Sampling from the fish, seafood products, fish food and fish habitat

1. General principles of sampling

In this chapter, the general principles of sampling from the fish and seafood products as well as from fish habitat are outlined. Some of these aspects are also covered in the chapter on qualitative and quantitative analysis. It should again be emphasized that the correct sampling and sample handling are essential for successful analysis.

The selection of sampling methods and sampling sites depends of the ultimate purpose. For chemical analysis different principles apply from those relevant to microbiological or veterinary studies. The samples should, of course, be representative both in quality and quantity. A sufficient number of replicates are required to obtain a reliable result. Three replicates are usually considered as a minimum for any statistical treatment of the results. As pointed out in the chapter on the microbiological analysis, the required number of microbiological samples is often five per production lot. This can be considered sufficient also for chemical analysis.

The person, who takes the samples, should also have sufficient knowledge of fish anatomy, food processing and environmental niches in the fish habitat to make sure that the samples are truly representative.

In the following sections these general principles are dealt in more detail.

2. Tools and materials needed for sampling

2.1. Sampling from fish

Fish samples are generally taken either from the skin and the mucus (slime), of muscle tissues and of various internal organs (gills, liver, spleen, gonads, various parts of the gastrointestinal tract. The general anatomy of fish, including the shape and position of the internal organs, is presented in Figure 1.

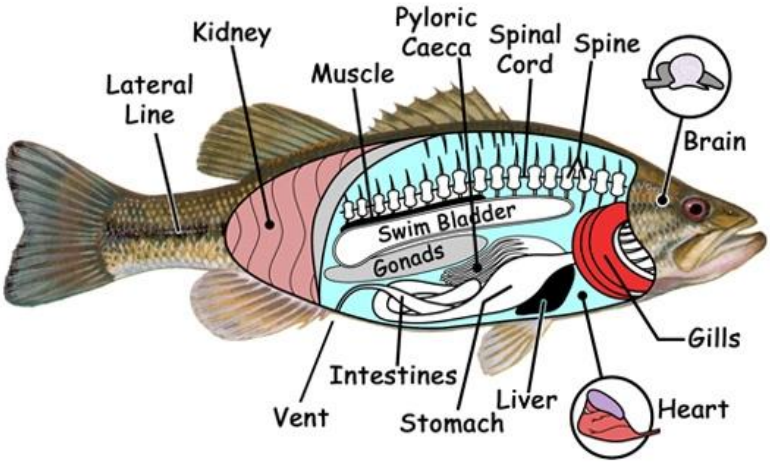

Figure 1: The general anatomy of fish and the relevant internal organs and their positions

2.1.1. Sampling tools

The following tools and materials are routinely used for fish sampling.

- substances or instruments for chemical or mechanical euthanasia of fish
- knives for scraping skin samples (slime, parasites)
- knives, scissors and forceps or tweezers for cutting fish muscle and internal organs (histology samples, samples for chemical analysis)
- biopsy needles for sampling fish organs such as kidney, gills, gut, slime (microbiological samples, samples for chemical analysis)
- Saline (0.6%), peptone water, fixatives (Histological samples)
- tools for handling, drying, freezing, homogenization of the tissue sample

For the sampling the fish in many cases have to be killed or in some cases anesthetized. Euthanasia should be done humanely applying either physical methods (quick decapitation with a suitable sharp hatchet, or using some chemical substance in the fish tank (clove oil, tricaine methane sulphonate etc). Detailed instructions for chemical euthanasia and anesthesia have been published by various authors (for example, Neiffer and Stamper, 2009)

The sampling knives are usually scalpel type (Figure 2), while scissors should be small and sharp and made of stainless steel. In case microbiological samples are taken, both scissors and scalpels should be sterile.

Figure 2: A typical scalpel that can be used in taking skin, slime and tissue samples from fish.

