. Based on hydrographic conditions, such as mean current velocities and minimum depth guidelines (Science Applications International Corporation 1986), the pen site would not normally be considered a good broodstock rearing location. Mean water temperatures can regularly exceed 15.5" C (60° F) through the summer, and other environmental stresses can accumulate to the point they threaten survival of the broodstock.

Transfer of smolts to SSNP--Each spring, the broodstock program receives 3,500 yearling fish (Table 1). These are selected randomly from the pond containing yearling smolts for release. Fish from the first two brood years (1987,1988) were from anadromous returns to Minter Creek (Hupp Springs). Starting with brood year 1989, smolts were progeny of anadromous females, but may have either anatlromous or captive broodstock male parents. Approximately 450 days of freshwater rearing are required to grow smolts to the transfer stage (45 g).

In an effort to control Bacterial Kidney Disease (BKD), all yearling smolts receive three Erythromycin (9%) treatments during rearing. Three weeks prior to salt water transfer, fish receive a Vibrio (type A) vaccination bath.

Table 1. Smolt transfers to SSNP.

| Year | Brood | Date | Number of Fish | Average Fish Wt. | % Transfer Loss |
|---|---|---|---|---|---|
| 1989 | 1987 | 4/20 | 3485 | 73.2 g | .014 |
| 1990 | 1988 | 4/4 | 3500 | 56.7 g | .714 |
| 1991 | 1989 | 3/25 | 3500 | 75.6 g | .857 |
| 1992 | 1990 | 4/9 | 3500 | 56.7 g | 2.875 |
| 1993 | 1991 | 4/14 | 3500 | 53.4 g | 1.428 |

Smolts are transferred by tanker truck to a saltwater boat launch site and loaded into a circular tank on a transport barge. The 7500 L tank is equipped with an oxygen supply (12-15 ml/L) and a seawater circulation system. Fish and freshwater are loaded from the tanker truck through irrigation pipe into the tank. The freshwater from the tanker truck mixes with an equal amount of seawater in the barge tank. This produces an initial salinity of about 12-15 ppt in the transport barge and buffers temperature between the fresh and saltwater.

The loaded tank and transfer barge are then moved to the pen site by tug. While in route, the barge circulates seawater into the tank. Over flow water is discharged to keep the container volume constant. Complete displacement of the initial fresh and seawater mix occurs during the one hour voyage to the site and ambient salinity (28 to 30 ppt) is achieved in the tank during this time. Survival during the transfer has been high, with little immediate or delayed osmoregulatory problems documented after the transfer.

## Growing Fish

Feeding Strategies--Diet type and feeding regimes, along with other husbandry practices and environmental cues can dramatically influence fish life cycles (Johnson 1993). The immediate culture goal is to provide adequate nutrition to grow fish to an appropriate size (as it relates to fecundity).

Early attempts at seawater captive broodstock rearing experienced low egg viability that was thought to be caused by poor feed quality or a lack of some nutritional component (Groves 1988). Although the formula composition and quality of feeds have greatly improved, additional research on diet and feeding strategies used on sahnonid broodstock is needed, particularly as it relates to egg and fry quality.

In some instances, programs have incorporated natural crustacean and forage fish organisms (R.Coleman, California Fish and Game, 1416 9th St., Sacramento, CA 95814. pers. commun.). Providing a natural food source in conjunction with fonnulated diets is intriguing, but limited by the difficulties of identification and procurement of food sources. Growing fish from smolt size to mature adults has been successfully accomplished with pelletized feeds. The WRSC program has relied, in-part, on feed manufactures and prevailing professional opinions for nutrition and feeding strategies.

Suggestions from feed manufacturers (Bio Products) and other ongoing broodstock programs have been fairly consistent in recommending feed with a high level of protein for the "growing" periods with a reduction in protein levels and feeding rates during the laner stages of maturation and egg development. A more detailed description of the feeding regimes used by year class is presented below and in Figure 1.

Feeding Program by Year Classes--Yearling ***(1.5) to 2-year-old fish***. Although fish will feed aggressively within hours of salt water transfer, food is introduced slowly over the first few days. By the following week, increasing amounts of feed are introduced to detennine satiation rates. Food is hand fed twice each day. Through the summer, fish can consume up to 3.5% of body weight per day (%body wt/day). By fall, with fish size increasing and cooling water temperatures, feeding rates are reduced to 2.0 %body wt/day. ln early winter, when water temperatures are less than 10° C, feeding rates can drop to 1.25 %body wt./day. During this time frame (late spring to early winter), weight gain is approximately 400 g per fish.

Both moist and semi-moist diets with high protein levels have been used. Feed sizes range from 4.0 mm to 6.0 mm. The choice of moist diets over dry fonnulations has been due to comfort and familiarity of the culturist and no attempt has been made to weigh the merits or economics between diet types.

***Two-to3-year-old fish.*** During this period of rearing, feeding rates fluctuate with seasonal water temperatures. When water temperatures drop in winter (< 7°C), feeding rates are reduced to 1.0 %body wt./day. Maximum feeding rates of 1.50-2.50% body wt./day are sustainable when temperatures are 10-15" C. When water temperatures exceed 15.5" C, feed is temporarily reduced to 0.75-1.0% body wt/day.

Larger fish (> 0.50 kg), are fed a higher protein (> 45%), moist diet that incorporates krill and synthetic carophyll red pigments in pellet sizes of 6.0 to 8.0 mm. There is an intuitive preference by fish culturists for fish with normally pigmented flesh and eggs, but reasons for this preference are not well researched (Groves 1989). Individual fish weight increases almost fourfold over the 12-month period from 450 g to 1,589 g each.

***Fish 4 years and older.*** After 650 days of saltwater rearing, the fish begin their fourth year of rearing. Feed management is viewed differently as the 4-year age group represents the dominant spawning age class. The feed program changes to reflect the fishes investment in gonadal development rather than growth.

A "grower" diet is used for the first three months of the year with "winter" feeding rates of 0.6-I .O% body wt./day. On 1 March, the diet is changed to a formulated broodstock diet based on feed manufacturers' recommendations. The broodstock diet is fed to satiation (0.8-1.25% body wt./day), in pellet sizes of 10-14 mm, ***until*** 1 June. Short-tenn environmental stress (temperature, phytoplankton blooms) may require a reduction of feeding rates to 0.50% body wt./day. At this time, a majority of the population is showing a bronze coloration. On 1 July, feeding is reduced to once a week (equivalent to 0.17% body wt./day). Feeding ceases on 1.5 August. A typical 4-year-old fish at this time averages 65-75 cm in length and range in weight from 3.0 to 7.0 kg.

After the transfer of mature fish to freshwater, during the first week of September, non-mature fish (age 4+) retained at the pens are placed back on the grower diet and fed at normal rates until the following March.

## Rearing in Net-Pens

Rearing densities--Providing plenty of space in the pens for growing broodstock is a management priority. Keeping fish rearing densities low, is believed to reduce the impacts of environmental stressors (low tides, water temperatures, phytoplankton blooms). The maximum allowable density for each age class is reduced as fish age (Table 2 and Figure 2). To alleviate handling stress, pen inventory and density adjustments occur only once each year (in conjunction with separating mature fish).

Net-pen dimensions are rectangular. 7.7 x 9.8 x 3.7 m, with an effective rearing volume of 228 $m^3$/pen. Currently, four brood years are maintained simultaneously in a total of 14 net-pens.

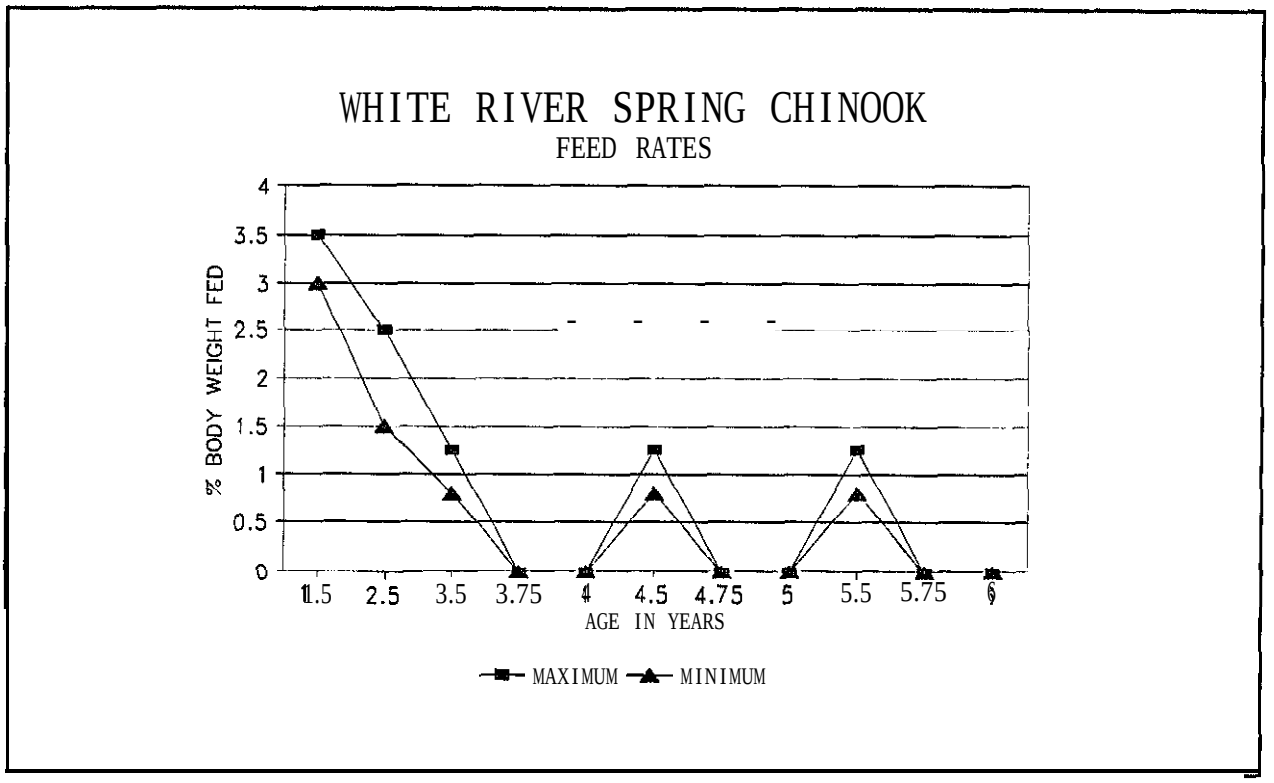



Figure 1. Feeding Rates for SSNP.

