I

Observation of external morphology

I 1 General guidelines for sketching fish

Unlike landscape painting and the preparatory drawing we learned in high school and earlier, sketches in ichthyology experiments are not ones for which artistic quality is demanded. They are rather understood as being similar to architectural and machinery designs in engineering, and "what you see" rather than "good-looking" works are demanded. In other words, sketching is a method to accurately depict the morphological characteristics of subject fish in detail. Demonstration of the ratio and/or the position of body parts will require observations from the front and rear, right and left, and top and bottom. Basically, sketching uses only sharp lines and stippling, but not techniques of "gradation", "shading", and the like. In other words, structures are represented by solid lines, whereas three-dimensional parts (unevenness) and characteristic markings are indicated by stippling.

1. Materials required

When sketching, ensure that you have a B-2B pencil with a soft lead for outlining the whole shape in the first rough sketch, and an H-3H pencil with a hard lead that can draw sharp lines in the finishing sketch. In addition, arrange for an eraser and a knife or a pencil sharpener to keep the pencil leads constantly sharp. Be sure to have a vernier caliper for the measurement of body parts. An approximately 30-cm ruler is also convenient to have for the measurement of larger material. It is also convenient if you have a simple calculator for scaling.

As for the paper you use for sketching, prepare at least 1 sheet of A4-size Kent paper with a smooth surface per fish species. It is preferable to prepare 2 sheets or more at all times because this quantity may be necessary depending on the contents of the experiment.

2. Before sketching

Depending on the fish species and the specimen condition, the fin rays may be folded inside a groove, and the opercle, preopercle, etc., may be stuck tightly in the opercular part, making the opercles unclear and undistinguishable at a glance in some cases. Therefore, confirm the borders between parts of the material given to you by not only looking at it but also by opening and closing the parts by touching them with your hand. It will help your sketching later if you determine whether the fin rays are spines or soft rays on this occasion. Ideally, specimens to be drawn will have a regular body form, be tagged with a specimen number, and be sorted by individual or species. However, if many specimens are handled, like in a student experiment, it is not always possible to use an ideal specimen. It is not uncommon that the specimen itself is deformed, such as being bent. It is difficult in most cases to correct the body form if specimens are preserved in formalin or alcohol. In the case of raw specimens, fix the form by closing the mouth, closing the opercular widening, and by other means. For fins, etc., take measures for extending them, including the use of specimen needles such as setting pins.

Usually in fish sketching, the left side is drawn by placing the fish so that it faces left. However, if the left side is heavily damaged, replace the specimen, or if no extra specimen is available, you may draw the right side. For extremely depressed species, such as rays, where it is difficult to show the overall shape of the fish if drawn laterally, sketch them from the top.

3. For accurate drawing

Regarding the sketch size of the fish body, draw the fish so that it fills approximately 70−90% of the width of the Kent paper in the horizontal direction. Be careful not to draw the fish body small just because the specimen is small. For drawing accurate lines and dots as you intend, pay attention to the following points as basic actions. Briefly, always keep your elbow to wrist touching the desk and the Kent paper, and move your hand as if you are lightly rubbing the desk and paper with the whole arm using your elbow as the basal point in drawing. If your wrist is above the Kent paper surface, you cannot draw accurate lines and dots as you intend. In addition, in the case of stippling in particular, maintain the condition in which the part from your little finger to wrist touches the Kent paper at all times, and move your thumb, index finger, and middle finger positioning the pencil up and down like you are putting a cap on it. It is common that you hear a loud "tap" sound while sketching. This is a sign that your hand is above the Kent paper (desk), with which you cannot draw a true dot (round), and a short drifted line will be made. Because a "line" is originally consecutive dots, it is not so difficult to draw lines by stippling if you follow the basic actions mentioned above.

4. Sketching procedure

1) Set the scale ratio of the fish to sketch so that it will occupy 70−90% of the whole Kent paper and is roughly at the center. Next, using projection (see the column), locate and measure the main proportions, such as the fin size, in the body parts including the head. Mark lines and points on the paper based on the sizes multiplied by the set scale ratio, and balance them on the whole (Figure 1A).
2) Draw a thin line along the marks to set the whole outline. In this case, the line does not need to be continuous but may be chopped if it is easier to set the shape (Figure 1B).
3) Once the outlines are set, draw the rays in the fins. In this case, draw them not only by discriminating spines and soft rays but also accurately in the number. In addition, for species with spines on the head such as scorpionfish and squirrelfish, you need to accurately draw these as well. Because these spines and soft rays are used as criteria for species identification in many cases, pay particular attention to their number and position (Figure 1C).

   As 1)−3) above are only the steps of rough sketching, use a B-2B pencil with a soft lead so that the drawing can be easily rubbed out with an eraser.
4) Using an H-3H pencil with a hard lead, draw each outline with a clear solid line along the line(s) drawn in 3). Next, lightly tap the lines with an eraser to rub out the lines in the rough sketch part (Figure 1D).
5) Next, represent the parts of concentrated pigments including unevenness and characteristic mottles and spots by stippling. Adjust the pigment intensity by dot density, and never use any solid lines (Figure 1E).
6) Lastly, enter the names of the parts. At this point, consider the position of the lines indicating the parts so that they do not cross each other if possible (Figure 1F).

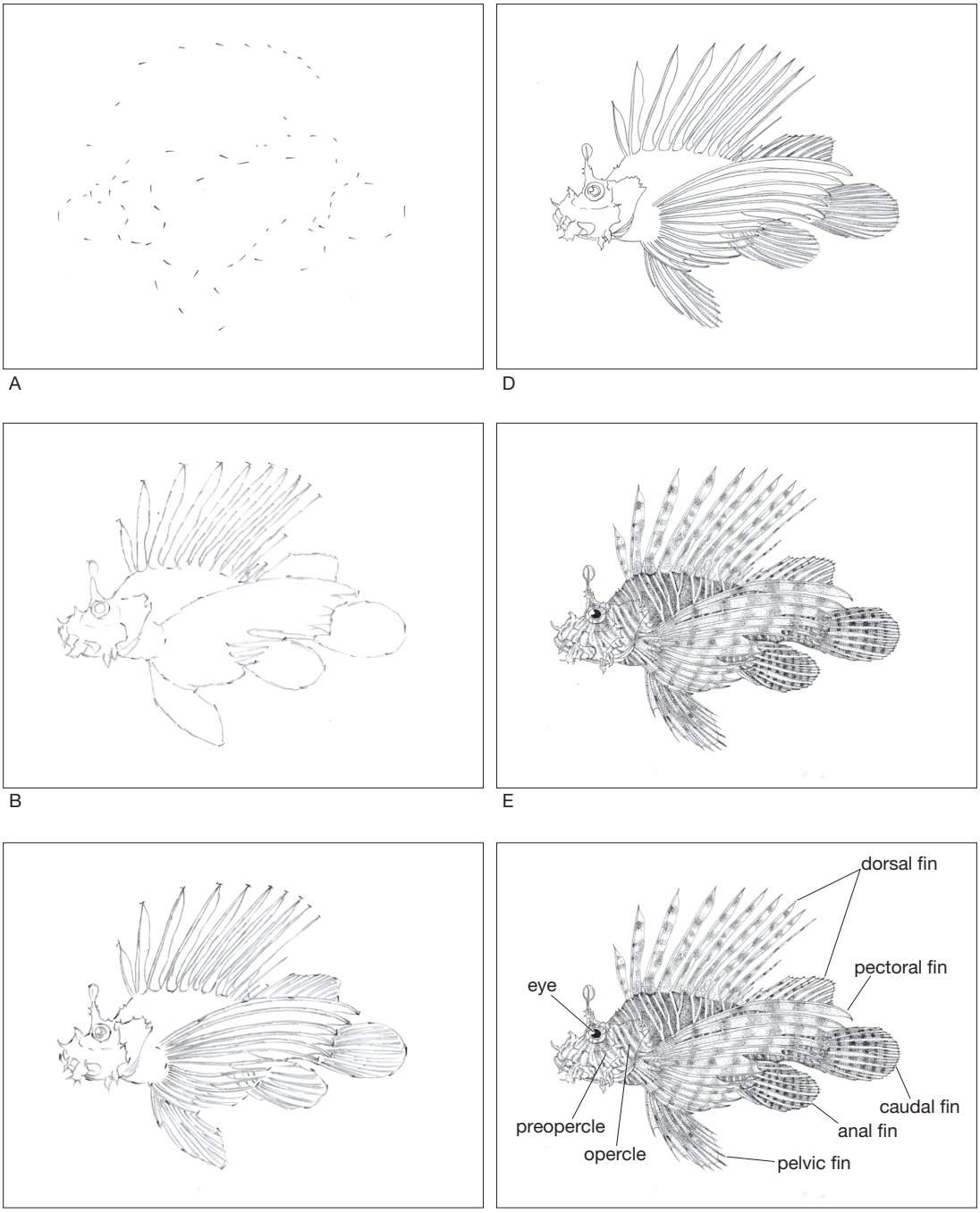


Fig. 1 Example of sketch of general fish.

I 2 External morphology of sharks (Chondrichthyes)

The skeleton of Chondrichthyes is formed of cartilage. Although the degree of calcification of the cartilages varies considerably depending on the species, the cartilaginous skeleton sufficiently protects the brain and supports the body and fins. The body (1) shows various morphologies based on this skeleton. Generally, sharks and rays are distinguished by having gill slits (external gill slits) (13) on the lateral sides and the ventral side, respectively. The body form of many sharks is fusiform or fusiform and elongated, but a few species are compressiform. The body (1) is covered by placoid scales and consists of the head (2), trunk (3), tail (4), and fins (15−23). The trunk accommodates the major digestive organs and urinary and reproductive organs. In sharks, mature females have higher ratios of trunk length to total length than males in some cases because they form large eggs and/or

