FRED Special Report

Alaska Department of Fish & Game
Division of Fisheries Rehabilitation,
Enhancement and Development

Regulation Changes, Policies and Guidelines for Alaska Fish and Shellfish Health and Disease Control

by

State Pathology Review Committee

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1985 - 1987

October 1988

TABLE OF CONTENTS

Section	Page
MISSION STATEMENT	1
CHANGES IN EXISTING REGULATIONS	1
Pursuant to 5 AAC 41.070(b)(2) (Importation of oysters permitted as long as they have no diseases not indigenous to Alaska or a disease not considered significant to oyster health or marketability)	1
Pursuant to 5 AAC 41.080(b) (Disinfection should be required of all finfish eggs coming into a hatchery regardless of origin)	2
Pursuant to 5 AAC 41.080(c) (Not all fish and shellfish hatcheries should be required to have inspections every year)	2
Pursuant to 5 AAC 41.080(d) (Reclassification of disease categories)	3
Pursuant to 5 AAC 41.080(e) (Change in disease control measures)	6
Pursuant to 5 AAC 41.080(f) (Change in disease control measures)	6
Pursuant to 5 AAC 41.080(g) (Change in disease control measures)	8
Pursuant to 5 AAC 41.100 (Expansion of "fish pathology section" and amended definition of "fish")	10
APPLICATIONS	11
Wild Fish Transplants	12
Fish Broodstock Screening	17
Disease History of Juvenile Fish Prior to Release	19
Transfer of Fish Between Hatcheries	25
Sockeye Salmon Culture	28

TABLE OF CONTENTS (continued)

Section	Page
Shellfish Culture	34
Oysters	34
APPENDIX - GENERAL GUIDELINES	39
Quarantine Unit Fish Hatcheries	41
Drugs and Other Chemicals Used in Aquaculture	43
Disinfection Procedures for Hatcheries	48
Inspection and Diagnostic Procedures	56
Finfish	56
Shellfish	65
Hatchery Inspections	66
Schedule I - Rationale	67
REFERENCES	71

MISSION STATEMENT

The following document includes proposed changes in state regulations, new policies, and recommendations to be used by recognized authorities and user groups for maintaining adequate finfish and shellfish health within the State of Alaska. These criteria include regulating and permitting protocols, diagnostic procedures, prophylactic measures, and treatments of infectious diseases of salmonid fishes and oyster species. The criteria are established for the purpose of regulating interstate and intrastate movements of the above live animals or their gametes for planting in natural waters, research and education purposes, and/or other interests not defined herein. The long-range goal of this document is to prevent dissemination of infectious finfish and shellfish diseases within or outside the borders of Alaska without introducing impractical constraints for aquaculture and necessary stock-renewal programs. In so doing, other established state criteria regarding genetic and aquaculture policies will be maintained.

CHANGES IN EXISTING REGULATIONS

The recommendations of this committee include suggested changes in existing regulations found within the Alaska Statutes and Regulations for Private Nonprofit Salmon Hatcheries (ASRPNSH).

<u>Pursuant to Article 3, 5 AAC 41.070(b)(2)</u>

There are several parasitisms in oysters, and other shellfish species, which may or may not be indigenous to Alaska, that do not affect their health or marketability. These are considered insignificant diseases that should not restrict oyster importation into Alaska. Article 3, 5 AAC 41.070(b)(2) should read[1]:

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[1] Proposed additions to regulations will be underlined, and proposed deletions will be bracketed in capital letters.

(2) the disease history or an inspection indicates no incidence of disease that is not indigenous to Alaska or is considered significant (by the Fish Pathology Section) to oyster health or marketability.

Pursuant to Article 3, 5 AAC 41.080(b)

Egg disinfection should be practiced on all eggs coming into a hatchery, regardless of their origin. Returning stocks originating at a hatchery can and do have disease prevalences which wax and wane from year to year which could be reduced by thorough external egg disinfection. Article 3, 5 AAC 41.080(b) should read:

(b) Within 24 hours of taking and fertilizing live fish eggs or transporting live fish eggs between watersheds, all eggs must be treated, for at least 10 minutes, with iodine solution of at least 100 parts per million of active iodine ingredient, with pH at least 6.0 or greater, or in a manner approved by the Fish Pathology Section of the Department. This requirement does not apply to shellfish eggs and may, at the discretion of the Commissioner or his authorized designee, also exclude eggs taken at certain large scale pink salmon facilities where the operational history shows that disease has not been a problem in returning stocks of fish.

Pursuant to Article 3, 5 AAC 41.080(c)

It is recommended that not all hatcheries need inspection every year. Some facilities have had no disease problems; consequently, if management and hatchery design remain the same, such facilities may only require inspection once every other year. Also, poor spring weather makes it extremely difficult to fly to and inspect hatcheries prior to release of fish. Also, prerelease inspections of fish are generally not necessary and should be eliminated unless warranted in certain instances by the fish pathology section. Article 3, 5 AAC 41.080(c) should be amended to read:

(c) Each fish hatchery or fish rearing facility must be inspected by the Department's Fish Pathology Section at least once [EACH] every other year [AT LEAST TWO WEEKS PRIOR TO THE TRANSPORT OR RELEASE OF FISH]. The Commissioner or his designee may require and conduct additional inspections if the disease history of the stock or facility is incomplete, or if the disease history or current condition of the stock evidences incidence of disease.

Pursuant to Article 3, 5 AAC 41.080(d)

All disease categories have been completely changed to reflect current understandings of disease problems and concerns. Article 3, 5 AAC 41.080(d) has been entirely replaced with this amended version:

(d) The occurrence of any of the following pathogens or diseases of fish must immediately be reported to the Department's Fish Pathology Section:

1. Finfish Disease Categories

a. Class I. Diseases of Critical Concern

1) VHS - Viral hemorrhagic septicemia
2) IPN - Infectious pancreatic necrosis
3) OMV - *Oncorhynchus masou* virus
4) *Herpesvirus salmonis*
5) Whirling disease (*Myxobolus cerebralis*)

b. Class II. Endemic Diseases of Concern

1) IHN - Infectious hematopoietic necrosis
2) BKD - Bacterial kidney disease (*Renibacterium salmoninarum*)
3) Furunculosis (*Aeromonas salmonicida*)
4) ERM - Enteric redmouth (*Yersinia ruckeri*)
5) ICH - Ichthyophthiriasis (*Ichtyophthirius multifiliis*)

c. Class III. Nuisance Diseases

1) Vibriosis (saltwater *Vibrio anguillarum*, *V. ordalii*, *V. alginolyticus*)
2) Cold water disease (*Cytophaga psycrophila*)
3) Columnaris (*Flexibacter columnaris*)
4) Trichodiniasis (*Trichodina*, etc.)
5) Ichthyobodiasis (*Ichthyobodo* = *Costia*)
6) Hexamitiasis (*Hexamita*)
7) Lymphocystis Virus
8) Helminth diseases
9) Fungal diseases (*Saprolegnia* sp.; *Phoma herbarum*)
10) Motile bacterial septicemias (*Aeromonas hydrophila*, *Pseudomonas*)

d. Class IV. Uncategorized Diseases

1) VEN - Viral erythrocytic necrosis
2) PKD - Proliferative kidney disease
3) Vibriosis (freshwater)
4) *Loma* (Microsporidan)
5) Reovirus
6) Ceratomyxiasis (*Ceratomyxa shasta*)
7) Finfish pathogens not defined in Category I, II, and III including non-salmonid agents.

2. Shellfish disease categories

a. Class I. Diseases of Critical Concern

1) European Iridoviruses
2) Oyster Herpesvirus
3) *Ostracoblabe implexa* (Foot disease fungus)
4) *Perkinsus marinus* and other like protozoa
5) *Haplosporidium* sp. (*nelsoni*; *costalis*)

6) *Marteilia* sp. (*refringens*; *sydnei* aber disease; QX)
7) *Bonamia ostreare* (protozoan microcell)
8) Velar disease (Iridovirus)
9) *Mytilicola* sp. (*intestinalis*; *orientalis*) Copepod
10) Malpeque Bay disease
11) Denman Island disease

b. Class II - Nuisance Diseases

1) Focal necrosis (Gram + bacteria, *Nocardia*-like)
2) Prokaryote inclusions (chlamydia; mycoplasma; rickettsia)
3) Bacillary necrosis (*Vibrio*; *Pseudomonas*; *Aeromonas*; others)
4) *Sirolpidium zoophthorum* (fungus)
5) Mycelial disease (Actinomycete-like)
6) *Hexamita* sp. (protozoan)
7) Ciliates (*Sphenophrya*; trichodinids; *Ancistrocoma*)
8) *Nematopsis* sp. (sporozoan)
9) Microsporidea (HEP and others)
10) Helminth parasites
11) Neoplastic disease
12) Ovacystis virus (papovavirus)
13) Symbionts
    a) *Polydora* (mudworm)
    b) *Diplothyra* (boring clam)
    c) *Cliona* (boring sponge)
    d) Bryozoa
14) Predators
    a) *Stylochus* (polyclad)
    b) drills
       Urosalpinx cinerea
       Ocenebra japonica
       Rapana sp.

Finfish diseases have been divided into four categories that may be handled differently when diagnosed. Only salmonid diseases have been specified. As addressed in Category IV, diseases of other fish will be examined on a case-by-case basis as the need arises.

Shellfish diseases have been divided into only two categories; only diseases of oysters have been considered. Other shellfish diseases will be considered on a case-by-case basis as the need arises.

Pursuant to Article 3, 5 AAC 41.080(e)

Although all fish and shellfish diseases should be reported, not all are important enough to merit cause for the Commissioner to prohibit stocking in new areas and to quarantine the permittee's facility until disinfected. Recommend omission of last sentence in (e), beginning with "Presence". Exotics are addressed in (f). Article 3, 5 AAC 41.080(e) should read:

> Diseases reported under (d) of this section, if found by inspection under (c) of this section, must be treated by taking steps acknowledged by the Fish Pathology Section to be effective in eliminating the disease. Containers or facilities must be disinfected by the permittee in a manner directed or approved by the Commissioner or his authorized designee. [PRESENCE OF ANY OF THESE DISEASES OR ANY OTHER DISEASE NOT PREVIOUSLY OBSERVED IN ALASKA MAY BE CAUSE FOR THE COMMISSIONER OR HIS AUTHORIZED DESIGNEE TO PROHIBIT STOCKING OF THE FISH IN NEW AREAS AND TO QUARANTINE THE PERMITTEE'S FACILITY UNTIL DISINFECTED.]

Pursuant to Article 3, 5 AAC 41.080(f)

The finfish diseases of critical concern listed in Class I are extremely serious, such that if detected more specific and drastic measures regarding containment and eradication need to be

addressed. Much of (f) has been reworded. This category includes five infectious agents that have not been detected in Alaska, two of which (OMV, VHS) are exotic to the North American Continent. Three of these agents (VHS, IPN, and M. cerebralis) can be extremely virulent and capable of killing whole populations of fish. The remaining two can also cause severe problems, making infected stocks unsuitable for any purpose. There is no known treatment for any of these agents, except prevention.

The shellfish diseases of critical concern listed in Class I include 11 infectious agents that are exotic to Alaska; two of these (1, 6) are exotic to North America. Any oysters for importation into Alaska having detectable Class I agents will be refused for entry. Any oysters within the state infected with agents exotic to North America should be considered for destruction or immediate marketing to protect the environment or other oyster stocks.

