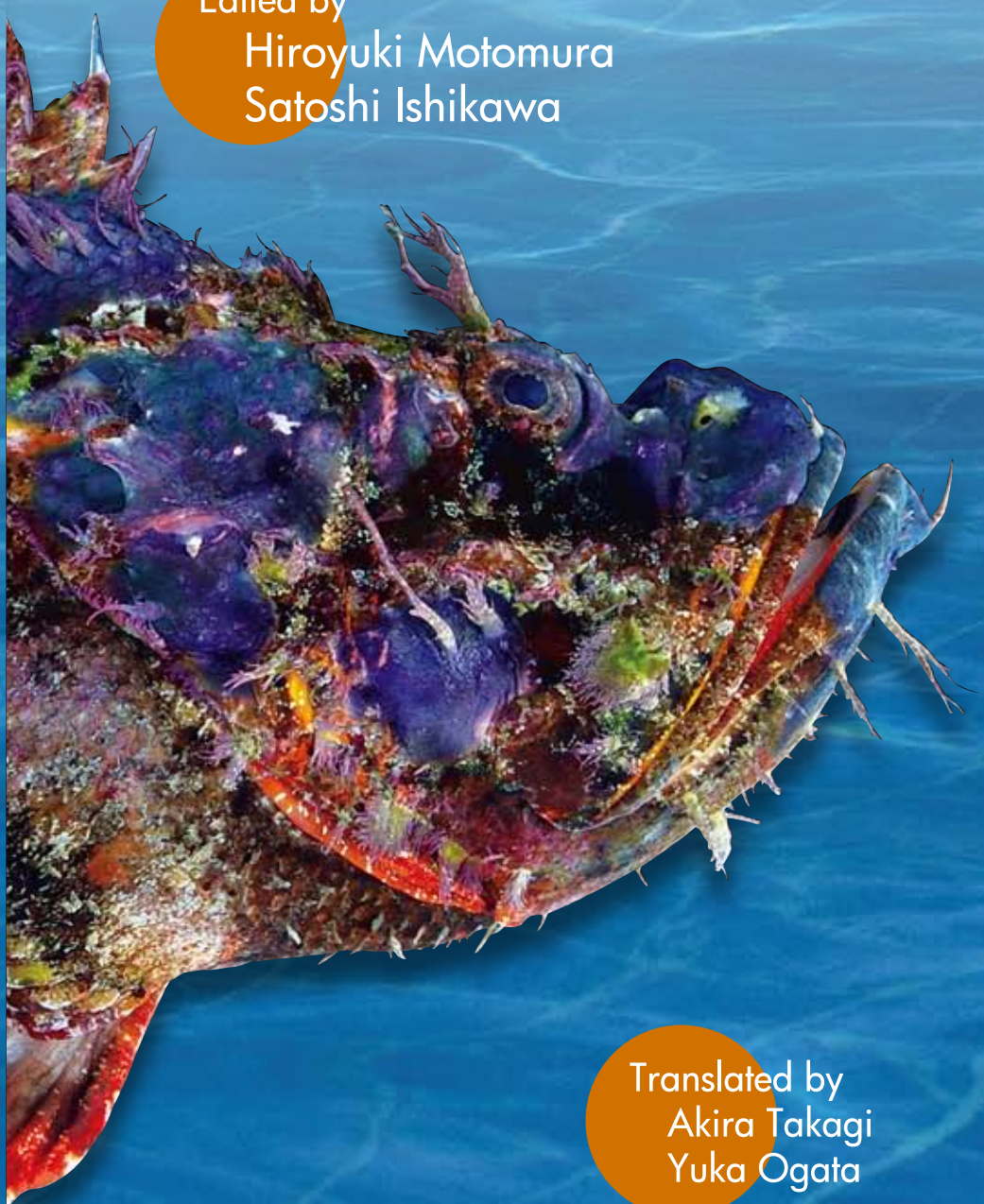
The Kagoshima University Museum / The Research Institute for Humanity and Nature

Fish Collection Building and Procedures Manual

English Edition

Edited by
Hiroyuki Motomura
Satoshi Ishikawa



Translated by
Akira Takagi
Yuka Ogata

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Hiroyuki Motomura and Satoshi Ishikawa

(translated by Akira Takagi and Yuka Ogata)

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The Kagoshima University Museum / The Research Institute for Humanity and Nature
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Project leader: Hiroyuki Motomura

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Preface

The recent destruction and deterioration of the environment has led to a worldwide decline in biodiversity. To conserve the current biodiversity, there is an urgent need to identify and catalog species. Therefore, natural history museums and biological research institutes, including universities, must keep primary sources, including voucher specimens and photographs, of organisms to record the current biodiversity and pass them on to posterity as humanity's common properties.

This book describes curatorial procedures for fish specimens and fish collections as practised by the Kagoshima University Museum. The curatorial and cataloging methods and procedures described here, while perhaps not ideal for large museums such as the Natural History Museum, London, or National Museum of Nature and Science, Tsukuba, are suitable for local small-sized museums or universities with lower budgets. This book also provides details of various equipment for photography, preservation and storage, including specifications and names of manufacturers. It also mentions methods for the creation of specimen databases and procedures for specimen loans.

The book was originally published in 2009 as a manual written in Japanese [Motomura, H. (ed.) 2009. Fish Collection Building and Procedures Manual. The Kagoshima University Museum, Kagoshima]. The English edition of the manual is herein published as a part of the editors' research projects, Kagoshima Fish Diversity and Coastal Area Capability Enhancement in Southeast Asia projects, and supported by the Kagoshima University Museum and the Research Institute for Humanity and Nature.

I am grateful to M. Aizawa, H. Endo, Y. Iwatsuki, S. Kimura, K. Matsuura, and H. Senou for sharing their extensive knowledge of curatorial procedures for fish specimens, M. McGrouther, D. Catania, J. Maclaine, A. Bentley, K. Murphy, D. Nelson, M. Sabaj, K. Swagel, E. Holm, G. Dally, Y.-C. Liao, L. Kelvin, and G. Yearsley for providing information on their fish collections, photographs of collection labels and jars, and comments on the book, and volunteers and students of the Kagoshima University Museum for their assistance.

Hiroyuki Motomura
The Kagoshima University Museum

Preface

Underway since 2012, "The coastal area capability enhancement in Southeast Asia" project, directed by the Research Institute for Humanity and Nature (RIHN) system, will continue through 2017. Its purpose is to realize "area capability" and to generate a new approach toward rural development evaluation based on the harmonization between ecosystem health conservation and improvement of local people's quality of life. Because rural people's lives are invested in the capital involving goods and services that ecosystems provide, we believe that strengthening the link between natural capital and local people is key for the sustainable development of rural areas. Thus far, however, the benefits of natural capital and services have not been fully recognized. As part of the RIHN project, we conduct a detailed field survey of environmental, biological, social, and economic aspects through a holistic joint approach to grasp the actual situation regarding ecosystem health, the livelihoods of local people, and the connection between the two.

The original *Fish Collection Building and Procedures Manual* was published by Kagoshima University Museum and edited by Prof. Motomura. We are pleased that the publication of this English edition marks one of RIHN's most memorable achievements. We are certain that it will contribute to the understanding of biodiversity and the variety of natural capital through the establishment of a sophisticated fish collection at museums and research institutes. Though Southeast Asian coastal fauna and flora hold high biodiversity, taxonomic studies and food web analyses, including population studies, have not been thoroughly investigated. We hope that this manual will promote biodiversity studies in the Southeast Asian coastal area and provide people with a unique opportunity to improve their research skills.

The RIHN project is based on the joint research efforts of Southeast Asian Fisheries Development Center (SEAFDEC), Faculty of Fisheries of Kasetsart University, the University of the Philippines Visayas (UPV), and Japanese researchers who are members of the RIHN project. Aklan State University and Eastern Marine Fisheries Research and Development Center of Department Fishery, Thailand, are active participants as well. Through this collaboration, we share the same vision for future biodiversity studies.

Satoshi Ishikawa
The Research Institute for Humanity and Nature

Editors

Hiroyuki Motomura, PhD
Professor, the Kagoshima University Museum
1-21-30 Korimoto, Kagoshima 890-0065, Japan
Ph: +81 99 285 8111; fax: +81 99 285 7267
E-mail: motomura@kaum.kagoshima-u.ac.jp
URL: http://www.museum.kagoshima-u.ac.jp/staff/motomura/motomura.html

Satoshi Ishikawa, PhD
Associate Professor, Research Institute for Humanity and Nature
457-4 Motoyama, Kamigamo, Kita-ku, Kyoto 603-8047, Japan
Ph: +81 75 707 2320; fax: +81 75 707 2507
E-mail: oounagi@chikyu.ac.jp
URL: http://www.chikyu.ac.jp/index_e.html

Translators

Akira Takagi, PhD
Senior Project Researcher, Research Institute for Humanity and Nature
457-4 Motoyama, Kamigamo, Kita-ku, Kyoto 603-8047, Japan
Ph: +81 75 707 2317; fax: +81 75 707 2507
E-mail: akirapt@chikyu.ac.jp

Yuka Ogata, PhD
Project Researcher, Graduate School of Frontier Science, the University of Tokyo
5-1-5 Kashiwanoha, Kashiwa, Chiba 227-8564, Japan
Ph: +81 3 5841 5018; fax: +81 3 5841 5018
E-mail: aogata@mail.ecc.u-tokyo.ac.jp

Authors

Kaoru Kuriiwa
Research Assistant, Department of Zoology, the National Museum of Nature and Science
4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan

Mizuki Matsunuma
PhD student, the United Graduate School of Agricultural Sciences, Kagoshima University
1-21-24 Korimoto, Kagoshima 890-0065, Japan

Masatoshi Meguro
PhD student, the United Graduate School of Agricultural Sciences, Kagoshima University
1-21-24 Korimoto, Kagoshima 890-0065, Japan

Hiroyuki Motomura
See Editors

Gota Ogihara
PhD student, the United Graduate School of Agricultural Sciences, Kagoshima University
1-21-24 Korimoto, Kagoshima 890-0065, Japan

Tomohiro Yoshida
PhD student, the United Graduate School of Agricultural Sciences, Kagoshima University
1-21-24 Korimoto, Kagoshima 890-0065, Japan

Contents

Collecting fishes by team of the Kagoshima University Museum

STEP 1 Transport

Hiroyuki Motomura

After fish samples are obtained, they should be transported in an appropriate way to the laboratory at the university or institute for preparing specimens for reference collection. Therefore, a suitable transportation method should be selected, depending on the conditions. In this section, methods of sample transportation are discussed in detail.

Live fish samples are the best for preparing beautiful specimens. To keep fishes alive, they should be carried in small, water-filled plastic bags, taking care that they are not hurt in any way. For example, if gobies and scorpionfishes are carried in the same plastic bag, the latter may prey on the former; even if the gobies survive predation by the scorpionfishes, their body surface and fin membranes will be frayed and torn.

Some fishes carried in a plastic bag.

Although live-fish transportation is the most appropriate way to prepare good specimens, this method exposes the fishes to the risk of being damaged. If live fish samples do not have access to abundant air, the fishes will suffocate and die. When suffocated, most fishes spread open their mouths and gill covers wide. In this condition, accurate measurements like those of standard length of the fishes cannot be obtained, since the open mouths and gill covers rigidify because of rigor mortis and it is difficult to restore them to the normal positions. Data for calculating accurate standard length are the most important in academic researches. Furthermore, if fishes die because of lack of oxygen in room-temperature water (including seawater), their body coloration when fresh cannot be recorded, since the body color fades rapidly and significantly.

Although live-fish transportation is very valuable for preparing beautiful specimens, it should be noted that transportation of some fishes alive, especially freshwater fishes, is legally prohibited. These laws differ among countries, and all laws in a country should be respected while collecting and transporting fish samples.

After successful transportation of live fish samples to the laboratory, the fishes

Largemouth bass (*Micropterus salmoides*), listed under the Invasive Alien Species Act of Japan.

Bluegill (*Lepomis macrochirus*), listed under the Invasive Alien Species Act of Japan.

Fresh fishes carried in ice-cold water.

Fish samples temporarily kept in ice-cold water to preserve their freshness.

should be sacrificed by immersion in ice-cold water or anesthetized.

If sample treatment cannot be initiated immediately after collection (live fishes from sampling sites or dead fishes from places like fish markets), the fishes should be transported in a cold box filled with ice-cold water (seawater for marine fishes and freshwater for freshwater fishes). It should be noted that if fishes are put on ice directly, the color of the body part touching the ice changes. Moreover, ice-cold water is crucial for preserving the freshness of the samples before treatment. Thus, it is better to temporarily keep fish samples in ice-cold water for at least several minutes before initiating treatment.

Freezing is a better method of preserving fish samples than keeping in ice-cold water, if the samples cannot be treated for a long time and/or they need to be transported over long distances. However, fishes begin decaying, especially the internal organs, on long-term chilled storage. Fish samples should be stored in a freezer if they cannot be treated within 1–2 days after collection. Methods of freezing fish samples are discussed in STEP 2.

1. Freezing of samples (for preservation and storage) → Step 2

2. Treatment of samples (for specimen preparation) → Step 4

STEP 2 Freezing

Masatoshi Meguro and Hiroyuki Motomura

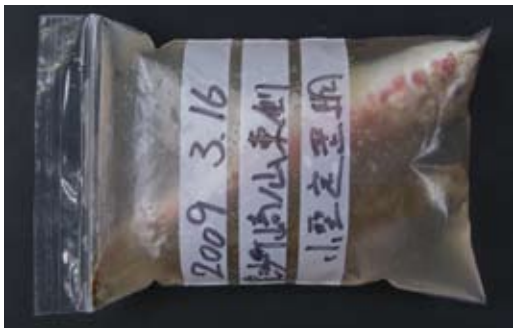
It is very important to obtain fresh fish samples for preparing beautiful specimens with a long shelf life, but specimens may not be prepared from fresh fish samples at any time. Freezing is the only way to keep fish samples fresh for a long time before treating them to prepare specimens. However, freezing 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 home-type 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.

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.

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. Freezer-burned 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 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 → Step 3

STEP 3 Defrosting

Masatoshi Meguro and Tomohiro Yoshida

Defrosting is the first step in preparing specimens from fish samples frozen for a long time. It does not involve simply thawing at any time but should be initiated at the right time.