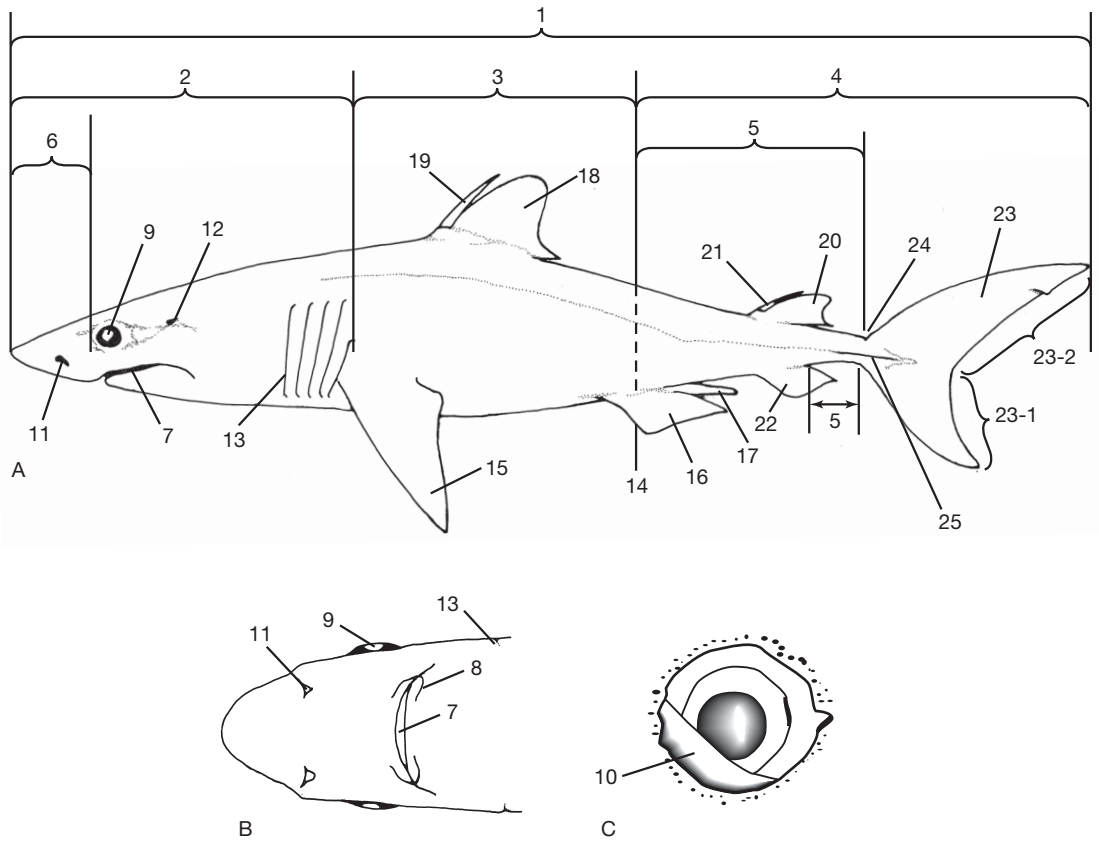


Fig. 1 Sketch of a fictional fish, "Spiny Porbeagle" (male). A, lateral view; B, ventral view of head; C, eye.

1. Body
2. Head
3. Trunk
4. Tail
5. Precaudal tail
6. Snout
7. Mouth
8. Labial-furrow
9. Eye
10. Nictitating eyelid
11. Nostril
12. Spiracle
13. Gill slits
14. Cloaca
15. Pectoral fin
16. Pelvic fin
17. Clasper
18. First dorsal fin
19. First dorsal fin spine
20. Second dorsal fin
21. Second dorsal fin spine
22. Anal fin
23. Caudal fin
    23-1 Lower lobe
    23-2 Upper lobe
24. Precaudal pit
25. Caudal keel

become pregnant. Because the posterior terminus of the vertebral column curves dorsally entering the caudal fin (23) in Chondrichthyes, the caudal fins are included in the tail. This structure of the caudal fins is called the heterocercal type. In sharks belonging to the genus *Lamna* that swim at a high speed, the lower lobe of the caudal fins (23-1) is developed, whereas in bottom-dwelling sharks, the lower lobe is not extended because they swim around immediately above the seafloor. In sharks belonging to *Alopias*, the upper lobe (23-2) is especially developed, accounting for approximately half of the total length. In *Lamna* sharks, keels (25) also seen in tunas are developed from the precaudal tail (5) to the caudal fin to stabilize the tail action without resistance during high-speed swimming. Some species possess precaudal pits (24) at the base of the caudal fin. The pectoral fins (15) are located posterior to the head and form a clear border from the lateral side. Some pelagic shark species are seen to submerge to nearly 500 m depth in a gliding condition in which they use their relatively large pectoral fins like the main wings of gliders and do not move their caudal fin. The dorsal fin (18, 20) is single in sharks of the order Hexanchiformes (Chlamydoselachiformes, Hexanchiformes) and double in other sharks. The tawny nurse shark *Nebrius ferrugineus* usually has two dorsal fins, but individuals with a single dorsal fin are uncommonly witnessed. There are also sharks with the two dorsal fins positioned posterior to the pelvic fins (16). Sharks belonging to Heterodontidae and Squaliformes (Dalatiiformes, Centrophoriformes, and Squaliformes), excluding a few genera, possess spines (19, 21) on the anterior margin of the dorsal fin. A cloaca (14) is located between the right and left pelvic fins. In males, the pterygiophores of pelvic fins are specialized and extended to be a copulatory organ (phallus) (17). The anal fin (22) is missing in sharks of the order Squaliformes (Echinorhiniformes, Dalatiiformes, Centrophoriformes, Squaliformes), Squatiniformes, and Pristiophoriformes. The gill slits (13) are located anterior to the pectoral fins, and at least a part of them are on the lateral side without exception. Sharks of the order Hexanchiformes (Chlamydoselachiformes, Hexanchiformes) and a species in Pristiophoridae have 6 or 7 pairs of gill slits, whereas other sharks possess 5 pairs. In sharks of the order Squatiniformes, the gill slits are hidden by the pectoral fins. The eyes (9) are on the lateral sides of the head. Some species have nictitating membranes (10) protecting the eyes. In many sharks, there is a spiracle (12) immediately behind the eye. There is a pair of nostrils (11) anterior to the mouth (7). The mouth of many sharks has labial furrows (8) on the edges. On the snout (6), ampullae of Lorenzini (Figure 2) are scattered as electroreceptors.

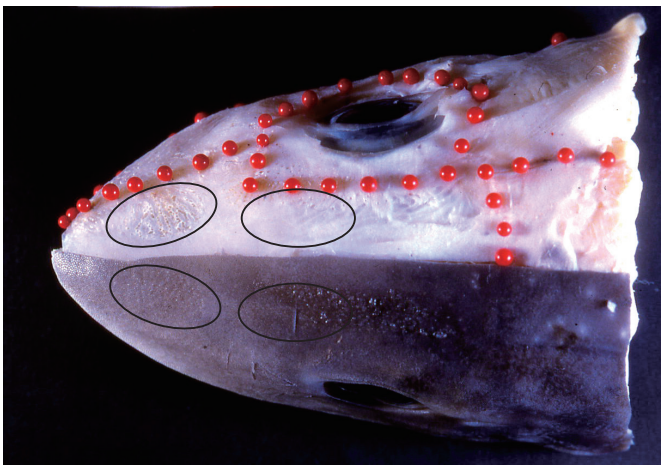


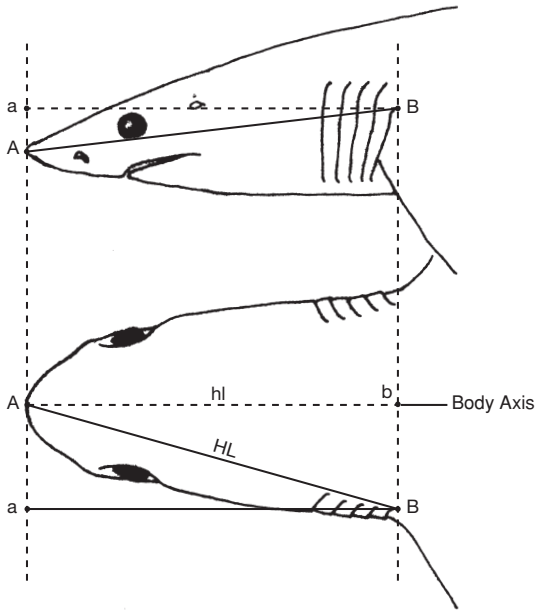
Fig. 2 Dorsal view of head of a shark, Starspotted Smooth-hound *Mustelus manazo*.

* The classification to the rank of order is according to Compagno et al. 2005 (Sharks of the World, Princeton Univ. Press). However, the order names given within parentheses are according to *Fishes of Japan with Pictorial Keys to the Species*, Second Edition (edited by Tetsuji Nakabo, Tokai University Press, 2000).

• Column: Two different measurement methods

(Hirokazu Kishimoto)

The measurement of parts of fish bodies is an important and basic requirement in morphological examination. Nevertheless, a difference exists between Chondrichthyes researchers who habitually use projection and Teleostei researchers who measure the shortest distance between two points. Either method gives the same value or nearly the same value in measurements along the body axis such as of the total length. However, the difference in measurement by the two methods is significant in the case of the head length and the snout length of depressed fish, and the predorsal length of compressed and deep fish. An example of the measurement of the head length of a shark is as follows: In projection, because the length is measured between the snout tip A and b which is the distance between A and B, the posterior terminus of the last gill slit, projected on the body axis, a ruler is practically placed at A vertically to the body axis to measure the distance from A to b (hl). On the other hand, in the measurement of the shortest distance between two points, the tips of a vernier caliper are placed directly on A and B to measure the distance obliquely to the body axis (HL). It is clear that hl is shorter than HL. Therefore, we must be fully aware that the head length may become consistent with the value of another species if a wrong method is chosen. Both methods have some advantages and disadvantages. Projection is an essential method for sketching, in which the ratios between the body parts are reflected as you see the material. However, it is likely to cause errors in measurement in the step of placing a vertical line to the body axis. On the other hand, distance measurement between two points causes little measurement error, but is not suitable for sketching due to the difference between the numerical and apparent ratios.