Because no baseline survey work has been done, there is no class including endemic diseases of concern for bivalve mollusks in the state of Alaska. Consequently, some of the diseases listed in Class I may actually be endemic and will have to be considered on a case-by-case basis when detected in cultured stocks within the state. Until that time, the state of Alaska will take the conservative approach with seed imports of Crassostrea gigas and consider all Class I diseases as exotic. Article 3, 5 AAC 41.080(f) should read:

> As determined by the Commissioner or his authorized designee, detection of any Class I disease in finfish stocks or Class I disease exotic to North America in shellfish stocks within a hatchery or rearing facility will require immediate action, including quarantine, stoppage of water flows to eliminate effluent release, complete destruction and proper disposal (caustic lime burial or incineration) of affected stocks within the facility, and a thorough disinfection of holding

areas and equipment. A facility so affected may be required to remain dry or out of production for one year and be certified free of the disease before continued production of fish or shellfish.

[STOCKS OF FISH IN HATCHERIES OR REARING FACILITIES IN WHICH A] If Class I diseases [HAS BEEN DETECTED MUST BE IMMEDIATELY DESTROYED] exotic to Alaska but not to North America are detected in Alaskan oysters, they may require destruction by the permittee if the Commissioner or his authorized designee determine that the disease. . . poses a threat to the health and perpetuation of native, wild, or hatchery stocks of shellfish in the [HATCHERY EFFLUENT WATERSHED] immediate area or the intended release location. In limited circumstances, the Commissioner or his authorized designee may allow retention or transportation of these diseased fish or shellfish under controlled conditions that pose no threat to native, wild, or hatchery stocks of fish and shellfish (e.g. movement to a disease laboratory having effluent depuration).

Pursuant to Article 3, 5 AAC 41.080(g)

The Finfish diseases of endemic concern listed under Class II include five obligate pathogens causing most of the serious finfish disease problems in Alaska. These are of concern because they currently affect fish transport within and outside the state and define sampling sizes, frequencies, and methodologies. All but two (IHN, BKD) are treatable to some degree, and depending upon the agent and the circumstances involved, fish in the diseased state may or may not require destruction and proper disposal followed by complete disinfection of the hatchery facility. (*See* Policy Section III A-D for treatment of different diseases under various circumstances.) Article 3, 5 AAC 41.080(g) should read:

Stocks of finfish in hatcheries or rearing facilities in which a Class II disease has been detected [MUST BE

IMMEDIATELY DESTROYED] may require destruction and complete disinfection of the facility by the permittee, [IF] depending upon the agent involved as determined by the Commissioner or his authorized designee [DETERMINES THAT] and if the disease poses a threat to the health and perpetuation of native, wild or hatchery stocks of finfish in the hatchery effluent watershed of the intended release location.

Article 3, 5 AAC 41.080(e) includes adequate action for finfish Class III and Class IV diseases and requires no additional amendment. Class III diseases include several agents that often are secondary to poor environmental conditions and/or finfish husbandry techniques. Some of these require movement restrictions based on prevalence of the disease and resultant fish mortality. In general, these do not constitute a major concern for finfish health in Alaska.

Class IV diseases include those entities as yet undiscovered, six other agents that remain obscure regarding their importance to finfish health in Alaska, and all other nonsalmonid diseases that could become concerns in the future. As the need arises, each entity will be evaluated on a case-by-case basis. Four of the agents (excluding *Loma* and *C. shasta*) are as yet exotic to salmonids in Alaska and may necessitate destruction of infected stocks if detected. *C. shasta* is a serious pathogen of salmonids in the Pacific Northwest, and has been reported in salmonids from tributaries of the Yukon River. This agent as yet has not been detected in the usual Alaska State finfish transport proceedings and will not be routinely searched for. If this agent or any other entity in this category become a serious problem in Alaska, they will be treated as Category II diseases and sampled for accordingly.

The symbionts and predators listed in Shellfish Class II Nuisance Diseases are not adequately treated in Article 3, 5 AAC 41.080(e). Some of these nontarget species are unwanted exotics and if

Class II shellfish diseases in general, the state reserves the right to refuse certification or restrict movement of oysters if there is oyster mortality or significant disease associated with the prevalence of any agent(s).

Consequently, the following new section is recommended: Article 3, 5 AAC 41.080(h) should read:

(h) The presence of predators recognized in Class II shellfish diseases which may be exotic to Alaska will result in refusal of shellfish import certification by the Commissioner or his authorized designee until resubmitted representative samples of the shipment are free of nontarget invertebrate species. The Commissioner or his authorized designee also will refuse certification or restrict movement of oysters if there is oyster mortality or significant disease associated with the prevalence of any infectious agent(s).

Pursuant to Article 3, 5 AAC 41.100

The definition "fish pathology section" needs to be expanded to include the new Juneau fish pathology laboratory. Definition (3) under Article 3, 5 AAC 41.100, should read:

(3) "Fish pathology section" means the Alaska Department of Fish and Game, Fisheries Rehabilitation, Enhancement and Development Division, Fish Pathology Section located at: 333 Raspberry Road, Anchorage, Alaska 99502, telephone (907) 344-0541; and 3333 Old Glacier Highway, Juneau, Alaska 99802, telephone (907) 465-3577.

Fish transport permits for in-state movement, possession, etc., are required for shellfish as well as finfish since fish are defined in AS 16.05.940(6) to include all invertebrates and amphibians. However, this is not made clear in the ASRPNSH and should be explained by defining "fish" in this definitions section as it is within the Alaska Statutes except when

designated otherwise. Article 3, 5 AAC 41.100 should include an additional definition with the following changes:

> (14) "Fish" means any species of aquatic fish, invertebrates and amphibians in any stage of their life cycle found in or introduced into the state <u>except where specifically designated "finfish" or "shellfish."</u>

APPLICATIONS

The State of Alaska has, within its boundaries, large areas of separated watersheds supporting wild fish stocks which have never been examined for diseases. Consequently, there is a risk of unknowingly transporting presently undiscovered finfish diseases (in Alaska) from one major geographic area to another which may not be detected at the 5% level in 60 adult fish examined prior to transport. To minimize this risk the Department of Fish and Game will not advocate the transplant of wild finfish stocks between the major geographic zones designated as Southeast, Kodiak Island, Prince William Sound, Cook Inlet, Bristol Bay, AYK, and interior. To maintain consistency with the Alaska Department of Fish and Game Genetic Policy, and because wild fish stocks are in several hatchery water supplies, this disease policy will include hatchery stocks of fish as well, with exceptions considered only on a case-by-case basis. Proposals to do so must be for gametes only and accompanied by adequate justification for using a non-local stock and a FRED pathology disease history based on cultured fish having no detectable diseases in at least the last two consecutive years of screening a minimum of 150 adult fish and no diseases during rearing of their progeny.

A. WILD FISH TRANSPLANTS	DISEASE CONSIDERATIONS
1. BETWEEN WATERSHEDS WITHIN A DESIGNATED GEOGRAPHIC AREA	
a) Transplant of adult fish to a watershed barren of salmonids	(1) Prior year sampling recommended to define year to year variability in disease prevalence.
	(2) Sampling required in same year but prior to transplant of adult fish of stock.
	(3) Class II disease criteria:[a/] Bacterial Kidney Disease (BKD) - Cannot exceed levels in Schedule I (See Section IV, Appendix E). Furunculosis - Carrier state cannot exceed levels in Schedule I Infectious Hematopoietic Necrosis (IHN) - No samples required unless proposed transplants are IHNV susceptible salmonids from a sockeye or kokanee watershed since IHN disease has not been prevalent in salmonid species other than sockeye - All sockeye and kokanee are presumed carriers. Detection of IHNV in any salmonid other than sockeye/kokanee precludes use for transplant. Ichthyophthirius (ICH) - Not applicable unless present as a clinical disease, in which case consideration would be on a case-by-case basis. Enteric Redmouth (ERM) - A rare disease in Alaska because of which fish are not routinely screened for *Yersinia ruckeri*. Consequently, its dissemination is a significant concern when detected. If diagnosed, transplant of those fish would be decided on a case-by-case basis.

[a/] Classes I, III, and IV finfish diseases are addressed sufficiently in the regulation section.

A. WILD FISH TRANSPLANTS	DISEASE CONSIDERATIONS
1. BETWEEN WATERSHEDS (continued)	
b. Transplant of juvenile fish to a watershed barren of salmonids.	Class II disease criteria: BKD - No significant (defined on page 55 __ 0.5%/day) mortality and immediate disease history of hatchery performance cannot exceed levels in Schedule I. Furunculosis - As indicated by fluorescent antibody technique with confirmation by isolation. If the disease state exists, treat and release when mortality becomes insignificant and prevalence does not exceed Schedule I. If prevalence of infection exceeds Schedule I, fish cannot be released. IHN (sockeye, kokanee) - Release if no disease. Clinical signs of IHN and isolation of virus will require destruction of affected lots. Lots which are virus negative may be released as soon as posible. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Detection of IHNV necessitates destruction. Operator of a facility that has IHNV detected must demonstrate that remaining stocks have been sufficiently isolated to prevent cross contamination; that is, the facility must have been qualified for acceptance at least as a PQU. ERM - Same as for adult fish except if diagnosed in the diseased state with significant mortality, destruction of the lot may be required. Ich - Saltwater release allowed. Freshwater release: treat and release as soon as practical to minimize exposure of other hatchery stocks.

1. BETWEEN WATERSHEDS (continued)

c. Transplant of adults, juveniles or eggs, to a watershed containing other "significant" stocks of salmonids.

(1) Stocks to be transplanted:

Juveniles and eggs
- If no disease history then prior year samples from spawning or post-spawned adult fish recommended.

Adults
- If no disease history then samples of adult fish (preferably post-spawned) stock to be transplanted required prior to transplant in year of transport.

(2) Stocks in receiving watershed:

If stocks to be transplanted are negative for finfish pathogens then there is no need to sample stock for disease in the recipient watershed. If pathogens are detected in donor fish or the intent is to establish a broodstock source then the following applies. Prior year sampling of resident fish is strongly recommended. Sampling should include all stocks determined to be significant by area biologists. In order to develop a disease history, stocks in receiving watershed should have 60 samples collected from adult fish (preferably post-spawned) for examination. If, for the purpose of transplanting fish stocks having a known carrier state of a fish pathogen, 60 resident fish are not available for examination, then the latter stocks are presumed negative for all pathogens. In any case Class II criteria below apply.

(3) Class II disease criteria:

BKD and Furunculosis - If stocks in receiving watershed have zero prevalence, then stock proposed for transplant must also have zero prevalence (min. sample size = 60). Responsibility for obtaining a 60 adult fish sample rests with the applicant. If adequate transplant sample numbers are unavailable, the transplant cannot be made.

A. WILD FISH TRANSPLANTS	DISEASE CONSIDERATIONS
1. BETWEEN WATERSHEDS (continued)	If any stock in the receiving watershed is positive for BKD or furunculosis, then the stock proposed for transplant must not exceed levels in Schedule I. (A. salmonicida in the receiving and donor watersheds must be confirmed by culture. This is due to non-specific fluorescence encountered in FAT.) IHN - No samples required for sockeye or kokanee except for establishment of a disease history: all stocks are presumed carriers. Transplant of sockeye or kokanee into non-sockeye systems having IHNV susceptible species is not advocated and will be evaluated on a case-by-case basis regarding: average titer and prevalence of virus in the stock to be transplanted and the resource value of the susceptible species at risk in the recipient or nearby watersheds. Transplant of IHNV-susceptible species to a watershed containing sockeye or kokanee would also be evaluated on a case-by- case basis and may not necessarily be rejected solely on the basis of fish health concerns. Applicant and resource managers must be willing to accept the possible loss of transplanted fish or condemnation of the stock due to IHNV. Transplant of chinook, chum, rainbow, steelhead, or cutthroat into a non-sockeye system from a system with sockeye will require virus sampling. Any virus positive stock would be disqualified. However, if virus negative, these species would be presumed IHNV carriers, and decision criteria for sockeye and kokanee transplants would apply. Ich - If there is a disease history of Ich then no transplant is permitted unless receiving waters also have a history of Ich. ERM - Same as for BKD and furunculosis except if diagnosed in the diseased state with significant mortality, destruction of the lot may be required.