Defrosting should be initiated considering the room temperature and the mass of the ice supplied with the samples. If thawing is started too early, the fishes will begin decaying, especially the internal organs. On the other hand, if thawing is delayed, sufficient ice will not melt. In this situation, however, the remaining unmelted ice should not be pulled from the fish bodies, since it will break the scales and surface skin.

In addition, scrupulous attention is required while thawing a few frozen samples together, to avoid mixing up of data notes.

Rinsing of samples → Step 4

Defrosting under running water.

STEP 4 Rinsing

Hiroyuki Motomura and Masatoshi Meguro

Defrosted fish samples should be rinsed to remove grime and mucous membranes. Rinsing requires scrupulous attention, since scales and fin membranes may easily break after long-term storage in the freezer. Usually, the body surface is gently rubbed with fingertips to remove extraneous material. If necessary, a soft-bristled paintbrush can be used. Some fishes have a mucous membrane on their body surface. This membrane becomes cloudy if formalin is applied directly (without rinsing well) for fin spreading. Furthermore, if the fishes are not rinsed enough, their body coloration cannot be recorded well. Therefore, mucous membranes should be completely removed. However, it is slightly difficult to ensure this, since the mucous membrane is colorless and transparent.

Assigning tags → Step 5

An imperfect catfish specimen, with mucous membrane not removed completely (KAUM–I. 3508, 451.8 mm standard length). The membrane is removed only around the nape, and the original body color can be observed.

A good catfish specimen, with mucous membrane removed well (KAUM–I. 4806, 72.9 mm standard length).

STEP 5 Assigning tags to specimens

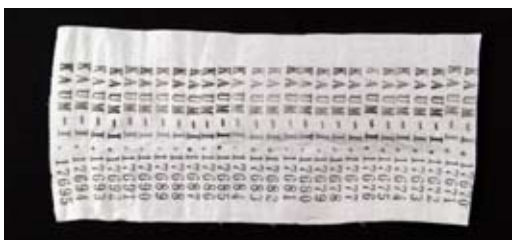
Hiroyuki Motomura

After rinsing, each cleaned fish is assigned an individual number for individual identification of the specimen. When a few samples are treated together, each sample needs to be individually identified; they should not be thrown into disarray, so that there is no confusion arising in subsequent processes.

All specimens should be assigned individual numbers, especially for obtaining tissue samples for DNA analysis and for taking photographs. However, the number tag should not be attached to the fish body before taking photographs. This is because the tag will need to be removed while photographing and reattached to the fish body after the specimen is photographed.

As an individual number is assigned to a specimen, a specimen list should be simultaneously prepared using the sampling-date note as reference.

A list prepared using a regular or a mechanical pencil is maintained almost permanently. The specimen list should include the scientific name of the species. However, quick species identification of some fishes is quite difficult. In this case, the scientific name need not be written at this stage. If a long time is taken for identification, it will not be possible to record body coloration, since the body color fades with every moment. Identification can be performed taking enough time after the specimen is photographed.



Specimen tags used in our museum. Each numbered tag is cut and used for an individual specimen.

■Preparation of a number tag

The number tag is made of cloth. Calico is the best cloth for the tag, since it absorbs inks well, is not a stretchy fabric, and does not tear easily. Serial numbers are printed on white calico by using a numbering machine with pigmented ink. The brevity code of the inventory location of the specimens is also printed in front of each individual number. A brevity

Left: numbering machine (D51, Lion Office Products Co. Ltd.). Right: inkpad.

Specimen tag. Serial numbers and museum abbreviation code are printed on calico (before applying collodion). Below: Collodion bottle.

code is an established code for the name of an international research institution. For example, the abbreviation code of the Kagoshima University Museum-Ichthyology is KAUM–I. The printed information on the calico tag is completely dried by air seasoning for at least 1 day, followed by a few more days. Thereafter, the calico tag is coated with a formulated concentrated collodion solution to make it waterproof. The wet calico tag is then hung outside to dry, like washed clothes, secured by a clothespin.

If collodion is applied before completely drying the ink, the print will be smudged and become unreadable. A tag with an unreadable or missing number will obviously have to be remade. When a number tag is remade, the same numbering machine is not used. This is because the numbering machine will automatically print the next serial number. Thus, the use of the same numbering machine for reprinting a number tag is certainly wrong, since it will cause either overlapping or omission of numbers. A

Tapewriter (DM1585-B, Orient Enterprise Co. Ltd.) and number-tag tape (9 mm width).

Its use is convenient. Cut the calico tag 30–50 numbers and rolled and preserved.

tapewriter is a useful tool for remaking a particular number tag.

After the calico tag is coated with collodion, it becomes hard and waterproof like a plastic board. It can be easily cut using a pair of scissors and the ink too does not get washed in water. However, collodion application does not confirm alcohol resistance.

1. Obtaining tissue samples for DNA analysis → Step 6

2. Pre-fixation (no tissue samples taken for DNA analysis) → Step 7

STEP 6 Obtaining tissue samples for DNA analysis

Hiroyuki Motomura and Kaoru Kuriiwa

In current research, DNA analysis is performed intensively by many scientists. Many museums collect and store tissue samples for DNA analysis, besides whole specimens.

In this section, methods of collection and storage of tissue samples in our museum are discussed.

Tissue samples should be collected after assigning the number tags (STEP 5) and before applying formalin (STEP 7) to the specimens, so that each tissue sample is assigned the same individual number as the specimen. Moreover, formalin constitutes a limiting factor for DNA analysis.

In taxonomic study of fishes, scientists examine the left side of the fish body. Therefore, muscle tissue samples should be collected from the right side of the body, so that the important characters for taxonomy such as the lateral line and pectoral fins are preserved. Enough genomic DNA can be extracted from at least a 5-mm^2 muscle tissue section. However, if possible, it is better to obtain a 1-cm^2 muscle tissue section, as reserve. In addition, the muscle tissues samples should not contain other material such as scurf, fat, and blood. This is because the scurf will produce a smear on the tissue sam-

Fish (right side of body) with tissues excised for samples. White part indicates the point of excision.

Screw-cap sampling glass bottles used for tissue samples.

Tissue sample and data label (specimen number and species name) in a screw-cap sampling glass bottle.

Data (specimen number, species name, and sampling site) written on a screw-cap.

ples when they are immersed in ethanol, and the fat and blood cells will hinder DNA purification.

It is difficult to collect muscle tissues from some small fish species. In this case, the right pelvic (abdominal) fin is collected as an alternative for muscle tissue. Fishes have a pair each of pectoral fins and pelvic fins. Pelvic fins have relatively low mutations as compared with pectoral fins and are not used as an alpha-level taxonomy. Thus, they can be excised.

The obtained muscle or fin tissue samples are put into screw-cap sampling glass bottles filled with 99.5% ethanol.

A 20-cc screw-cap sampling glass bottle is well suited for a 1-cm^2 muscle tissue section. A large tissue sample should not be put into a small bottle to prevent penetration of ethanol into the whole tissue. A small note indicating the individual number and species name should be included with each tissue sample. The note should be written with a regular or a mechanical pencil on waterproof paper.

For better storage of the tissue samples, ethanol should be changed regularly, as it becomes hazy because of water oozing out from the tissues.

In our museum, the screw-cap sampling glass bottles containing the tissue samples are stored in boxes. This arrangement makes it easy to locate a particular sample in order to write the same individual number as the specimen and the species name on the cap. For this purpose, an ethanol-proof pen should be used, since the ink of a normal felt pen will get washed away by ethanol, erasing all information. The tissue sample boxes are stored in the deep freezer for subsequent analysis.

Previously, the liver was used to extract enough DNA from fresh fish samples, since it contains the largest amount of DNA. However, it contains a large amount of fat, as well as sugar and protein. Therefore, nowadays, a liver tissue sample is not suitable for conducting DNA analysis.

Tissue samples in screw-cap sampling glass bottles in a box. The box is placed in deep freezer after closing the lid.

Pre-fixation → Step 7

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

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).

■Equipment

1. Pins

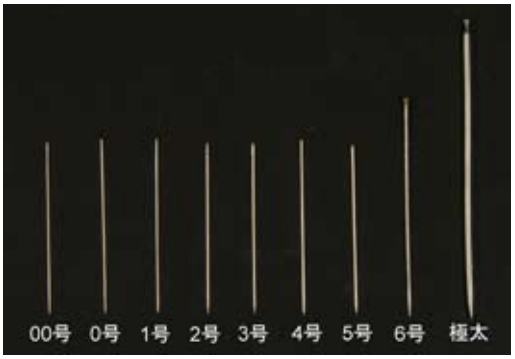
Pins are the most important tools for spreading fish fins. Normally, insect pins are used for this purpose. Shiga Insect Pins are the best choice for spreading fins. They are particularly useful as they are available in many sizes. We usually use No. 00 to No. 6 pins and micropins. The thickness of the 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.

Sprays to prevent dehydration of fishes during sample treatment.

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.

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 Channidae (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 free 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.

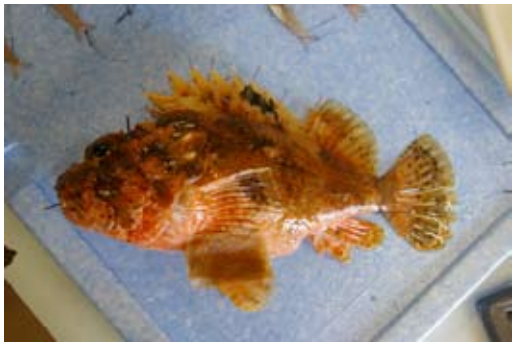
Formalin fixation normally takes 5–10 min for small fish specimens, *e.g.*, smaller than 10 cm. Large fish specimens can be fixed in approximately 15 min.

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.

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

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.

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.

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

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.

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

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

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

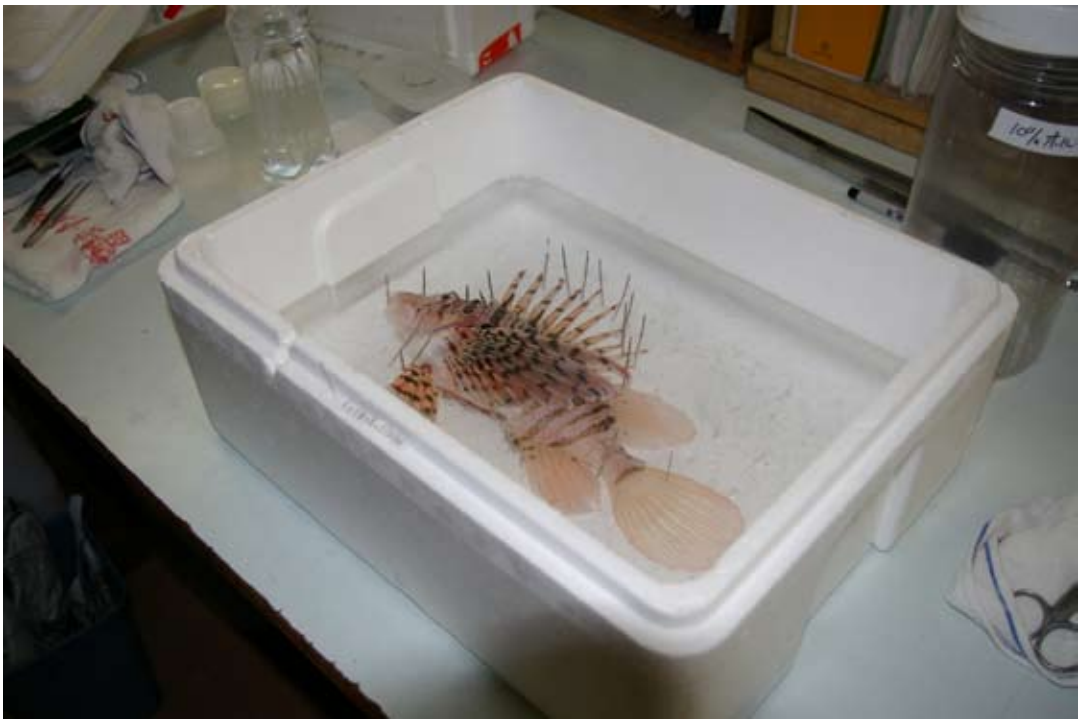
In the case of fishes in which pectoral fin is a taxonomic character, the right pectoral fin should be removed, spread, and photographed. This makes it easy to identify the species for taxonomic purpose.

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.

Photography → Step 8

STEP 8 Photograpy

Hiroyuki Motomura

Photography of specimens needs to be performed very carefully. Fish specimens are fixed in formalin (STEP 12) and preserved in alcohol. However, in the course of the fixation and preservation procedures, most of the original body color of a fish, except black, fades away. Therefore, we have only one chance to record the body color of a specimen.

In this section, the photography procedure is explained, along with the introduction of the equipments used in the Kagoshima University Museum. Refer to Information 1 for the basic technical information about specimen photography and cameras.

■Close-up copy stand

Fish specimens have body width. Hence, for good specimen photography, the depth of field should be increased (see Information 1 for details). This requires stopping down the camera lens. However, stopping down the lens slows the shutter speed. Therefore, it is necessary to use a close-up copy stand for specimen photography.

■Lighting

Fish specimens are photographed indoors, and the lens requires to be stopped down. Therefore, lighting is a very important element of good specimen photography. In our museum, a photo-reflector lamp was used until 2007. Thereafter, it was replaced with a fluorescent lamp because of high heat generation (making photography in summer difficult) and short life (requiring to be turned on and off frequently) of the photo-reflector lamp. The following fluorescent lamps are used for specimen photography in our museum.

Close-up copy stand, light, and glass aquarium for medium specimens.

Close-up copy stand, light, and glass aquarium for small specimens.

For medium to large specimens: Copy light
Company: LPL Co., Ltd.
Model: FL-217 L18527
Japanese Article Number (JAN) code: 4988115, 185309

For small specimens: Web dot studio light
Company: LPL Co., Ltd.
Model: WL-230 L18552
JAN code: 4988115, 185521

■Glass tank (aquarium)

After pre-fixation (STEP 7), the photography procedure should be initiated immediately. The specimen should be re-washed according to STEP 4 and immersed in a glass tank filled with water. In our museum, 3 different sizes of tanks are used, depending on the sizes of fish specimens.

