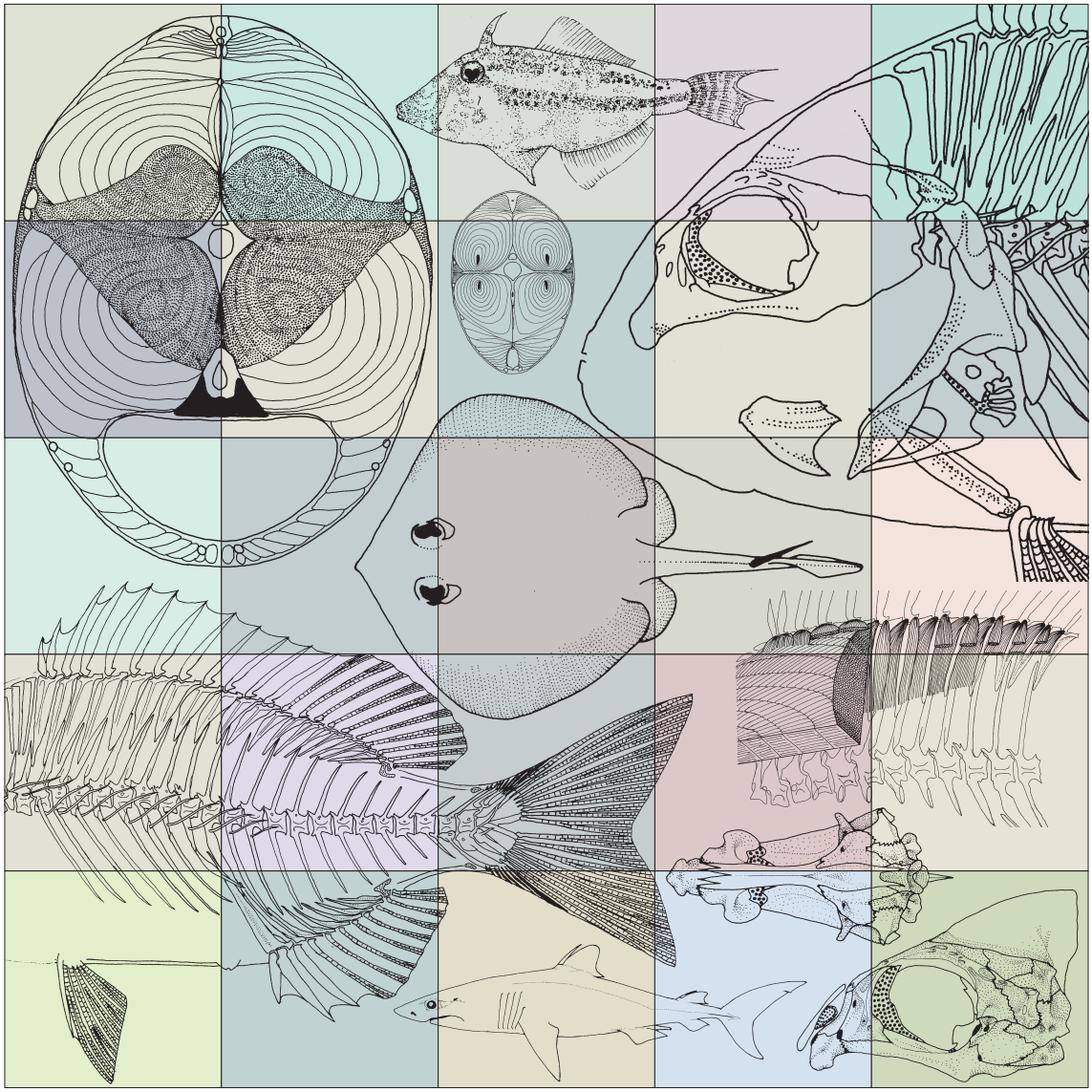
Laboratory Manual on Fundamental Ichthyology

Edited by Hirokazu KISHIMOTO, Nobuhiro SUZUKI and Izumi AKAGAWA
This selected English version is translated & edited by Benjamin F. MUTO



Tokai University Press

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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).

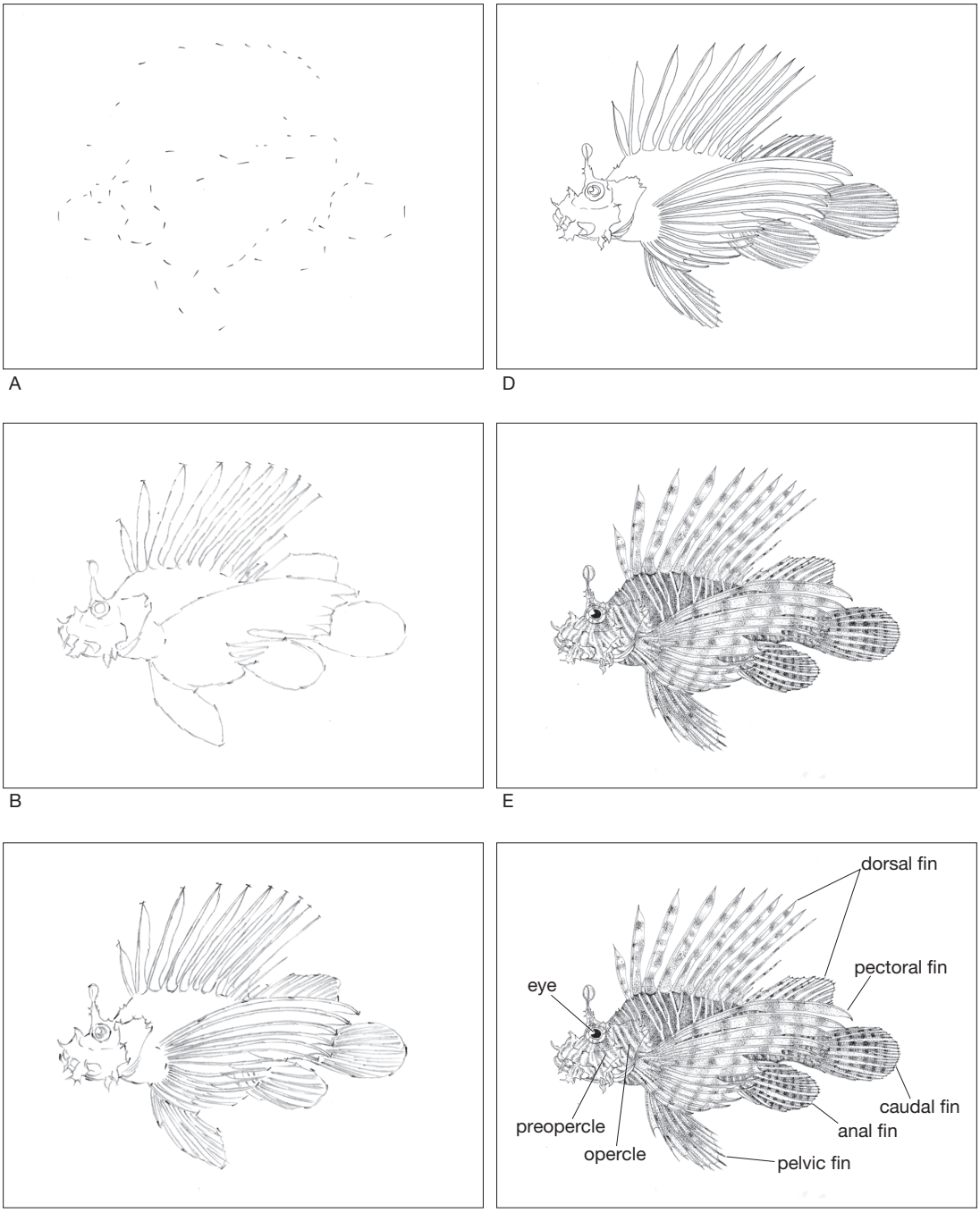


Fig. 1 Example of sketch of general fish.

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

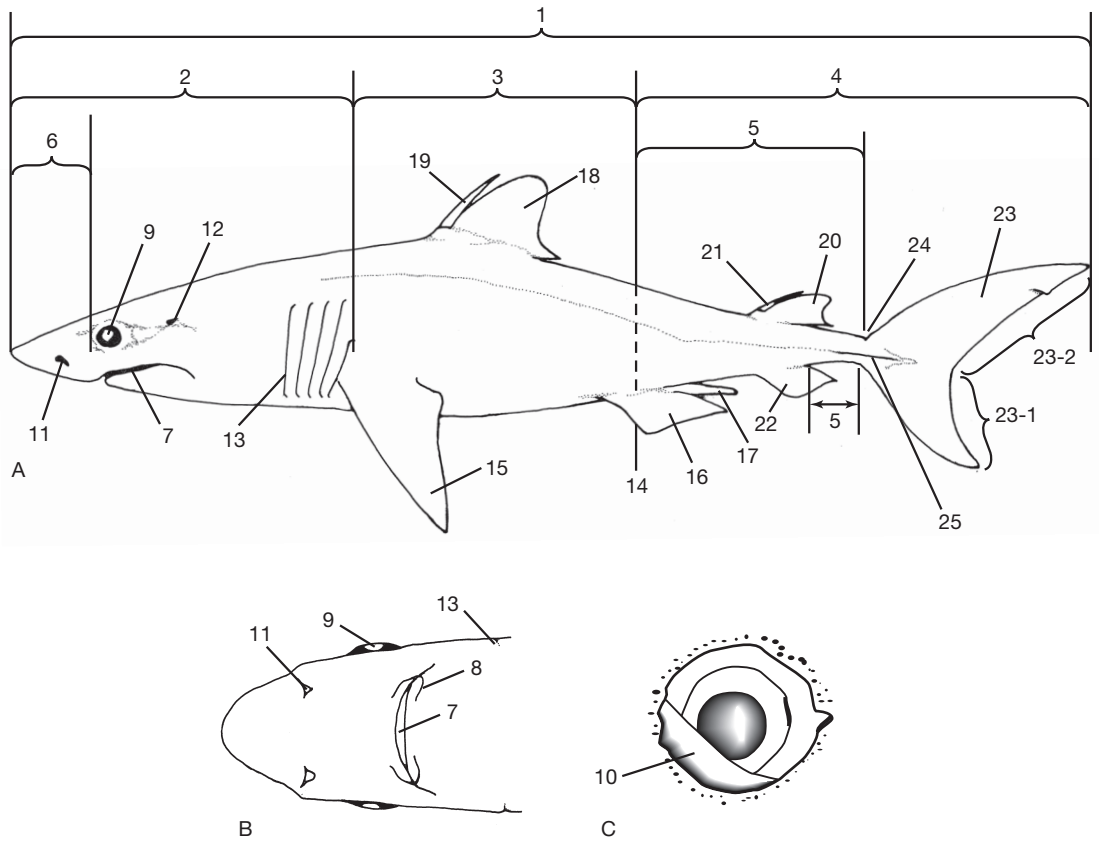


Fig. 1 Sketch of a fictional fish, "Spiny Porbeagle" (male). A, lateral view; B, ventral view of head; C, eye.

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.

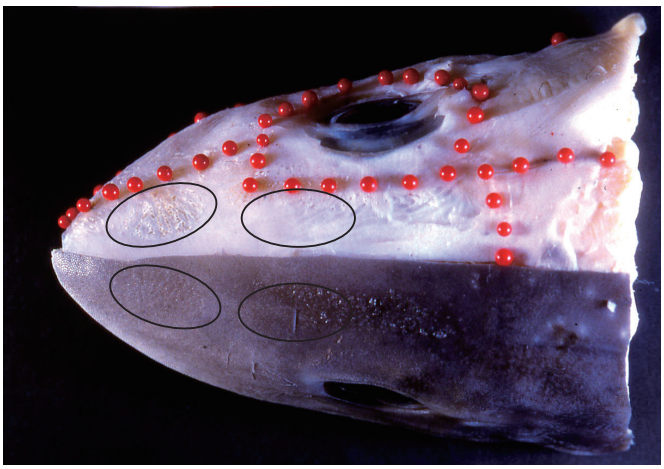


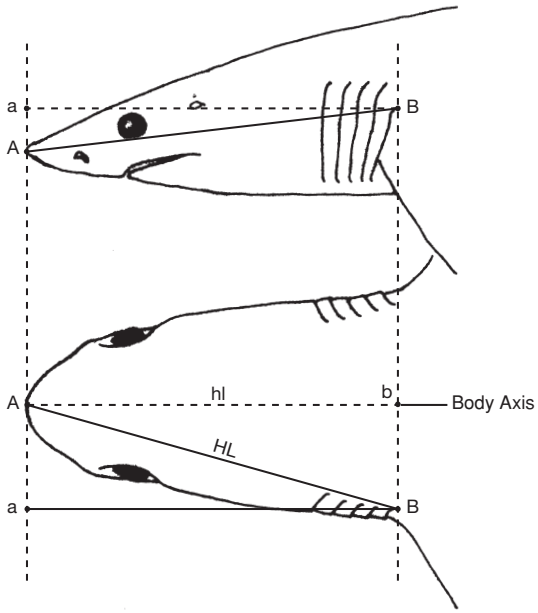
Fig. 2 Dorsal view of head of a shark, Starspotted Smooth-hound *Mustelus manazo*.

