ALASKA SOCKEYE SALMON CULTURE MANUAL

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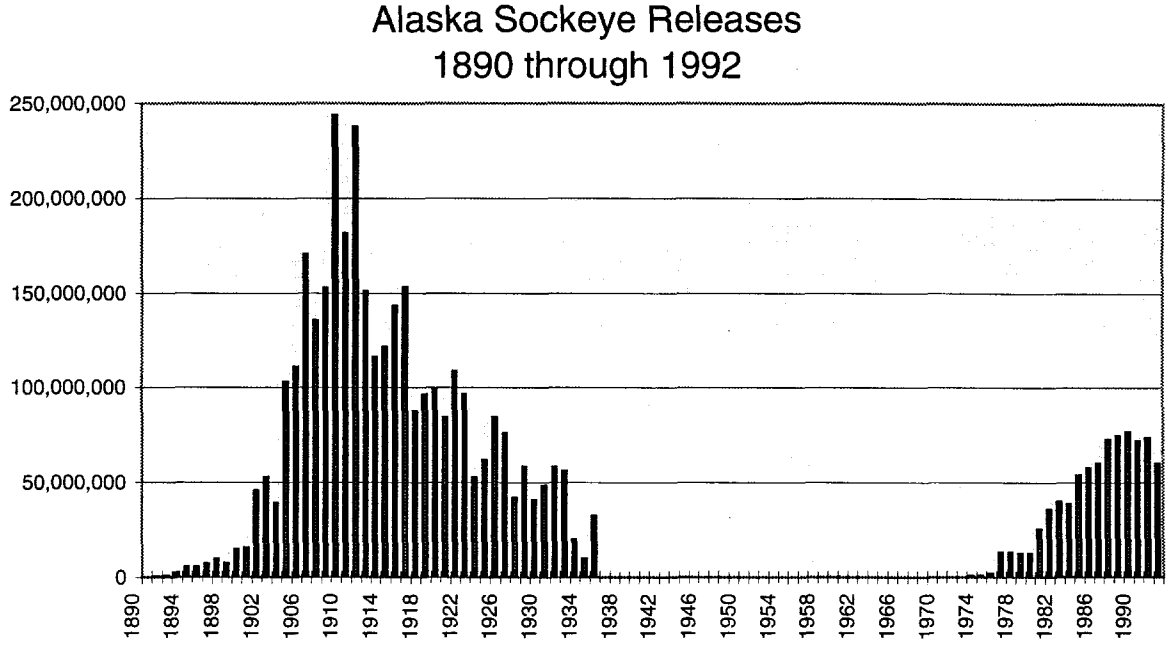
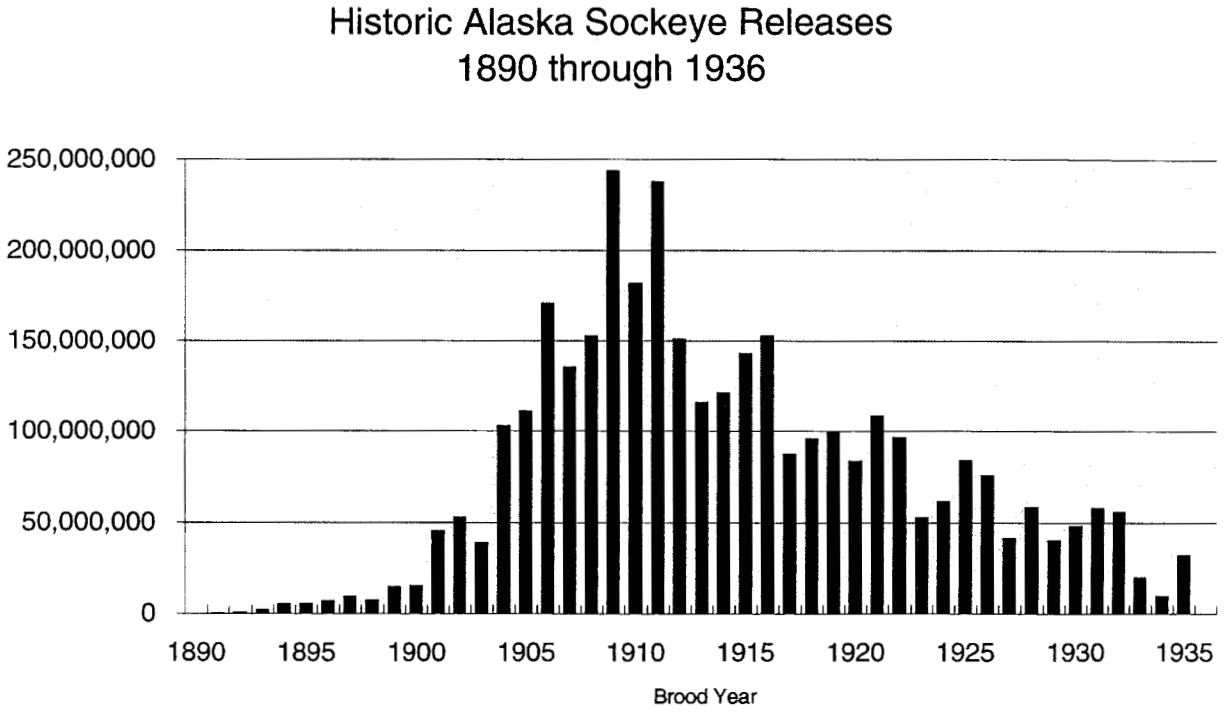


Introduction

The intent of this manual is to review the "Sockeye Salmon Culture Policy" of the Alaska Department of Fish and Game (ADF&G) and to discuss, in detail, the application of the policy together with technical fish culture procedures required for the successful culture of sockeye salmon in hatcheries. These procedures, which have been used successfully in ADF&G hatcheries for a number of years, build upon previously established general fish culture practices and include specific requirements for process water quality, disinfection and compartmentalization during incubation and rearing. This manual is directed towards journeymen fish culturists and biologists involved in sockeye culture and assumes that users have a working knowledge of basic salmon culture procedures.

Comparatively, the culture of sockeye salmon is not difficult. In fact, fish culturists find sockeye salmon to be quite adaptive to the coldwater incubation and rearing conditions found in most Alaskan hatcheries. Unlike other species of salmon, all anadromous stocks of sockeye in Alaska are reservoirs of infectious hematopoietic necrosis virus (IHNV). In order to reduce fish losses caused by IHNV, successful culture of sockeye salmon has relied on the use of specific fish culture procedures outlined in the ADF&G's *Regulation Changes, Policies and Guidelines for Alaska Fish and Shellfish Health and Disease Control, 1988.*



Background

Infectious hematopoietic necrosis virus (IHNV) is a rhabdovirus infecting certain salmonid fishes and may have been the causative agent behind reports of disease in sockeye salmon in the Pacific Northwest as early as 1925 and again in 1946. Additional reports of epizootics in sockeye salmon followed in the early and mid 1950s. In 1965 the advent of fish tissue culture allowed isolation of a virus from diseased sockeye salmon fry which later was identified as IHNV. Rhabdoviruses are bullet-shaped of which the most noted member is the rabies virus in warm blooded vertebrates. As a group, these viruses have a fragile lipid outer envelope that confers viability making them very susceptible to environmental extremes such as heat, drying and chemical disinfection. Since the mid 1960s, strains of IHNV have caused epizootics in chinook, chum and Atlantic salmon, rainbow trout, cutthroat trout and steelhead in North America. The virus was also inadvertently exported to Japan via sockeye salmon eggs from Alaska and subsequently caused epizootics in chum salmon and two species of landlocked salmon (amago and yamame) which occur only in Japan. The virus typically causes high mortality in juvenile fish by destroying major organs, especially the blood forming tissues of the kidney. Hence, the name of the disease describing this condition is infectious hematopoietic necrosis or IHN. Earlier synonyms for IHN are Columbia River sockeye disease and Sacramento River chinook disease.

The causative virus is transmitted horizontally (fish to fish contact) and growing evidence also supports vertical transmission from parent to progeny via the egg. The biological characteristics of IHNV facilitate viral destruction and containment through adequate disinfection and compartmentalization. Generally, IHNV is only isolated from juvenile fish undergoing mortality due to IHN disease or some other stressor and from mature spawning or post-spawned adult fish. It is unknown whether a true virus carrier state exists in juvenile fish that survive infection to become spawning adults. However, in the case of Alaskan sockeye culture, several unexplained occurrences of IHN disease could be circumstantial evidence for a carrier state. At this point, it is important to note that while all the authors agree with the procedures in this manual and their importance, they do not necessarily agree on the nature of the dynamic relationship between the virus and its host. Regardless, our culture practices and disease policies are based, in part, on the assumption that a carrier state exists.

Strains of IHNV that infect chinook and chum salmon, steelhead and rainbow trout in the "Lower 48" do not appear to occur naturally in Alaska where only the sockeye strain has been detected. However, because the sockeye strain of virus is capable of infecting and possibly adapting to susceptible non-sockeye species, disease policies pertaining to IHNV in Alaska are stringently designed to minimize this possibility. Currently, there is no commercially available vac-

cine or therapeutic method to control IHNV. Avoidance procedures and containment of viral infections in the hatchery appear to be the most effective measures for controlling losses.

