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

by Ted Meyers

REGIONAL INFORMATION REPORT[1] NO. 5J03-07

Alaska Department of Fish and Game
Division of Commercial Fisheries
P.O. Box 25526
Juneau, Alaska 99802-5526

Revised March 2003

[1] The Regional Information Report Series was established in 1987 to provide an information access system for all unpublished divisional reports. These reports frequently serve diverse ad hoc informational purposes or archive basic uninterpreted data. To accommodate timely reporting of recently collected information, reports in this series undergo only limited internal review and may contain preliminary data, this information may be subsequently finalized and published in the formal literature. Consequently, these reports should not be cited without prior approval of the author or the Division of Commercial Fisheries.

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FORWARD

The 2003 revisions made in this document have not altered the intent, principals or rationale on which the disease policy was originally formulated by the State Pathology Review Committee (Meyers et al. 1988). The changes made include: editorial improvements; additional clarifications where necessary; updated lists of pathogens; omission of the Sockeye Culture Policy and Diagnostic Procedures sections that have been published as separate documents; updating of the Shellfish Culture section; updating of the Appendix regarding current drug usage in aquaculture with addition of new sections describing the responsibilities of the ADF&G Fish Pathology Section, good fish culture practices to reduce disease, recognition of disease at the hatchery, a partial list of finfish and shellfish pathogens in Alaska and investigation of fish kills. This revised document better reflects the current fish health program in Alaska.

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MISSION STATEMENT

This document includes proposed changes in state regulations, policies, and recommendations used by recognized authorities and user groups for maintaining adequate finfish and shellfish health within the State of Alaska. These criteria include evaluation protocols for regulating and permitting fish transports, prophylactic measures and therapeutic treatments for infectious diseases of salmonid fishes and shellfish species. The criteria are established for the purpose of regulating interstate and intrastate transports of live finfish and shellfish or their gametes for transplanting into state waters, research and education purposes, and/or other interests not defined herein. The objective of this document is to prevent dissemination or amplification of infectious finfish and shellfish diseases within or outside the borders of Alaska without introducing impractical constraints for aquaculture and necessary stock-renewal programs while maintaining other established state criteria regarding genetic and aquaculture policies.

CHANGES IN EXISTING REGULATIONS

The recommendations of this committee included suggested changes in existing regulations found within Title 16 of the Alaska Statutes and the Alaska Statutes and Regulations for Private Nonprofit Salmon Hatcheries (ASRPNSH), 1996 edition.

Pursuant to Article 3, 5 AAC 41.070(b)(2)

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

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

Pursuant to Article 3, 5 AAC 41.080(b)

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

> (b) Within 24 hours of <u>taking and fertilizing live fish eggs or</u> transporting live fish eggs between watersheds, all eggs must be treated, for at least 10 minutes, with an 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 <u>and may, at the discretion of the Commissioner or his authorized designee, also exclude eggs taken at large scale pink and</u>

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

<u>chum salmon facilities where the operational history shows that disease has not been a problem in returning stocks of fish.</u>

Pursuant to Article 3, 5 AAC 41.080(c)

Not all hatcheries require inspection **every** year. Some facilities have no significant disease problems. Consequently, if management and hatchery design remain the same, such facilities may only require inspection once every other year. Also, unpredictable spring weather often prevents travel in small aircraft for inspection of remote hatcheries prior to release of fish. Additionally, prerelease inspections of fish are usually not necessary and are not done unless specifically required by the Fish Pathology Section. Therefore, 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] <u>every other</u> year [AT LEAST TWO WEEKS PRIOR TO THE TRANSPORT OR RELEASE OF FISH]. The Commissioner or his authorized 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), the disease classifications, have 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 Pathogen Categories

a. Class I. <u>Pathogens of Critical Concern</u>

1. Piscirickettsia
2. VHSV – Viral hemorrhagic septicemia virus (European)
3. IPNV – Infectious pancreatic necrosis virus
4. OMV – Oncorhynchus masou virus
5. Herpesvirus salmonis
6. Whirling disease (*Myxobolus cerebralis*)
7. ISAV – Infectious salmon anemia virus

b. Class II. <u>Endemic Pathogens of Concern</u>

1. IHNV – Infectious hematopoietic necrosis virus
2. VHSV – Viral hemorrhagic septicemia virus (North American)
3. BKD – Bacterial kidney disease (*Renibacterium salmoninarum*)
4. Furunculosis (*Aeromonas salmonicida*)
5. ERM – Enteric redmouth (*Yersinia ruckeri*)
6. ICH – Ichthyophthiriasis (*Ichthyophthirius multifiliis*)

c. Class III. Nuisance Pathogens

1. Vibriosis (marine *Vibrio* (Listonella) *anguillarum*, *V. ordalii*, *V. alginolyticus*)
2. Cold water disease (*Flavobacterium psychrophilum*)
3. Columnaris (*Flavobacterium columnare*)
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* sp.)

d. Class IV. Uncategorized Pathogens

1. VENV – Viral erythrocytic necrosis virus
2. PKD – Proliferative kidney disease (*Tetracapsula bryosalmonae*)
3. *Vibrio salmonicida* (cold water marine Hitra)
4. *Loma* sp.(Microsporidian)
5. Reovirus
6. Paramyxovirus
7. *Ceratomyxa shasta* (Myxosporidian)
8. Emerging finfish pathogens not defined in Category I, II, and III including non-salmonid agents (togaviruses, nodaviruses, etc).

2. Shellfish Pathogen Categories

a. Class I. Oyster Pathogens-Critical Concern

1. European Hemocyte and Gill Iridoviruses (HIVD, GNVD)
2. Oyster Herpesvirus
3. Ostracoblabe implexa (shell disease fungus)
4. Haplosporidium sp. (*nelsoni*; *costalis*)
5. *Perkinsus marinus*, *P. atlanticus* and other similar protozoa
6. *Marteilioides chungmuensis*
7. *Marteilia* sp. (*refringens*; *sydnei*; *branchialis*; Aber disease; QX)
8. *Bonamia ostreare*, *Bonamia* sp.(microcells)
9. Velar disease virus (OVVD Iridovirus)
10. *Mytilicola* sp. (*intestinalis*; *orientalis*; parasitic copepods)
11. Malpeque Bay disease (unknown etiology)
12. Denman Island and Australian winter diseases (*Microcytos mackini*, *M. roughleyi*)

b. Class II – Nuisance Oyster Pathogens or Pests

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. (flagellate protozoan)
7. Ciliates (*Sphenophrya*; thigmotrichs; trichodinids; *Ancistrocoma*)
8. *Nematopsis* sp. (sporozoan)

9. Microsporidea (HEP and others)
10. Helminth parasites
11. *Pseudomyicola* sp. (parasitic copepod)
12. Gregarines (protozoa)
13. Neoplasia (germinomas)
14. Ovacystis virus (gametogenic papovavirus)
15. Symbionts
    a) *Polydora* sp. (polychaete mudworm)
    b) *Diplothyra* sp.(boring clam)
    c) *Cliona* sp.(boring sponge)
    d) Bryozoa
16. Predators
    a) *Stylochus* sp. (polyclad worm)
    b) Drills
        Urosalpinx cinerea
        Ocenebra japonica
        Rapana sp.

c. Class III – <u>Endemic Pathogens of Concern in Bivalves **Other than** Oysters</u>

1. Herpesviruses – littleneck clams, rock scallops
2. Histozoic coccidian – littleneck clams
3. Hemic neoplasia – littleneck clams, blue mussels
4. *Pseudoklossia* coccidia – littleneck clams, basket cockles, blue mussels

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 species will be examined on a case-by-case basis as the need arises.

Disease agents for oysters consist of only two categories with no Endemic Pathogens of Concern identified after many years of inspections. However, Endemic Pathogens of Concern have been identified in bivalve species other than oysters and are listed in Class III. 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 may be of significant importance to justify that the Commissioner prohibit stocking in new areas and to quarantine the permittee's facility until disinfected. The last sentence in (e) should be omitted, beginning with "Presence". Exotics are addressed in (f). Thus, Article 3, 5 AAC 41.080(e) should read:

> Diseases reported under (d) of this section, or 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 must be addressed. Much of (f) has been reworded. This category includes seven infectious agents that have not been detected in Alaska, two of which (OMV and European VHSV) are exotic to the North American Continent. Five of these agents (European VHSV, IPNV, *Piscirickettsia*, ISAV and *M. cerebralis*) can be extremely virulent and capable of decimating populations of fish. The remaining two may also be severe problems, causing infected stocks to become unsuitable for any purpose. There is no known treatment for these agents, except prevention.

The shellfish diseases of critical concern listed in Class I include 12 infectious agents that are exotic to Alaska; three of these (1, 6, 7) are exotic to North America. Any oysters for importation into Alaska having detectable Class I agents will be refused for entry. Any oysters or other invertebrates within the state that are infected with agents exotic to North America would be considered for destruction or immediate marketing to protect the environment or other shellfish stocks. Therefore, Article 3, 5 AAC 41.080(f) should read:

> As determined by the Commissioner or his authorized designee, detection of any Class I disease agent in finfish stocks or Class I disease agent 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 agent before continued production of fish or shellfish.
>
> STOCKS OF FISH IN HATCHERIES OR REARING FACILITIES IN WHICH A] If Class I disease agents [HAS BEEN DETECTED MUST BE IMMEDIATELY DESTROYED] exotic to Alaska but not to North America are detected in Alaskan oysters, destruction of those oysters by the permittee may be required if the Commissioner or his authorized designee determine that the disease agent [. . .] 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 disease agents of endemic concern listed under Class II include six obligate pathogens causing most of the serious finfish disease concerns in Alaska. These pathogens are of concern because they limit fish transport within and outside the state and define sampling sizes, frequencies, and methodologies. All but two (VHSV, IHNV) are treatable to some degree. 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. Thus, Article 3, 5 AAC 41.080(g) should read:

> Stocks of finfish in hatcheries or rearing facilities in which a Class II disease agent 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 or the intended release location.

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

Class IV disease agents include unknown pathogens that may emerge in the future, seven other agents that are questionable regarding their importance to finfish health in Alaska, and all other non-salmonid fish pathogens that could become concerns in the future. As the need arises, each entity will be evaluated on a case-by-case basis. As an example, *C. Shasta* is a serious pathogen of salmonids in the Pacific Northwest, and has been detected as sub-clinical infections in isolated salmonid stocks from Bristol Bay and tributaries of the Yukon River. Because this agent has not been detected in hatchery fish or in the mainstream of Alaska State finfish transport inspections, samples will not be routinely tested for this pathogen. If this agent or any other entity in this category becomes a significant fish health problem in Alaska, each will be considered a Category II disease agent and routine samples will be examined accordingly.

The symbionts and predators (pests) listed in Shellfish Class II Nuisance Pathogens are not adequately treated in Article 3, 5 AAC 41.080(e). Some of these non-target species are unwanted exotics and the state reserves the right to refuse certification or restrict movement of oysters if there is oyster mortality, significant disease or generally poor shellfish health/condition associated with the prevalence of any of these agents.

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

> (h) The presence of pests recognized in Class II nuisance pathogens 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 non-target 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" should omit reference to the nonexistent FRED Division and include the 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, Commercial Fisheries [REHABILITATION, ENHANCEMENT AND DEVELOPMENT] Division, Fish Pathology Section located at:333 Raspberry Road, Anchorage, Alaska 99502, telephone (907) 267-2244 [344-0541]; and 3333 Old Glacier Highway, Juneau, Alaska 99802, telephone (907) 465-3577.

Live finfish cannot be imported into Alaska for the purposes of stocking or rearing in waters of the State (Article 3, 5 AAC 41.070). Fish transport permits for export and in-state movement, possession, etc. are required (Article 3, 5 AAC 41.005) for shellfish as well as finfish since fish are defined in AS 16.05.940(6) to include all invertebrates and amphibians. However, this distinction is not clear in the ASRPNSH and should be clarified by defining "fish" in this definitions section as it has been done within the Alaska Statutes except where designated otherwise. Article 3, 5 AAC 41.100 should include an additional definition (5) with the following changes:

> (5) "Fish" means any species of aquatic fish, invertebrates and amphibians in any stage of their life cycle found in or introduced into the state except where specifically designated "finfish" or "shellfish."

TRANSPORT APPLICATIONS FOR FINFISH

The State of Alaska has large areas of separated watersheds supporting wild fish stocks that have never been examined for disease agents. Consequently, there is a risk of unknowingly transporting presently undiscovered (in Alaska) finfish disease agents from one major geographic area to another that may not be detected at the 5% level in 60 adult fish examined prior to transport. This risk will be minimized by the Department's policy to discourage the transport 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 Genetics Policy, and because wild fish stocks are in several hatchery water supplies, this disease policy will also include hatchery stocks of fish, 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. There also must be a hatchery disease history for cultured fish that demonstrates no detectable disease agents of transport significance for the last two consecutive years of screening a minimum of 150 adult broodfish and no detection of such agents in progeny fish.

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

- Prior year sampling recommended to define year-to-year variability of disease prevalence.
- Sampling <u>required</u> in same year but prior to transplant of the adult fish stock.

Class II disease criteria [a]

Bacterial Kidney Disease (BKD): Agent *Renibacterium salmoninarum* (Rs) cannot exceed levels in Schedule I (See Appendix pg 60).

Furunculosis: Carrier state cannot exceed levels in Schedule I.

Infectious Hematopoietic Necrosis Virus (IHNV): No samples required unless proposed transplants are IHNV-susceptible salmonids from a sockeye or kokanee watershed since IHNV has not been prevalent in salmonid species other than anadromous sockeye salmon. All sockeye salmon and most kokanee stocks 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): An infrequent disease in Alaska caused by *Yersinia ruckeri* (Types 1 & 2). 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 (0.5%/day) mortality and immediate disease history of hatchery performance cannot exceed levels in Schedule I. Furunculosis: Indicated by FAT with confirmation by isolation. If the disease state exists, fish must be treated for release when mortality is insignificant and prevalence does not exceed Schedule I. If prevalence of infection exceeds Schedule I, fish cannot be released. Withdrawl period after drug therapy may be required. IHNV (sockeye, kokanee): Release if no disease. Clinical signs of IHN and/or detection of virus will require destruction of affected lots per ADF&G Sockeye Salmon Culture Policy. Lots that are virus-negative may be released as soon as possible. IHNV (chum, chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction. Operator of a facility where IHNV is detected must demonstrate remaining stocks have been isolated to prevent exposure; ie, the facility must be minimally qualified as a partial quarantine unit (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: Seawater release allowed. Freshwater, treat and release to minimize exposure of other hatchery stocks.

