CHAPTER 7

COLLECTING TISSUE SAMPLES

This chapter describes guidelines for collecting biological tissue samples (fish, crabs, crayfish, oysters, mussels). The tissue sampling guidelines have been standardized among the resource agencies in Texas.

Guidelines for Tissue Sampling in Texas

Various federal, state, and local agencies collect and prepare tissue samples from surface waters in Texas. The purpose of these guidelines is to ensure that tissue data collected by the different agencies are comparable. Tissues are sampled for a variety of reasons, including the assessment of both ecological and human-health risks, background conditions, the impacts of pollution, and long-term status monitoring.

However, tissue sampling and analysis are costly and time consuming, and decisions based on these data can have various effects on the public. For these reasons, it is important to maximize the comparability of data from tissue analysis by following a set of sampling guidelines used by all authorities that conduct tissue sampling in Texas.

These tissue monitoring guidelines are intended to be general and may not apply to all special-purpose studies. Variations from these guidelines must be detailed in quality-assurance-project plans.

Different sampling strategies are required, depending on the objectives. The four main objectives for collecting tissue data are assessing:

- background conditions
- long-term trends
- ecosystem health
- human-health risk

Background Conditions

The objective in sampling background conditions is to determine the level of contaminants in fish from areas least affected by pollution or pollutants. Sampling sites should be limited to freshwater or tidal streams. The Texas Parks and Wildlife Department (TPWD) already maintains fish-tissue data from least affected bays and many reservoirs (for example, Christmas Bay, Espiritu Santo Bay, and South Bay). Contrary to a common assumption, many ecoregion sites are not suitable for tissue sampling. Sampling for background conditions is typically done by the TPWD.

Where to sample—Areas of minimal or no impact (ecoregion sites).
Type of sample—Whole-body composites of mature fish.
Number of species—Two to three, including both predators and bottom-feeders
Number of individuals—Three to five, preferred, of the same species composited.
Size of individuals—Larger fish are preferred, due to bioaccumulation, with individuals varying in total length by no more than 25 percent within the sample.
Sampling frequency—All specimens may be collected in a single event, if possible.
Example species—*Freshwater:* bass, sunfish, buffalo, crappie, catfish, carp. *Estuarine*: catfish, seatrout, sheepshead, oysters.

Long-Term Trends

The objective of long-term-trend sampling is to monitor accumulation of contaminants in fish from sites where a major cleanup has occurred or where future impacts are likely. The sampling would be conducted once a year for an indefinite period. Therefore, it is imperative that the target species be common and easily collected year after year to facilitate data analysis. Long-term-trends for ecological health are assessed by taking samples from whole, mature fish. A long-term-trend study can include fillets if there is a need to assess the status of a human-health issue.

Where to sample—Sites with historical data, sites where a major cleanup has occurred, or where expected future impacts are likely to occur.
Type of sample—Whole body composites of mature fish for ecosystem health; fillets for health issues (for example, assessing the status of a consumption advisory).
Number of species—Two to three species.
Number of individuals—Three to five of the same species composited.
Size of individuals—By no more than 25 percent variance in total length.
Sample frequency—Once a year indefinitely.
Example species—*Freshwater:* bass, buffalo, catfish, carp. *Estuarine:* oysters, catfish, seatrout, sheepshead.

Ecosystem Health

The objective of ecosystem health sampling is to monitor bioaccumulation of contaminants in fish from sites affected by point or nonpoint sources. This type of sampling may be combined with sampling for human-health risk. Whole-fish samples are analyzed.

Where to sample—Areas affected by point or nonpoint sources.
Type of sample—Whole-body composites.
Number of species—Two to three for each sampling event.
Number of individuals—Three to five of the same species composited.
Size of individuals—Larger fish are preferred, due to bioaccumulation,
with individuals varying in total length by no more than 25 percent within the sample.
Sampling frequency—All specimens may be collected in a single event, if possible.
Example species—*Freshwater:* bass, freshwater drum, catfish, carp. *Estuarine:* catfish, flounder, drum, seatrout, sheepshead.

Human-Health Risk

The objective of this sampling strategy is to monitor the bioaccumulation of contaminants in fish at sites affected by point or nonpoint sources that are the target of recreational fishing. This type of sampling may be combined with sampling for ecosystem-health risk. Target species may differ based on regional preferences. If a problem is detected, the information will be referred to the DSHS for further studies or consumption advisories.