Small tank: 20 cm (length) × 15 cm (width) × 5 cm (height)
Medium tank: 30 cm (length) × 20 cm (width) × 10 cm (height)
Large tank: 60 cm (length) × 30 cm (width) × 20 cm (height)

The tanks are custom made, but they are relatively inexpensive (large: about ¥10000; medium and small: about ¥5000). At the time of ordering a glass tank, it is important to ask the manufacturer to use transparent silicone for gluing each glass panel. If normal white silicone is used, photography lighting will produce shadows, marring the clarity of the photographs.

Glass aquarium should be placed a little above the white board on the close-up copy stand. Here, square wooden blocks are used as legs for the aquarium.

An acrylic tank is lighter and more convenient than a glass tank. However, its surface easily develops scratches (which will be visible in the photographs). Therefore, an acrylic tank is not recommended. On the other hand, a glass tank is heavy and breakable. In our museum, a glass tank is used in the laboratory, and an acrylic tank is used in the field. This is because a glass tank is so fragile that it can break by slight carelessness. If the tank breaks, it will not be possible to record the body color of the specimen in the fresh state, even when a rare specimen is obtained. To mitigate the risk, we arrange for 2 glass tanks each of the large, medium, and small sizes in our museum.

After the aquarium is filled with water, it is placed on a close-up copy stand. Prior to this, a clean white board (matte type) is placed on the close-up copy stand, following which 4 blocks are placed on the board as legs for the aquarium. The white board will serve as a white background for the photographs (details explained later). If the aquarium is directly placed on the white board, a shadow of the specimen will appear on the board. Therefore, the aquarium should be about 3–10 cm above the white board.

The swimbladder is not evolved in the members of Scorpaenoidei and abyssal fishes, and therefore, they can easily and stably sink to the bottom of the aquarium. On the other hand, fishes such as those of Perciformes live in shallow water, and therefore, it is often difficult to sink them to the bottom of the aquarium. In such a case, the right side of the abdomen should be punctured with a needle to let

Specimen is stably lying on the bottom of the water-filled aquarium. Above: view from the back. Below: view from the front.

A slight tilt in the fish position can be adjusted using a rubber piece (*e.g.*, an eraser piece).

the air out of the swimbladder, and the fish should then be re-immersed in water. If the fish still floats, the right side of the abdomen should be incised to let the air out of the abdominal cavity. Small fishes may not sink even after incising the abdomen, or even if they sink, they may lie tilted. In such a case, a thick needle should be inserted in the abdomen or the pectoral area to stabilize the body by the weight of the needle. However, it should be ensured that the needle does not overlap the pectoral and pelvic fins. It will not be possible to process the image (STEP 16) later if the needle is visible through the fin membrane.

Specimen is not stable. The body is leaning backward. Further, the photograph is not taken from the right angle. This is an example of a poor photograph.

Specimen is stabilized using a needle. Ensure that the needle does not overlap the pelvic fin.

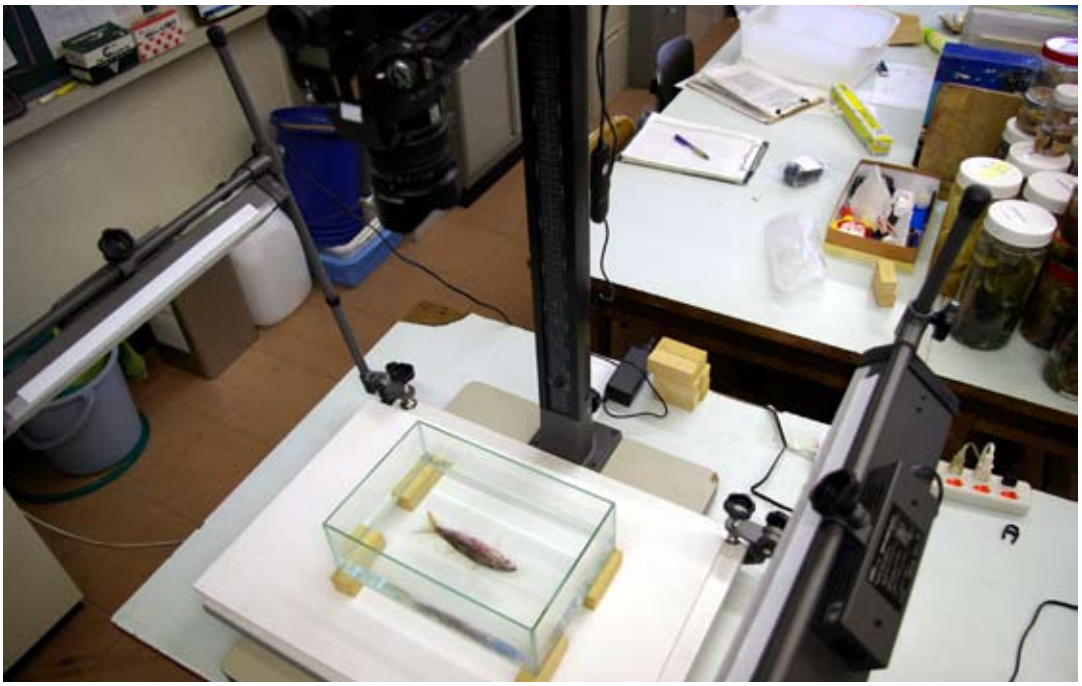
■Photography technique

Refer to Information 1 for the general specimen photography technique and camera characteristics. Here, the actual photography technique is explained, taking a digital single-lens reflex (SLR) camera (Pentax K100D Super) as an example.

We use 2 kinds of lenses: general zoom lens (DA 18-55mm F3.5-5.6 AL) and micro lens (D FA 50mm F2.8 Macro). With one camera body and 2 different lenses as one set, we always prepare 3 sets, so that the situation can be managed when multiple groups of students and volunteers want to go to the field at the same time.

The photography technique involves the following steps.

1. Fix the camera on the pole of the close-up copy stand. Ensure that the camera is not tilted and has a memory card. Replace the lens with the micro lens while photographing small fishes. Con-



Specimen (*Upeneus tragula*) ready for photography (light not turned on). Remote release is hanging behind the pole of the close-up copy stand.

The first photograph should be taken with the tag.

Photography with a black background. A black board is placed between the white board and the aquarium base. In order to avoid reflection of roof barre and lamp in the glass of the aquarium base, it is necessary to hold a large black board above the camera.

nect the alternate current (AC) adapter to the external power port of the camera. If a battery is used, the photographs will not be clear when the battery reaches exhaustion, and further, the camera settings may not be saved due to battery exhaustion. Therefore, we recommend the use of external power for the camera. The AC adapter is compatible with 240 V, and therefore, it can directly be used outside Japan.

2. Set the aperture priority mode (Av) to stop down the lens to focus the fish body. Configure the aperture value setting with the mode dial (the extent of stopping down the lens depends on the size and thickness of the fish body; see Information 1 for details.)

3. Turn on the light and adjust the white balance. If the light in the photograph is the same as that in the previous photographs, it need not be re-adjusted (the setting does not change even when the camera is switched off). A flash lamp may not be used.

4. Push the "Function (Fn)" button on the side of the monitor, and use the Auto Bracket function (this setting needs to be configured every time). The Auto Bracket function allows 3 successive shots by clicking the shutter button only once. When this function is used for the first time, go to "Settings" in "Menu" and set the increment level of the exposure of the 3 serial shots as 0.5 steps (this setting does not change even when the camera is switched off).

5. Configure Remote Release on the camera. Since the aperture priority mode is used, the shutter speed becomes slow, and the photograph can be blurred because of a little camera shake when the shutter button is pushed. Further, since 3 successive shots are taken using the Auto Bracket function, the shutter button needs to be kept pressed. Therefore, it is necessary to configure Remote Release.

6. Adjust the camera lens and pole of the close-up copy stand to bring the fish on the bottom of the aquarium inside the viewfinder. In the case of most cameras, the view from the viewfinder and the actual photograph do not match completely—a point that should be considered while photographing the specimen. The viewfinder field of view in Pentax K100D Super is 96%, which means 4% of the actual photograph (the outer edge) is not observed from the viewfinder.

7. Take the first shot such that it includes both the fish body and the specimen tag (STEP 5). Next, take 3 serial

shots, using the Auto Bracket function. When a white background is used, set the initial Auto Bracket level as 0 and then take the photographs (the increment level should be set as 0.5 steps in point 4, so that the photographs are taken at 3 exposure levels: 0, +0.5, and +1.0).

8. After 3 photographs are taken with the white background, place a black board (or black velvet fabric) between the aquarium base and the white board, and take 3 successive shots with the black background. Each specimen should be photographed with both white and black backgrounds. This is because white parts (*e.g.*, fins) will not be clearly visible against a white background, whereas black parts (*e.g.*, black spots on the fin membrane) will not be visible against a black background.

If the specimen is photographed with a black background, with the initial Auto Bracket level set as 0, the photograph may be too bright and overexposed. Therefore, set the initial level as –1.5, and then take the photographs (the specimen will be photographed at the 3 exposure levels of –1.5, –1.0, and –0.5). Note that this setting depends on the model of the camera and the brightness of the light.

When a black background is used, a roof lamp and/or patterns of roof may be reflected in the glass of the aquarium base and on some part of the fish body. Some measure should be adopted to avoid the reflection. In our museum, we hold a large black board above the camera fixed on the close-up copy stand to avoid the reflection.

The above steps describe the actual photography technique. With regard to saving the photographs in the memory card, 7 photographs for each specimen should be saved as follows: one photograph of the specimen with the tag (with a white background), 3 specimen photographs (taken in 3 steps of exposure compensation) with a white background, and 3 specimen photographs (taken in 3 steps of exposure compensation) with a black background. These photographs are obviously digital images and can be modified on the computer, but the overexposed parts cannot be modified because they do not contain any graphic information. The body color of fishes like those of Carangidae and Trichiuridae is silver gray, which is easily reflected. Therefore, overexposure should particularly be avoided in their case. For these types of fishes, it is safe to take photographs by using the 3 steps of exposure compensation.

Very large specimens (*e.g.*, sharks and hairtails), which cannot fit on the close-up copy stand, can be placed on the floor and photographed. When we photographed a 2-m-long largehead hairtail, we placed it on the floor and clicked the photograph from the second-floor balcony. Extreme caution should be exercised while photographing specimens outdoors, since cats may target the specimens.

1. Details about specimen photography → Information 1

2. Photography in field → Information 2

3. Tagging → Step 9

4. Image processing → Step 16

INFORMATION 1 Details about specimen photography

Kaoru Kuriiwa

■Choice of camera and lens

1. Compact or SLR camera

The most commonly used cameras can be divided into 2 main types: (1) compact digital camera, which has an integrated lens and (2) SLR camera, which has a replaceable lens. The question is which of the 2 types is suitable for specimen photography.

The basis of selecting a compact camera will obviously be convenience. A compact camera is small, light, and can be used anywhere. No complicated setup is required, and most people can easily handle it. Basically, it captures a deep-focus shot (everything from the objects in the background to those in the foreground are evenly focused), without adjusting the aperture or shutter speed. On the other hand, the lens of an SLR camera needs to be replaced depending on the purpose; furthermore, the body is large and heavy as compared with that of a compact camera. Exposure needs to be set according to the aperture and shutter speed, and the depth of field (the depth and thickness that come into focus) should be carefully selected. Thus, it requires some knowledge and skill to take a good photograph. The performance of both types of cameras is improving with time, but there still exist significant differences in 2 basic parts, irrespective of the extent of sophistication of the machines. The difference is in the size of the light-receiving element (sensor) and that of the aperture of the lens.

A camera receives optical information through the sensor, and there is considerable difference in the sensor size between compact and SLR cameras. Suppose there are 2 cameras—a compact camera and an SLR camera—with the same resolution, *i.e.*, ten megapixels (10 MP). In a 10-MP camera, the optical information received by the sensor is delimited into 1 × 106 dots, and an image is produced. The sensor of a commonly used compact camera is 5.7 mm × 4.3 mm (1/2.5-inch-type charge-coupled device [CCD] sensor), whereas that of a general SLR camera is approximately 24 mm × 16 mm (advanced photo system [APS] sensor). As evident, there is a difference of more than 15 times. In fact, despite both being 10-MP cameras, there is a significant difference in the optical information of each pixel. Thus, an SLR camera has more optical information than a compact camera, allowing it to duplicate the object faithfully and describe it in more detail. Imagine sharing a table-sized cake and a 20-cm-wide cake, each with 10 people. It is needless to say a piece of which cake will be larger (consider cake size as sensor size, number of people as pixel value, and quantity of the cake as optical information). Furthermore, there is a difference in the bore diameter of the lens between compact and SLR cameras, and an SLR camera, which has a larger bore diameter, has higher resolution.

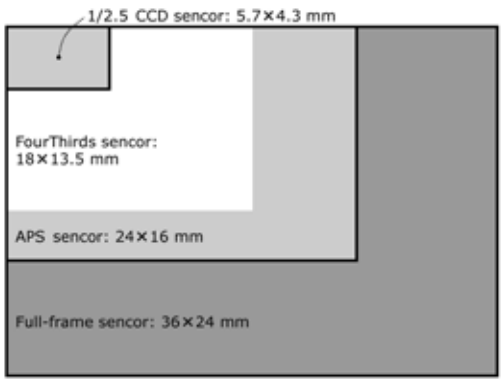


Figure 1. Comparison of the light-receiving element (sensor) of compact and SLR cameras.

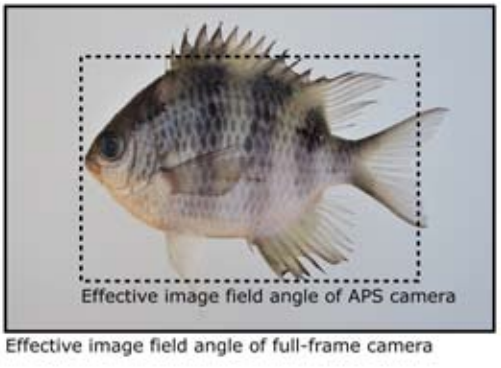


Figure 2. Difference in the effective image field angles of full-frame and APS cameras.

Specimen photography for academic purpose should be taken under better photographic equipments to take a high-resolution image. Therefore, it is preferable to use an SLR camera.