A. WILD FISH TRANSPLANTS		DISEASE CONSIDERATIONS
2. TO A HATCHERY		
a. Quarantine Unit (QU) (see Section IV, Appendix A)	(1)	Class II disease criteria. No constraints for pathogens in carrier state since they will be in isolation.
b. Other than a QU	(1)	Class II disease criteria. If no other stocks are present at hatchery, criteria in Section A.1.a.(3) apply. If other stocks are present in the hatchery and their disease histories are negative for pathogens, then the transplanted stock history must be negative. If other stocks are present in the hatchery and they have a history of BKD, furunculosis or ERM, then the transplanted stock must meet the criteria for Schedule I. If a pathology-approved Partial Quarantine Unit (PQU-Section IV, Appendix A) is to be used, then other stocks at the hatchery are not a concern. In either case (except effluent depuration (page 38) in a PQU), if there are wild salmonids present in the hatchery watershed criteria in A.1.c apply.

B. BROODSTOCK SCREENING FOR EGG-TAKES [i/]	DISEASE CONSIDERATIONS
1. EGG-TAKE AT HATCHERY (indigenous stock)	
a. For release of progeny at hatchery.	As long as an acceptable disease history (Schedule I) within the broodstock has been established and fry performance has indicated no disease concerns, no disease screening required, but recommended every other year. Disease outbreaks in juveniles and/or significantly high levels of a Class II pathogen in broodstock may require corrective action and more sampling.
b. For release of progeny at another site.	Samples can be taken in year prior to initial eggtake.
	Class II disease criteria:
	BKD - Prevalence in brood source cannot exceed levels in Schedule I. Depending upon the circumstances, Family Tracking[ii/] may be an acceptable alternative.
	Furunculosis and ERM - Not considered (B.2.a) unless (1) there has been recent problems within the disease histories or (2) it is a new stock without prior disease history, in which case screening should be done for a disease history.
	IHN (Sockeye, Kokanee) - Sample size = 60 adult (postspawned) fish in prior year for establishing population prevalence - ripe fish can be used thereafter at the eggtake. For small-scale eggtake Family Tracking Method preferred, with elimination of eggs from high titer ($\geq 10^4$) females or fertilized by high titer males. In rare instances, when numbers permit, eggs from any mating of virus positive parents should be destroyed.
	IHN (chum, chinook, steelhead, rainbow, cutthroat) - Screening for IHNV would not be routine in indigenous non-sockeye hatchery stocks unless IHN disease or other virus exposure is suspected. For large scale eggtakes, sampling in year prior is recommended. Any detection of IHNV would disqualify the broodstock.

[i/] Note: The following "Disease Considerations" regarding BKD are in lieu of the preferred use of fish stocks having no history of the agent causing this disease. Toleration of the Schedule I minimal levels of this disease agent in stocks used at any facility is condoned only if: an alternative stock(s) is unavailable; other circumstances specific to on-going programs leaves no practical alternative; other corrective procedures such as Family Tracking are practiced to mitigate disease concerns. (see Section IV, Appendix E, Schedule I Rationale)

[ii/] For small populations of less than 1,000 where a sample of 60 adult fish in one year would constitute a significant loss, alterative arrangements can be made with Pathology. The recommended procedure is to sample fish over a period of years prior to the proposed egg-take. Under well justified circumstances another method might be approved. This is called Family Tracking and requires sampling at the time of eggtake. Family tracking involves keeping egg lots separate during water hardening, disinfection, and incubation in Heath Trays until testing of individual parents is completed. Egg lots from disease-positive parents are discarded.

B. BROODSTOCK SCREENING FOR EGG-TAKES	DISEASE CONSIDERATIONS
2. EGG-TAKE AT A SITE REMOTE FROM HATCHERY	
a. For stocking of progeny back to system of origin	Class II disease criteria. - approved QU - no constraints. - Non-QU (sampling required but recommended in year prior to egg take). BKD - Prevalence in brood source cannot exceed levels in Schedule I. For hatcheries requiring reuse or recirculation of water, the consequences of bringing BKD in from outside could not be tolerated. If feasible, Family Tracking would be used or a known BKD-negative stock would be required. Furunculosis and ERM - No specific limitation. Pathology does not recommend that high-risk stocks be used if there are alternatives. Egg disinfection is required; Pathology may monitor/assist at eggtake, and may require fry samples prior to release depending upon fry performance. At present, there is no evidence to indicate that vertical transmission of either A. salmonicida or the ERM agent occurs WITHIN the eggs of salmonids. Consequently, eggs from a low number of carrier brood should pose no additional risk if rigorous external disinfection is practiced. However, the risk of inadequate egg disinfection would increase with increasing numbers of carrier broodfish. IHN (sockeye, kokanee) - Sample size = 60 post-spawning adult fish in year prior, required for disease history information; specific precautions to be recommended by Pathology will relate to facility type, location, and fish handling capabilities. All sockeye are presumed carriers. Once a disease history is established for a particular stock, subsequent sampling may only include 60 of those fish actually used in the eggtake in order to monitor the virus prevalence actually brought into the hatchery.

B. BROODSTOCK SCREENING FOR EGG-TAKES	DISEASE CONSIDERATIONS
2. EGG-TAKE AT A SITE REMOTE FROM HATCHERY (continued)	IHN (chum, steelhead, rainbow, chinook, cutthroat) - In a system with sockeye - 60 samples from the desired susceptible species (post-spawners) are required in year prior. Any incidence of IHNV in sample precludes using that stock for eggs. Ich - Not applicable.
b. For release at the hatchery or c. For release at a remote site	Same criteria as B.1.b. Also, IHNV susceptible species other than sockeye from sockeye systems are not advocated for use and will be considered on a case-by-case basis.
d. Stock originating from hatchery fish at remote site for release into barren system.	Same criteria as A.1.b and C.3.b.
e. Stock originating from hatchery fish at remote site for release to a system with salmonids.	Same criteria as A.1.c. and C.4.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
1. AT THE HATCHERY SITE	Pre-release examination of juvenile fish will not be performed as a general rule unless: mortality or another clinical disease sign or otherwise poor performance anytime prior to release warrants concern by the Fish Pathology Section; the broodstock disease history at eggtake was positive for BKD at levels greater than Schedule I and Family Tracking was not practiced. Class II disease criteria: BKD - If no significant mortality, no restriction; A total cumulative mortality equal to or greater than 5% in 90 days prior to release attributable to BKD will preclude release. It is the long-range goal that all

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
1. AT THE HATCHERY SITE (continued)	facilities surpass or meet the minimal subclinical detection criteria in Schedule I. Facilities that do not meet Schedule I limits but have total cumulative mortalities of less than 5% in the 90 days immediately prior to release can release provided they develop a plan that will alter the physical plant and/or operation to assure meeting the limits of Schedule I within 6 years from date of adoption of these policies. Furunculosis - Must be treated until mortality reaches background level (.03%/day/lot of fish), then release will be allowed. IHN - (sockeye, kokanee) Infected lots, as determined by clinical signs and/or detection of IHNV must be immediately destroyed. Lots which are negative for virus may be released as soon as possible. Any further outbreaks will require destruction of additional affected lots. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Same as sockeye except finding of IHNV in fry is sufficient cause for destruction of the inventory of that stock unless demonstrated that lots within that stock have been sufficiently isolated and unexposed to the virus. Also, operator of a facility that has a positive diagnosis of IHNV must demonstrate that sufficient isolation has been maintained to assure that other susceptible stocks have not been contaminated. Otherwise, the disposition of the exposed stock(s) may also be in jeopardy. ERM - If diagnosed in disease state with significant mortality, elimination of a stock may be required, depending upon circumstances. Ich - Treat prior to release.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
2. RETURN TO SYSTEM OF ORIGIN	Class II disease criteria: BKD -If broodstock was negative, juveniles are assumed negative unless examination prompted by mortality or other poor hatchery performance reveals otherwise. In this case, release will not be recommended unless the broodstock and not the hatchery water supply (such as in a QU or PQU) is determined to actually have been positive in which case release will be considered on a case-by-case basis. If the broodstock were screened and had positive samples exceeding Schedule I and resultant egg lots were not culled by Family Tracking then a 60 fish prerelease sample of juveniles will be required and cannot exceed Schedule I for release authorization. Furunculosis - If the disease state is present, treat and release when mortality returns to background level and prevalence does not exceed Schedule I. However, if brood source had no confirmed history of A. salmonicida, release (to the system of origin) of positive juveniles in the carrier state will not be authorized. IHN (sockeye, kokanee) - Infected lots with clinical signs of disease or detectable virus must be destroyed. Virus negative lots may be released as soon as possible. Further diagnosis of IHNV or increases in mortality in additional lots will necessitate their destruction. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Same as sockeye except finding of IHNV in fry is sufficient cause for destruction of the inventory of that stock unless demonstrated that lots within that stock have been sufficiently isolated and unexposed to the virus. Also, operator of a facility that has a positive diagnosis of IHNV must demonstrate that sufficient isolation has been maintained to assure that