Table 2. Net-Pen Rearing Density

| Fish Age | Pens | Individual Fish[b] | Fish/Pen | Fish/wt.[c] | Start kg/m³ | End kg/m³ | Max" kg/m' |
|---|---|---|---|---|---|---|---|
| 1.5-2.0 | 1 | 3400 | 3400 | 400 g | 0.89 | 5.96 | 6.5 |
| 2.0-3.0 | 4 | 2800 | 700 | 1362 g | 1.48 | 4.53 | 5.0 |
| 3.0-4.0 | 8 | 800 | 100 | 4540 g | 1.59[d] | 2.00 | 3.0 |
| 4.0-5.0 | 1 | 120 | 120 | 6356 g | 2.62' | 1.60 | 2.5 |

[a] Represents maximum target density managed.
[b] Typical September inventory.
[c] Typical September fish Wt.
[d] Figured at initial # fish stocked, significant loss of inventory (3.0-4.0 age fish) occurs by the following Sept.
[e] Figured at initial #fish stocked, significant loss of inventory (4.0 + age fish) occurs by the following Sept.

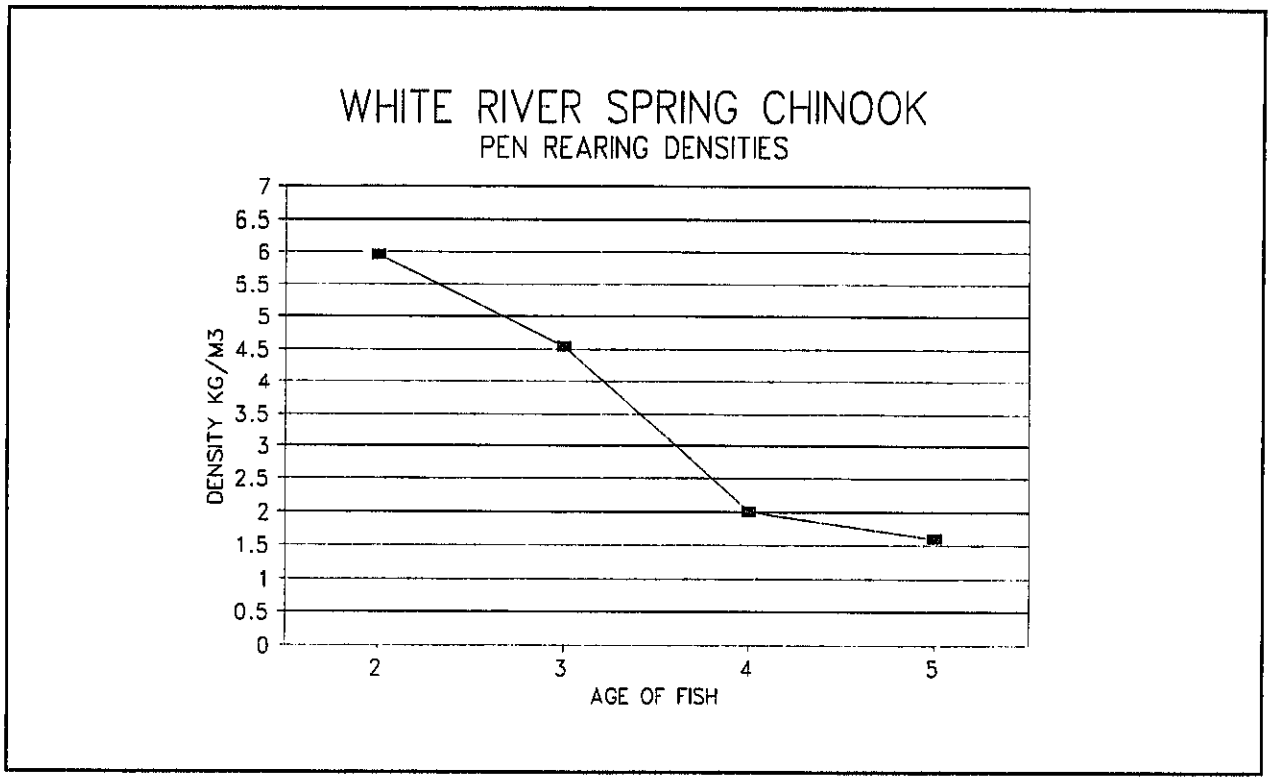



Figure 2. Pen rearing densities at SSNP.

Pen environment--Netting used to construct pens can be of nylon or polyester fabrics in a variety of strands and weaves. Pens can be dipped in water soluble treatments to toughen the material or to administer antifouling properties (waxing, flexdip). Polyester fabric is currently the only material used at SSNP. Besides defining the limits of confinement, pen systems can sometimes effect fish in negative ways. Consequently, the, physical characteristics of the pens are changed as fish grow, in order to minimize stress on broodstock.

***Mesh*** Size. As the fish increase in size and age, the nets are replaced with those having larger mesh openings. Fish from 1.5 to 2 years of age are reared in pens having 2.0 cm mesh (stretch). Fish 3 years old are reared in 5.85 cm (stretch) mesh. Pens for fish of 4 and 5 years of age have mesh sires up to 7 cm. During the summer, rapid exchange of water in and around the pens is critical. The larger mesh insures complete water exchanged during tidal movements. Fish are normally reared in pens with as large a mesh size as possible. This allows better dispersal of metabolic wastes and reduces surface area available for fouling organisms.

***Shading***. Unlike Atlantic salmon (*Salmo salar*) and Steelhead (*Onchorynchus mykiss*) which are frequently observed hovering in the upper water column (Squaxin Seafarm Observations), spring chinook adults (3+ and older) appear to favor the bottom of the water column. Fish come to the surface when feeding, but quickly retreat to the deepest water possible.

To provide some relief from sunlight in the comparatively shallow pens, shade screens (1 mm mesh) have been used to cover a portion (40%-60%) of the existing bird net covering. Completely shading the pens would require the removal of covers during each feeding period. Field observations suggest fish prefer the shaded portions of the pens.

***Billowing.*** Moderate billowing of the net-pen side walls during tidal exchange indicates the strength of the current and the amount of water passing through individual pens. Concrete fiied containers (1 gallon) are suspended 'Song the inside walls of the pens in order to maintain a rectangular configuration during tidal exchanges.

Excessive billowing, when a side or bottom panel is forced to the surface, has been observed during extreme tidal surging or as the result of organisms fouling the mesh. We believe this stresses fish and requires action (either cleaning or replacing nets, or adding more weight bottles) to reduce it. This is especially true if fish are forced to the surface or continually have to negotiate folds in the net-pen. During these events, adults have been observed "porpoising" out of the water (significance unknown).

Retrieving Mortalities-Floating mortalities are removed as they occur, mortalities that sink to the pen bottom are removed by dip nets when visible. At times, one side of a net-pen must be lifted to access the bottom. During this procedure, care is taken not to overcrowd fish and the activity is timed to avoid other environment stress (bright sun, high temperature, slack tide).

Constant net-pen replacement during the spring and summer is required due to growth of marine organisms. Dealing with this fouling problem provides a regular opportunity to retrieve mortalities on the pen bottom. Divers are used to retrieve mortalities when disease is suspected, although regimented daily diving to retrieve mortalities is avoided.

When minimal daily mortality is occurring (< 0.05%), much of it decomposes rapidly or is consumed by red rock crabs (*Cancer productus*), that are purposely stocked within the net-pens for this purpose. A distribution of the documented mortality by cause is presented in Figure 3.

Pathology--Fish that become lethargic or cease feeding show symptoms of physiological developmental problems (such as crinkle back, lack of tolerance for seawater), or are hosting debilitating pathogens. When moribund fish are observed, they are collected for examination by a pathologist.

Of the five brood years reared at the pens, one clinical outbreak of Bacterial Kidney Disease (*Renibacterium salmoninarum*) occurred. This outbreak occurred in the 1990 brood, when the fish were two years old. Mortality in excess of five percent was documented. It is believed that horizontal transmission from an adjacent fall chinook pen was the cause. In addition. medicated feed (Romet) has been fed in response to a outbreak of Vibriosis (type A) (*Vibrio anguillarum*) occurring in the 1987 brood fish.