1. BETWEEN WATERSHEDS (continued)

c. Transplant of adults, juveniles or eggs, to a watershed with other “significant” (resource value) 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 (spawning) 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 (spawning) for examination. If fish stocks having a known carrier state of a fish pathogen are to be transplanted and 60 resident fish are not available for examination, then the resident stocks are presumed negative for all pathogens. In any case, Class II criteria apply.

(3) Class II disease criteria
BKD and Furunculosis: If stocks in receiving watershed have zero pathogen prevalence, then stock proposed for transplant must also have zero prevalence (min. sample size = 60). The applicant is responsible for obtaining a sample of 60 adult fish. If adequate sample numbers of transplanted fish are unavailable, the transplant cannot be made.

A. WILD FISH TRANSPLANTS	DISEASE CONSIDERATIONS
1. BETWEEN WATERSHEDS (continued)	If stocks in the receiving watershed are positive for the agents of BKD or furunculosis, then the transplanted stock must not exceed levels in Schedule I. *A. salmonicida* in the receiving and donor watersheds should be confirmed by culture due to potential non-specific fluorescence by FAT. IHNV: No samples required for anadromous sockeye except to establish a disease history: all stocks are carriers. Stocks of kokanee may be negative for IHNV and must be sampled. SSCP procedures are required for spawning all sockeye salmon and kokanee. Transplant of sockeye or kokanee into non-sockeye systems having IHNV-susceptible species is discouraged. Evaluation is on a case-by-case basis regarding 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 be evaluated on a case-by-case basis and may not be rejected on the basis of fish health concerns. Applicant and resource managers must accept the possible loss of transplanted fish or condemnation of the donor 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. If virus-negative, these species would be potential IHNV carriers, and decision criteria for sockeye and kokanee transplants would apply. Ich: If there is a disease history of Ich then transplant is not 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 Appendix pg 39)	Class II disease criteria No constraints for pathogens in carrier state since they will be in isolation.
b. Other than a QU	Class II disease criteria If no other stocks are present at hatchery, criteria in Section A.l.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 agents then the transplanted stock must meet the criteria for Schedule I. If a pathology-approved Partial Quarantine Unit (PQU Appendix pg 37) is to be used, then other stocks at the hatchery are not a concern. In either case (except effluent depuration in a PQU), if there are wild salmonids present in the hatchery watershed criteria in A.l.c apply.

A. WILD FISH TRANSPLANTS	DISEASE CONSIDERATIONS
3. TO A FLOW-THROUGH RESEARCH FACILITY/AQUARIUM	
a. Local fish and invertebrates	No restrictions provided animals and water source are from “local” waters adjacent to the research facility or aquarium
b. Non-local fish and invertebrates	Effluent depuration or treatment required with no release of animals and proper disposal of dead animals by incineration or landfill.

B. BROODSTOCK SCREENING FOR EGG-TAKES [a]	DISEASE CONSIDERATIONS
1. EGG-TAKE AT HATCHERY (indigenous stock)	
a. For release of progeny at hatchery.	Provided an acceptable disease history 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 the 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 egg-take. Class II disease criteria BKD: Prevalence of Rs in a brood source may require Family Tracking[b] as an acceptable control measure. 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 is done to establish a disease history.

[a] Note: The following "Disease Considerations" regarding BKD are an alternative to the preferred use of broodstocks having no history of the Rs agent. Toleration of minimal levels of this disease agent in stocks used at any facility is allowed only if: an alternative stock(s) is unavailable; other circumstances specific to ongoing programs leave no practical alternative; other mitigating procedures such as Family Tracking are practiced to reduce disease risk.

[b] For small populations of less than 1,000 where a sample of 60 adult fish in one year would constitute significant loss, alternative arrangements with the Pathology Section may include sampling fish over a period of years prior to the proposed egg-take. Under well justified circumstances an alternative is Family Tracking that requires kidney samples during the eggtake. Family Tracking requires keeping egg lots separate during water hardening, disinfection, and incubation in Heath Trays until testing of individual parents is completed. Egg lots from Rs-positive parent fish are destroyed.

B. BROODSTOCK SCREENING FOR EGG-TAKES	DISEASE CONSIDERATIONS
1. EGG-TAKE AT HATCHERY (indigenous stock)	
b. For release of progeny at another site. (continued)	IHNV (Sockeye, Kokanee): Sample size = 60 adult (spawning) fish in prior year for establishing population prevalence; spawned fish can be used thereafter at the egg-take to determine annual IHNV risk. SSCP procedures must be used for spawning all sockeye salmon and kokanee. IHNV (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 egg-takes, sampling in year prior is recommended. Any detection of IHNV would require the destruction of the broodstock and any eggs spawned and condemnation of the broodstock as a future source of eggs.

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: Rs prevalence in brood source requires Family Tracking. For hatcheries requiring reuse or re-circulation of water, the consequences of introducing Rs from outside cannot be tolerated. Family Tracking must be done or a known Rs-negative stock is required. Furunculosis and ERM: No specific limitation. High-risk stocks should not be used if low risk stocks are available. Egg disinfection is required; Pathology staff may monitor/assist at egg-takes, and may require fry samples prior to release depending upon fry performance. There is no evidence that vertical transmission of *A. salmonicida* or the ERM agent occurs WITHIN the eggs of salmonids. Consequently, eggs from a low number of carrier broodfish pose no additional risk if rigorous external disinfection is practiced. However, the risk of inadequate egg disinfection increases with increasing numbers of carrier broodfish. IHNV (sockeye, kokanee): Sample size = 60 spawning adult fish in year prior are required for disease history information; specific precautions recommended by Pathology staff will depend on facility type, location, and fish handling capabilities. All anadromous stocks of sockeye are carriers. SSCP criteria must be used for spawning all sockeye and kokanee. After establishment of a disease history, subsequent sampling may include 60 fish used in the egg-take to monitor the prevalence of IHNV brought into the hatchery with gametes.

B. BROODSTOCK SCREENING FOR EGG-TAKES	DISEASE CONSIDERATIONS
2. EGG-TAKE AT A SITE REMOTE FROM HATCHERY (continued)	IHNV (chum, steelhead, rainbow, chinook, cutthroat): In a system with sockeye, 60 samples from the desired susceptible species (spawners) are required in year prior. Any detection of IHNV in samples prohibits use of 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.l.b. Also, IHNV-susceptible species other than sockeye salmon from sockeye systems are not recommended 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 or terminal seawater release site (no watersheds)	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.l.c. and C.4.

Disease History of Juvenile Fish Prior to Release

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
1. AT THE HATCHERY SITE	Pre-release examination of juvenile fish is not done unless mortality or other clinical signs of disease or otherwise poor performance prior to release warrant concern by the Fish Pathology Section; and/or the broodstock disease history at egg-take was positive for Rs and Family Tracking was not done. 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 prohibit release. It is the long-range goal that all facilities meet the detection criteria in Schedule I. Those that do not but have total cumulative mortalities of less than 5% in the 90 days prior to release can release provided there will be future alterations in the physical plant and/or operations to achieve the limits of Schedule I within 6 years from date of problem occurrence. Furunculosis: Must be treated until mortality reaches background level (.03%/day). A withdrawl period after drug therapy may be required before release. IHNV: (sockeye, kokanee) Infected lots, as determined by clinical signs and/or detection of IHNV must be immediately destroyed per SSCP. Lots that are negative for virus may be released as soon as possible. Additional virus detection or clinical signs will require destruction of affected lots. IHNV: (chum, chinook, steelhead, rainbow, cutthroat): Same as for sockeye except detection of IHNV in fry will require destruction of the inventory of that stock unless demonstrated that lots within that stock have been isolated and not exposed to the virus. It also must be demonstrated that isolation has been maintained for other susceptible stocks on site to

C.	DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
1.	AT THE HATCHERY SITE (continued)	assure they have not been exposed to IHNV. Otherwise, the destruction of the other exposed stock(s) will be required. ERM: If diagnosed as clinical disease with significant mortality, elimination of a stock may be required, depending upon circumstances. Ich: Treat prior to release.
2.	RETURN TO SYSTEM OF ORIGIN	Class II disease criteria BKD: If broodstock was Rs-negative, juveniles are assumed negative unless found to be Rs-positive by examination. In this case, release cannot occur (to the system of origin) unless the broodstock, not the hatchery water supply (such as in a QU or PQU), is determined to actually have been positive whereby release will be considered on a case-by-case basis. If the broodstock had Rs-positive samples and progeny egg lots were not culled by Family Tracking then a prerelease sample of 60 juvenile fish will be required and cannot exceed Schedule I for release authorization. Furunculosis: If clinical disease is present, treat and release when mortality returns to a background level and prevalence does not exceed Schedule I. A withdrawl period after drug therapy may be required. However, if the brood source had no confirmed history of *A. salmonicida*, release of positive juveniles (to the system of origin) in the carrier state will not be authorized. IHNV (sockeye, kokanee): Infected lots with clinical signs of disease and/or detectable virus must be destroyed per SSCP. Virus-negative lots may be released while further detections of IHNV or observed clinical signs in additional fish lots will require their destruction.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
2. RETURN TO SYSTEM OF ORIGIN (continued)	IHNV (chum, chinook, steelhead, rainbow, cutthroat): Same as for sockeye except detection of IHNV in fry requires destruction of the inventory of that stock unless demonstrated that lots within that stock have been isolated and not exposed to the virus. It also must be demonstrated that isolation has been maintained to assure that other susceptible stocks on site have not been exposed to IHNV. Otherwise, the destruction of the other exposed stock(s) will be required. ERM: If clinically diseased 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: Seawater release allowed. Freshwater release may be allowed on a case-by-case basis after treatment to minimize exposure of other hatchery stocks.
3. TO BARREN SYSTEMS (no salmonids)	
a. Closed system (landlocked lake)	A landlocked lake has no outlet with direct or indirect connection to another watershed. Class II disease criteria ERM: If detected in a carrier state, transplant would be decided on a case-by-case basis. If clinically diseased with significant mortality, destruction of the lot(s) may be required.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
3. TO BARREN SYSTEMS (no salmonids) (continued)	All other Class II diseases: No restriction for pathogens 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.
	Furunculosis: As indicated by FAT with confirmation by isolation. If clinically diseased, 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. A withdrawl period after drug therapy may be required before release.
	IHNV (sockeye, kokanee): Release if no disease. Clinical signs of IHN and/or detection of virus requires destruction of affected lots per SSCP. Release virus-negative lots as soon as possible. Subsequent to release, mortality and/or detection of virus from additional lots will require their destruction.
	IHNV (chum, chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction of that stock unless demonstrated that unaffected lots have not been exposed and that remaining stocks on site have been isolated to prevent virus exposure, i.e. the facility must qualify as a PQU.
	ERM: Same as for C.3.a.
	Ich: Seawater release allowed. Freshwater release, treat and release to minimize exposure of other hatchery stock.

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
4. TO SYSTEMS WITH OTHER "SIGNIFICANT" (resource value) STOCKS OF SALMONIDS a. Closed system (landlocked lake) b. Open system	Class II disease criteria BKD: If Rs 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 Rs. In this case, the carrier state in released juveniles cannot exceed levels in Schedule I. Release is not allowed if clinically diseased as indicated by significant Rs related mortality occurring within 90 days prior to release date. Furunculosis: If detected in the present inventory of juveniles prior to release then those fish cannot be released unless other species or stocks at release site or upstream in the tributary of release also have histories of the causative agent. In this case released juveniles cannot exceed levels in Schedule I. If clinically diseased, fish must be treated until mortality is insignificant and carrier state does not exceed Schedule I. A withdrawl period after drug therapy may be required before release. IHNV (sockeye, kokanee): Release allowed provided no clinical signs of IHN and/or virus is detected. Release into non-sockeye systems having IHNV-susceptible species not recommended and will be evaluated on a case-by-case basis. IHNV (chum, chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction of affected lot(s) and entire inventory of that stock and others on site unless isolation from virus exposure can be demonstrated. Transplant of chinook, chum, rainbow or steelhead into a

C. DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
4. TO SYSTEMS WITH OTHER "SIGNIFICANT" SALMONIDS (continued)	non-sockeye watershed from a hatchery on a sockeye watershed will be evaluated according to sockeye transplant criteria **if** such a stock has not been isolated and/or has been exposed to a water supply containing rearing or spawning sockeye during any part of the life cycle. ERM: Same as for furunculosis except if clinically diseased with significant mortality, destruction of the lot may be required depending upon circumstances. Ich: Seawater release allowed. Freshwater release may be allowed on a case-by-case basis after treatment to minimize exposure of other hatchery stocks.

C.	DISEASE HISTORY OF JUVENILE FISH PRIOR TO RELEASE	DISEASE CONSIDERATIONS
5.	REMOTE SEAWATER 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 with large inventories of pre-smolts for release into a "mop up" terminal harvest fishery. Depending upon the fishery, natural stocks are exposed to negligible disease risk when hatchery returns are completely harvested. Release of smolts is not permitted when clinically diseased as indicated by a ≥5% cumulative mortality occurring within 90 days prior to seawater release.

Transfers Between Hatcheries

D.	TRANSFERS BETWEEN HATCHERIES	DISEASE CONSIDERATIONS
1.	EGGS	Class II disease criteria BKD: Transfer not allowed unless the receiving hatchery has a history of Rs in resident stocks and/or Family Tracking is done. Eggs from Rs-positive parent fish from the donor facility are destroyed before transport or while in isolation at the receiving facility. Family Tracking may reduce or prevent amplification of the carrier rate within the broodstocks returning to both facilities. Furunculosis: Eggs from high risk stocks not recommended if low risk stocks are available. However, no restrictions for criteria as previously stated (B.2.a). IHNV (sockeye, kokanee): If the receiving facility is qualified to take eggs directly from a broodstock, then the same facility can receive eggs from another facility.

