

STEP 7

Pre-fixation

Mizuki Matsunuma and Hiroyuki Motomura

Fish specimens are usually made for scientific research. Measurement and counting of traits are quite convenient when the fish body is mounted straight and all the fins are spread well. It is difficult to obtain the real, discrete value of an imperfectly fixed specimen.

Some fishes have significantly long bases of the dorsal and/or anal fins, such as those of Muraenesocidae (pike congers) and Ophichthidae (snake eels). Thus, it is really difficult to count the number of fin rays if the fins are fixed in a folded condition. Perfectly fixed speci-



An imperfect specimen of *Scorpaenodes evides*. The fish is fixed with the mouth and gill cover open, and therefore, body length cannot be measured correctly.



An ideal specimen of *Scorpaenodes evides* (KAUM-I. 4371, 48.2 mm standard length).

mens obviously have a higher academic value than imperfectly fixed ones.

■Equipment

1. Pins

Pins are the most important tools for spreading fish fins. Normally, insect pins are used for this purpose. Shiga Insect Pins are the best choice for spreading fins. They are particularly useful as they are available in many sizes. We usually use No. 00 to No. 6 pins and micropins. The thickness of the pin increases with its number; for example, the thickness increases from 0.3 mm to 0.65 mm for No. 00 to No. 6 pins. The most appropriate pin should be selected according to the fish size and density of its fin membrane. A micropin is thinner than a No. 00 pin. It is used for very small fishes with very weak fin membranes, such as those of Gobiidae and Tripterygiidae. Since micropins are very thin and small, it is preferable that they are handled with forceps.

Sometimes, bamboo skewers are used to spread the fins of large fishes such as scombrids. In our museum, we use a long and thick pin to spread the fins of a large-scaled fish. We use an E979 needle supplied by Australian Entomological Supplies, which has a 70-mm stainless-steel solid head and a thickness of 1.37 mm.

The sizes of a micropin and different Shiga Insect Pins are provided below.



Shiga Insect Pin No. 5 and outer packaging.



Fin spreading in formalin solution in a plastic food tray.



Shiga Insect Pin No. 00 to No. 6 and long needle made by Australian Entomological Supplies.

- Micropin (stainless steel)
0.18-mm thick, 17.5-mm long

- Shiga Insect Pin (stainless steel)
No. 00: 0.30-mm thick, about 40-mm long
No. 0: 0.35-mm thick, about 40-mm long
No. 1: 0.40-mm thick, about 40-mm long
No. 2: 0.45-mm thick, about 40-mm long
No. 3: 0.50-mm thick, about 40-mm long
No. 4: 0.55-mm thick, about 40-mm long
No. 5: 0.60-mm thick, about 40-mm long
No. 6: 0.65-mm thick, about 40-mm long

2. Plastic foam boards and food trays

Plastic foam boards and trays are used as the base for pinning of spread fins. Boards and trays of different sizes should be arranged to suit different sizes

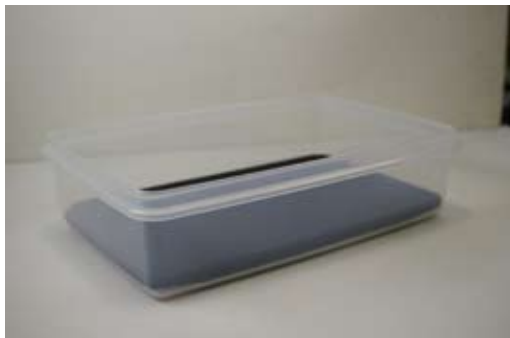
of fishes. The ideal thickness of a board is at least 3 cm. A small fish can be fixed in formalin solution in a food tray. One should collect a diverse range of flat-bottomed food trays on a regular basis for fixation. Sometimes, when a tray is used several times, formalin leaks from the tiny holes created by the pins. This can be prevented by overlapping trays of the same size and/or placing the trays with formalin on hard plastic trays during fixation.

3. Soft sponge boards

Thin and weak pins like micropins bend easily and sometimes break the fin membrane when pulled out from the plastic foam boards and/or food trays. Boards made of a soft material, *e.g.*, sponge boards, reduce the risk of damage to the specimen and pins. A black sponge board is better than a white one, since it facilitates examination of the transparent fin membrane. The sponge board is fixed on a flat-bottomed ceramic casserole or a tupperware.