I 3 External morphology of rays (Chondrichthyes)

The body (1) of rays, Chondrichthyes, is depressed in all species. The head (3) and the pectoral fins (17) are fused and no border is present in most species. The anal fin is missing. In the case of rays, the head is from the snout tip to the posterior terminus of the last gill slit (external gill slit) (15), the trunk (4) is from the posterior terminus of the last gill slit to the center of the cloaca (16), and the tail (5) is from the center of the cloaca to the posterior terminus of the caudal fin (23), or the terminus of the whiptail if there is no caudal fin. The external morphology of rays is continuous and diverse—from

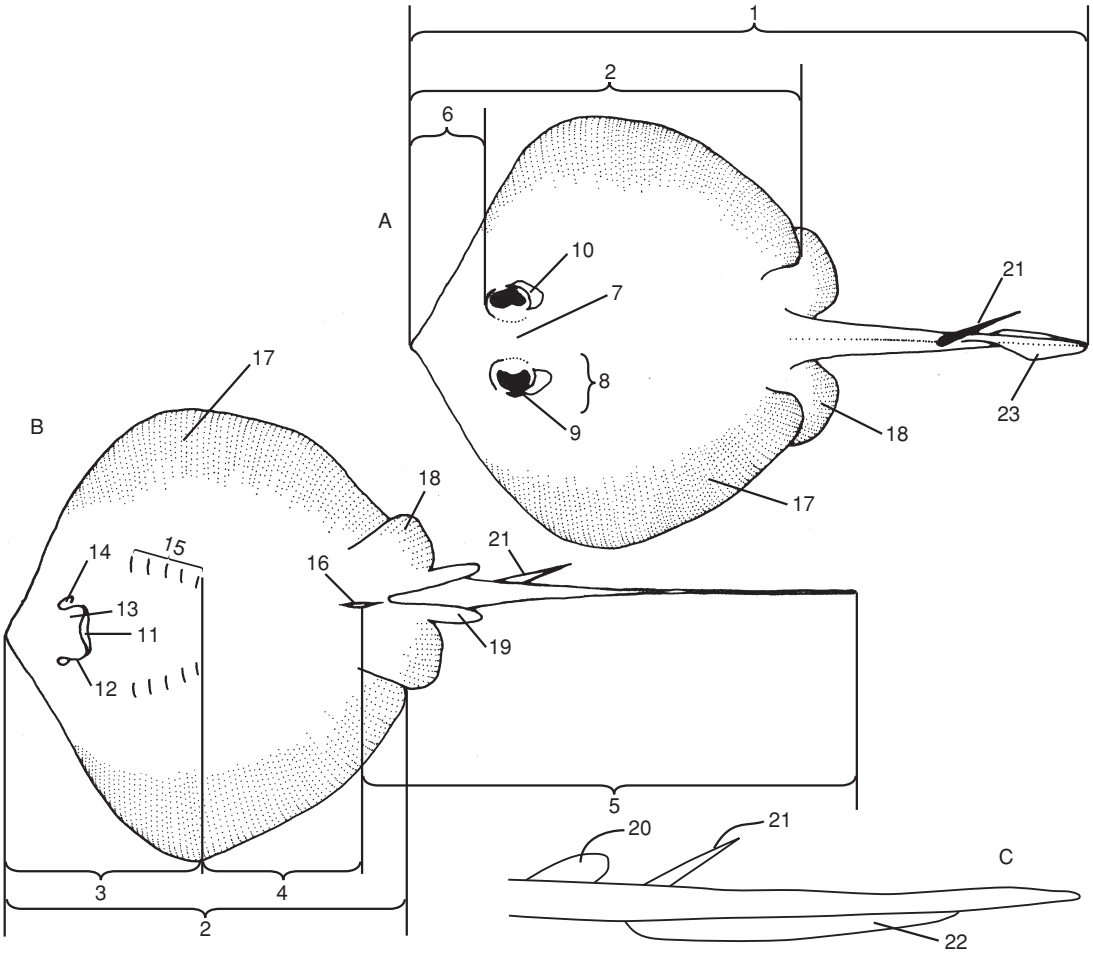


Fig. 1 A, dorsal view of female Sepia Stingray *Urolophus aurantiacus*; B, Ventral view of male Red Stingray *Dasyatis akajei*. C, Lateral view of whiptail of Red Stingray.

1. Body
2. Disc
3. Head
4. Trunk
5. Tail
6. Snout
7. Interorbital space
8. Shoulder
9. Eye
10. Spiracle
11. Mouth
12. Nasal groove
13. Nasal flap
14. Nostril
15. Gill slits
16. Cloaca
17. Pectoral fin
18. Pelvic fin
19. Clasper
20. Dorsal fin
21. Caudal spine
22. Ventral fold
23. Caudal fin

Pristoidei and Rhychobatoidei species that have a shark type body form, two dorsal fins (20), and a clear caudal fin, to the Dasyatidae and Myliobatidae species that have a whiptail instead of a caudal fin. In the species without a shark-type form, the part where the head and pectoral fins are fused is called the body disc (2). These species have thorns on the mid-dorsal line of the body surface, between the eyes (7), in the pectoral girdle part (8), etc., or a tail spine (21) on the dorsal surface of the tail in some cases. Among the species without a caudal fin, there are species that possess a cutaneous fold (21) on the mid-ventral line of the whiptail. There are 0−2 dorsal fins, and they are small in many species. The pelvic fins (18) are clearly distinguished from the pectoral fins in many species. In rays in the superfamily Rajoidea, the pelvic fins are divided into the anterior and posterior lobes. In males, the pterygiophores of the pelvic fins differentiate and extend to form a copulatory organ (phallus) (19). These species have 5 pairs of gill slits on the ventral surface of the head, with only the sixgill stingray *Hexatrygon bickelli* (6) being an exception. The eyes (9) are on the lateral surfaces of the head in rays in Myliobatidae, and on the dorsal surface of the head in other rays. The spiracles (10) are present immediately posterior to the eyes without exception and are well developed in many species. Except for the giant oceanic manta ray *Manta birostris*, in which the mouth (11) opens in the anterior margin of the head, the mouth is located on the ventral surface of the head. The nostrils (14) are immediately anterior to the mouth, and a nasal flap (13) covers the nostril groove (12). The ampullae of Lorenzini are developed in the snout (6). In rays in Rajidae, the ampullae develop all over the ventral surface of the head and a part of the ventral surface of the trunk in some cases.

Nelson (1994)

Phylum Chordata
- *Subphylum Calcichordata
- Subphylum Urochordata
- Subphylum Cephalochordata
- *Subphylum Conodontophorida
- Subphylum Vertebrata
  - Supercalss Agnatha
    - Class 1 Myxini
    - *Class 2 Pteraspidomorphi
    - Class 3 Cephalaspidomorph
  - Supercalss Gnathostomata
    - *Class 4 Placodermi
    - Class 5 Chondrichthyes
      - Subclass Holocephali
      - Subclass Elasmobranchii
    - *Class 6 Acanthodii
    - Class **8** Actinopterygii
      - Subclass Chondrostei
      - Subclass Neopterygii
        - Other Division
        - Division Teleostei
    - Class **7** Sarcopterygii
      - *Subclass position uncertain
      - Subclass Coelacanthimorpha
      - Subclass unnamed (Dipnoi and etc.)
      - *Subclass Rhizodontimorpha
      - *Subclass Osteolepimorpha
      - Subclass Tetrapoda

Osteichthyes

Pisces

reference: Nelson, J. S. (1994) Fishes of the World. 3rd edition* Academic Press. 600 p.
*4th edition .....

• Column: The reason why "Osteichthyes" disappeared (Hirokazu Kishimoto)

Thus far, in a class on ichthyology or related matters, it has been explained that the extant fish on the earth include Chondrichthyes and Osteichthyes, and in more detail, a group called Cyclostomes lacking a bone in the jaw is also dealt with in ichthyology. However, as used in this book, a name, Teleostei, similar to but distinct from Osteichthyes, has recently become remarkable. This is solely because many ichthyologists follow the taxonomy in *Fishes of the World*, Version 3 by Nelson (1994) that aggressively adopts recent study results. In his system, most of the commonly seen fish, including Japanese eel *Anguilla japonica*, common carp *Cyprinus carpio*, tunas, Japanese sea bass *Lateolabrax japonicus*, righteye flounders, and puffers, are grouped in Teleostei. If ancient fish such as gars and bowfin *Amia calva* are added to this, it is a group called Neopterygii. Moreover, if sturgeons and Polypteridae are added, it is called Actinopterygii. There is a group called Sarcopterygii, which is on the same level as Actinopterygii, and it includes those in the shape of a fish but with lobed pectoral and pelvic fins, such as coelacanths and lungfishes, as well as Tetrapoda including up to humans and monkeys. In other words, coelacanths and lungfishes are dealt with as a group more closely related to Tetrapoda than common fish. In such a case, it is impossible to group them in a taxon "Osteichthyes", as previously done by excluding only Tetrapoda from Sarcopterygii. For the same reason, it is impossible to broadly classify Vertebrata into fish excluding coelacanths and Tetrapoda including coelacanths; therefore, the taxonomic unit "fish" cannot be used in a strict sense.

In this book also, in compliance with the way of thinking by Nelson, the use of Teleostei for those other than Chondrichthyes serves its purpose in most cases. Use of the taxonomic name Actinopterygii, including sturgeons, would only make it difficult to gain understanding. Moreover, mentioning those by including coelacanths and lungfishes as a wider taxon would make it impossible to find a name for the taxon.

On the other hand, fish researchers generally drive coelacanths and lungfishes away into a group of Tetrapoda by thinking that they are animals far from fish. However, does this ever convince Tetrapoda researchers? I cannot help but feel that Coelacanths and lungfishes are in the shape of a fish rather than Tetrapoda. Thus far, in this book, I have ignored taxonomic ranks due to their complexity and used fuzzy "groups" instead. I also have a question regarding how I should deal with the groups. In the taxonomy proposed by Nelson, Tetrapoda as a whole is dealt with as a subclass in Sarcopterygii. In line with this, when looking at how the breakdown is provided into subordinate ranks, the conclusion is postponed. In all likelihood, if it is the detailed classification inside a subclass, they are supposed to be an amphibian superorder, reptilian superorder (including birds), and mammalian superorder. The question, "Is it acceptable to make them such a low taxon?" rises in me, accustomed as I am to the previous taxonomy. There is something I find difficult to believe when I think of the balance between fish and the larger taxonomic picture.

I 4 External morphology of Teleostei

For the identification of diverse species of fish, it is necessary to accurately understand their complicated morphology. Detailed expression of behaviors of fish through observation of their ecology requires an accurate understanding of their body forms and correct expression of the orientations. Therefore, for structural observations, it is suitable if the material is a fresh specimen so that the joints can be moved. However, fish can not keep their freshness during long-time observation, causing a putrid odor, and their surface dries. For advanced studies wherein you may need to observe valuable specimens that require permanent preservation, you should prepare the specimens to set to hold a normal body form for materials according to the method in Motomura and Ishikawa (2013, http://www.museum.kagoshima-u.ac.jp/staff/motomura/CollectionManual_lowres.pdf). If the specimen is preserved in formalin, water-rinsing starting from the day before the observation can reduce the irritating odor. However, rinsing for any longer is harmful for specimens because the decalcification of the skeleton occurs in freshwater. In the case of specimens preserved in ethyl alcohol, you should observe them as soon as you have taken them out while paying close attention to their rapid drying, because it is not adequate to soak them in water. You do not have to be worried about the odor of ethyl alcohol, even though it is intense, because it is not harmful. On the other hand, you need to handle formalin with caution.

A fish body is usually composed of the following four parts.