D.	TRANSFERS BETWEEN HATCHERIES	DISEASE CONSIDERATIONS
1.	EGGS (continued)	IHNV (chum, chinook, steelhead, rainbow, cutthroat): Eggs from IHNV-susceptible 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 the 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 Rs agent has been detected within the previous two years of stock disease history unless receiving facility has a history of Rs. In the latter 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 the latter case, the detection level in the juveniles to be transferred cannot exceed Schedule I and no significant furunculosis-related mortality can have occurred.
		IHNV (sockeye, kokanee): Transfer to another sockeye facility allowed unless there are clinical signs of IHN confirmed by virus isolation. Not permitted to a non-QU that contains non-sockeye susceptible species.

2. FISH (from hatchery to hatchery, excluding a QU) (continued).

IHNV (chum, chinook, steelhead, rainbow, cutthroat): Can be transferred from a non-sockeye facility to a sockeye facility if the latter is a QU where fish can be reared on an IHNV-free water supply and are not intended for adult return to the same site as the sockeye returns. Screening for IHNV in non-sockeye susceptible species is not necessary when from non-sockeye water supplies unless there is clinical disease suggestive of IHN. 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 the receiving facility is also a QU.

ERM: Same as for 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 POLICY

Issue

Artificial propagation of sockeye salmon is seriously limited by infectious hematopoietic necrosis virus (IHNV) occurring naturally in all anadromous sockeye salmon stocks in Alaska. The disease (IHN) has caused catastrophic mortality of juvenile sockeye salmon in hatcheries throughout Alaska. The causative agent is a novirhabdovirus that can adapt to and infect other salmonid species other than sockeye salmon. Consequently, the virus has been isolated from Alaskan chinook and chum salmon and has caused serious mortality of chinook, chum and Atlantic salmon and rainbow and steelhead trout in the Pacific Northwest as well as rainbow trout and chum salmon in Japan. Stringent control methods are required in Alaska to help prevent 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 an ADF&G Sockeye Salmon Culture Policy[3] (SSCP). This policy has undergone some revision since its origin but the key criteria remain unchanged. These criteria include: 1) virus-free water supply; 2) rigorous disinfection procedures; 3) compartmentalization of eggs and fry during incubation and rearing; and 4) immediate destruction of fish infected with IHNV followed by disinfection to contain the spread of the virus within the hatchery and prevent exposure of wild fish stocks. Additional rationale and procedures for avoiding IHNV in sockeye culture are published under separate cover in the Alaska Sockeye Culture Manual by McDaniel et al. (1994).

SHELLFISH CULTURE

Importation of live shellfish species into Alaska for mariculture purposes
{Article 3, 5 AAC 41.070(b)(d)}

Pacific oyster (*Crassostrea gigas*)
Spat (seed) less than or equal (≤) to 20 mm shell diameter are permitted for importation only from ADF&G-certified sources on the Pacific coast of North America and Hawaii.

Weathervane scallops (*Patinopectin caurinus*)
Weathervane scallops originating from wild stocks or cultured stocks in the Southeastern Alaska and Yakutat areas may be imported and released only into the same area waters and only from ADF&G certified sources.

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[3] These guidelines were developed by staff within the ADF&G Fisheries Rehabilitation, Enhancement and Development Division (FRED) and included R. Burkett (Chairman), R. Saft, J. Burke, J. Sullivan and B. Kepshire.

Certification requirements for shellfish importation

1. Separate broodstocks must be from the same sources and locations from year to year (stock for certification cannot be composed of multiple stocks from different locations) and must be physically or geographically isolated from non-certified stocks during all stages of culture.

2. There must be no detection of disease or pathogens of transport significance in the stock to be imported. *Mytilicola* sp. is indigenous to Pacific oyster stocks in the Pacific Northwest and requires a maximum size limitation of spat (≥20 mm) to reduce the likelihood of successful establishment of the parasite in Alaskan shellfish.

3. There must be no detection of disease or pathogens of transport significance in other shellfish stocks from the certified facility or in the hatchery water supply. Depuration of the water supply to standards established by the ADF&G Fish Pathology Section may be required.

4. The seed stock proposed for certification must be physically or geographically isolated from non-certified stocks through all stages of culture.

5. There must be a written proposal with an operational plan providing details of the isolation facility, seawater source and procedures of physical separation of the stock identified for certification.

6. There must be a schematic layout of the facility and a map locating the facility, broodstock location, and any nearby hatcheries or shellfish beds.

7. Disease history information must be provided from past production experience including laboratory reports from any pathology examinations.

8. Live samples for histological examination must be submitted to the ADF&G fish pathology laboratories or other laboratories approved by the ADF&G Fish Pathology Section. This must be done at least 60 days prior to transport permit application and approval to allow processing and pathology examination.

    - 60 adult animals from the parent broodstock
    - 200 spat from the cohort of animals proposed for import
    - 1,000 larvae (if applicable)

9. Renewal of certification is done annually and requires histological examination of 60 spat from the year class to be imported accompanied by an updated disease history and a statement of hatchery performance reviewing success, problems, mortality, etc during the previous growing season. Certification is generally valid for a year from the date of sample collection for pathology examination.

10. Certification will become invalid if: a) a Class I pathogen and/or other pathogen causing significant disease or mortality is detected at the facility; b) an uncertified shellfish stock is brought into the rearing or grow-out areas utilized for the certified stock.

11. All lots of imported spat must be free of pests and other non-target species. This can be accomplished by immersion of spat in a freshwater solution containing 10 ppm of chlorine bleach for 15 minutes.

12. Each Alaskan buyer must have a valid shellfish transport permit issued by the ADF&G mariculture coordinator and a copy of the permit must be on file at the certified facility before shipment of oyster spat into Alaska.

13. Certification applications, certification renewals and other required information are submitted to the Mariculture Coordinator, ADF&G, Commercial Fisheries Division, P.O. Box 25526, Juneau, AK 99802-5526.

Requirements for transport of live oysters and other indigenous shellfish species within Alaska for mariculture purposes

1. An approved shellfish transport permit is required.

2. An acceptable disease history is required for the shellfish stock to be transported, *from the donor site*, regardless of whether the stock originated from a certified source or whether a disease history exits for the stock at another site. Disease histories are site and stock specific.

3. If no disease history is on record, then at least 30 live animals must be submitted to the ADF&G fish pathology labs for histological examination at least 60 days prior to issuance of a shellfish transport permit.

Grow-out sites

If only juveniles are present at a grow-out site and are to be transported, then juvenile animals are submitted for examination.

When adults and juveniles are present on the same site:

a. If animals are to be transported to establish broodstock elsewhere, then adult animals are required for examination.

b. If juveniles are to be transported to multiple sites for grow-out and market, then adults are required for samples. If this continues on a regular basis then the site will qualify as a seed distribution facility requiring annual juvenile examination.

c. Transport of native shellfish species collected on culture gear require juveniles for samples unless 30 adult animals are available.

Hatcheries and seed distribution facilities

- Shellfish hatcheries require annual or every other year inspections of the hatchery facility and complete disease histories on all adult broodstocks with yearly histological examinations of juveniles from each stock that are shipped to various grow-out sites.

- Seed distribution facilities having no adult animals on site will require annual histological examination of juveniles shipped to various grow-out sites.

4. The definition of how far transport must be to require pathology examination is defined by the discreetness of stocks or populations with regard to the planktonic drift zone of larval dispersal by ocean currents, etc. If this cannot be resolved to the satisfaction of the Department, any movement, regardless of distance, will require submission of samples for histological evaluation.

5. Shellfish samples for histological examination will be required from any grow-out site, shellfish hatchery or seed distribution facility when there is unusual shellfish mortality exceeding the expected background levels of if clinically abnormal animals are observed.

6. Detection of any Class I disease agents exotic to North America will require quarantine, stoppage of effluent discharge, complete destruction of affected shellfish stocks with proper disposal and disinfection of the facility. Detection of Class I pathogens exotic to Alaska, but not North America, may require all of the above if the agent poses a threat to wild or hatchery shellfish stocks.

Requirements for export of live shellfish

1. An approved shellfish transport permit is required.

2. Authorization from receiving state authorities is required prior to issuance of an approved ADF&G shellfish transport permit.

3. The Fish Pathology Section will provide a disease history for the stock if one is on record but will not certify a stock as “disease free” and is not obligated to provide additional pathology examination should that be required by the receiving state authorities.

APPENDIX: GENERAL GUIDELINES

Responsibilities of the ADF&G Fish Pathology Section

Mission Statement

The Fish Pathology Section monitors and controls finfish and shellfish diseases statewide (according to Title 16 of the Alaska Statutes) by oversight of wild and hatchery fish and shellfish health, conducting diagnostic surveys, developing finfish and shellfish disease policies and by advising the Commissioner of the Alaska Department of Fish and Game and other state and federal authorities on fish disease issues.

Facilities and Staff

Facilities

There are two state-of-the-art laboratories fully equipped for complete diagnostic capabilities encompassing the disciplines of bacteriology, virology, serology, histology, DNA probe, PCR, immunocytochemical staining and transmission electron microscopy.

Anchorage diagnostic laboratory: strategically located in Region II to adequately service approximately one half (12) of the Alaskan hatchery facilities and other user groups located in the Anchorage, Kodiak, Cook Inlet, Kenai Peninsula, Prince William Sound and Fairbanks areas. The Anchorage laboratory also has a small wet-lab space to hold live fish for disease transmission studies and performs the PCR work.

Juneau diagnostic laboratory: strategically located in Region I to adequately service the other existing one half (19) of the Alaskan hatcheries and other user groups located in the Southeastern panhandle including the Juneau, Petersburg, Wrangell, Ketchikan and Baranof Island areas. The Juneau lab has additional capabilities for the enzyme-linked immunosorbent assay (ELISA) and transmission electron microscopy.

Staff

Most Fish Pathology staff members have several years of experience in the fish health or medical technology disciplines. Collectively, professional degrees and staff training are in the fields of microbiology, virology, finfish/shellfish pathology and veterinary medicine. There currently is a total of 6 full time staff positions.

Anchorage laboratory: staff consists of a Laboratory Technician, a Microbiologist I and a Fish Pathologist II in charge of day-to-day functions.

Juneau laboratory: staff consists of a Laboratory Technician, a Microbiologist II and a Fish Pathologist III that administers the fish pathology program statewide and supervises both laboratories.

Program Responsibilities

Diagnostic Services

The fish pathology laboratories provide complete diagnostic services according to Bluebook standards of the Fish Health Section of the AFS to all user groups statewide for examination of wild and hatchery finfish and shellfish. The caseloads for both laboratories are generated by the following user needs or duty requirements:

1. Fish health problems at approximately 31 fish rearing facilities statewide.

2. Wild fish or shellfish health problems reported by agencies or private user groups. Notable examples include VHSV in Prince William Sound herring, Bitter Crab Syndrome in Tanner crabs, fish kills, fish abnormalities or product quality control problems discovered by fish processors, commercial or sport fisherpersons.

3. Finfish or shellfish transport permits (FTPs) for instate movement that require establishment of a disease history. Many permitted shellfish farms move or sell shellfish across the state requiring pathology examination by ADF&G.

4. Occasional out-of-state requests by other agencies or laboratories for fish or shellfish diagnostic services. Example: Alaska is a cooperator in the National Fish Health Survey conducted by the USF&WS.

Caseload Effort

In FY 2002 the fish pathology labs processed 141 accession cases with a total of 7,820 animals examined requiring a total of 12,829 diagnostic tests. Each case accession # requires a written laboratory report issued to the client submitting the samples and appropriate distribution of copies. This FY 2002 effort compares to a 10-year annual average of 175 cases, 11,102 animals examined and 18,500 tests performed.

Two year (2001–2002) Average Percentile of Testing Effort by User Group

- State Sport Fish = 10.6%
- Other State = 1.0%
- PNP = 53.0%
- Federal = 26.8%
- Sci-Ed = 1.7%
- Miscellaneous = 6.9%

Hatchery Support

1. Advise and supervise fish health activities at 31 fish rearing facilities statewide.

2. Provide on-site fish health and physical plant hatchery inspections and advise on proper sanitation procedures. Each hatchery inspection requires a written report issued to the hatchery manager and appropriate distribution of copies.

3. Provide diagnostic services, recommend appropriate preventative measures and therapy to control fish disease problems.

4. Assist hatchery personnel with collection of disease samples when appropriate.

5. Conduct fish health workshops in finfish and shellfish disease recognition to train Alaskan hatchery personnel. Generally 25–30 students attend representing most hatchery facilities statewide. Lectures, notebooks and laboratory training are provided.

   Staff at several remote hatcheries have used fish pathology workshop training to develop a health condition profile where fish are periodically examined for general appearance and disease conditions before serious mortality can occur. Some of these facilities have necropsy areas where basic microscopy and bacteriology are performed to make preliminary descriptive observations of any problem prior to consulting with fish pathology staff. The turn-around time for a diagnosis in such cases has been much reduced because of this training.

6. Project Coordination for FDA Investigational New Animal Drug (INAD) permits for state hatchery facilities using OTC, formalin and erythromycin.

7. Review of Annual Management Plans from all permitted hatcheries in the state.

<u>Finfish and Shellfish Disease Management Through Regulatory Authority</u>
The Fish Pathology Section of the Department of Fish and Game has regulatory responsibilities as outlined in Title 16 of the Alaska Statutes. Specifically these duties fall within the general categories listed:

1. Review of all transport permits for instate movement and export of finfish, invertebrates and aquatic plants to evaluate health concerns that could occur due to animal or plant movement (5AAC 41.005, Permit Required; 5AAC 41.030, Permit Issuance or Denial; 5AAC 41.050, Permit Conditions).

   In 2002 Pathology staff reviewed approximately 200 permit applications for fish and shellfish transport or possession.

2. Develop and maintain a current finfish/invertebrate statewide disease history data base for the purpose of evaluating FTPs and use in other policy decisions (5AAC 41.020, Inspection for Disease of Brood Stock; 5AAC 41.080, Reporting and Control of Fish Diseases at Egg-Take Sites, Hatcheries and Rearing Facilities).

   The Fish Pathology Section maintains an extensive aquatic animal disease history data base that extends back to the mid 1970s.