* 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

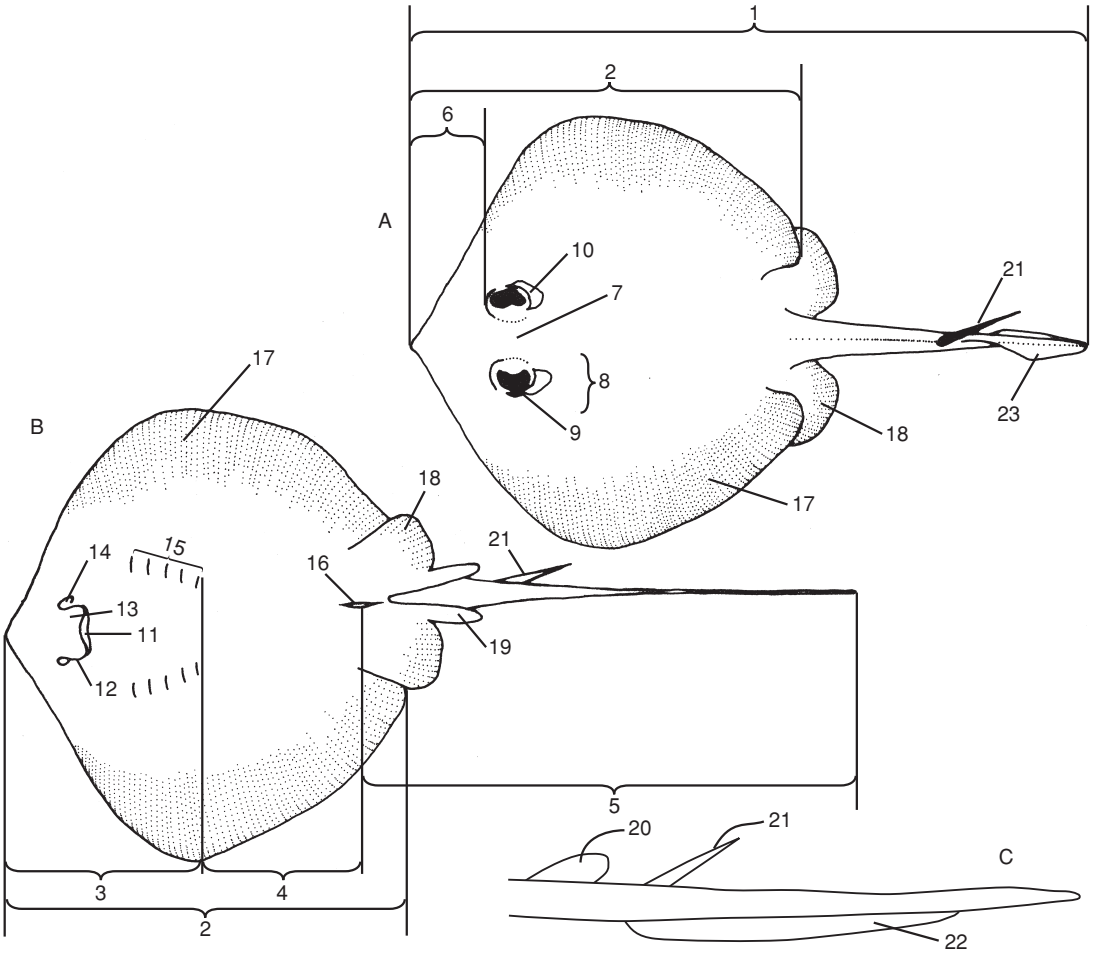


Fig. 1 A, dorsal view of female Sepia Stingray *Urolophus aurantiacus*; B, Ventral view of male Red Stingray *Dasyatis akajei*. C, Lateral view of whiptail of Red Stingray.

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)

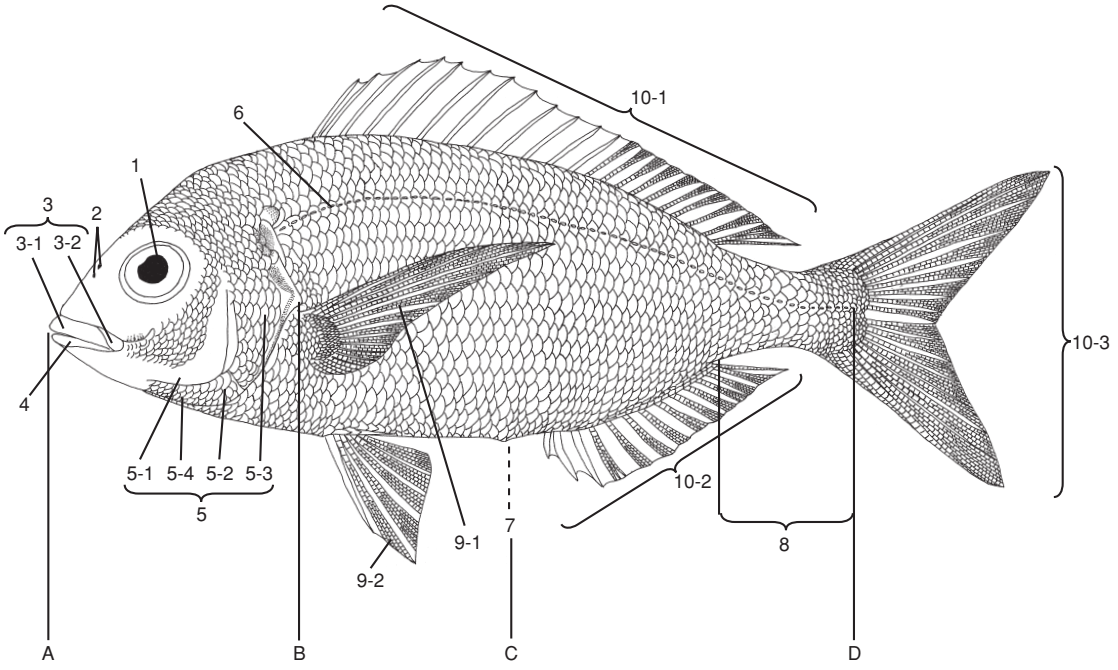


Fig. 1 Red Pandora *Pagellus belottii*, from Africa.

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

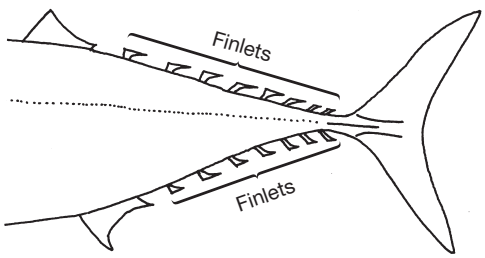


Fig. 2 Finlets of scombrid fish.

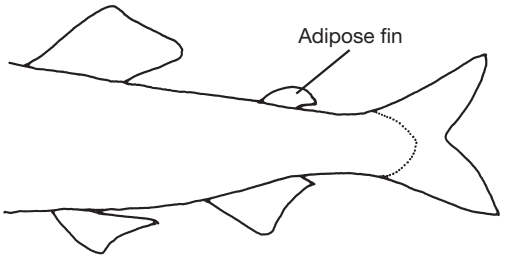


Fig. 3 Adipose fin of Ayu *Plecoglossus altrivelis altivelis*.

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

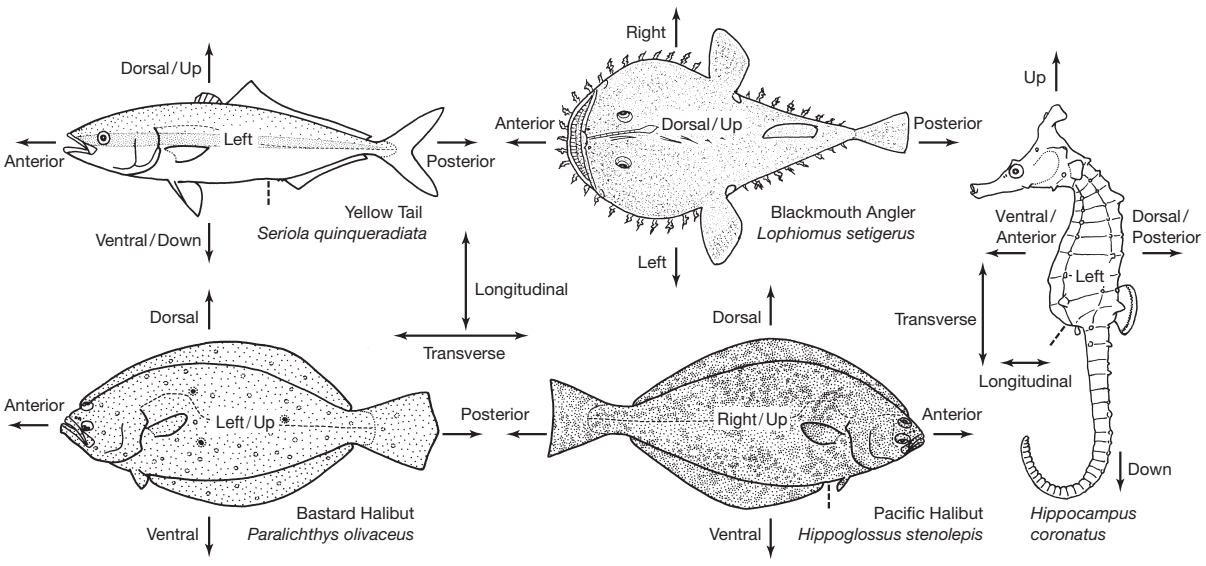


Fig. 4 Direction of fishes. (Illustrations from Nakabo (2000)

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

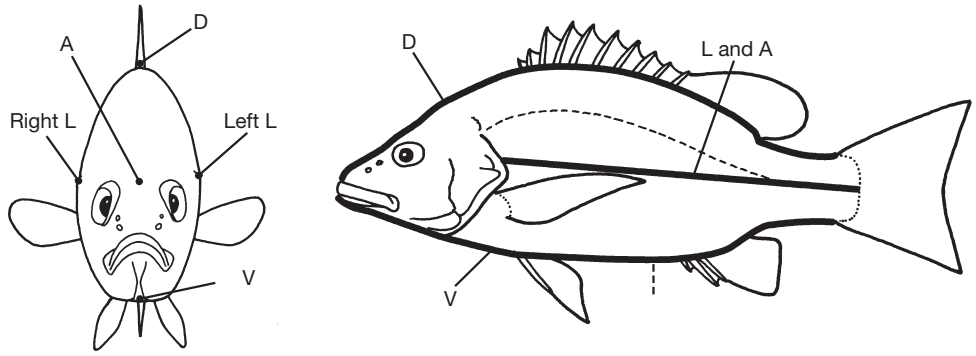


Fig. 5 Medial lines of fishes. (Illustrations from Nakabo (2000)