Although clinical mortalities due to some infectious diseases have been documented, it is impossible to determine the cause of death in most cases due to the decomposition of mortalities.

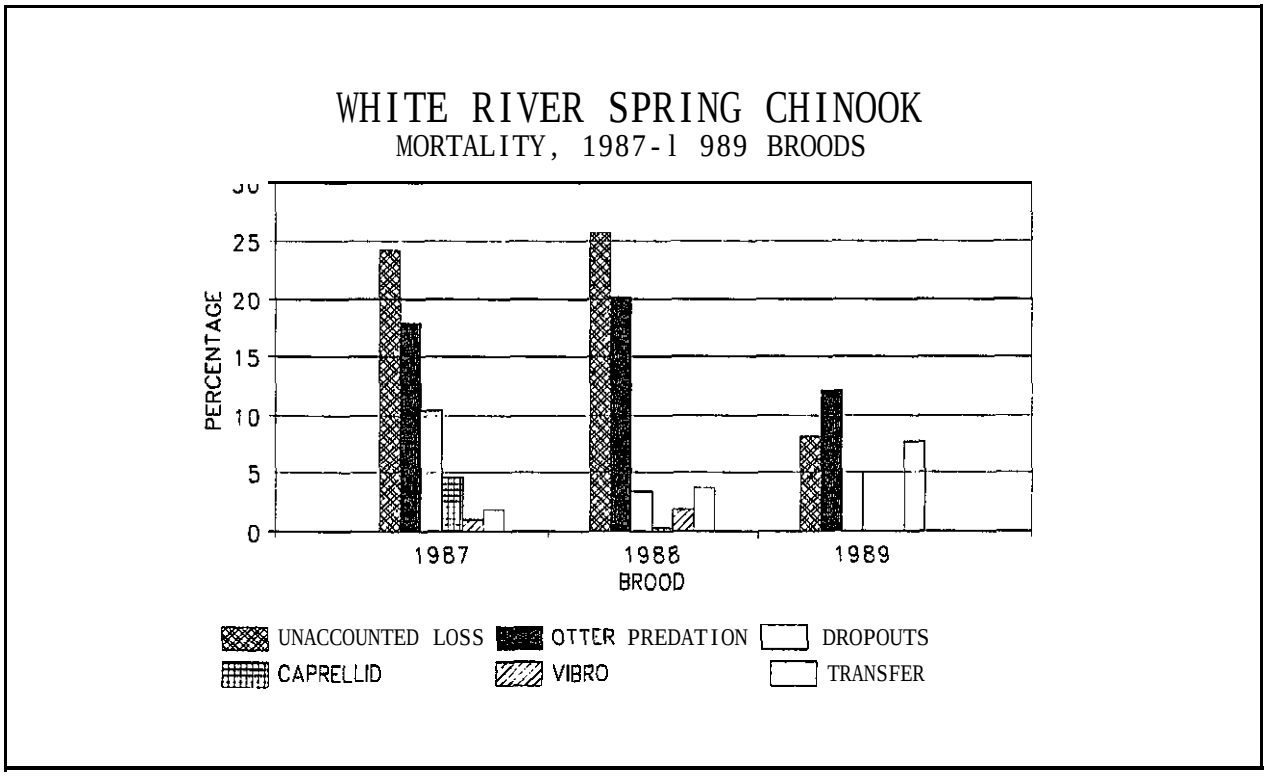



Figure 3. Source of Mortality for Spring Chinook Broodstock.

## Environmental Problems

**Caprellids**--During the early spring and summer months, serious infestations of caprellid amphipods (*Caprella sp.*) have been observed. This organism attaches to the sides of net-pens. Densities as high as 60,000/m$^2$ have been estimated. High densities of caprellids block water flow through the pens and many end up floating or swimming through the water within the pens. In 1989, caprellids were observed attached to fish, causing open wounds that resulted in significant mortality (4.6%) to the 1987 brood. To remove attached caprellids around the dorsal and caudal areas, all fish were bathed in a 1:2000 solution of formalin (F) for 20 minutes.

To reduce the impact of caprellids, clean nets are installed every two weeks during the spring and summer months. The overall extent of caprellid infestations are believed to be species/site specific, as they don't appear to pose the same problems for adjacent pens of yearling coho.

Predation-When rearing salmon in net-pens for extended periods of time, culturists realize a certain portion of the population will be lost to predators. Some commercial growers have estimated loss to predation to range from 10% to 30% (Lindsay 1980, Coche 1983 in Moring 1989). Mortality caused by predation at Puget Sound net-pen sites ranged from 8.4% to 38% after 214-260 days of rearing (Moring 1989).

With broodstock programs extending to 1,200 days of rearing, plenty of opportunity exists for predation. Even with significant time and labor investments to secure pens, predators can still be successful.

A small mesh cover netting (5 cm stretch) is stretched over the pen to prevent birds such as kingfishers (*Megaceryle alcyon*) from forcing their way in to the pens. Mounting this cover up to 1 m above the water level (by extending the height of the pen side) will prevent birds such as herons (*Ardeidae sp.*) from sitting on the cover and stabbing fish. Avian predation is minimal and generally targets fish smaller than 450 g).

Predation by aquatic mammals is consistently a problem. River otters (Lutra canadensis), from adjacent Squaxin Is., can be very damaging They can consume all sizes of fish, from 50 g up to the largest individuals (> 6 kg). Mink (*Mustela vison*) have also been observed on the pen walkways. These animals gain access into the pens by chewing or lifting the cover netting. Fish mortality is suspected when openings are observed in the cover nets.

Mortality is confirmed by observing fish scales or blood stains on the walkways. Even when the cover is physically tied down to the pen rails (electrical ties, rope, nails and staples have all been used), animals will chew through the cover netting to gain access to the fish. This behavior can also create holes in the net-pen sides. If a hole is created just under the water surface, immediate detection is difficult and can cause additional loss due to fish escaping.

Harbor seal (*Phoca vitulina*) activity at the net-pen site has been infrequent but is always possible. Predator nets (15 cm stretch mesh) that encompass the pens below the water line are used when possible. However, with the need to regularly replace pens to avoid excess fouling, the use of predator nets has been limited. Predator nets frequently entangle pens being changed and are also subject to fouling. Even when predator nets are used, spiny dogfish (*Squalus acanthias*) still gain access the pens by chewing holes through the bottom panels. As many as 46 dogfish have been removed from an individual pen in a single day.

Alternative mesh materials that can withstand most predatory animals are being investigated (Dilonet, Super Mesh). The ultimate protection, a metal sea cage system, has not been considered for this program. However. it may be warranted in other programs if success is based on reducing the impact of predators.

Unaccounted-for loss--Even when preventive measures appear to have successfully eliminated predators, and mortality is collected in a regular manner, approximately 20% (5% per

year) of a cohort (brood year) is unaccounted for after the life cycle is completed (Fig. 4). We believe this unaccounted-for loss is due to variations in original inventory, underestimated mortality and underestimated predation, although it is unknown which factor plays the most significant role.

**Phytoplankton--In** Puget Sound, mortality of pen reared salmon has been caused by several dinoflagellates (*Certatium fusus, Gymnodinium sipendens*), (Rensel and Prentice, 1980). In addition, some diatoms (***Chaeticeros*** *convolutus*, C. *concavicornis*) and a microflagellate (*Heterosigma akashiwo*) have been implicated in mortalities from British Columbia to Manchester Bay, Washington (Gains and Taylor 1986, In Moring 1989, Manken and Harrell, NMFS, Port Orchard, WA. personnel communication, April 1990).

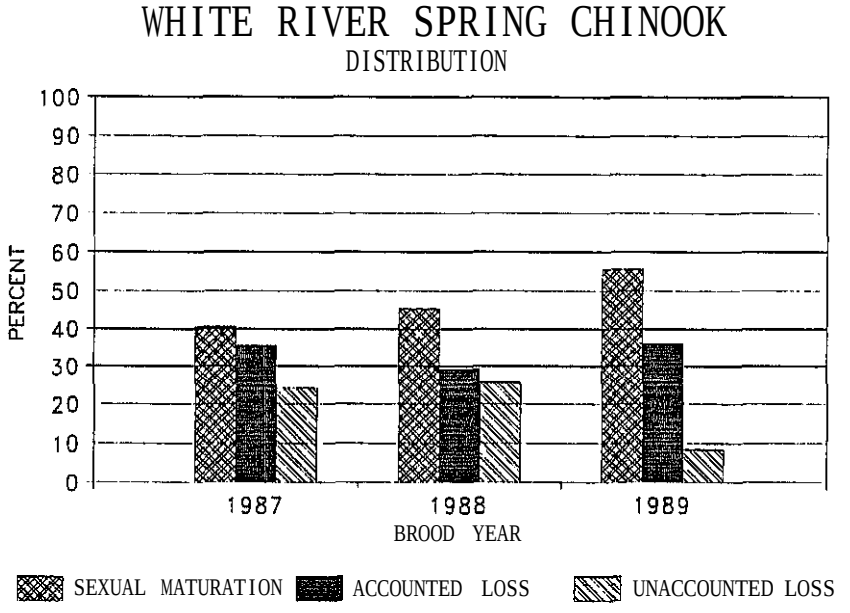



Figure 4. Distribution of Spring Chinook cohort,

Other species of noxious algae occur in Puget Sound with unknown impacts to aquaculture operations.