Additional information about IHNV in Alaskan sockeye culture can be found in Meyers et al. (1990) and a complete review of IHNV can be found in Wolf (1988).

Sockeye Salmon Culture in Alaska

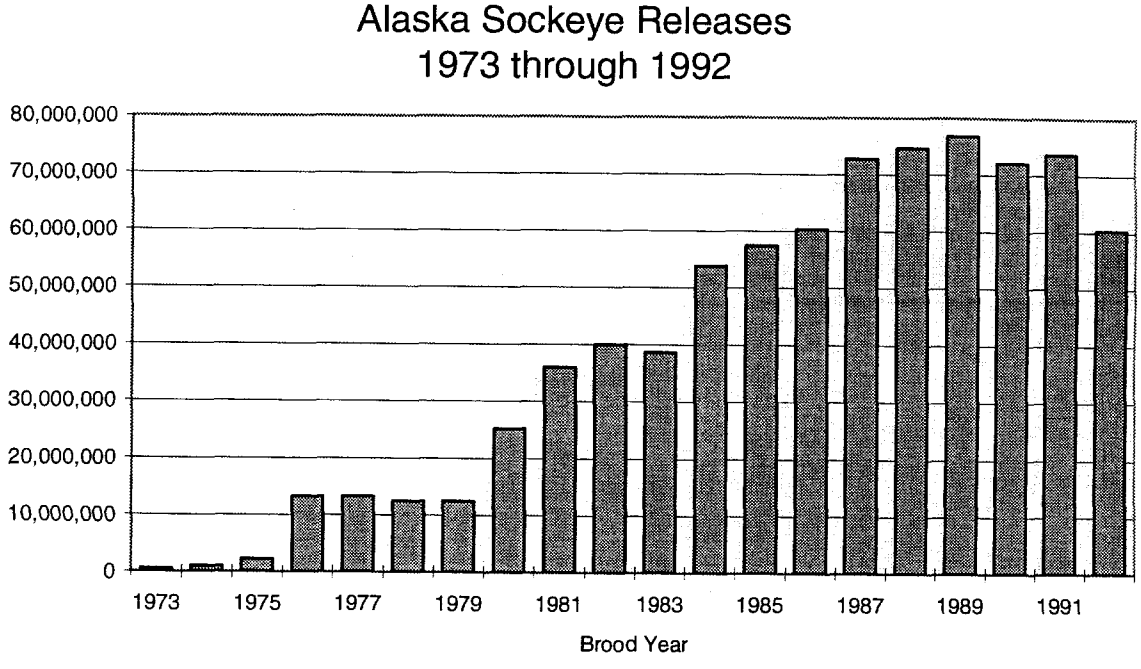
Sockeye salmon have been cultured in Alaskan hatcheries for over 100 years (Roppel 1982). The first sockeye salmon hatchery was built by cannery operators at the mouth of the Karluk River in 1891. Hatchery production of sockeye salmon continued to expand in the early 1900s as native stocks were subjected to increased commercial fishing pressure. Early production peaked in 1911 when 270 million eggs were taken statewide. Production declined steadily from 1911 until 1936 when all sockeye salmon hatcheries around the state closed.

ADF&G initiated a hatchery-based sockeye production program in the early 1970s to support a number of enhancement and rehabilitation projects around the state. Unfortunately, conventional culture methods continued to be complicated by IHNV. In 1978 and 1979 nearly 40% of all sockeye incubated in state hatcheries were lost due to IHNV. The importance of hatchery produced sockeye fry to the enhancement program compelled the Fisheries Rehabilitation, Enhancement and Development (FRED) Division staff to experiment with different fish culture procedures designed to "farm around" IHNV. The only effective methods to control IHNV were avoidance of the virus and compartmentalization of small units of fish in the hatchery. Consequently, procedures had to be developed to reduce the catastrophic mortality common in hatcheries during the early life history of this species. Application of these procedures resulted in widespread, successful incubation of sockeye salmon and were formally adopted into ADF&G's "Sockeye Salmon Culture Policy Statement", which was implemented in 1981.

The sockeye culture program has rapidly expanded in recent years; between 1987 and 1992 an average of 106.7 million sockeye eggs were taken annually at Alaskan hatcheries. Since implementation of the Sockeye Culture Policy, losses due to IHNV have been greatly reduced, averaging 4% annually over the last 10 years. However, in the spring of 1993 IHNV occurred at several sockeye salmon hatcheries causing significant overall fish losses exceeding 14% of the total statewide sockeye production. In one facility, 58% of the sockeye salmon being cultured either died from IHN disease or were destroyed due to detection of the virus. A critique of statewide hatchery losses revealed certain factors common to several facilities that have played a role in the high fish losses; some of the basic procedures for sockeye culture were either not

being employed or were being compromised during application. Hence, the 1993 experience with IHNV in Alaska has prompted the drafting of this manual.

The methods that were developed to control fish losses from IHNV in hatcheries are quite straightforward as discussed in the "Concepts" section of this manual. The concepts were designed around the principle of risk management in that the procedures employed reduce the risk of large scale production losses. It could be argued that this approach to "culturing around" the virus is much more efficacious than developing a magic vaccine. Realistically, it must be emphasized that these procedures do not and cannot eliminate IHNV from the hatchery environment; quite simply, IHNV is present in all anadromous stocks of sockeye salmon at all life stages. It is certain that a small percentage of cultured sockeye salmon will be lost to IHNV on an annual basis. These losses can be minimized but should be considered a cost of doing business in sockeye culture.



Concepts of the Alaskan Sockeye Culture Policy

Prior to 1980, IHNV was a formidable obstacle to successful sockeye salmon culture in Alaska. At that time, the ADF&G, FRED Division devised a policy to minimize the negative effects of this virus on sockeye culture. This policy includes procedures for taking and incubating eggs and rearing of fry and smolts that are based upon known and suspected biological parameters of the virus-host relationship. These criteria, which are common sense approaches, have allowed Alaskan hatcheries to "farm around" sockeye salmon culture problems relating to IHNV in at least 10 different facilities. These criteria involve three basic cornerstones:

A) A VIRUS-FREE WATER SUPPLY

B) SPECIFIC DISINFECTION PROCEDURES

C) CONTAINMENT BY COMPARTMENTALIZATION AND ISOLATION

The specific criteria of these cornerstones in the sockeye policy and their purposes include:

Water Supply

• IHNV-free water supply via wells, depurated or fishless water is required for all phases of sockeye culture. Depuration of hatchery effluent may be necessary to maintain a virus-free water supply for saltwater rearing containers downstream of the outfall (prevents horizontal exposure to the virus).

For some hatcheries the water supplies contain wild salmonids but no sockeye or IHNV. So far in Alaska, IHNV has rarely been found to spontaneously occur in non-sockeye species. Over 100 anadromous sockeye stocks have been examined in Alaska and all at some time have had detectable IHNV.

Disinfection

• Stringent disinfection of utensils, facilities, external surfaces of brood stock, etc. during and after the egg take. Use of disinfectant footbaths, steam and separate gear (prevents external contamination by virus).

• Separate water hardening of each family of eggs in 100 ppm iodophor for 60 minutes with replenishment and adequate mixing (reduces the potential virus contamination of other eggs by high titered gamete fluids; kills virus on the surface of the egg and in the perivitelline space; allows for more adequate disinfection of the smaller egg mass).

Containment

• Separate fertilization of eggs from each female using 1 or 2 males (reduces the potential virus contamination of other eggs by high virus titered seminal fluid).

• Eggs are pooled into separate upwelling Kitoi box incubators or stacks of NOPAD trays at densities of 250,000 to 300,000 eggs (80 to 100 females). Each incubator or stack is considered an expendable unit.

Smaller expendable units have been used, depending upon the number of eggs taken. Stacks usually contain a particular lot of eggs designated by day of egg take and are usually five trays high. Using the modified egg take procedures, an average of 2 million sockeye eggs per day can be taken with crews of 5 to 7 people with total egg takes reaching 20 to 30 million at certain facilities.

• Each sockeye stock physically isolated by barriers and disinfectant footbaths from any non-sockeye species as well as other sockeye stocks (protects other IHNV-susceptible species as well as separate sockeye stocks).

• Rearing containers for fry and fingerlings adequately separated by distance or physical barrier to maintain containment by compartmentalization and isolation. Reared fry are pooled in start tanks or raceways according to discrete production segments such as common incubator stacks or day/time of egg take.

Virus prevalence and/or titer can vary with each egg take or IHNV can occur in a group of fish due to handling during a particular egg take. Thus, fish are grouped according to common IHNV risk.

• Adequate exclusion of birds and other predators from outside rearing containers (maintains effective isolation through compartmentalization).

As an example, successful IHNV containment by limiting bird predation has been achieved by Clear Springs Trout Hatcheries in Idaho.

• Immediate destruction of suspected or confirmed IHNV-positive incubators or raceways of fish and disinfection regardless of mortality level.