3. Oversight and periodic inspection of hatcheries (5AAC 41.080, Reporting and Control of Fish Diseases at Egg-Take sites, Hatcheries and Rearing Facilities) to advise, prevent and control fish diseases in hatcheries and to prevent pathogen exposure of wild fish stocks.

   In conjunction with this responsibility the statewide ADF&G fish disease policy was established in 1987 to govern day-to-day fish health concerns, assist pathology review of transport permits and provide additional guidelines on shellfish health. A separate document, "Alaskan Sockeye Salmon Culture Manual" published in 1994 provides details of the ADF&G sockeye culture policy to control IHNV that was first implemented in 1981.

4. Advise departmental staff and other user groups regarding compliance with the current ADF&G fish disease policy when developing stock management plans, fish transplant strategies and policies, or other enhancement projects to establish populations of fish.

5. Disease certification of Pacific oyster seed for import into Alaska from the Pacific Northwest and Weathervane scallop seed from stock originating from Southeast Alaska and Yakutat (5AAC 41.070, Prohibitions on Importation and Release of Live Fish). These are the only aquatic animal species allowed for import into Alaska except for ornamental fish not raised for human consumption.

6. Require the destruction of diseased fish when mandated by the severity of the pathogen as determined by 5AAC 41.080 (Reporting and Control of Fish Diseases at Egg-take Sites, Hatcheries, and Rearing Facilities) and by the current ADF&G Fish Disease Policy.

Research

The Fish Pathology Section conducts applied research to achieve the objectives listed:

1. Disease transmission studies using on-site wet-lab facilities to determine pathogenicity and mode of transmission of new or poorly described disease agents (VHSV, Phoma).

2. Morphological description and biochemical characterization of new or poorly described disease agents (Bitter Crab Syndrome, VHSV, Phoma).

3. Evaluation of new techniques for the detection of finfish and shellfish disease organisms (ELISA, Dot Blot, DNA probe, PCR).

4. Distribution surveys of specific disease agents in finfish and shellfish stocks statewide (IHNV, VHSV, Bitter Crab Syndrome, VEN, BKD).

5. Maintain a current knowledge of existing and emerging research findings on new finfish and shellfish diseases and diagnostic methods through review of scientific literature, professional development training and attendance of professional meetings and workshops.

   Two staff of the Fish Pathology Section received FRED Division awards for technical achievement in 1988 and 1990 for the discovery and characterization of VHS virus in Alaska and for the development of the ELISA to screen Alaskan fish for the BKD agent.

Public Education

As part of the ADF&G, the Fish Pathology Section provides information to the public regarding finfish and shellfish disease issues and inquiries. This has been accomplished in the following manner:

1. Provide one-on-one information to fisherpersons, processors, other government agencies and the media regarding finfish and shellfish abnormalities, mortality, etc. by telephone, email, laboratory reports and scheduled meetings.

2. Conduct informational laboratory tours to elementary through college-level student groups.

3. Support local schools with mentoring of students for science fair projects.

4. Publish research results in peer review journals to disseminate new information on finfish and shellfish diseases (see attached list of laboratory publications).

5. Distribution among resource agencies and user groups of an informational color brochure describing the ADF&G Fish Pathology Section program.

6. Distribution of a Fish Pathology Laboratory Procedures Manual to Alaskan hatcheries and to several fish pathology laboratories nationwide that have requested the document as a reference. This manual has been used as a template for a procedures manual by the USF&WS for their 9 fish health centers and the National Wild Fish Health Survey (True 2000).

7. The Principal Fish Pathologist serves as finfish/shellfish disease technical representative/expert for the State of Alaska for participation in fish health issues with other state and federal agencies inside and outside of Alaska.

<u>Affiliations with Other Fish Health Laboratories, Agencies and Organizations</u>
Over several years the Fish Pathology staff have networked with other fish health laboratories outside of Alaska in various government agencies and organizations through different activities including co-authored publications in peer reviewed journals (see publications list), professional committees and societies and the rare need for specialized diagnostic tests that are not routine or practical for the ADF&G laboratories. The ADF&G Fish Pathology Program is well recognized by the fish health profession within and outside of the United States.

<u>Professional Activities Outside the ADF&G</u>
Fish Pathology staff:

1. Serve as the Alaska technical representative on the Pacific Northwest Fish Health Protection Committee (PNFHPC) and Chaired the committee from 1994–1995.

2. Are certified as Fish Health Inspectors and Fish Pathologist by the Fish Health Section (FHS) of the AFS.

3. Are members of the FHS of the AFS.

4. Served as President of the National Fish Health Section of the AFS from 1994 to 1995.

5. Are members of the European Association of Fish Pathologists.

6. Are members of the Society for Invertebrate Pathologists.

7. Hosted two PNFHPC meetings in Juneau (1995, 1997).

8. Hosted the 1997 National Meeting of the FHS in Juneau attended by 80 fish pathologists nationwide including Spain and Portugal.

9. Are technical advisors on fish health in Alaska for the NMFS offices in Silver Springs, MD and Washington D.C. regarding the previous Australian and New Zealand trade embargos on fresh/frozen US and Canadian salmon products.

10. Review about 20 manuscripts per year for peer reviewed journals and/or proposals for outside funding from USDA, Saltonstall-Kennedy (NOAA), Sea Grant, Whirling Disease Initiative, etc.

11. Attend 3-4 out-of-state fish health meetings annually to present research and remain current with new discoveries and technological advancements in the fields of fish and shellfish health.

12. Were among technical advisors invited by the NMFS and Idaho Fish and Wildlife for determination of disease screening protocols for the endangered Red Fish Lake sockeye salmon program.

13. Technical advisor for the USF&WS planning of the National Wild Fish Health Survey and are co-operators whereby large numbers of Alaskan samples are being examined in the Anchorage lab.

Quarantine Unit Fish Hatcheries

Introduction

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

Disease screening and disinfection play major roles in reducing the risk of spreading fish pathogens. However, testing is usually limited to a few disease agents of highest concern and testing may fail to detect low carrier-state levels of a pathogen. To provide additional protection for other hatchery stocks, the operational plan for the hatchery should provide for the isolation of a remote stock from others in the facility during 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. However, no design is failsafe; the 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 to provide barriers against the transfer of pathogens within the hatchery and to local wild stocks beyond facility perimeters. The most effective design is the Quarantine Unit (QU) that 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 of 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 recommend the development of quarantine units in hatcheries that use remote fish stocks. If disease occurs in a facility without quarantine capability, fish releases may not be authorized. At the very least, extensive testing and waiting periods may be required before fish can be certified for release. Development of quarantine facilities is an important investment for controlling pathogen spread, especially when wild fish stocks are at risk.

Classification

Hatcheries with offsite projects will be classified according to the level of quarantine criteria that have been satisfied. An ADF&G fish pathologist will determine the facility's classification based on an on-site inspection. The Fish Pathology Section recommends either ultraviolet or chlorination-dechlorination systems for effluent depuration. Ultraviolet units must have a minimum rating of 30,000 microwatt seconds/cm 2 after 7,500 hours of lamp operation. Any chlorination system must deliver at least a 2 ppm residual level of chlorine with a 5 minute contact time before beginning dechlorination with sodium thiosulfate or sulfur dioxide gas. The hatchery operator is responsible for ensuring that procedures necessary for quarantine culture are followed. Failure to do so will result in reclassification of the facility.

Drugs and Other Chemicals Used in Aquaculture

Regulation of Drugs and Chemicals used in Aquaculture

Chemicals and therapeutic drugs are used in aquaculture to improve water quality, remove or control aquatic algae or vegetation, eradicate nuisance fish species or aquatic invertebrates, immobilize fish (anesthetics), prevent infectious diseases and to control fish pathogens when disease occurs. Disinfectants are those chemicals that destroy a pathogen on contact on an inanimate surface or in water. If a disinfectant is placed into the water for the purpose of treating the external surfaces of fish then it is classified as a drug. Hence, a drug is defined as any article intended for diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals, and articles (other than food) intended to affect the structure or any function of the body of man or other animals (Stefan 1992). More familiar categories of drugs used in aquaculture are vaccines to immunize fish against diseases by oral, immersion or injection routes or antibiotics administered internally to fish either by diet or by injection. Antibacterial efficacy is usually accomplished by disruption of bacterial metabolism, such as cell wall synthesis. A few antibiotics can be effective when used as a bath for external infections but this application for most is not cost effective or efficacious due to the high fish densities and water volumes. When the use of any chemical or compound in aquaculture may affect human health and safety either directly or indirectly such as an animal drug, a feed additive or as a veterinary device then it is regulated by the Federal Food, Drug and Cosmetic Act enforced by the Food and Drug Administration (FDA). Drug or chemical uses that may also affect animal safety or the environment are further regulated by the federal Environmental Protection Agency (EPA) as well as the Alaska State Department of Environmental Conservation (DEC). In some cases a compound may be regulated by all three agencies.

All life stages of salmonid fishes are considered by the FDA to be "food fish" for potential human consumption, hence chemical/drug use in salmonid aquaculture is regulated by that agency. At this writing, concerns by the FDA over food safety, human health and environmental impacts has resulted in increasingly strict interpretation and enforcement of existing regulations. The FDA has reconsidered and rescinded previous rulings that allowed the use of many common chemicals/drugs for fisheries management. Procedures for obtaining investigational new animal drug (INAD) permits for experimental use of compounds under consideration by the FDA have also been restricted further. Registration of previously used as well as new drugs and chemicals for aquaculture use is not a high priority for the drug/chemical manufacturers because of the low potential market demand or value of these products. Total annual U.S. market demand per compound is estimated at $10 mil compared to a required demand of $60 mil per compound to make registration profitable for private pharmaceutical companies.

Currently, there are only three compounds approved for treating salmonid diseases (oxytetracycline, Romet 30, formalin) and one anesthetic (MS-222). Sulfamerazine, although registered, is not currently marketed by its sponsor and is unavailable. Registered compounds still have species, pathogen or environmental restrictions according to their registered labels that limit their use. For example, oxytetracycline (OTC) is approved for salmonids against furunculosis or motile Aeromonas and Pseudomonas infections at water temperatures above 9oC. Thus, the drug cannot legally be used against enteric redmouth or cold water diseases or in lower water temperatures that exist in Alaska during much of the year without an INAD permit. Romet is only approved for use against furunculosis but it is also effective against enteric redmouth isolates that are resistant to OTC. Obviously, extensions of these labels are necessary to allow these other uses that will incur additional expenditures and investigational effort.

Additional compounds have been classified as low regulatory priority (LRP) that do not currently require an INAD. These compounds include hydrogen peroxide, acetic acid, carbon dioxide, sodium bicarbonate, sodium chloride, sodium sulfite, povidone iodine and calcium chloride. Iodine compounds can be used for both surface disinfection and water hardening of eggs. Low regulatory priority means these compounds can be used for now, but this ruling by FDA is neither an approval nor a guarantee of their safety and effectiveness. This means the FDA may take a different position on any or all of these compounds at a future date.

Two INAD permits have been granted to the University of Idaho for data collection mandatory for the registration of injectable (# 6340) or dietary (# 6013) administered erythromycin for control of Bacterial Kidney Disease (BKD). Salmonid hatcheries in the Pacific Northwest and Alaska can participate on these permits provided the proper paperwork and data reporting is completed and on file with the University of Idaho coordinators. The INAD for malachite green was withdrawn with no legal option now available for its use and there is not likely to be one. The U.S. Fish and Wildlife Service also administers INADs for several other compounds by application through the Bozeman office in Montana.

FDA-Approved Drugs For Use On Food-fish (Including INAD Permitted Use)

The following is a description of the current FDA-approved drugs commonly used in Alaskan salmonid hatcheries. Although the legal suppliers for fisheries use have been provided when known, changes occur periodically and must be investigated by potential users. Medicated feeds must be obtained from approved suppliers since “top dressing” of feeds with drugs is not approved by the FDA. Extra-label use of drugs requires an INAD permit or a prescription from a licensed veterinarian.

1. *Oxytetracycline* (OTC, Terramycin, Pfizer, Inc, New York, N.Y.) is an antibiotic approved for the dietary treatment of furunculosis (*Aeromonas salmonicida*), motile Aeromonas (*A. liquifaciens*) and Pseudomonas (*Pseudomonas* sp.) bacterial hemorrhagic septicemias and infections by *Hemophilus piscium* in salmonids and catfish. The dosages are 2.5–3.75 g/45 kg of fish per day for 10 days with a withdrawal period of 21 days prior to slaughter or

release. Lobsters are also included on the label for dietary treatment of gaffkemia (*Aerococcus viridans*) at a dose of 1 g/lb of feed per day for 5 days with a withdrawal period of 30 days. Terramycin has also been effective and widely used for treatment of vibriosis (*Vibrio anguillarum*), enteric redmouth (ERM, *Yersinia ruckeri*) and cold water disease (*Flavobacterium psychrophilum*) and has shown some efficacy against vertical transmission of BKD when injected into broodstock. However, these are not yet FDA-approved uses of the drug. Label claims nearing approval include use for systemic columnaris and cold water diseases in all freshwater-reared salmonids and for otolith marking of all finfish by immersion.

2. *Sulfamerazine* (American Cyanamid Co, Princeton, New Jersey) is an antibiotic approved for the dietary treatment of furunculosis in rainbow, brook and brown trouts at a dose of 10 g/45 kg of fish per day for 14 days with a 21 day withdrawal period. This drug has been illegally used for many food and non-foodfish species not on the registration label including the illegal use of generic sources of the drug. Consequently, the demand and cost recovery to the legal sponsor of the drug has been inadequate, forcing them to discontinue marketing of the product. Sulfamerazine is presently unavailable for fishery use.

3. *Romet-30* (Type A) and *Romet B* (Type B) (Hoffman-LaRoche, Inc, Nutley, N.J.) are two forms of the same potentiated antibiotic approved for use in salmonids for furunculosis and in catfish for enteric septicemia caused by *Edwardsiella ictaluri*. Romet-30 contains 25% sulfadimethoxine and 5% ormetoprim per pound and is added to medicated fish feeds available from various feed suppliers. Romet B contains 18.75% sulfadimethoxine and 3.75% ormetoprim. Romet-30 is the currently available form of the drug administered at a dose of 50 mg/kg fish for 5 days for both salmonids and catfish with withdrawal times of 42 days and 3 days, respectively. The drug has been effective in treating OTC-resistant furunculosis as well as ERM but the latter is not an FDA approved label use.