Generally, fish samples should not be allowed to dry, especially before microbiological analysis. Therefore, they usually are immersed in 0,6% saline solution or peptone water and kept refrigerated until analysis (which should happen within a few hours of sampling). Sometimes quick deep-freezing of the sample is preferable (for chemical analysis or if there will be an extended time before the actual analysis can be performed).

With histological and cytological samples a quick fixation is essential to prevent autolysis or putrefaction and to keep the microanatomy intact. Typical fixatives include formaldehyde (usually appr 4 % in phosphate buffered saline), methanol- glacial acetic acid and ethanol-glacial acetic acid mixtures (most of the fixatives are nowadays available commercially). A commonly used fixative for fish is Bouin's solution (5 % acetic acid, 9 % formaldehyde and 0.9 % picric acid in aqueous solution). The fixation times depend of the type of fixative and of the sample. The amount of fixative is usually at least ten times of the volume of the sample. Detailed instructions for the histopathological sampling are presented, for example, in the guidance document of the National Research Institute of Japan (http://nria.fra.affrc.go.jp/RCFD/histological-methods_e.html).

Homogenization of the samples is often necessary before the actual analysis. This can be done either immediately after sampling using a suitable blender or other type of homogenizer. However, usually the homogenization is done in laboratory immediately before the analytical procedures.

2.2. Sampling of fish and seafood products

The tools for sampling the seafood products depend of the type of the product. For intact or minimally processed fish the instruments are the same that are used for live fish. From processed fish and seafood products the instruments include spatulas, knives, tweezers, sample containers etc., depending on the food type. Again, for microbiological analysis the tools should be sterile. The storing conditions should prevent any microbiological or chemical changes in the samples. In practice this means storage in closed, sterile and chemically inert containers (made of glass, stainless steel or suitable plastics). Deep-freezing is the method of choice, if the samples cannot be analyzed within a few hours of sampling.

2.3. Sampling of fish feed

Generally, the same procedures as described for fish and seafood products apply. In cases of dry feeds, no specific pretreatment is usually necessary. Of course, any microbiological or chemical contamination should be avoided during the sampling by using the general precautions described above.

2.4. Sampling from the fish habitat (sea, lake, river, fish pond)

2.4.1. Water analysis

For the collection of samples for laboratory studies a suitable water sampler such as Ruttner-sampler (Figure 3) can be used.

Instead laboratory analyses, field measurements and monitoring are nowadays more often used in aquaculture, as follows:

- Automatic analyzers to monitor water quality, flow etc. (multi-purpose diagnostic kits for main parameters are available, but quite expensive)
- Automatic dissolved oxygen (DO) and temperature meters combined with alarm-device.

- Portable temperature recorders; oxygen probes; secchi disc to analyse water transparency; pH meter,

Figure 3: Ruttner sampler or a closable PVC cylinder that can be lowered and closed in certain depth; sample is taken up on-board the vessel.

2.4.2. Plankton sampling

Several devices exist for the study and analysis of phyto- and zooplankton, such as:

- Phytoplankton sampler (large sized water sampler to collect algae and for primary production analyses)
- Zooplankton sampler (large sized water sampler to collect zooplankton) to study zooplankton composition and biomass
- Zooplankton towing net (cone shaped net with 100-250 ug mesh size) to study zooplankton composition
- Zoobenthos sampler (grabs, box-corers, e.g. Ekman, Van Veen, dredges and hand nets) to study benthic infauna and epifauna

3. Sampling from fish and seafood products

3.1. Sampling from fish:

3.1.1. Microbiological sampling

The standard sampling sites for microbiological analysis are usually the skin and mucus as well as gills. Microbiological analysis from fishes obtained from catch or from aquaculture sites are usually associated with veterinary sampling (See 3.1.3.). Otherwise microbiological

analysis of intact fish is usually limited to cases where there is a reason to suspect spoilage or improper handling (such as too high storage temperatures).