2. APS or full-frame camera

There are actually 3 main sensor sizes for digital SLR cameras (Figure 1). One is full-frame sensor, the size of which is the same as that of the sensor of a 35-mm film camera (full-frame camera). Another is APS sensor, the size of which is the same as that of the sensor of an APS camera. The third type is called FourThirds sensor, which is mainly adopted by OLYMPUS. Full-frame sensors have so far been adopted only by a few companies (*e.g.*, Nikon, Canon, and SONY) in their top models, and APS sensors are adopted by other companies in their SLR cameras. Here, we focus on full-frame and APS cameras.

Full-frame and APS cameras have different field of view (field angle) even when lenses with the same focal length are used (Figure 2). This is due to the difference in sensor size. For example, when a lens with 50-mm focal length is used, the field angle of a full-frame camera becomes 50 mm, but that of an APS camera becomes approximately 75 mm, which is 1.5 times smaller (effective image field angle). In general, field angle is expressed using that of a 35-mm film camera (full-frame camera) as a reference. That is why the field angle of an APS camera lens is often indicated as "35-mm camera conversion: __ mm." In other words, if a 50-mm focal length lens is used in an APS camera, the field angle corresponds to that of a 35-mm film camera with an approximately 75-mm focal length lens. However, the field angle is not actually reduced by 1.5 times, but the visible 50-mm field angle of a full-frame camera is reduced to make it approximately 75 mm for an APS camera.

Whether the photographer chooses a full-frame or an APS camera depends on his preference based on the characteristics of both. The advantages of a full-frame camera include high sensitivity because of its large sensor, broad range of tones, and wide field angle. However, it is expensive, large in size, and may produce limb darkening. On the other hand, the advantages of an APS camera include telephotography, ability to achieve high resolution in the center of an image because of reduction of field angle, small

size, light weight, and cost effectiveness. Its disadvantage is that the focal length of the lens is not the same as the field angle, but is a 35-mm format equivalent (for example, a 50-mm focal length lens cannot be used for 50-mm field angle). Further, to get the same aperture value from the same effective image field angle, the aperture diaphragm in a full-frame camera needs to be adjusted to narrow down the aperture by about one level. In short, it should be noted that there is a difference in the depth of field at the same aperture level (f-number, described later with Figure 8).

3. Zoom or micro lens

There are 2 kinds of lenses: zoom lens and fixed-focal-length lens. The difference lies in that the focal length of the former can be changed, whereas that of the latter is fixed. Zoom lens is very convenient because it does not require the position of the camera to be changed to change its focal length. However, it should be noted that its release f-number (maximum aperture, described later with Figure 8) is generally darker and its resolution is lower than that of fixed-focal-length lens. Dark release f-number is disadvantageous for specimen photography and often requires narrowing down of the aperture. Since resolution is important in specimen photography, low resolution of zoom lens is also a disadvantage. In addition, zoom lens can best reduce aberration around the center of the focal area; the aberration increases at the edges of the focal area (barrel distortion occurs on the wide-angle side, and pincushion distortion occurs on the telescopic side). Thus, fixed-focal-length lens, called micro lens, appears to be the best choice for specimen photography.

The characteristics of micro lens are as follows: (1) high resolution in close-up photography, (2) high magnification (can photograph with life-size or increased magnification), and (3) minimum aberrations (especially distortion). In terms of resolution of a close-up photograph, micro lens shows much better performance than zoom lens. There are micro lenses with zoom function (zoom micro lenses) and zoom lenses with micro function, but they are quite inferior to fixed-focal-length micro lenses. In addition, the maximum magnification of micro lens is 1:1 (life-size magnification) or 1:2, while that of zoom lens with micro function is only approximately 1:4.

Many companies sell various kinds of micro lenses such as standard (focal length, approximately 50 mm), medium telephoto (focal length, 100 mm), and telephoto (focal length, 150 mm or more). In specimen photography, the suitable focal length of lens differs depending on the size of the specimens. This is because overhead photography is conducted using a tripod and camera base. Furthermore, shooting distance is an important factor. The distance between the sensor and the subject is called shooting distance (Figure 3). A lens with shorter focal length has a longer minimum shooting distance, and vice versa. When a large specimen is photographed using a lens with long focal length, the field angle lacks width (short

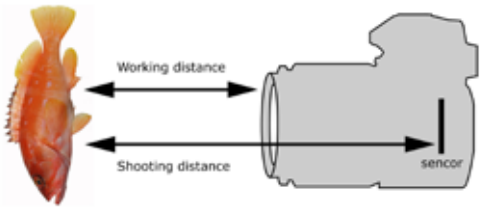


Figure 3. Shooting distance and working distance.

Figure 4. Difference in the effective image field angles of full-frame and APS cameras. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/16).

Figure 5. Difference in the effective image field angles of full-frame and APS cameras. Nikon D60, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/8).

shooting distance), and the image does not fit in frame. This should be borne in mind that the actual field angle (effective image field angle) of an APS camera is converted into 35-mm format. The distance from the front of the lens to the object is called working distance (Figure 3). The description of lens performance by a company mentions the minimum shooting distance, but not working distance. Note that even though the minimum shooting distance is the same, working distance can be different among lenses with different body length. In addition, to what extent a lens can enlarge the image of an object is expressed as the maximum magnification, and this is not related to focal length. When lenses with the same maximum magnification and different focal lengths are compared, the difference is found to be only in the shooting distance for maximum magnification.

As mentioned above, the effective image field angle of an APS camera becomes equal to that of 90-mm medium telephoto macro lens in 35-mm format, even though standard macro lens is used. When a specimen measuring more than 40–50 cm is photographed using 90-mm medium telephoto macro lens, the image does not fit in frame because of short shooting distance (Figure 2). Figures 4 and 5 show how difference in field angle due to difference in sensor size affects the actual photograph. The photographs are taken with a full-frame camera and an APS camera, with both fixed in position and having the same focal length lens (60 mm) (standard length of the specimen: 26 cm). Figure 4 shows the photograph taken with a full-frame camera, and the effective image field angle is 90 mm in 35-mm format. To photograph the same area as in Figure 4 by using an APS camera, a lens with 40-mm shooting distance (35-mm format equivalent: 60 mm) needs to be used or the camera needs to be positioned comparatively high. If a large specimen is taken, an APS camera will not photograph it as a full-frame camera because of narrow field angle. If an even larger specimen is taken, macro lens will not allow its image to fit in frame, regardless of which camera is used. In this case, zoom lens should be used. As stated above, aberrations of zoom lens increase at the edges of the focal area. Therefore, with zoom lens, the photograph should be taken being as close to the center of the focal area as possible.

■Shooting procedure and settings

1. Storage formats of digital images

Photographs taken with a digital camera are stored in RAW, TIFF, and JPG formats. RAW format, as the name suggests, is a raw image data file. It is "developed" into image files in TIFF and JPG formats. The word "developed" implies that a RAW file is "converted" into a TIFF or JPG file on the computer. A TIFF image does not deflate during development; thus, the image quality is good, although the file size increases. On the other hand, a JPG image is compressed during development; thus, the file size decreases but the image quality is low. As explained, each format has its own characteristics. The question is which is the best format for storing specimen photographs.

As stated above, a RAW file contains raw image data, and the image is simply a digitized output signal from the sensor. The data are not adjusted by the image processor engine of the camera, and hence, the image can be retouched without deteriorating its quality. On the other hand, TIFF and JPG files contain processed image data, and hence, the image quality deteriorates with every retouch. Allegorically, raw ingredients collected for cooking correspond to a RAW image, and cooked food corresponds to TIFF and JPG images. The concept is the same that cooked food cannot really be re-cooked.

Many characteristics of a RAW image, such as exposure level, white balance, noise, and color tone, can be changed; thus, RAW format is undoubtedly more flexible than TIFF and JPG formats. If retouch is presupposed, the image should definitely be saved in RAW format. If a specimen photograph is to be used in a research paper, the background often needs to be processed and the photograph size also needs to be changed. For this purpose, the image should be saved in RAW format. On the other hand, data in RAW format are very voluminous and require extra effort for conversion into JPG format. Therefore, when many specimen photographs are taken, the images may be saved in JPG format, and only when it is decided beforehand that a particular image has to be used in research papers, it may be saved in RAW format. In terms of tint of specimen photographs, white balance is very important. This point needs to be considered while saving an image in JPG format.

2. Camera installation and lighting

There are mainly 2 ways of placing a specimen for photography: (1) the specimen can be placed in a transparent glass tank filled with water (Figure 6) and (2) the specimen can be placed on anti-reflex glass and photographed on land (Figure 7). Fishes with many skin flaps, such as those of Scorpaenoidei and Lophiidae, should be photographed in water in order to correctly visualize the dimensions of the skin flaps. A very large specimen that cannot fit in the aquarium should be photographed by the latter method.

When an immersed specimen is photographed, lighting plays a very important role. Shine flashlights from either side of the specimen, taking care that there is no reflection or shadow on the fish body. It often works well if a tracing paper is fixed in front of the light to soften it. Measure the exposure with the flashlights on, and photograph the specimen with only the flashlights on and the room lamp off. Suitable background color—whether black or white—should be decided depending on the skin and scale color of the fish, although the best way is to photograph the specimen with both backgrounds. Furthermore, if there

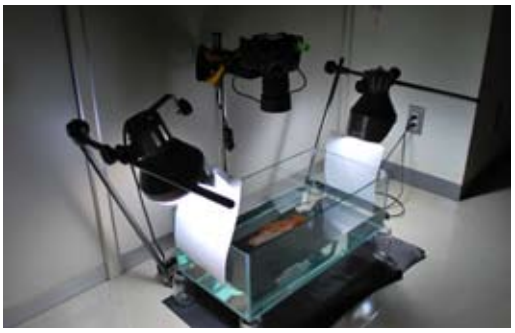


Figure 6. Photography technique for a specimen placed in water.

Figure 7. Photography technique for a specimen placed on land.

is air inside the abdominal cavity, the specimen may float on the water surface or heel over in the aquarium. In this case, puncture the right side of the abdomen with a surgical knife to remove the air. Be careful not to damage the organs, so that the water remains clean. If an organ is accidentally damaged, wash with running water. A glass aquarium is better than an acrylic aquarium, because it has resistance against scratches and tarnish.

For photography on land, the specimen should be placed outdoors in a shaded area. Even in a shaded area, sometimes a different shade of the specimen may be recorded. This should be taken care of. Pay attention to white balance too, and configure the settings such that, as much as possible, the real color of the specimen is recorded. Mist the fish frequently with water to prevent dehydration. However, wipe the body surface lightly while photographing in order to avoid refection due to the water adhering to the body surface.

3. Minimization of shaking

Shaking should be minimized while photographing. For this purpose, a tripod or camera base and remote release should be used. If possible, use a camera level vial to position the camera horizontally. It has earlier been stated that the lens aperture often needs to be narrowed down, and this decreases shutter speed (described later with Figure 8). Slow shutter speed increases camera shake, and therefore, photographs should not be taken with a handheld camera. Nowadays, many companies equip cameras with a function of hand-shake correction. However, this function can only "alleviate" hand shake, and there is a limit of the degree of correction. In specimen photography, slow shutter speed exceeds the limit of the hand-shake correction function. Further, hand-shake correction should be turned off while using a tripod, because it will cause another shake if kept turned on. Release is used to avoid shake when the shutter button is pushed. If release is not available, a self-timer (set at approximately 2 s) or a remote control can be used.

An SLR camera is equipped with an in-built mirror. At the moment when the shutter button is pushed, the mirror flips up and light is received by the sensor behind the mirror. The flipping up of the mirror generates a slight shake (mirror shock). This shake cannot be avoided by using a tripod, camera base, release, self-timer, or even the hand-shake correction function (this is a different kind of shake). To avoid mirror shock, raise the mirror before pushing the shutter button. This is called mirror lock-up photography and is

a very good technique for specimen photography. However, all cameras do not have the feature of mirror lock-up photography. Incidentally, Nikon provides the exposure-delay mode (not available in lower models). With this mode activated, the mirror flips up when the shutter button is pushed, and then after approximately 0.4 s, the shutter is released. This, like mirror lock-up photography, is a method to control mirror shock.

■Photography

1. Exposure

Exposure depends on aperture and shutter speed. The aperture value, which correlates with the depth of field, is very important. Therefore, exposure is decided using manual setting or aperture priority autosetting. In manual setting, the photographer decides the exposure by setting the aperture value and shutter speed. In aperture priority autosetting, the photographer decides the aperture value, and then, the camera automatically decides shutter speed that suits the aperture. Please decide the correct exposure, referring to the histogram.

Figure 8 shows a correlation chart of aperture, depth of field, light entering the aperture, and shutter speed. The aperture value (f-number) starts from 1 and increases in multiples of 1 and √2 (1.4) in sequence (that is, a sequence of multiples of 1 and 1.4 in alternation). As the aperture is narrowed down by one level, the amount of light entering it decreases 2 times. Accordingly, for the same exposure after narrowing down the aperture by one level, shutter speed should be decreased to half (shutter speed should be expressed as multiples of 2).

Overexposure (too much light) causes clipping, and underexposure (too less light) . Be careful that clipping particularly tends to occur with a digital camera. If an image is saved in RAW format, the clipped or suppressed part can be retouched, and information can later be retrieved (this technique cannot be adopted with an image saved in JPG format). However, if an image is completely white or black, the color in each part is saturated, and in this case, no information can be retrieved even from RAW format. The view through the viewfinder differs with each camera model and manufacturer. Therefore, a few photographs should be taken at each exposure level from light to dark. It is also useful to use the Auto Bracket function, with which a camera automatically takes a few photographs at each exposure level (this function is called the automatic exposure bracketing [AEB] function in Canon).

2. Aperture and depth of field

Fishes have body width. Hence, they should be photographed by narrowing down the aperture to increase the depth of field (Figure 8). In a general portrait, the focus is on the eyes; similarly, in a fish specimen photograph, the focus should be on the eyes of the fish. If the depth of field is not enough and each fin is not in focus, the specimen photograph cannot be used for academic purpose. Therefore, the aperture generally needs to be narrowed down. Note that if the aperture is narrowed down too much, resolution becomes low because of diffraction of light.

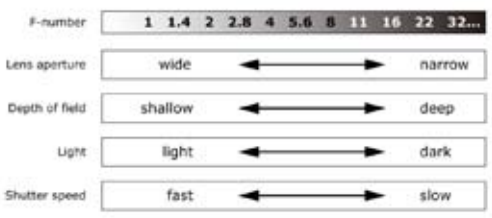


Figure 8. Correlation chart of aperture, depth of field, light entering the aperture, and shutter speed.