Head: From the snout tip to the posterior margin of the operculum (A−B in the figure)

Trunk: From the posterior margin of the operculum to the anus (B−C in the figure)

Tail: From the anus to the base of the caudal fin (tail includes the caudal fin in some cases) (C−D in the figure)

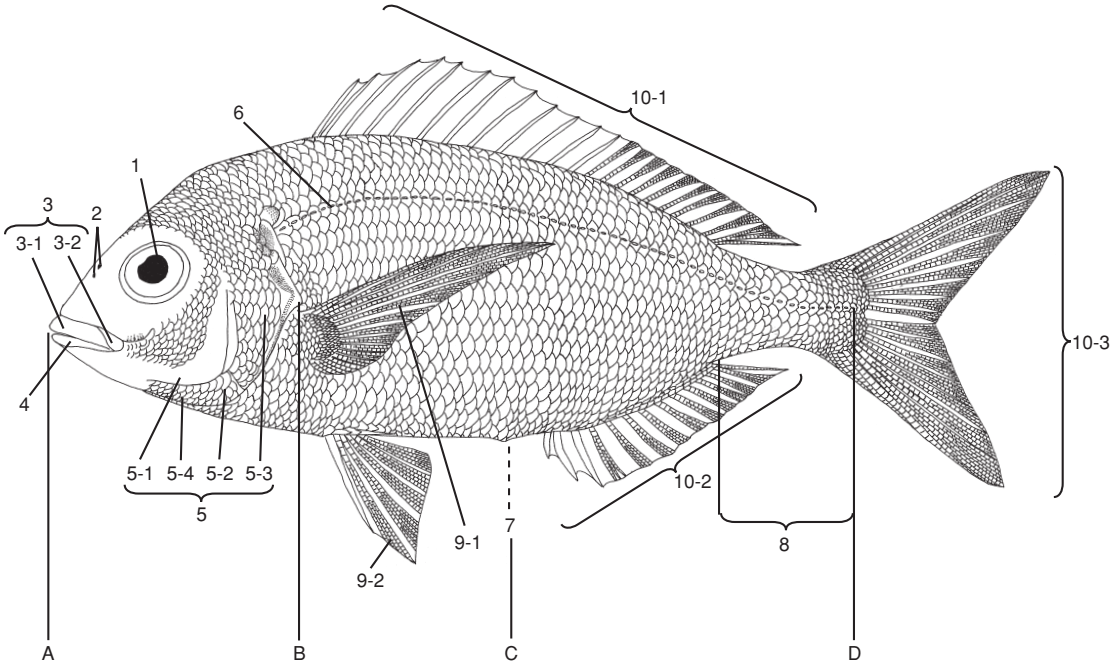


Fig. 1 Red Pandora *Pagellus belottii*, from Africa.

Fins: All fins (9 and 10 in the figure)

The parts are further composed of a variety of respective organs. The name of the body parts is as follows in an example of a fish in Sparidae, Teleostei.

1. **Eye**: Although the eyes cannot be closed due to the lack of the eyelids, they are not significantly different from human eyes. Fish in Mugilidae and Carangidae have a cover called the adipose eyelid that covers the eye from the anterior and posterior sides, but it is difficult to identify due to its transparency in most cases.
2. **Nostril**: In Teleostei, it is common that there is a pair of anterior nostrils and a posterior nostril on each side. However, there are also species with only one nostril, such as Pomacentridae and sticklebacks.
3. **Upper jaw**: This is formed by three types of bones, premaxillary (3-1), maxillary (3-2), and supramaxillary, which exist in varying numbers, from many to none, depending on the fish species. In primitive fish, such as Clupeidae, the premaxillary is small and the oral margin of the upper jaw is occupied mainly by the maxillary. In many advanced fish, including Perciformes and Scorpaeniformes, the premaxillary is large, occupying all margins of the upper jaw, whereas the maxillary is recessive.
4. **Lower jaw**: The dentary is the only bone involved in the oral margin of the lower jaw. The dentary and teeth on the premaxillary have variable morphologies related to the feeding habit.
5. **Operculum (gill cover)**: This is composed of four types of bones, the preopercle (5-1), the opercle (5-3), the subopercle (5-2), and the interopercle (5-4). The size and/or number of spines around the preopercle, and the presence or absence of any spine on the posterior margin of the opercle among these bones are frequently used for species identification because they can be observed from outside (for details, see III-6). The branchiostegals hidden by the posterior inferior margin of the operculum, which support the branchiostegal membrane, look like the frame of an umbrella. The number and shape are characteristic of each species. In a functional aspect, it significantly contributes to gill ventilation by cooperating with the operculum.
6. **Lateral line**: This is a characteristic sensory organ most fish have, whereas only a few other vertebrate animals, such as amphibian larvae, have this organ. Normally, it is located a little superior to the mid-lateral line and looks like a dotted line. This is because the scales, each of which has a pore (pored lateral line scale, see Figure 4 in I-5), form a line. The double-structured tunnel from the opening on the exposed external surface of the scale connects to the branch of the subcutaneous lateral-line organ running longitudinally along the body at the opening of the internal surface of the covered part. In some fish, the body surface has no scales and the lateral scales are covered by the skin, exposing only their pores, which form a line on the surface. Some other fish lack a lateral line. In addition, lateral lines are remarkably variable. Some fish have several lateral lines on one side, a winding lateral line, or lateral lines running like a net.
7. **Anus**: This is a terminal opening of the digestive tract and is located in conjunction with the

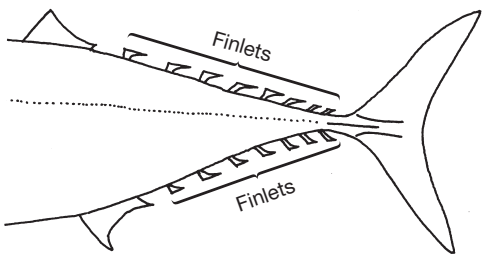


Fig. 2 Finlets of scombrid fish.

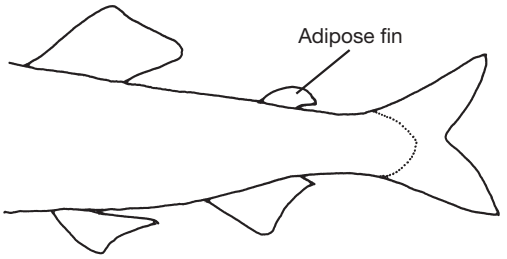


Fig. 3 Adipose fin of Ayu *Plecoglossus altrivelis altivelis*.

urogenital pore immediately anterior to the anal fin. However, there are exceptions, including firefly-fish *Acropoma japonicum*, in which the anus is located in the vicinity of the pelvic fins.

8. **Caudal peduncle**: This is a part from the posterior terminus of the anal fin base to the caudal fin base or the terminus of the vertebral column.
9. **Paired fins**: These are left-to-right pairs of fins of two types, pectoral fins (9-1) and pelvic fins (9-2). The former is composed of only soft rays. However, pectoral fins of fish in Siluriformes have the spiny soft ray in the superior margin. The latter is composed of 6 or more soft rays in the primitive fish group, whereas in the advanced fish group, many fish have 5 soft rays or fewer and a spine at the most lateral side (anterior margin) (only fish in Siganidae have a spine each in the medial and lateral (anterior and posterior) margins) (see Figure 4 in II-2). These are considered the organs homologous to the anterior limb and the posterior limb of Tetrapoda, respectively.
10. **Vertical fins** = unpaired fins = median fins: These are the fins that are located at the median of the body's outline, do not form a left-to-right pair, and open and close vertically.
    - **10-1. Dorsal fin**: This is equipped with rays with the pterygiophores, and is arranged on the mid-dorsal line from the trunk to the caudal peduncle. If it is divided into two or three parts longitudinally by a clear indentation(s), they are distinguished as the first dorsal fin, second d. f., and third d. f. in order posteriorly, and represented by the abbreviations D1, D2, and D3, respectively. This is composed of only soft rays in the primitive fish group, whereas the advanced fish group is equipped with spines in the anterior part (all of the first dorsal fin) in many cases. Moreover, when this is followed by individually separated soft rays, like in Scombridae and Carangidae, they are called finlets (Figure 2). In addition, when a small fin without rays lies posterior to the dorsal fin, as seen in Salmonidae, it is called an adipose fin (Figure 3).
    - **10-2. Anal fin**: This is the same as the dorsal fin except that it is arranged on the mid-ventral line in the tail.
    - **10-3. Caudal fin**: This is a fin located at the posterior terminus of the body, and composed of only soft rays. It is common that the part located in the upper and lower halves of the median of the body is called the upper and lower lobes of the caudal fin, respectively. Because this is

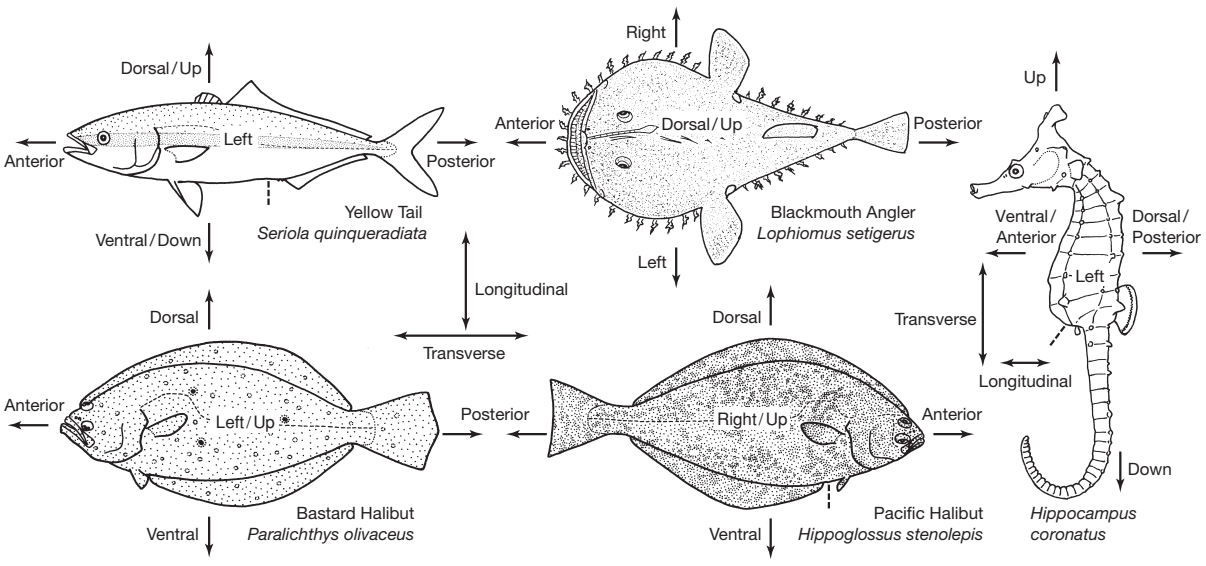


Fig. 4 Direction of fishes. (Illustrations from Nakabo (2000)

the source of thrust in swimming, the shape varies depending on the swimming ecology.

The expression of orientation for the fish body is based on the following guidelines (Figure 4).

Dorsal side: The half of the body to the vertebral column, in which the spinal cord is arranged.