This accomplishes three objectives:

1. Protection from virus exposure for remaining lots or stocks of fish on site.

2. Prevention of release of IHNV-positive fish into a watershed where other juvenile sockeye or salmonid species may be impacted by virus exposure.

3. Reduction in the number of returning adult fish carriers of IHNV which, depending upon the circumstances, should not increase virus prevalence in stocks returning to the release site, be it a hatchery or remote watershed.

In most cases eventual high mortality will occur after the detection of IHNV in juvenile fish. However, this mortality may take longer to occur in larger older juveniles at colder water temperatures.

Other Considerations

• Fry released unfed or after short-term rearing (4-6 weeks).

Smolt programs underway at Main Bay, Beaver Falls, Kitoi Bay, Snettisham, Crooked Creek, Trail Lakes and Eklutna hatcheries are a major deviation of the earlier concern about transporting healthy sockeye out of the hatchery as soon as possible. Long-term culture of sockeye to smolts can increase the risk of IHNV occurring at a facility due to later expression of virus carrier fish and loss of control in containment if unprotected outside raceways or saltwater rearing pens are used. It is true that most IHNV problems in Alaska have occurred in yolk-sac or swim-up sockeye fry. However, this was before smolt production and does not mean that IHNV cannot occur in older fry, presmolts or smolts. Major mortality of large smolts in saltwater from IHNV is possible and occurred in Alaska in 1993. In many cases, individual incubators or rearing containers of sockeye tested virus-negative only to become virus-positive later during prerelease inspections.

• Stress of any kind can contribute to IHNV outbreaks as has been observed several times with sockeye in Alaska.

Additional Disinfection Procedures

Specific disinfection procedures have evolved that appear to reduce the potential of IHNV exposure and subsequent outbreak of the disease during the culture process. Rinsing eggs in iodophor solution immediately after fertilization can eliminate virus particles present in ovarian fluids and remove organic material. Flushing eyed eggs with iodophor after picking and prior to reseeding into incubators can reduce or eliminate virus particles that may have been released by dead eggs or survived the initial disinfection process. Periodic formalin flushes to control fungus may achieve the same result.

Importance of Containment

Virus-free water and adequate disinfection are very necessary rules for successful sockeye culture but the containment precautions can still allow for success when these other two criteria fail to prevent IHNV from occurring in

one or multiple incubators or raceways. Such occurrences of IHNV are likely due to vertical transmission, a purely random event, which increases with increasing proportions of high titered parent fish and increasing numbers of eggs taken.

If more containment strategies are used, less fish inventory will be lost when IHNV occurs. However, containment can be interpreted in many ways or degrees and is often where major effort and cost is involved to start or expand a sockeye program. Thus, it is easy to become complacent on containment procedures to save money and labor.

In most cases where IHNV has been a problem, lack of adequate containment has been a major factor in the outcome of these events. These inadequacies have included; insufficient physical separation of freshwater raceways, lack of predator bird control, failure to contain IHNV in hatchery effluent passing over saltwater net pens of fish, inability to contain the virus in saltwater net pens (whether this is possible remains to be seen), and procrastination in killing fish infected with IHNV. Remedies to all of these shortcomings are difficult and expensive but require attention for future success.

Containment procedures for IHNV are just as important in the sockeye culture policy today as they were at the inception of the program. Hence, complacency on this or the other two cornerstones for sockeye culture can and will result in significant fish losses; maybe not this year or next, but at some point it will happen.

Horizontal Transmission

Clearly, the major mode of freshwater transmission demonstrated for IHNV in sockeye salmon and other salmonids has been by horizontal contact in which virions from infected live or dead fish are either waterborne or adsorbed onto fomites such as organic sediments (Wingfield and Chan 1970, Mulcahy et al. 1983, Mulcahy and Pascho 1986, LaPatra et al. 1987, Yamamoto et al. 1989).

IHNV can remain viable for months in the sediments of freshwater rivers (Wedemeyer et al. 1978, Mulcahy et al. 1983). The viability of IHNV in estuarine and saltwater is more short-lived at 27 and 22 days, respectively (Toranzo and Hetrick 1982). Nonetheless, in saltwater net pen situations IHNV has been transmitted to both Atlantic and sockeye salmon presmolts by cohabitation with virus infected fish and/or waterborne exposure (Traxler et al. 1993). More recently an IHNV epizootic in production saltwater net pens of sockeye smolts at Main Bay Hatchery in Alaska was documented in 1993.

Vertical Transmission

Vertical transmission of IHNV, i.e. within the egg, has been demonstrated by the isolation of virus from the contents of unfertilized and fertile sockeye salmon eggs incubated in a virus-free water supply (Mulcahy and Pascho 1985). Other strong circumstantial evidence that is supportive of vertical transmission includes: strong adsorption of IHNV particles to salmonid sperm, suggesting one mode of entry into the egg (Mulcahy and Pascho 1984); and documented IHN disease or detection of virus in Alaskan sockeye salmon hatcheries following rigorous egg disinfection, isolation and incubation on virus-free water supplies (Meyers et al. 1990).

However, the concept of vertical transmission of IHNV within the egg still remains controversial. Laboratory investigations have been unable to show unequivocally that vertical transmission occurs in either chinook salmon (Engelking et al. 1991) or steelhead trout (LaPatra et al. 1991). Japanese investigators also reported that the egg contents of uneyed masu and chum salmon may inactivate IHNV particles making vertical transmission in these species unlikely (Yoshimizu et al. 1989). Conflicting results were obtained by Burke and Mulcahy (1983) in which IHNV viabilities in egg yolk from sockeye salmon and in ovarian fluid from rainbow trout were comparably high, such that virus was not inactivated by egg yolk from sockeye salmon.

Collectively, these reports emphasize the conclusions of Mulcahy and Pascho (1985) that apparent vertical transmission of IHNV as manifested by mortality or detectable virus is rare and does not occur with the spawning of every infected female (or male) fish. Also, when such vertical transmission occurs within a single family of eggs, a lethal dose of the virus is not passed on to every egg. The same investigators also mentioned the concept of vertical transmission of sublethal levels of virus which, as we surmise, could go virtually undetected in most instances even with rigorous screening (blind passages) of considerable sample numbers. The infrequency of lethal instances of suspected vertical transmission makes confirmatory laboratory studies very difficult in that millions of eggs from hundreds to possibly thousands of parent fish may be necessary to demonstrate a few instances of progeny infected by parent fish. Examination of such numbers of parent fish and resulting progeny are not feasible in a laboratory or even larger scale hatchery experiments.

The point, however, is not to require unequivocal scientific evidence prior to implementing procedures that will protect the production lots of sockeye salmon in a facility from vertical transmission of IHNV. It is operationally simple and efficient to accept the notion that both horizontal and vertical transmission occurs and take the appropriate steps to avoid IHNV outbreaks. Why put your hatchery production at risk over the nuances of a scientific debate when it is so easy to achieve a high degree of protection from both routes of transmission simultaneously by following the procedures outlined in this manual?

Egg Take Procedures

Brood Stock

In order to successfully culture sockeye salmon, a project plan listing goals, objectives and procedures must be developed with input from various program disciplines including pathology, limnology, genetics, and management as well as fish culture staff. In choosing a brood stock for a particular program careful consideration should to be given to the following: geographic location in proximity to the project, run timing and stock strength, disease history, type of spawners (inlet, outlet, shore, etc.) and the migratory pattern if known.

Once a brood stock has been chosen, appropriate permits, such as the Fish Transport Permit (FTP), must be obtained. Remember, an FTP application can not be used to initiate a project. An approved project plan must be submitted with a fish transport permit application. This needs to be done months ahead of any proposed egg take.

Select an egg collection site as close to a natural spawning area as possible to eliminate having to transport adults. Also, a survey should be done to find the closest source of virus-free water. If none can be found, then clean water will need to be transported in. Another thing to consider are traffic patterns, both human and animal. For example, try to avoid gravel bars heavily used by bears feeding on spawning salmon. Consider how carcasses will be disposed of after spawning and what would likely happen at the site in case of heavy rains (floods).

Adults may be captured by a number of different methods such as weirs, seines, gill nets, hook and line and electric shocker. Whatever method is used, the goal is to reduce stress on the fish as much as possible. Brood stock should be collected over as much of the run as possible in order to maintain genetic variability (refer to ADF&G, *Genetic Policy*, 1985). From experience we have learned that collecting "bright" fish and holding them in some type of containment results in high mortality. Fish naturally mature at a faster rate when not in captivity. Every attempt should be made to collect only fish that are "ripe" or "near ripe", preferably within four to five days of spawning.

Fish held for spawning should not be overcrowded, especially if they are being held for more than two to three days. Dead and moribund sockeye inside holding units should be removed daily and disposed of at a distance downstream or removed from the egg take site. The same applies to the water source the fish are being held in. If there are naturally spawning fish upstream, carcasses should be removed on a daily basis if feasible. Standard procedures call for the water temperature to be monitored daily. Fish mature slower in cold water, but can be held at higher densities.