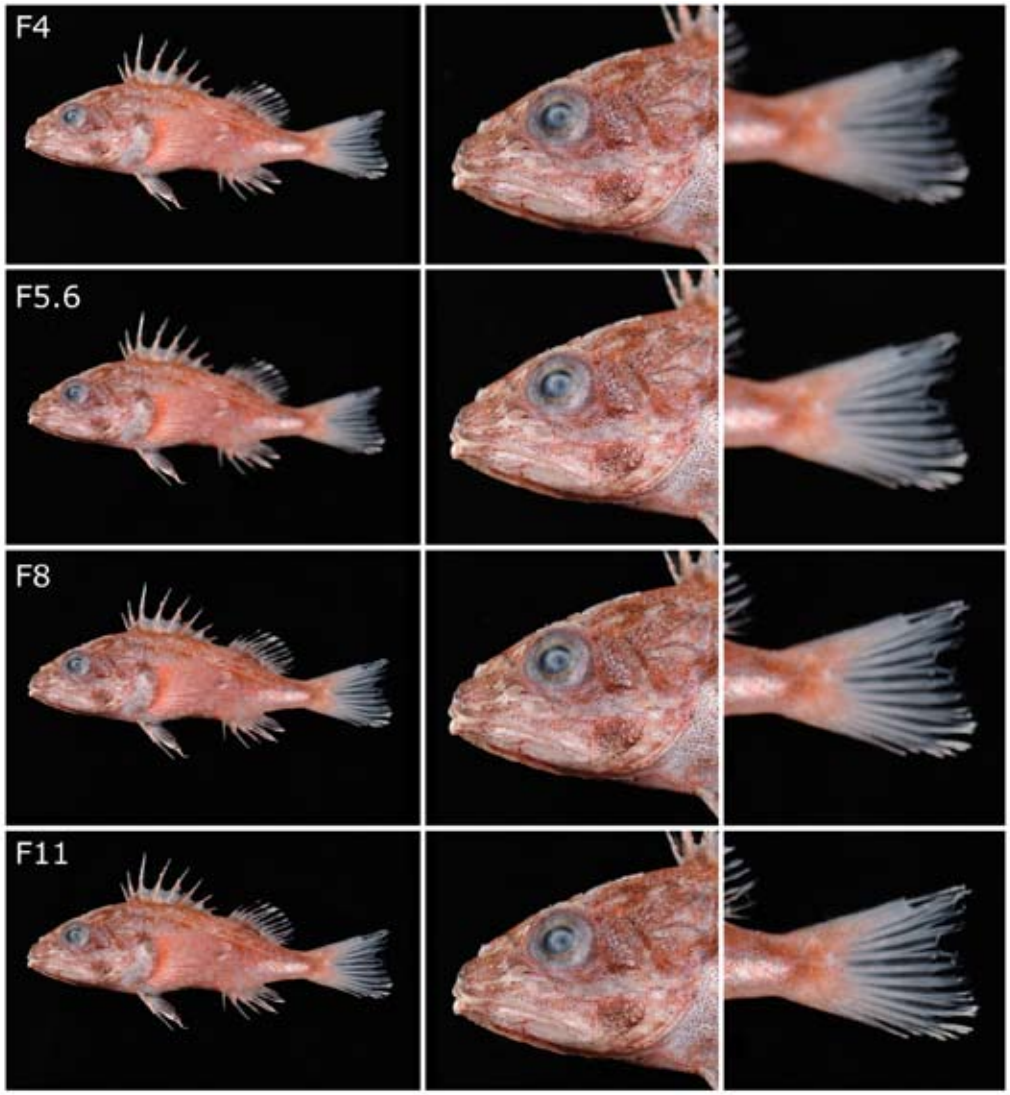


Figure 9. Difference in depth of field depending on f-number. Standard length of the specimen, 5.6 cm; total length of the specimen, 7.1 cm; shooting distance, approximately 30 cm. Nikon D3, AF-S Micro Nikkor 105mm f/2.8G.

This is called narrow-aperture defocus. When the aperture is narrowed down, the path of light entering the lens becomes narrow. If the aperture is narrowed down too much, the light travels around the back of the aperture diaphragm, and because of interference of this light, resolution becomes low. Diffraction occurs independently of the camera, whether film or digital, but the effect is more notable in the case of a digital camera. This is because the smaller the sensor is, the more it can be affected by diffraction. An APS camera is particularly affected severely. Degradation of resolution due to diffraction can be interpreted as degradation of contrast, to be precise. In other words, a photograph in "white - black - white" changes to one in "white - gray - white."

Depth of field depends not only on aperture but also on shooting distance. Long shooting distance increases the depth of field, whereas short shooting distance decreases it. That is why the depth of field

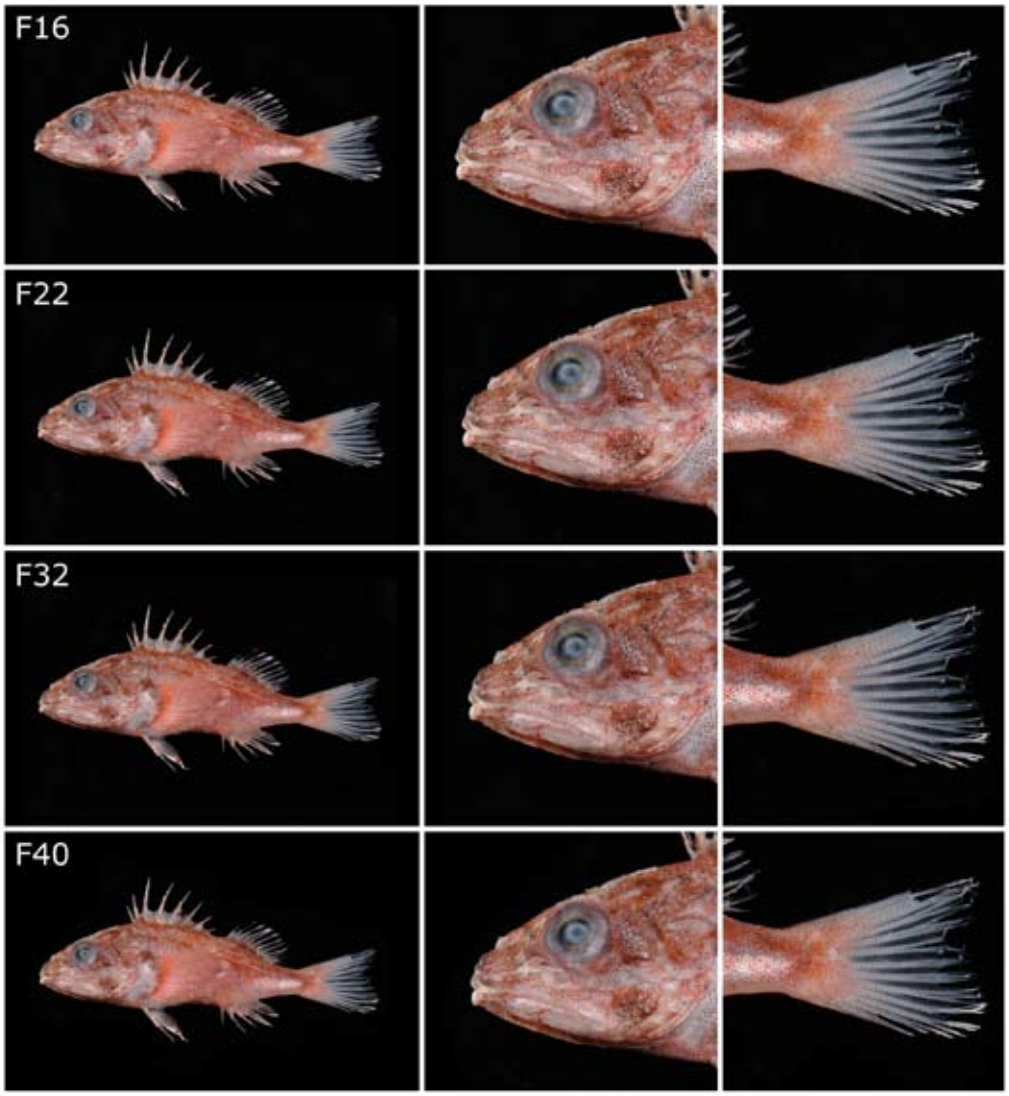


Figure 10. Difference in depth of field depending on f-number. Shooting conditions and equipment are the same as indicated for Figure 9.

becomes extremely shallow in close-up photography, in which macro lens is used. The resolution peak of the lens itself is generally at f/5.6–f/8; however, although the fish eyes or head are in focus around that f-number, the caudal fin and soft rays of the anal fin sometimes appear blurred. In this condition, the aperture may need to be narrowed down to as much as f/32. However, this decreases the lens resolution, and at the same time, degradation of resolution also occurs because of diffraction due to an excessively narrowed aperture. Check the focus by scaling up the viewfinder, and look for the appropriate f-number and depth of field. The depth of field also depends on focal length. The shorter the focal length is, the deeper the field becomes (pantoscope), and the longer the focal length is, the shallower the field becomes (telescope).

3. Examples

Let us take a look at examples to un-

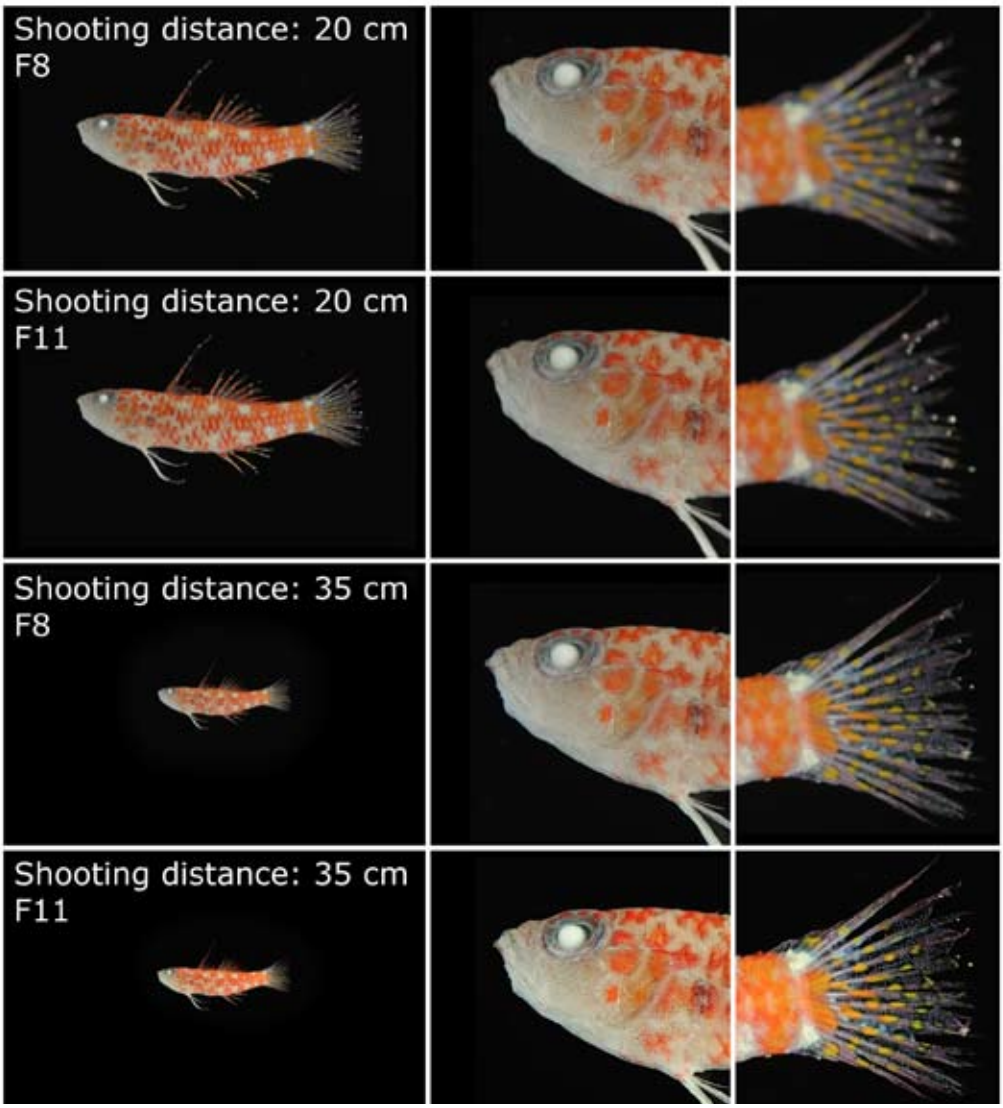


Figure 11. Difference in depth of field depending on f-number and shooting distance. Standard length of the specimen, 2.2 cm; total length of the specimen, 2.7 cm; shooting distance, approximately 20 or 35 cm. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G.

derstand the correct f-number and depth of field for photographing a specimen. Figures 9–11 show photographs taken with a full-frame camera. The f-number of a full-frame camera is about one level lower than that of an APS camera, which has a different sensor size, to generate the same aperture values from the same effective image field angle. Thus, in the following explanation, f/8 of a full-frame camera corresponds to f/5.6 of an APS camera.

Specimen photographs taken at f/4–f/40 (APS: f/2.8–f/36) are shown in Figures 9 and 10. The standard length of the specimen is 5.6 cm (total length, 7.1 cm), and in all the photographs, the focus is on the eyes. This is almost the limit of close-up photography (shooting distance, approximately 30 cm). The left side shows the complete image; the middle shows the image at the same f-number, with

the cephalic part cropped; and the right side shows the image with the caudal fin cropped as well. The photograph obviously lacks the depth of field at f/4–f/8 (APS: f/2.8–f/5.6). The camera is beginning to focus on the caudal fin at f/11 (APS: f/8), and focuses it enough at f/22 (APS: f/16). In the cephalic part, on the other hand, degradation of resolution due to diffraction becomes noticeable at f/22 (APS: f/16). It is particularly notable at f/40 (APS: f/36). The effects of the depth of field and diffraction are only noticeable in the original image in large size, not when the image size is scaled down as shown here. Nevertheless, even in the reduced image, the difference in the depth of field is quite obvious. Recent digital camera models may be able to control the effect of diffraction well. Figure 11 shows the photographs taken with the lens at two distances and f-numbers from a small specimen (shooting distances, 20 and 35 cm; f-numbers, f/8 and f/11). When the distance from the object was increased, large depth of field was possible even with small f-number.