Ventral side: The half of the body to the vertebral column, in which the digestive tract is arranged

Left/right: In most fish, the surface appearing on the top is left and the surface coming in contact with the chopping board is right when the fish is placed on the board so that the ventral side faces your side (down) and the head is on the left.

You will understand and better relate to the above-described orientations if you imagine yourself swimming with your face pointing down.

Anterior/posterior: The direction where the head lies is anterior (front), whereas the opposite direction, where the tail lies, is posterior (rear). Exceptionally, in the sea horse, the ventral side is anterior and the dorsal side is posterior.

Longitudinal/transverse: The orientation from the head to the tail is longitudinal whereas the orientation from the dorsal side to the ventral side is transverse. Therefore, in most fish, the horizontal orientation is longitudinal whereas the vertical orientation is transverse. Beware that these are the reverse for sea horses and humans.

Top/bottom, up/down: The orientation toward the core of the earth is down whereas the orientation toward the sky is up. Therefore, generally, the dorsal side is the top and the ventral side is the bottom.

In the same position, if the fish is flattened from both the right and left sides, like a porgy (Figure 5), it is called compressiform, whereas if the body form is flattened from the top (dorsal side), such as monkfish and rays, it is called depressiform. However, the fish in Pleuronectiformes are not depressed, but extremely compressed, because they are rotated sideways with their colored side with eyes (left side in bastard halibut *Paralychthis olivaceus* and right side in halibut, = eyed side) up and their opposite side, white and without eyes (right side in bastard halibut and left side in halibut, = eyeless side), down.

The midlines of a fish body running from the head to the tail (longitudinally) come in 5 types below. Although these are substituted by median with an adjunctive in some cases, it is preferable to distinguish these as follows.

A: axis or axial line: the midline of a fish body (axial part)

L : mid-lateral line: the midline of the lateral surface (right and left)

D: mid-dorsal line: the midline in the dorsal side

V: mid-ventral line: the midline in the ventral side

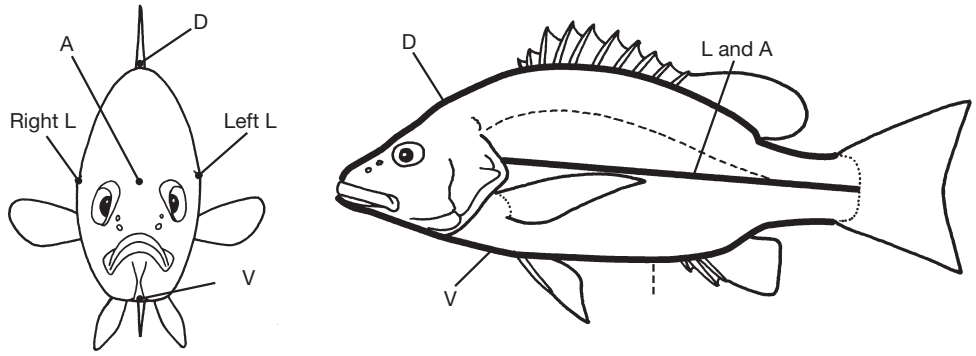


Fig. 5 Medial lines of fishes. (Illustrations from Nakabo (2000)

I 5 Scales and lateral line canals

1. Scales of Teleostei

Except for contemporary Agnatha (Cyclostomata), many fish are equipped with scales on their skin. Scales are derived from the dermis. Because scale morphology is variable among species and nearly constant within species, it is a taxonomic character. Scale patterns appearing on the scale surface allows us to estimate the age, stock, etc. Cosmoid scales, which are covered by thick layers of hard dentine, are seen in many fossil species and coelacanths, etc., and are regarded as primitive scales. Hence, fish scales tend to become thinner as fish evolve. The scales of contemporary sturgeons, etc. are called ganoid scales. The scales characteristic to Chondrichthyes are called placoid scales and are covered by a relatively hard enamel layer. They are also called dermal denticles in some cases because their structure is similar to that of teeth. In sharks, the body surface is densely covered by placoid scales forming a so-called sharkskin. On the other hand, in rays, the placoid scales are as degenerative as being scattered on the surface, making the skin relatively smooth. The scales of Teleostei are called bony scales, and they are arranged as if the body surface is tiled. Scales of the Japanese sardine *Sardinops melanostictus* (Figure 1C), chum salmon *Oncorhynchus keta*, cherry salmon *O. masou* (Figure 1A), common carp *Cyprinus carpio*, Carassius, dotted gizzard shad *Konosirus punctatus* (Figure 4B), etc. are cycloid scales, which are smooth when exposed on the body surface (Figure 1A). Scales of red seabream *Pagrus major* (Figure 1D), Japanese sea bass *Lateolabrax japonicus*, sabre squirrelfish *Sargocentron spiniferum* (Figure 4A), etc. are ctenoid scales equipped with small spines

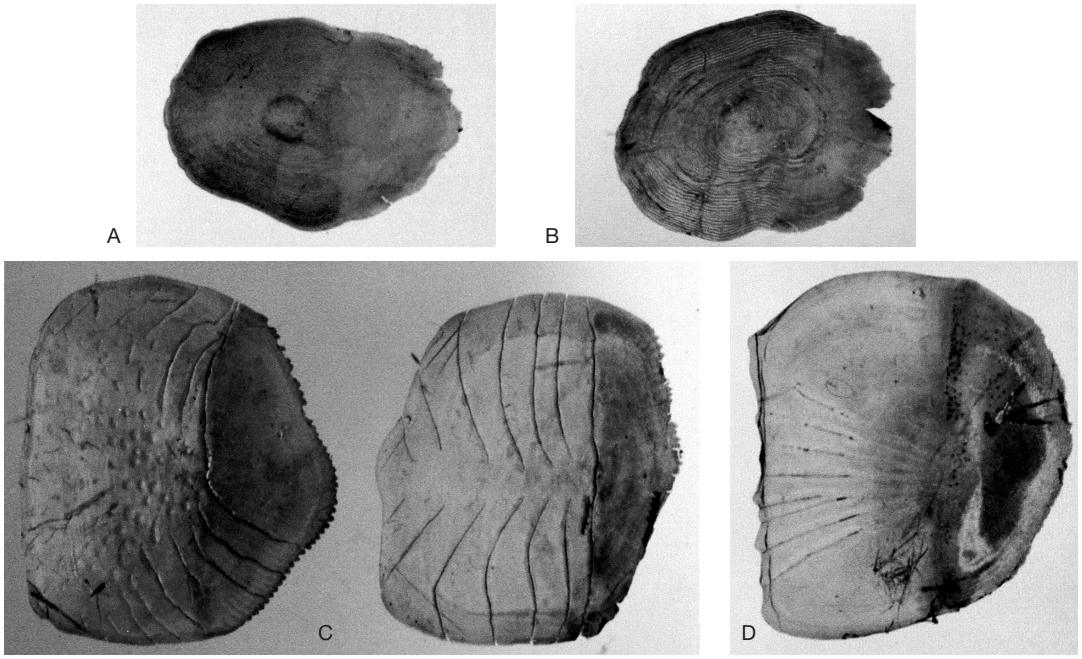


Fig. 1 Scales of teleosts.
A, Cycloid scale of Masu Salmon *Oncorhynchus masou masou*; B, Regenerated cycloid scale of Masu Salmon *Oncorhynchus masou masou*; C, Cycloid scale of Japanese Pilchard *Saldinops melanosticus*. Left, normal scale. Right, regenerated scale; D, Ctenoid scale of Red Sea Bream *Pagurus major*.

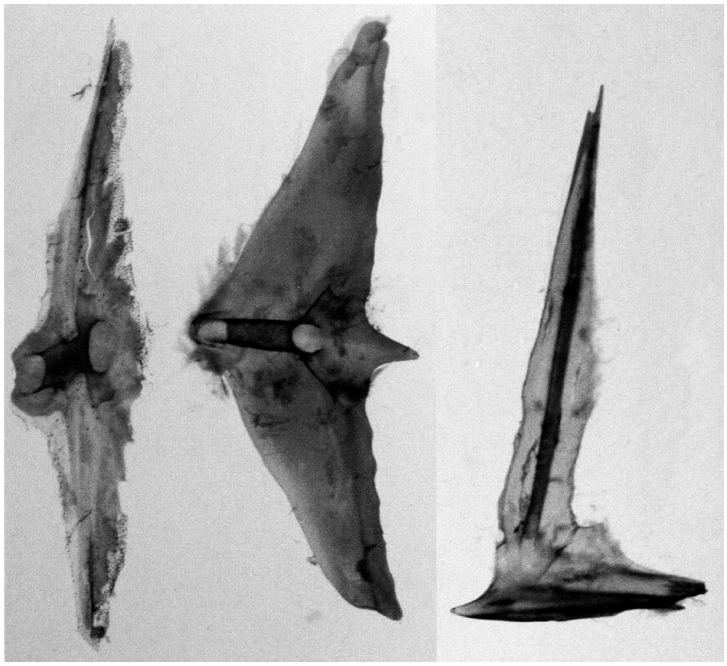


Fig. 2 Scutes.
A, Japanese Jack Mackerel *Trachurus japonicas* (Left, from trunk. Right, from caudal peduncle);
B, Dotted Gizzard Shad *Konosirus punctatus* (From ventral).

in the part exposed on the body surface. In addition, there are plate-like scales with bony spines or keels on the lateral line of jack mackerel and the ventral body margin of sardine, which are called scutes by comparison of them to mountain ridges (Figure 2). Figure 3 shows the name of scales of the spotbreast angelfish *Genicanthus melanospilos*. Figure 4 shows the name of the lateral side scales of sabre squirrelfish *Sargocentron spiniferum* and spotted gizzard shad *Konosirus punctatus*. On the bony scales, various scale patterns are seen in the layer of bony plate containing calcium. A scale pattern is formed by the array of the grooves arranged radially from the focus or longitudinally and by the ridges arranged like rings or transversally. As the fish grows, an interval is formed between the ridges. The interval forms a wide growth zone when growth is rapid, whereas it forms a narrow resting zone when growth is slower. Because the resting zone is formed with a cycle of approximately one year, it is used as a guide for determining the age by estimating that this is an annual ring (Figures 4 and 5). Generally, the formation of the annual ring is due to the yearly appearance of the difference in growth speed on the scale. However, it should be noted that because growth is influenced by changes in the environment such as water temperature and physiological changes such as those for spawning, the annual rings become unclear in artificially reared fish, and a pseudo annual ring (also called a spawning mark) is formed when a fish has spawned. In addition, scales come off when a physical force is applied to them from outside in some cases. A scale is regenerated in the place where the previous scale came off, and this is called the regenerated scale (Figure 1). Because no scale pattern in the process of growth before the drop off is formed on the regenerated scale, it is not eligible for use in age determination. In addition, lateral scales on the lateral line are also not eligible because it is difficult to see their center and ridges.