4. Paintbrush

A paintbrush is used to apply formalin on the fins to spread them. A brush



Black soft sponge board fixed on a plastic storage case.



Concentrated formalin solution in small bottles, used for fin spreading.



Paintbrushes with different sizes.

with moderate softness and good water-retention quality is the best choice. A hard-bristled brush will abrade the body surface and/or even rip some scales. Therefore, the softness of bristles should be checked while purchasing a brush from the stationery. Brushes with tips of different diameters, around 3–10 mm, should be arranged to suit different sizes of fishes.

5. Formalin

A formulated concentrated formalin solution is used for spreading the fins to prepare a specimen. It is convenient to use formalin packaged in small bottles, which can be hermetically sealed, before starting fin spreading in a number of specimens. Formalin has serious toxic consequences, and therefore, the air should be cleared of its fumes after using it indoors. A 10% dilute solution of formalin is used to spread the fins of a small fish in a food tray.



Sprays to prevent dehydration of fishes during sample treatment.

6. Atomist spray

Atomist spray is used to prevent damage due to dehydration. It can be purchased from garden supply shops.

In addition to the above-described equipment and reagent, we use commonly used laboratory instruments, including forceps and trays, for fin spreading.

■ Preparation for fin spreading

Before starting fin spreading, the fishes are rinsed in water to remove the mucilaginous solution and dirt adhering to the body surface (STEP 4).

Some fishes like those of Mullidae are rinsed gently, because their scales can easily exfoliate. On the other hand, sharks and rays and members of Chanidae (snakeheads) have a hard body surface and can therefore be rinsed vigorously. Mucilaginous solution adhering to the body surface should particularly be removed taking extra care, since it often remains at the gill pores and around the mouth and fin bases.

For effective removal of the mucilaginous solution, a dishwashing sponge and household detergent may be used. The bodies of some defrosted fishes continue to rigidify after death. They should be softened gently at the time of rinsing to facilitate their fixation.

After rinsing the fish body, the moisture should be wiped off the body surface by using a paper towel. If formalin is applied on wet body surface, it will spread on the plate and/or tray, causing discomfort to us by its odor.

When there are many fishes lined up for fin fixation, they are considered in the order of precedence. Fishes like herrings, anchovies, and small gobies decay easily and therefore should be treated before tough fishes like those of Scorpaenidae and Holocentridae (North Pacific squirrelfish). Fishes that are to be treated later should be stored in ice-cold water in an ice chest to prevent spoilage.

■ Fin spreading

There are numerous types of fishes in the world, having different body shapes. Thus, a different approach for fin spreading needs to be adopted for different

fishes. A general method of fin spreading is provided below, following which specific methods for different fish shapes are discussed.

1. Positioning of the fish

In a conventional fish specimen, the fish body is laid with the left side facing up for fin spreading. The fish is laid in a coolite tray in such a position that the body axis is horizontal, with the left side of the body facing up and the right side facing down. An exception includes anglerfishes and flatfishes, which are laid with the dorsal surface facing up. After the fish is laid straight in the tray, slightly thick insect pins should be inserted at the 4 corners around the fish. If the mouth is open, it should be closed by pinning under the jaw. In the case of fishes with a slender and round body shape, like herrings and mackerels, the head often inclines downward. To keep the body axis straight, the fish head should be held in a slightly raised position by using a pin and/or placing a small piece of coolite under the head. Fishes with long bodies, like sea snakes and pike congers, are usually laid in an S or inverted C shape. Moreover, the tails of fishes like seahorses are laid in their normal round shape.

2. Fin spreading

As much as possible, thin pins should be used during fin spreading to prevent damage to the fin membrane. The pins should be inserted near the fin base along the fin rays and not in the center of the fin. A good specimen photograph cannot be obtained if there are distinct holes in the fins.

After fixing the body axis, the caudal fin (tail) should be pinned at both the ends and spread naturally. The order of spreading the different types of fins is im-



1. Fixed body axis.



2. Spread and fixed caudal fin.



3. Spread and fixed dorsal fin ray from the rear.



4. Spread and fixed anal fin.



5. Spread and fixed dorsal spiny ray.



6. Spread and fixed pelvic fin.

Process of fin spreading for specimen of *Seriola dumerili*.



Dragonets (Callionymidae). Above: before fin spreading. Below: after fin spreading.



Fin spreading.