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE		DISEASE CONSIDERATIONS
2. RETURN TO SYSTEM OF ORIGIN (continued)		other susceptible stocks have not been contaminated. Otherwise, the disposition of the exposed stock(s) may also be in jeopardy. ERM - If diagnosed in the disease state with significant mortality, elimination of a stock may be required depending upon circumstances. If detected in the carrier state and the brood source had no confirmed history of the ERM agent, release of juveniles back into the system of origin will not be authorized. Ich - Saltwater release allowed. Freshwater release may be allowed on a case-by-case basis as quickly as practical after treatment to minimize exposure of other hatchery stocks.
3. TO BARREN SYSTEMS (no salmonids)		
a. Closed system (landlocked lake)	(1)	A closed or landlocked lake has no surface drainage or connection to an anadromous stream.
	(2)	Class II disease criteria: ERM - If detected in a carrier state, transplant would be decided on a case-by-case basis. If diagnosed in a disease state with significant mortality, destruction of the lot(s) may be required. All other Class II diseases - no restriction for pathogen in carrier state. Release of fish in the diseased state (excluding ERM) would be considered for research purposes only.
b. Open system		Class II disease criteria: BKD - No significant mortality and immediate disease history of hatchery performance cannot exceed levels in Schedule I.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
3. TO BARREN SYSTEMS (no salmonids) (continued)	Furunculosis - As indicated by fluorescent antibody technique with confirmation by isolation. If the disease state exists, treat and release when mortality becomes insignificant and prevalence does not exceed Schedule I. If prevalence of infection exceeds Schedule I, fish cannot be released. IHN (sockeye, kokanee) - Release if no disease. Clinical signs of IHN and isolation of virus will require destruction of affected lots. Release virus negative lots as soon as possible. Subsequent to release, destroy any additional lots that show high mortality and clinical signs of IHN or yield virus on isolation. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Detection of IHNV necessitates destruction. Operator of a facility that has IHNV detected must demonstrate that remaining stocks have been sufficiently isolated to prevent cross contamination; that is, the facility must have been qualified for acceptance at least as a PQU. ERM - Same as for C.3.a. Ich - Saltwater release allowed. Freshwater release: treat and release as soon as practical to minimize exposure of other hatchery stocks.
4. TO SYSTEMS WITH OTHER "SIGNIFICANT" STOCKS OF SALMONIDS a. Closed system (landlocked lake) b. Open System	Class II disease criteria: BKD - If detected within the prior 2 years of stock disease history or within the present inventory of juveniles prior to release then those juveniles cannot be released unless other species or stocks at release site or upstream in the tributary of release also have a history of BKD, in which case the carrier state in released juveniles cannot exceed levels in Schedule I. Release is not allowed if the disease state exists as indicated by significant BKD related mortality occurring within 90 days prior to release date.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
4. TO SYSTEMS WITH OTHER SALMONIDS (continued)	Furunculosis - If detected in the present inventory of juveniles prior to release then those juveniles cannot be released unless other species or stocks at release site or upstream in the tributary of release also have a history of the causative agent, in which case released juveniles cannot exceed levels in Schedule I. If disease state exists fish must be treated until mortality is insignificant and carrier state does not exceed Schedule I. IHN (sockeye, kokanee) - Release allowed as long as no clinical signs of disease are present or virus can be isolated. Release into non-sockeye systems having IHNV susceptible species is not advocated and will be evaluated on a case-by-case basis. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Detection of IHNV will require destruction of lot and possible entire inventory of that stock and others unless operator can demonstrate sufficient isolation from infected lots to prevent cross contamination. Transplant of chinook, chum, rainbow or steelhead into a non-sockeye system from a hatchery on a sockeye system will be evaluated according to sockeye transplant criteria <u>if</u> such a stock has not been adequately isolated and/or has been exposed to a water supply containing rearing or spawning sockeye during any period of its life cycle. ERM - Same as for furunculosis except if diagnosed in a disease state with significant mortality then destruction of the lot may be required depending upon circumstances. Ich - Saltwater release allowed. - Freshwater release may be allowed on a case-by-case basis as quickly as practical after treatment to minimize exposure of other hatchery stocks.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
5. REMOTE SALTWATER RELEASE FOR TERMINAL FISHERIES	Class II disease criteria: BKD and Furunculosis - An exception to the Schedule I carrier rate criteria may be made on a case-by-case basis when involving large inventories of presmolts destined for release into a "mop up" terminal harvest fishery. Depending upon the fishery, there may be little disease risk to natural stocks since the surviving adult returns are almost completely harvested by the commercial fleet before entering freshwater. Release of smolts would not be allowed if clinical disease exists as indicated by a ≧5% cumulative mortality occurring within 90 days prior to saltwater rearing.

D. TRANSFERS BETWEEN HATCHERIES	DISEASE CONSIDERATIONS
1. EGGS	Class II disease criteria: BKD - Not allowed unless the receiving hatchery has a history of BKD and the donor broodstock disease history must meet Schedule I. An exception would be the use of a BKD positive broodstock from one facility as a source of eggs for several other facilities in which case the Family Tracking method would be used. Eggs from BKD positive parents would be destroyed before transport or while in isolation at the receiving facility. This methodology should also reduce the carrier rate to acceptable limits within broodstock returning to the parent facility within 2 to 3 years. Furunculosis - Eggs from high risk stocks not recommended if alternative sources exist. However, no restrictions for reasons previously stated (B.2.a).

D. TRANSFERS BETWEEN HATCHERIES	DISEASE CONSIDERATIONS
1. EGGS (continued)	IHN (sockeye, kokanee) - If receiving facility would qualify to take eggs directly from broodstock, then it would qualify to receive eggs from another facility. IHN (chum, chinook, steelhead, rainbow, cutthroat) - Eggs from IHNV susceptable species from a sockeye facility are not recommended for transfer to a non-sockeye facility unless the receiving facility is a QU or the stock has been adequately isolated and not exposed to a water supply containing rearing or spawning sockeye during any period of its life cycle. ERM - Same as for furunculosis. Ich - Not applicable.
2. FISH (from hatchery to hatchery, excluding a QU).	Class II disease criteria: BKD - Not allowed if fish to be transferred have had BKD or if the agent of BKD has been detected within the previous two years of stock disease history unless receiving facility has a history of BKD, in which case the detection level in the juveniles to be transferred cannot exceed Schedule I and no significant BKD related mortality can have occurred. Furunculosis - Not allowed if fish to be transferred have had furunculosis unless receiving facility has a history of furunculosis, in which case the detection level in the juveniles to be transferred cannot exceed Schedule I and no significant related mortality can have occurred. IHN (sockeye, kokanee) - Can be transferred to another sockeye facility unless there are clinical signs of IHN confirmed by virus isolation. - Not permitted to a non-QU which contains susceptible species (chum, chinook, steelhead, rainbow, or cutthroat).

D. TRANSFERS BETWEEN HATCHERIES	DISEASE CONSIDERATIONS
2. FISH (from hatchery to hatchery, excluding a QU). (continued)	IHN (chum, chinook, steelhead, rainbow, cutthroat) - Can be transferred from a non-sockeye facility to a sockeye facility if a QU where they can be reared in an IHN-virus-free water supply and are not intended for return to the same site as the sockeye returns. Screening for IHNV in susceptible species other than sockeye is presently not necessary from non-sockeye water supplies unless clinical disease suggestive of IHN is present. Clinical disease with isolation of IHNV will result in the destruction of any fish stocks. - IHNV susceptible stocks cannot be transferred from a non-QU sockeye facility to a non-sockeye facility having other susceptible species or stocks unless this facility is also a QU. ERM - Same as furunculosis except diseased fish sustaining significant mortality may have to be destroyed depending upon circumstances. Ich - Not allowed if the fish to be transferred have had an outbreak of Ich unless the receiving facility also has a history of Ich in its water supply. In the latter case, the fish for transfer must not be sustaining significant mortalities, otherwise treatment and holding of fish will be necessary at the donor facility until mortalities fall within background levels.

Sockeye Salmon Culture

Issue:

Artificial propagation of sockeye salmon is seriously limited by infectious hematopoietic necrosis (IHN). This disease has caused catastrophic mortalities of sockeye salmon in the State. IHN is caused by a rhabdovirus which can adapt to and infect other salmonid species besides sockeye salmon. Consequently, the virus has been isolated from Alaskan chinook and chum salmon and has caused mass mortalities of chinook salmon and rainbow and steelhead trout in other states as well as rainbow trout and chum salmon in Japan. Careful monitoring is needed in Alaska due to the potential for the virus to adapt and infect other IHNV susceptible fish species as well as sockeye salmon.

Policy:

Following the 1980 IHN epizootics, the most logical disease control concepts and techniques applicable to sockeye salmon culture were assembled into a departmental Sockeye Salmon Culture Policy Statement[a]. This policy has undergone some revision since then but in many instances remains unchanged.

Understanding the following policy statements will minimize future mortalities due to IHN and enhance the success of sockeye salmon hatcheries. Much of the policy is based on the following knowledge gained through IHN virus (IHNV) disease-control research, and will be periodically revised as data and technology suggest.

1. Water-borne transmission occurs. Fish shed IHNV from the anal vent and in reproductive products during spawning.

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[a] These guidelines were developed by a team of FRED staff including R. Burkett (Chairman), R. Saft, J. Burke, J. Sullivan and B. Kepshire.

Water-borne virus can enter previously uninfected salmon via infection of the gills or skin. Crowding sockeye facilitates this horizontal infection between fish, which increases IHNV prevalence.

2. The quantity of IHNV in gonadal fluids can range from no detectable virus to $\geq 10^8$ infectious viral units per ml. The current assumption is that the more virus present, the greater the disease risk. Eggs from a single female containing large amounts of virus may transmit the virus, upon hatching, throughout a common incubator. Compartmentalization and good environmental sanitation can limit the magnitude of mortality when IHN occurs.

3. Sockeye stocks are carriers of IHNV which can be vertically transmitted. Iodophor disinfection of eggs does not kill all virus associated with eggs or ovarian fluids that contain higher quantities of virus. Hatcheries with sockeye stocks will normally have some covert virus within the facility. Excessive stress may precipitate a change from the carrier state to the disease state. This change usually results in mass mortality among the fish involved. Actions or events causing stress include poor incubator performance, marginal water quality or supplies, and excessive handling, grading, and marking.

Hatchery Water Supply:

Virus-free water is required and may be achieved by use of well water, hanging lakes having no resident salmonids or depuration of a suspect water supply.

Species Mix Within a Hatchery:

In Alaska, the disease state (IHN) is becoming more frequent in salmonid species other than sockeye. Combinations of chinook and chum salmon, and steelhead or rainbow trout are allowed within a

facility. However, when sockeye are present, none of these other known susceptible species will be allowed in the same facility unless the Department determines that the design and operation of the facility precludes interspecies transmission of the virus. This is to prevent virus infection and possible adaption with resultant mortality of species other than sockeye, both in the hatchery and in local feral populations.

Equipment, Supplies, and Personnel Movement:

1. Equipment between hatcheries: Hatcheries containing sockeye salmon will have little exchange of equipment to or from other hatcheries. Only items that can be adequately disinfected and must be moved from a sockeye hatchery will be moved. These items must be cleaned and disinfected, using at least 200 ppm chlorine or iodophor solutions for 10 minutes or live stream, at the shipping hatchery and similarly disinfected at the receiving hatchery.

   Equipment that cannot be effectively disinfected will not be moved from a hatchery culturing sockeye salmon.

2. Equipment within hatcheries: No movement of equipment within a hatchery between established compartments, where one compartment or several compartments contain different sockeye stocks, will be allowed without adequate disinfection (as above). A stock is defined for the purpose of this document as a distinct spawning population having the same water supply.

   Equipment that cannot be effectively disinfected, such as that containing wood will not be moved between stock compartments.

3. Supplies: Most supplies and materials are not readily disinfected and will not be moved from facilities culturing sockeye salmon.

4. Personnel: Personnel entering or leaving a hatchery that cultures sockeye salmon will go through a disinfection foot bath (same strength as above). Protective clothing, such as rain gear and spawning gloves will be kept separate from other clothing used in the field, etc., and will be left in the work place before leaving through the footbath. This procedure will be used by all visitors and hatchery personnel.

Egg-take Procedures:

The following procedures will be incorporated into any sockeye salmon egg-take:

1. Eggs and sperm of individual fish will be collected in separate disinfected containers or disposable bags or combined immediately in the same container in the desired fertilization ratio. If numbers of males permit, a 1:1 fertilization ratio is recommended.

2. Eggs will be fertilized, water hardened, and rinsed (if necessary) in virus-free water such as well water, depurated (UV or ozone) water, or surface water not exposed to sockeye salmon at any time during the year. This will require either separate collection and transportation of gametes from remote egg-take sites to a suitable processing site (a hatchery) or the use of known virus-free water transported to or available at the site.

3. Eggs will be disinfected during water hardening in a 100 ppm iodophor solution for 1 hour.

4. Adult sockeye salmon will not be severely crowded in any holding structure. Crowding causes stress and facilitates the spread of IHNV to all of the contained fish. Adult exposure to IHNV at remote areas will be minimized by removing dead and moribund sockeye from areas of broodstock holding with disposal of carcasses at a remote distance

downstream from the egg take. The ventral surface of all fish will be disinfected prior to egg or sperm stripping with a solution of iodophor (100 ppm). This may be applied with a sponge or paper towel. Disinfectant must be wiped from these surfaces immediately prior to spawning with a clean paper towel.

5. Egg takes may be restricted to the early and middle portions of the run because in some stocks, the later spawning fish tend to have higher prevalences as well as titers of IHNV. Depending upon genetic and virus prevalence concerns, egg-takes from each sockeye stock can be evaluated on a case-by-case basis.