Spawning

The egg-take crew should be divided into working groups. Each group works at a specific set of tasks in a general area throughout an egg collection day. For example, the people sorting and killing fish work in one area and do not go into the area where disinfected eggs are handled. These procedures, along with disinfection measures employed during the spawning process, reduce the risk of transferring virus from brood stock to fertilized eggs. Generally, people doing different tasks switch positions every few days and thus learn how to do all the tasks involved in the egg take process. All waters that come in contact with eggs must be IHNV-free, with one exception; water that is acceptable for general fish culture use can be used to make the iodophor solution. If ice is used to control the temperature of water used for fertilization or iodophor solution, it must be made from virus-free and unchlorinated water.

There are many brands of iodine based disinfectants available. However, they are not all the same and not all are compatible with use on fish eggs. Brands that are labeled as 1.0% available iodine (Betadine) are diluted with 100 parts of water to give a 1:100 iodophor solution (38 ml to 1 gallon or 190 ml to 5 gallons). Brands that are labeled as 1.5% available iodine (Wescodyne) are diluted with 150 parts of water to give a 1:100 iodophor solution (25 ml to 1 gallon or 125 ml to 5 gallons). Iodophor solutions should be buffered to obtain a pH of 7.0 by adding 1.5 grams of baking soda to each gallon of solution.

Do not use hot water in making the iodophor solution. Use of cold water will minimize vaporization of the iodine into the air and the inhalation of these vapors by workers. The use of protective gloves and eye wear is recommended when working with iodophor solution because misuse or overexposure may result in health problems. Monitor the temperature of the solution and keep it below 10 °C.

Anesthetics such as Finquel (MS-222) are useful in sedating fish when checking for ripeness or collecting sperm. Anesthetic solutions need to be changed frequently to maintain adequate oxygen levels and reduce the buildup of IHNV.

The same general fish culture procedures used for sorting and killing other species as outlined in the ADF&G *Fish Culture Manual* also apply to sockeye. Bleeding fish is not necessary. If a small crew requires that individuals are involved in all egg-take procedures it is important that they thoroughly disinfect hands and rain gear between the different stages of the egg take. All fish are treated as infected individuals and are not stacked or overlapped. Each fish is disinfected, either by swabbing the ventral area with iodophor or dipping the fish into iodophor. The vent area is then wiped dry with a clean paper towel prior to spawning. Care should be taken that iodophor is not dripped into any spawning container.

Crew members collecting gametes must make sure that each fish has been disinfected & wiped dry prior to spawning. Eggs & sperm of individual fish are then collected into separate disposable containers using standard methods described in the ADF&G *Fish Culture Manual.* A fertilization ratio of one male to one female is recommended. Any eggs or seminal fluids that are of questionable appearance should be discarded. Crew members collecting eggs and sperm must disinfect all implements and hands in iodophor solution and rinse with virus-free water between handling each fish. At this point the mixture of eggs & sperm is activated using virus-free water and allowed to sit for one to two minutes. All egg containers should be handled from the outside only.

Egg Handling

Fertilized eggs should be rinsed with a 1:100 iodophor solution one to two minutes after the eggs and sperm have been activated with virus-free water. The iodophor should be added forcefully to the fertilized eggs to dislodge them from the bottom of the container and insure thorough contact with all eggs. Discard the initial iodophor rinse to remove organics and excess milt that otherwise binds up free iodine and dilutes the iodophor solution during water hardening. Refill the egg container with cool, clean 1:100 iodophor solution. Maintain the original dark brown color for a minimum of one hour by adding additional iodophor if needed as the eggs water harden. Do not move containers of eggs during the hardening process. Monitor the temperature of the iodophor solution and keep it below 10 °C by adding small amounts of ice. Ideally, all water used during the egg take and transport process should be maintained within a three to four degree temperature range below 10 °C. Do not change temperature at a rate greater than 5 °C per hour.

After the one hour iodophor treatment, the solution should be poured off and replaced with cool virus-free water. At this point eggs from several females may be mixed as they go into larger transport containers or incubators. Even though the eggs are now water hardened and less sensitive, careful handling is required. Disposable egg containers can then be discarded.

Transport

Standard transport methods as described in the ADF&G *Fish Culture Manual* apply to sockeye. However, when sockeye eggs are received at an incubation facility different treatment procedures are required to insure that IHNV is not brought into the hatchery. The exterior of any transport container should be disinfected with an iodophor and rinsed with virus-free water before it is taken inside. Likewise, any rain gear, gloves, boots, etc. that have been at the egg take site should be disinfected.

A checklist of sockeye egg take procedures can be found in Appendix A.

Incubation Procedures

Introduction

When sockeye are being cultured, none of the IHNV susceptible species will be allowed in the same facility unless the ADF&G determines that the design and operation of the facility precludes interspecies transmission of the virus.

Once again, the three main points to be considered during the incubation of sockeye eggs, alevins and fry are: 1) a hatchery water supply free of IHNV as well as other serious fish disease organisms; 2) the rigorous disinfection of everything brought into the area where sockeye are incubated as well as the disinfection of equipment used within this area and between incubators and 3) the isolation of eggs and fry into as many small sized lots as is reasonable.

If IHNV is detected at any time during incubation, then a fourth point that would be applied is; take immediate actions to destroy any focus of infection. This means all individuals in that unit, be it an incubator, start tank or raceway. This is a critical procedure to contain the virus and prevent it from contaminating other units.

It is also important that the hatchery personnel have a basic understanding of the disease organism, how it might be spread in the facility and a set list of procedures to follow that specifically address that hatchery situation. Without a basic knowledge of this disease the list of procedures to follow might be unknowingly compromised. Also learning to recognize the clinical signs of IHNV (see page 25) and taking immediate action is very important.

Preparation for Incubation

The sockeye module where the eggs will be incubated and all incubation equipment should be thoroughly cleaned and disinfected prior to the arrival of any eggs. If there is not a separate module for sockeye, then the incubation area should be partitioned off with nonporous material. This is to remind people that procedures used in this area of the hatchery are different.

All personnel entering or leaving the work area mentioned above, should go through a disinfectant footbath. Not only foot gear, but any protective clothing such as rain gear, gloves, etc. must either be properly disinfected or left outside this area.

Each incubation unit is set up with a separate inlet and outlet water line with as much space as possible left between individual incubation units to eliminate the possibility of viral cross- contamination from aerosol. During incubation each incubator is considered a separate unit filled with infected eggs.

Fertilized Eggs

When receiving and seeding eggs, every effort should be made to utilize all incubators available, rather then heavily loading some while leaving others unused. The concept is to get the eggs into as many small sized lots as is reasonable. This will reduce overall loss if an outbreak of IHNV occurs in one or more incubators. Never mix two different lots of eggs in the same incubator. All eggs taken during one day are considered a lot.

The most commonly used incubator for sockeye in Alaska is the Kitoi Box. These boxes can be loaded with 50,000 to 250,000 eggs, but exceeding 250,000 eggs per incubator is not recommended. Remember, the lower the number of eggs per incubation unit, the lower the number of eggs or alevins lost if an IHN outbreak occurs and is contained to that unit.

As eggs arrive they are checked for temperature to see if any tempering is required. The temperature of the eggs and the incubation water temperature should be within 3 to 4 °C of each other. Pour eggs underwater from the disinfected transport containers into the incubators. If egg bags are used for transport, they should be discarded when they have been emptied.

Use only currently approved fungus control measures. Hands and tools are disinfected after being used in any incubation unit. Effluent water flowing from an incubator should not be reused for further fish culture.

Eyed Eggs

Incubating ("green") eggs should be shocked and picked when the "eyed" stage is reached, usually around 300 C.T.U.'s. The method for shocking eggs is described in the ADF&G *Fish Culture Manual*, page 41. It is important to remove all dead eggs as they may be a source of IHN virus for sac fry and they provide a medium for the growth of fungus. Sorting of live and dead eggs is performed with electronic egg sorters. All equipment and tools used in the process are disinfected between each lot of eggs. A flush treatment of iodophor is recommended after eggs are picked to reduce numbers of potential residual IHNV particles released from infected dead eggs.

The incubators are cleaned and disinfected once again while the eggs are being sorted. As the eyed eggs are reseeded into the incubator, they are mixed with substrate that has been previously cleaned and disinfected. Remember, the lower the number of eggs per box, the lower the IHNV loss potential. Approximately five cubic feet of plastic substrate (saddles) are used per incubator resulting in a substrate depth of ten inches.

Periodic (once per week) floor cleaning with steam or a disinfectant is recommended throughout incubation. Do not use hot water with an iodophor as this produces vapors which present a health hazard to hatchery personnel.

Fish culture equipment is not generally moved between incubation modules, though such things as egg sorters may be moved between modules after they have been disinfected. Since it is not easy to disinfect wood, this material is not used in a sockeye incubation module.

Heating of water for egg or alevin incubation is not recommended, because it is potentially stressful and increases the risk of creating gas supersaturation problems.