Spread fins just before applying formalin solution.

portant. The body does not move forward if the caudal fin is spread and fixed before the dorsal and anal fins. Small fishes, in particular, tend to float in formalin because of the buoyant force. Therefore, the caudal fin should be spread and fixed first to prevent the floating and moving of the fish body.

After the caudal fin, the dorsal and anal fins are spread. The fins will be wrinkle free and beautifully spread if they are spread from the rear fin rays toward the front. Next, the pelvic fin on the left side is spread completely and fixed naturally. The pelvic fin on the right side is along the body line with pins and completely hidden. Finally, the left pectoral fin can be spread naturally by using the fingers. Some fishes have soft rays on the pectoral fins. These soft rays, found in fishes of the order Scorpaeniformes, including Scorpaenidae and Triglidae, are not spread. On the other hand, the soft rays are an important taxonomic character for threadfins, and therefore, they are spread for these fishes.

Goatfishes and catfishes have barbels on their chin. All the barbels are spread and barbel angles are adjusted such that the barbels do not overlap when photographs are taken.

3. Application of formalin

A concentrated formalin solution is applied around the fins after spreading all the fins. In the case of large fishes, which have thick and tough fin membranes, formalin is applied on the entire fin. On the other hand, it should be very carefully applied in the case of tiny fishes and/or fishes having thin and weak fin membranes. If it is over applied, fin membranes will break because of shrinking after fixation. Thus, it should only be applied at the bases of the fins. In addition, it is applied around the mouth to keep it closed.

Formalin fixation normally takes 5–10 min for small fish specimens, *e.g.*, smaller than 10 cm. Large fish specimens can be fixed in approximately 15 min.

However, it is difficult to fix a large fish, which has thick muscle tissue (especially, the pelvic fin and/or jaw), by simply applying a layer of formalin. To properly fix each body part, the target parts should be covered with a formalin-soaked paper towel.

To prevent the fish body from drying after application of formalin, it should be covered with a paper towel and sprayed with plenty of water.

Some fishes have elongated pelvic-fin rays, such as threadfin bream (*Nemipter-*



Application of formalin solution.



It is difficult to spread the pelvic fin of a large fish. Therefore, the fin is covered with a formalin-soaked paper towel.

us), which may come out from under the wetted paper towel. In this case, a long pin should be inserted around the fin tip.

Extreme care is required to pull out the pins after fixation. The fins may be fixed successfully, but irreversible damage may be caused if the pins are pulled out roughly. Furthermore, the pins should be carefully pulled out such that they do not create wide holes in the fins. After formalin fixation, the next treatment should be initiated before the eyes become clouded and body color changes.



Fish body covered with a wet paper towel, sprayed with water to prevent drying of the fish.

4. Rinsing

After formalin fixation, the fish body is re-washed and prepared for obtaining photographs. To prevent any damage before the photographs are taken, the fish specimen is stored in ice-cold water. However, the specimen should not be stored in ice-cold water for a long time.

■Special cases of fin spreading

For fin spreading, some fishes essentially require the most suitable treatment according to their morphological characters.

1. Small fishes [*e.g.*, Gobiidae (gobies), Tripterygiidae (triplefins), and Adrianichthyidae (ricefishes)]

Small fishes and larval fishes have very weak fin membranes, which may



Fin spreading in formalin solution in a plastic food tray.



Right pectoral fin (inner side) of *Chelidonichthys spinosus*.



Fin spreading on a black soft sponge board fixed on a plastic storage case.



Right pectoral fin (inner side) of *Inimicus japonicus*.

easily break on dehydration. For small fishes, fin spreading should be performed in 10% diluted formalin solution in a plastic food tray or a plastic case bedded with a soft sponge mat. The fish body (especially, the bodies of gobies) tends to move by the buoyant force in the formalin solution. Thus, the fish mouth should be held using a relatively thick pin to prevent the body from moving or floating. After all the fins are spread and fixed, the pin holding the mouth in place is removed and the mouth is pinned using new pins. For small fishes, the thinnest possible pins should be used and these pins should be handled with forceps.

2. Fishes with ornamental pectoral fins [e.g., Triglidae (sea robins), Exocoetidae (flying fishes), and Synanceiidae (stonefishes)]

In the case of fishes in which pectoral fin is a taxonomic character, the right pectoral fin should be removed, spread, and photographed. This makes it easy to identify the species for taxonomic purpose.