Small fishes, *e.g.*, a few centimeter long ones, should be photographed from a very close distance, so that they occupy the full frame. Therefore, the optimal f-number tends to be large. On the other hand, a large fish needs to be photographed from a long distance, and hence, the aperture need not be narrowed down too much to obtain the optimal depth of field. Figure 12 shows the photograph of a specimen with less body depth (standard length, 18.6 cm; total length, 22.7 cm), taken with a full-frame camera (shooting distance, approximately 56 cm). The aperture value is f/11 (APS: f/8). Figure 13 shows the photograph of a specimen with greater body depth (standard length, 13.9 cm; total length, 17.1 cm), taken with an APS camera (shooting distance, approximately 68 cm). The aperture value is f/16 (full-frame: f/22).

The examples mentioned above indicate that the optimal f-number for fishes longer than a few centimeters is mostly acceptable up to f/22 of a full-frame camera and about f/16 of an APS camera (microscope photography is better for fishes smaller than a few centimeters, not SLR photography). Body depth differs depending on species. Therefore, every time a photograph is taken, the view through the viewfinder should be checked to ascertain the optimal f-number. With practice, one can roughly estimate the standard value from the specimen size, body shape (body depth), and shooting distance before pushing the shutter button.

Figure 12. Specimen without much depth. Standard length, 18.6 cm; total length, 22.7 cm; shooting distance, approximately 56 cm. Nikon D700, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/11).

Figure 13. Specimen with good body depth. Standard length, 13.9 cm; total length, 17.1 cm; shooting distance, approximately 68 cm. Nikon D60, AF-S Micro Nikkor 60mm f/2.8G (aperture: f/16).

INFORMATION 2 Specimen photography in field

Hiroyuki Motomura

To photograph a specimen in field, whether in the native country or abroad, facilities of local research organizations such as local museums and universities can be used. If they have the equipments for specimen photography, such as close-up copy stand and appropriate lighting system, the specimen can be photographed at the organization as explained in STEP 8. However, if there is no such organization, specimen photography may have to be conducted elsewhere such as at the hotel of stay. In this case, a small foldable close-up copy stand and a small light need to be carried along to the field. Photography equipments can then be set up using the desks and chairs in the hotel room.

Photography using a desk in hotel room.

Photography using the chairs and a table in hotel room.

Sometimes, even though facilities of local research organizations can be used, the organizations do not have perfect photography equipments. If the stay is short, photography can be conducted at the hotel, as indicated above. However, if the stay is supposed to be long, as in our case at the Kagoshima University Museum, a different approach should be adopted. We brought some acrylic boards cut beforehand, and framed them in the field. Acryl is convenient since it is light, splinterless, and easy to frame. In addition, we brought a dismantled close-up copy stand and purchased 2 stand lights found in the local area. There was no need to arrange for an electric transformer for them.

Assembling of acrylic aquarium. Use vinyl tape to instantly join the acrylic boards, and apply glue with the cylinder. You can use it from the next day.

INFORMATION 3 Side-view photography

Hiroyuki Motomura

Overhead specimen photography is explained in STEP 8 and Information 2. A specimen can also be photographed from the side, and the technique for side-view photography will be explained in this section. As shown in the figure, the fish specimen is stabilized by placing between a glass board (or acrylic board) and front side of the aquarium and then photographed from the side. The glass board should be fixed using clips, like clothes-pins, so that it can easily be adjusted to change its angle and position depending on the thickness of the fish.

Dirt and incrustations from the fish settle at the bottom of the aquarium. Thus, this photography technique has some benefits such as low frequency of water replacement as compared with that in overhead photography and stabilization of the fish body without removal of air from the bladder and/or abdominal

Specimen photography room at the Museum Support Center, Smithsonian Institution National Museum of Natural History. Side-view photography of the type specimen in progress. Camera is fixed on a tripod, and flash lamp is used for lighting. The photographed image is scanned directly into the computer.

Glass aquarium seen from the front. The black board at the back of the aquarium is used for a photograph with black background.

Glass aquarium seen from the side. Fish is stabilized by placing between a glass board and front side of the aquarium.

Acrylic aquarium with white background.

Acrylic aquarium with black background.

Acrylic aquarium with black background.

cavity. However, the drawbacks are that it is difficult to stabilize very small fishes in the right position and that the glass board appears in the photograph since the fish body is placed between the glass board and the front side of the aquarium. The latter is especially troublesome in the case of a fish with dermal flap on the surface of pectoral fins, because the dermal flap is pressed against the surface of the glass board.

This photography method is adopted by the Smithsonian Institution National Museum of Natural History, the Kagoshima University Museum, and the Fisheries Research Laboratory at Mie University.

STEP 9 Tagging

Mizuki Matsunuma and Hiroyuki Motomura

Management of specimens varies depending on the organization possessing them. Specimens can be stored individually in bottles, with the corresponding data note included in each bottle, or specimens can be stored in bottles grouped by species/families, with register number tags. The latter method is adopted in our museum; specimens are tagged and stored in bottles, which are grouped together by species. In this section, tagging of specimens is explained.

After photographs are taken (STEP 8), the specimens should immediately be assigned tags with register numbers printed on them. Refer to STEP 5 for the materials required and method used to prepare specimen tags.

In general, a specimen tag should be threaded through the mouth from a right gill slit, trying as much as possible not to damage the fish body. In our museum, "Daruma" home-sewing, thick thread (white) by Yokota Co., Ltd., Japan, is used. Other types of textile threads can also be used, provided they are strong enough for the purpose. For threading, a general surgical needle, 1/2 circle and spring eyed (#1–#10), by Natsume Seisakusho Co., Ltd., Japan, is used. The thread is easily drawn through the needle eye, as it simply needs to slide down the groove in the needle (from spring eye);

Home-sewing, thick thread (white) by Yokota Co., Ltd., Japan. Considering its strength and price, this is the most suitable thread for tagging.

General surgical needle, 1/2 circle and spring eyed (#1–#10), by Natsume Seisakusho Co., Ltd., Japan. The spring eye (needle hole) and curve are perfect for tagging.

Typical method of tagging. The threaded needle is passed through the right gill slit to the mouth.

moreover, the half arc makes it easier to pass the needle through the gill slits.

Prepare the needle, thread, and specimen tag. Open a right gill slit with fingers, and pass a needle threaded with a tag through the gill slit to the mouth. Finally, cut the thread at a reasonable length and tie a knot to ensure that the tag does not come off. Sometimes, it is difficult to pass the needle through the gill slits to the mouth depending on the types of fishes. Different methods for different types of fishes are explained below.

■Large fishes/fishes with a long distance between gill slits and mouth

When it is difficult to pass a needle through the gill slits to the mouth because of large size of the fish, the tag can be attached through the lower jaw. For tagging in this case, the soft tissue lining around the teeth should be pierced.

Tagging through the lower jaw. The soft tissue between dentaries should be pierced.

For tagging fishes with small gill slits and a long distance between the gill slits and the mouth, such as those of Tetraodontidae, the gill slit area can be pierced.

Specimen in a Ziploc bag for preservation. Tags should be inside the bag, not attached to the fish.

Specimens preserved in screw-cap vials with tags.

For tagging sharks and rays, the base of the right pelvic fin should be pierced.

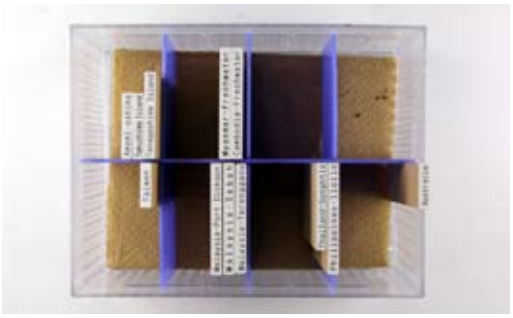
For tagging members of Syngnathidae, tie the thread at the sulcus of arthromeres.

Alternatively, for tagging members of Tetraodontidae and Lophiidae, which have small gill slits and a long distance between the gill slits and the mouth, the gill slit area can be pierced.

■Small fishes

When the body of the fish is too small and there is a risk of damage to the lower jaw while trying to pass a needle through the gill slits to the mouth, the tag can be enclosed with the fish body in plastic food-storage bags (*e.g.*, Ziploc, which is a brand of polyethylene bags with zippers). For the large range of sizes, "Unipac" by Seisannipponsha Ltd. (Seinichi) is suitable. Furthermore, specimens of high academic value and fragile larvae/fingerlings should be preserved in storage bags. In addition, screw-cap vials and test tubes can be used for storage.

A sampling-site tag is prepared in the same manner as a number tag in the Kagoshima University Museum.



Rubber stamp for sampling-site tag.

■Members of Chondrichthyes

Since sharks and rays are generally large and it is difficult to tag the mouth through the gill slits, the base of the right abdominal fin should be pierced for tagging.

Specimen with a number tag and a sampling-site tag. The latter is useful to know sampling locality of the specimen.

■Elongated fishes (such as those of Syngnathidae)

Fishes with extremely small gill slits and mouth, such as those of Syngnathidae, need to be tagged on the cauda, with a knot tied in the thread. The risk of losing tags can be reduced by tying the thread at the sulcus of arthromeres.

The unique approach adopted by our museum, that is, the use of sampling-site tags, besides number tags, can be used. Local museums (or local universities) play an important role by providing information about the local environment. It would not be worthwhile to collect fishes from the Atlantic Ocean for Kagoshima University. It is important to collect local fishes from Kagoshima and manage them. Thus, the sampling site becomes confined to an extent, and sampling-site tags can be prepared beforehand.

At central and large museums (such as National Museum of Nature and Science, Tokyo) and Western museums, specimens in bottles are managed by including labels inside the bottles (STEP 5), and thus, information about the sampling site of the specimen is instantly available. However, in our museum, the bottles are sorted by the species of the specimens, in which case it will not be possible to know the sampling site of the specimen without noting the specimen number and searching the database. Therefore, we prepare sampling-site tags prior to our main surveys and attach them to the specimens together with number tags. This method is very useful because the sampling site of the specimen can be instantly known. A sampling-site tag (rubber stamped) can be purchased for about ¥105. It is delivered within 24 h when ordered online, and thus, sudden requirement at the field can be managed.

Measurement → Step 10

STEP 10 Measurement

Gota Ogihara

After photography (STEP 8) and tagging (STEP 9), measure the size of the specimen with a caliper. Use millimeters (mm) as the measuring unit, and record up to one decimal place.

Measurement methods used for fishes vary depending on the taxonomic groups. The projective method, which measures the three-dimensional figure as flat figure, is used for sharks; the total length (distance between the tip of the rostrum and the end of the caudal fin) is also measured for sharks. In case of rays, the tip of the tail is often removed (especially in markets), which does not allow accurate measurement of the total length. In this case, the disk width (the longest distance between the right and left pectoral fins) is also measured. For teleosts, the standard length (the shortest distance between the lower jaw and the end of the hypural) is measured. The end of the hypural can be ascertained by flexing the caudal fin to the left side and observing the line on the peduncle. In fishes with the lower jaw longer than the upper jaw, such as those of Synanceiidae, Serranidae, and Hemiramphidae, measurement should be taken from the top of the upper jaw. In fishes with scutes, such as those of Carangidae, the end of the hypural cannot be easily ascertained; therefore, fork length (the shortest distance between the top of the upper jaw and the most indented part of the caudal fin) is alternatively measured.

Caliper used in the Kagoshima University Museum. Above: VC-30, M-type standard caliper by Mitutoyo, Japan. Below: CD-SC, series 500 ABS digimatic solar caliper by Mitutoyo, Japan.

Measurement data are eventually input and registered into a computer database. Therefore, it is very useful in many ways to measure and process specimens simultaneously. For instance, if it is known how big a particular specimen is, it can easily be identified from the numerous individual specimens in bottles with the registered size information. Further, if a request is received to investigate the specimen collection, the specimens will not require to be physically rechecked, but database search will provide the answers. Furthermore, by checking body length and sampling date in the database, valuable information such as growth speed and appearance time of a particular species can be deduced at one glance. For large specimens, it is particularly better to measure the size while processing them, since such specimens cannot be removed from and placed in bottles frequently.

Identification → Step 11

STEP 11 Identification

Gota Ogihara and Hiroyuki Motomura

Identification involves determination of the name (scientific name) in biological classification. In this section, the identification method used in our museum is discussed. If the specimen could be identified anytime between STEP 5 (Assigning tags to specimens) and STEP 10 (Measurement), the species name should be noted in a notebook. However, if the specimen cannot be easily identified at an early time point, identification should be postponed so as to preserve the freshness of the specimen for better photography.

Pepole who is not quite familiar with fishes, should first identify the taxonomic group of the specimen (family, genus, etc.). We call this method "matching identification," for which pictorial books with colored pictures are used. The specimen is compared with the pictures in the book, searching for similar image (figure) and color (pattern). This method does not require any professional knowledge. Following books are useful for matching identification of fishes in Kagoshima.

The fishes of the Japanese Archipelago (Tokai University Press)
Sea fishes of Japan (Yama-kei Publishers Co., Ltd.)
Fishes of the Kuroshio Current, Japan (Osaka Aquarium Kaiyukan)
A photographic guide to the gobioid fishes of Japan (Heibonsha)

Once the family (or genus) is roughly identified by matching identification, search for the species on the page of the family (or genus) by using "Fishes of Japan with pictorial keys to the species (Tokai University Press)". This book shows a graphic representation of classification characters (characters used to distinguish a taxonomic group) and is easy to use, but this requires some knowledge of fish taxonomy.

If the species cannot be identified despite using the above book, refer to scientific papers (often written in English) on each taxonomic group. This may be difficult for an inexperienced volunteer. When the species cannot be identified even by referring to scientific papers, a researcher who specializes in the taxonomic group to which the fish belongs should be requested to identify the specimen. If even the specialist cannot identify the specimen, there is a possibility that the fish has not been taxonomically described (*i.e.*, it will be a new species).

Although external morphology is mainly used for identification, there are some taxonomic groups in which external morphology is not as clear as in others (such as, Muraenidae and Sciaenidae). In this case, check the difference in the number of vertebrae by examining an X-ray image and in internal morphology (such as otolith and swimbladder) by studying the anatomy of the fish.