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

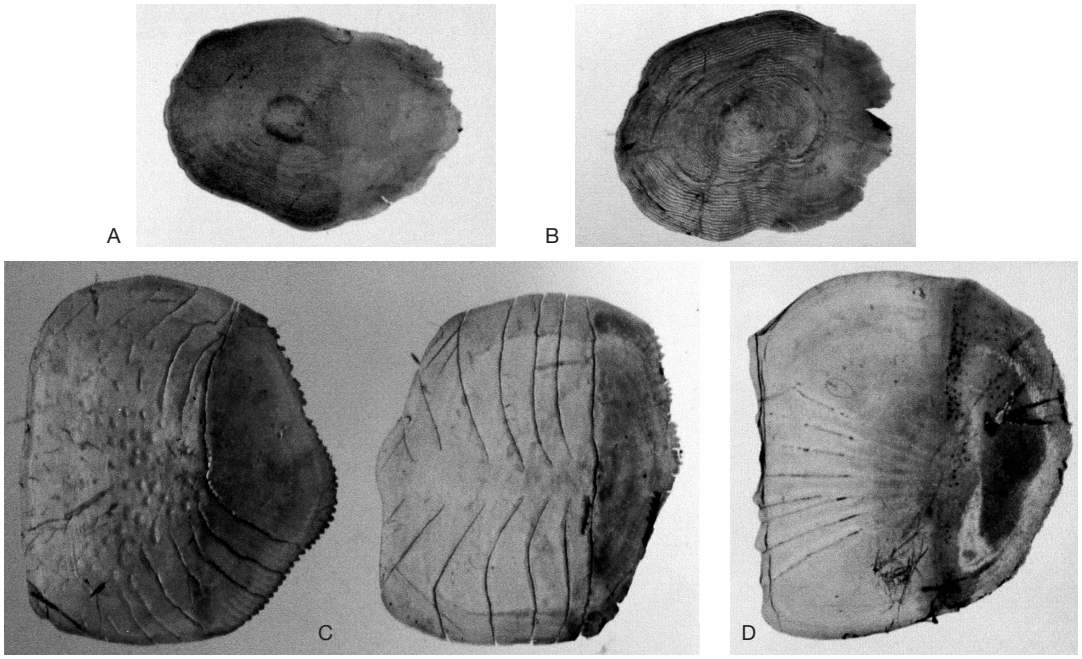


Fig. 1 Scales of teleosts.
A, Cycloid scale of Masu Salmon *Oncorhynchus masou masou*; B, Regenerated cycloid scale of Masu Salmon *Oncorhynchus masou masou*; C, Cycloid scale of Japanese Pilchard *Saldinops melanosticus*. Left, normal scale. Right, regenerated scale; D, Ctenoid scale of Red Sea Bream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

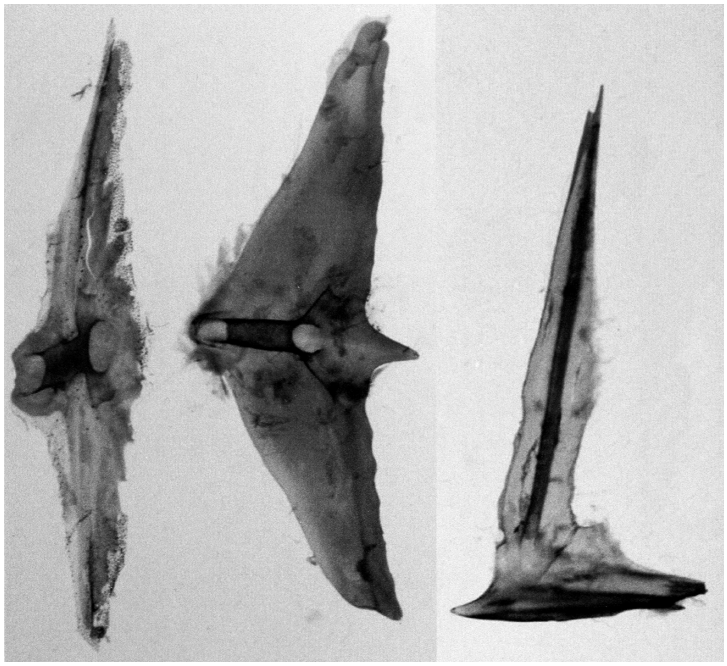


Fig. 2 Scutes.
A, Japanese Jack Mackerel *Trachurus japonicas* (Left, from trunk. Right, from caudal peduncle);
B, Dotted Gizzard Shad *Konosirus punctatus* (From ventral).

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

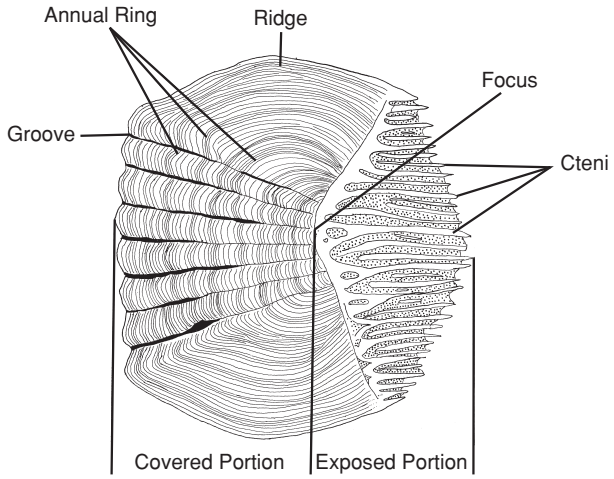


Fig. 3 Parts names of ctenoid scale of Spotbrest angelfish *Genicanthus melanospilos*. Sketch by Katsunori Nakamura.

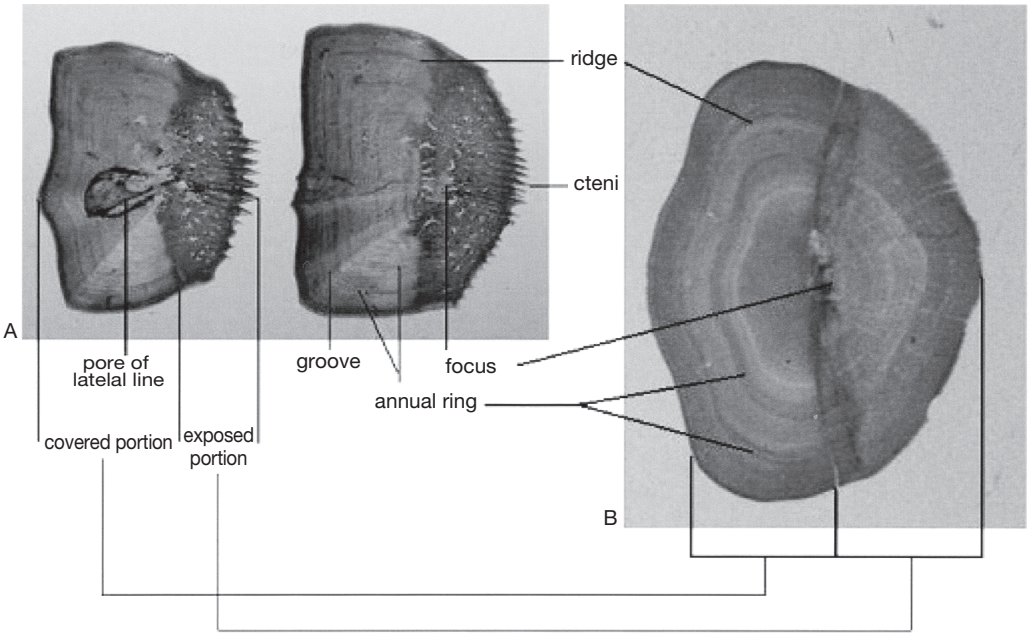


Fig. 4 Parts names of scales.
A, Sabre Squirrelfish *Sargocentron spiniferum* (Left, pored lateral line scale);
B, Dotted Gizzard Shad *Konosirus punctatus*.

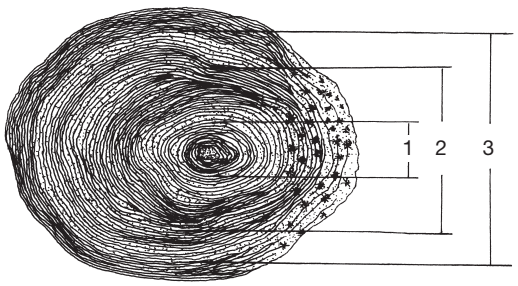


Fig. 5 Annual rings of Masu Salmon *Oncorhynchus masou masou*.

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).

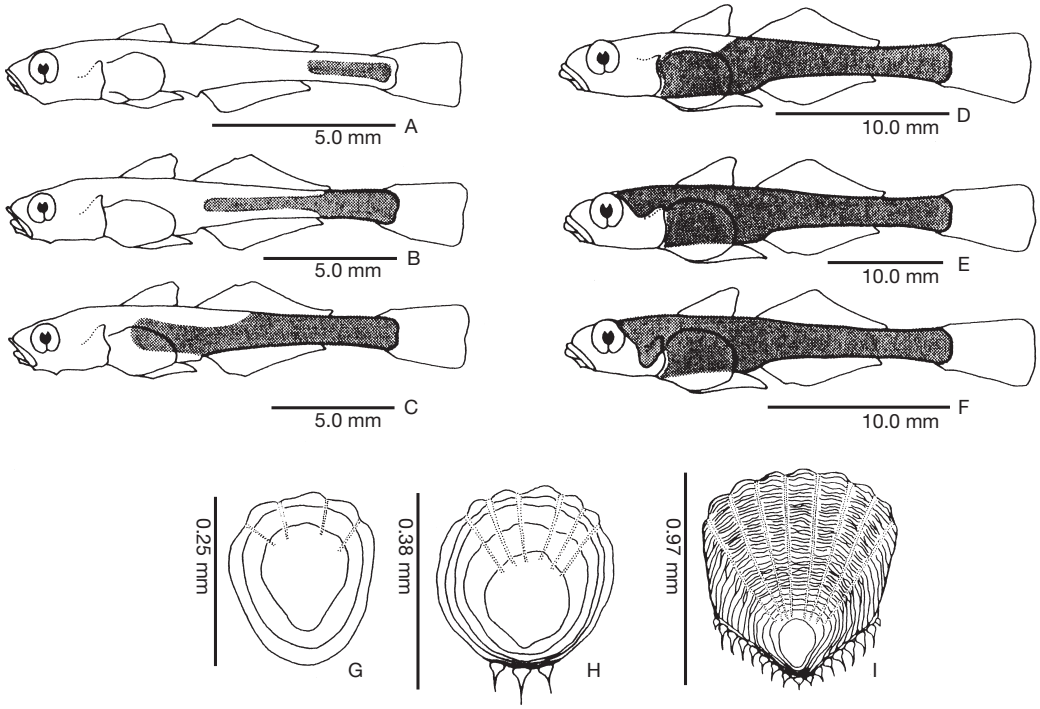


Fig. 6 Area expansion of distribution (A-F), and morphological change (G-I) of first scales of Yellowfin Goby *Acanthogobius flavimanus*. G, first scale; H, developping scale; I, developed adult scale.

(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.)

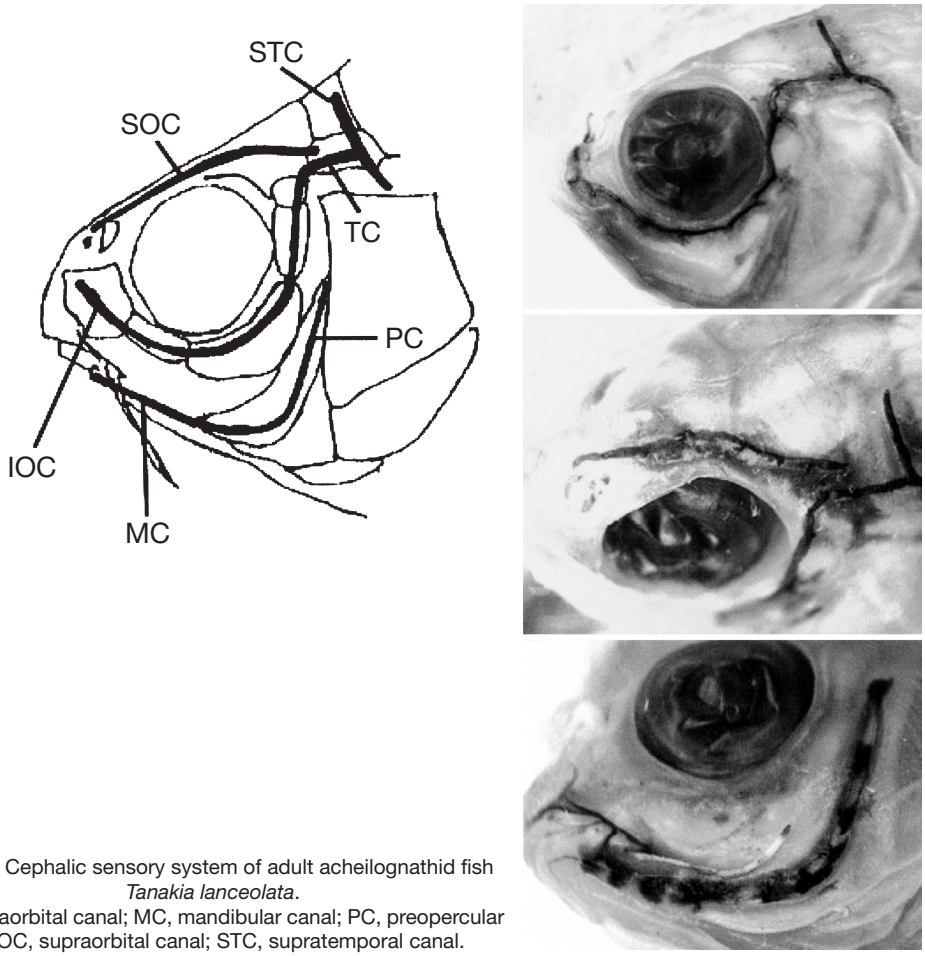


Fig. 7 Cephalic sensory system of adult acheilognathid fish *Tanakia lanceolata*.
IOC, infraorbital canal; MC, mandibular canal; PC, preopercular canal; SOC, supraorbital canal; STC, supratemporal canal.