The SSNP site has had only one verified occurrence (1973) of mortalities attributed to noxious phytoplankton, *Ceratium fusus*, (Rensel, 1989). The SSNP site does not have extensive documentation of either occurrence or abundance of various phytoplankton populations which occur in Peale Pass. It is possible that phytoplankton blooms may add to the stress on the captive brood and contribute to the chronic, low level of mortality that occurs each summer.

Routine sampling is conducted at the SSNP site in order to document blooms and information is shared with the University of Washington, School of Oceanography phytoplankton hotline (206) 685-3756. This program is seeking to add to the known phytoplankton data base for Washington waters.

State and Tribal staff regularly take secchi disc readings, measure dissolved oxygen and temperatures from May to October. Qualitative phytoplankton sampling is accomplished by weekly 2 meter and 6 meter net tows (20 micron mesh). When species of interest are noted, quantitative densities (cells/L) are determined by taking 100 ml fiied volume samples from a depth sampler (Van Dom style). Species are identified and counted from a Palmer/Maloney 0.1 ml chamber slide. Various stratified layers are sampled to determine the depth of blooms. When species of concern are present, fish cultural activities are temporarily reduced to lower stress (full ration feeding, pen changes, etc.).

Contingency plans for dealing with blooms of known toxic phytoplankters have been explored for the WRSC broodstock program. Early transfers of adults to freshwater, transfer of adults to other saltwater facilities, circulating water from below stratified phytoplankton layers and towing the pen complex to areas without toxic levels of phytoplankton have all been considered. Currently, emergency transfers of adults to other facilities with salrwater and physically towing the net-pens to other areas of Puget Sound are the two acceptable strategies for dealing with this situation. Transferring adults to other facilities has the best chance of success but could only save a limited number of fish (only 4 year old females). Since these pen structures have never been moved from their current location, it is unknown if this can be accomplished without causing additional fish loss (through stress or unintentional release of a large number of fish).

**Identification** of Mature Fish and Transfer Practices

Seawater or Freshwater Spawning--WDFW performed an informal field experiment to test the feasibility of seawater maturation and spawning in the fall of 1991. After completing the main freshwater transfer segment, 13 mature females (4 year old) were retained at the pen site to complete oogenesis. Of the 13 females, only 6 survived to a stage of complete maturity. Out of the 6 spawned, only 2 fish provided eggs. (Approximately 2500 eggs each that were 80% viable). The other four had visibly poor egg quality and much reduced fecundity. This experience, plus similar results from another saltwater chinook broodstock program, Big Qualicum B.C., confirmed our decision to continue final egg maturation in freshwater (Redfern, 1988). All WRSC are now transferred to freshwater facilities for final maturation and subsequent spawning.

Timing of Transfers--Although sexual dimorphism is fully evident in maturing fish by early summer. it was not clear when to transfer maturing adults to freshwater. Results from the NMFS Manchester Research Pens WRSC broodstock program in the mid 1980's, recommended delaying transfer of the fish until late summer or early fall (Report on the 1985 brood White

River Spring Chinook. Eltrich 1986, memo to files, WDFW 600 Capitol Way N., Olympia, WA 98501).

WDFW conducted an informal field trial using 23 fish (4 year olds) exhibiting slight coloration (bronzing). These fish were transferred from the SSNP complex to freshwater holding ponds on June 20, 1991. This trial was in response to a contingency plan developed by staff in case noxious phytoplankton blooms during the summer required the immediate transfer of all mature broodstock to freshwater. Of the 23 fish transferred, few survived to maturation (Report on the 1991 brood White River Spring Chinook. Eltrich 1992, memo to files, WDFW 600 Capitol Way N., Olympia, WA 98501). The results convinced salmon culture staff to reject this option for dealing with algae blooms.

Currently, all maturing fish (age classes 3,4,5) are transferred during the first week of September. Catastrophic mortality caused by phtyoplankton blooms are still a concern and levels of specific algae populations are monitored in conjunction with the University of Washington. This time frame takes into consideration water temperatures (seawater temperatures are decreasing to less than 16" C) and occurs before complete ovulation renders the females too fragile to handle.

Logistical Operation--Successful handling and freshwater transfer of 900-1200 maturing broodstock is a critical step. The operation requires a significant investment in equipment and labor. Fish are placed in fish transport trucks (tanker) that have been driven on board Washington National Guard LCM's (Landing Craft:Medium). LCMs are moored parallel to the pen complex where crews can load the adult fish into the tankers. The LCMs are used to ferry the trucks back and forth from the pen site to a mainland access ramp. Four, 3,750 L tank trucks and two LCM's are required for the most efficient operation.

Tanker trucks carry 3,750 L of freshwater (from Hupp Springs @ 10° C) with a 5% salt solution. Oxygen is added at 1.0 L for each 135 kilograms of adult fish. Approximately 50-70 adults are hand placed into each tanker truck. The LCMs transport the trucks to the boat ramp where they drive to their destination (Hupp Springs, White River Hatchery). Adults are unloaded from the tanker truck by tilting the truck bed and sliding the fish down a ramp into the pond. Travel time is 1 S-2.5 hours depending on destination. This system requires that broodstock be handled only once during the entire transfer process.

Handling Procedures--The operation is coordinated to move all fish out of a given pen as quickly as possible. Fish that are crowded in the net-pen are continually stressed while waiting to be moved. The last 20% of the adults handled in each pen show obvious signs of stress (lethargic, change in skin color, etc.). Once fish are placed into the tanker trucks, they acclimate to the cooler freshwater (10-12° C.) and calm significantly. Sporadic mortality occurs with the last few fish remaining in the pen (0%-6%/pen). Non-mature older fish (known as 4+ brights) are exceedingly fragile when handled in conjunction with mature fish.

"Sanctuary dipnets" are used to move fish. These dipnets are constructed with a vinyl pouch that contains the fish in a portion of water and prevents net mesh chaffing during dip netting. Two separate dipnet and injection teams concentrate on a single pen at a time to speed up the process. After dipping out 1 or 2 adults at a time, the sanctuary net is placed directly in a watered tote. While in the sanctuary net, mature fish are injected in the dorsal sinus with Erythromycin (ethro-200 @ 0.5 ml/10 lb). After injection, fish are placed into a watered inner tube carrier and taken to the truck on-board the LCM.

Non-mature fish are carried to designated pens in the inner tube carriers. This process attempts to keep fish in a watered environment most of the time to ensure maximum survival (Flagg and Harrell 1990). With approximately 100 adults (4 yr) per pen, a coordinated, intense effort of about 20 minutes is required to remove all fish from each pen. The total transfer takes 1.5-3 working days.

The use of anesthetics (MS 222-Tricaine Methane Sulfonate) has been tried, but discontinued. The time spent waiting for the drug to take effect was better used by reducing the tune fish spent under stress in the pen, waiting their mm.

Mature fish and Eggs

Captive broodstock are transferred to Hupp Springs facility and White River Hatchery. Both systems currently combine net-pen broodstock adults with available anadromous WRSC returns to make up the total brood year escapement.

The majority of captive broodstock are ready for spawning approximately IS-30 days after transfer. The majority of the anadromous WRSC spawning occurs from 10 to 30 Sept. The captive broodstock spawning overlaps at a slightly later date (20 Sept.-10 Oct.). The percentage of sexually maturing fish by age class is presented in Figure 5.

Egg Comparisons--Although the captive broodstock program has greatly increased the number of viable WRSC eggs (800,000 plus yearly), fecundity and egg viability have been less than those from anadromous sources (Table 3). Individual broodstock egg quality varies greatly from fish to fish within the spawning population.

Table 3. Comparisons of Fecundity and Egg Viability.

| | ANADROMOUS | | | CAPTIVE BROOD | | |
|---|---|---|---|---|---|---|
| | Fecundity[a] | Eggs/lb. | %Viable[b] | Fecundity | Eggs/lb. | %Viable |
| 1991 | 3668/fish | 1840 | 94.0 | 2700/fish | 1820 | 63.4 |
| 1992 | 3268/fish | 1750 | 96.0 | 2536/fish | 1775 | 70.5 |
| 1993 | 3220/fish | 1695 | 95.0 | 2200/fish | 1650 | 65.7 |

[a] Breakdown of ape class fecundity not available.
[b] Viability does not take into account eggs that are discarded.

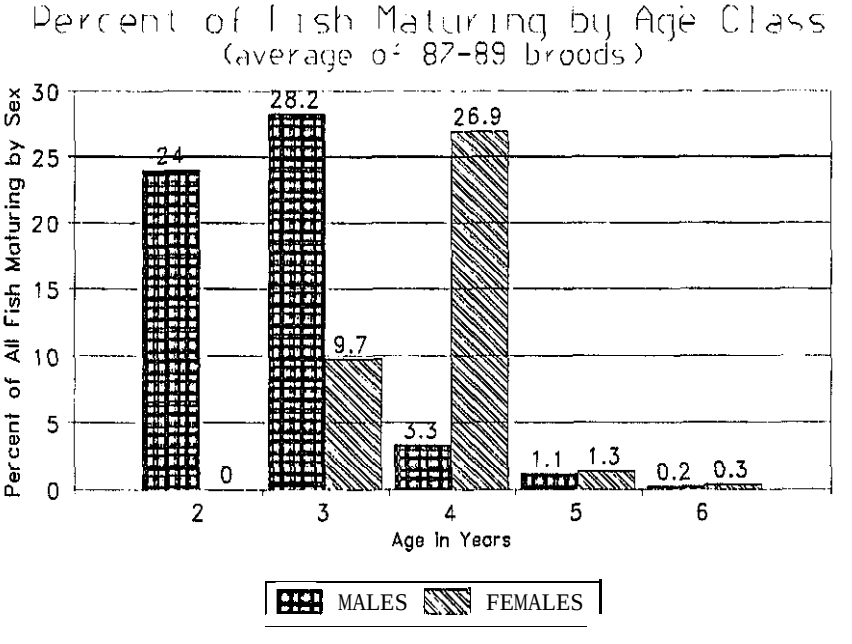



Figure 5. Percent of Sexually Maturing Fish by Age Class.