Fixation → Step 12

STEP 12 Fixation

Hiroyuki Motomura

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 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.

Formalin fixation of small specimens.

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

Replacement of formalin with alcohol

→Step 13

STEP 13 Replacement of formalin with alcohol

Hiroyuki Motomura

After fixation, replace formalin with alcohol for long-term storage of specimens. There are several reasons to replace formalin with alcohol, a couple of which are that 1) formalin produces formic acid on oxidation, which demineralizes hard tissues such as fish bones and 2) formalin causes nose irritation and is very poisonous, which makes prolonged examination of specimens difficult. However, alcohol is more expensive than formalin, and many research organizations actually preserve large specimens, which need a large amount of alcohol, in formalin solution (without replacing with alcohol). When a specimen is to be retained in formalin, add sodium hydrogen carbonate (baking soda) or hexamethylenetetramine in formalin to neutralize formic acid, and then use it to avoid degradation of bony tissue.

If a formalin-fixed specimen is directly placed in alcohol, it can simply be assumed that the same amount of formalin as the volume of the fish will percolate into alcohol, which will later create problems while examination of the specimen. Therefore, the specimen should be first immersed in real water to exude formalin from it. Thereafter, it should be placed in "substitutive alcohol" for several days; used alcohol can be used as substitutive alcohol. At this stage, formalin and real water are completely removed from the specimen and replaced with alcohol. Alcohol used in our museum is "general alcohol; ethyl alcohol, 99 vol%; synthesis, unmodified; 18L TNS."

Storage → Step 14

As the first step of replacing formalin with alcohol, the formalin-fixed specimen is soaked in real water for 1 day to exude formalin from it.

STEP 14 Storage

Hiroyuki Motomura

After replacement of formalin with alcohol, the specimen should be stored in a specimen bottle filled with alcohol. In foreign museums, glass jars are often used as specimen bottles, but in Japan, glass jars of reasonable size and price are not readily available. Therefore, in many Japanese museums, specimen bottles made of polyvinyl chloride are used. In our museum, we choose the bottle size depending on the specimen size. We use a 3-L wide-mouthed T-type bottle, which is useful for fishes with large body width, such as blue-striped angelfish, although air circulation is more in this bottle. Sometimes, the lid can slip while carrying the bottles; therefore, they should be carefully handled. Following is a list of specimen bottles used in our museum.

Specimen bottles by AS ONE Corporation. From the left: 0.25-L, 1-L, and 2-L bottles.

AS ONE Corporation

Wide-mouthed T-type bottle with rubber seal (clear vinyl chloride)
0.3 L (width, 75 mm; height, 92 mm)
0.5 L (width, 90 mm; height, 118 mm)
1 L (width, 97 mm; height, 167 mm)
2 L (width, 112 mm; height, 255 mm)
3 L (width, 134 mm; height, 263 mm)

Wide-mouthed bottle with inner lid (clear vinyl chloride)
0.1 L (width, 48 mm; height, 82 mm)
0.25 L (width, 61 mm; height, 119 mm)

Wide-mouthed BB-type bottle without stopcock
20 L
Plastic container 75 L

Sanko Co., Ltd.

Plastic drum (open type)
25 L (width, 296 mm; height, 522 mm)
30 L (width, 312 mm; height, 502 mm)
120 L (width, 490 mm; height, 792 mm)
210 L (width, 580 mm; height, 974 mm)

Power drum (open type)
60 L (width, 400 mm; height, 618 mm)

Specimen bottles are labeled with family number and species name and arranged according to the number. Specimen preparation and arrangement are done by volunteer staff members. Therefore, we use standard Japanese names

Storage containers for medium to large specimens. Upper row: 20-L wide-mouthed BB-type bottle without stopper cock. Lower row: 25-L plastic drum. Label the front side with white vinyl tape, and write the family number and species name with alcohol-resistant Sharpie Fine Point pen (sold in Europe and the United States).

Thermoelectric printer (Name Land; CASIO) for printing labels for specimen bottles. We use black ink for normal specimens and red ink for type specimens. Further, we use 12-mm-wide tape for 0.5- to 3-L specimen bottles and 9-mm-wide tape for 0.25-L specimen bottles.

Specimen fixed in 99% ethanol (for DNA analysis). Label is printed using a thermoelectric printer, which prevents deterioration or loss of print.

Specimen bottle labeled with family number and species name (2-L wide-mouthed T-type bottle).

instead of scientific names as species names. Labels are printed using “Name Land” by CASIO, which is a type of thermoelectric printer. A thermoelectric printer provides excellent resistance to alcohol (see Information 4), which prevents loss of label print because of alcohol.

Shelf for storage of specimen bottles.

INFORMATION 4 Specimen data labels

Hiroyuki Motomura

Because of the storage capacity of the specimen room in the Kagoshima University Museum, we allocate one number tag to one immersion specimen, and store/control it in a specimen bottle that is sorted by species. Other museums, however, including many foreign museums of natural history and the National Science Museum in Japan, store one lot of specimens in one specimen bottle. In this case, various information regarding the specimen is written on the label (specimen label), which is placed together with the specimen in the bottle. In this section, I will explain the pertinent details regarding specimen labels.

USNM 373429
Blenniella caudolineata
(261) Wallis and Futuna

USNM 373429 Fam: 261 Spec: 1
Blenniella caudolineata
Prep Loc Count
photo
Exped.Name: Wallis Island-2000
Field #: JTW 2000-03
Date Coll: 4 Nov 2000
Collectors: J. Williams, P. Sasal & E. Jobet
Accession Num: 619502 Date Cat: 20 Aug 2003
Pacific Wallis and Futuna
Lat: 13 21 50 S Long: 176 13 30 W
Depth: 0.00-1.00 M
Precise Location: Wallis Islands, Ile Uvea, Rocky Shore At S. Tip of Nukutapu Islet, Off SE End of Ile Uvea.
Ecological Habitat: Rocky shore in surge zone. rotenone.
Remarks: female

Specimen label used in the Smithsonian National Museum of Natural History, USA.

Because immersion specimens are stored in alcohol, which is highly volatile, the label and ink that are use must be sure to take this into account. Moreover, since specimens are intended to be passed down from generation to generation as assets shared by all human beings, they must be preserved properly not just on a scale of years or decades, but on a scale of centuries.

Presently, there are four types of printers that are commercially available for the printing of labels.

■**Ink jet/ Bubble jet printer** (In this technology, the ink is sprayed onto the paper)

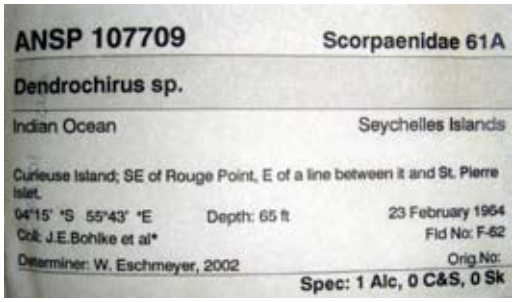
Normal ink will blur if it is soaked in alcohol, but a specifically alcohol-resistant ink has recently been introduced to the market. In the Natural History Museum, London, for example, specimen

Specimen label printed by an ink jet printer using alcohol-resistant ink, in the Natural History Museum, London (photo by J. Maclaine).

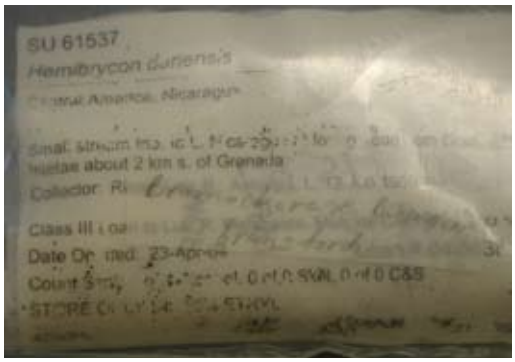
labels are printed using an ink jet printer. However, it is an open question whether today's alcohol-resistant ink will remain legible into the long-term future.

■**Laser printer** (This kind of printer projects the picture via a laser onto a photoconductive drum. The drum then prints the picture onto paper in the form of toner, via direct contact and heat)

Since the script or image is not printed under pressure, the ink will come off if the paper is soaked in alcohol. In the Australian Museum, Sydney, and the Museum and Art Gallery of the Northern Territory, Darwin, printed specimen labels are heated in an oven and soaked in alcohol after the ink has been completely released onto the paper. Acrylic-coated paper, as we will explain later, is not suitable because it will not accept the ink.



Specimen label printed with laser printer and general ink used in the Academy of Natural Sciences, Philadelphia. This label is acrylic-coated.



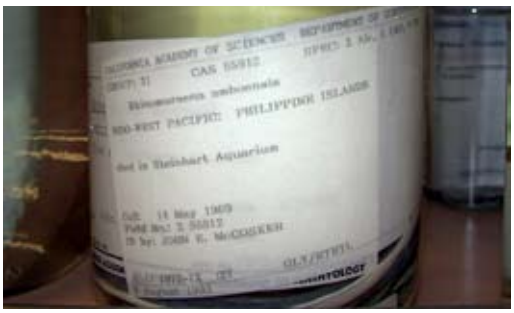
Specimen label printed with laser printer and general ink. The print has become illegible (photo by D. Catania).

In the Field Museum of Natural History, Chicago, they print labels using alcohol-resistant ink and use specimen labels without subjecting them to heat treatment. At the University of Michigan Museum of Zoology, Ann Arbor, and the Academy of Natural Sciences, Philadelphia, on the other hand, they print specimen labels using a general ink toner, acrylic-coat the labels using spray (Krylon Matte Finish), and then apply them. Acrylic coating, however, can be dissolved by fat that is exuded from specimens (especially Chondrichthyes), and thus is not practical for such applications.

In the Academia Sinica, Biodiversity Research Center, Taipei, specimen labels are printed using normal ink, and are then laminated so they can be soaked in alcohol. The problem is that the laminate will eventually come unstuck. This method is also not recommended due to the cost and the labor involved.

■**Dot impact printer** (This kind of printer applies pressure to a ribbon that is saturated with ink, and thus prints words and images using thousands of tiny dots)

In this case, the printed label does not blur and come unstuck, but one cannot expect clear printing because this is a low-resolution technology. A dot impact printer was traditionally used in the Cali-



Specimen label printed with dot impact printer and alcohol-resistant ink. The print is too light and hard to read (photo by D. Catania).

Thermoelectric printer: Datamax I Class Thermal Printer (photo by D. Catania).

Specimen label printed by Datamax I Class Thermal Printer (photo by D. Catania).

Datamax I Class Thermal Printer. In the photo, the black roll is the ink ribbon and the white roll is acrylic-coated paper (Photo by D. Catania).

fornia Academy of Sciences, San Francisco, but the alcohol-resistant ink ribbons it uses are no longer available. Therefore, this method is practical only in museums that still have a considerable stock of alcohol-resistant ink ribbons.

■**Thermoelectric printer** (This kind of printer transcribes words to paper by heating an ink ribbon on film)

This printing technology is characterized by its resistance to blurring and coming unstuck, and is thus the best printer for the printing of specimen labels. It has the disadvantage, though, of the overall cost of the ink ribbons it uses, which are expensive. In American museums, thermoelectric printers are predominantly used for the production of specimen labels.

As mentioned above, various printing methods are used in different museums to produce specimen labels. There are drawbacks and advantages involved in the choice of a given product, due to the rapid development of printers and ink, and the discontinuation of existing products. That said, the safest (since the print will endure for the long-term) and clearest (since the print can easily be read) method at the moment is printing on acrylic-coated paper (a thick, shiny, water-resistant paper) using a thermoelectric printer. The Datamax series of thermoelectric printers, made by Alpha System, in the U.S., is the most widely used printer in American museums.

Three different kinds of ink ribbon can be used in a thermoelectric printer: waxed, plastic, and a combination wax/plastic ribbon. The plastic ribbon is for light printing, and the waxed ribbon can come unstuck, so the combination ribbon is regarded as being best for the printing of specimen labels.

STEP 15 Creation of database

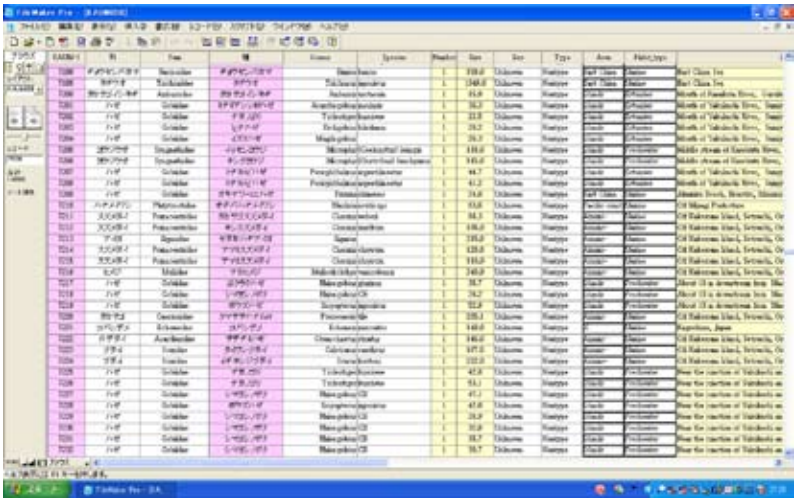
Hiroyuki Motomura

Input data immediately after completing a series of specimen preparation procedures. In our museum, we use FileMaker Pro 9 as the database software and set up the database to enable people to access it, input data into it, and search it from any computer on the campus.

In our museum, there is a dictionary file in which fish name (Japanese name and Latin name) and species name (standard Japanese name and scientific name) are linked. If the standard Japanese name of a species is input, the Japanese and Latin names of the family and the scientific name of the species will automatically be generated. For automated input system, the correct standard Japanese name should be input, and not the scientific name, which is mistakable. The settings can completely prevent input error. We use pop-up menus for selecting sampling site (Pacific Ocean, Sea of Japan, etc.), habitat environment (saltwater, freshwater, brackish water, etc.), sampling method (use of fill net, set net, angling, etc.), and the option of "with or without picture." This selection system saves the trouble of inputting information.

In order to manage the data safely for a long period, we print thousands of copies of the input data and bind them for storage.

Screen shot of list-style display. If the standard Japanese name is input in the second row from the top, family name and species name will automatically be generated in the first row and third row, respectively. This figure shows selection of habitat environment from the pop-up menu.