• 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.

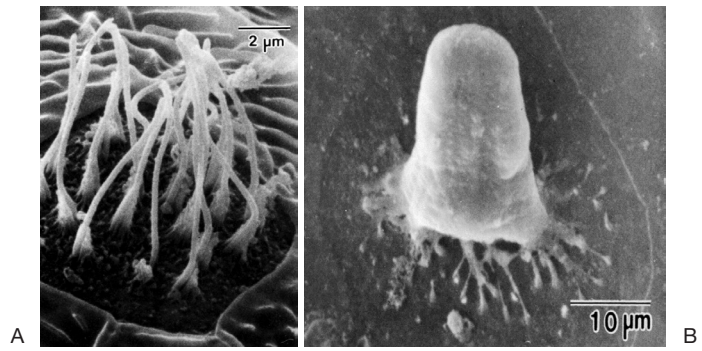


Fig. 8 A: Free neuromast of larva of Hong Kong Grouper *Epinephelus akaara*.
B: Cupula of Japanese Rockfish *Sebastes inermis*.

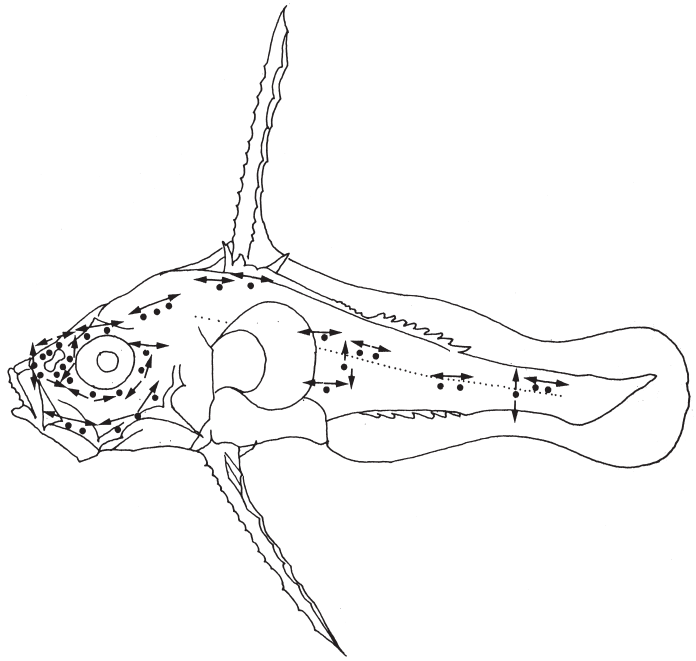


Fig. 9 Distribution of cupula on pelagic larva of Hong Kong Grouper *Epinephelus akaara*.

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.

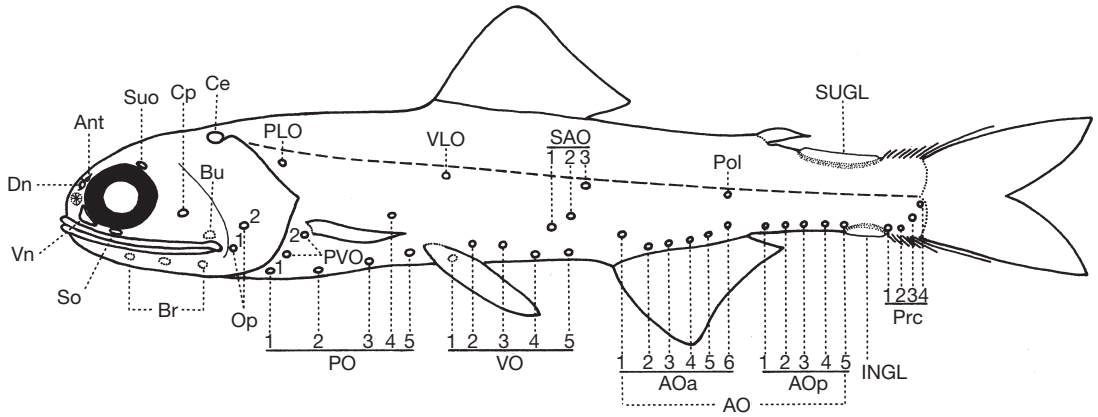


Fig. 1 Distribution of luminescent organs of myctophid fish (modified from Fujii (1084)).
Ant, antorbital organ; AOa, anterior anal organ; AOp, posterior anal organ; Br branchiostegal organ; Bu, buccal organ; Ce, cervical organ; Cp, cheek photophore; Dn, dorsonasal organ; INGL, infracaudal luminous gland; 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,

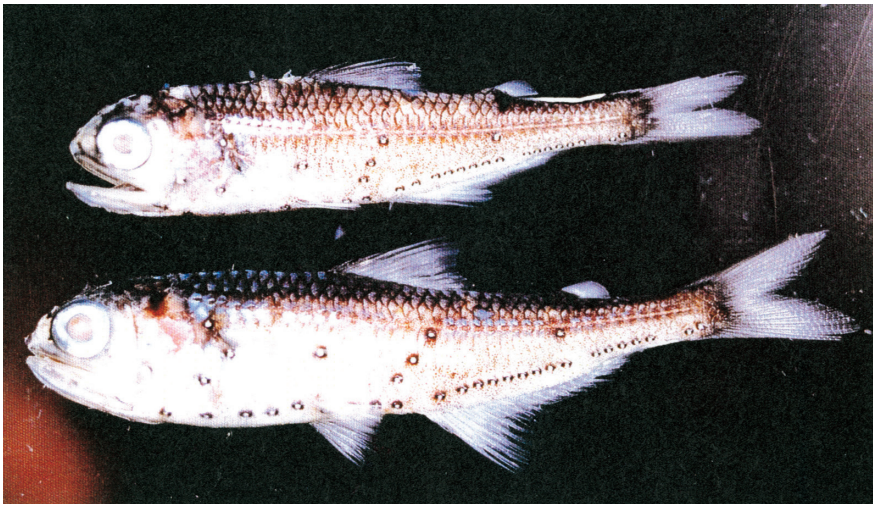


Fig. 2 Pearly Lanternfish *Myctophum nitidulum*.
Upper, male with SUGL; Lower, female with INGL.

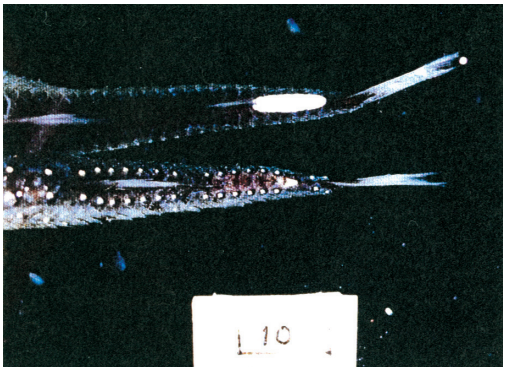


Fig. 3 Prickly Lanternfish *Myctophum asperum*.
Upper, dorsal view of male with SUGL; Lower, ventral view of female with INGL.

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.

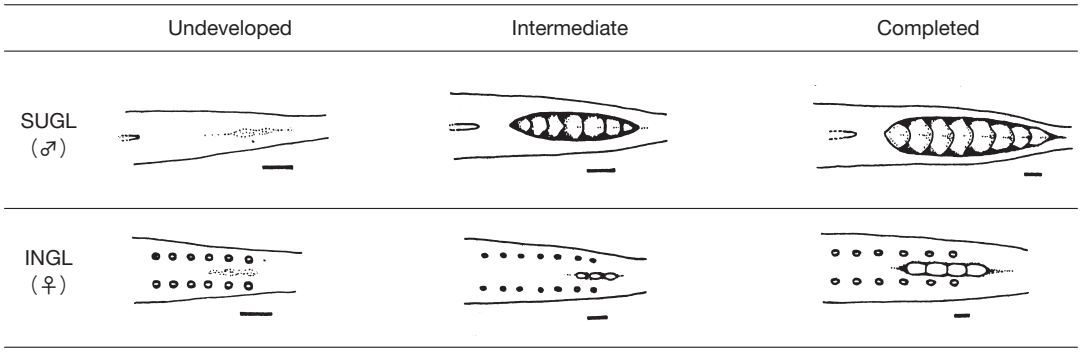


Fig. 4 Ontogenic change of SUGL (upper) and INGL (lower) of Pearly Lanternfish Myctophum nitidulum. Scales indicate 1 mm.

II

Fish measurements

II 1 Measuring equipment

Equipment for measuring the size of parts of a fish body varies depending on the size of the fish, and each type of equipment has a distinct precision. For measuring huge fish such as sharks, a tape measure is used, whereas fish larger than 30 cm are usually measured using a straightedge (wooden ruler) in millimeters. If the size is <30 cm, a vernier caliper is used to accurately measure the size down to the first decimal point (in tenths of millimeters). Vernier calipers include dial calipers and digital calipers, the latter of which have a variety of types, including ones to which recording equipment can be attached. Every caliper can read down to the second decimal point. However, precision to the first decimal point is sufficient for the measurement of organisms. In the case of a microscopic size such as the case of larvae and juveniles, a micrometer is used. However, because the measurements use projection (see the column) in most cases, it is inevitable that the measurement angle of each part becomes a little inaccurate.

Division reading and principle of vernier calipers

You need to carefully understand the method and principle of vernier calipers because an error in the reading of divisions of a vernier caliper frequently used in measurement of fish bodies can lead to a serious mistake.

The ways to divide scales on vernier calipers

Normally, a vernier has n divisions that are equal to n−1 divisions on the main scale. Depending on the precision, there are two forms for the divisions, as shown in the table below.

The minimum division on the main scale (mm)	The way to divide the vernier	The minimum read value (mm)
(1) 1	19 mm are equally divided by 20 (Fig. 1) 39 mm are equally divided by 20	1/20=0.05
(2) 0.5	12 mm are equally divided by 25 24.5 mm are equally divided by 25 49 mm are equally divided by 50	1/50=0.02

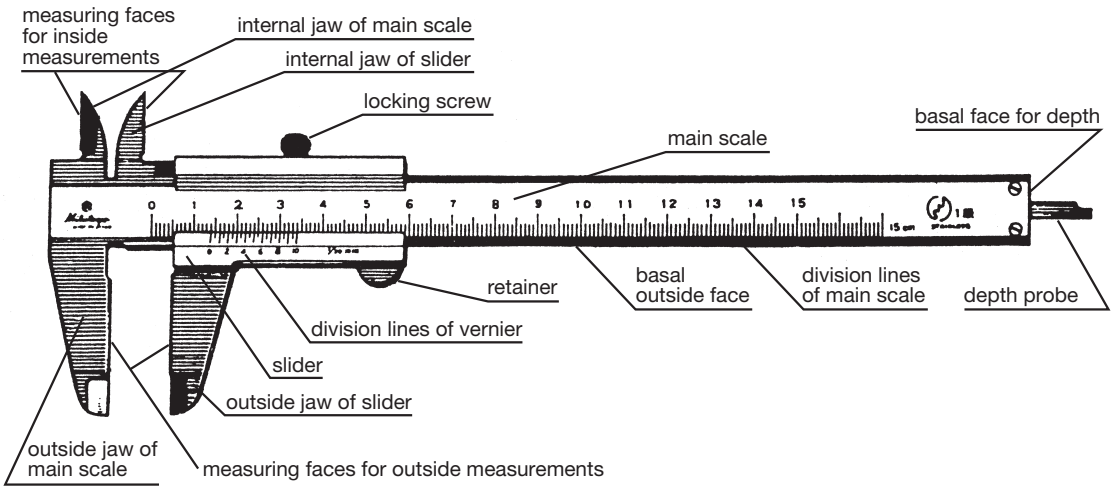


Fig. 1 Legends of vernier caliper.