4. *Erythromycin thiocyanate* can only be used by facilities that have successfully been approved for use of INAD permit # 6013 administered by the University of Idaho through the FDA or by extra-label veterinary prescription. The drug is an antibacterial against *Renibacterium salmoninarum* (Rs) used as a feed additive that has been available from various feed companies including BioProducts, EWOS, Moore-Clark and Nelson's Sterling Silver Cup at doses of 100-200 mg/kg fish (0.3 to 3% of diet) originally administered for 14 days with 5 days of withdrawal and 7 more days of treatment. Additional regimens have been used including continuous 28 day feeding and intermittent feeding strategies of every other day for up to 60 days to increase drug absorption at colder water temperatures The current target species on this permit include coho, chinook, pink and sockeye salmon as well as 8 other trout species.

5. *Erythromycin injectable* (Erythro-200, Merial Ltd., Athens, GA) only for approved users of INAD # 6340 to be injected (IP or dorsal sinus) into adult salmonid broodstock (species listed above) no later than 15 days prior to spawning in 1 or more doses each of 5 to 40 mg/kg body weight. Bactericidal levels of the drug accumulate in the egg yolk if injected prior to ovulation and appear effective in killing Rs cells in the egg. This regimen in concert with prophylactic feeding of the drug to swim-up fry hatching from eggs of injected parents

has been successful in controlling vertical transmission of the Rs bacteria. Injected broodfish cannot be used for human consumption.

6. *Tricaine methanesulfonate* (MS-222; Tricaine-S–Western Chemical, Inc.,1269 Lattimore Road, Ferndale, WA 98248: Finquel-Argent Chemical, Redmond, WA) is a general anesthetic for amphibians, fish and other cold-blooded aquatic animals for temporary immobilization as an aid in handling, during manual spawning, weighing, measuring, marking, surgical operations, transport, photography and removal. The drug is dissolved in ambient water at a concentration of 15 ppm to 330 ppm depending upon the degree of sedation desired, species and size of fish, water temperature and softness of the water all of which determine drug efficacy. For other aquatic cold- blooded animals the drug is used at 50 ppm to 1,000 ppm depending upon the species and life stage of the animal. There is a withdrawal time in fish of 21 days and use in fish intended for food is restricted to Ictaluridae, Salmonidae, Esocidae and Percidae at water temperatures exceeding 10oC (50oF). Use in other fishes and cold-blooded animals is limited to hatcheries or laboratories.

7. *Formalin* (Formalin F, Natchez Animal Supply Co., MI; Paracide-F, Argent Laboratories, Redmond, WA; Parasite-S, Western Chemical, Inc., Ferndale, WA) (aqueous solution of 37% by weight formaldehyde gas) used as a parasiticide on all finfish and penaeid shrimp for external protozoa (*Ichthyophthirius* sp., *Chilodinella* sp., *Ichthyobodo* sp., *Schyphidia* sp., *Epistylis* sp., *Trichodina* sp.), monogenetic flukes (*Cleodiscus* sp., *Gyrodactylus* sp., *Dactylogyrus* sp.) and as a fungicide for Saprolegniaceae on all finfish eggs. As a parasiticide, the dose used is 15 ppm (1:67,000) up to 25 ppm (1:40,000) in earthen ponds for an indefinite period; for raceways and tanks, a 1 hour drip used at 170 ppm or about 1:6,000 (when > 50°F) up to 250 ppm or 1:4,000 (when < 50°F). Treatment can be daily until parasite control is achieved, however every other day treatments are often necessary to minimize gill hyperplasia from formalin exposure. Eggs are treated with a drip of 1000 ppm (1:1000) up to 2000 ppm (1:500) for 15 min. No withdrawal time is required for formalin, however a 4–7 day withdrawal period prior to egg hatching and prior to seawater introduction of smolts may be necessary to assure successful transitions through these life stages. An additional label claim nearing approval is use on all finfish for Saprolegniasis.

   Formalin should be in a closed container and stored in a safe area as described in the FRED manual "Safer Chemical Use In Alaskan Aquaculture". If stored at temperatures below 45°F formalin will form a white precipitate of paraformaldehyde that is more toxic than the parent chemical. When this happens, the aqueous portion is still usable but less potent. Formalin is a strong oxidizer and should not be used on fish when dissolved oxygen levels are 5 ppm or less.

Chemicals of Low Regulatory Priority: Not Approved by The FDA but Currently Allowed for Use On Food-fish Without an INAD

1. *Povidone iodine compounds* are used widely in fisheries as general disinfectants for utensils and as drugs when used for disinfection of eggs during or after water hardening. Products such as Wescodyne, Betadine and Argentyne are effectively used at 25–50 ppm for general disinfection and at 100 ppm for 10 minutes as external egg disinfectants or for 1 hour for both external and internal disinfection of eggs during water hardening. Argentyne is sold as a

buffered compound but all other iodophors must be buffered to pH 7.0 with sodium bicarbonate. Alaska Title 16 regulations (5 AAC 41.080(b)) require iodophor disinfection of all fish eggs within 24 hours when transported between watersheds for at least 10 minutes with 100 ppm of active iodine ingredient at a pH of 6.0 or greater. The ADF&G fish disease policy requires that all eggs taken into the hatchery be surface disinfected as above regardless of watershed source. Exemptions include multi million pink and chum egg facilities where surface disinfection of so many eggs from the hatchery watershed may be impractical and unnecessary if egg-associated diseases have not been a problem in the broodstocks.

2. *Sodium bicarbonate* is used (0.05%) to buffer certain unbuffered iodophor compounds to a pH of 6.0 to 7.0 when used at the working dilution of 100 ppm for egg disinfection. Sodium bicarbonate dissolved in ambient water at concentrations from 142 ppm to 642 ppm is also used as a means of introducing carbon dioxide into the water to anesthetize fish after a 5 min exposure.

3. *Acetic acid* can be used as a parasiticide at 1000 ppm to 2000 ppm for 1–10 minutes as a bath.

4. *Carbon dioxide gas* bubbled through the water column can be used as an anesthetic most commonly used for euthanasia of Pacific salmon broodstocks.

5. *Sodium chloride* (NaCl) and seawater have proved useful for the following:
    a. NaCl is used as an osmoregulatory enhancer or antistressor at 0.5% to 1% dissolved in freshwater for an indefinite period or at 3% for 10 to 30 minutes as a parasiticide.
    b. NaCl mixed 1:1 with calcium chloride (CaCl) has been used as a formalin replacement for treating egg fungus at the Robertson Creek Hatchery in Port Alberny, British Columbia. Hatchery staff used the equal mixture of NaCl and CaCl dissolved in freshwater at a final concentration of 20 ppt for a 1 hour static bath on coho and chinook salmon eggs. Results were successful based on green egg to swim-up survivals when eggs were treated three times a week. However, the treatment costs were said to be expensive which may be prohibitive at most facilities.
    c. Seawater is more feasible in cost than NaCl for treating egg fungus at those facilities near saltwater access. Seawater has been used successfully for this purpose in at least two facilities in Alaska, ie Kitoi Bay and Armin Koernig Hatcheries. Raw seawater of 20 ppt to 30 ppt is pumped to replace freshwater in the headboxes supplying incubators and then allowed to flow for a 1 hour exposure of pink and chum salmon eggs. Experienced temperature differences between fresh and seawater of 4–6°C have not caused any adverse effects and dissolved oxygen levels have been adequate. Some amount of fungus appears to buildup but not significantly enough to cause excessive egg mortality. Survivals from green egg to hatch in seawater treated incubators have been equal to those in incubators treated for fungus by other means. Vibriosis or other fish diseases potentially originating from raw seawater have not occurred. However, a refinement for better control of potential diseases and nuisance organisms would be UV depuration of the incoming seawater.
    d. Seawater at flows of 1–2 gpm mixed with 270 gpm of freshwater has also been used successfully to harden soft water from 0 to 300–500 units of conductivity at the Wally Noerenberg Hatchery. This has reduced coagulated yolk (whitespot) and facilitates

dissolving of eggshells at hatching for pink and chum salmon. Recommended levels of water hardness for elimination of whitespot are generally given in ppm ranging from 75 ppm to 100 ppm. Raw seawater should be depurated with UV light prior to mixing in the hatchery water lines to prevent introduction of marine fish pathogens.

6. *Calcium chloride* is also used to increase the hardness of water and has been used successfully at Deer Mountain (chinook, coho, steelhead), Crystal Lake (steelhead) and Snettisham (sockeye) Hatcheries to improve egg survival at hardness levels of 75 ppm to 100 ppm. However, the cost is significant.

7. *Sodium sulfite* has been used (not in Alaska) at a 15% solution for 5 to 8 minutes to improve hatchability of eggs. Further information on this use is vague.

8. *Hydrogen peroxide* (35%, Eka Nobel, Inc., Marietta, GA) can be used as a parasiticide and external antibacterial treatment for skin and gill infections as well as a fungicide for fish and eggs of all fish species. Approved dosages should not exceed 500 ppm for fish and 1000 ppm for eggs for 15 minute to 1 hour every other day.

Drugs Used That Are Not FDA-Approved

1. *Quaternary ammonium compounds* (Hyamine 1622, Roccal, Purina 4 Power) are used as footbath or utensil disinfectants at 600 ppm and have been used to treat bacterial gill disease of salmonid fishes at 1.0 ppm up to 4 ppm for a 1 hour flush for 2–3 consecutive days.

2. *Diquat* (1, 1'-ethylene-2, 2'-dipyridylium dibromide; Bipyridilium; Reglone) is an herbicide that has been used to treat bacterial gill disease of salmonids, generally at 2 ppm for a 1 hour flush for 2–3 consecutive days. Previously, Diquat was federally approved for use as an herbicide with food fish at 0.25 ppm to 2.5 ppm having a withdrawal period of 14 days before treated water could be used for other purposes. Currently the compound is not FDA-approved but there is an INAD for its use and it is EPA-approved for use on fungus with a 21-day withdrawl period.

3. *Calcium oxide* (Quick lime) and calcium hydroxide (slaked lime) are under consideration by the FDA as low regulatory priority compounds. Presently, these chemicals are not approved by FDA. Previously, these compounds were "generally regarded as safe" (GRAS) by the FDA and approved by the EPA as pond sterilants used at 1,338 lbs and 1,784 lbs per acre, respectively.

4. *Clove oil* and *Eugenol* may not be used in any form on fish that could be available for human consumption. *Isoeugenol* (AQUI-S) is a possible substitute under INAD exemption that requires a 21 day withdrawl period.

Surface Disinfectants Not Used on Fish

1. *Didecyl-dimethyl ammonium chloride* (Net-Dip) has been EPA approved as a general disinfectant and sanitizer used for fish holding equipment at 3.5 fl oz. in 4 gallons of water for 10 minutes. The chemical is not to be used directly on fish or in water containing fish.

2. *Calcium hypochlorite* (Olin HTH chlorinator) has been EPA approved as a disinfectant and sanitizer used for fish holding equipment and utensils at 200 ppm of available chlorine on contact to disinfect and sanitize fish tanks, raceways and utensils. Substrate disinfection may require overnight exposure. HTH has also been used to destroy and disinfect fry that have undergone infection by IHNV. In this case the chlorine is administered to the raceway or tank of fish for a 6 hour exposure (FRED manual Safer Chemical Use in Alaskan Aquaculture). Other uses have been to disinfect (depurate) effluent and rarely influent water used in fish culture. Effluent water is usually treated at 2 ppm residual chlorine for a minimum of 5 minutes contact time prior to dilution into surface water. Influent water has been treated successfully for viruses and bacteria at 1.2 ppm to 1.6 ppm free chlorine for at least 1 minutes contact time prior to dechlorination by sodium thiosulfate used at about 0.56 g per 1 gallon of chlorinated water. As a margin for error this is about 10 times more sodium thiosulfate needed to neutralize 1 gallon of water containing 2 ppm free chlorine (FRED manual Safer Chemical Use in Alaskan Aquaculture). Compressed sulfur dioxide gas has also been injected into chlorinated water lines as a dechlorinator.

NOTE:

- Many of the compounds listed above are dangerous to human as well as fish health if used incorrectly. For additional information on safe chemical use in aquaculture refer to Wood (1979), OSHA guidelines, MSDS forms supplied with all chemical products and the FRED manual of Safer Chemical Use in Alaskan Aquaculture.
- Drug treatment calculations should be checked by at least two people as assurance against possible mathematical errors.
- External drug treatments, such as the use of formalin, should be done on a small group of fish first as another check for the accuracy of calculations and to reveal any unexpected adverse reactions of fish due to unknown variables.
- Application of any drug for the treatment of a suspected fish disease should not be done without positive identification of the problem and a recommendation from ADF&G fish pathology staff.

Non-Chemical Disinfection

Whenever possible, steam cleaning should be substituted for chemical disinfection of raceways, fish tanks, floors and walls of buildings. Substrates and incubators have been disinfected by steam as well as by industrial washing equipment and detergent. Thorough rinsing must be observed when detergents are used.

Disinfection Procedures for Hatcheries

Egg Disinfection

Introduction: Disinfection is necessary to control the spread of pathogens carried on the surface of eggs. Disinfection is done immediately after fertilization and during or after water hardening upon arrival and prior to exposure to running water at the receiving station. Eggs that have not been disinfected must not be placed into water at the receiving station unless the water can be held and sanitized before release. Otherwise, eggs should be returned to the point of origin or destroyed. Disposal should be by burial in dry ground or in wet ground with quicklime. Disinfection should also occur when eggs are taken at any 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, ADF&G Staff, 1983).

- Betadine or Argentyne: 1:100 dilution of jug strength for 10 minutes (100 ppm iodine).
- Wescodyne: 1:150 dilution of jug strength for 10 minutes (100 ppm 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, with respect to a pathogen or event in the influent hatchery water, as a group of fish of the same species and age that originated from the same discrete spawning population and share a common water supply within the hatchery. It may become necessary to compartmentalize a single lot as defined above into separate lots based on separate water manifolds to individual rearing containers having separate utensils.