## Anadromous Broodstuck Program

White River spring chinook returning to Minter Creek are captured at the hatchery trapping facility. The mid-point of escapement (50%) has ranged from the week ending July 21st to the week ending August 10th (1983-1993 escapement records). The earliest hatchery recoveries occurred on June 7th in 1984. Returns have been as late as July 12 (1983). It should be noted that 1983 escapement was quite low relative to the ensuing years and may not adequately reflect overall run timing.

The final arrival time probably extends into October as evidenced by the final spring chinook arrival time of October 30th, (1986 brood). The opportunity to define final arrival time is constrained by operational necessities of handling coho adults. When adult coho begin to arrive, the opportunity to separate spring from fall chinook is lost. Before the arrival of coho, however, spring chinook can be handled individually and ponded separately from fall chinook even though their return timing overlaps.

Hatchery escapement records demonstrate that spring chinook have been identified and separated upon arrival as late as September 23rd (1986). That same year, spring chinook were subsequently recovered from among fall chinook adults as late as October 30th. Evidence demonstrates with certainty that spring chinook can arrive at least through the third week in September and probably well into October.

The adults are inoculated for BKD and Furunculosis (using erythromycin and liquimycin) and transported immediately by tanker to Hupp Springs hatchery. Thereafter they are held to maturation under the same conditions as adults from South Sound Net-pens.

## Performance of Anadromous Production

In an effort to understand the role cultural practices have on the survival of White River spring chinook yearlings released from Hupp Springs Hatchery the data base of coded-wire tag information was analyzed. The following areas were investigated: 1) effect of fish size at release (expressed in fish per pound) and date of release (expressed in days reared) on total survival (total catch plus total escapement), 2) effect of fish size and date of release (both as defined above) on escapement of adults to Minter Creek hatchery (adults being 3 years old or older).

Survival data were regressed using the actual percentages as the dependent variable and also the arcsine transformed equivalent. Since the relationships did not change, this discussion will concentrate only on those regressions using the actual percent survivals. Some comparisons were made graphically. Only yearling releases of spring chinook were included. The returns of some zero age releases have not been completely analyzed at this time. Brood years up to and including 1985 were used.

Size at Release--Size at release, expressed in fish per pound, was analyzed graphically and the results are presented in Figure 6. Survivals for a single size of fish at release were averaged before graphing. Results from this analysis suggest survival increases as fish grow toward 7 or 8 fish per pound, then decrease as fish grow above this level. This is somewhat counter intuitive. All things being equal, experience at other hatcheries would suggest survival should do no worse than remain constant as fish grow larger before release. Thus, there must be a factor masking the survivals of those larger fish.

As noted on this graph, those groups showing the highest survivals were from fish released in late April or early May. A regression analysis of percent survival on size (in fish per pound) at release found no relationship between variables. When escapement (as defined below) was regressed on size at release no relationship was detected either.

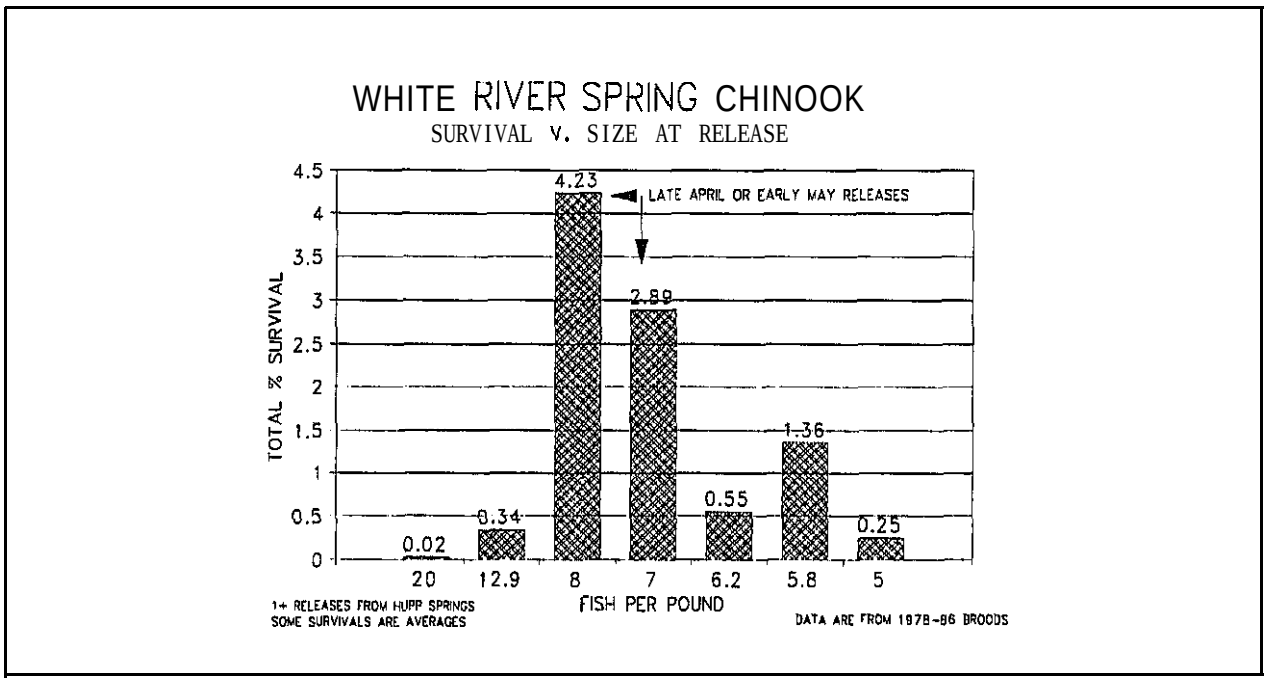



Figure 6. Survival of White River Spring Chinook v. size at release.

Date of Release--Survival v. date of release was graphically analyzed (Figure 7). Survivals for each release date were averaged before graphing.

A trend toward higher survival for later release dates was found and is very similar to that found at other Puget Sound spring chinook hatcheries (Performance of Spring and Summer Chinook Hatchery Programs in Puget Sound. A. Appleby, unpub. data. 600 Capitol Way N. Olympia, WA 98501). A regression analysis of data produced the following equation:

$$\text{Survival} = -35.5 + 0.0789 \times \text{time}$$

where time is expressed as days reared; df = 12; $r^2 = 0.48$; and ($p = 0.005$).

Escapement v. date of release was also analyzed using simple liner regression. Escapement is defied as percent return to the hatchery rack as 3 year old or older fish. The regression produced the following equation: Escapement= -7.46 + 0.0166 x date; df=12; $r^2$= .43; (p=.025). Based on this analysis, attempts at increasing survival by changing date of release should also increase escapement. Extreme caution is advised when analyzing this variable. Changes in harvest regulations could have a large influence on the results.

A multiple regression of date of release and size at release on survival reduced the amount of variation in the survival that can be explained by these two variables. This was expected given the corresponding $r^2$ values calculated for each regression.