Where to sample—Areas impacted by point or nonpoint sources that are commonly used for recreational fishing.
Type of sample—Individual fillets of muscle tissue.
Number of species—Two to four.
Number of individuals—Three to five of the same species (individual analysis).
Size of individuals—At least the legal limit, however larger individuals are most preferable. Individual fish should vary in length by no more than 25 percent.
Sampling frequency—All specimens may be collected in a single event, if possible.

Example species—*Freshwater:* bass, crappie, catfish, freshwater drum. *Estuarine:* oysters, Atlantic croaker, sand trout, speckled trout, red drum, black drum.

Site Description

Site descriptions include the latitude and longitude (measured to the nearest second), the county, and detailed description of the site so it can be located on a county road map. The sites are described as primarily used for sport fishing, commercial fishing, or a combination of both, or not used for fishing. Permanently maintained field notes describe the specific sampling location, the date, and if appropriate, the chain-of-custody tag numbers. Field notes also describe the method of collection, including electrofisher settings and number of seine hauls, time of collection, hydrologic conditions (for example, high or low flow, incoming or outgoing tide), water temperature, days since last significant rainfall, and if collected, other physicochemical field measurements.

Notifying the TPWD in Advance of Collecting

A TPWD Scientific Collection Permit requires **notification of before sampling. A confirmed response from the local game warden is required prior to collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal** . Be prepared to give information on when, where, and why fish will be collected. This is required. Obtain phone numbers for regional offices from the TPWD Communications Center: Austin, 512-389-4848, or Houston, 281-842-8100. Always carry a copy of the permit when collecting biological samples.

Sampling Techniques for Fish

Collect samples using active procedures such as electrofishing, trawling, or seining. Passive capture techniques (gill nets and trammel nets) for fish can be used, as long as gear is clearly marked with permittee's name and permit number, and is checked on a frequent basis. Samples can begin to deteriorate in as little as two hours in warm water and six hours in cold water.

Collection Methods

The method by which tissue samples are collected will depend on several factors, including characteristics of the water body, the number of sampling personnel, and the availability of sampling equipment. For example, electrofishing does not work in estuarine waters, so a trawl or gill net might be used. In places where access is limited, hook and line may be the best approach. Chemicals, such as rotenone, should not be used to collect tissue samples. The following are methods generally used in tissue collection:

- trawl
- boat-mounted electrofisher
- hook and line
- bag seine
- gill net

Continue collection until the appropriate species, size, and number of individuals are caught.

Reference for collection methods. Murphy and Willis (1996).

Required Equipment

See Chapter 9 for the list of basic SWQM equipment.

Safety

See Chapter 11 for safety considerations.

Characterizing Fish Specimens

Scrub the coolers with soap and rinse them with tap water, distilled water, or ambient water prior to use. Hold live fish in clean live wells or in an ice chest until specimens are chosen for analysis. If necessary, rinse debris from the fish with ambient or clean water and immediately ice them in clean coolers.

Collecting Information in the Field

While in the field, or as soon as possible after collecting specimens, record the following information.

Length and weight of fish. Measure and record the total length of each fish to the nearest millimeter and the weight, to the nearest gram.

Sex. If a visual determination can be made, record the sex of the specimen if an individual whole fish is submitted for analysis. This is optional if composite samples are submitted, or if edible tissues are to be analyzed.

Anomalies. Record any unusual deformities, wounds, or infections (for example, fin erosion, tumors, scoliosis, parasites).

Sample Preparation for Edible Portions

Preparing Fish Samples in the Laboratory

Ship whole fish specimens to the laboratory and request that fillets be prepared by laboratory personnel. Keep in mind that this adds to the cost of analyzing samples. Follow the procedures for packaging and shipping whole fish samples. **Field personnel with appropriate facilities and experience are encouraged to prepare fillets in the field or office laboratory.**

Preparing Fish Samples in the Field

Equipment

When processing samples in the field, a clean working area is required when filleting fish, removing internal organs, or preparing shellfish samples. Fillet samples on a polypropylene cutting board that has been covered in aluminum foil with the dull side of the foil toward the fish.