Equipment Sanitization

Introduction: The prevention of serious diseases caused by infectious agents at any hatchery is of utmost importance. Fish disease agents occur in hatcheries by the introduction of pathogens from egg, fish or equipment transfers and from populations of resident fish in the hatchery water supplies. Therefore, any inter-hatchery activities increase the concern for maintaining adequate disinfection and control of endemic diseases that may occur at those facilities.

Methods (Hnath 1983): 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 fish rearing containers, incubators, nets, fish pumps, utensils, raingear, waders, boots, egg sorters, fish transport vehicles or anything else that may have had contact with fish, eggs, or culture waters. If fish transport motor vehicles are exchanged between facilities, they must be disinfected accordingly in a thorough manner to ensure efficacy.

1. *Rearing containers*: 200 ppm active chlorine in liquid bleach (sodium hypochlorite, 5.25% active ingredient) or calcium hypochlorite (HTH, registered, 65% active ingredient chlorine) for a minimum of 10 minutes. 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, by using 2 lb sodium thiosulfate/lb HTH or 1.5 gm sodium thiosulfate/liter of 200 ppm chlorine. Chlorine is corrosive to metal and should be thoroughly rinsed 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.

2. *Fish transport vehicle exterior*: The exterior of motor vehicles including chassis and undercarriage is decontaminated with high temperature (115–130°C) steam or with 20 ppm 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.

3. *Fish transport vehicle interior*: Interior surfaces of motor vehicles, aircraft, or boats that have been contaminated during transport by contact with fish, eggs, or culture waters should be scrubbed with non-corrosive 600 ppm quaternary ammonia compounds, i.e., Hyamine or Roccal using 1.5 ml of 50% stock solution/liter water; Iodophors at 100 ppm or Roccal at 800–1000 ppm for 30 minutes are the disinfectants of choice for transport tank interiors rather than chlorine solutions that adversely affect pumps and aerators.

4. *Other equipment*: Incubators, utensils, fish pumps, nets, egg sorters, waders, boots, raingear, etc., can be disinfected with 200 ppm chlorine for 10 minutes; or 600 ppm quaternary ammonium compound for 30 minutes; or 100 ppm iodophor solution for 10 minutes. It may be necessary to scrub the disinfectant onto the surface. Disinfected equipment should be thoroughly rinsed with clean, uncontaminated water and dried before use.

5. *Personnel*: All individuals involved in transport operations should wear outer protective garments (rubber gloves, rain gear, boots, waders, etc.) when handling fish, eggs, or cultural water. Hands should always be disinfected before handling culture 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 culture water at a station can be disinfected by soaking for 30 minutes in 600 ppm quaternary ammonia compound and then rinsed thoroughly before being worn.

   Disinfectants are toxic to humans as well as fish. Care and common sense must be applied in their use to avoid upper respiratory irritations and/or contact dermatitis from continued overexposure. All containers of disinfectant must be capped or have lids on when not in use. The recommended levels for disinfection must not be exceeded. Disinfectants should be applied with brushes rather than aerosolized in a closed area. Goggles and respirators appropriate for the chemical used are necessary if aerosolization or splash will occur during chemical application. 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 include 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:

1. *Cleaning*: Most pathogens are removed from environmental surfaces by cleaning. Surfaces must be cleaned of dirt and organics for disinfectants to be effective.

2. *Drying*: Most fish pathogens (except IPNV) are destroyed by drying, thus, most anything that is clean and dry is generally free of viable agents. Some materials 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.

3. *Design*: A hatchery should be designed to allow maximal cleaning and drying of surfaces. The use of wood must be avoided and concrete floors should be sloped for adequate drainage and drying. 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, different fish stocks and age groups of fish also help prevent pathogen spread via water.

4. *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 disinfection is still unreliable because minor delamination or cracking of the fiberglass is often difficult to detect.

5. *Concrete raceways*: Raceway sanitization 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 least 48 hours.

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

7. *Fiberglass containers*: These should be considered as semi-porous due to cracks that are often too small to be noticed. Spraying disinfectant may not be sufficient and soaking is preferred.

8. *Artificial substrate*: Saddles or bio-rings should be pre-cleaned 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, substrate will develop a surface scum that can be removed prior to disinfection by: (1) agitation with sand in a cement mixer; (2) pressure spraying with water using commercial equipment or; (3) soaking in a citric acid solution for 24–48 hours.

9. *Disinfectants*:
   a. Steam: Steam should be used whenever possible to minimize use of toxic chemicals.
   b. Chlorine (with adequate respirator): 200ppm chlorine can be used as a soak or as a spray for disinfection. 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 200 ppm chlorine in the raceways and lines for 1 hour and at least 100 ppm for several hours. Letting the raceways soak overnight is the safe way to do this. Sodium thiosulfate (0.7g/1) provides the necessary quantity of sodium ions needed to neutralize the chlorine ions at 200 ppm strength after disinfection. Sufficient sodium thiosulfate should be on hand before 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 recommended level of 1.5 g of thiosulfate/liter errors on an excessive concentration to ensure complete neutralization of the chlorine. Measuring the residual chlorine (orthotolidine reagent or iodometric titration) after neutralization is recommended to be absolutely sure that toxic levels are not released into the environment. Drinking water often contains 0.l ppm that is sufficient to kill fish. Chlorine should not be detectable in effluent water.
   c. 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.
   d. Iodophors: Disinfection with iodophor solutions containing 100 ppm available iodophor will suffice for walls, floors, and other non-porous surfaces.
   e. Quaternary Ammonium Compounds (Roccal, Hyamine, etc.): Follow manufacturer's recommendations for use, but these compounds can be very toxic to fish and must be thoroughly rinsed from equipment before use.

10. *Respirators/Protective Clothes*: Should be worn whenever formalin, iodophor, chlorine, or other toxic chemicals are used, particularly in any manner that might cause aerosolization or splash. Respirators may be necessary during formalin treatments of eggs for fungus control. Knowledge of proper respirator use and assurance of proper function must be established before an individual performs tasks that require respirators. The correct respirator cartridges must be selected with regard to the toxic substances used.

11. *Environment*: Prior to sanitizing a raceway or any structure that will require large quantities of toxic chemicals, a failsafe plan must be designed that prevents environmental contamination. A second person should independently assess the plan and repeat the mathematical calculations.

Finfish and Shellfish Diagnostic Procedures

Diagnostic procedures for the detection of finfish and shellfish diseases are described in a separate document, the ADF&G Fish Pathology Section Laboratory Manual (Meyers 2000).

Hatchery Inspections

Annual or biannual hatchery inspections made by a fish health professional 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 inspector 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.

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

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

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

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

5. Methods of fish movement from incubators to rearing to release.

6. 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.

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

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

9. 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 number .

Good Fish Cultural Practices and Environment to Reduce Diseases

Many diseases, both infectious and noninfectious, can be prevented by good fish cultural practices and a clean adequate water environment. Both of these requirements either eliminate pathogens or reduce stressors which predispose fish to diseases. This discussion assumes use of a water supply having adequate physical and chemical parameters for rearing salmonids. Some variables that can and should be optimized include:

1. *Adequate water flows* without "dead spots" or air pockets, especially in incubators. Any areas of no or low flow within incubators can result in localized egg or fry death and fungus buildup. Mortality and fungus continues to spread resulting in excessive ammonia levels which promotes more fungus and mortality that can destroy an entire incubator of fish and those below if in a stack.

2. *Proper egg and fish loading densities* for various incubators and rearing containers as determined by volume and flow and/or other water quality parameters that might be unique to certain facilities.

3. *Proper feed pellet size* for the fish life stage and percentage of body weight fed according to water temperature. Feed with too many fines or too much feed too often can cause serious gill irritation, especially in chinook salmon. Dry feeds are generally too abrasive for starting chinook fry. Overfeeding fish when water temperatures are very low is another common mistake made by hatchery staff. When water temperatures are 1–2°C fish can be fed very low levels every other day without any adverse effects. Longer periods of starvation have been tested without problems occurring. Overfeeding at low water temperatures can cause gill irritation and excessive body or visceral fat that can result in organ dysfunction and sudden death under stress.

4. *Adequate conditions for feed storage* and use before expiration of its shelf-life to prevent loss of vitamins and nutritional deficiencies, mold growth and rancidity.

5. *Adequate dissolved oxygen* concentrations for sustaining life without excessive amounts (> 100% saturation) of total dissolved gases (TDG) to prevent gas bubble disease (GBD). Gas

supersaturation causes GBD which often predisposes fish to many other diseases that would otherwise not occur or remain subclinical. Any manipulation of hatchery plumbing, snow melt, hydroelectric turbines, extreme high barometric pressure are but some of the causes of gas supersaturation at hatcheries. Routine periodic monitoring of TDG should be done with a saturometer at various points in the water flow at all facilities. Supersaturation quite often is transient, producing spikes that cannot be measured unless frequent monitoring is done. Oxygen contacting systems can be used to displace dissolved nitrogen but overall TDG should not exceed 100%, regardless.

6. *Increase the hardness* of very soft incubation water (as described above) to prevent white spot in eggs and resulting dropout in the fry later on.

7. *Adequate fungus control* on eggs using saltwater or formalin. Excessive formalin treatment can cause white spot as well.

8. *Adequate disinfection of eggs* using iodophor compounds prior to incubation or placing into the hatchery water supply. Exceptions would be certain multimillion egg pink and chum salmon facilities where disinfection would offer no benefit due to lack of any significant egg-associated pathogens in the disease histories of such facilities.

9. *Use of disinfectant footbaths* between fish stocks and between incubation and rearing areas. Footbaths are especially necessary for preliminary isolation of a diseased group of fish.

10. *Use of separate utensils* for each fish stock and better yet for each separate lot of fish. Alternatively, utensils can be kept in disinfectant at various stations such that their common use will not spread diseases among the various lots or stocks of fish.

11. *Stringent use of the sockeye culture policy* where sockeye are concerned; the key elements being a virus-free water supply, adequate general disinfection including water-hardening eggs in iodophor for 1 hour and compartmentalization of eggs and fry to contain losses when IHN occurs. More details are given under "Sockeye Salmon Culture" in the ADF&G Special Publication No. 6 “Alaska Sockeye Salmon Culture Manual” (McDaniel et al. 1994).

12. *Use of disinfectable materials* for incubating eggs and rearing fry. This does not include anything made of wood.

13. *Adequate cleaning of raceways* to eliminate detritis, feces and saprophytic fungi.

14. *Limit fish access to the hatchery water supply* as much as possible, especially anadromous species. If obligate fish pathogens are present that routinely cause disease problems, depuration of the hatchery water should be considered.

15. *Avoid significant pinheading*. Mechanical removal of fry from bulk incubators may be necessary if fish will not outmigrate volitionally with enough yolk (about 3–5%) to successfully start on feed. This is especially true for certain stocks of chum salmon.

16. *Fish Health/Condition Assessment*; Periodic Examination of Moribund and Healthy Fish.

The first line of defense against disease or poor fish performance in general is a regular examination of fish by species and by lot within a species. This involves more than just casual observation during feeding. Such observation is important for noting behavior and mortality levels, water flows and water quality, etc but closer examination requires the sampling of fish, both healthy and moribund, for external as well as internal scrutiny. If this is done routinely when fish are apparently healthy, then the hatchery staff will be well on top of any problem if and when it develops. In fact, such routine examinations will allow hatchery staff to fine tune their feeding and other environmental parameters such that healthier fish are produced. When this occurs there is less stress and certain diseases may never occur. The procedures for this fish health assessment have been developed for the past 20 years by Goede (1997) and are simple to perform. Briefly, 20 live fish are collected from each lot within a stock, blood is examined for hematocrit (% packed red cell volume), buffy coat (white blood cells) and plasma protein content followed by length/weight measurements for body condition. Next, fish are examined externally for appearance of eyes, gills, pseudobranchs, thymus, fins and opercula. Internal examination follows for observations of mesenteric fat, spleen, hindgut, kidney, liver, bile and gonads. From these observations the general health and quality of the fish can be determined by comparison to a large data base of information. A computer program is available for entering the data, computing results and reporting of the fish health/condition assessment.

The importance of this kind of routine fish examination cannot be overemphasized for staying on top of dietary or water quality problems and the production of the best quality fish. An added benefit is that fish will be healthier with fewer disease problems. If any disease does occur it will be discovered early so that corrective action may be taken before the problem is out of control. Fish health/condition assessment procedures should be practiced at all hatcheries statewide.

Also, dead fish should be examined as well since they are often the best source of clinical signs if a disease is present.

Vaccines

Occasionally prophylactic drugs are necessary to prevent clinical infectious disease when the risk is high. There are several vaccines commercially available for prevention of bacterial diseases in salmonids such as vibriosis, enteric redmouth, and furunculosis. Most of these are applied by immersion but injection has often been more effective for furunculosis vaccines. On the horizon are vaccines against other fish pathogens such as IHNV. Currently, DNA vaccines for IHNV have shown very good protection but they require injection and are still in the experimental phases of laboratory and field testing (LaPatra et al. 2001).

The most commonly used vaccine in Alaska is the immersion type for vibriosis used to reduce fish losses once in seawater netpens. Generally, the risk of vibriosis becomes significant when seawater temperatures reach 8°C and beyond. If vaccination is planned, the following variables should be considered. Although these are based upon available laboratory and field results, users should always consult product information from the manufacturer for specific details regarding dosage, optimum fish size and immunization period at a suggested water temperature.

1. Ideally, immunization should occur about 30 days prior to seawater introduction such that adequate time has elapsed for immunity to develop at a water temperature of 10°–12°C.

2. The larger the fish (≥ 4g), the greater the immunological competence.

3. Variables such as small fish size, stress, smoltification, disease, dramatic fluctuations in water temperature, cold water temperatures, high suspended solids, improperly formulated diets and algal blooms can impair the development of adequate immunity.

4. Under optimum field conditions immunity may last from 9 month to 1 year, but generally the protective period is much less due to stress, etc. This is particularly true for chinook salmon.

5. Despite vaccination, fish losses of up to 10% can occur from vibriosis even if ideal immunization conditions were apparent.

6. Revaccination in seawater may be necessary, especially for chinook salmon.

Recognition of Disease at the Hatchery

1. Keep containers of sick fish as isolated as possible, reducing potential exposure and spread of the disease to healthy lots if the cause is infectious.