How to read divisions on vernier calipers

(1) Principle: In the case of Figure 2, because the division line 0 on the vernier is over 7 on the main scale and the 4th division line coincides with a division line (regardless of the position) on the main scale, the size is as follows:

$$7 + (0.05 \times 4) = 7 \text{ mm} + 0.2 \text{ mm} = 7.2 \text{ mm}$$

Convenient method: Add (the division line on the vernier coinciding with a division line on the main scale) × 0.1 to the division line on the main scale over which the division line 0 on the vernier lies. That is, read it as follows:

$$7 + (2 \times 0.1) = 7 \text{ mm} + 0.2 \text{ mm} = 7.2 \text{ mm}$$

Fig. 2

(2) Principle: In the case of Figure 3, because the division line 0 on the vernier is over 4.5 on the main scale and the 11th division line coincides with a division line (regardless of the position) on the main scale, the size is as follows:

$$4.5 + (0.02 \times 11) = 4.72 \text{ mm}$$

Convenient method: Add (the division line on the vernier coinciding with a division line on the main scale) × 0.1 to the division line on the main scale over which the division line 0 on the vernier lies. That is, read it as follows:

$$4.5 + (2.2 \times 0.1) = 4.72 \text{ mm}$$

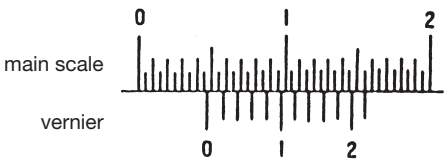


Fig. 3

II 2

How to measure Chondrichthyes; sharks

Measurements of Chondrichthyes are generally done on projection on lateral axis. These customs are different from those on Teleostei, done on point-to-point lengths.

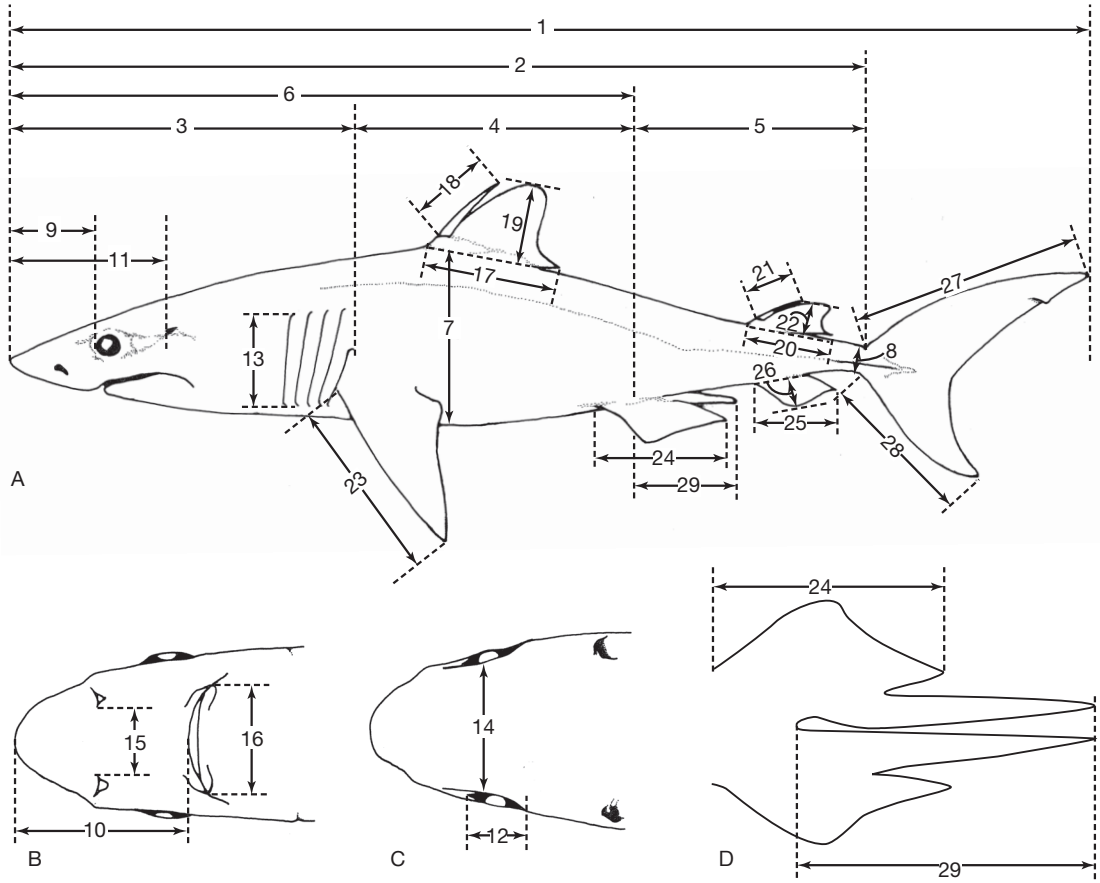


Fig. 1 Common measurements on sharks.
A, lateral view; B, ventral view of head; C, dorsal view of head; D, ventral view of pelvic fin.
See text for numbers on the figures. Drawn by Yumi Kawai.

1. **Total Length; TL**

   Length from anterior-most point of body to posterior-most point of caudal fin. Anterior most point has both cases on upper and lower jaws. Generally this length has taken to the endpoint of upper lobe of caudal fin with normal position, but sometimes taken with most end point by pulling anterior direction. In some species the end of upper lobe is not be able to detect, and precaudal lengs as below is used instead of total length.

2. **Precaudal Length; PCL**

   Length from anterior-most point of body to origin of upper lobe of caudal fin, or if present, to deepest point of precaudal pit, incision between caudal peduncle and upper lobe of caudal fin.

3. **Head Length; HL**

   Length from anterior most of body to posterior most point of last gill slit.

4. **Trunk Length**

   Length from anterior-most point of body to midpoint of insertion of vent (cloaca).

5. **Tail Length**

   Length from midpoint of insertion of vent to origin of upper lobe of caudal fin, or if present, to deepest point of precaudal pit.

6. **Snout-vent Length**

   Length from anterior-most point of body to midpoint of insertion of vent.

7. **Body Depth; BD**

   Depth on deeper most part of body, without fin parts.

8. **Depth of Caudal Peduncle.**

   Depth of lower most part of caudal peduncle. Generally measured on origin of caudal fin.

9. **Preorbital Length**

   Length between anterior-most points of body and orbit.

10. **Preoral Length**

    Length between anterior-most points of body and mouth.

11. **Prespiacular Length**

    Length between anterior-most points of body and spiracle (if exists).

12. **Orbit Diameter**

    Horizontal diameter of orbit.

13. **First Gill Slit Length**

    Length between upper and lower-most points of first gill slit.

14. **Interorbital Width.**

    Shorter-most width between right and left orbits, from dorsal view.

15. **Internarial width**

    Shorter-most width between right and left nasal pores, from ventral view.

16. **Mouth Width**

    Width between right and left ends of mouth.

17. **First Dorsal-fin Length**

    Length between origin and posterior most point of first dorsal-fin.

18. **First dorsal-fin Spine Length**

    Length of exposed part of first dorsal-fin spine from proximal origin to tip. Record in cases of broken and/or worn down.

19. **First Dorsal-fin Hight.**

    From base to distal most point of first dorsal-fin.

20. **Second Dorsal-fin Hight.**

    From base to distal most point of second dorsal-fin.

21. Second Dorsal-fin Spine Length

Length of exposed part of second dorsal-fin spine from proximal origin to tip. Record in cases of broken and/or worn down.

22. Second Dorsal-fin Hight

From base to distal most point of first dorsal-fin.

23. Pectoral-fin Length

From base to tip of pectoral-fin.

24. Pelvic-fin Length

From anterior origin to posterior tip of pelvic-fin.

25. Anal-fin Length

From anterior origin to posterior tip of anal-fin.

26. Anal-fin Hight

From base to higher-most point of anal-fin.

27. Dorsal caudal-fin length

From origin to posterior most point of upper lobe of caudal.

28. Preventral caudal-fin Length

From origin to posterior most point of lower lobe of caudal-fin.

29. Clasper Length

From anterior most point of insertion of vent (cloaca) to tip of clasper.

II 3

How to measure Chondrichthyes; skates and rays

Measurements of Chondrichthyes are generally done on projection on lateral axis. These customs are different from those on Teleostei, done on point-to-point lengths.

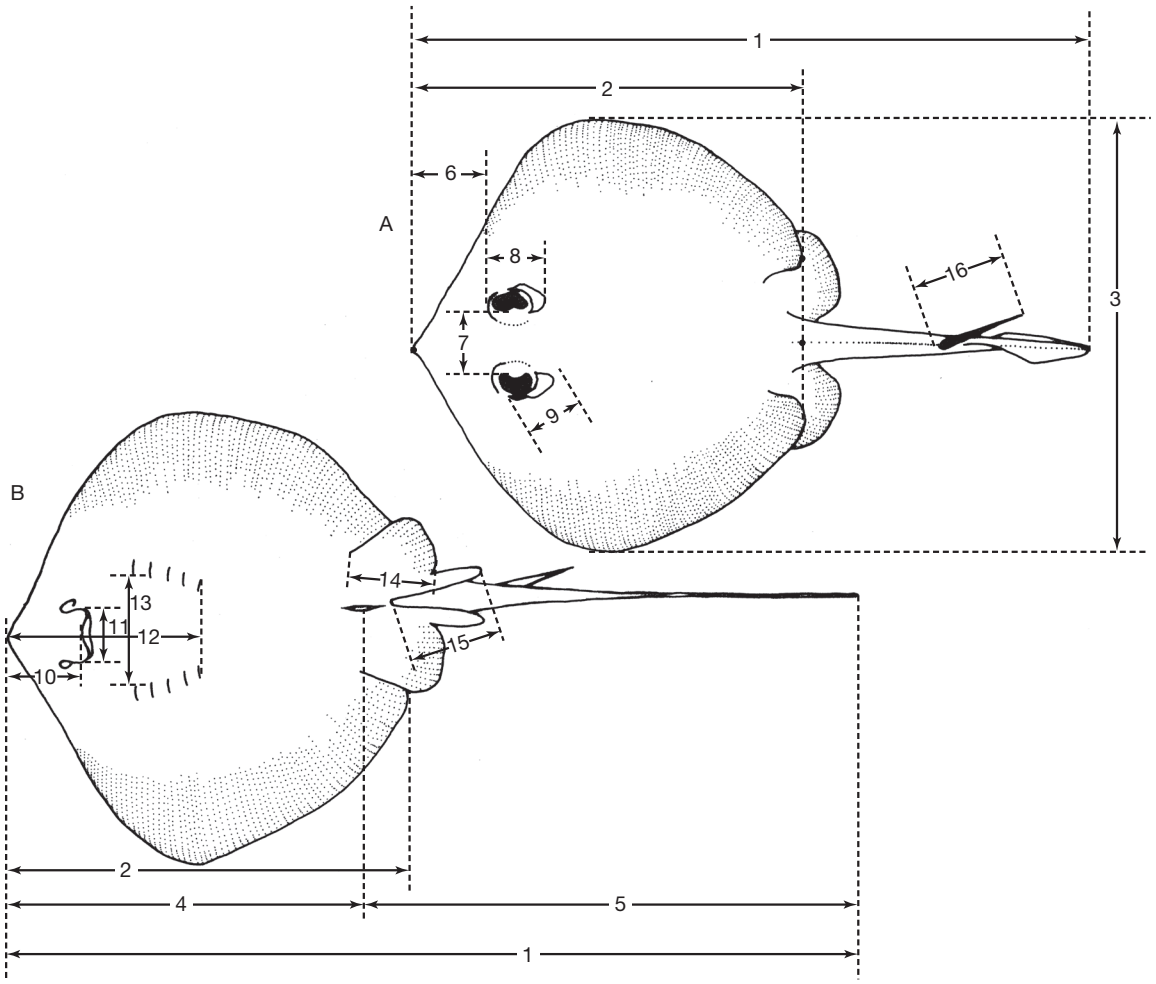


Fig. 1 Common measurements on rays and skates.
A, dorsal view of Sepia Stingray *Urolophus aurantiacus*; B, ventral view of Wip Stingray *Dasyatis akajei*.
See text for numbers on the figures. Drawn by Yumi Kawai.