- Thoroughly, clean the plastic cutting board, glass jars, and stainless steel knives with plastic handles and scales (for weighing) with distilled water and allow to air dry.
- Use electric knives only when necessary (due to hard scales) because they are difficult to keep clean.
- Replace the aluminum foil covering the cutting board between each sample.
- Rinse the knife and cutting board with distilled water between samples.

Preparation

- Fillet the fish and remove the skin unless a special study requires that the skin be left on.
- Scale the fish if the skin is left on.
- Fleecing (slicing off some of the underlying skin with a knife) may be substituted for scaling carp and buffalo, rather than the more typical method of knocking off scales. Use of this alternative method should be recorded in the field notes.
- Use the fillet from the left side of the fish. The fillet includes the muscle tissue beginning at the mid-dorsal line (all large bones removed except for intramuscular bones).
- If there is a sufficient sample from the left side, the fillet from the right side of the fish may be kept separate as a duplicate or backup sample.
- For very large specimens, collect only a posterior and anterior portion of the left fillet. *Do not puncture any of the internal organs.* Do not rinse the fillet with water—the rinsate may contaminate the sample or wash away pollutants of concern.

Observations

Note unusual conditions of the internal organs and record any in the field notes.

Packaging

- Wrap the fillet in aluminum foil with the dull side toward the fillet, twice. At a minimum 200 grams of tissue is needed for analysis.
- Avoid using tape, since the sample may be contaminated if the foil tears. Tape may be used to secure the outer plastic bag.
- Place the foil-wrapped fillets to be composited together in the same plastic bag and label them appropriately. A glass jar can be used for small specimens. A plastic bag or glass jar affords the best protection from melting ice.
- Use fish and fillets or portions of fillets with similar weights (within ±25 percent). The fillets can then be composited in a jar or wrapped in the same piece of aluminum foil.
- When unequal sized fillets are composited, they are homogenized in the laboratory, equal aliquots of homogenate from each fillet must be used for the composite to minimize the potential influence of unequal sizes.

Special Samples

Special studies may, on rare occasions, require internal organs—such as the liver, kidneys, or gills—or eggs. Remove internal organs or eggs and prepare them in a manner similar to that for fillets. The laboratory may request a different sample weight for these kinds of sample.

Preparing Crab Samples

Characterizing Specimens

Measure the total width of the carapace from the tip of one lateral spine to the tip of the opposite lateral spine, and record to the nearest millimeter. Weigh crabs individually.

Laboratory Preparation

For edible portions, ship specimens to the laboratory whole and request that the sample be prepared by laboratory personnel. This reduces sample contamination.

Field Preparation

If samples must be prepared in the field, a clean working area is required. For edible portions, pull away the top of the carapace of the crab (called "backing"), and remove the internal organs by shaking. The remaining crab can be folded and put in a jar.

Preparing Whole-Organism Samples

Fish

Packaging

- Rinse fish, if necessary, with ambient, tap, or distilled water.

- Use excess aluminum foil to carefully wrap and rewrap the fish with the dull side of the foil toward the fish. Avoid using tape, since the sample may be contaminated if the foil tears. For spiny-rayed specimens, clip the dorsal and pectoral spines before wrapping to avoid puncturing the aluminum foil.

- Place the foil-wrapped sample in a plastic bag. For large specimens, a plastic tie may be used to secure the plastic bag.

- Whole fish samples consisting of numerous small fish may be placed in glass jars that have been rinsed with pesticide-grade acetone (or methylene chloride) and allowed to air dry. Lids must have Teflon liners or be lined with aluminum foil, with the dull side toward the sample.

Crabs

Field Observations

Measure the total width of the carapace from the tip of one lateral spine to the tip of the opposite lateral spine, and record it to the nearest millimeter. For whole crabs, use the entire crab for the sample. Weigh crabs individually.

Packaging

Place samples in glass jars. Lids must have Teflon liners or be lined with aluminum foil, with the dull side toward the sample. If the specimens are too large for jars, they can be wrapped in foil.

Crayfish and Prawns

Field Observations

- Weigh crayfish or prawns individually. If the specimens are small, an average weight can be calculated.

- Record the total length from the tip of the rostrum to the tip of the telson, to the nearest millimeter.

- Use whole crayfish or prawns for the sample, unless the study requires tails only.

Packaging

Place samples in pre-cleaned glass jars. Lids must have Teflon liners, or be lined with aluminum foil, with the dull side toward the sample.

