Appendix 3. Applicable Excerpts from the Department of Fish and Game Operations Manual.

Fish Stocking Program 2163.2

Fish stocking is generally viewed as one of many fishery management tools. Hatchery produced fish are used to introduce species into new and existing waters, supply year-classes when natural reproduction is absent or has failed, or to produce or maintain a recreational or commercial fishery.

Anadromous Fish Planting

Anadromous fish planting to maintain or enhance recreational fisheries, or restore native populations will be conducted in accordance with established hatchery guidelines, mitigation agreements, or policies established by the Commission. The FPB has the responsibility of ensuring that anadromous fish planting in inland waters is in keeping with those policies.

Use of Hatchery Produced Fish

Trout and salmon produced in Department hatcheries may be stocked at different sizes and waters to meet specific fisheries management objectives.

Native Anadromous Salmon and Trout in Inland Waters

Anadromous salmon or trout from sources within this State may not be stocked in inland waters without prior approval of the FPB Chief. This stocking may be done only if the fish are surplus to the needs of the regular stocking program.

Native Anadromous Salmon and Trout in Anadromous Waters

Serious disease problems occur in several drainages in California. To prevent the spread of these diseases, restrictions on movement of fish between drainages may be imposed. It is also important to protect the genetic integrity of fish in drainages that have been relatively unaffected by past stocking. Movement of native anadromous salmon and trout between drainages must have prior written approval of the FPB Chief.

FISH HEALTH POLICIES AND PROCEDURES 2180

No formal policies have been adopted by the DFG or Commission. A working policy is to minimize the impact of diseases on fish, amphibians and aquatic invertebrates within California. Implementation of this working policy is achieved through:

1. inspecting imported fish and aquatic species, or their gametes, obtained from other states and countries;
2. inspecting aquatic species raised in State, private and cooperative program hatcheries prior to approval for planting into public waters;
3. inspecting wild fish and aquatic species captured for transport to a different location;
4. inspecting wild fish and aquatic species to acquire information, useful for fishery management decisions, on the geographical distribution of pathogens;
5. recommending therapies and corrective measures, or stock destruction to minimize disease impacts.

Fish Disease General 2181

When catastrophic diseases occur at private aquaculture facilities the Director is notified, and the Aquaculture Disease Committee may meet to discuss remedial actions, as specified in Title 14, Section 245. Recommendations of this committee are provided to the Director, who decides on the disposition of affected fish.

When catastrophic diseases occur at State hatcheries or cooperative programs, the Fish Disease Review Committee may meet to advise the Director on the recommended disposition of diseased fish. This committee is composed of the FPB Chief, Hatchery Operations Committee Chairman, Fisheries Management Committee Chairman, Fish Health Laboratory Supervisor, Lands and Facilities Branch Chief, and the appropriate regional manager.

Fish diseases also occur in wild populations, and it is important to determine which diseases or parasites are present when significant mortalities occur or sick fish become apparent. Knowledge of the occurrence and distribution of diseases and parasites throughout the State is of significant value to fishery managers, and reports of their occurrence are encouraged.

Diagnostic Procedures 2182

Diagnostic procedures for pathogen detection follow American Fisheries Society professional standards as described in "Bluebook: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, Fourth Edition, 1994, John C. Thoesen, Editor".

Collection of Samples 2183

Fish tissues deteriorate rapidly and many parasites disintegrate shortly after the fish dies, especially with dry or warm conditions. Post-mortem examinations can provide valuable information if properly handled and rapidly transported to the Fish Health Laboratory. The following procedures are recommended for sample collection for disease diagnosis. Although this section is specifically prepared for the collection of fish tissues, many of the procedures for amphibians and reptiles are similar. For nonfish tissue collections, Fish Health Laboratory personnel should be consulted.