Screen shot of chart-style display. Information can be input/searched in the database in 3 styles, namely, list style, chart style, and form style, with FileMaker Pro 9.

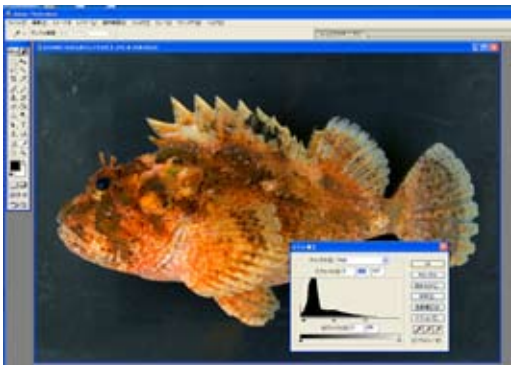
STEP 16 Image processing

Hiroyuki Motomura

In this section, image processing method is explained. The following steps explain the actual procedure employed in our museum. We use the Adobe Photoshop 7.0 software, but basically any version may be used for this method. The following procedure may appear cumbersome, but once one gets used to it, an image can be processed in about 3 min.

Steps 4 and 5 can be omitted for an image with a white background.

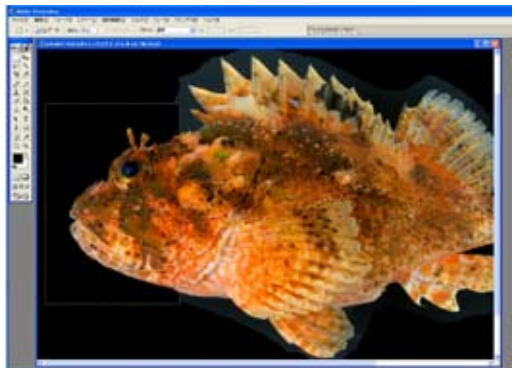
1. Unprocessed original image.



2. Fine tune the image. Select “Image” → “Compensate Color Tone” → “Level Compensate.” A dialog box will appear. Adjust the triangle and click “OK.”

3. Rotate the image such that the specimen is horizontal. Select “Image” → “Rotate Canvas” → “Input Angle.” A dialog box will appear. Type the angle value and click “OK.”

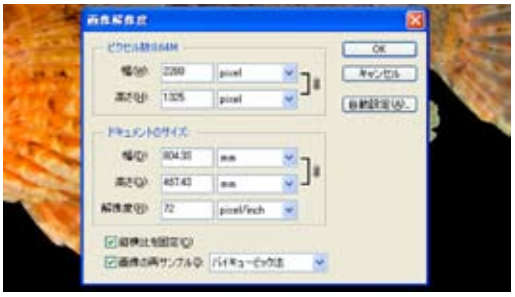
4. Roughly paint the background black. Select “pencil tool” and increase the diameter of the brush. The area close to the fish need not be painted.



5. If needed, zoom in and paint the background black. Roughly select the borderline of the image and background by using “rectangle select tool” and click on the selected area with “fill paint tool.” If this automatically paints the specimen black, reduce “Latitude” and click the selected area again.

6. After painting the background black, crop the excess background.

7. The image after cropping the excess background.



8. Before saving the image, adjust resolution and image size. Select “Image” → “Image Resolution.” A dialog box will appear. In the case of this image, the resolution, width, and pixel value are 72 pixels/inch, 804.33 mm, and 8.64 MP, respectively. In the dialog box, change the resolution from 72 to 300 pixels/inch.

9. Pixel value can be increased by increasing resolution. To decrease the pixel value to as small as it was before changing resolution, decrease the width (image quality cannot be improved beyond that of the original image even by increasing the pixel value). In this image, by changing the width from 804.33 mm to 190 mm, the pixel value was increased to 8.37 MP, which was almost the same as it was before changing the width (8.64 MP). Image quality at 190-mm width and 300 pixels/inch resolution is acceptable for almost any type of printing.

10. Finally, select “File” → “Save as.” Set the image quality to 12, and confirm that the compression is set as “Highest (Low Compression Rate).” Select the “Baseline (Standard)” option.

Unprocessed original image.

Processed image with black background.

Processed image with white background.

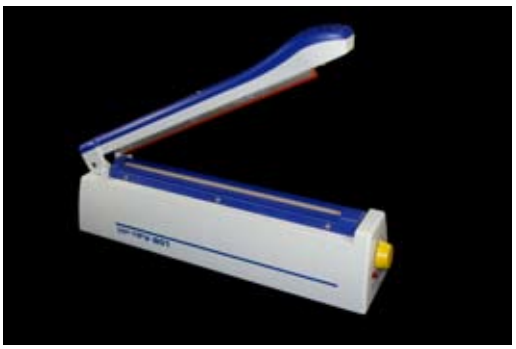
STEP 17 Loan

Hiroyuki Motomura

Stored and managed specimens are not simply maintained in the collection but utilized for various research purposes such as for biodiversity, taxonomic, and ecological research. Some researchers visit organizations and examine specimens, but most of the others borrow specimens to examine them at their organization. In our museum, there were 52 cases of loan requests (681 specimens) only in the fiscal year 2008 (which does not include a huge number of requests for on-campus use).

In this section, mailing of specimens is discussed. Wrap each specimen carefully in a gauze dampened with 20% alcohol. Transportation of highly concentrated alcohol is prohibited by regulations such as Special Provision A58 in the International Air Transport Association (IATA) Dangerous Goods Regulations; therefore, alcohol is diluted to 20%. Pack the wrapped specimen in 3 folds of thick plastic bag. Use sealer to seal the package, and try to enclose as less air as possible while sealing. Ziploc or any other thin vinyl should not be used, because alcohol can leak even on a small change in atmospheric pressure.

Specimen is wrapped in a gauze dampened with alcohol and packed in vinyl.



FV-801 sealer by Hakko Corporation, used for sealing specimens in vinyl.

Packed specimen is placed in a cardboard box filled with cushioning materials.

Place the hermetically sealed specimen in a cardboard box filled with cushioning materials. Ensure that the cardboard box is fairly strong.

Before shipping the box abroad, attach a clear Ziploc bag on the top or side of the box and enclose an invoice (specific list of the content) in the bag. This helps to confirm at the Customs that the content is not a Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)-listed species or (tax-free) product. There is a column for the price of the content on an international package, and an object that costs more than US $20 is subject to taxation. NVC (which means "no commercial value") or a price of US $20 or lower can be written in the price column.

Even when the invoice is included, the content is sometimes checked at the Customs. In this case, there is a possibility that the vinyl package is opened. Therefore, a written warning should be enclosed with the box. The following points should be mentioned: (1) the content is a formalin-fixed specimen for research purpose and has no trouble with quarantine; (2) the species is not listed in CITES; (3) the specimen is preserved in 20% alcohol; and most importantly (4) the package should be immediately resealed.

Two same invoices should be mailed with the package while shipping. One of them should remain with the borrower of the specimen, whereas the other should be signed by the borrower and returned to the lending organization as receipt. The borrower usually writes the result of re-identification on the copy of the invoice. For general specimens, the loaning period is normally for 1 year.

The Kagoshima University Museum
1-21-30 Korimoto, Kagoshima 890-0065, Japan
Telephone: +81 99 285 8141
Facsimile: +81 99 285 7267
E-mail: jmu@kaum.kagoshima-u.ac.jp
URL: http://www.museum.kagoshima-u.ac.jp/

THE KAGOSHIMA UNIVERSITY MUSEUM

SPECIMEN LOAN INVOICE

To: Dr. XXXX XXXX
CSIRO Marine & Atmospheric Research,
GPO Box XXX, Hobart,
Tasmania 7001, Australia

Tel.: +61 3 62XX XXXX
E-mail: XXXX@XXX.au

Loan number: Zool.2006-004
Date sent: 27 Nov. 2006
Loan period: 12 months
Due Date: 27 Nov. 2007
Transaction: Loan
Sent by: Airmail
No. of package: 1
Authorized by: Kimihiko Oki
Packed by: Hiroyuki Motomura

Mammals / Birds / Reptiles / Amphibia / (Fishes) / Crustacea / Molluscs / Insects / Others

KAUM number	Species	Specimen count	Remarks
KAUM-I.	Gymnuridae		
278	Gymnura japonica	1	
392	Gymnura japonica	1	Taken tissue for DNA analysis
616	Gymnura japonica	1	Taken tissue for DNA analysis
815	Gymnura japonica	1	Taken tissue for DNA analysis
985	Gymnura japonica	1	
986	Gymnura japonica	1	
1066	Gymnura japonica	1	Taken tissue for DNA analysis
1067	Gymnura japonica	1	Taken tissue for DNA analysis
1068	Gymnura japonica	1	Taken tissue for DNA analysis
1069	Gymnura japonica	1	Taken tissue for DNA analysis

Remarks: Total 10

Curator of the Collection: Hiroyuki Motomura

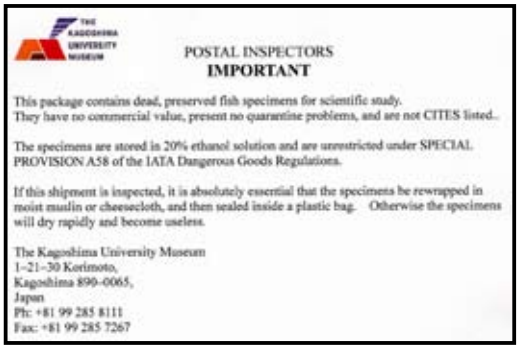
Loan received in good order (except as noted):

Signed _____ Date _____

1) Please sign and return upon receipt of specimens.
2) Please make a copy of this invoice to keep for your records before the return of the invoice.
3) If work on the borrowed material is published, the borrower should forward a reprint to the Kagoshima University Museum.

Page 1 of 1

Loan invoice for specimen. Information such as specimen data and loan period is listed.



THE KAGOSHIMA UNIVERSITY MUSEUM

POSTAL INSPECTORS
IMPORTANT

This package contains dead, preserved fish specimens for scientific study.
They have no commercial value, present no quarantine problems, and are not CITES listed.

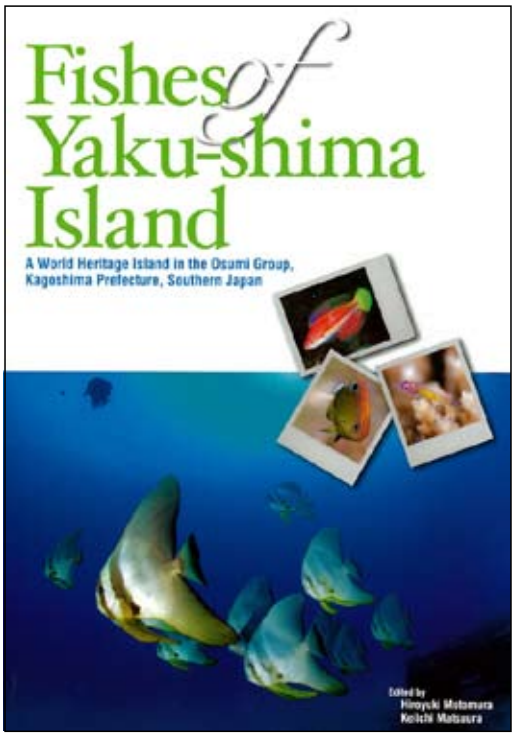
The specimens are stored in 20% ethanol solution and are unrestricted under SPECIAL PROVISION A58 of the IATA Dangerous Goods Regulations.

If this shipment is inspected, it is absolutely essential that the specimens be rewrapped in moist muslin or cheesecloth, and then sealed inside a plastic bag. Otherwise the specimens will dry rapidly and become useless.

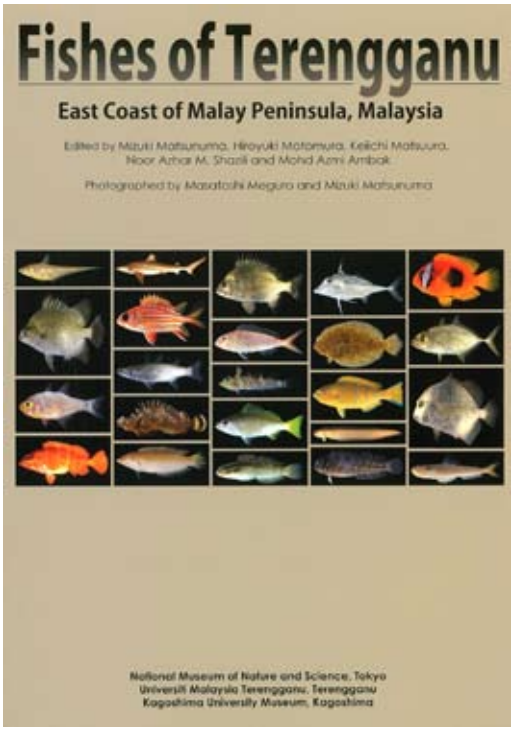
The Kagoshima University Museum
1-21-30 Korimoto,
Kagoshima 890-0065,
Japan
Ph: +81 99 285 8111
Fax: +81 99 285 7267

Important memorandum to be enclosed with the specimen in the cardboard box.

Fish photographs, taken with methods described in this manual, were published in the following books. These books can be downloaded as PDFs from: http://www.museum.kagoshima-u.ac.jp/staff/motomura/dl_en.html



Motomura, H. and K. Matsuura (eds.). 2010 (Mar.). Fishes of Yaku-shima Island – A World Heritage Island in the Osumi Group, Kagoshima Prefecture, Southern Japan. National Museum of Nature and Science, Tokyo. viii + 264 pp., 704 figs.



Matsunuma, M., H. Motomura, K. Matsuura, N. A. M. Shazili and M. A. Ambak (eds.). 2011 (Nov.). Fishes of Terengganu – East Coast of Malay Peninsula, Malaysia. National Museum of Nature and Science, Tokyo, Universiti Malaysia Terengganu, Terengganu, and Kagoshima University Museum, Kagoshima. viii + 251 pp., 678 figs.

Front page left images: Fishes of Kagoshima Bay, Japan
Front page central image: Devil Scorpionfish, *Scorpaenopsis diabolus*, from Tanegashima, Kagoshima, Japan
Back page right images: Fishes of Yakushima, Kagoshima, Japan


RIHN


Systematic Laboratory of Fish
Kagoshima University Museum


THE
KAGOSHIMA
UNIVERSITY
MUSEUM