2. List the clinical signs observed.

3. Note the environmental history, i.e., can these signs be related to water-quality, handling, feeding, prior treatment for disease, etc., that hatchery staff can correct or account for?

4. Make an external examination of affected fish noting any gross lesions. Include wet mounts of gills, skin scrapes, and lesion material (if present) for examination with a compound microscope.

5. Make an internal examination of affected fish noting any gross lesions in the viscera, i.e., hemorrhage, pale coloration, discolored or white foci, ascites, foreign bodies. Include impression smears of lesion material, gut contents and blood for examination with a compound microscope.

6. Note any organisms observed during the external and internal examination of affected fish, i.e., protozoa, bacteria, helminth parasites, etc.

   Several manuals are available that describe and illustrate normal fish anatomy and the common fish health problems. Any of these would be helpful in directing preliminary fish health examinations. Recommended sources are “Diseases of Hatchery Fish” by James Warren (1991), the ADF&G Pathology Short-course Notebook and the Fish Pathology Section Laboratory Manual that provide detailed protocols for necropsy, sample collection and shipment as well as descriptive notes on common salmonid diseases in Alaska.

7. Contact an ADF&G fish pathologist.

8. Be prepared to provide a complete recall of events (anamnesis) to the pathologist in charge. Fill out a case data report. Information could include:
   A. Environmental history
      a. Water-quality
         (1) physical parameters (temperature, pH, DO, salinity, runoff, etc.)
         (2) source of water (well, river, reservoir)
         (3) any re-circulation or alternative water source used.
      b. Nature of containment for fish (raceway, VR, pen, etc.) and hatchery layout regarding the number of different fish lots.
      c. Other aquatic species present in the water source and their relative abundance.
      d. Any new change of hatchery procedure (new equipment, different disinfectant, change in diet, etc.)
      e. Any recent treatment for a fish health problem.
      f. Any recent importations of fish or fish eggs onto the hatchery premises.
      g. Type of diet used and storage practices.

   B. Present Clinical History:
      a. Fish species, life-stage, brood year, source of stock, how many lots affected and loading densities.
      b. Nature of disease
         (1) acute or chronic
         (2) clinical signs
            (a) behavioral
            (b) mortality rate
            (c) external lesions
         (3) necropsy exam
            (a) how many fish examined
            (b) external observations, gross and microscopic
            (c) internal observations, gross and microscopic

Provision of as much information as possible by hatchery personnel may determine whether the fish health problem requires a personal visit by a qualified fish pathologist for collection of samples or whether fish samples may be shipped to the pathology laboratory. In either case, complete preliminary information facilitates a more rapid response time in the diagnosis of a fish health problem. This is especially important for the many remote hatcheries in Alaska.

9. Disease Diagnosis by the Fish Pathologist is Based on:
   - Isolation of an infectious agent (bacterial or viral) if present in the samples examined, followed by biochemical and/or serological identification (definitive evidence). Protozoa and helminth parasites are generally identified according to their morphologies.
   - Clinical signs of disease (gross and microscopic tissue morphologies) and other anamnesis information (presumptive evidence).
   - Histopathology may be done if other observations and tests prove negative (usually presumptive evidence).
   - Transmission electron microscopy, optional and not usually routine (can be definitive evidence).

10. Treatment, if appropriate, is determined by identification of the etiological agent or noninfectious cause and is recommended by the pathologist in charge.

Partial List of Common Pathogens for Finfish and Shellfish in Alaska

Finfish

Bacteria
1. *Renibacterium salmoninarum* – Bacterial Kidney Disease (BKD)
2. *Aeromonas salmonicida* (typical and atypical) – Furunculosis
3. *Aeromonas hydrophila/liquefaciens* – Motile Bacterial Septicemia
4. *Pseudomonas fluorescens* – Motile Bacterial Septicemia
5. *Pseudomonas* sp. – Motile Bacterial Septicemia
6. *Vibrio (Listonella) anguillarum* – Vibriosis
7. *Yersinia ruckeri* (types 1 & 2) – Enteric Redmouth
8. *Serratia liquefaciens* – Bacterial Septicemia
9. *Flavobacterium psychrophilum* – Coldwater Disease (sequela myeloencephalitis)
10. Unidentified Flavobacteria – Superficial skin and gill infections

Fungi
1. *Saprolegnia* sp. – External egg and body fungus, internal systemic mycoses
2. *Phoma* sp. – Internal infections of air bladder and other organs

Protozoa
1. *Trichodina* sp.– External gill and skin infections
2. *Trichophrya* (*Capriniana*) – External gill infections (commensal)
3. *Ichthyobodo* (*Costia*) *necatrix* – External gill and skin infections
4. *Epistylis* sp. – External gill and skin infections
5. *Myxobolus* sp. – Skin and internal infections in both fresh and saltwater fish species
6. *Henneguya* sp. – Skin and internal infections in both fresh and saltwater fish species
7. *Ceratomyxa shasta* – Internal infections of salmonids
8. *Ichthyophonus* sp. – Internal granulomatous disease of marine species

Viruses
1. Infectious Hematopoietic Necrosis Virus (IHNV) (sockeye salmon and rarely, chum and chinook salmon)
2. Viral Erythrocytic Necrosis Virus (VEN) (Pacific herring)
3. Viral Hemorrhagic Septicemia Virus (VHSV) (Pacific herring, cod, hake, pollock)
4. Aquareovirus (chinook salmon)
5. Paramyxovirus (chinook salmon)

Non-infectious diseases or causes of mortality
1. Gas Bubble Disease (air entrapment; drop in barometric pressure; heating of very cold water)
2. Gill hyperplasia (feed or particulate abrasion; ammonia or formalin toxicity)
3. White Spot Disease (handling; soft water and/or aluminum toxicity)
4. Drop Out (too little yolk at swimup; sequela to white spot or not osmocompetent in seawater situations)

5. High egg or yolksac fry mortality (mechanical failure of incubator accompanied by ammonia toxicity and *Saprolegnia*; overloading, blank eggs or other developmental problem)
6. Excessive fat in body cavity and/or fatty liver (overfeeding during cold water temperatures)
7. Bloat (excessive feeding in seawater)

Shellfish: Bivalves

Bacteria
1. *Nocardia* organism (FIB) in vesicular connective tissues - not common (Pacific oyster)
2. Rickettsial intracellular organisms in vesicular connective tissue cells, digestive tubule cells (Pacific oyster), gill epithelium (weathervane scallop, blue mussel, clam species), and various other tissues

Protozoa
1. *Ancistrocoma*-like ciliate in the digestive tubules and gut (Pacific oyster)
2. Unidentified small eosinophilic thigmotrich ciliate on the gills (Pacific oyster)
3. *Sphenophyra*-like ciliate on the gills (Pacific oyster)
4. Unidentified gregarine-like organism within vesicular connective tissue (Pacific oyster)
5. *Trichodina* sp. on gill and mantle epithelial surface (Pacific oyster)
6. *Hexamita* sp. within the tissues as secondary invaders (Pacific oysters)
7. Coccidia-like organisms in connective tissue and kidney (native littleneck clam, basket cockle).
8. Unidentified gregarines in gut, gills or otherwise histozoic (oysters, littleneck clams, cockles, blue mussels, scallops)

Metazoa
1. Unidentified arthropods on the gills, in the digestive tubules and intestine (*Pseudomyicola*) (oysters, littleneck clams, blue mussels, cockles)
2. Helminths encysted in tissues or in gut: turbellaria, trematode metacercariae or sporocysts, nematode larvae, tapeworm plerocercoids (oysters, littleneck clams, cockles, blue mussels)

Non-infectious anomalies (Pacific oyster)
1. Pearls
2. Hermaphroditism
3. Summer Mortality – stress related due to prolonged near-mature condition of gonads in both sexes but primarily females

Viruses
1. Ovacystis – papova-like viruses in oocytes and germinal cells of gonads (Pacific oyster)
2. Intranuclear Cowdry-type A inclusions of digestive tubule cells or mantle epithelium caused by a herpes-like virus (native littleneck clam, rock scallop)

Shellfish: Crabs

Bacteria
1. Bacteremia, possibly from injury or stress (red, blue, golden king crabs; Dungeness crab; *bairdi* Tanner crab)
2. Rickettsial intracellular organisms (blue and golden king crabs)

Viruses
1. Herpes-like virus in red, blue and golden king crabs

Protozoa
1. Bitter Crab Dinoflagellate Syndrome in Tanner crabs (*opilio* and *bairdi*)
2. *Paranophrys* ciliate in blue and golden king crabs

Metazoa
1. Rhizocephalan barnacle parasitism by *Briarosaccus callosus* in red, blue and golden king crabs
2. *Carcinonemertes* nemertean worm egg predators of Dungeness, king and Tanner crabs

Investigation of Fish Kills

Objective

Pathological examination of fish/shellfish can establish whether an infectious or parasitic cause of death is present and may be able to estimate time of animal death based on gross and microscopic tissue changes. These changes may provide clues to determine the cause of the fish kill if it is due to non-infectious environmental trauma or intoxication. Successful pathology interpretation is contingent upon receiving animal tissues that are in good condition and fresh. All animal tissues decompose after death, which quickly masks any abnormal tissue changes that might have been present in the living animal.

Habitat Assessment

1. Date and time of day.

2. Site location: Description of area affected including identifying landmarks and recent excavation, construction, or other activity present.

3. Name, address, telephone number of person who first noted the fish kill.

4. Names of other witnesses.

5. Time when fish kill first reported.

6. Estimated time when fish kill began.

7. Water quality characteristics:
    - Dissolved oxygen concentration (DO)
    - pH
    - Water temperature
    - Conductivity
    - Color of water
    - Odor of water

- Presence of algal blooms
- Salinity if seawater or estuary

8. Characteristics of the fish:
   - Condition of fish observed: live, moribund, dead, decaying
   - Size and species distribution of affected fish
   - Condition of the dead or moribund fish: gills flared, gaping mouths, fins extended, external lesions present on gills and skin, external parasites, excessively dark or abnormal coloration, spinal curvatures, excessive mucus, chemical odor, normal but dead, etc.
   - Behavior of live or moribund fish: listless, prostrate, corkscrew swimming, convulsive, attempting to escape from water, flashing, gasping at surface, normal, etc.

9. Characteristics of invertebrates:
   - Condition of invertebrates observed: live, moribund, dead
   - Species
   - Coloration and visual abnormalities
   - Behavioral abnormalities

10. Characteristics of plants (dead, discolored, normal, etc.) and sediments (discolored, bad odor, etc).

11. Presence of obvious chemical or other foreign materials. Description and sample of foreign condition.

Collection of Fish or Invertebrate Samples and Sample Materials

1. The best samples to collect are moribund (sick) fish/shellfish that must be kept on ice (do not freeze). If moribund fish are not available freshly dead will suffice. Package and label separately if both live and dead animals are collected (10 per group is usually sufficient). Decomposed animals are not useful for pathological examinations.

2. Place bagged animals into a cooler on blue ice with newspaper or other material in between for insulation.

3. Live (moribund) animals that are 15 cm total length or less can be placed in jars with 10% buffered formalin for immediate fixation (5 animals). Abdomens should be dissected open and internal organs pulled out slightly. Allow 10 times more volume of fixative than tissue for proper fixation.

4. Larger fish require on site excision of major tissues and internal organs. Approximately 1 cm square pieces of tissue are placed into 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts fixative.

5. Live and fresh dead animals should be placed into Whirl-Pak or Ziploc bags. Samples must be kept cold in transit.

6. A black waterproof magic marker is used to label plastic bags and tape on the outside of fixative jars. A label must also be included inside each fixative jar using a lead pencil to mark a square of paper. All labels should include collection date, location of collection, and contents.

7. A pathology sample submission form must be included with each group of samples submitted. Place all paperwork and forms into a Ziploc plastic bag to keep dry and legible. Place in cooler with the samples.

8. Always call the fish pathology section staff at the nearest laboratory before sending samples. Send iced and/or fixed samples to:

Anchorage Fish Pathology Laboratory
333 Raspberry Road
Anchorage, AK 99518
Phone: (907) 267-2244

or

Juneau Fish Pathology Laboratory
3333 Old Glacier Highway
Juneau AK 99801
Phone: (907) 465-3577

Common Mistakes to Avoid When Submitting Pathology Samples

1. Do not freeze the samples. Unintentional freezing can occur on blue ice if samples are not insulated with newspaper or other material. Freezing destroys tissue structure making certain pathological interpretations impossible.

2. Do not put too much tissue into fixative jars. Allow for 10 times more fixative than tissue.

3. Abdomens of fish must be dissected open before putting into fixative to adequately preserve internal organs and tissues.

4. Decomposed samples are not acceptable. If tissues are discolored, soft and pull apart easily or have a putrid odor, they are decomposed and of no value for pathological examination.

5. Habitat assessment information and a pathology submission form must be included when submitting samples. This information is often the most important for solving the cause of a fish kill and can only be provided by the person collecting the samples.

6. Excessive numbers of animals should not be placed in a single sample bag. This can result in crushed tissues and incomplete chilling. Use common sense- 5 fish per bag if small or single fish per bag if larger. Keep bags equally distributed in the cooler for shipment.

7. Live and dead animals should not be mixed. Keep them in separate bags and make sure everything is labeled properly.

8. Always call an ADF&G fish pathology staff member before submitting samples.

Schedule I For Fluorescent Antibody Test – Rationale

Detection of disease-causing agents in fish populations becomes more difficult with covert existence in a carrier state. Sub-clinical infection produces no obvious external or internal signs of disease. Thus, destructive sampling of larger numbers of fish is required to reduce the risk of not detecting a disease organism with acceptable statistical confidence. The efforts and cost required to process such samples are 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, Simon and Schill 1984). This model was used to compute Schedule I for all finite sample sizes. The binomial approximation to the hypergeometric distribution was used for the infinite population case (populations greater than 25,000).