3.1.2. Histological sampling

There is a defined standard protocol for histological sampling of fish. A 20-25 mm anterior-posterior incision is made with a sharp scalpel through the ventral area of the body wall. The scalpel is withdrawn to ensure that the incision is not obstructed. For sex identification, visual examination of ovary and testis are made and a small biopsy sample (5-8 mm) is collected by fine forceps. In this case, forceps were carefully inserted into the body cavity until reaching the gonad. Tissue sample is then cut by sharp scissors and fixed in Bouin's solution for next histological assay.

Samples of gonadal histology are kept in Bouin's solution. Then after 48 h, samples are embedded in paraffin following a schedule to put them into alcohol series for dehydration and clearing. The blocks are sectioned with the thickness of 6 micrometer and stained with hematoxylin and eosin for histological examination.

If the procedure is applied to a live fish the gills are kept wet. The abdominal incision is closed with one or two simple sutures, using a needle and a clamp. Fish are injected intramuscularly 1ml 5% oxytetracycline per fish and then place of incision is disinfected with povidone-iodine solution and chloramphenicol spray. No deep anesthesia is used during the biopsy process.

3.1.3. Veterinary sampling

To carry out the measure prescribed by the ichthyo-pathologist farm personnel should be able to carry out certain measures prescribed by the ichthyo-pathologist as part of fish health management. To this effect, sampling equipment are kept sterilized, clean and available as soon as they are needed.

In case of any signs of fish diseases, parasites or other abnormalities in fish behavior are noted, the farm personnel should take independently immediate samples of fish, water and/or plankton community to primarily identify type and occurrence of fish diseases. Samples of fish diseases are taken in mucus on fish skin and gills or from internal organs.

Basic sampling for example on fish skin should be taken independently and in more demanding cases, the veterinary authorities should be assisted.

Sampling mucus on fish skin and gills is done as follows:

- remove some slime below the pectoral fin and place a thin layer on the petri dish;
- remove gills on one side of the head and put into small amount of water on an object glass, rinse the slim, avoid blood and cover with a cover glass

Study fish surface in order to notify parasites and to make preliminary analyses of defined bacterial, viral or parasite fish diseases.

Assist veterinary authorities in sampling internal organs of fish and preparing a bacterial sample from fish skin, gills and kidney:

- open the ventral side of fish with scissors or knife with a longitude cut from head towards anus
- remove vertical flesh if necessary to see internal organs
- remove viscera (stomach, gut, organs) and swim bladder
- take a sample from the kidney by a sterilized tool and place the piece of kidney on a plate with agar nutrient medium in order to start bacterial culture of possible disease organisms

The sending of the fish to the veterinary authorities should be done as follows:

- send a few live fish suspected for a disease; plastic bag with 2/3 of water and 1/3 of oxygen
- kill sample fish by breaking the neck (< 5 cm fish), cutting vertebrate column (<15 cm fish) or by hitting the head (large fish)
- wrap the sampled fish in moist and thick newspaper
- cover the package with ice
- label the package with contact information and details of sample, species, size, age, symptoms

4. Sampling from seafood products

The sampling of seafood products either for microbiological or chemical analysis depends of the product, and no specific guidance can be given. Naturally the samples should be kept sterile and any microbiological or chemical changes or contaminations should be prevented. Certain physical measures might be needed, such as removing of the shells of certain species of seafood (clams and oysters).

5. Sampling from fish habitat

Water sampling is made with water sampler or portable field recorders (DO, temp). Vertical profile sampling in the lake and sea to study temperature stratification should be done during all seasons, if possible, but mainly during critical seasons when oxygen deficiency becomes likely (summer stratification, middle winter). Sampling program should be designed according to the production scale: small farm needs less sampling and less parameters, bigger farms frequent sampling and more parameters. Physical-chemical environment monitoring is normally required once a year, if the farm production has not changed. Central parameters include: DO, temp, total-P, total-N, NH4-N; pH, conductivity, turbidity, BOD, COD, fecal bacteria. Ecosystem studies, such as phytoplankton/primary production, zooplankton, zoo benthos, are required very seldom; only in case of Environmental Impact Assessment (EIA) of large-scale industrial fish farming. Ecosystem studies are repeated usually once in every three years.