(Observation and preparation of specimens)

1. Collect several scales from Japanese sardine *Sardinops melanostictus* (T. & S.), cherry salmon *Oncorhynchus masou* (Brevoort), and red seabream *Pagrus major* (T. & S.). At this time, exclude

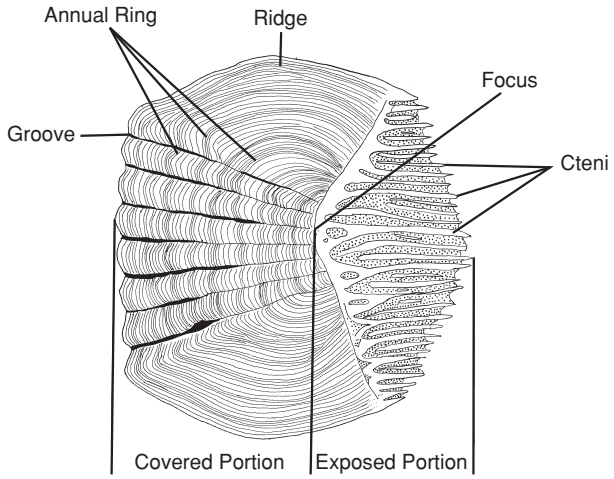


Fig. 3 Parts names of ctenoid scale of Spotbrest angelfish *Genicanthus melanospilos*. Sketch by Katsunori Nakamura.

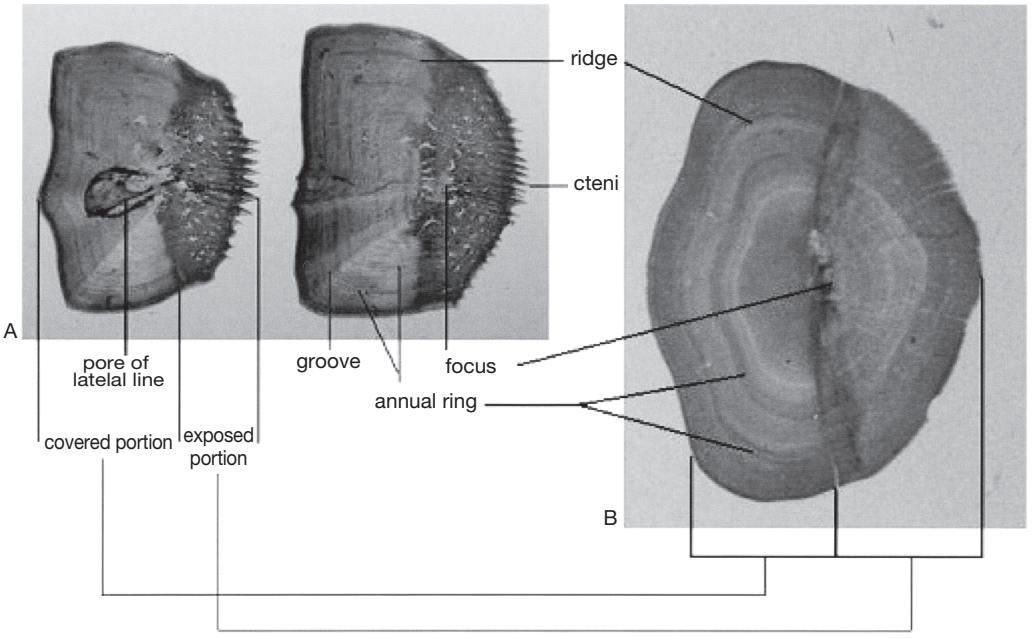


Fig. 4 Parts names of scales.
A, Sabre Squirrelfish *Sargocentron spiniferum* (Left, pored lateral line scale);
B, Dotted Gizzard Shad *Konosirus punctatus*.

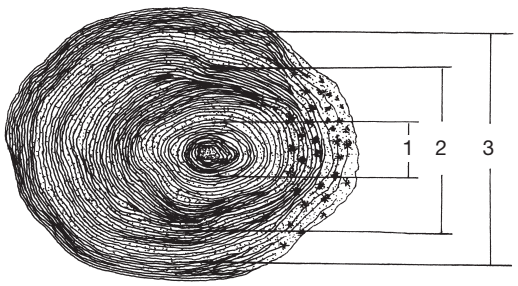


Fig. 5 Annual rings of Masu Salmon *Oncorhynchus masou masou*.

regenerated scales, lateral scales, and scutes. At the time of the collection, lightly pinch the scale using forceps, etc. However, beware that pulling it too hard may cause a crack on the cover of the scale or damage small spines. If you can pinch the scale to some extent with your fingers, you may remove the scale by pulling with the fingertips.

2. Wash the scales in tap water with gentle rubbing with your fingertips to remove the slime and stain.
3. Place the three types of scales separately on a glass slide in the same orientation. At this time, wipe away the moisture sufficiently.
4. Overlap the glass slide on which the scales are placed with another glass slide before the scales become dry and curled, and seal both ends of the glass slides with paper tags. At this time, be careful not to make the scales dry. Enter your student No. and full name on the tags.
5. Sketch the three types of scales on Kent paper while paying attention to the ridges, grooves, growth zones, resting zones, and scale periphery. As required, observe the scales by magnifying them using a stereomicroscope, etc. At that time, observe the detail of the scales using reflective light and transmission light properly. In addition, though beginner students frequently attempt to express resting zones by writing them more densely than growth zones, this is wrong. Correct sketching is to express it by writing the interval narrower because the interval of the scale pattern becomes narrower in the resting zones.
6. Enter the experimental theme, data of the material fish, etc. on the Kent paper.
7. Determine the age using the resting zones of the scales.
8. Referring to Figure 3, enter the name of the parts of the scales (write both Japanese and English names).

2. Lateral line canals in head

The lateral line is an organ present in both Chondrichthyes and Teleostei, and it functions not only as a receptor of mechanical stimuli, including water flow and pressure, but also as a chemical receptor for univalent ions. In many fish, the lateral line on the lateral side runs longitudinally under the skin of the lateral side, and connects to the body surface through a pore or a canal pore at nearly every segment. In fish species in which scales are developed, the pores of the lateral line are exposed on the scales in some cases, and these are called the pored lateral line scales. In many fish species, the lateral line on the lateral side is a canal that goes from the upper terminus of the operculum to the caudal peduncle on both sides of the body and runs nearly along median part of the lateral side longitudinally. However, it is wavy or runs along the ventral side of the body in some species. In addition, the number of lateral lines varies from 5, as in fat greenling *Hexagrammos otakii*, to 2, as in Japanese jack mackerel *Trachurus japonicus*, to none, as in Japanese sardine *Sardinops melanostictus*.

The lateral line canal organs in the head are partly or completely embedded under the dermal bone of the head and connect to the external environment through canals opening on the surface of the dermal bone. The lateral line canal organs in the head of Teleostei are broadly classified into three elements, the supraorbital canal (SOC), the infraorbital canal (IOC), and the operculomandibular canal. Moreover, the operculomandibular canals are further classified into the mandibular canal (MC) and the preopercular canal (PC). Because the auditory vesicle exists in the connection part from the infraorbital canal to the temporal canal, the lateral line canals in this connection part are further classified into the otic canal and the postotic canal in some cases. The major canals, the supraorbital canal, infraorbital canal, and operculomandibular canal, branch to the supratemporal canal and the trunk canal in the temporal region via the otic and postotic canals to link to the lateral line canal on the lateral side. Thus, there are seven left-to-right pairs of canal organs at the maximum on the head

of Teleostei. However, some of these elements are missing depending on the fish species or in the developmental stages of individuals even in the same species in some cases. Each sensory cell of these lateral lines is the terminus of the nerve, such as the nervus buccalis, nervus glossopharyngeus, nervus mandibularis, nervus opercularis, and nervus agus. Figure 7 shows the lateral line canals on the head of adult (over 40 mm total length) *Tanakia lanceolata*. In the experimental observation, observe the lateral line canals on the head of a bitterling, sketch them by paying attention to the number of pores of each lateral line canal organ opening on the surface, and enter each name.

• Column

(Nobuhiro Suzuki)

In addition to the fact that fish scales are diverse morphologically, in fish with a very slimy surface such as eels, degenerative small scales are embedded in the skin, and there are also fish species without scales. Scale morphology also differs depending on the body part. For example, in bastard halibut *Paralichthys olivaceus*, the colored surface of the side with eyes is covered by ctenoid scales, whereas the white surface on the side without eyes has cycloid scales. In cultured bastard halibut, it is often seen that the white surface on the eyeless side is black-pigmented. On the body surface of this part, the cycloid scales develop small spines to change into scales resembling ctenoid scales in some cases. Scales are not present on larvae immediately after hatching, but appear in the juvenile stage. Even in fish species equipped with ctenoid scales, the scales close to their appearance (scales in early development) are small and round, and as the individual grows they develop small spines on the exposed part (Figure 6).

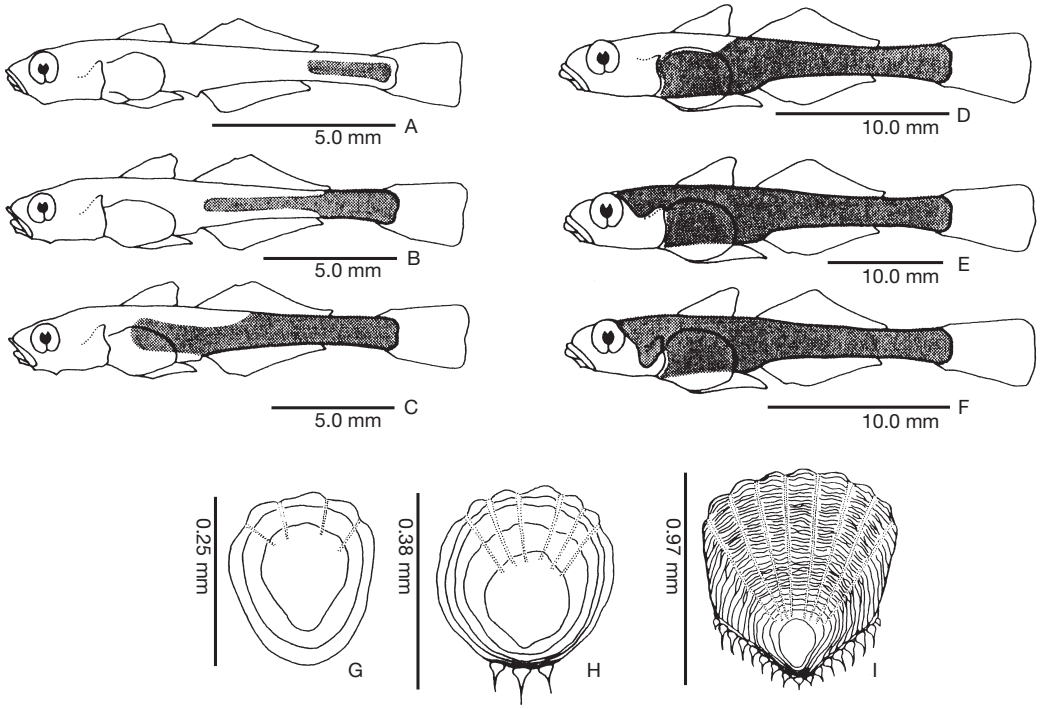


Fig. 6 Area expansion of distribution (A-F), and morphological change (G-I) of first scales of Yellowfin Goby *Acanthogobius flavimanus*. G, first scale; H, developping scale; I, developed adult scale.