Fin spreading in *Pisodonophis zophistius*.

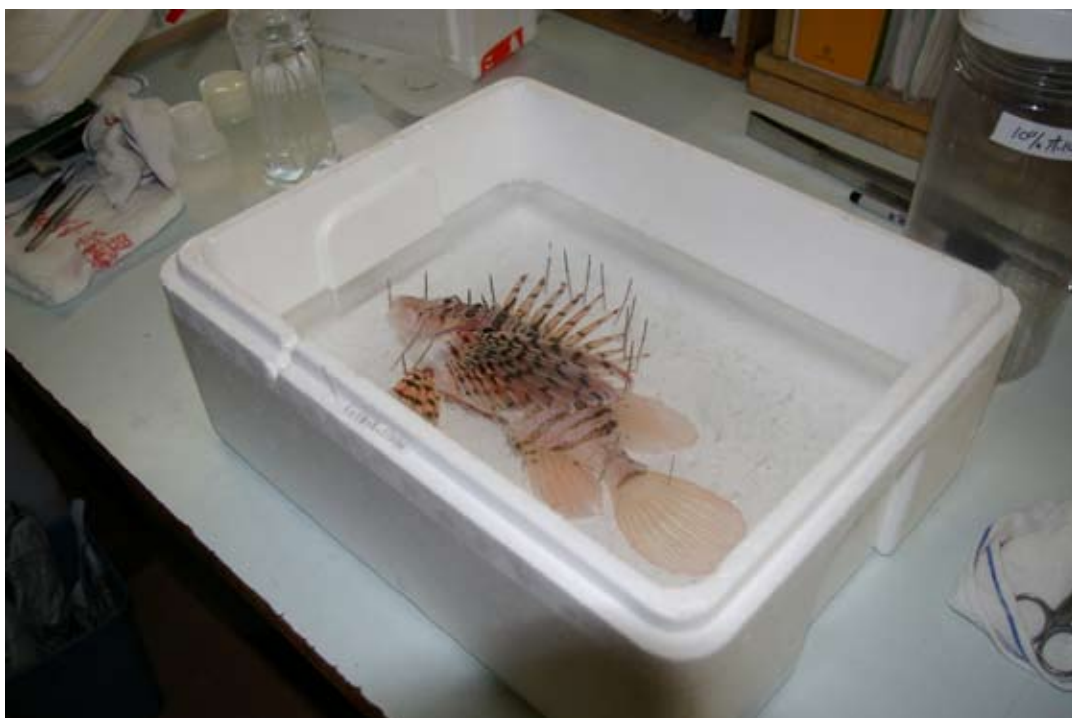


Fixed specimen of *Gymnothorax kidako*.

3. Fishes with long bases of the dorsal and anal fins [e.g., Hydrophiidae (sea snakes), Muraenesocidae (pike congers), and Trichiuridae (hairtails)]

In the case of fishes with long bodies and long bases of the dorsal and anal fins, it is easy to spread the fins by individually spreading the fin rays from the rear

toward the front. While spreading the fins, dorsal fin and anal fin rays are fixed alternately on the 2 sides, not on one side after the other.



Fin spreading in *Pterois lunulata* in formalin solution in a styrene foam box. It is a good way of fin spreading for fishes with skin flaps and long fin rays.



Fin spreading in *Pterois volitans*. Fin membranes and skin flaps spread beautifully.

4. Flatfishes [*e.g.*, Paralichthyidae (flounders) and Soleidae (soles)]

In the case of flatfishes (Heterosomata), spreading of the anterior parts of the dorsal and anal fins is often forgotten. The number of fin rays is an important taxonomic character for flatfishes. Thus, the fins should not be fixed in a folded condition. Before fixing the fins, the base and tip of each fin should be checked carefully. Since it is difficult to spread the fin rays on the front perfectly, forceps should be used to manipulate the fin.

5. Fishes with long fin rays and/or skin flaps [*e.g.*, Pteroinae (lionfishes) and species of the carangid genus *Alectis*]

Members of Scorpaenidae (especially, lionfishes) have skin flaps and long fin rays, such as larval stages of threadfishes. Their fins are best spread in 10% diluted formalin solution in a plastic case.



Sharks and rays do not require fin spreading.

6. Sharks and rays

Members of the shark and ray families do not require fin spreading.