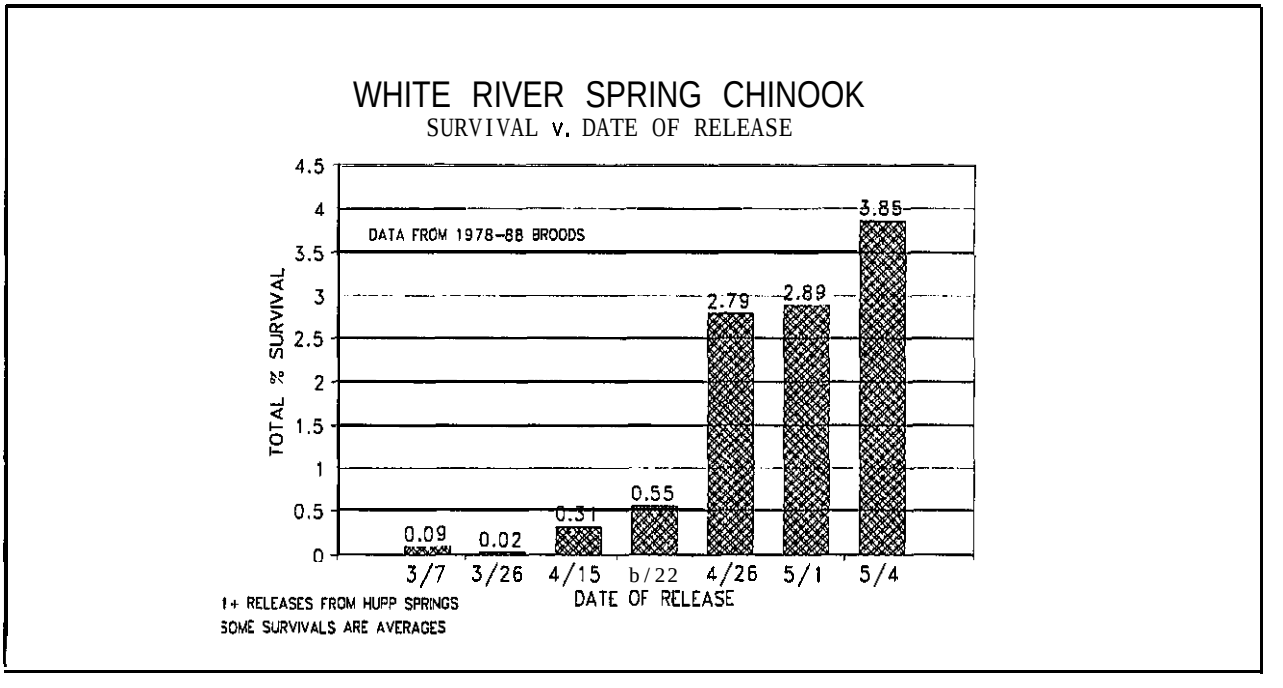
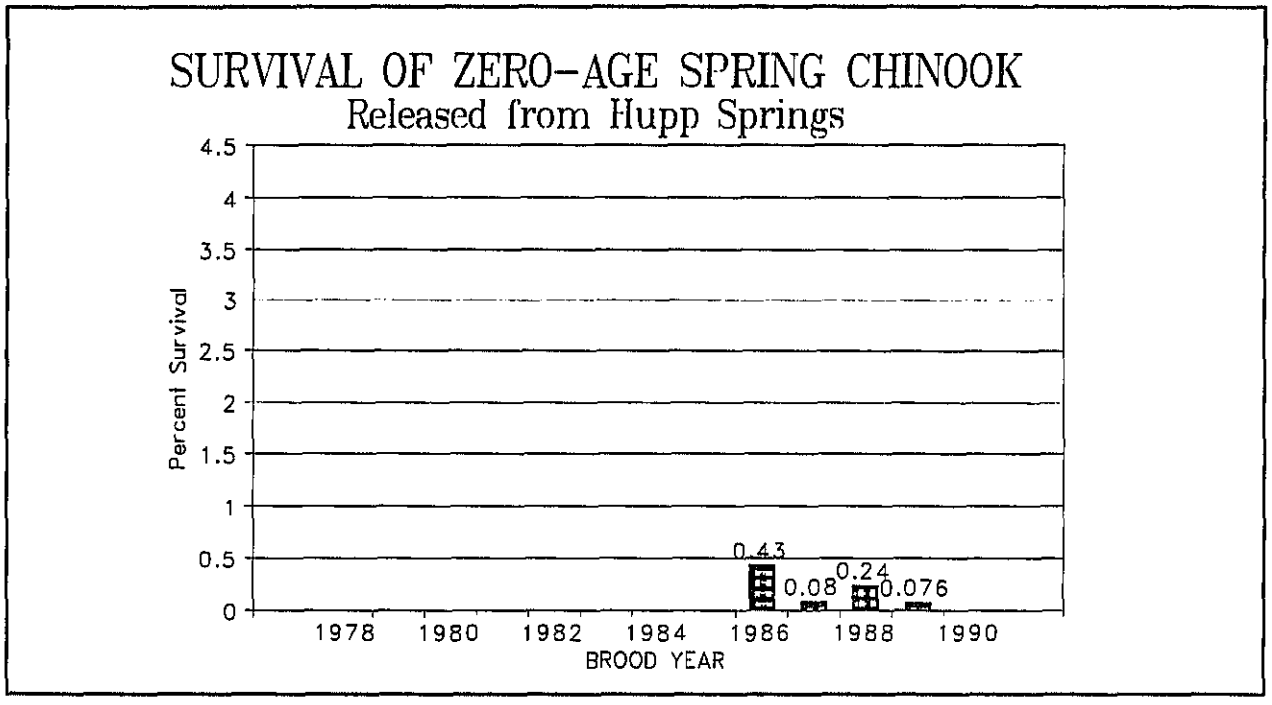



Figure 7. Survival of White River Spring Chinook v. date of release

**Conclusions--The** current program at Hupp Springs calls for production of a fiied number of yearling and zero-age White River spring chinook. The production of the zero-age component requires that we release the yearling group earlier than the current analyses would recommend. The past few brood years (1986 to current) releases have contained tagged groups of both yearling and zero-age fish. The returns of these marked fish will allow additional analyses. Preliminary estimates of the survival of these groups are presented in Figures 8 and 9. The two aspects which will continue to he examined are: 1) total percent survival and 2) percent survival as escapement. These analyses will allow adult and egg production to be maximized.




**Survival** of zero-age at release White River Spring Chinook.

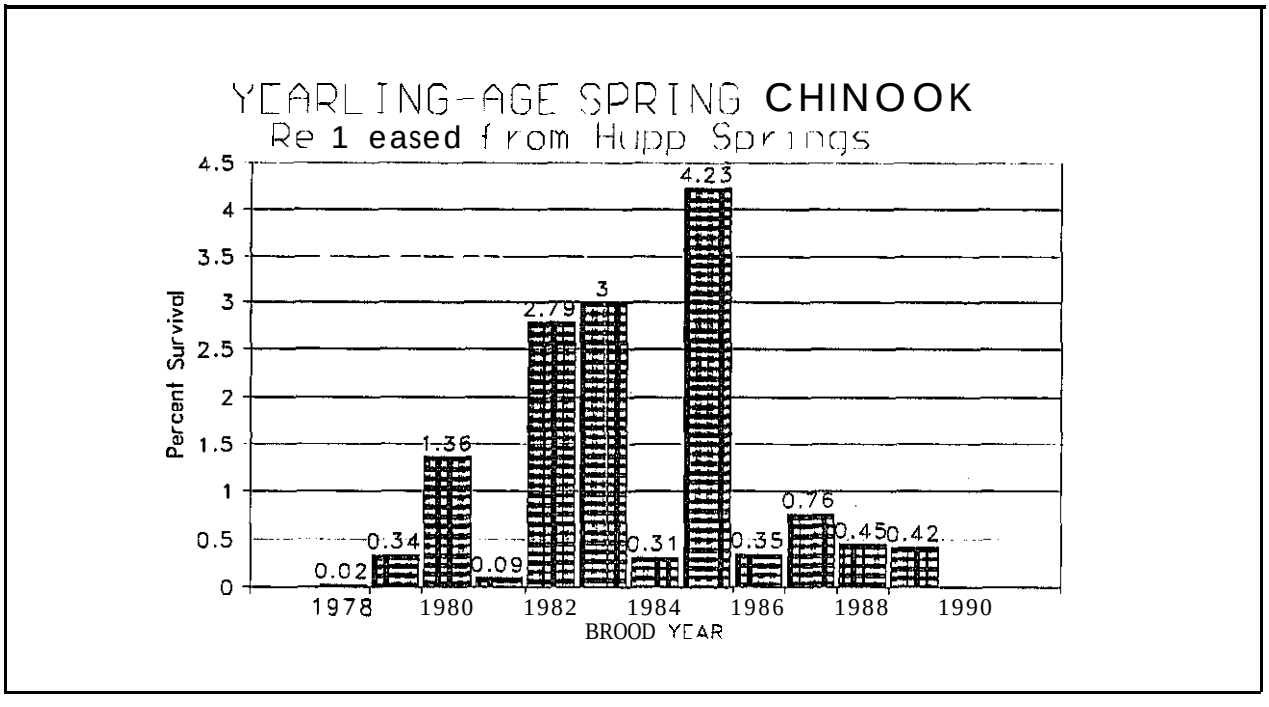



Figure 9. Survival of yearling age at release spring chinook.

## Summary of Current Spawning/Rearing Operations for Captive Brood and Anadromous Programs

At the time of spawning, anadromous females and captive brood females are held in separate ponds. The males of each group are together in one pond at Hupp Springs, with additional net-pen males held at Minter Creek. All anadromous fish are marked with a hole punched through the operculum at the time of transfer.

The eggs from each group are kept separate throughout spawning and incubation. All adult males and jacks are randomly selected for use in fertilizing eggs from both groups, producing the following possible matings:

1) anadromous male x anadromous female
2) captive brood male x captive brood female
3) anadromous male x captive brood female
4) captive brood male x anadromous female

A maximum of thirty females are taken at a time. The fish are assigned a number, the length is recorded, and fish are spawned. Each bucket containing a female's eggs is marked with her corresponding number. Both the fish and eggs are checked for any obvious abnormalities (gross kidney lesions, water hardened eggs, etc.). Snouts of anaclromous fish are removed and transferred to the coded wire tag recovery lab in Olympia for tag recovery and analysis (all juveniles in the anadromous program are 100% coded wire tagged prior to release). Eggs from adults with either coded wire tag uncertainties (lost or no-tags) or with tags identifying them as being from other stocks (most often fall chinook), are removed from the population prior to the eyed stage of development.

Historically, all tags were read at the time of spawning (prior to combining of gametes). This prevented the creation of fish of uncertain parentage. As the program has grown to its current level, the need for reasonable speed in handling large numbers of fish has outweighed the need for real time analysis.

After the females have been spawned, 30 males are selected and killed. Lengths and origin (anadromous or captive) are recorded with the corresponding female's number they are spawned with. A one to one male to female ratio is the goal on each spawning day, however, on some days a shortage of ripe males may alter this (the spawning protocol is currently under review). After fertilization, eggs are combined into 2 fish pools and are transferred to Minter Creek hatchery for water hardening (in iodine at a 1: 100 ratio) for one hour prior to being place into incubation units.