Incubators are checked on a regular basis for indications of an existing problem. Signs such as early emergence of sac fry, oil droplets on the water surface, etc., demand close monitoring and are often associated with start of an IHNV epizootic. Immediate action to destroy any suspected focus of disease is strongly recommended. When the sockeye culture policy was initiated, it was mandated that the hatchery manager destroy any unit of eggs or fry that appeared to be infected with IHNV. Though the hatchery manager was required to discuss the situation with management and fish pathology staff, if possible prior to killing the suspected fish, it was agreed that killing a suspected lot of fish that did not prove to be infected would not be considered a negative act. In hindsight, the track record for this expeditious approach to IHNV control has proven invaluable.

Fry Rearing Procedures

Introduction

Following specific fry holding and rearing procedures will help insure optimal survival of fry and minimize the risk of loss due to IHNV. As with egg take and incubation procedures, virus control during rearing is centered around virus-free water, compartmentalization, and disinfection but a strong focus on preventing horizontal transmission is also required. Even using all the proper procedures will not guarantee that IHNV outbreaks will not occur. However, use of proper rearing procedures will reduce spread and minimize losses.

IHNV is often detected late in incubation or early in emergence. Extreme care is required to guarantee early detection and prompt destruction of fry suspected of being IHNV positive.

Fry Holding

Many sockeye programs rely on unfed fry planted in rearing lakes each spring, shortly after ice-out. Fry holding is defined as holding emergent fry in the hatchery up to seven days prior to release. Feeding is usually not initiated. Incubation temperature manipulation can be used to control time of emergence and release.

Volitional emergence is preferred as fry emerge when they are nearly ready to feed. After emergence, fry should be held a minimum of 24 hours, to facilitate transfer with minimal loss. Stock specific differences do exist and not all stocks will volitionally emerge readily, particularly stocks that spawn along lake shores as opposed to inlet spawners.

Successful non-volitional programs do make up a portion of the sockeye programs in Alaska. Non-volitional emergence requires very careful monitoring of yolk content to body mass ratios in order to determine incubator unloading time. Care must be taken to minimize physical damage and stress on fry.

If emergence is non-volitional and fry are not free swimming or ready to feed a longer holding period may be required. Holding non-volitional fry may require special containers with upwelling flow and large screen areas so that fry do not smother and are not forced against outlet screens. Some programs use modified incubators to hold fry for short periods.

Holding fry without feeding minimizes metabolic requirements of the fish. Less dissolved oxygen is required and no solid wastes are produced to foul the container or water. The need for feeding is eliminated if the fry are stocked before they have exhausted the yolk reserve. Proper release timing is critical.

At cold water temperatures, 4 °C or less, the transplant window lasts for several days but becomes shorter as water temperatures increase.

Fry Rearing

Short-term rearing has become the normal practice for the majority of the Alaska sockeye programs. Longer rearing, to over one gram, is applied to only about 5% of the statewide production. Most programs now feed the fry for a short (7 to 45 days) period before transplanting. The feeding results in larger fry which have learned to feed and have stronger swimming abilities than unfed fry. Rearing provides a much longer transplant window so weather, ice-out and other logistical variables are not as program threatening as they are with unfed fry programs. However, the cost of rearing facilities and operational requirements are significantly higher than for holding-only programs. Benefits of rearing are larger, stronger fish at the proper time for release into lakes. This should result in higher survival to adult.

Many sockeye culturists believed the risk from IHNV increases when fish are reared. In the early days of sockeye culture in Alaska, from 1981 to 1986, most program managers minimized rearing as part of the effort to avoid IHNV loss. The strategy was to get the fish out of the high density confines of the hatchery as soon as possible. Gradually the rearing time was extended and culturists found that sockeye reared easily. Losses due to IHNV occurred but were insignificant until 1993 when major epizootics and fish mortality were sustained at several hatcheries. Reduction of compartmentalization and containment during rearing accounted for much of this loss. This episode emphasized that rearing programs must be continually evaluated for disease control measures to minimize possible IHNV loss.

Water Source

As previously discussed, only virus-free water must be used for IHNV-susceptible species including sockeye salmon. The best choice is well or spring water. Second choice is a fishless water supply such as a "hanging" lake. If this is not available, a water source with fish but free of virus-susceptible species is acceptable. However, this does expose the hatchery fish to other types of fish pathogens. If a virus-free source can not be found, depuration of rearing water is possible but at considerable cost. The Kitoi Bay sockeye incubation and early rearing system is an example of a depurated water program in Alaska.

Containers

As with any salmonid rearing program, sockeye rearing containers should be carefully cleaned and disinfected before use. Rectangular start tanks of various designs have been used successfully but, as a rule of thumb, dimensions

should remain close to a ratio of 10 to 1, length to width. Use of circulating containers such as circular tanks or Swedish ponds is discouraged as IHNV particles will be circulated for longer periods in such tanks. Container size should be adequate to rear fish to release size (short-term rear) or to a size adequate for transfer into larger production raceways (1 gram).

Sockeye fry should be spread out to utilize as many containers as are available and each container is treated as a separate unit. A designated group of incubators (or incubator) should be assigned to a single rearing unit that will serve only that incubator grouping. Ideally, assigning one incubator to one start tank minimizes the risk of disease loss. Large scale programs are forced to use large raceways as production units. In these cases, fry from several egg takes and/or incubators must be combined into one raceway.

Use of baffles is recommended to clean out excess organics and separate out weak and possibly diseased fry. The exchange rate (R) should be at least two when fish are large enough to tolerate resulting velocities. As with any species, flow requirements are established by consideration of three biological requirements: adequate dissolved oxygen, adequate dilution to remove wastes, and a quality rearing environment. Rearing densities should be maintained at less than one pound per cubic foot per inch (6.3 kg per cubic meter per cm).

Physical Layout

A rearing unit refers to a single start tank or raceway. There may be several units for each fish stock and it is particularly critical that each unit be treated separately. Each rearing unit must have a separate inlet and outlet. There should be no connection between units that would allow effluent or backflow from one unit to flow into another. Physical separation of rearing units is essential to prevent transfer of virus. There should be at least 18 to 24 inches on each side between adjacent rearing units. If this is not possible, some type of waterproof barrier should be installed. Sockeye may not be reared in the same facility with other IHNV-susceptible species such as rainbow and steelhead trout or chinook and chum salmon unless the ADF&G determines that the design and operation of the facility will preclude interspecies transmission of the virus.

Heated water may increase stress and virus multiplication. Therefore, it should not be used unless absolutely necessary. Heated water must be degassed after mixing to prevent gas bubble disease. Gas stabilization with oxygen contactors can be used in sockeye fry rearing systems to keep dissolved nitrogen below 100% and dissolved oxygen in the range of 100 to 120% saturated. Preferred values are 98% nitrogen and 100% oxygen.

Outdoor raceways should be fenced to exclude small mammals and potential human trespass. Outdoor raceways should also be covered; minimally with bird netting to prevent cross-contamination by bird predation. Not only will birds and mammals travel from unit to unit but they may bring pathogen-contaminated water or other items from nearby streams or lakes into the rearing units. It is also possible that IHNV is transmitted as an aerosol. At windy sites, fencing should be constructed such that it also serves as a windbreak.

Wood cannot be adequately disinfected and should not be used for dam boards, walkways or any other structures that come into contact with fish rearing water. Aluminum, plastic and other easily disinfectable materials should be used in sockeye rearing areas. Disinfectant footbaths will be located at the entrance and exits of a sockeye rearing area and between units of different stocks to prevent cross-contamination.

General Daily Care

For practical reasons, fish culturists should assume that sockeye fry carry IHNV and the culturist's goal is to minimize the spread and accumulation of virus. Procedures must be established at each facility that reinforce isolation of each group or unit. Some general procedures for maintaining separation and reducing viral accumulation include:

1. Use separate utensils for each unit.

2. Disinfect protective gear between working with each unit.

3. For utensils that must be placed in a disinfectant solution, allow a contact time of at least five minutes for adequate treatment.

4. Walkways and floors around rearing units should be disinfected at least weekly and whenever rearing water drips on the floor i.e. during sampling or picking of dead fish.

5. Equipment should not be moved to other compartments within a facility or to other facilities. If equipment must be moved, it must be thoroughly disinfected.

6. Supplies should not be moved from sockeye facilities to other facilities containing IHNV-susceptible species.

7. Dead fish can act as point sources of virus infection and must be removed from rearing containers daily.

8. Careful mortality records must be kept to identify fish health problems early.

Feeding

Rearing sockeye fry requires careful attention to training the fish to eat and then feeding to achieve growth. Cold water often adds an additional challenge by slowing metabolism and feeding response. The small size of the emergent fry make feed size selection especially important. Careful early feeding and changing to larger particle size at the correct time is critical.

Recommended reading: *Feeds and Feeding Practices for Improved Fish Health* from Abernathy Salmon Culture Technology Center. This publication summarizes feeding information that applies to all salmon including sockeye. Proper feeding is extremely important. High fish losses can occur from failure to feed. For start-up feeding, use a high quality semi-moist diet, regardless of cost. Both BioProducts and Moore-Clark provide quality feed. Moist and semi-moist feeds are more palatable but dry feeds are more economical. Sockeye fry can be switched to a dry diet when they weigh approximately one gram.