6. Any eggs or seminal fluids that are of questionable appearance will be discarded.

7. Utensils, spawning gloves, knives, and other items coming in contact with fish will be disinfected (200 ppm chlorine or 100 ppm iodophor) between uses for each fish. Each utensil should then be rinsed in water after disinfection and before its next use.

8. Eggs, when seeded for hatching, should be distributed with substrate (saddles recommended) at densities well below the known optimal number for sockeye in whatever incubator type used. These low densities will optimize survival and minimize the number of eggs in each incubator (i.e., eggs from a given stock should be spread as equally as possible throughout all incubators available to that stock, and not crowded into several incubators while leaving others unused). Production goals will be adjusted accordingly.

Isolation of Stocks, Incubation and Rearing:

1. Physical separation or compartmentalization of sockeye stocks will be provided to the maximal extent practical

during all stages of incubation and rearing. The emphasis of sockeye culture will be on the quality of fish produced, not production numbers alone.

2. Each incubator unit or stack and rearing container will be serviced by a separate incurrent and efferent water flow.

3. If fish are reared, a designated group of incubators (or incubator) will be assigned to a single distinct rearing container that will serve that incubator grouping alone.

4. Direct release or minimal rearing will be practiced when possible.

5. Heating of water for egg incubation or fry rearing is potentially stressful and is not recommended, unless inadequate temperature units make this necessary.

6. Disinfection of utensils, gloves, dip nets, etc., will occur between contacts with different incubation, holding, or rearing containers.

7. A flush treatment of iodophor (100 ppm) is recommended after eggs are picked to reduce numbers of potential residual IHN virus particles released from infected dead eggs. Periodic formalin drips as recommended for fungus control would also be an effective alternative treatment.

8. Periodic floor cleaning with steam or disinfectant (200 ppm chlorine or 100 ppm iodophor) will be done as needed to maintain high levels of environmental sanitation. Do not use a combination of steam and disinfectant as this will present a major health hazard to hatchery personnel. Periodic disinfection will eliminate organic debris that may retain virus.

9. Containers, pumps, hoses, and other devices coming in contact with or containing salmon will be disinfected after use.

10. The hatchery will be disinfected after an IHNV-induced mortality (see IV C. Disinfection for Hatcheries). Portions of a facility that are physically and operationally separate, including the water supply, may be considered a different hatchery.

Transplanting of sockeye salmon.

1. Sockeye salmon will not be transplanted to any watershed upstream of a hatchery water intake or allowed to enter there naturally if sockeye or other susceptible salmonids are cultured in the hatchery. Exception would be allowed if the water is depurated with ultraviolet radiation or ozone prior to exposure to hatchery fish, or if an alternate virus-free water source exists.

2. Any stock of sockeye salmon experiencing clinical signs and mortality related to IHNV or from which virus can be isolated will be destroyed immediately to facilitate containment of the disease and prevent contamination of other stocks or lots of fish.

Shellfish Culture

Oysters:

1. Importation of oysters from outside Alaska.

   a. Only oyster stocks from those vendors having a hatchery broodstock will be evaluated for certification of imported oyster spat. A continual change in brood source or use of multiple sources practiced by some vendors would require a

complete certification of all stocks or complete certification every year rather than a certification renewal. This would be a needless cost of time and expense to the Fish Pathology Section and an unnecessary additional risk that pathogens would be missed when other single developed stocks with an established disease history are available.

b. Certification sample sizes for adults and juveniles will follow the American Fisheries Society procedures manual (Amos 1985). These will require a random sample of 200 spat (and approximately 1000 larvae if available) and 60 adult brood stock (numbers sufficient to determine carrier prevalence of approximately 2-5% at a 5% error) prior to shipment.

c. Certification renewal will be on a yearly basis and will require examination of 60 spat and/or larvae from the year class to be imported and an updated disease history and hatchery performance review of the hatchery stocks from the vendor for the previous growing season. A certification will become invalid if a disease outbreak occurs within stocks at the facility or if an uncertified stock is brought into the rearing facility or grow-out areas.

d. Any disease agent listed in Class I or known to be causing mortality or significant disease will disqualify the lot and prevent issuance of a Fish Transport Permit (FTP).

e. All lots must be free of predators.

f. Each stock should have a disease history (preferably testing from previous transports outside Alaska).

g. Live oysters from Korea, the Gulf of Mexico, and the Atlantic Coast of North America may not be imported into Alaska for cultural purposes pursuant to Article 3, 5 AAC 41.070(b).

2. Movement of oysters or indigenous shellfish stocks within the state for cultural purposes.

a. 30 live animals will be examined before movement is authorized.

b. Such would also include subsequent movement of animals imported into Alaska; i.e., stock would be sampled prior to import, and then re-sampled if they were to be moved again at a later date. If a setting station or hatchery is established for wide distribution of spat or juvenile shellfish then the seawater influent should be depurated to reduce risk of disseminating indigenous shellfish diseases.

c. The definition of what constitutes "movement" (how far) relative to the need for primary or additional testing is defined by the discreteness of stocks or populations with regard to dispersal by ocean currents, etc. If this cannot be determined, any movement regardless of distance will require pathology evaluation. Any movement will require an FTP.

d. Additional criteria for approval following disease outbreaks, etc., are the same as described under disease control for shellfish in II-F.

3. Oyster Facility Inspections

a. Annual sampling of 30 animals/lot/year class will not be required but is recommended for establishment of endemic disease histories.

b. Additional sampling will be required if mortalities exceed usual background levels or if abnormal animals are observed.

c. In either case, if a disease agent exotic to North America is present, the stock would be considered for destruction and if any other Class I diseases are detected, the disposition of those infected stocks will be decided on a case-by-case basis.

4. PMFC Agreement (copy attached)

Alaska is a signatory to this agreement. Final decisions must be consistent with this agreement.

5. Shellfish other than oysters

Importation of any shellfish species other than oysters into Alaska is prohibited.

APPENDIX

APPENDIX - GENERAL GUIDELINES

Quarantine Unit Fish Hatcheries

Introduction:

Hatcheries are often used to support projects that require transport of fish or gametes from remote sites to the hatchery. Any movement of fish between areas raises concern that pathogens may be spread. Consequently, such risk dictates that measures be taken to minimize the inadvertent dissemination of diseases.

Disease screening and disinfection play major roles in reducing the risk of spreading pathogens. However, testing is usually limited to a few diseases of highest concern and testing can be ineffective in the detection of carrier-state levels of disease. To provide additional protection for other hatchery stocks, the hatchery should be able to isolate the remote stock from others in the facility through incubation and rearing. Varying levels of isolation can be achieved through use of physical barriers and other safeguards in the hatchery's design. Isolation capability falls into three categories ranging from almost none to quarantine levels. It should be stressed, however, that no design is fail-safe; its efficacy is determined by the operating procedures and the commitment of the hatchery personnel to carrying out these procedures.

Definitions:

Three levels of isolation are described based on the efficacy of the hatchery design in providing barriers to the transfer of pathogens within the hatchery and outside to local wild stocks. The most effective design is the Quarantine Unit (QU) which provides strict isolation. The second design has significant safeguards and is called a Partial Quarantine Unit (PQU). Those hatcheries that cannot meet the criteria of the two isolation units fall into the third category: conventional hatchery. If disease appears in any stock within a conventional hatchery, all stocks are at a higher risk of being exposed than if they were in a quarantine unit.

	Quarantine Unit	Partial Quarantine Unit
Water Source	well, spring, or depurated having no Class I or II pathogens.	no Class I or II pathogens detected in water source, not accessible to anadromous fish; i.e., barriered lakes or streams.
Isolation Measures	-stocks separated by physical barrier during incubation.	-no physical separation of stocks by a barrier during incubation.
	-no water transfer between stocks during incubation or rearing.	-no water transfer between stocks during incubation or rearing.
	-rearing units will be in separate rooms for each stock.	-physical separation between rearing units.
	-thorough disinfection of unit and its equipment prior to introduction of new stock.	-thorough disinfection of unit and its equipment prior to introduction of new stock.
	-Separate footwear and outerwear to be left in each isolation unit/rearing room. Footbaths used when necessary.	-disinfection of footwear using footbaths upon entering and exiting isolation unit.
Effluent	-depuration	-depuration may or may not be required.
Equipment	-separate for each incubation and rearing unit	-separate for each incubation and rearing unit

Pathology guidelines clearly encourage development of quarantine units in hatcheries supporting remote projects. If disease occurs in a facility without quarantine capability, releases may not be authorized. At the very least, extensive testing and waiting periods may be involved before fish can be certified for release. Development of quarantine facilities is an important investment in controlling pathogen spread, particularly in Alaska, where wild stocks are so valuable.

Classification:

Hatcheries involved in offsite projects will be classified according to their ability to meet the quarantine criteria. A FRED pathologist will determine the facility's classification after making an on-site visit. The Pathology Laboratory recommends either ultraviolet or chlorination-dechlorination depuration systems. Ultraviolet units should have a minimum rating of 30,000 microwatt seconds/cm^2 after 7,500 hours of lamp operation. Any chlorination system should deliver at least a 2 ppm residual level of chlorine with a 1 minute contact time before dechlorination with sodium thiosulfate or sulfur dioxide gas. The hatchery operator will be responsible for ensuring that operational procedures necessary for quarantine culture are followed. Failure to do so will trigger the potential for reclassification.

Drugs and Other Chemicals Used in Aquaculture

Drugs and other chemicals are used in aquaculture for a number of purposes. Many uses include treatment of the water to improve water quality, remove or control aquatic algae or vegetation, eradicate nuisance fish species or aquatic invertebrates, or immobilize fish (anesthetics). However, this discussion will address only those chemicals recommended by the FRED Pathology Section for use in the State of Alaska to control salmonid fish pathogens and to improve fish health. These chemicals fall into

the general categories of drugs and disinfectants. Disinfectants include chemicals that destroy the pathogen on contact whether the pathogen is on the fish or an inanimate object. Drugs are normally fed to fish to treat systemic infections but some are also used to treat external infections.

The use of drugs is generally regulated by the Food and Drug Administration (FDA) while other chemicals are controlled by the Environmental Protection Agency (EPA). Generally, any use that has a medical claim or could affect the safety of the food consumed is regulated by the FDA. Uses that affect animal safety or environment are regulated by the EPA. Because there is some overlap of these uses in the aquatic environment, there is some confusion as to which agency approves a drug or other chemical use. In either case, the compounds must be shown to be effective and safe. Safety applies to the welfare of the fish being treated, non-target fish, and other aquatic plants and animals. The FDA also requires that treated fish are safe for human consumption.

There are a few chemicals that do not require registration by either agency; such chemicals are "approved" because they have been in use for a number of years and are considered "generally regarded as safe" (GRAS).

The following is a description of the drugs and other chemical compounds advocated for fisheries use in Alaska. The suppliers, when provided, are those approved to distribute specific compounds for fisheries use and are the legal sources. Some of the compounds listed below (Quaternary Ammonias and Iodophors) are not yet federally approved or prohibited for general or specific uses in aquaculture and do not have satisfactory substitutes. Consequently, the state will continue their use.

Antibiotics:

The only FDA approved antibiotic for foodfish use is oxytetracycline (Terramycin, Pfizer, Inc., New York, NY). Terramycin is approved only for treatment of Aeromonas, Pseudomonas, and Hemophilus infections at 2.5-3.75 g/45 kg of fish per day for 10 days in the feed. It is approved only for salmonids and catfish and there is a 21-day withdrawal period before the fish can be slaughtered or released for stocking or potential immediate human consumption. Terramycin has also been used widely for treatment of Vibrio, sp., and systemic myxobacterial infection, but these are not federally approved uses of the drug.