Oysters, Clams, Rangia, and Mussels

Laboratory Preparation

Ship specimens to the laboratory intact and request that samples be prepared by laboratory personnel. This reduces sample contamination.

Preparing Samples in the Field

If samples must be prepared in the field, a clean working area is required.

- Scrub unopened specimens with ambient, distilled, or tap water, and shake them to remove excess water. In order to facilitate opening clamshells, it may be useful to wrap the scrubbed specimens in pre-cleaned aluminum foil, or to place them in jars and cool or freeze them until they open slightly.

- With clean hands, insert the point of a pre-cleaned knife between the shells on the ventral side of the specimen.

- Cut the adductor muscle from the upper shell half (the flat shell half for oysters) and pry the shell open enough to drain the liquor into the sample container.

- Discard the upper shell half, cut away the meat, and drop it into the sample container.

- Avoid cutting into the soft-tissued organism and spilling the internal contents in or on the shell.

Containers for Samples

Pre-cleaned glass jars are the recommended sample containers. Lids must have Teflon liners or be lined with aluminum foil, with the dull side toward the sample. The jar must be weighed before and after adding the sample in order to obtain the sample weight and the average specimen weight. Record the number of specimens.

Storing and Shipping Samples

Labeling

Label the container with pencil or waterproof marker with the sample type, tag or sample number, date, and analyses requested.

Preservation

Whenever possible, analyze tissue samples that have not been frozen. The samples should not be kept on wet ice for more than 24 hours. Keep samples frozen until chemical analyses are performed.

Shipping

Pack fish in ice and ship to lab as soon as possible. Samples may be kept on ice overnight and shipped the next day, if necessary. If possible, effort should be made to immediately ship tissue samples. If there is to be a delay in shipping, tissue samples may be frozen. Check with the laboratory conducting the tissue analysis for its preference; some labs may prefer receiving frozen samples.

Due to increased shipping restrictions, samples being sent by a freight carrier require additional packing. No matter how much care is taken in sealing the ice chest, leaks can and do occur. For shipping, samples and ice should be placed in a large plastic bag inside the ice chest. The bag can be sealed by simply twisting the bag closed (while removing excess air) and taping the tail down. Leaking ice chests can cause samples to be returned or to arrive at the lab beyond the holding time. Place laboratory analytical request forms corresponding to samples in the ice chest in a Ziplock bag and tape it to the inside of the lid.

If shipping samples containing dry ice by public carrier, be sure to label them properly and notify the carrier that dry ice is being shipped.

Selecting Specimens

Time of Collection

For many objectives it is preferable to collect specimens outside of the active spawning season. Concentrations of some pollutants are not as stable during spawning season. Specimens should be sexually mature, if possible and if compatible with study objectives.

Sample Size

Sometimes this may not be possible; in such a case, alert the laboratory to the less-than-optimal sample size. Using present analytical methods, 200 grams is considered the minimum. Large fish are preferable—a consideration when choosing fish for a composite sample.

Species Selection

Select species suitable to the sampling objective. Try to sample fish that are permanent residents of the area, when possible. This might not be practical if a particular species is being evaluated for human-health risk.

Composite Samples

In composite samples, the smallest fish in the composite should be no more than 25 percent smaller than the largest. It is best to select specimens in the same size range, even if the total number of individuals in the sample will be fewer.

Composite samples should consist of the largest fish possible.

Composite samples should consist of at least three fish, preferably five.

Composite samples should consist of specimens from the same species and, if possible, the same sex.

Other Tissue Types

Special studies concerning possible pollution sources may require the collection of other tissues (for example, from the liver, kidney, or gills) for specific contaminants of concern. The same procedures described for edible tissue can generally be followed for these other tissue types.

Selecting Species Specific to a Study or Investigation

Different species have varying food habits, and rates of bioaccumulation differ among organic compounds, fish age, and life-cycle stage. The focus of a project (for example, human health, pollution uptake, environmental impacts) will determine which species are best for sampling. The value of a fish species for sport and human consumption differs in various regions of the state.

Several species (and suggested minimum size for each) are recommended because of their abundance, sport or commercial importance, position in the food chain, and potential for bioaccumulation. Table 7.1 lists target species and suggested minimum size.