The eggs are treated daily with a formalin flush (10ml/ 1/2 gal/mm of inflow) to control fungus and soft-shell (soft-shell is more common in the captive brood eggs). Well water is used throughout the incubation period. After the eggs reached the "eyed" stage, they are "shocked," dead eggs are removed (picked) and the live eggs are placed in vertical incubators containing a rugose substrate. Tray loadings are approximately 7,000 eggs/tray.

Rearing Procedures

Hupp Springs--After hatching, fry are transferred directly from incubators to raceways at Hupp Springs, normally in December or January. Rearing procedures are routine and consistent with current technology and practices. The typical program at Hupp is to rear as many fish as possible to yearling smolts (currently about 80,000). An additional 250,000 zero-age smolts are reared and released as well.

During the early rearing phase fingerlings are held in raceways (10 x 100). Each release group receives a unique coded-wire tag (every fish is tagged) in order to monitor the performance of each rearing strategy (Figures 8,9). Each year 3,500 yearlings are transferred to the South Sound Net-pen complex in order to maintain the captive brood program. The remaining yearling smolts are released in April or May and the zero age fish are released in late May or early June.

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Appendix 6-A

ADULT PRODUCTION BY SOURCE AND EGG TAKE FOR WHITE RIVER SPRING CHINOOK:
(WDFW hatchery records unpubl. data. 600 Capitol Way N. Olympia, Wa 98501-1091).

| Brood Year | Source | Adults Spawned Males | Females | Eggs Taken |
|---|---|---|---|---|
| 1972' | White | 40 | 6 | 19,400 (plus hybrids) |
| 1973 | no program | | | |
| 1974 | White | 8 | 5 | 20,000 |
| 1975[2] | White / Puyallup | 22 | 13 | 49,300 |
| 1976' | White | 6 | 27 | 116,000 |
| 1977' | White | 10 | 7 | 40,000 (1,271 smolts to Manchester) |
| 1978 | White | 4 | 4 | 11,500 |
| 1979 | White | 12 | 18 | 81,500 |
| 1980 | white | 21 | 11 | 71,195 (744 smolts to Manchester) |
| 1981[5] | White/ Manchester | 4 | 18 | 81,118 (1,155 smolts to Manchester) |
| 1982[6] | White | 9 | 4 | 28,233 (1,090 smolts to Manchester) |
| | Manchester | 7 | 7 | (combined) |
| 1983' | White | 16 | 6 | 17,800 (500 smolts to Manchester) |
| | Minter | 12 | 8 | 19,900 (combined) |
| 1984 | White | 1 | 1 | 5,429 (530 smolts to Manchester |
| | Minter | 17 | 16 | 42,800 combined). |
| | Manchester | 28 | 16 | 27,200 |
| 1985 | Minter | 17 | 11 | 29,600 (1,857 smolts to Manchester) |
| | Manchester | 25 | 21 | 58,659 (1,648 smolts to Manchester) |
| 1986 | White | | 2 | 7,000 |
| | Minter | 47 | 43 | 122,850 |
| | Manchester | 0 | 60 | 103,700 |
| 1987 | Minter | 52 | 56 | 177,270 (3,485 smolts to SSNP) |
| | Manchester | 0 | 5 | 11,350 |
| 1988 | Minter | 88 | 68 | 206,603 (3,500 smolts to SSNP) |
| | Manchester | 0 | 9 | (combined) |
| 1989 | Minter | 117 | 219 | 689,000 (3,500 smolts to SSNP) |
| | Manchester | 0 | 50 | 98,000 |
| 1990 | Minter | 74 | 105 | 341,800 (3,500 smolts to SSNP) |
| | SSNP | 0 | 68 | 161,700 |
| 1991 | Minter | 94 | 95 | 348,500 (3,500 smolts to SSNP) |
| | SSNP[8] | 405 | 493 | 1,388,565 |

Appendix 6-A, Continued.

| | | | | | |
|---|---|---|---|---|---|
| 1992 | Minter | 113 | 139 | 451,000 | (3,500 smolts to SSNP) |
| | SSNP[8] | 494 | 477 | 1,212,500 | |
| | Muckleshoot' | 20 | | 24,000 | |
| 1993 | Minter | 172 | 181 | 579,700 | (expect 3,500 smolts to SSNP) |
| | SSNP[8] | 268 | 503 | 1,068,759 | |
| | Muckleshoot | 154 | | 234,544 | |

Adult numbers include only those used for spawning, for total adults/year, see text.
FOOTNOTES:

Several groups of hybrid chinook using White River spring chinook were released in other rivers.

[2] Adults returned to Puyallup Hatchery (most likelv from an unrecorded on-station release of 1971 brood White River spring release and were used for spawning.

[3] No coded-wire tags were applied because of bacterial kidney disease.

[4] Includes five adults that returned to Puyallup hatchery and were used for egg take. Smolts released into Minter Creek without imprinting. Coded-wire tags were not applied.

[5] A small group of smolts (670) resulted from eggs provided by NMFS from Manchester. These were tagged separately.

[6] Four adults,presumably males, were transferred back to Manchester.

[7] Jacks from Manchester consisted of 53 three-year-olds and 21 two-year-olds.

[8] Includes fish and resulting eggs transferred to the Muckleshoot hatchery on the White River.

[9] Returns from the 1989 brood released from Muckleshoot hatchery.

**1971--Fifty-two** adults were taken to the Department of Fisheries' Puyallup Hatchery to produce sperm for the hybridization program. Approximately 19,000 eggs were taken from seven females captured incidental to the male collection program. The exact history of those pure stock springs is clouded, but they were probably planted on-station at Puyallup Hatchery judging from an unexpected return to the hatchery in 1975. White River males were crossed with females from Green River, Issaquah, and Cowlitz and hybrids were planted in Soos Creek, the Hoko River, Whidbey Island net-pens, and the Sultan River. All the hybrid groups were coded-wire tagged.

**1972--Fifty-three** adults were captured to provide sperm for the hybrid program. Six females were taken incidently and produced 19,400 eggs. The pure stock progeny were released in Minter Creek and into the White River. Each group had a unique coded-wire tag. Several hybrid crosses were made with White River spring chinook males including: Cowlitz River spring chinook released in Finch Creek (Hood Canal), Hood Canal fall chinook released in Finch Creek, Hood Canal fall chinook released in the Hoko River, Hood Canal fall chinook released in Capitol Lake (Olympia), Green River fall chinook released in Issaquah Creek, and Issaquah fall chinook released in Issaquah Creek. All groups were uniquely identified with coded-wire tags.

**1973--** No program.

**1974--During** the spring of 1974, 29 adults and 9 jacks were transferred from the White River to Puyallup Hatchery. Eight males and eight females died prior to spawning. Five females were eventually spawned and approximately 20,000 eggs were taken. The fish were raised at the Department of Fisheries' Minter Creek Hatchery and 8,340 were planted as yearlings into the White River. The group was represented by a unique coded-wire tag (Table 1).

**1975--Twenty-one** adults were transferred from the Buckley trap to Puyallup Hatchery. Twenty-two spring chinook returned to Puyallup Hatchery, most likely the return from an unrecorded on-station plant of the 1971-brood White River springs. Six females and two males died during the holding period and thirteen females provided 49,300 eggs. The fingerlings were raised at Minter Creek Hatchery and 40,580 yearlings were planted in the White River represented by a unique coded-wire tag.

**1976--Forty-four** adult White River spring chinook were captured at the Buckley trap and transported to Puyallup Hatchery. Eight females and three males died during the holding period. Twenty-seven females were spawned, producing 116,500 eggs. After losing 36,000 eggs, probably from a disturbance by a visitor during the critical stage of development, 8 1,000 fry were transferred to Minter Creek Hatchery for rearing. A total of 47,525 yearlings were released in the White River. Tagging was precluded because fish were infected with bacterial kidney disease (BKD). Due to the disease problem a low survival rate was anticipated.

**1977--The** 1977-brood was made up of 14 adults from. the White River and 5 returns to Puyallup Hatchery. Adults were trapped at the Buckley site by sport fish staff from the Department of Fisheries and spawned at Puyallup Hatchery. The resulting fry were transferred to Skagit Hatchery with the expectations of reducing disease problems that had plagued the program in earlier years. Smolts were to be released into the White River.

Citing dismal performance from enhancement efforts within the White River, a decision was made within the Department of Fisheries to discontinue smolt plants into the White River in favor of releases into Minter Creek. The change occurred late in the rearing period for the 1977

brood juveniles and resulted in the reprogramming of this brood for release into Minter Creek. As a result of this decision, 20,461 yearlings were planted from Skagit Hatchery directly into Minter Creek in March 1979. During the same period of tune WDFW agreed to transfer 1,000 smolts to the National Marine Fisheries Service (NMFS) for captive saltwater rearing at their Manchester Bay site. The NMFS had, for several years, been involved in captive brood rearing programs for Atlantic salmon.

lY78--At the tune the 1978 brood adults were trapped (all13, 6 females, 7 males, from the White River), the production plan was still aimed at putting the progeny back into the White River. The detennination to release smolts in Minter Creek was made in 1979, after the trapping period for the 1978 brood stock was complete. Note that the 1977-brood fingerlings were still on hand at Skagit Hatchery and were affected by this decision as described above.