(Observation and preparation of specimens)

1. Prepare a specimen sufficiently fixed by 10% formalin and preserved in 70% ethyl alcohol (hereinafter referred to as simply alcohol). Because skin pigments of the specimen cause trouble in observations, decolorize (blanch) the pigments first. To blanch, remove alcohol with running tap water and then immerse the specimen in 5% H2O2 to sufficiently decolorize the pigments.
2. Remove H2O2 from the specimen with running tap water and immerse it in 2% KOH to make it transparent until you can see through the muscle.
3. Remove KOH with running tap water and immerse the specimen in 70% alcohol solution.
4. For observation of the lateral line canals on the head, drop a staining solution prepared by dissolving Suminol Cyanine 5% Extra to saturation in 70% alcohol in a pore of the lateral line using something pointed, such as a toothpick, to stain the inside of the lateral line canal (wiping away alcohol remaining inside the lateral line canal with a gauze beforehand helps the staining solution to go into the canal). Observe this under a stereomicroscope. The use of incident light helps the observation. Because the lateral line canals on the head are formed along with the growth of the individual, an adult fish (an individual with a total length over 40 mm or developing a secondary sexual character such as tubercles and an ovipositor) needs to be used for observation of completed lateral line canals on the head of bitterling. (Because the stain is decolorized once the specimen is placed in 70% alcohol after observation, it can be reused as a specimen many times. Preserve the specimen in 70% alcohol again.)

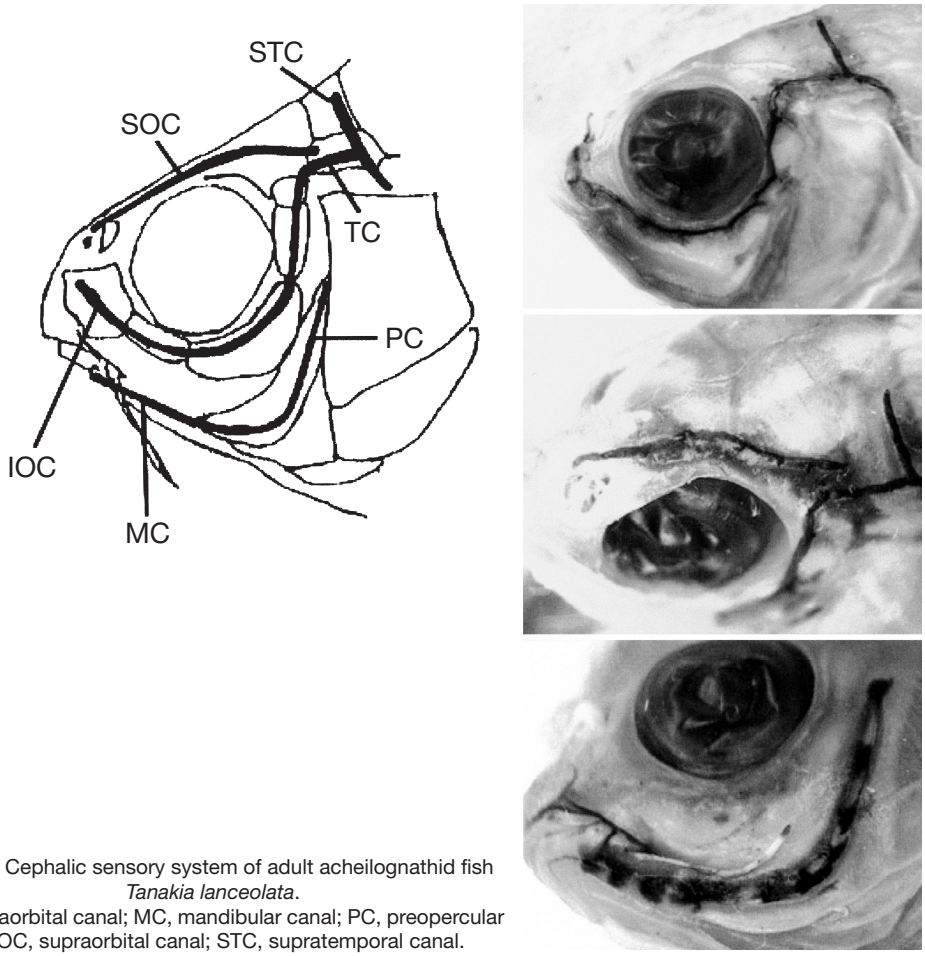


Fig. 7 Cephalic sensory system of adult acheilognathid fish *Tanakia lanceolata*.
IOC, infraorbital canal; MC, mandibular canal; PC, preopercular canal; SOC, supraorbital canal; STC, supratemporal canal.

• Column

(Nobuhiro Suzuki)

A sensory cell called a neuromast is found at the individual terminus of a lateral line pore. Those on the surface not being embedded in the skin are called free neuromasts, whereas those located at the bottom of the canal organ embedded in the skin are called canal neuromasts. A neuromast contains many hair cells. The hair cell is composed of a long kinocilium and dozens of stereocilia, which are arranged with polarity in a neuromast (Figure 8A). Upon stimulation, the kinocilium tail, and the stimulus is amplified by the stereocilia to sense the direction and intensity of the stimulus in the mechanism. The neuromast is in a plate-like or rod-like form if it is covered by a jelly-like substance, and is called the cupula (Figure 8B). There are already neuromasts on the epidermis of larvae immediately after hatching. At this time, they take the form of free neuromasts, and they change to the cupulas by increasing in number as the individual grows. Figure 9 shows the position of the free neuromasts (black circles) and the direction of a stimulus (arrow) sensed by each neuromast on the surface of a larva of redspotted grouper *Epinephelus akaara* in the pelagic stage.

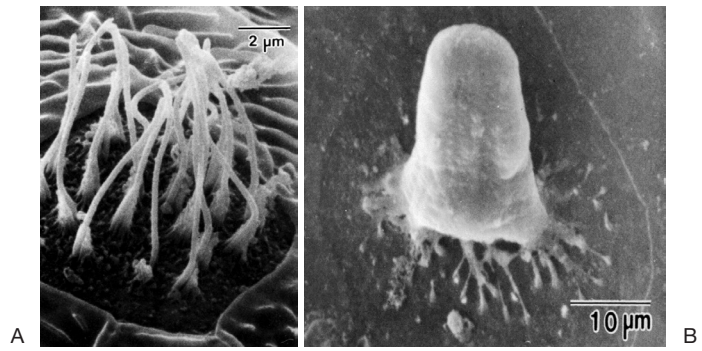


Fig. 8 A: Free neuromast of larva of Hong Kong Grouper *Epinephelus akaara*.
B: Cupula of Japanese Rockfish *Sebastes inermis*.

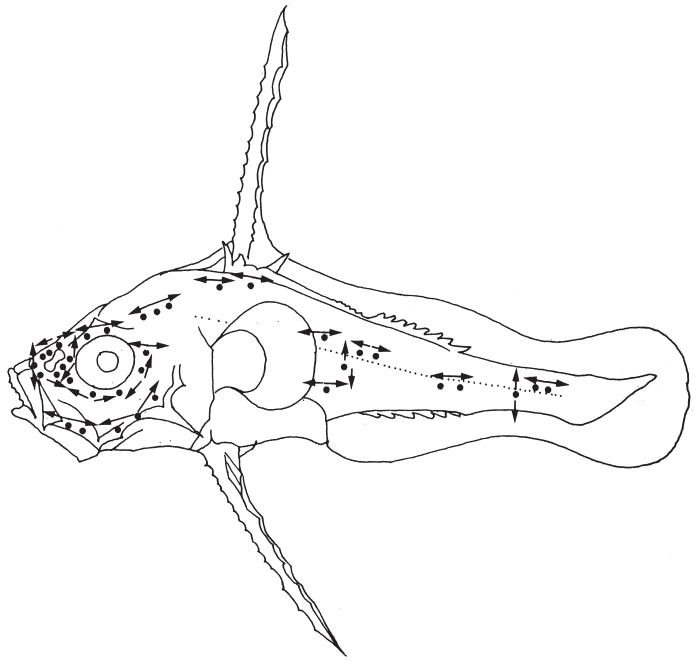


Fig. 9 Distribution of cupula on pelagic larva of Hong Kong Grouper *Epinephelus akaara*.

I 6 Luminescent organ

Many fish species have luminescent organs, which can have a wide variety of structures. However, the organs can be broadly grouped into those formed in the integument and those formed through differentiation from the digestive tract. In addition, the luminescence forms also have several types. In a broad grouping, there are the type that emits light by means of luminescent bacteria forming a symbiosis in the luminescent organ, and another type that emits light due to a chemical reaction between luciferin and luciferase occurring inside the luminescent organ. The latter luminescent organ is rich in variation in the morphology, arrangement form, etc. depending fish species. As mentioned above, fish species with luminescent organs include many species treated as deep-sea fish. Currently, those are broadly classified into two groups based on their adaptation process*.

Particularly, on the body of meso- and bathypelagic deep-sea fish (apart from ancient deep-sea fish), such as fish in Myctophidae, Neoscopelidae, Gonostomatidae, Sternoptychidae, Chauliodontidae, and Stomiidae, spherical or oval luminescent organs are arranged from the lateral surface to the ventral surface, and the number and arrangement form are important taxonomic characters. Although the structure of these luminescent organs varies depending on the fish species, basically, a lens, an emitter, a reflection layer, chromatophores, etc. are arranged from the body surface to the inside of the body. In all cases, it is spontaneous and intracellular luminescence.

Here, we deal with (easily obtainable) fish in Myctophidae distributed universally in the meso- and bathypelagic waters among the fish groups above.

Myctophidae: In addition to the spherical luminescent organs on the lateral side, there are large luminous glands on the top and bottom of the snout and/or the caudal peduncle. These luminescent organ groups form a small group as shown in Figure 1, which is important as a taxonomic character. Approximately 250 species are known from the seas in the world. The scales easily come off in many of the species. This group is the small deep-sea fish distributed in the meso- and bathypelagic waters. Their habitat depth differs between the daytime and the nighttime because they practice diel vertical migration in which the unit is a day. Additionally, they are foraged by many marine animals including skipjack/tuna, salmon/trout, dolphin, and other sea animals.