Fish should be hand fed initially, preferably two to three times per hour. Frequency of feeding should be decreased & size of food increased as soon as possible to prevent gill problems. Manufacturer's recommendation for feed size should be used. Mix smaller & larger feeds together when changing sizes. The temperature should be no less than 4 °C for a good rearing environment.

Overfeeding is dangerous. Feeding food with small particle size, such as starter mash, in excess or for long periods can result in serious gill problems. Once fish are feeding, only feed what the fish consume immediately. Change to feed without fines as soon as practical. The skilled culturist not only feeds the fish but also makes sure the food is eaten and modifies feeding amounts to meet the fish and rearing program needs.

Dust and fines are common in fish food at hatcheries in Alaska because extensive shipping and handling tends to break down food pellets. Careful shipping and handling can minimize breakdown of feed particles. This is a potentially serious problem since sockeye are on small feed for a considerable time. In many cases, more fry are lost due to gill problems than IHN disease.

Raceway Maintenance

Increased attention to keeping containers, tools and work areas clean are an important part of sockeye fry programs. Rearing containers that provide continuous cleaning are desirable. Rectangular containers with baffles do an excellent job of continuous cleaning. Daily cleaning and mortality picking should supplement the use of baffles. Adequate flow rates help remove all sizes of suspended particles and the use of baffles will eliminate the need for drawdown cleaning.

If the containers do not flush well, a swimming pool type vacuum system can be used to frequently remove feces and excess food. In baffled containers, brushing is only required at the few spots where material builds up such as in front of the screens. It is particularly important to keep sockeye raceways clean to prevent fish from eating excess food and debris in the bottom of the raceways which can lead to internal fungal infections and gut impaction.

Disease Detection and Correction

Diseases can be identified before they cause large mortalities by observing the fish for behavioral or physical abnormalities. The following check list should be posted and referred to frequently by all hatchery staff participating in rearing activities.

1. Feed the fish first thing in the morning and observe their behavior. Look for abnormal swimming, gasping, refusal of feed, listlessness, or resting on the top or bottom of the tank.

2. Regularly remove a few moribund fish and examine externally for abnormal body color, cloudiness of skin, excess mucus, frayed fins or tail, lesions, protruding eyes, bleeding at the anal vent, fecal casts, or hemorrhaging. Fecal casts may appear as whitish strands in the water. Use a magnifier to examine fish for hemorrhaging at the base of fins which is commonly associated with IHN disease.

3. When disease is suspected, try to determine the probable cause and notify the fish pathology section as soon as possible. These signs of disease are not specific to IHNV disease and it should not be assumed that their presence can only indicate IHNV.

4. A hatchery manager may decide to destroy a group of sockeye fry suspected of having IHN disease in order to protect the remaining fish in the facility. It is not necessary to wait for results of pathology testing to destroy suspect fish but it should normally be done in consultation with a pathologist. The decision should be based on the degree of isolation of the suspect unit and the knowledge and experience of the manager. A sample of 24 live moribund fish should be collected prior to destruction for later confirmation of the diagnosis.

5. The current Sockeye Salmon Culture Policy stipulates that if fish are infected with IHNV, all the fish in that unit will be destroyed.

6. If a unit is positive for virus, samples of moribund fish from other units will be requested to rule out horizontal transmission.

Smolt Rearing Procedures

Introduction

The primary risk of IHNV when rearing one gram fry to smolts is usually associated with horizontal transmission of the virus because the virus is less likely to come from vertical transmission within a group of fish after they have been reared to a weight of approximately one gram. The key factor is continued vigilance against both routes. The virus cannot move on its own; its movement is passive and associated with water or fish. When sockeye fingerling become infected, the virus is usually physically introduced to the fish through human activity or allowed to come in contact with the fish through inappropriate facility design. However, there have been outbreaks of IHNV in larger juvenile sockeye in hatcheries that suggested the fish had been carrying the virus without clinical signs of disease.

Hatchery Water Source

At most sockeye facilities the final rearing phase involves combining one gram fingerlings from individual start tanks into large production raceways. The general rearing process does not differ significantly from smolt rearing techniques for other salmon species with the exception of a few specific principles.

First, as previously discussed, the hatchery water must be IHNV-free. The optimal situation is a hatchery water source without fish. If this is not possible, the culturist must be aware that sockeye are susceptible to many pathogens that cause problems with other salmonids. Other diseases such as BKD may be as limiting to sockeye culture as IHNV. Regardless, it is certain that if IHNV is in the water supply, sockeye cannot be successfully reared.

Secondly, the temperature of the water must be considered. Sockeye can be reared in water that is considered too cold for other species. They will feed and grow at temperatures as low as 2.5 °C and perhaps lower. That the fish do not either grow or metabolize rapidly at these temperatures is advantageous to the culturist. Since the target release size for a sockeye smolt in Alaska is only 6 to 8 g, they need not grow rapidly during the cold winter months. When the water is cold and metabolism slows, the raceways can be loaded quite densely without negatively impacting the fish. In several instances at Main Bay Hatchery the density of rearing fish in raceways was about 90 kg per cubic m without loss in feed conversion or aberrant fish behavior. As the water warms in the spring (4 to 5 °C) the most dense raceway groups can be split, possibly transferring some portion of the fish to saltwater net pens.

Employing good fish culture practices relative to the hatchery water is recommended. This may include gas stabilization, oxygen supplementation, moni-

toring total dissolved gas saturation, or monitoring dissolved oxygen at both the head and tail of the raceway.

Rearing Containers

Rectangular raceways have been most commonly used in Alaska to rear sockeye to smolt size. If properly set up, the single pass flow-through design of rectangular raceways will provide for a cleaner rearing environment than circular ponds. Raceways have generally been made of concrete and it has become apparent that sockeye rearing causes more rapid deterioration of concrete raceways. The frequent brushing and between-use steam cleaning and pressure washing seems to cause flaking or cracking and subsequent leaks between units. This could prove important in isolation associated with disease. In the long term, aluminum raceways may prove more advantageous.

Headbox design and dam board construction are important to raceway isolation. The headbox should not be common between raceways to prevent the back pressure and eddies in the current from carrying debris or live fish into the headbox and subsequently between raceways. The dam boards should be water tight and made of some material that can be disinfected between use. The hatchery should be designed so that water entering a raceway has not been used for rearing upstream of the raceway. Water leaving a raceway also must not be used for rearing elsewhere in the hatchery or net pens.

Physical separation of raceways by barriers or adequate space is ideal and supplementing good culture practices that confine all activity and tools within each discrete raceway will functionally accomplish isolation of each rearing unit. Raceways should either be enclosed in a building or otherwise protected from predators, including birds as small as dippers. In the majority of cases when IHNV has infected fish in raceways used for rearing sockeye to smolt size, predators have been the prime suspect in the spread of disease between units.

Raceways should be pressure washed and disinfected with steam after use. They should then be dried for two weeks to two months and later disinfected with steam immediately prior to the next use.

Loading Raceways

Successful sockeye culture is the result of careful risk management. This is another critical point where the overall risk of the endeavor can be decreased. Place fish from as few start-up raceways as possible into a single production raceway.

It is important, for several reasons, that fish be enumerated as they are moved from incubator units to the raceways. The overall productivity of a hatchery may be determined by the application of an intensive rearing model while at the same time maximizing the density of fish in the raceway. In order to accomplish either of these things, the number of fish in each raceway must be known so that an accurate rearing model can be developed. It is often quite easy to passively move the fish through a counting device as they are moved from incubator units to raceways.

The peak density at which sockeye smolt can be successfully reared in a raceway depends on many things. If the water is cold (2 to 6 °C) and saturated with oxygen, sockeye may be reared at densities up to 100 kg per cubic m in raceways. Unlike coho and chinook, sockeye do not seem to exhibit aberrant behavior or aggression toward one another at these densities. It is possible that eventual survival to adult might be affected by the density at which the fish were reared in the raceway. In the single instance when this was examined at Main Bay, the rate of survival from the denser raceways was less, but the overall benefit of releasing more fish from the denser raceways was greater and resulted in increased production of adults.

Each hatchery is different and various rearing densities should be tested in order to optimize production at a specific facility. One way to maximize production is to reach a peak raceway density in the spring and move a portion of the fish to net pens in sea water. Fish in the raceway can then be reared concurrently with fish in net pens, magnifying the rearing capacity of the hatchery. However net pens should not be placed such that they receive effluent from hatchery raceways.

Feeding

The culture of sockeye fry to smolts has been considered partly experimental, and it may be beneficial to look at this work the same way for some years. Initial production of smolt used a feeding model based on the hatchery constant method that can be found in the ADF&G *Fish Culture Manual*. The hatchery constant model was developed at water temperatures considerably warmer than those used in sockeye culture. The actual metabolic and growth potential of the fish may not remain on the same scale at these cooler water temperatures. From the work at Main Bay and Auke Creek facilities, it appears that feeding rates can be reduced to about 80% or less of the model. Apparently the sockeye growth rate at lower temperatures exceeds that of other salmonids. Crude feed conversions, the simple weight of semi-moist or dry feed to the wet weight gain of the fish, should remain close to or under 1.0 over the long term.