Sulfonamides:

Two sulfonamides are FDA approved for foodfish use, sulfamerazine (American Cyananimid Co., Princeton, New Jersey) and a potentiated sulfonamide which is a combination of sulfadimethoxine and ormetoprim (Romet-30, Hoffman-LaRoche, Inc., Nutley, New Jersey).

Sulfamerazine. Sulfamerazine is approved for the treatment of furunculosis in salmon at 10g/45 kg of fish per day for 14 days in the diet. It is approved only for salmonids and there is a 21-day withdrawal period before the fish can be released or slaughtered. Although the FDA approval is limited, the drug has been used widely in fisheries for both foodfish and non-foodfish. Furthermore, generic sources of sulfamerazine are widely available, which has resulted in substitution of other sulfonamides. Consequently, the demand and cost recovery for the approved product have dropped, forcing the manufacturer above to discontinue producing the drug for fisheries use.

Romet-30. Romet-30 was registered in October 1984 for treatment of furunculosis in salmonids at 50mg/kg of fish per day for 5 days, and there is a 6-week withdrawal period. Romet-30 has broader activity than sulfamerazine and experimentally shows activity for other bacterial infections in a variety of fish and is effective against most strains of *Aeromonas salmonicida* resistant to oxytetracycline and sulfamerazine.

Disinfectants:

Three chemicals have FDA approved theraputic claims and three have EPA disinfection approval. Acetic acid and salt (sodium chloride) are FDA approved as GRAS for all foodfish. Acetic acid is approved as a parasiticide at 1000-2000ppm for 1-10 minutes, and salt is approved as an osmoregulatory enhancer at 0.5-1% indefinitely or 3% for 10-30 minutes. Uniodized salt and seawater have also been used to treat fungus infestations on incubating fish eggs (see procedures in FRED Fish Culture Manual).

Formalin. Formalin (Formalin-F, Natchez Animal Supply Co., Natchez, Mississippi) is approved for salmonids, catfish, largemouth bass, and bluegill as a parasiticide at 25ppm in ponds or up to 250ppm for 1 hour (not on consecutive days) in tanks or raceways, or at 1000-2000ppm for 15 minutes to treat fungus on eggs. No withdrawal time is needed for formalin, although a 4-7 day withdrawal prior to smolting may be necessary to assure adequate saltwater adaption in presmolts. Use of formalin for fungus control on eggs has been an adequate substitute for malachite green in Alaska State hatcheries. Consequently, because of its teratogenic potential and subsequent refusal for federal approval, malachite green is not acceptable for use by state facilities and is not recommended for use by private hatcheries.

Didecyl-dimethyl ammonium chloride. Didecyl-dimethyl ammonium chloride(Net-Dip, Aquasciences Research Group, Inc., North Kansas City, Missouri) and calcium hypochlorite (Olin HTH chlorinator granules, Olin Corporation, Stamford, Connecticut) are both EPA approved as general disinfectants and sanitizers and are not to be used directly on fish. Net Dip is for fish holding equipment at 3.5 fluid oz. in 4 gallons of water for 10 minutes, and HTH is to be used at 200ppm available chlorine for 1 hour to disinfect and sanitize fish tanks, raceways, and utensils. HTH can also be used to disinfect water to be used for fish at 5-10ppm chlorine for 12-24 hours.

Calcium oxide. Calcium oxide (Quick lime) and calcium hydroxide (slaked lime) are approved by FDA as GRAS and EPA has limited their approval as pond sterilants at 1,338 lbs and 1,784 lbs per acre, respectively.

Iodophors:

Iodophors are also used widely in fisheries as general disinfectants for utensils and as egg disinfectants. Products such as Wescodyne, Betadine, and Argentyne have been used. Iodophors are very effective and are generally used at 25-50ppm for general disinfection and at 100ppm for 10 minutes as external egg disinfectants or for 1 hour for internal disinfection during water hardening of eggs. Iodophors can be toxic to eggs unless buffered to about pH 7.0 and this is easily done with sodium bicarbonate (Argentyne is prebuffered).

Quaternary ammonium compounds. Quaternary ammonium compounds (quats) such as Hyamine 1622, Roccal, or Purina 4 Power are used at 2-4ppm for 1 hour to treat bacterial gill diseases and can be used as a general disinfectant following the manufacturer's recommendations, usually at 600ppm.

Diquat. Diquat (1, 1'-ethylene-2, 2'-dipyridylium dibromide) (Lubar Company, Kansas City, Missouri), also known as Bipyridilium and Reglone is used to treat bacterial gill diseases at 2ppm final active concentration for 1 hour. Diquat is federally approved to be used as an herbicide with foodfish at 0.25 to 2.5ppm having a withdrawal period of 14 days before treated water can be used for other purposes.

For detailed reference and recommended procedures in chemical treatment of fish diseases, refer to the FRED Fish Culture Manual (1983), Wood (1979) and Schnick, Meyer and Gray (1986).

Disinfection Procedures for Hatcheries

Although the compounds discussed below are commonly used as disinfectants in hatchery practices, they are also toxic to human health if misused. Precautions should be taken in their handling as directed by OSHA guidelines, and the FRED Safer Chemical Use in Alaska Aquaculture Manual (1988).

Egg Disinfection:

Introduction. To control the spread of pathogens carried on the surface of eggs, disinfection is necessary. This is done immediately after fertilization and during or after water hardening upon arrival and prior to exposure to running water at the receiving station. If preparations have not been made, under no circumstances should eggs be placed in water at the receiving station unless the water can be held and sanitized before release. If preparations cannot be made, eggs should be returned to the point of origin or destroyed. This can be done by burial in dry ground or in wet ground with quicklime. Disinfection should also occur when eggs are taken at the site where incubation will occur (Wood 1979).

Products. (The Alaska Department of Fish and Game does not endorse any particular supplier or brand except in those instances where they are the only distributor or product approved for fisheries use.)

Betadine - (VF Grace, Anchorage). Non-detergent, with 10% povidone iodine, aqueous polyvinyl pyrrolidone-iodine (1%). Not buffered. (Amend 1974; Vestal Laboratories, 1974)

Wescodyne - (West Chemical Co.). Detergent, with 1.6% active iodine in ethanol-iodine complexes. Not buffered. (Amend 1974; Vestal Laboratories, 1974)

Argentyne - (Argent Chemicals). Non-detergent polyvinyl pyrrolidone iodophor similar to Betadine, but buffered.

Methods. (Wood 1979, FRED Staff, 1983).

Betadine or Argentyne: 1:100 dilution of jug strength for 10 minutes (100ppm iodine).

Wescodyne: 1:150 dilution of jug strength for 10 minutes (100ppm iodine).

Disinfect before exposing to running water at the receiving station, even when the egg take occurs at the receiving station.

Comments. To avoid the toxic acidifying effect from soft water, buffer Betadine and Wescodyne with 0.05% sodium bicarbonate.

Change iodophor solution between lots of fish or when it begins to lighten in color. A lot is defined as a group of fish of the same species and age that originated from the same discrete spawning population and that always have shared a common water supply within the hatchery.

Equipment Sanitization:

Introduction. The prevention of contamination or recontamination of a hatchery on disease-free status is of the utmost importance. Infectious fish diseases do not occur at a fish cultural station unless pathogens have been introduced or occur naturally among resident fish in the water supply. The increase of inter-hatchery activities in Alaska raises concern about the importance of maintaining adequate disinfection and control of endemic diseases at those facilities.

Methods. (Hnath 1983)

Equipment: All equipment used in one hatchery should not be allowed to enter any other hatchery until that equipment has been sanitized. Ideally, sanitation should occur before equipment leaves its resident station and again on its arrival at a second station. Equipment includes nets, fish pumps, utensils, raingear, waders, boots, egg sorters, fish transport vehicles or anything that may have contact with fish, eggs, or cultural waters. If fish transport motor vehicles are exchanged between facilities, they should be disinfected according to fish transport vehicle disinfection instructions. Disinfection must always be done thoroughly and properly to be effective.

Fish tanks. 200 ppm active chlorine in the form of liquid bleach (sodium hypochlorite, 5.25% active ingredient) or calcium hypochlorite (HTH, registered, 65% active ingredient chlorine) for 10 minutes minimum. After disinfection, the solution should be dumped at a safe site where it will not directly drain into natural waters. Neutralization of chlorine is recommended, and can be done by using 2.2 lb sodium thiosulfate/lb HTH or 1.5gm sodium thiosulfate/liter of 200ppm chlorine. Chlorine can be corrosive to metal and should be thoroughly rinsed following use with clean, uncontaminated water. Raingear should be worn to prevent/reduce chlorine contact with clothing. Because organic substances

will readily inactivate chlorine and limit its effectiveness, dirty equipment should be cleaned before it is disinfected with chlorine.

Fish transport vehicle exterior. The exterior of motor vehicles including chassis and undercarriage is decontaminated with high temperature (115-130°C) steam or with 20ppm chlorine. Chlorine should be thoroughly rinsed with clean, uncontaminated water to minimize corrosion. It is not necessary to disinfect the exterior of aircraft or boats used for transporting fish or eggs.

Fish transport vehicle interior. Interior surfaces of motor vehicles, aircraft, or boats that have been contaminated during transport by contact with fish, eggs, or cultural waters should be scrubbed with noncorrosive 600ppm quaternary ammonia compounds, i.e., Hyamine or Roccal using 1.5 ml of 50% stock solution/ liter water; Roccal at 800-1000ppm for 30 minutes is the disinfectant of choice for transport tank interiors rather than chlorine solutions which adversely affect pumps and aerators.

Other equipment. Utensils, fish pumps, nets, egg sorters, waders, boots, raingear, etc., can be disinfected with 200ppm chlorine for 10 minutes; or in 600ppm quaternary ammonium compound for 30 minutes; or 100ppm iodophor solution for 10 minutes. If necessary, the disinfectant should be scrubbed onto the surface. Disinfected equipment should be thoroughly rinsed with clean, uncontaminated water and dried before use.

Personnel. All individuals involved in transport operations should wear outer protective garments (rain gear, boots, waders, etc.) when handling fish, eggs, or cultural water. Hands should always be disinfected before handling cultural water at another station. When work is completed at the station, hands and protective garments should be properly

disinfected. Natural cotton and wool fabrics that contact cultural water at a station can be disinfected by soaking them for 30 minutes in 600ppm quaternary ammonia compound and rinsed thoroughly before being worn.

Disinfectants are not only toxic to fish, but also to human beings. Care and good sense should be applied in their use to avoid upper respiratory irritations and/or contact dermatitis from continued overexposure. All containers of disinfectant should be capped or with lids on when not in use. The recommended levels for disinfection should not be exceeded. On a routine basis, disinfectants should be applied with brushes rather than aerosolized in a closed area. Aerosolization of disinfectants in a closed area may be necessary on occasion (i.e., to sanitize a facility after a disease outbreak) and is acceptible if personnel wear adequate protective respirators equipped with the appropriate filters, goggles and other outerware. If possible, a better alternative would be to fog closed areas with disinfectant (not formalin) using an automated device. Live steam from a portable steam generator should be used for disinfection whenever possible to reduce chemical use.

Complete Hatchery Sanitization:

Introduction. Plans for sanitizing a hatchery should be incorporated into the design of the facility such that, when and if necessary, disinfection can be accomplished easily and effectively.

Planning. Personnel designated to conduct the sanitization should formulate a detailed plan prior to the operation. This should incorporate inspection of the facility, discussions with the manager, methods, materials, safety, training, and adequate follow-up. Methods should include drying, elimination of water leaks or potential sources of contamination, volumetric measurements of the buildings, purchase of chemicals, initial cleaning, ventilation, and preventive maintenance.