Myctophidae is divided into the following three groups based on their distribution form at night.

1) Surface migrants (there are luminous glands on the upper or lower part of the caudal peduncle): This group ascends to the surface layer of less than 10 m depth, mainly 0−1 m, at night. For that reason, they can be collected by a surface horizontal tow using a juvenile net at night. They descend to a depth of 200−400 m in the daytime and inhabit the layer with little light. *Myctophum*, *Symbolophorus*, *Centrobranchus*, etc. are the major genera.
2) Midwater migrants: This group inhabits mainly depths of 400−700 m in the daytime, but ascends to 100−200 m depth. However, they do not ascend to immediately below the surface. This group includes the fish ascending to the deeper layer and the fish ascending to the shallower layer.

* 2 large types in deep sea fishes.
1. Ancient type (or primary or true deep sea fishes): appeared from old geological (early Cenozoic or before) era, and adapting to the deep-sea by relatively long period. Such as Lanternfishes (or Myctohphidae), Swallowers (*Saccopharhynx* spp.), and Footballfishes (*Himantolophus* spp.).
2. New type (or secondary or continental shelf type): advanced into deep-sea in glacial ages of Quaternary period or later, from inland waters, coastal areas, or continental shelves. Thus, new type deep-sea fishes have related species with coastal areas. Such as Eelpouts (or Zoarcidae), Sculpins (Cottidae), Snailfishes (Liparidae), Cusk-eels (Ophidiidae), or Rattails (Macrouridae), etc.

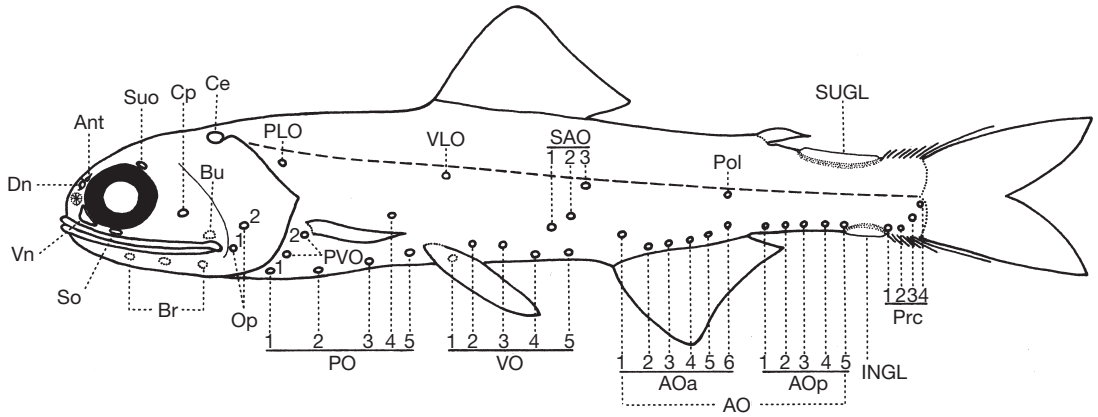


Fig. 1 Distribution of luminescent organs of myctophid fish (modified from Fujii (1084)).
Ant, antorbital organ; AOa, anterior anal organ; AOp, posterior anal organ; Br branchiostegal organ; Bu, buccal organ; Ce, cervical organ; Cp, cheek photophore; Dn, dorsonasal organ; INGL, infracaudal luminous gland; Op, opercular organ; PLO, suprapectoral organ; PO, pectoral organ; Pol, posterolateral organ; Prc, precaudal organ; PVO, subpectoral organ; SAO, supraanal organ; So, suborbital organ; SUGL, supracaudal luminous gland; Suo, supraorbital organ; VLO, supraventral organ; Vn, ventronasal organ; VO, ventral organ.

Diaphus, *Diogenichthys*, *Lampanyctus*, *Benthosem*, etc. are the major genera.

3) Non-migrants: The habitat depth changes little between the daytime and the nighttime. *Stenobrachius* is included.

Observation points

1. Arrangement of luminescent organs

The schematic diagram of the arrangement of luminescent organs shown above is of the group belonging to the abovementioned surface migrants. The luminescent organs of Myctophidae gather in small groups that are named individually. The number of the groups and positional relationships among the groups differ depending on the species.

2. Identification of species

Because the arrangement and number of luminescent organ groups rarely differ between the right and left, those on the left side should be used for observation and counting in principle. I advise you to focus on the following luminescent organ groups and scales in the specimens of Myctophidae belonging to the abovementioned surface migrants.

a. Are the 3 luminescent organs (1−3) of SAO in a straight line or a bent line?
b. Positional relationships among SAO1−3, VO1−5, and VLO.
c. Number of AOa.
d. Number of AOp.
e. Total number of AOa + AOp.
f. Relative positional relationship between Pol and the adipose fin.
g. Which type of scales, cycloid scales or ctenoid scales (several species included in *Myctophum* have ctenoid scales)?

3. Determination of the sex (Figures 2 and 3)

Among Myctophidae, in the species belonging to the surface migrants, males have a large SUGL that is, however, missing in the lower part of the caudal peduncle. Females have a small INGL that is,

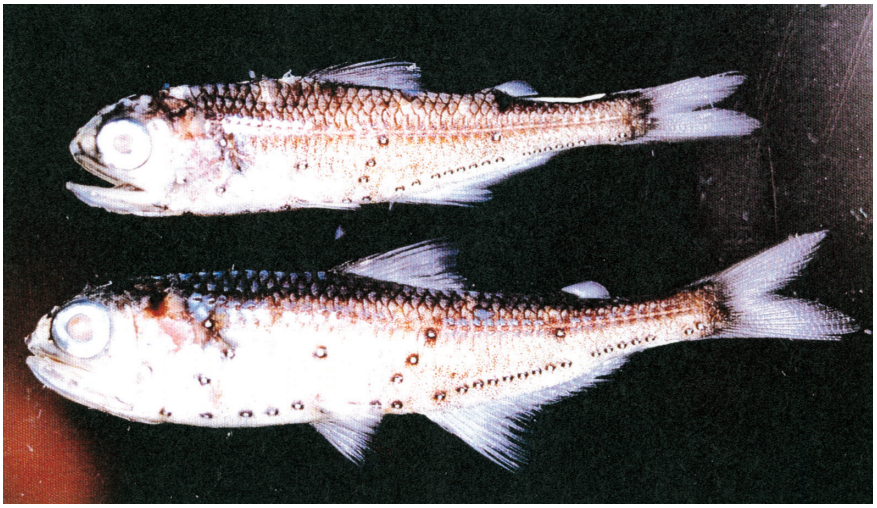


Fig. 2 Pearly Lanternfish *Myctophum nitidulum*.
Upper, male with SUGL; Lower, female with INGL.

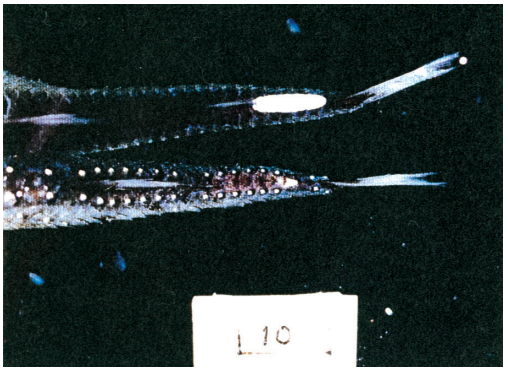


Fig. 3 Prickly Lanternfish *Myctophum asperum*.
Upper, dorsal view of male with SUGL; Lower, ventral view of female with INGL.

however, missing in the upper part of the caudal peduncle. Exceptionally, in *Tarletonbeania*, males have it in the both upper and lower parts, whereas females lack it in the both parts. The appearance of this luminescent gland is an effective character for the determination of sex only in adults in which it is completely developed as a secondary sexual characteristic.

These differences between males and females are said to help them to identify the other sex in their spawning season, when males swim in the lower layer whereas females swim in the upper layer.

4. Development of the caudal luminous gland (Figure 4)

With regard to the outline of the caudal luminous gland of adults, the length and width differ depending on the sex. In females, small plates of the same size are arranged, whereas in males, plates of slightly varying sizes overlap each other in the structure. The number and shape of these plates are effective taxonomic characters of each species because these are nearly constant in each species.

In the case of *Myctophum nitidulum* in Figure 4 below, the number of plates of adults is 3−4 in females and 6−8 in males, showing a higher number in males. After their appearance, this caudal luminous gland becomes larger in shape and increases in number to completion as the fish grows,

and the whole shape becomes unique to the species. For that reason, the sex of an individual can be identified from the appearance even if the luminescent gland is incomplete (see the intermediate type in Figure 4).

This gland appears once the body length reaches around 35 mm in female individuals, and completes its development when the length reaches around 65 mm. On the other hand, in males, it appears once the body length reaches around 28−29 mm, and completes its development when the body length reaches around 60 mm, earlier than in females. In addition, because the caudal luminous gland is a secondary sexual characteristic, the developmental process and development of the gonad (ovary or testis) progress almost simultaneously. Therefore, I advise you to observe them in parallel. However, you may miss the testis of immature males unless you pay attention because it is small, even though it leads to the anus.

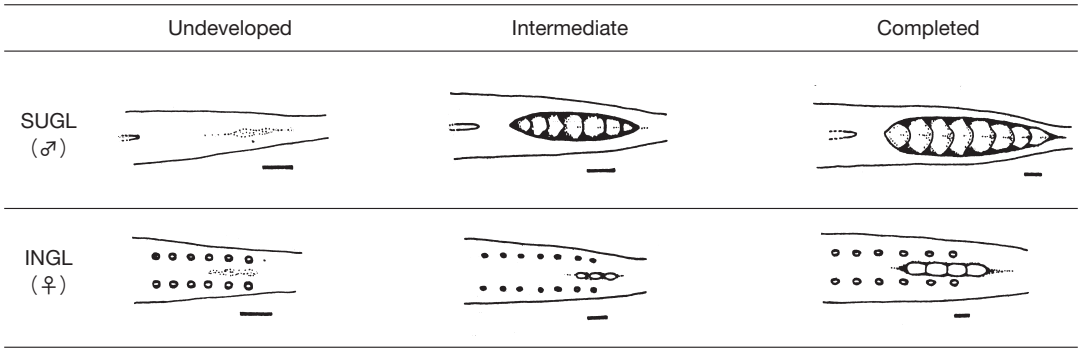


Fig. 4 Ontogenic change of SUGL (upper) and INGL (lower) of Pearly Lanternfish Myctophum nitidulum. Scales indicate 1 mm.