Raceways should be sampled every week to adjust the feeding rate based on the growth, size of the fish and the water temperature. In accordance with the model, all the feed given the fish must be weighed prior to feeding.

Sockeye can be successfully reared to smolt on moist, semi-moist, and dry feeds of known name brands considered to be "quality" feeds such as Moore-Clark or Biodiet. Generally feed costs necessitate the fry being reared on dry diets after initial feeding, as previously discussed in the fry rearing section. The fish should be fed up to four times a day when the water is warmer, but at cooler water temperatures (2.5 to 3.0 °C) the entire ration can be fed during a single feeding. Minimizing the actual number of feedings per day will help keep each lot of fish at fairly uniform size.

Daily Care

The daily care during smolt rearing is much the same as what is done with fry. Dead and obviously moribund fish should be removed daily. The numbers of these should be recorded in an inventory for each raceway. Some fish culturists are reluctant to remove moribund fish from a raceway. It is very rare that a moribund or obviously sick fish recovers. It is far better to take an obviously sick fish away from the well fish than to leave the fish there in the hope that it will somehow get better when all the while it could be exposing healthy fish to pathogens.

Culturists are going to be very concerned about IHNV when first doing sockeye culture. Every dead fish need not be sent to the Pathology lab if the mortality in a raceway is within acceptable limits and there are no specific clinical signs of disease. Experienced fish culturists will generally know when something is really wrong, and it is no different with sockeye than with any other species.

Treat each raceway as if the fish in that raceway were infected with disease. Each raceway should have its own fish culture tools. Utensils such as raceway vacuum heads and suction hose, that are used in more than a single raceway, should be passed through disinfectant between uses. Sweep excess feed from walkways and raceway areas daily. It is especially important to avoid or at least minimize movement between smolt rearing areas with yearling fish and rooms with incubators and start tank units filled with eggs, sac fry, or fry. It will certainly help as a reminder, if as well as for actual disinfection, to have a foot bath at the entrances and exits to smolt rearing areas.

Raceway Cleaning

Maintaining a clean rearing environment is an important part of sockeye culture. There are a number of ways raceways can be operated to aid in this

process such as maintaining a flow rate of at least two exchanges per hour and use of baffles. Although baffles can greatly reduce the work associated with raceway maintenance they will not do the entire job. Raceways must be cleaned frequently when baffles are in place but the cleaning will take a lot less work. When the fish have reached about 3 or 4 g and a density of about 30 kg per cubic m in the raceway, the normal swimming action of the fish will accomplish most of the cleaning. If baffles have been installed, they are sometimes removed at this point. Again, frequent cleaning will still be required but the work will be reduced.

Disease Detection

Monitoring fish for the detection of disease during this phase of rearing is similar to monitoring rearing fry, which is discussed in the previous section of this manual. The primary difference between fry and juveniles rearing to smolt size is that very little mortality is expected among the older fish and actual problems may be even easier to notice.

The sockeye salmon culture policy stipulates if fish are infected with IHNV, all fish in that rearing unit will be destroyed. If the fish in any unit are destroyed, the unit should be adequately cleaned and disinfected prior to any subsequent use.

Seawater Rearing

Net pens can be used in seawater to rear sockeye smolts prior to release with the primary advantages being to gain space and increase rearing temperature. With most other species, there would be an increase in survival to adult associated with rearing in seawater prior to release. This correlation has not been proven for sockeye and in fact the only time it was tested, the fish released directly from freshwater returned slightly better than those released from saltwater net pens.

The pens should be an appropriate mesh size related to the size of the fish. For example, with fish between 4 and 6 g, either 3/16 or 1/4 inch mesh would be appropriate with the 1/4 inch mesh being easier to clean.

For both rearing and disease considerations it is advantageous to separate pens or clusters of pens as much as possible. The pens should not be placed directly in the hatchery effluent. Smolt rearing in pens in front of Main Bay Hatchery were probably infected with IHNV from hatchery effluent in the spring of 1993. The virus came from several infected incubator/start tank units in the hatchery. Once fish in the pens were infected, the disease spread rapidly between the different net pens of smolt and all the fish were eventually destroyed.

Sockeye smolts should be given an appropriate seawater challenge prior to moving fish to net pens. Even when fish seem to pass a seawater challenge, there may be a small mortality (several percent) within 48 hours of transfer to seawater with the first groups transferred in the spring (early April). When fish are moved to seawater later in the spring (late April or May) mortality may be minimized. Each facility will have to determine their optimum time for transfer.

Sockeye smolts are fragile and should be moved from raceways and transferred to net pens as gently and passively as possible. As an example, the fish can be gently crowded toward an Archimedes screw type fish elevator which removes them from the raceway. The fish might then be fed into a large diameter (8 inch) plastic hose where they are moved by gradual gravity flow to the net pens in seawater adjacent to the hatchery.

It is convenient to enumerate smolts when moving them to net pens, and this is the last time this task will be logistically reasonable. There are a number of fish counters on the market that could be placed in the transfer system to passively count the fish as they flow down to net pens. If programs are going to be evaluated, it will be important to establish a final prerelease number. Sockeye smolts have been reared at densities as high as 18 kg per cubic m in net pens (40 ft x 40 ft x 12 ft deep) at Main Bay Hatchery. Each net pen site will be unique; culturists might first start with fairly conservative densities (perhaps 10 kg per cubic m) and if successful, increase these over time.

Smolt Release

Both the size of a smolt at release and the time a smolt is released can have a significant impact on the success of a yearling smolt program. Experience to date indicates that to maximize smolt to adult survival the target size for smolts at release should be between 6 and 8 g. Size of a smolt at release may be site specific and this recommended size at release is a starting point when examining this parameter. Much more work is needed in determining optimum smolt size at release.

Optimum release time for hatchery produced sockeye smolts will probably be site or area specific. It is recommended that hatchery releases match the peak out-migration timing of the nearest population of wild sockeye. It might also prove wise to further examine this parameter at each specific site so in order to eventually maximize facility production.

Current work indicates that it is not necessary to rear sockeye smolts in seawater prior to release in order to maximize marine survival. Rearing fish in seawater prior to release is a good operational strategy for increasing the rearing space available at a hatchery.

IHNV Disease - General Considerations

Alaskan sockeye hatcheries normally lose more fish to other causes than to IHNV. Presence of some signs may not indicate IHNV but should compel the hatchery personnel to rapid action. This should include quarantine of the suspect unit, more thorough examination of the fish and consultation with a fish pathologist. Samples should be submitted expeditiously if IHNV is suspected. Frequently, the disease will only be present in the moribund fish so proper selection of samples is critical. The rate of mortality due to IHN is dependent on the age of the fish and the water temperature. An IHNV outbreak may be occurring with mortalities of less than 0.1% per day if the water temperature is less than 5 °C. This is particularly true in older fish. Mortality, however, will usually start to increase as the disease spreads.

In some cases, hatchery personnel may decide, based on their observations and experience, to destroy a rearing unit prior to completion of pathology testing for IHNV. This should only be done after consideration of the disease risk to the facility and the experience and ability that hatchery personnel have in recognizing the clinical signs of IHNV. This should be encouraged to prevent infection of additional units. Submitting samples from the unit prior to destruction will facilitate the learning process for hatchery personnel but should not necessarily delay fish destruction if the outcome appears obvious.

Fish Destruction

Production of sockeye in a hatchery always entails the risk of an IHNV outbreak and, invariably, the hatchery operator will be required to destroy infected fish. It is important that this be done expeditiously and effectively in order to contain the epizootic. There are several ways of decontaminating infected fish:

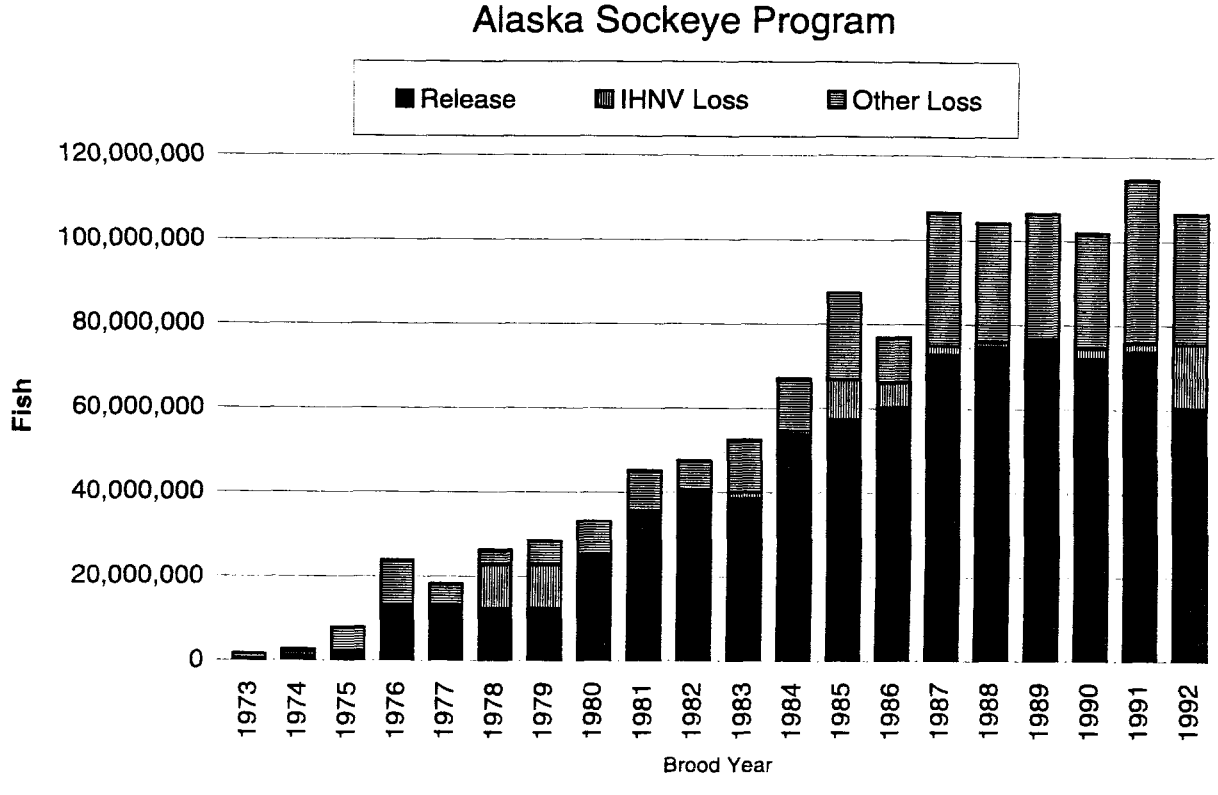
1. Use 200 ppm chlorine at least overnight. The fish tissue will turn into a slurry. When large amounts are needed, chlorine is usually purchased as sodium hypochlorite, HTH. If the chlorine is to be released into fish-bearing waters, it will have to be neutralized. This can be accomplished by the addition of commercial sodium thiosulfate at a rate of 5.6 grams for each gallon of 200 ppm chlorine solution or 2.2 lb per lb of HTH. Following decontamination, the fish should be transported to a landfill or buried. Fish treated with chlorine should not be incinerated due to release of toxic chemicals.

2. Bag infected fish in plastic bags and layer with lime in a pit. Cover the pit with dirt to prevent birds or animals accessing the buried fish. When bagging and transporting infected fish, be careful not to allow spread of contaminated water or fish.

3. If the proper equipment is available, infected fish may be incinerated.

4. Any disposal of fish needs to be approved by the appropriate regulatory agency. Other disposal methods may be acceptable if approved.

5. Incubation, substrate, rearing units and hatchery equipment should be scrubbed to remove remaining organics then disinfected and allowed to dry. All floors and other areas that may have come in contact with infected fish or water should be disinfected. Formalin fogging of the facility is no longer recommended as this procedure poses unacceptable health hazards.



Summary

Sockeye salmon have been successfully cultured in Alaskan hatcheries for a number of years and this endeavor has certainly been a learning process. We have learned that hatchery site characteristics such as water quality and temperature and physical plant layout can influence program success. Likewise, stock specific characteristics such as IHNV prevalence and proportion of brood stock having high virus titers almost certainly influence the probability of a disease outbreak in the hatchery.

The sockeye culture cornerstones of virus-free water, adequate use of disinfectants, compartmentalization, and isolation simply cannot be compromised. Good fish culture practices are also essential. Most importantly, we have learned that regardless of how strictly fish culture and disease control procedures are followed, IHNV will continue to affect mortality of sockeye salmon cultured in hatcheries. Staff training in the area of disease recognition and facility redesign to accommodate compartmentalization and isolation techniques are important steps at all sockeye production facilities. By using proper compartmentalization and isolation techniques, IHNV related mortality can be managed. Our philosophy has been to assume some small annual percentage loss due to IHNV and factor it into facility production plans as a cost of doing business.

Our knowledge base concerning the culture of sockeye salmon is relatively limited compared to other species of Pacific salmon. Comprehensive evaluation of the sockeye program must continue. Applied research studies designed to determine the optimum size and timing of smolt releases will have positive benefits in terms of increased marine survival of hatchery smolts. Continued refinement of feeding models and pre-smolt rearing densities will maximize in-hatchery production.

All work to date with age-0 smolt production has met with marginal success in terms of producing adults and feasibility may be largely dependent on specific brood stock characteristics and release location. Stocking sockeye pre-smolts into lakes in late fall has been successful and can greatly increase the production capacity of hatcheries with limited rearing space.

Although we now know more about the biology of IHNV than when the Alaskan sockeye policy was initiated, there are still many features of the virus life cycle, such as vertical transmission and the carrier state, that remain to be conclusively demonstrated by future research. While this information would be nice to know, it isn't necessary to have in hand in order to successfully culture sockeye salmon. This is being proved every day in Alaska.

Acknowledgments

Many individuals, too numerous to list, have had a part in developing, testing and refining the sockeye salmon culture procedures presented in this manual. But, among all the fish culturists, biologists, fish pathologists and program managers involved in this endeavor over the years, one person must be acknowledged as having made it happen; former Fisheries Rehabilitation, Enhancement and Development Division (FRED) Director Robert S. Roys. Bob Roys, recognized the importance of sockeye salmon to Alaska's fisheries enhancement program and provided the direction and funding support that enabled the FRED Division technical staff to develop successful sockeye salmon culture procedures.

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Appendix A

Sockeye Salmon Egg Take Procedures

The following step by step procedures were taken from the egg take section of Alaska Department of Fish and Game's *Alaskan Sockeye Culture Manual* and are intended to be used as a field checklist to insure proper egg take procedures are followed.

1. Using a ***200 ml plastic graduated cylinder***, make up a 1:100 iodophor solution by adding 38 milliliters of jug strength ***Betadine (1% active iodine)*** to one gallon of chlorine-free water (190 ml per 5 gallon water). Buffer by adding 1.5 gram of ***baking soda*** per 1 gallon of solution. Pre-weigh 1.5 gram packets of baking soda prior to the egg take.

2. Place killed males and females on a ***spawning rack***, ventral side up, and maintain physical separation between individual fish. Use a ***sponge*** to swab the ventral area of each fish with 1:100 iodophor solution.

3. Wipe the vent area dry with a clean ***paper towel*** as you pick up each fish to spawn.

4. Disinfect hands and ***zak knives*** before handling each male or female. ***Thin disposable latex gloves*** can be worn to protect hands from iodophor.

5. Collect eggs from individual females into separate ***disposable egg containers***. Do not pool eggs from more than one female into separate containers.

6. After spawning three females into separate containers, add equal amounts of milt from one male into each egg container. Quickly repeat this procedure using second and third males if adequate males are available. The goal is to maintain a minimum spawning ratio of one male to one female. It is critical that the elapsed time for milt addition not exceed 30 to 45 seconds prior to mixing and/or water addition as sperm motility and viability deteriorates rapidly after approximately 60 seconds. Remember to disinfect hands between each fish.

7. Gently stir each container using a separate ***disposable tongue depressor***. Discard the depressors after stirring.

8. Activate the milt using ***virus free water (vfw)*** or a 0.7% saline solution made by mixing 7 grams of ***non-iodized salt*** in 1 liter of vfw water (26.5 g salt per gallon of water). Water or saline solution should just cover the top of the

eggs. Gently swirl the container, being careful not to splash or spill, then let it sit for one to two minutes.

9. Rinse the eggs with 1:100 iodophor solution, discarding the rinse. Repeat this procedure until the rinse solution is relatively clear of organic material. This will remove excess milt, blood, etc. that will otherwise dilute the iodophor solution during the one hour water-hardening process.

10. Refill container by forcefully adding (fall of about 12 inches) clean iodophor solution. Maintain the original dark brown color for one hour by periodic addition of more iodophor solution. A disinfected thermometer should be used to keep track of the temperature of the iodophor solution. Either ice made from chlorine free water or frozen 1:100 iodophor can be added to maintain a relatively constant temperature during this one hour period.

11. After one hour, pour off the iodophor solution, rinse eggs with vfw and place eggs into egg bags filled with vfw. At this point, eggs from several females can be pooled into plastic bags with water for transport. Leave at lease two inches of water over the eggs to allow for water absorption during transport. Place ice in bottom of cooler and around bags to maintain temperature. Use burlap to eliminate direct contact between eggs and ice.

12. The outside of coolers and bags of eggs should be disinfected with a 1:100 iodophor solution before being brought into the hatchery. No further disinfection of eggs is required prior to loading into incubators. Eggs may need to be tempered until they are within 3 to 4 °C of incubation water temperature.

NOTE: The ***bold*** items constitute a list of required supplies in addition to the normal list of egg-take gear such as rain gear, hip boots, dip nets, egg bags, coolers, etc..