Freshwater Species		**Background Long-Term Trends Ecosystem Health Human-Health Risk[a]**
Common Name	**Scientific Name**	**Minimum Length or Size Range (mm)**
Bass, hybrid striped	*Morone saxatilis* × *M. chrysops*	457
Bass, largemouth	*Micropterus salmoides*	356
Bass, striped	*Morone saxatilis*	457
Bass, white	*Morone chrysops*	254
Catfish, blue	*Ictalurus furcatus*	305
Catfish, channel	*Ictalurus punctatus*	305
Catfish, flathead	*Pylodictus olivaris*	457
Common carp	*Cyprinus carpio*	No minimum
Crappie, white	*Pomoxis annularis*	254
Crappie, black	*Pomoxis nigromaculatus*	254
Freshwater drum	*Aplodinotus grunniens*	No minimum
Gar, longnose	*Lepisosteus osseus*	No minimum
Gar, spotted	*Lepisosteus oculatus*	No minimum
Gray redhorse	*Moxostoma congestum*	No minimum
River carpsucker	*Carpiodes carpio*	No minimum
Shad, gizzard	*Dorosoma cepedianum*	No minimum
Shad, threadfin	*Dorosoma pentenense*	No minimum
Smallmouth buffalo	*Ictiobus bubalus*	No minimum
Sunfish	*Lepomis* spp. (do not mix sunfish species)	No minimum
Walleye	*Sander vitreum*	No minimum
Crayfish	*Procambarus* spp.	No minimum

Estuarine Species		Background Long-Term Trends Ecosystem Health Human-Health Risk[a]
Common Name	**Scientific Name**	**Minimum Length or Size Range (mm)**
Catfish, hardhead	*Arius felis*	No minimum
Atlantic croaker	*Micropogonias undulatus*	No minimum
Drum, black	*Pogonias cromis*	356–762
Drum, red	*Sciaenops ocellatus*	508–711
Sheepshead	*Archosargus probatocephalus*	No minimum
Spot	*Leiostomus xanthurus*	No minimum
Southern flounder	*Paralichthys lethostigma*	356
Spotted gar	*Lepisosteus oculatus*	No minimum
Trout, spotted sea	*Cynoscion nebulosus*	381–635
Trout, sand	*Cynoscion arenarius*	No minimum
Trout, gulf	*Cynoscion nothus*	No minimum
Blue crab	*Callinectes sapidus*	No minimum
Oyster	*Crassostrea virginica*	No minimum
Shrimp, brown	*Penaeus aztecus*	No minimum
Shrimp, white	*Penaeus setiferus*	No minimum
Shrimp, pink	*Penaeus duorarum*	No minimum

[a] Minimum sizes and size ranges are based on length limits set by TPWD.

Submitting Tissue Data

Information on submitting tissue data and details on necessary parameter, anatomical, and EPA species codes are located in the *SWQM DMRG*. Submit field data, and if scheduled, chemical data associated with the sampling events, as described in the *SWQM DMRG*.

Use the Fish-Collection Reporting Form (see Figure 7.1) to record data from each collection event. This data must be included in an annual report to TPWD. For additional information on scientific collection permit reporting requirements see Appendix A.