1. Live Fish. Live fish are the best samples for examination. If a fish kill is occurring, select only live fish showing typical symptoms common to the population. Those near death but still alive are best. Try to select fish which will arrive at the laboratory alive, and not die en route. Between 5 and 10 fish showing symptoms should be collected. If possible, also collect 5 fish not showing symptoms into a separate container. Bring or ship these fish to the Fish Health Laboratory alive. For transport, fill a doubled plastic bag 1/4 full of pond water, overlay with oxygen, and seal with rubber bands. Place these into a container (styrofoam or plastic coolers) to protect from sunlight and temperature. Add ice or ice substitute to the cooler (not to the water in the bag) if needed to keep cool. Do not exceed 50 grams of fish per liter of water. Contact your nearest pathologist to arrange for examination. If shipped, use an express carrier (UPS, Federal Express, etc.) which will deliver fish in 24 hours or less. Seal containers tightly and label "UP, LIVE FISH / PERISHABLE".
2. Dead Fish. Some information may be obtained upon examination of dead fish if the fish have been dead only a short time. Fish dead longer than 24 hours in cool water, or 8 hours in warm water are virtually useless. Place fish in separate plastic bags and overlay with ice. Notify your nearest pathologist and transport or ship fish within 24 hours.
3. Frozen Fish. Very little information is recovered from frozen fish. Some viral and bacterial pathogens, or larger metazoan parasites can be recovered. Freeze only one fish per package. Do not use dry ice. Samples should arrive at the laboratory still frozen.
4. Preserved Fish. Histological evaluation of tissues from live, freshly euthanized fish can provide valuable information on diseases, toxic reactions, or nutritional deficiencies. Dead or frozen fish are unsuitable for histology. Ten percent neutral buffered formalin, Davidson's fixative, or Bouin's fixative are suitable for most field applications. Davidson's provides the best results for most cases. Fish smaller than one inch can

be placed directly into fixative. Larger fish which fit into the container must be prepared. Make an incision from the anus to the gill isthmus and gently pull the viscera out of the abdominal cavity. Puncture the swimbladder. Slit the intestine and liver (if larger than 1/2 x 1/2 x 1/4 inch). Make an incision along the back of the fish. Slice off the operculum to expose the gill to the fixative. Place the prepared fish into the container with fixative. For larger fish (too large to fit into the container) tissue samples must be prepared. Select any abnormal appearing tissue, and any lesion material. Very useful are: gill, liver, kidney, heart, intestine, spleen. Tissue pieces should be small, not to exceed 1/2 inch square by 1/4 inch wide.

Prepare tissues from sick, moribund fish and, in separate containers, healthy appearing fish from the same location. Five to ten fish per sample.

The volume of fixative to tissue should be at least 10:1 (i.e., a six inch fish per pint of fixative). Use of too little fixative results in unusable tissues which must be discarded.

Since you may be unable to recollect these samples, be sure to use plenty of fixative. Err on the generous side of 10:1. Leave tissues in fixative for 48 hours. Change solution to 70% ethanol (isopropyl alcohol is suitable) after 48 hours. Always ship samples in 70% ethanol, not fixative. Label each container with date, species, location. Do not re-use fixatives. Discard appropriately after use. Containers should be plastic (Nalgene, etc.). No glass.

To prevent contact of fixative with your eyes or skin wear gloves and protective eyewear.

5. Fixatives.

   Neutral Buffered Formalin: formalin 100 ml, sodium phosphate monobasic (NaH2PO4H2O) 4 grams, Sodium phosphate dibasic (Na2HPO4) 6 grams, distilled water 900 ml.

   Davidson's Fixative: 95% ethanol 300 ml, formalin 200 ml, glacial acetic acid 100 ml, distilled water 300 ml.

   Bouin's Fixative
   Call FHL if you want to use this. Recommended for bony tissues.

6. Fish Health Laboratories:

   2111 Nimbus Road
   Rancho Cordova, CA 95670
   916-358-2822 or 358-2827

601 Locust Street
Redding, CA 96001
530-225-2300

407 West Line Street
Bishop, CA 93514
760-872-1171