Methods.

Cleaning: Most pathogens are removed from environmental surfaces by cleaning. For disinfectants to be effective, surfaces must be cleaned of dirt and organics beforehand.

Drying: Since most fish pathogens (except IPNV) are destroyed by drying, anything that is clean and dry is generally free of viable agents. Some things may be dry on the surface but not within. For example, wood is often surface dry, but wet internally. Concrete raceways can have cracks where water remains.

Design: A hatchery should be designed to allow maximal cleaning and drying of surfaces. The use of wood should be avoided when possible. Concrete floors should be sloped so that adequate drainage and drying occurs. Gravel floors cannot be adequately sanitized. Walls sealed with waterproof paint would also make later sanitation easier. Separate water manifolds supplying egg and rearing containers and different stocks of fish also help prevent pathogen spread via water.

Wood: Equipment and containers made of wood or other porous material used in the hatchery cannot be adequately disinfected and should be burned rather than attempting to reuse after sanitizing. Wooden incubators or rearing containers coated with fiberglass resin, although better than uncoated wood, should also be eliminated since their disinfection is still unreliable because of often unnoticed delamination or cracking of the fiberglass.

Concrete raceways: Raceway sanitation is best accomplished by soaking in chlorine. First, assess the raceway for cracks and leakage into and from other raceways and repair accordingly. Any significant amount of curing compounds, sealer or new concrete applied to a raceway surface for repair may require an undefined amount of time to leach out toxic compounds in running water before fish can safely inhabit the raceway. When in doubt, test a small number of fish in the raceway for at lease 48 hours.

Aluminum raceways: Outside spraying with chlorine (and use of a proper respirator) rather than soaking should suffice since aluminum is non-porous. However, soaking is preferred. Gasoline or electrically powered high pressure sprayers have been very effective at some facilities for cleaning raceways (and other equipment) prior to disinfection.

Fiberglass containers: These may have some cracks and therefore may be at least semi-porous. Spraying disinfectant on them may not be sufficient. Soaking is preferred.

Artificial substrate: Saddles or bio-rings should be precleaned of organic debris and disinfected in chlorine for at least 30 minutes, rinsed in clean water and thoroughly dried before reuse the following season. After prolonged use, sub- strate will develop a surface scum which can be removed prior to disinfection by either of two methods: (1) agitation with sand in a cement mixer or; (2) pressure spraying with water using commercially made equipment for this purpose.

Chlorine (adequate respirator use recommended): 200ppm chlorine should be used as a soak or for spray disinfecting. Active available chlorine from HTH is about 65% (check label). Hnath (1983) recommends filling a raceway halfway and then adding half the HTH while stirring. The raceway is then filled to within 5 cm of the top and the final half of the HTH is stirred in. Fill all raceways in the same manner and include chlorination of all pipelines, especially drains. If possible, the entire raceway system should be disinfected at the same time. If the hatchery is too large to allow simultaneous disinfection it can be done in sections, being careful not to permit contaminated water to backflow into areas or pipelines already disinfected. The goal is to retain a level of 200ppm chlorine in the raceways and lines for 1 hour and at least 100ppm for several hours. Letting the raceways soak overnight is the safe way to do this. Sodium thiosulfate (0.7g/l) provides the necessary quantity of sodium ions needed to neutralize the chlorine ions at 200ppm strength after disinfection. Sufficient sodium thiosulfate should be on hand <u>before</u> chlorination begins so that an accident can be neutralized before an environmental disaster occurs. Allowing the chlorine solution to sit longer will permit enough chlorine molecules to escape into the atmosphere so that mixing or solubility variables will be more than compensated for. A level of 1.5g of thiosulfate/liter has been recommended in the past as an overkill concentration but the cost of the thiosulfate can be prohibitive. Measuring the residual chlorine (orthotolidine reagent or iodometric titration) after neutralization is recommended so that toxic levels are not released into the environment. Drinking water often contains 0.1ppm and this level will still kill fish. Chlorine should not be detectable in effluent water.

Formalin fogging: Formalin fogging or fumigation is not recommended for human health reasons. Formalin fogging will produce a precipitate on every surface that dries, leaving a paraformaldehyde film. Paraformaldehyde sublimates slowly into the atmosphere as formaldehyde gas, leaving hazardous fumes in

the hatchery for unpredictably long periods of time. Formalin fumigation using potassium permanganate can potentially produce a violent explosion and resultant formaldehyde gas is extremely dangerous in closed areas.

Iodophor: Disinfection with iodophor solutions containing 100ppm available iodophor will suffice for walls, floors, and other non-porous surfaces.

Quaternary Ammonium Compounds (Roccal, Hyamine, etc.): Follow manufacturer's recommendations for use, but remember that these compounds can be very toxic to fish and must be thoroughly rinsed from equipment before use.

Respirators/Protective Clothes: Should be worn whenever formalin, iodophor, chlorine, or other toxic chemicals are used, particularly in any manner that might cause vaporization or splash. Respirators may be in order when formalin treatments for fungus control on eggs are performed. Knowledge of proper use or respirators, in addition to the assurance that the respirators are functioning properly, must be established before requiring an individual to perform tasks that require respirators. The correct respirator cartridges must be selected with regard to the toxic substances used.

Environment: Prior to sanitizing a raceway or anything else that will require large quantities of toxic chemicals, devise a failsafe plan that prevents environmental contamination. Have another person independently assess it and repeat the mathematical calculations.

Inspection and Diagnostic Procedures:

Finfish Diagnostics. Diagnostic procedures used for detection of fish disease agents will be according to the American Fisheries Society Fish Health Bluebook (Amos 1985). Additional specific

procedures may be found in the FRED Report #29 (Fried 1984) and the unpublished Fish Pathology Section procedures manual.

The major purpose of this section is to clarify the proper fish sampling procedures to be carried out at the facilities by hatchery personnel when disease problems arise. This is an absolute necessity in order that samples received by the pathology lab are adequate to allow a definitive disease diagnosis. The following discussion is a modification of the Fish Health section from the FRED Fish Culture Manual.

Disease Recognition and Action: Whenever abnormal behavior patterns of fish, external abnormalities, or high mortalities occur at a hatchery, an immediate response from the hatchery manager is imperative. Assistance should be requested from the regional project manager and the Fish Pathology Section (FPS) of FRED whenever mortalities appear excessive. An epizootic is occurring when mortalities reach 1.5% per day. This requires immediate attention. A total commitment of the facility staff and appropriate personnel is needed to save the remaining fish.

Mortalities less than 1.5% down to 0.5% indicate that a fish health problem is present. Notify the regional project manager and FPS.

Mortalities of less than 0.5% per day but greater than .03% should be investigated. Hatchery personnel should attempt to remedy the situation by modifications of environment or feeding. Inform the regional project manager and the FPS.

The percentages given above are for total mortalities. It is no less a matter of concern, however, if one lot of fish is dying at 1.5% per day while the others remain healthy. Contact your supervisor immediately and isolate the sick fish as much as possible to prevent transmission of the disease to other lots.

In order to reduce the spread of fish disease, make sure that dead fish are incinerated or soaked in a solution of 200ppm of chlorine or iodine (active ingredient) for 12 hours before disposal.

Sample Collection and Shipment: Prior to collecting any samples, contact the Fish Pathology Section to discuss the appropriate type of sample and numbers of fish needed. The following instructions are general guidelines but some samples need special treatment and the pathology personnel will be able to provide details. Samples that are not in an adequate condition upon arrival will not be processed. All proposals for sampling (Southeast Region, Southcentral Region and AYK-Westward Regions) should be cleared through pathology by contacting the appropriate lab personnel assigned to a particular facility.

Preparing Samples: Different procedures are followed in sampling for bacteriological, virological, parasitological, and histological analyses. Further details regarding the procedures below will be provided to hatchery personnel upon initial contact with the FPS.

In clinical cases of disease (≧0.5% mortality/day) 10 moribund fish are generally a sufficient sample size to make a diagnosis. In situations where no excessive mortality or clinical disease is apparent, a larger sample size of 60 fish may be necessary. However, depending upon individual circumstances, sample sizes may vary between 10 and 60. Samples should be examined from each affected lot, incubator, or rearing container. Consult with the FPS for specific sampling requirements in each situation.

Bacteriology. Small fish must be received either alive or freshly dead (within 1-2 hours) on ice in a cooler. Fish should not be frozen.

Live fish are preferred for diagnostic samples. Place at least 10 moribund fish in one or more large leak-proof plastic bags containing hatchery water. Seal the bags so space for

air remains and leakage will not occur. Label bags with fish status (moribund or healthy), incubator or raceway number, stock and species. If oxygen is available, add to bags before sealing. Addition of an oxygen tablet to each bag is recommended particularly for samples that must be shipped. Make a similar bag containing 10 healthy fish. Again, if the fish are large fingerlings or smolts, the amount of fish per bag should be adjusted accordingly.

In addition, enclose 10 moribund fish in a smaller dry plastic bag. Do not add water. If the live fish do not survive transport, then the dry fish, which will have undergone less deterioration and contamination from the water and its bacterial flora, will be processed instead. In a disease outbreak, 30 fish per lot of affected fish will be required for shipment (10 moribund, 10 healthy and 10 moribund, but dry).

Virology. Clinical Disease: In clinical disease outbreaks of suspected IHNV in sockeye salmon, 10 moribund or freshly dead fish are sufficient to isolate the virus for a confirmed diagnosis. In other salmonid species, 60 moribund fish may be required to establish an etiology. For alevins, fry and fingerlings, whole fish should be sent.

For suspected viral outbreaks in juvenile fish primarily in sockeye and chum salmon: 1) follow instructions given above, and 2) enclose additional moribund fry, 5 per bag, (10 fish/lot), but do not add water.

Broodstock and Disease History Examination: For establishing a disease history in adult fish or in broodstock screening, 60 samples from adult fish will be required. Samples of choice are from postspawning female fish consisting of ovarian fluids collected from each fish and shipped in separate sterile containers with lids (factory sterilized disposable centrifuge tubes). When required, samples from post-spawning males should consist of livers and spleens from each fish,

asceptically removed and pooled in individual sealed plastic bags. Tissues from more than one fish should not be combined in one bag. All tissues and fluids for virus assays should be shipped to the FPS on ice (4°C) but never frozen. Freezing and subsequent thawing inactivates IHNV producing lower titers, which in some samples may be too low to detect routinely. Virus samples on ice should be sent to the FPS lab as soon as possible within 72 hours of collection.

These sampling procedures are applicable to assays for other finfish viruses should the need arise.

Ovarian fluids for virology testing: Obtain instructions from the lab regarding whether you should take ovarian fluids from ripe fish used in the egg take or from postspawning fish. Disinfect the external ventral surface and either rinse with clean, pathogen-free water or wipe dry with paper towels.

For postspawners, partially strip a single fish's ovarian fluids into a paper cup (recommend 4 oz pleated portion cups but paper drink cups can be used), avoiding the extrusion of blood and fecal material. For ripe fish, you may either extrude a small amount of fluid prior to taking eggs or remove eggs and pour fluid off the eggs. Two ml of fluid is adequate for ripe fish, but 3-5 ml should be obtained if sampling post-spawners since these fish may take on some water diluting the ovarian fluids.

Crimp edges of cup to form a spout and pour fluid into a 15 ml centrifuge tube with cap, "straining out" any eggs. Avoid contaminating the rim with your hands. Discard cup after each fish.

