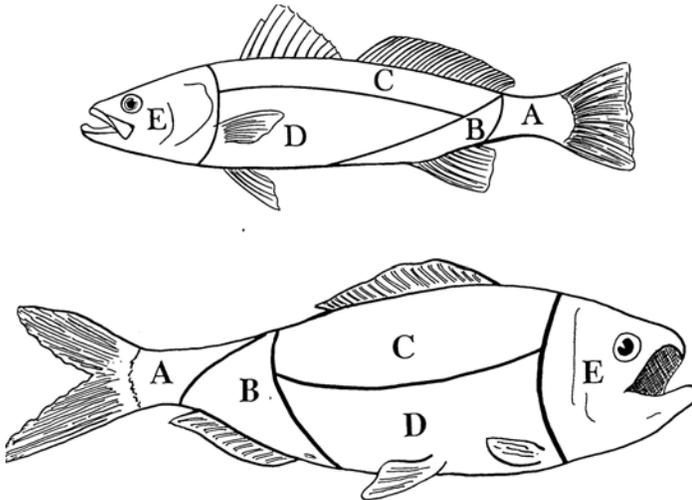


Collection and Handling of Fish Pathology Samples

The following is a set of instructions for handling fish specimens destined for postmortem examination, including euthanasia, proper fixation, and transport.

The best specimen to examine is a fish collected alive which was exhibiting the clinical signs most common to the population of diseased fish from which the specimen came. Whenever possible, a fish should be examined immediately after removal from the water. If not, the fish should be kept chilled and examined as soon as possible. After euthanasia, either put the fish in a 10% solution of buffered formalin, or store fish in individual plastic bags and place in a cooler or refrigerator. Do not freeze, as freezing creates artifacts, making evaluation of lesion morphology difficult, if not impossible.

Post mortem changes commence immediately after a fish is dead. These changes occur in both fish tissues and in parasites, making the task of assessing pathological conditions more difficult. Moreover, migrations of helminths after the



death of the fish often occur, so that the parasites true location becomes uncertain. ***For these reasons, fish collected dead may be of little use for diagnostic purposes.***

Fish Collection

Record species, fork length, lesion prevalence, and lesion location on all fish caught, when possible (see diagram above). Also record sample location, date,

and time of day, since these data will be correlated with water quality data. Note method of capture; e.g., whether a school of fish was sought by cast net. Even the smallest observation could turn out to be critical to determining the pathogenesis of these lesions.

Euthanasia and Fixation of Fish

If the fish is still alive, it will be necessary to humanely euthanize it before preparing it for transport. This may be done by placing the live fish into a solution of the anesthetic MS-222 at 500 mg/liter (about 2 teaspoons per gallon of water). This solution may be re-used for multiple fish.

After gill flaring has ceased, make a ventral cut from the gills to right above the anal pore (so as to open the body cavity for proper fixation). HOWEVER, avoid cutting into an obvious lesion area (e.g., do not cut into an ulcer around the anal pore). Place the fish into a 10% solution of neutral buffered formalin in a sealable container. There should be about ten parts formalin to one part tissue. If this is not possible, or formalin is unavailable, place each fish in an individual, sealed plastic bag and place on ice. Some fish will be too large for the container (e.g. flounder). In this case, cut out the lesion(s) with wide (at least 2 cm) margins of normal surrounding tissue and place in formalin. Also, include a 2 cm wide piece of a non-lesioned skin with its underlying body wall/muscle. Include the viscera when possible.

SUMMARY OF FISH COLLECTION:

1. Collect fish as fresh as possible. Dead or decaying fish generally have no diagnostic value.
2. Euthanize live fish humanely.
3. Record fork length, lesion prevalence, and lesion location.
4. Make a ventral slit to open body cavity.
5. Place in 10% neutral buffered formalin in a sealed container

Contact Mark Hale or Elizabeth Fensin of the DWQ Environmental Sciences Section for shipment of fish samples (919 743-8400). Fish preserved in formalin may be shipped at a convenient date. Fish chilled or refrigerated should be shipped within 24 hours.