1. **Total Length; TL**

   Length from anterior most part of body to posterior end of caudal-fin or tail. Rays of the Superfamily Dasyatoidea generally do not have caudal-fin, but with tail like a wip. Remind that such tale sometimes lost their tips.
2. **Disc Length; DL**

   Length from anterior-most point of body to posterior end of pectoral-fin. Posterior end of pectoral fin is decided by point of left or right side of fin, or intersection of line between both end of pectoral fin and body medial axis.
3. **Disc Width; DW**

   Distance between distal ends of both pectoral fin.
4. **Snout-vent Length**

   Length from anterior-most point of body to midpoint of insertion of vent.
5. **Tail Length**

   Length from midpoint of insertion of vent to posterior most of caudal-fin or tail.
6. **Preorbital Length**

   Length between anterior-most points of body and orbit.
7. **Interorbital Width.**

   Shorter-most width between right and left orbits.
8. **Orbit Diameter**

   Horizontal diameter of orbit (soft part) as sharks or teleosts.
9. **Spiracle Length**

   Length between anterior and posterior most points of spiracle.
10. **Preoral Length**

    Length between anterior-most points of body and mouth.
11. **Mouth Width**

    Width between right and left ends of mouth.
12. **Snout-last gill slit length (= Head Length; HL)**

    Anterior most point to intersection of lines between both end of right and left last gill slit and body medial axis.
13. **Inter-first Gill Slit Width**

    Distance between right and left first gill slits,
14. **Pelvic-fin Length**

    Length from anterior origin to posterior tip of pelvic-fin. In some rajid skates, pelvic-fin has anterior and posterior lobes, and should be measure both of them.
15. **Clasper Length**

    Length from anterior most points of insertion of vent (cloaca) to tip of clasper.
16. **Caudal Spine Length**

    Length of exposed part of caudal spine from proximal origin to tip. Record in cases of broken and/or worn down.

II 4 How to measure Teleostei

Teleostei have many bones, many of which can be outlined from the body surface. Fins are constituted of many foldable bony fin rays. The body surface is covered by bony scales in general. Because many of these structures become traits for counting and measurement, Teleostei have many more measurement parameters, particularly counting parameters, than do Chondrichthyes.

Measurement method: As opposed to measurements of Chondrichthyes, which generally use projection, in measurements of Teleostei, the distance between two points is measured by directly applying a vernier caliper to both ends of the measurement site, as shown in the figure below. Therefore, the axis of measurements is independent of the body axis, and oblique measurements are frequently used. However, this is only the method for making measurements; therefore, it is understood that sketching uses projection.

To compare measurement values between species or individuals, it is typical to calculate and examine the ratio or percentage of body parts to the standard length or the head length. In addition, the percentage of one body part to another body part, for example, the eye diameter to the upper jaw length, is also a valid examination parameter. When a graph is prepared with the vertical axis representing such a ratio or percentage of the length of a body part and the horizontal axis representing the body length, the plots are distributed horizontally if the species shows no change during growth, whereas an inclined distribution means that the rate changes with growth. If any difference is seen in the distribution range of the plots in a comparison of graphs of the same parameter between fish species, it is a valid taxonomic character, and the less the overlap is, the higher the validity is estimated.

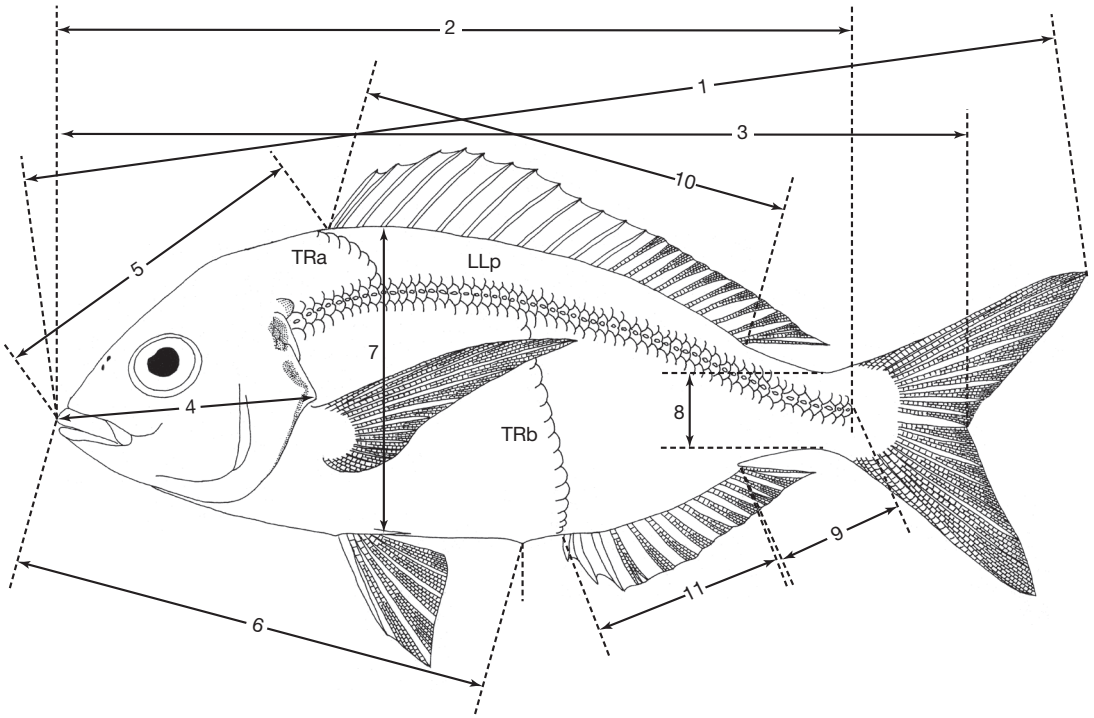


Fig. 1 Common counts and measurements on teleosts.
See text for numbers on the figures.

1. **Total length (TL)**

This is the length from the most anterior terminus of a body to the most posterior terminus of the caudal fin. The anterior terminus may be on either the upper or lower jaw as long as it is the most anterior. For the posterior terminus of the measurement, use the most posterior terminus of either the upper or lower lobe of the caudal fin, including a thread-like extension. The official definition prescribes to measure it by compressing the caudal fin if it is a fresh fish, the caudal fin of which can be opened and closed. However, in the case of a specimen with fixed fins or of caudal fins that cannot be opened and closed, like those of fish in Scombridae, use the posterior terminus of the caudal fin extended in the normal form. Although this is a measurement valued in market and fisheries science, it is not valued very much in ichthyology because the posterior terminus of caudal fins is damaged in many cases.

2. **Standard length (SL) or body length (BL)**

This is the length from the snout tip to the terminus of the vertebral column (posterior margin of the caudal skeleton) (see Figure 4 in III-7). Because the most anterior terminus of the head varies depending on the fish species, it is not necessarily consistent with the snout tip. Even if the lower jaw protrudes more than the upper jaw (superior mouth), as in Scorpaeniformes, Serranidae, and Hemiramphidae, the lower jaw should not be included. When the edges of both jaws are at the same position (terminal mouth) or the upper jaw is at the most anterior terminus (subterminal mouth) as in Carangidae and Sparidae, measure from the most anterior terminus of the upper lip. If the mouth is located on the ventral surface (inferior mouth) as in Engraulidae and Polynemidae, measure from the snout tip located superior to the upper lip. Because the terminus of the vertebral column, the posterior terminus of the measurement, is also the base of the caudal fin, use the folding line made by bending the caudal fin hard as the terminus of the measurement. This is the parameter valued most in ichthyology as the measurement representing the size of fish.

3. **Fork length (FL)**

This is the length from the snout tip to the point where the posterior margin of the upper and lower lobes of the caudal fin meet. This is used for the measurement of fish with a crescent or fork type of caudal fin. The start point of measurement is the same as that of the standard length in 2. The posterior terminus is the most concave part at the center of the posterior margin of the caudal fin. This is regarded as an important measurement parameter as an alternative for the standard length in fish in Carangidae and Scombridae in which joint parts of the caudal fin are so rigid that it is difficult to bend the fin.

4. **Head length (HL)**

This is the length from the snout tip to the most posterior margin of the branchiostegal

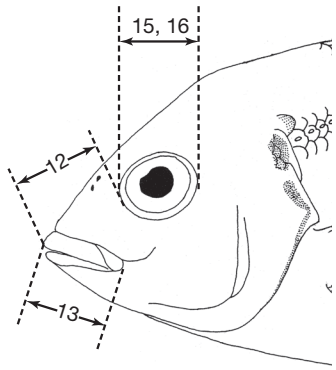


Fig. 2

Fig. 3

membrane. In some cases, the measurement terminus is the posterior terminus of the skeleton constituting the operculum. However, because it is difficult to ascertain in many cases, measure this length by including the membranous part.

5. **Predorsal length (PDL)**

   This is the length from the snout tip to the start point of the dorsal fin or the first dorsal fin.

6. **Preanal length (PAL)**

   This is the length from the snout tip to the center of the anus. Alternatively, there is also a method that measures the length to the start point of the anal fin.

7. **Body depth (BD) or body height (BH)**

   This is the height at the highest part of the body. Measure this by excluding the fleshy part and scales belonging to fins, and additionally note the measurement point by the ray number of the dorsal fin, etc. Because the position of the highest part varies among individuals, or depending on the physiological condition even in the same individual, the measurement site is often prescribed, such as the start point of the first dorsal fin and the position of the median spine of the dorsal fin.

8. **Depth of caudal peduncle**

   This is the minimum height of the caudal peduncle (from the posterior terminus of the anal fin base to the caudal fin base).

9. **Length of caudal peduncle**

   This is the length from the posterior terminus of the anal fin base to the center of the caudal fin base.

10. **Basal length of dorsal fin**

    This is the length from the start point of the dorsal fin (anterior terminus of the base of the first ray) to the posterior terminus of the base (posterior terminus of the base of the last ray). The fin membrane part following this should not be included. If the dorsal fin is separated into two or three parts, measure the size of each of them.

11. **Basal length of anal fin**

    This is the length from the start point of the anal fin to the posterior terminus of the base. The notes for this length are the same as above.

12. **Snout length**

    This is the length from the snout tip to the anterior margin of the orbit.

13. **Maxillary length or length of upper jaw**

    This is the length from the most anterior point of the upper lip (premaxillary) to the posterior terminus of the maxillary. Note that this is not the length of only the maxillary.

14. **Interorbital width**

    This is the minimum width of the bony part between the orbits when viewed dorsally. The minimum width including the fleshy part is measured in some cases.

15. **Orbit diameter (OD) ≒ eye diameter**

    This is the maximum diameter of the fleshy orbit, measured horizontally where possible. The bony orbit diameter is measured by applying a vernier caliper hard in some cases. The orbit refers to a groove in which an eyeball is located.

16. **Eye diameter (ED)**

    This is the maximum horizontal diameter of the eyeball across the cornea. This is equal to the fleshy orbit diameter in many fish.

17. **Length of fin ray**

    Normally, the maximum ray length of each fin is measured. However, a ray is specified using an ordinal number in some cases. However, in the case of the caudal fin, the soft ray at the median is usually measured.

In the case of spines, a thread-like part attaching to the edge and a part extending like a soft ray should not be included. In the case of soft rays, measure the length to the tip including these extensions. Enter the number of the ray which was measured in ( ).

Counting method:

1. **Number of fins**

   If the dorsal fin (D) and the anal fin (A) among the vertical fins are divided longitudinally, count them as the first dorsal (anal) fin, the second dorsal (anal) fin, etc.

2. **Number of fin rays**

   The number of rays constituting each fin is the most basic and important countable trait for the identification of the species. At the time of the counting, you need to have sufficient knowledge of the basic structure.

   There are two kinds of Teleostei fin rays formed by any type of bony material, the non-paired spine at the median of the fin (Figure 4A) and the left-to-right paired soft rays (Figure 4B and C). Moreover, another difference between the types is that the soft rays have many transverse segments whereas spines lack them. In addition, when the soft rays are observed from the side, branched soft rays (Figure 4B) originating from a base and ramifying to several branches in the middle, and unbranched soft rays (Figure 4C) that have no branching to the tip (Figure 4C) are found. To distinguish such ray types on small specimens, you need to observe them under a stereomicroscope with transmission light. Even in the case of a large specimen, it is better to observe them with a transmission light using a fluorescent lamp or sunlight. In the case of even larger specimens, the fins of which are covered by a thick fin membrane and/or have many black pigments making ray identification difficult unless modified, compress them hard with your finger pad, or if this does not work, somewhat peel off the fin membrane.