Cap tubes <u>tightly</u> making sure that the cap is not cross-threaded. Place tubes in a rack and label with stock of fish, sampling location and date. Place upright in cooler with cold packs or ice. <u>Do not freeze</u>.

Tissue samples from males for virology testing: Disinfect the external ventral surface and either rinse with clean, pathogen-free water or wipe dry with paper towels.

Carefully cut open fish, taking care not to cut the gastro-intestinal tract which would contaminate tissues with bacterial flora.

Aseptically remove the spleen and a portion of the liver about the size of the spleen and place into a single plastic bag using a spoon, knife or forceps.

Seal each bag and keep cool (4°C).

Between the sampling of each fish, clean dissecting utensils with ethanol or iodine and dry with a clean paper towel. Organic matter will affect the working ability of disinfectants so any tissue should be wiped off utensils with a separate paper towel prior to disinfecting. Disinfect hands between the sampling of each fish.

When done sampling place all sealed sample bags in a large plastic bag. Label the bag with number of samples, stock of fish, sample location, sample type and date. Place in cooler on cold pack.

Fluorescent antibody testing (FAT). BKD, ERM, and *Aeromonas salmonicida*. In disease outbreaks involving small fish, 10 moribund or freshly dead fish per affected lot(s) shipped in plastic bags on ice (not frozen) are sufficient for a disease diagnosis. Frozen samples are not desired because a presumptive diagnosis of *A. salmonicida* cannot be confirmed by bacteriologic culture of such material. Sampling would be according to A.(1) and (2) described above. An additional sample of 60 randomly selected apparently normal fish from

the same lot(s) may be required at a later date to determine the prevalence of subclinical disease within a given group of fish before release is approved.

In situations where a disease history and/or broodstock screening is desired, a minimal sample size of 60 fish will be required. Family tracking will require screening of all parent fish involved in the egg take. Whole fish should be sent when sampling alevins, fry and fingerlings. In situations where large fish are to be examined, only kidney tissues are required. Sampling procedures are similar to those described in 4(a) to (f) except kidney would be taken instead of liver and spleen. Also, the kidney sample from large fish should be larger, about 6-8 cm in length.

Although fresh-on-ice samples are necessary for successful isolation of certain disease agents, freezing would become necessary if there will be excessive delay in getting the samples to the FPS.

In situations where it is more practical for field personnel to prepare the slides for FAT rather than mail tissues, the appropriate materials will be provided by the FPS. Briefly, after collection of kidney tissues the procedure requires:

1) Homogenization of the kidney sample from each fish by kneading within the plastic sample bag.

2) A sterile wooden applicator stick is touched to an individual homogenized kidney sample and then mixed with a drop of phosphate buffered saline (PBS) deposited in a single numbered well on a multiple well slide.

3) The samples are allowed to air dry at room temperature and the slides may be mailed to the FPS in slide boxes.

Each kidney sample requires a separate applicator stick and well. Slides are prepared in duplicate for parallel testing if fish are to be screened for both BKD and A. salmonicida. Homogenization of the kidney is important to break open BKD pustules and distribute the organism for easier detection. It is also important to not make kidney smears too thick within the depressions which makes interpretation difficult. Also, such smears may wash off the slide during processing.

Parasitology and General Necropsy. The same sampling procedures as in Bacteriology [A.(1)(2)] apply here. Live fish are preferred to frozen or preserved fish. This is especially true for detection of external protozoan parasites and general gross tissue lesions, which are usually lost during freezing. Fish may be fixed in 10% buffered formalin if live fish are not available. Fish longer than 6 cm should be opened along the belly to ensure adequate formalin fixation of all tissues.

The FPS discourages routine submission of large numbers of fish (≧20) for purposes of establishing parasite (helminth) prevalences since the effort does not justify the value of the resulting information.

Histology. Histological samples should be fixed in Bouin's solution. Fix live fish. Use 10 moribund fish and 10 that are apparently normal from the same lot. Dead fish are not suitable for histology. The volume of fixative should be 10 times the volume of the tissue. For fish longer than 6 cm, slit the belly, detach the intestine at the anus, and pull the internal organs out slightly. For large fish, send only specified organs in fixative. Call the FPS for specific instructions prior to fixing.

Sample Shipment Instructions (for all samples)

1) Pack samples in a small ice chest made of plastic or sturdy styrofoam which will not be damaged in transit. Ice chests (other than styrofoam) will be returned to the hatchery.

2) Add ice in sealed, leak-proof plastic bags or use pre-packaged ice substitutes. To prevent freezing, separate the samples from the ice with newspaper or other insulative material.

3) Place completed Case Data Report(s) (forms available from FPS) for each stock sampled within a waterproof plastic bag and enclose in ice chest.

4) Close, seal, and label the ice chest with "refrigerate but do not freeze" (unless samples are frozen F.A.T. specimens) and "perishable". Label with mailing address and the name of the person contacted in the lab. The mailing addresses for the pathology labs are:

Fish Pathology Lab	Juneau Fish Pathology Lab
ADF&G, FRED Division	ADF&G, FRED Division
333 Raspberry Road	3333 Old Glacier Highway
Anchorage, AK 99518-1599	Juneau, AK 99801

5) Ship via express air or air freight (if you know it will not get bumped off the flight) as soon as possible. Instruct airline to refrigerate sample upon its arrival in Anchorage. If sent early in the week, fewer air freight and delivery problems are encountered. Avoid shipping on Fridays.

6) Contact the courier services currently used by the FPS in Anchorage or Juneau to have the sample(s) delivered.

7) Phone the Fish Pathology Lab to notify that the sample is enroute. Please provide the flight number, airbill number and expected time of arrival. Subsequently check to see if it has arrived. It is the responsibility of the sender to ensure that the sample arrives in the laboratory and in satisfactory condition.

Shellfish Diagnostics:

Rationale. Invertebrate pathology is still in its infancy, with diagnostic technology far behind that used for finfish. Consequently, the diagnostician is limited to the rudimentary detection of shellfish diseases through the use of histology because almost none of the recognized pathogens have been cultured on artificial media. The exceptions are the *Perkinsus* species of protozoa which can be cultivated in thioglycollate broth. Based on the above information, shellfish certification procedures in Alaska will consist of a 2-way approach; histological examination for shellfish pathogens, and thioglycollate screening for *Perkinsus* organisms.

Out-of-State vendors of oyster spat who desire to market their products in Alaska must contact the FPS and arrange shipment of shellfish samples for disease certification as designated below. Those oyster growers in Alaska desiring to purchase out-of-state oyster spat must do so only from ADF&G certified stocks and are required to submit an application for a Fish Transport Permit for prior approval of oyster importation.

Sample Collection. Randomly select animals through entire lot. Need nonbiased samples from each lot being shipped.

Sample Type and Numbers.

1) Motile larvae - 200-1000
2) Spat - 200
3) Adult - 60+

Sample shipment should be as described in the finfish section. All oyster samples shipped to the FPS should be dry and on ice except that larvae will have to be in a sealed container of seawater.

Hatchery Inspections:

Annual or biannual hatchery inspections made by a fish pathologist are for the purpose of observing facility design and practices as they relate to the control of fish and shellfish diseases. The function of the pathologist is to offer advice to correct perceived fish health problems. A hatchery inspection includes an on-site visit and a written report submitted to the hatchery manager addressing the criteria listed below.

Fish stocks at facility (eggs or rearing fish). 1) number, 2) brood year, 3) source, 4) release date, and 5) release location.

Incubator types. (fish species, loading densities and % survival to eyed stage)

Rearing containers. (fish species, size, and loading densities)

Water flow. 1) volume, 2) single pass, 3) re-use - details (treatment, # passes, etc.), 4) recirculation - details (treatment, # passes, etc.), 5) water source, 6) resident fish, 7) depuration (in or out and method), 8) water temperature (at time of inspection), 9) source for water hardening of eggs, and 10) total dissolved gas.

Methods of fish movement from incubators to rearing to release.

Disinfection procedures. (methods and dose) 1) eggs (before entering hatchery), 2) substrate (after each season), 3) utensils (between stocks), 4) equipment and incubators (between stocks or after each season), 5) footbaths - in and out of facility, and 6) mortality disposal.

Current type of feed. 1) brand, 2) method of storage, and 3) turn over time (expiration dates, lot #s).

Health problems observed in eggs and/or fish at facility. 1) stock-lot, 2) age, and 3) signs.

Previous problems. 1) water quality (pH, temperature, sediment, DO, TDG, hardness, etc.), 2) percent egg or fish mortality/stock or lot/day, 3) previous treatments: a) fungus control (chemical, dose, schedule), and b) other prophylactic or therapeutic treatments (reason, when, lot or stock, drug or chemical, method of application, dose, and results), and 4) feed: a) feed type, b) problem (odor, texture, palatability to fish, etc.), c) date, and d) lot #.

Schedule I - Rationale

Detection of disease-causing agents becomes more difficult when they covertly exist within fish populations in a carrier state. This condition produces no obvious outward signs of disease. Thus, destructive sampling is required of larger numbers of fish to provide prevalence data such that the risk of not detecting a disease organism is at an acceptable statistical level. The effort and expense involved in processing such samples is considerable, and proportional to the number of samples. Consequently, it is imperative that sample numbers be as small as possible, but still provide statistically reliable prevalence data. The model that best fits most situations encountered in sampling fish for disease detection is the hypergeometric distribution (Ossiander and Wedemeyer 1973). This model was used to compute Schedule I from a program written in Turbo Pascal for the IBM/PC microcomputer. The hypergeometric distribution was used for all finite sample sizes. Populations greater than 25,000 fish may be assumed to be infinite. The binomial approximation to the hypergeometric distribution was used for the infinite population case.

The Schedule I used in this document for BKD, A. salmonicida and ERM screening consists of the bottom sub-table where population size is infinite. Note that there is little change in the schedule as population sizes increase from 1000 to infinity. Sixty fish is the sample size which still provides 95% confidence that at least a single diseased fish will be detected in the sample if disease is present within 5% of the population. Currently, BKD and ERM agents and A. salmonicida are detected in fish using fluorescent antibody testing (FAT) in which results are recorded on a scale of 1+ to 4+ according to the intensity of fluorescence. This intensity is based upon relative numbers of organisms within a given number of microscope fields. The most conservative approach would be to reject a fish population if one fish tests positive in a sample of 60. However, a more practical compromise is necessary between the ideal situation of no disease and a more realistic one where some disease in the carrier state is frequently present and must be tolerated to some degree. That degree of tolerance (acceptable percent of positive FAT categories within the population) is arbitrarily determined in Schedule I whereby at a 5% risk of no detection in a 60-fish sample the population is rejected (i.e., limitations may be placed upon the disposition of those fish as determined on a case-by-case basis) if: 7 or more fish are 1+ in FAT (Population prevalence of 20%); 2 or more fish are 2+ (population prevalence of 10%); 1 or more fish are 3+ (population prevalence 5%), i.e., no 3+ or 4+ fish are allowed because of the large numbers of disease organisms carried and potentially shed into the environment.

SCHEDULE I. Rejection numbers for different population and sample sizes when the risk is 5% (0.05).

Population Size = 1000

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	32	51	90
2+	10	1	3	6	7	14	23	42
3+	05		1	2	3	6	10	19
4+	01						1	2

Population Size = 2000

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	31	50	87
2+	10	1	2	5	7	14	22	41
3+	05		1	2	2	5	9	18
4+	01						1	2

Population Size = 5000

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	40
3+	05		1	2	2	5	9	18
4+	01						1	2

Population Size = 10000

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

Population Size = 25000

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

Population Size = infinite

FAT	% Disease Prevalence	Sample Size 30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	85
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

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