STEP 2

Freezing

Masatoshi Meguro and Hiroyuki Motomura

It is very important to obtain fresh fish samples for preparing beautiful specimens with a long shelf life, but specimens may not be prepared from fresh fish samples at any time. Freezing is the only way to keep fish samples fresh for a long time before treating them to prepare specimens. However, freezing is not suitable for all fishes such as those of Gobiidae and Blenniidae, which have very weak fin membranes, and of Clupeidae, whose scales readily exfoliate on freezing. It should also be carefully considered whether or not to choose freezing for interim storage.

We use "National NR-FC28FG" deep freezers built by Panasonic Corporation for freeze preservation in our museum, the Kagoshima University Museum. Fish samples are stored at -20°C in NR-FC28FG deep freezers; ideally, samples should be stored at -80°C.

Preservation temperature is an important factor to be considered to prevent clouding of the eye lens. Regular hometype refrigerators can also preserve fish samples, although their humidity level is higher than that of deep freezers. However, regular home-type refrigerators can-



Deep freezer for preservation of fish samples.



Freezer-burned fish specimen. It is difficult to spread each fin, since the specimen gets dehydrated and becomes hard.



Specimen freeze preserved in seawater. Tip of the caudal fin is bent, and hence, it is not a well-preserved specimen.

not prevent clouding of the eye lens, and therefore, they are not suitable for preserving fishes, especially those of Labridae.

If fish samples are left in a freezer for a long time, the fish bodies become rigid (not frozen) and are not restored to the normal state after defrosting. This condition is called "freezer burn." Freezer burn occurs when the tissues are damaged by dehydration and oxidation, because of air reaching them. Freezer burn causes irreversible denaturation of samples. Freezerburned samples cannot become normal again even if immersed in sufficient amount of water. The fins cannot spread well, which makes it difficult to record fin color and pedicel length correctly.

It is very difficult to prevent freezer burn completely; however, the use of appropriate water type can reduce the risk of symptom development. Seawater should be used for marine fishes and freshwater for freshwater fishes during freeze preservation of fish samples.

A note indicating the sampling date should be included with the sample fish before freezing. It is very important that all the data are recorded before the information slips from the mind. All available information about the samples should be written down in detail, including the names of the people who collected



Specimen freeze preserved in seawater. It is a well-preserved specimen. This specimen had been preserved since 2007. It could well be treated in 2009.

the samples, the places of origin of the samples, the collected data, the sampling methods, and the depth at which the samples were collected.

The data should be written with a regular or a mechanical pencil on waterproof paper. Ballpoint pen and ordinary paper are not suitable for recording the data to be included with the freezing samples. This is because the information will be lost on thawing the samples, since the paper will tear and the ink will get washed away. Moreover, important fish samples will be rendered worthless without the relevant background information. Thus, data recording is very important.

Nowadays, all specimens used for experiments, even those for molecular biological research, registered in research institutes, including museums and universities. Sometimes, fish samples are stored in a freezer for later DNA analysis. However, prolonged freezing of samples not only deters preparation of beautiful specimens but also causes freezer burn, which inhibits species identification. It is preferable to first obtain some tissues for DNA analysis and then immediately proceed to sample preservation.

Defrosting of samples \rightarrow Step 3