   When counting fin rays, their bases are supposed to be counted. However, the counting of branched soft rays requires particular care. An important rule is that the last two soft rays adjacent at the base should be regarded as a branched soft ray in the dorsal and anal fins. This is based on the fact that such apparently two soft rays are supported by only one bone (pterygiophore). Do not add the number of the rudimentary ray located at the start of the dorsal fin and top and bottom

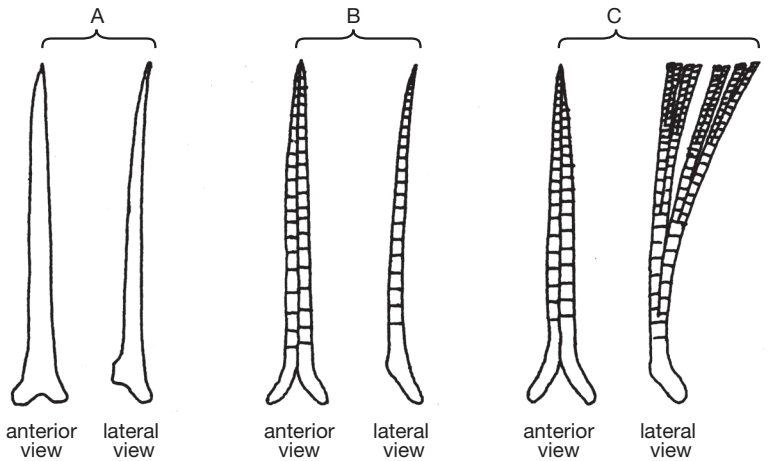


Fig. 4 Skeletal fin rays of teleosts.
A, spine; B, unbranched soft ray; C, branched soft rays.

of the caudal fin base to the number of rays. In the caudal fin (see III-7-5 and Figure 4 in III-7), only the number of all branched rays is described separately in the upper and lower lobes in some cases, and in other cases, soft rays of both the upper and lower lobes reaching nearly the posterior terminus are counted and mentioned as the number of principal rays separately in the upper and lower lobes. Generally, the number of principal rays is equal to the number of branched soft rays plus an unbranched soft ray in the upper and lower lobes.

The counting of rays of paired fins uses fins on the left side except in the case of damage and the like. Because the starting part of the pectoral fin (P_1) has a rudimentary ray and/or an unbranched ray in some cases, not only the number but also the difference in the condition is important in some cases. Regarding pelvic fins (pelvic fin; P_2), because a spine is close to the first soft ray in some cases, you must count it by separating them using a needle, etc.

The number of rays counted according to the criteria above is represented as follows:

Represent spines by capital Roman numerals (I, II, …V, … X, see p. 40), unbranched soft rays or rudimentary rays by lower case Roman numerals (i, ii, … v, …), and branched soft rays by Arabic numerals (1, 2, 3, …). In addition, represent the intervals between multiple fins separated in a vertical fin by a hyphen (-), and the border between spines and soft rays in a fin by a comma (,). The ordinal number of a fin and the number of spines or soft rays of a fin are expressed by the following fin formulas.

a) If a vertical fin composed of only soft rays is clearly separated:

D. 13-18-16; A. 21-27 (Figure 5A-example of Pacific cod *Gadus macrocephalus*)

b) If the dorsal fin is completely or nearly completely separated, and any vertical fin composed of both spines and soft rays is included:

D. XIII-1, 11; A. III, 7; P2. I, 5 (Figure 5B-example of Korean rockfish *Sebastes schlegeli*)

3. Number of scales in the lateral line (LL)

All scales arranged on the lateral line should be counted. However, if any scales are on the caudal fin, exclude them.

4. Number of pored scales in the lateral line (LLp)

Count all pored scales by excluding the poreless scales among the scales arranged on the lateral line. Exclude scales on the caudal fin.

5. Scales in a longitudinal series

In cases of lateral line not exit, running far from mid sagittal line, or breaking, scales in a longitudinal series, excluding on caudal fin, are counted, from pectoral girdle to the end of vertebrae.

6. Number of transverse scales; TR

a) Scales above lateral line; TRa: vertical number of scales on a line from base of dorsal fin to lateral line. Lateral line scale is not included. In some cases this count starts from medial spine of dorsal fin etc. with special definition.

Fig. 5 Skematic figures of teleosts.
A, Pacific Cod *Gadus macrocephalus*. B, Korean Rockfish *Sebastes schlegelii*.

b) **Scales below lateral line; TRb**: vertical number of scales on a line from base of anal fin to lateral line. Lateral line scale is not included.

Lateral line scales and transverse scales are expressed by scale formula as below.

Scales: 7+70+11

This formula means TRa is 7, LL is 70, and TRb is 11.

7. Predorsal scales

Count of scales on mid dorsal line from anterior most of dorsal fin base to front.

8. Number of gill rakers; GR, see Figure 1 in III-2.

Gill rakers are aligned in two lows, and the outer low is longer. Generally the count is taken from the outer side row of 1st gill arch, but sometimes count only on lower limb of arch with remarks.

Counts on upper limb and lower limb are joint by "+" and generally the raker on the joint is counted on lower raker as 8+12. However sometimes counted and expressed as 8+1+11 as for "Figure 2 in III-2".

Rakers on upper limb have basal structure for fordable to ventral angle, and those of lower limb have such to dorsal angle. Also the raker on the angle is fordable to both angles.

9. Number of branchiostegals; Br

Number of limb-like bones under head, appearing when mouth closing.

10. Number of pyloric caeca

Count number of all tips, except in cases of branching.

correspondence of Arabian and Roman Numbers

Arabian Numbers	Roman Numbers	Arabian Numbers	Roman Numbers
1	I	21	XXI
2	II	22	XXII
3	III	···	···
4	IV	28	XXVIII
5	V	29	XXIX
6	VI	30	XXX
7	VII	40	XL
8	VIII	50	L
9	IX	60	LX
10	X	70	LXX
11	XI	80	LXXX
12	XII	90	XC
13	XIII	98	IIC
14	XIV	99	XCIX
15	XV	100	C
16	XVI	101	CI
17	XVII	126	CXXVI
18	XVIII	200	CC
19	XIX	300	CCC
20	XX	400	CD
		500	D

III

Observation of internal morphology

III 1

Formation of viscera

Fresh fish viscera (sg. viscus) have peculiar solidity and touch. These characters are not available from preserved specimens, and need fresh specimens to observe them. The positions of viscera in abdominal cavity are often dislocated by dissection in fresh specimens, so need formalin fixed (and ethanol preserved) are used for such observations. In this chapter digestive organs and other viscera of Blue Mackerel *Scomber australasicus* are shown in line figure (Fig. 1) and color photographs (Fig. 2).

Operation starts from cutting off opercle, by cutting from lower most part of gill membrane (widely united with throat in some species) to both side of joint of lower jaw. Lifting up opercle and cut off the joint with neurocranium, and continuously cut off postorbital region to both joints of upper jaw. Or, just cut off suborbital region by heavy-duty scissors (= bone scissors).

The next is removing lateral muscles, by inexperienced students, cutting from anus (16) to ventral medial line by scissors. Experienced students start cutting around anus by surgical knives without

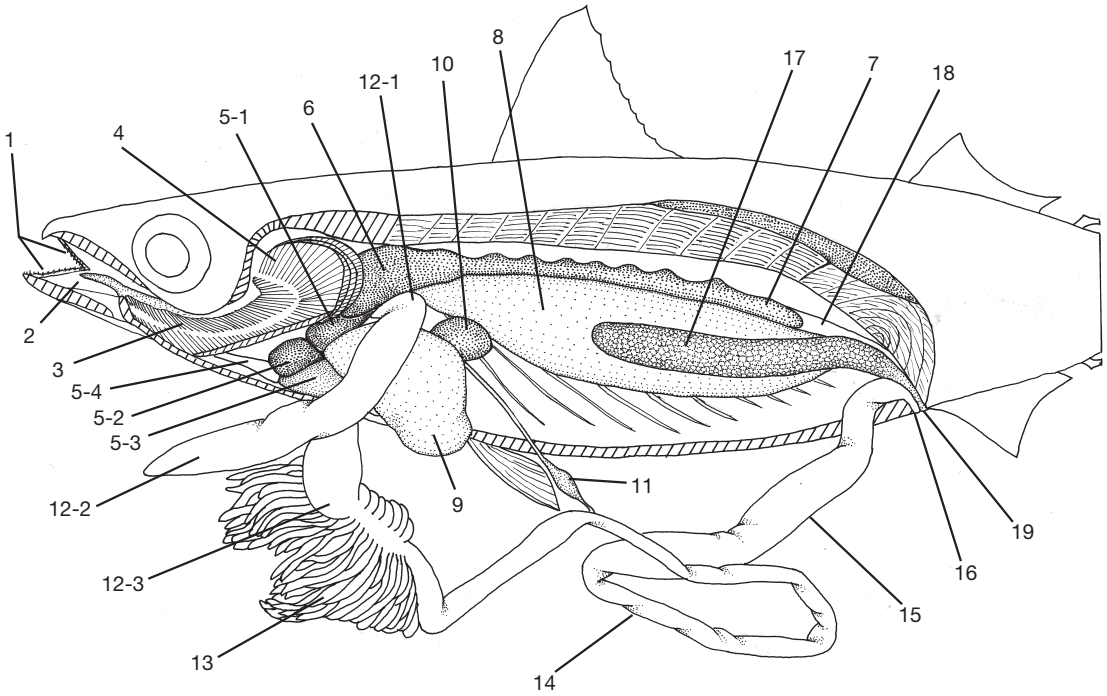


Fig. 1 Viscera of Blue Mackerel *Scomber australasicus*.

1. mouth (jaws)
2. oral cavity
3. gill raker
4. gill filament
5. heart
   - 5-1 venosus sinus
   - 5-2 auricle
   - 5-3 ventricle
   - 5-4 arterial bulb
6. head kidney
7. body kidney
8. air (gas) bladder
9. liver
10. spleen
11. gall bladder
12. stomach
    - 12-1 cardia
    - 12-2 blind sac
    - 12-3 pylorus
13. pyloric caeca
14. intestine
15. rectum
16. anus (=vent)
17. gonad
    - ovary
    - testis
18. urocystis
19. urogenital orifice

damaging digestive organs, and next, with scissors, continue cutting forward. Further, cut with scissors from anus by dorso-anterior angle of left side to near pectoral fin base. Carefully remove kidneys (7) one by one, hidden under lateral process of vertebrae. Before doing this, swim bladder (absent in Atlantic Mackerel *S. scombrus*) appears but is easily broken in dissecting process, and hard to observe or draw.

Fig. 2 Anatomical steps of Blue Mackerel *Scomber australasicus*.
Upper, removing opercula and lateral muscles; Middle, removing pectoral girdle and base of ribs;
Lower, Pulling off of gastrointestinal tract.

The last of this serial dissection is removing firm bones of pectoral girdle (Fig. 2, middle). This step should be very careful to avoid injure soft parts connected nearby underside of the bone, such as head kidney (6) or sinus venosus (5-1). Cut off the connection of upper (=dorsal) side of pectoral girdle and neurocranium. Also cut off lower (=ventral) side connection of both side of cleithrum.

Pull down viscera in abdominal cavity for observation and drawing (Fig. 2). To avoid damage by dissecting instruments on viscera, this step should be done by hands, and carefully cut peritoneum by fingers, and keep attaching of start and end of digestive organs with body.