Table 7.2. Quick Reference Guide—Procedures for Collecting Fish Tissue

Routine Fish Tissue							
Objectives	**Where to Sample**	**Type of Sample** **How Much?**	**Number of Species**	**Number of Individuals**	**Size of Individuals**	**Sampling Frequency**	**Example Species**
Background conditions	Areas of minimal or no impact	Whole body composites > 300 grams	2 to 3	3 to 5, same species, composited	≤ 10% variance in total length of fish in sample	Once a year for two years Typically done by TPWD	***Freshwater:*** bass, catfish, sunfish, carp ***Estuary:*** hardheads, oysters
Long-term trends	Sites with historical data, where a major cleanup has occurred or future impacts are expected	Whole body composites of mature fish > 300 grams	One common species, preferably a bottom feeder	3 to 5, same species, composited	≤ 10% variance in total length of fish in sample	Once a year indefinitely	***Freshwater:*** bass, catfish, sunfish, carp ***Estuary***: hardheads, oysters
Ecosystem health	Areas affected by point or nonpoint sources	Whole body composites > 300 grams	2 to 3	3 to 5 per species composited	≤10% variance in total length of fish in sample	Once a year for two years	***Freshwater:*** bass, catfish, sunfish, carp, forage fish ***Estuary:*** forage fish, hardheads
Human health	Areas affected by point or nonpoint sources commonly used for recreational fishing	Muscle tissue fillets > 300 grams	2 to 4	1 to 2 per species	Large; > legal limit if possible	Once a year for two years; sampling continues indefinitely if there is an advisory	***Freshwater:*** bass, catfish, crappie, suckers ***Estuary:*** oysters, Atlantic croaker, sand trout, speckled trout, red drum, black drum
Sampling techniques	**Active:** Electrofishing, trawling, seining (preferred). **Passive:** Gill nets and trammel nets. This gear must be checked frequently. Sample deterioration can occur in as little as two hours in warm water, and six hours in cold.						
Sample information	Record total length; weight in grams, sex of fish; and note any deformities, wounds, tumors, or infections for each fish.						
Equipment	Aluminum foil, scale, measuring board, plastic bags, tape, marking pen.						
Sample preparation	**Human Health**—Edible tissue samples are prepared in the field or by the lab performing the analysis. If samples are prepared by the lab, specify that the lab is to fillet the sample, and the fillet is to be analyzed. It is best to contact the lab prior to shipping.						
Sample handling	**1.** Fish may be held in live well (or ice chest) in native water until specimens are chosen. **2.** Rinse fish, if necessary, with ambient water. **3.** Double wrap fish in aluminum foil with dull side toward the fish. **4.** Before wrapping clip dorsal and pectoral spines, if necessary. **5.** Put fish wrapped in aluminum foil into plastic bag and tape shut. **6.** Label with tag number, station location, type of sample (muscle or whole), species, number of fish in composite. **7.** Pack fish in ice and ship to lab ASAP. Samples may be kept on ice overnight and shipped the next day, if necessary. If possible, ship tissue samples immediately. If there is to be a delay in shipping, tissue samples may be frozen. Check with the laboratory conducting the tissue analysis for its preference.						
Selection of sample type	• Determine in advance what is being looking for, what is the concern—human health or ecosystem health? • Select species and type of sample (edible tissue or whole fish) that best fits the concern. • Determine what parameters need to be analyzed. • Consider limiting parameters requested, if the contaminant(s) is known. • Try to select species that are common to the area of concern.						
A list of methods used to analyze fish tissue appears in Appendix D.							

Figure 7.1. Example of a Fish-Collection Reporting Form

Texas Commission on Environmental Quality FISH-COLLECTION REPORTING FORM Scientific Collection Permit No. ________________			
Water body:*		**Date:***	**Time:***
Location:*			
Station no.:		**County:***	
Weather:		Lat/Long:	
Secchi depth (m):	Flow (cfs):	Avg depth:	Max depth:
Water temp (0.3m):	DO (0.3m):	Spec cond (0.3m):	pH (0.3m):
Collectors:**			
Gear Used			
Boat-Mounted Electrofisher	Low range:	High range:	AC or DC?
	Pulses/sec:	% on:	
	Amps: _____A	Duration: ______sec	
Backpack Electrofisher	Voltage ______V	Frequency ____pps	
	Pulse width ____msec	Duration _____sec	
Gill net	Mesh size:	Length:	Duration of set:
Trawl	Width:	No. Hauls: _______	Duration of haul:
Seine	Length:	No. Hauls: ________	Duration of haul:
Cast net	Diameter:	No. Casts: or Duration of casting:	
Other (specify)			
Habitat(s) sampled:			
Observations/comments:			
* **Required Information** when reporting fish collection data to the Texas Parks and Wildlife Department. Holders of scientific-collection permits are required to submit an annual collection summary to the TPWD. ** Collectors must be listed in Appendix I of the Scientific-Collection Permit. Each permit contains detailed requirements.			

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Figure 7.1. Example of a Fish Collection Reporting Form (continued)

Nekton Collection Data Form

Common Name/Scientific Name*	Total Length, ____(units)	Weight, _____(units)	Total Number*	Number Saved for Analysis*	Physical Defects (See below)*

Physical Defects

1—Discoloration	2—Deformities	3—Eroded Fins	4—Excessive Mucus	5—Excessive Ext. Parasites	6—Fungus
7—Poor Conditions	8—Reddening	9—Scoliosis	10—Tumors	11—Ulcers	12—Other

*** Required Information**

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