## STEP 12

## Fixation

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After identification (STEP 11), the specimen should be immediately fixed in formaldehyde solution (formalin). The stores usually sell approximately 37% formalin, and we call it "formulated formalin." For fixing fish specimens, decuple-diluted formulated formalin is used. This is called "10% formalin," but the actual formaldehyde content of this solution is 3.7%.

For fixation, the specimens should be completely soaked with 10% formalin. For medium specimens, incise the right side of the abdomen to facilitate absorption of formalin. For large specimens, additionally incise the right side of the dorsum. At many research organizations, formalin is injected into a body cavity by using a syringe. However, in our museum, surgical knife and syringe are not used because of the presence of many general museum volunteers. Surgical knife and syringe are not preferable themselves, and there is a risk of formalin spill if excessive pressure is exerted on the specimen. Formalin is assigned to the category of poisonous and deleterious substances (specific chemical substances, second category) by Occupational Safety and Health regulation on disability prevention from specific chemical substances and therefore needs to be handled with extreme care.

Small specimens can be soaked directly, but to avoid damage, they should not be soaked together with medium specimens in the same container. In our museum, we fix very large fishes such as giant mottled eel and oarfishes, which need to be fixed straight, in a large tank placed on the roof. For medium and small specimens, we use large plastic containers and Tupperware, respectively.

Fish body consists mostly of water. Therefore, the longer the specimens stay in a formalin fixation container, the more the formalin gets diluted. Formulated formalin needs to be added regularly, depending on the size and number of newly added specimens. If too many specimens are added without adding formalin, they will not be fixed and begin to decay. Pres-



Formulated formalin: 37% formalin solution. The product is labeled as "third type," but it is currently designated as "second type" by Occupational Safety and Health regulation.



Formalin fixation of medium to large specimens.

ence of bubbles on the surface of formalin solution, even only a few, is an initial sign of contamination. Once the specimen begins to decay, formalin contamination cannot be inhibited even by adding highly concentrated formalin. As a strategy, the decaying specimen can be fixed in 98% ethanol; this will fix the specimen well



Formalin fixation of small specimens.



A tank for fixation of a large specimen. It is placed on the roof and locked.

and the problem of contamination will be resolved. However, ethanol has a very bad odor, and it cannot be reused. Therefore, it is important to monitor the density of formalin in order to avoid contamination.

The period of soaking a specimen in formalin (fixation period) varies depending on research organizations. Mostly, it is 7 days to 3 weeks; in our museum, it is approximately 10 days. In 7 days, the specimen is not fixed well, and it will dehydrate on alcohol treatment (STEP 13). Dehydration will produce many wrinkles on the body surface and make the specimen quite hard (especially fishes of Carangidae). In our museum, we regularly prepare a large number of new specimens, and we do not have enough number of (and space to place) formalin fixation containers to soak specimens for weeks. Therefore, 10 days is the best duration for both complete fixation and fast rotation

Replacement of formalin with alcohol →Step 13