The sampling strategy in any ecosystem study comprises a series of procedures aimed at selecting the most favorable sampling locations and determining the appropriate sampling density for accurate description and quantification of target assemblages in a given geographical area during a given period of time. The choice of strategy depends mainly on the distribution of the organisms belonging to the different species, on the characteristics and the number of habitats present in the investigated area, and on the spatial and temporal variability of environmental parameters. It also depends on the feasibility, including the cost–effectiveness ratio. (As said before, the scale and size of EIA depends on the scale of farming operations).

The main strategies for spatial allocation of sampling sites are:

- systematic sampling based on a regular distribution of the sampling sites over the whole investigated area,
- random sampling with sampling sites randomly distributed over the whole area
- Stratified sampling is based on the identification of an area presenting reasonably homogeneous characteristics (stratum), for example vegetated bottom patches, bare substrate, etc.

Once the sampling strategy and design has been defined according to the aim of the study, it is necessary to select the most suitable sampling instruments, determine the sample size and number of replicates, and select sampling techniques and treatments for samples.

6. Sampling from gaseous materials

The need of samples of atmospheric or gaseous materials is not particularly typical for fish production and aquaculture. In some cases the need to study the dusting potential of fish feed might be necessary for the occupational health reasons. For these purposes there are several instruments, also suitable for on site investigation.

The sampling of volatile organic compounds in the ambient air is described in the chapter on quantitative and qualitative analyses. The standard method is based on the use of certain absorbents (resins) through which the air sample is pumped of sucked, and from which they can be extracted using organic solvents.

The analysis of dissolved gases from the natural waters or water used in aquaculture tanks is usually done by specific potentiometric methods, the most important of which is the analysis of DO.

7. Traceability of samples taken from fish, seafood products and fish habitats

Correct labeling and documentation is the absolute necessity to ensure the traceability. Every sample from fish, seafood products and fish habitats should be labelled clearly indicating date of sampling, number of sample site (name of sampling area), persons who took the sample, and any other relevant data affecting the analytics and results. This information should be properly recorded in both the laboratory journals and at the site of the

sampling (if the site was an establishment, such as fish farm or food processing plant). In addition to electronic storage of data, it is recommended to keep also hard copies of the journals, which are duly inspected and signed by the chief of the laboratory. Some specific aspects that should be taken into consideration include:

- Labelling with water proof ink on the label or sampling bottle
- Keep a journal indicating the sampling procedures, samples taken, data on environmental conditions, weather, time of the day, and any other relevant information possibly affecting the analytics and results
- Take photos or otherwise describe of the appearance of sampled water, plankton, mud, fish, seafood, etc. as an additional file of information.

8. Storage and transportation of samples

The general principles of the storage of samples have already been outlined in previous sections. Some special aspects related to certain types of samples or otherwise need to be emphasized include:

- Plankton samples are fixed with 4% formaldehyde solution and kept in stable conditions
- Water samples are kept in shade, not exposed to sunlight and in insulated cool box (possibly on ice)
- Fish samples are kept alive or on ice depending on the coming analytics
- Primary samples of fish skin, gills, slime etc. are taken on-board the vessel and stored wet and refrigerated for the microbiological analyses
- Seafood samples are kept alive or on ice depending on the coming analytics
- Samples are transported to the harbour/ laboratory during the same day if possible and the cold-chain kept up all the way
- Long-term storage of the samples should be avoided (particularly for hygienic or safety diagnostics) but fixed tissue, plankton, and fish samples keep well for several weeks or months storage

Further reading:

Neiffer, DL., Stamper, MA. 2009. Fish sedation, anesthesia, analgesia and euthanasia: Consideration, methods and the types of drugs. ILAR Journal 50: 343-360

Miva, S. Sampling Methods of Histopathology for Fish and Shellfish. National Research Institute of Aquaculture,Fisheries Research and Education Agency

(http://nria.fra.affrc.go.jp/RCFD/histological-methods_e.html)