The adults were hauled to Puyallup Hatchery by WDF Salmon Culture Division personnel and, after spawning, eyed eggs were transferred to Skagit Hatchery as in 1977. Following the decision to plant smolts at Minter Creek, the 1978-brood fingerlings were transferred to the Department of Fisheries' Garrison Springs Hatchery and men to Minter Creek Hatchery where 4,220 smolts were planted after five months of rearing.

1979--This brood of adults was the first to be taken with the planned objective of releasing smolts into Minter Creek from WDFW's new Hupp Springs Hatchery. The hatchery has a spring-fed water supply with excellent water temperatures for holding and rearing spring chinook (10 C). During the period of adult collection at the Buckley trap (33 total, 21 females, 12 males) ), Hupp Springs was under construction. Garrison Springs Hatchery, a spring-fed facility located near the Hupp Springs site, was chosen as the interim adult holding and spawning site. The quality spring water was thought to be the best available in the project area.

After spawning the unfertilized eggs were transferred to Minter Creek Hatchery where they were fertilized, incubated and hatched. The fingerlings were transferred to Hupp Springs Hatchery for final rearing and 48,575 smolts were released into Minter Creek in March, 1981.

1980--Adults were trapped at the Buckley site (42 total, 21 females, 21 males) and delivered by Corps of Engineers personnel to Garrison Springs Hatchery Eggs were sent to Minter Creek Hatchery for incubation and hatching. Fry were started at Minter Creek and fingerlings were sent to Hupp Springs in March, 1981. Tbese fish were reared to yearlings and 19,600 smolts were released in March, 1982. The NMFS received 744 smolts for rearing as captive broodstock.

1981--Adults from the Buckley trap (22 total, 19 females. 3 males) were held at Garrison Springs Hatchery. The eggs were incubated and hatched at Garrison Springs rather than Minter Creek Hatchery. The NMFS program provided the first group of eyed eggs produced from the saltwater captive brood stock program, derived from the 1977-brood. All fry were transferred from Garrison Springs to Hupp Springs. NMFS received 1.155 yearling smolts and 37,300 yearlings were released into Minter Creek.

1982--Beginning with the 1982 brood year, adult holding was shifted to Hupp Springs Hatchery. representing the last facility change leading to the present production strategy. Also of note, in 1982, the first transfer of mature adults from the saltwater captive brooclstock program al

Manchester (21 total, 13 females, 8 males) to the freshwater holding site at Hupp Springs was complete. Adult spring chinook from the White River were again captured at the Buckley (13 total, 3 females, 10 males) trapping facility and transported to Hupp Springs.

Eggs were incubated and hatched at Minter Creek Hatchery. Fry were returned to Hupp Springs for yearling smolt release. NMFS received 1,090 smolts and 21,000 were released into Minter Creek.

1983--Broodstock came from three complimentary components of the program including the first significant 4 year old returns from the Miter creek release program (28 total, 9 females, 18 males) and 3 year old brood stock from the 1980 brood at Manchester (74 total, all jacks). This was also the last major contribution from the Buckley trapping facility (24 total, 6 females, 18 males). All adults were spawned at Hupp Springs and the eggs were incubated and hatched at Minter Creek Hatchery. Fry were returned to Hupp Springs and released as yearling smolts in May, 1985. WDFW released 34,500 smolts and 500 smolts were provided to NMFS for seawater rearing.

1984--Adult brood production was provided from previous Hupp Springs on-site releases (45 total, 21 females, 24 males) and from the Manchester brood program (65 total, 20 females, 45 males). There was also a small return to the White River (7 total, 5 females, 2 males). As in recent years, adults were held and spawned at Hupp Springs and eggs were moved to Minter Creek Hatchery. The fry were returned to Hupp Springs and reared for yearling release. There were 47,300 smolts released into Minter Creek in June 1986.

1985--Two brood sources provided the 1985 egg take, Minter Creek returns (35 total, 12 females, 23 mates) and Manchester saltwater brood (66 total, 32 females, 34 males). Transfer of adults captured at the Buckley facility was discontinued after a disagreement between WDFW and the Puyallup and Muckleshoot tribes concerning the removal of spring chinook from the White River and the protocol for reinstatement of the fish. Manchester received 3,505 smolts and 45,986 yearlings smolts were released into Minter Creek on May 1, 1987.

1986--Adult broodstock returned as a result of on-station releases at Minter Creek (186 total, 70 females, 114 mates) and from the saltwater captive brood program at Manchester (100 total, 73 females, 27 males). Additional adults were provided from trapping operations initiated by the Muckleshoot tribe at Buckley (3 total, 3 females, 0 males). The egg take from all sources was 236,350.

An agreement between the Muckleshoot tribe and the Department of Fisheries provided for return of a number of progeny from this brood to the White River system and 5,296 fingerlings were planted in the White River on May 15. 1987. The yearling rearing capacity at Hupp Springs (80,000) was exceeded for the first time. triggering a release of 91,825 zero-age smolts on May 19, 1987. An additional plant of 1,100 occurred on July 10, 1987. There were 86.755 fish planted as yearling smolts on April 22. 1988.

1987--Broodstock came from Manchester (19 total, 7 females, 12 males), which produced about 11,500 eggs) and from returns to Minter Creek (144 total, 68 females, 77 males), which produced about 177,270 eggs. The total number of eggs was 188,620. Of the resulting smolts. X3.074 were released as yearlings in 1989, and 84,250 were released as zero-age smolts in 1988. This brood year marked the first year smolts (3,500) were transferred to the South Sound Net-pen

Complex at Squaxin Is. (near Olympia) rather than Manchester for captive brood rearing.

**1988--Broodstock** came from returns to Minter Creek (504 total, 77 females, 426 males) and Manchester (9 total, 9 females, 0 males). The number of eggs taken totaled 206,850. Of the resulting smolts 89,737 were released as yearlings, 3,500 were transferred to the South Sound Net-pens (spring of 1990), and 95,524 were released as zero-age smolts in June, 1989.

**1989--Broodstock** came from returns to Minter Creek (355 total, 232 females, 123 males) and Manchester (86 total, 52 females, 34 males) from Manchester (all 4 year olds). The number of eggs taken totaled 98,000 Manchester plus 689,000 Minter = 787,000. Of the resulting smolts, 91,172 were released as yearlings, 3,500 were transferred to the South Sound Net-pens. Also, 384,500 fingerlings were transferred to the new Muckleshoot Hatchery on the White River, and 249,773 zero-age smolts were released into Minter Creek in May, 1990. All the Manchester eggs were transferred to the White River Hatchery.

**1990--Broodstock** came from returns to Minter Creek (242 total, 116 females, 119 males) and from the South Sound Net-pens (580 total, 68 females, 5 12 males, all 3 year olds). The number of eggs taken totaled 161,700 SSNP plus 341,800 Mimer = 503,500. Of the resulting smolts, 81,023 were released as yearlings, and 3,500 were transferred to the South Sound Net-pens (both in the spring of 1992). Also, 16,300 Mimer origin plus 125,000 SSNP origin (total 141,300 eggs/fingerlings) were transferred to the Muckleshoot Hatchery on the White River and 189,800 zero-age smolts were released into Minter Ck. in June, 1991.

**1991--Broodstock** came from two sources; returns to Minter Creek (236 total, 113 females, 119 males,) and the South Sound Net-pens (974 total, 532 females, 442 males; 3 and 4 year olds). The number of eggs taken totaled 1,581,400 (1,232,900 SSNP plus 348,500 Minter). Of the resulting smolts, 84,493 were released as yearlings and 3,500 were transferred to South Sound Net-pens. Also, 13,400 Minter origin plus 193,000 SSNP (total 206,400) were transferred to the Muckleshoot Hatchery on the White River as eggs or fry and 266,030 were released as zero-age smolts into Minter Ck. in June 15, 1992.

**1992--Broodstock** came from three sources; returns to Minter Creek ((463 total, 179 females, 286 males) and SSNP (985 total, 477 females, 494 males, 3,4 and 5 year olds). The number of eggs taken totaled 1,504,000 (1,052,500 SSNP plus 45 1,500 Minter). An additional 24,000 eggs were taken from a small number of 3 year old chinook which returned to the Muckleshoot hatchery. Of the resulting smolts, about 90,000 are being held for yearling release in 1994 and 3,500 will be transferred to SSNP. Also 112,000 Minter origin plus 447,500 SSNP origin (total 559,500 eggs/fish) were transferrecl to the muckleshoot Hatchery on the White River and 85,330 Minter origin and 168,664 SSNP origin (total 253,994) zero-age smolts were released into Minter Ck. in 1993.

**1993--Broodstock** came from three sources: returns to Minter Creek (332 total, 177 female, 155 males,) and SSNP (742 total, 485 females. 257 males, 3, 4, and 5-year-olds). Number of eggs taken was 1,648,459 (1,068,759 SSNP plus 579.700 Minter). An additional 234,544 eggs were taken from 3 and 4-year-old fish that returned to the Muckleshoot hatchet-y. Of the resulting smolts, about 90,000 will be held for yearling release and 3,500 will he transferred to South Sound Net-pens (spring 1995). Also, 179,600 Minter origin plus 295,700 SSNP origin (total 475.300 eggs/fish) have been transferred to the Muckleshoot Hatchery. A release of about 250.000 (combination of Minter and SSNP) zero-age smolts was conducted in June 1994 into Minter Creek.