The Schedule I used in this document for Rs (BKD), *A. salmonicida* and ERM agent screening consists of the last 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 providing a 95% confidence that at least a single diseased fish will be detected in the sample if disease is present within 5% of the population. Pre-release evaluations for BKD, ERM and *A. salmonicida* agents are performed with juvenile fish using the fluorescent antibody test (FAT). Results are recorded on a scale of 1+ to 5+ according to the intensity of fluorescence and relative numbers of organism in 30 microscope fields at 1000 X magnification. 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+ by 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+,4+ or 5+ fish are allowed due to the large numbers of disease organisms carried and potentially released into the environment.

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

Population Size = 1,000

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 = 2,000

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 = 5,000

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 = 10,000

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 = 25,000

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

REFERENCES

Amend, D. F. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. Trans. American Fish. Soc. 103(1):73–78.

ADF&G (FRED) Staff. 1983. Fish Culture Manual. Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement, and Development.

ADF&G (FRED) Staff. 1988. Safer Chemical Use In Alaskan Aquaculture. Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement and Development.

Elston, R. A. 1990. Mollusk diseases: guide for the shellfish farmer. Washington Sea Grant Program, Seattle.

Elston, R. A. 1999. Health management, development and histology of seed oysters. World Aquaculture Society. Baton Rouge, Louisiana.

Goede, R. 1997. Goede health condition profile. pp. 21–44 *in* Fish Health Workshop Notebook, ADF&G, Fish Pathology Section, Anchorage, Alaska.

Hnath, J. G. 1983. Hatchery disinfection and disposal of infected stocks. pp. 121–134 *in* A guide to integrated fish health management in the Great Lakes basin (eds. F. P. Meyer, J. W. Warren and T. G. Carey)

Kennedy, V. S., R. I. E. Newell and A. F. Eble. 1996. The eastern oyster, *Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland.

LaPatra, S. E., S. Corbeil, G. R. Jones, W. D. Shewmaker, N. Lorenzen, E. D. Anderson and G. Kurath. 2001. Protection of rainbow trout against hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. Vaccine 19:4011–4019.

Ossiander, F. J. and G. Wedemeyer. 1973. Computer program for sample sizes required to determine disease incidence in fish populations. J. Fish. Res. Bd. Canada 30:1383–1384.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraven, L. G. Fowler, and J. R. Leonard. 1982. Fish Hatchery Management. U.S. Dept. of Interior, Fish and Wildlife Service, Washington, D.C.

Simon, R. C. and W. B. Schill. 1984. Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. J. Fish Dis. 7:515–520.

Stefan, G. E. 1992. FDA regulation of animal drugs used in aquaculture. Aquaculture Magazine 18:62–67.

Stoskopf, M. K. 1993. Fish medicine. W.B. Saunders Company, Philadelphia, Pennsylvania.

True, K., editor. 2000. National Wild Fish Health Survey Laboratory Procedures Manual. First edition, U.S. Fish and Wildlife Service.

Vestal Laboratories. Notes on V.I.S. and other iodophors. 1978. Technical bulletin 85–201. St. Louis, Missouri.

Warren. J. W. 1991. Diseases of hatchery fish, 6^{th} edition. U.S. Fish and Wildlife Service, Pacific Region.

Wood, J. W. 1979. Diseases of Pacific salmon, their prevention and treatment, 3rd edit. State of Washington Department of Fisheries, Hatchery Division.

FISH PATHOLOGY SECTION PUBLICATIONS

Boustead, N. C., T. R. Meyers and S. Short. 1993. Absence of infectious haematopoietic necrosis virus (IHNV) in New Zealand sockeye salmon, *Oncorhynchus nerka*. New Zeal. J. Mar. Freshwater Res. 27:55–60.

Boustead, N., Pascho, R., Elliott, D., Meyers, T. 1999. Data on New Zealand salmon samples examined for *Renibacterium salmoninarum* by United States laboratories. National Institute of Water & Atmospheric Research Ltd, Christchurch, NZ. Client report No. CHC99/8, Project No. SCF9053, May, 1999.

Burke, J., D. Mulcahy. 1983. Retention of infectious haematopoietic necrosis virus infectivity in fish tissue homogenates and fluids stored at three temperatures. J. Fish Dis. 6:543–547.

Burke, J., R. Grischkowsky. 1984. An epizootic caused by infectious hematopoietic necrosis virus in an enhanced population of sockeye salmon, *Oncorhynchus nerka* (Walbaum), smolts at Hidden Creek, Alaska. J. Fish Dis. 7:421–429.

Carls, M. G., Marty, G. D., Meyers, T. R., Hinton, D. E., Thomas, R. E., Rice, S. D. 1998. Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. Can. J. Fis. Aquat. Sci.55:2300–2309.

Eaton, W. D., M. L. Kent, T. R. Meyers. 1991. Coccidia, X-cell pseudotumors and *Ichthyophonus* sp. infection in walleye pollock (*Theragra chalcogramma*) from Auke Bay, Alaska. J. Wildl. Dis. 27:140–143.

Eaton, W. D., D. C. Love, C. Botelho, T. R. Meyers, K. Imamura, and T. Koeneman. 1991. Preliminary results on the seasonality and life cycle of the parasitic dinoflagellate causing bitter crab disease in Alaskan Tanner crabs (*Chionoecetes bairdi*). J. Invertebr. Pathol. 57:426–434.

Emmenegger, E. J., Meyers, T. R., Burton, T., Kurath, G. 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Dis. Aquat. Org. 40:163–176.

Follett. J. E. and T. O. Burton. 1995. Epizootics of infectious hematopoietic necrosis virus in an enhanced population of sockeye salmon *Oncorhynchus nerka* smolts at Chenik Lake, Alaska. AK Fish. Res. Bull. 2:137–142.

Follett, J. E., J. L. Geesin and T. O. Burton. 1994. Detection of *Ceratomyxa shasta* in Alaskan chum salmon, *Oncorhynchus keta*. AK Fish. Res. Bull. 1:97–98.

Follett, J. E., T. R. Meyers, T. O. Burton and J. L. Geesin. 1997. Comparative susceptibilities of several salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). J. Aquat. Anim. Health 9:34–40.

Follett, J. E. and M. K. Schmitt. 1990. Characterization of a cell line derived from inconnu. J. Aquat. Anim. Health. 2:61–67.

Follett, J. E., J. B. Thomas, A. K. Hauck. 1987. Infectious haematopoietic necrosis virus in moribund and dead juvenile chum, *Oncorhynchus keta* (Walbaum), and chinook, *O. tshawytscha* (Walbaum) salmon and spawning adult chum salmon at an Alaskan hatchery. J. Fish Dis. 10:309–313.

Grischkowsky, R. S., D. F. Amend. 1976. Infectious hematopoietic necrosis virus: prevalence in certain Alaskan sockeye salmon, *Oncorhynchus nerka*. J. Fish. Res. Bd. Canada 33:186–188.

Hawkes, C. R., T. R. Meyers, T. C. Shirley and T. M. Koeneman. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of Southeastern Alaska. Trans. Amer. Fish. Soc. 115:252–257.

Hawkes, C. R., T. R. Meyers and T. C. Shirley. 1987. Growth of Alaskan blue king crabs, *Paralithodes platypus* (Brandt), parasitized by the Rhizocephalan, *Briarosaccus callosus* Boschma. Crustaceana. 52:78–84.

Hershberger, P. K., Kocan, R. M., Elder, N. E., Meyers, T. R., Winton, J. R. 1999. Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Alaska, USA. Dis. Aquat. Org. 37:23–31.

Kocan, R., M. Bradley, N. Elder, T. Meyers, B. Batts and J. Winton. 1997. North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. J. Aquat. Anim. Health 9:279–290.

Marty, G. D., E. F. Freiberg, T. R. Meyers, J. Wilcock, T. Farver and D. Hinton. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32:15–40.

McDaniel, T. R., K. M. Pratt, T. R. Meyers, T. D. Ellison, J. E. Follett, and J. A. Burke. 1994. Alaska Sockeye Culture Manual. Spec. Fish. Rpt. No. 6, Alaska Dept. Fish and Game, Juneau.

Meyers, T. R. 1998. Healthy juvenile sockeye salmon reared in virus-free hatchery water return as adults infected with IHNV: a case report and review of controversial issues in the epizootiology of IHNV. J. Aquat. Anim. Health 10:172–181.

Meyers, T. R. 1989. Apparent chronic bacterial myeloencephalitis in hatchery-reared juvenile coho salmon *Oncorhynchus kisutch* in Alaska. Dis. Aquat. Org. 6:217–219.

Meyers, T. R. 1990. Diseases of crustacean—diseases due to protistans and metazoans, 350–389 *in* Kinne, O. (editor) Diseases of Marine Animals Vol. III, Biologische Anstalt Helgoland, Hamburg, Germany.

Meyers, T. R., editor. 2000. Fish Pathology Section Laboratory Manual. Special Publications No. 12, Alaska Department of Fish and Game, second edit., CFMD Division, Juneau, Alaska.

Meyers, T. R., C. Botelho, T. M. Koeneman, S. Short and K. Imamura 1990. Distribution of bitter crab dinoflagellate syndrome in southeast Alaskan Tanner crabs *Chionoecetes bairdi*. Dis. Aquat. Org. 9:37–43.

Meyers, T. R., A. K. Hauck, W. D. Blankenbeckler and T. Minicucci. 1986. First report of viral erythrocytic necrosis in Alaska, USA, associated with epizootic mortality in Pacific herring, *Clupea harengus pallasi* (Valenciennes) J. Fish. Dis. 9:479–491.

Meyers, T. R., T. M. Koeneman, C. Botelho and S. Short. 1987. Bitter Crab Disease: a fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs *Chionoecetes bairdi*. Dis. Aquat. Org. 3:195–216.

Meyers, T. R., D. Korn, T. M. Burton, K. Glass, J. E. Follett, J. B. Thomas, J. Sullivan, N. Starkey, S. Short and K. Lipson. 2003. IHNV in Alaskan sockeye salmon culture from 1973 to 2000: annual virus prevalences and titers in brood-stocks compared to juvenile losses. J. Aquat. Anim. Health 15:21–30.

Meyers, T. R., D. Korn, K. Glass, T. M. Burton, S. Short, K. Lipson and N. Starkey. (*in Press*). Retrospective analysis of antigen prevalences of *Renibacterium salmoninarum* (Rs) detected by ELISA in Alaskan Pacific salmon and trout from 1988 to 2000. J. Aquat. Anim. Health.

Meyers, T. R., P. Krasnowski, D. Amend, B. Bachen, J. Cochran, K. Hauck, K. Rawson and R. Saft. 1988. Regulation changes, policies and guidelines for Alaska fish and shellfish health and disease control. Spec. FRED Report, Alaska Dept. Fish and Game.

Meyers, T. R., D. V. Lightner and R. M. Redman. 1994. A dinoflagellate-like parasite in Alaskan spot, *Pandalus platyceros*, and pink, *P. borealis*, shrimp. Dis. Aquat. Org. 18:71–76.

Meyers, T. R., J. F. Morado, A. K. Sparks, G. H. Bishop, T. Pearson, D. Urban and D. Jackson. 1996. The distribution of bitter crab syndrome in Tanner crabs (*Chionoecetes bairdi, C. opilio*) from the Gulf of Alaska and the Bering Sea. Dis. Aquat. Org. 26:221–227.

Meyers, T. R., S. Short, W. Eaton. 1990. Diseases of five commercial crab species in Southeast Alaska, 407–420 *in* Cheng, C. and Perkins, F. (editors) Pathology in Marine Science, Academic Press, New York.

Meyers, T. R., S. Short and W. Eaton. 1990. Summer mortalities and incidental parasitisms of cultured Pacific oysters in Alaska. J. Aquat. Anim. Health. 2:172–176.

Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger and R. Gates. 1993. Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of *Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, USA. Dis. Aquat. Org. 16:181–189.

Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger and R. Gates. 1993. Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaskan Pacific salmon. Dis. Aquat. Org. 16:191–197.

Meyers, T. R., Short, S., Lipson, K. 1999. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fishes. Dis. Aquat. Org. 38:81–86.

Meyers, T. R., S. Short, K. Lipson, W. N. Batts, J.R. Winton, J. Wilcock and E. Brown. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. 19:27–37.

Meyers, T. R., J. Sullivan, E. Emmenegger, J. Follett, S. Short, W. N. Batts and J. R. Winton. 1991. Isolation of viral hemorrhagic septicemia virus from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, pp. 83–91 *in* Proc. 2nd International Symposium on Viruses of Lower Vert, Corvallis, Oregon.

Meyers, T. R., J. Sullivan, E. Emmenegger, J. Follett, S. Short, W. N. Batts and J. R. Winton. 1992. Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 12:167–175.

Meyers, T. R., J. B. Thomas, J. E. Follett and R. R. Saft. 1990. Infectious hematopoietic necrosis virus: Trends in prevalence and the risk management approach in Alaskan sockeye salmon culture. J. Aquat. Anim. Health. 2:85–98.

Meyers, T. R., F. Thrower, S. Short, K. Lipson, J. Joyce, C. Farrington and S. Doherty. 1999. Different prevalences of *Renibacterium salmoninarum* detected by ELISA in Alaskan chinook salmon *Oncorhynchus tshawytscha* spawned from freshwater and seawater. Dis. Aquat. Org. 35:101–105.

Meyers, T. R. and J. R. Winton. 1996. Viral hemorrhagic septicemia virus in North America. Ann. Rev. Fish Dis. 5:3–24.

Oshima, K. H., K. H. Higman, C. K. Arakawa, P. de Kinkelin, P. E. Vestergard Jorgensen, T. R. Meyers and J. R. Winton. 1993. Genetic comparison of viral hemorrhagic septicemia virus isolates from North America and Europe. Dis. Aquat. Org. 17:73–80.

Rutecki, L., T. R. Meyers. 1992. Mortality of juvenile sablefish captured by hand-jigging and traps. N. Amer. J. Fish. Mgmt. 12:836–837.

Winton, J. R., C. N. Lannan, J. L. Fryer, R. P. Hedrick, T. R. Meyers, J. A. Plumb and T. Yamamoto. 1987. Morphological and biochemical properties of four members of a novel group of reoviruses isolated from aquatic animals. J. Gen. Virol. 68:353–364.

Weiland, K. A. and T. R. Meyers. 1989. Histopathology of *Diphyllobothrium ditremum* plerocercoids in coho salmon *Oncorhynchus kisutch*. Dis. Aquat. Org. 6:175–178.

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