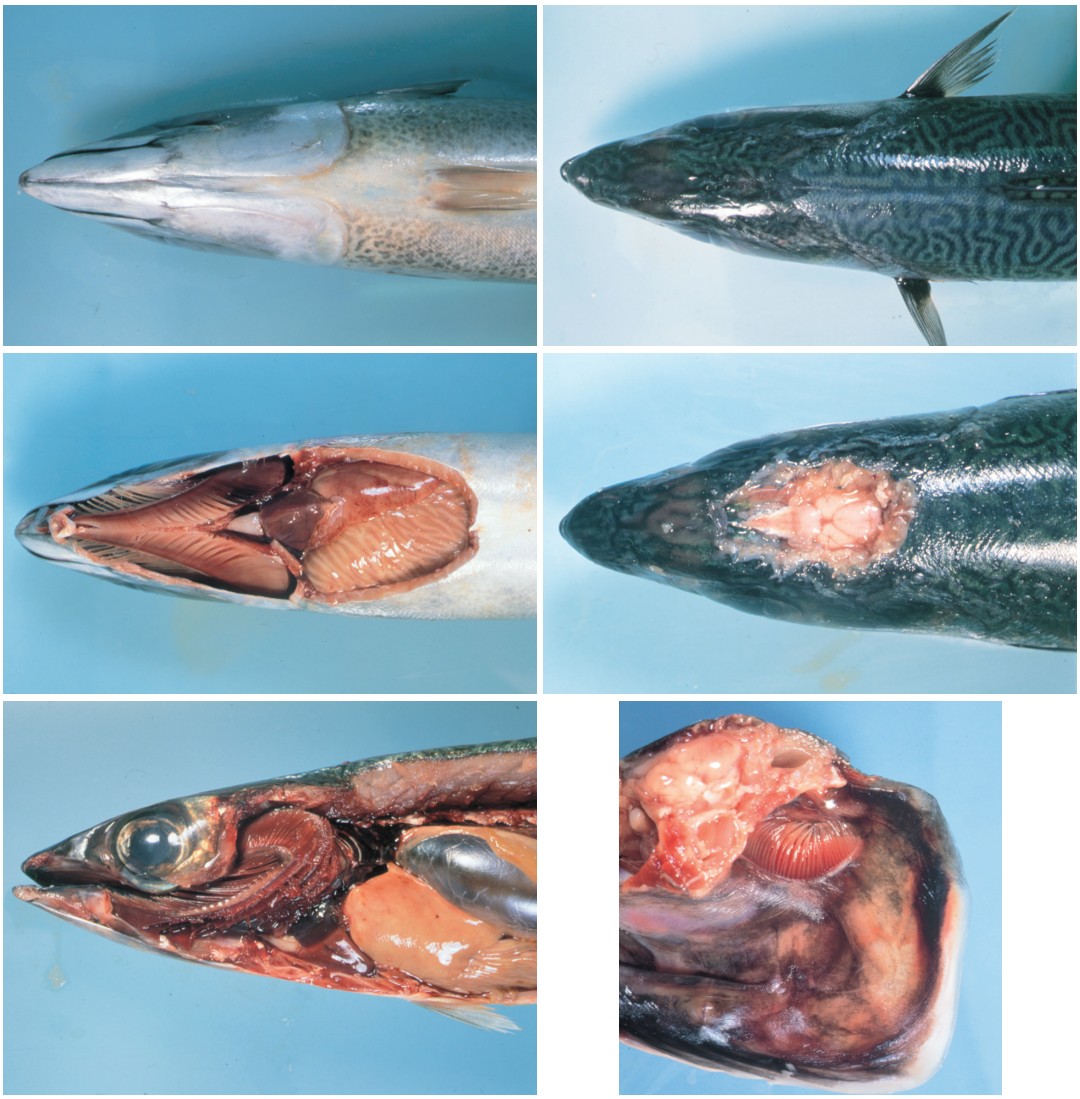


Fig. 3 Dissecting Blue Mackrel Scomber australasicus.
Left upper: ventral view of head and thorax. Left middle: dissection of ventral view of head and thorax. Left lower: lateral view of dissected head and thorax.
Right upper: dorsal view of head and parietal region. Right middle: Dissection of head and parietal region. Right lower: inside of opercula, showing parabranchia.

III 2 Observation of gills

Gills are common in fish, occupy wide space under neurocranium, and are easily recognized red organs by opening gill slit. One set of gill, the main part is thin curved bone, or gill arch, with tubercular or rodlike process on anterior part, or gill rakers. The posterior part is with numerous soft red flaps, or gill filaments.

One gill arch consist of > shaped upper limb, and lower limb. The stem of upper limb is epibranchial (No.60), and the stem of lower limb consists of two bones, hypobranchial (No. 57) for anterior part, and ceratobranchial (No. 58) for posterior part. Both left and right sides of gill arch are connected by a lined several bashibranchials (No. 56), and its anterior tip is homologous with human tang, including basyhyal (No. 48) in hyoid arch. The anterior tip of upper limb is hanged down from neurocranium by suprapharyngobranchial (No.61). On the way of evolution, original 5 pairs of gill arches modified in recent fishes, as upper surface of lower branch of 5th gill arch with lower pharyngeal teeth, and called as lower pharyngeal (No. 59). Also upper branch of 4th and both branch of 5th arches are modified to upper pharyngeal (No. 63) with lower pharyngeal teeth on the lower surface. Pharyngeal teeth of fishes have rich morphological variation, and used important taxonomic keys for some taxon such as the order Cypriniformes and family Scaridae.

Generally there are gill rakers on the anterior surface of 4 pairs of gill arch, generally those on the 1st (most outside, most anterior) are conspicuously developed. Each of gill rakers is covered with densely minute spines on surface, and the epithelium has taste buds. Gill rakers are filtering organs for aspirated water, and plankton feeder has many long rakers, but those of swallowing carnivores are lost or rudimental. The shape or number of rakers related with feeding habitat, and are used for important taxonomic key characters. Count of gill rakers is done on the outside row of 1st gill arch (see II-2).

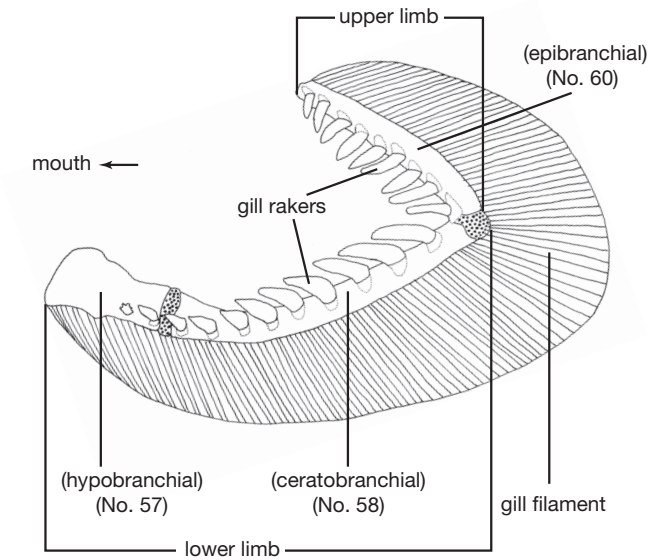


Fig. 1 Anterior most (= external most) gill arch of Red Sea Bream *Pagrus major*. Numbers on figure indicate names of skeletons, III-3. Dotted lines mean joints.

Gill filaments are gas exchanging organs of oxygen and carbon dioxide between brad and environmental water, and also keep salinity balance of body. There are 2 rows of gill filaments on posterior end of 1st to 4th gill arches. In elasmobranchs, 2 rows on each gill arches are divided by interbranchial septum, and 5 – 7 pairs of external gill slits are opened. In teleosts, interbranchial septum are degenerated and lost, and gill filaments are arranged in parallel. Each gill filaments has many vertical (secondary) gill lamella on upper and lower sides, and such expanded surface area is effective for gas and mineral exchanges. Length and number of gill filaments are related with fish activity. Pelagic fishes have developed long filament, and benthic and slow swimming fishes have undeveloped filaments. Thus, relative length of the filaments is a material to consider fish activities.

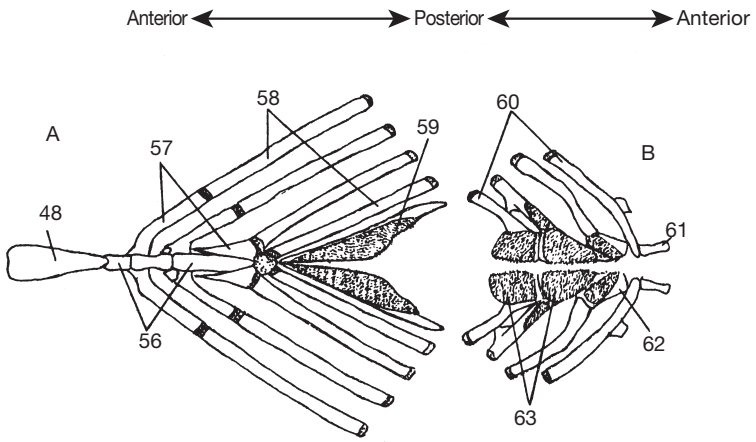


Fig. 2 Gill arch of Japanese Sea Bass *Lateolabrax japonicus*.
A, lower half. B, upper half. From Mastsubara et al. (1979). See III-3 for names and numbers of bones.

Fig. 3 Gills of various fishes.
A, pelagic fish, Frigate Tuna *Auxis thazard*; B, carnivorous fish, Humpback Red Snapper *Lutjanus gibbus*; C, plankton feeder, Midnight Snapper *Macolor macularis*; D, mesopelagic fish, Long Snouted Lancetfish *Alepisaurus ferox*; E, benthic fish, Yellowfin Stargazer *Uranoscopus chinensis*.

III 3 Names and classification of skeletons

Numbers of text indicate those on figures of III-2~III-7.

- Endoskeleton
  - Axial Skeleton
    - Cranium
      - Neurocranium ····· Nos. 1-23
        Protecting central nerve systems and sensory organs
      - splanchnocranium ····· 24-63
        Supporting and protecting both jaws, hyoid arch, branchial arch, palate, and gills
    - Nothochord
      Running below spinal cord, and supporting it
    - Vertebral Column ····· 64-86
      Covering and protecting spinal cord and notochord
  - Appendicular skeleton
    - Pectoral Girdle ····· 87-96
      Supporting pectoral fin
    - Pelvic Girdle ····· 97
      Supporting pelvic fin
    - Pterigiophore ····· 98-104
      Supporting rays of each fins
- Exoskeletin ·····
  - Fin Ray ····· 105-112
  - Scale

No.	English Names
1–23	**neurocranium (= cranium)**
1 (III-6, Fig. 1)	ethmoid
2 (III-6, Fig. 1)	ethmoid cartilage
3 (III-6, Fig. 1)	lateral ethmoid (= prefrontal)
4 (–)	preethmoid
5 (–)	supraethmoid (= mesethmoid)
6 (III-6, Fig. 1)	prevomer
7 (III-5, Fig. 1; III-6, Fig. 1)	nasal
8 (III-6, Fig. 1)	rostral (cartilage)
9 (III-5, Fig. 1)	sclerotic
10 (III-6, Fig. 1)	frontal
11 (III-6, Fig. 1)	pterosphenoid
12 (III-6, Fig. 1)	basisphenoid
13 (–)	orbitosphenoid
14 (III-6, Fig. 1)	sphenotic
15 (III-6, Fig. 1)	pterotic
16 (III-6, Fig. 1)	epiotic
17 (III-6, Fig. 1)	prootic
18 (III-6, Fig. 1)	exoccipital
19 (III-6, Fig. 1)	supraoccipital
20 (III-6, Fig. 1)	parietal

No.	English Names
21 (–)	intercalar (= opisthotic)
22 (III-6, Fig. 1)	basioccipital
23 (III-6, Fig. 1)	parasphenoid
24–63	**splanchnocranium (= visceral skeleton)**
24 (–)	supraorbital
25 (III-5, Fig. 1)	lachrymal (= preorbital)
26 (III-5, Fig. 1)	infraorbital
27 (–)	suborbital
28 (III-5, Fig. 1)	premaxillary
29 (III-5, Fig. 1)	maxillary
30 (–)	supramaxillary
31 (III-5, Fig. 1)	dentary
32 (III-5, Fig. 1)	angular
33 (III-5, Fig. 1)	retroarticular
34 (–)	articular
35 (–)	coronomeckelian
36 (III-5, Fig. 1)	mentomeckelian cartilage
37 (III-5, Fig. 1)	palatine
38 (III-5, Fig. 1)	ectopterygoid (= pterygoid)
39 (III-5, Fig. 1)	endopterygoid (= mesopterygoid)
40 (III-5, Fig. 1)	metapterygoid
41 (III-5, Fig. 1)	quadrate
42 (III-5, Fig. 1)	symplectic
43 (III-5, Fig. 1)	hyomandibular
44 (III-5, Fig. 1)	preopercle
45 (III-5, Fig. 1)	opercle
46 (III-5, Fig. 1)	subopercle
47 (III-5, Fig. 1)	interopercle
48 (III-2, Fig. 2; III-5, Fig. 1)	basihyal (= glossohyal)
49 (III-5, Fig. 1)	upper hypohyal lower hypohyal
50 (III-5, Fig. 1)	ceratohyal
51 (III-5, Fig. 1)	epihyal
52 (III-5, Fig. 1)	interhyal
53 (III-5, Fig. 1)	branchiostegal
54 (–)	gular plate
55 (III-5, Fig. 1)	urohyal
56 (III-2, Fig. 2)	basibranchial
57 (III-2, Fig. 1; III-2, Fig. 2)	hypobranchial
58 (III-2, Fig. 1; III-2, Fig. 2)	ceratobranchial
59 (III-2, Fig. 2)	lower pharyngeal
60 (III-2, Fig. 1; III-2, Fig. 2)	epibranchial
61 (III-2, Fig. 2)	suprapharyngobranchial (= suspensory pharyngeal)
62 (III-2, Fig. 2)	infrapharyngobranchial (= pharyngobranchial)
63 (III-2, Fig. 2)	upper pharyngeal
64–86	**vertebral column**
64 (III-7, Fig. 1)	abdominal vertebra
65 (III-7, Fig. 1; III-7, Fig. 4)	caudal vertebra
66 (III-7, Fig. 4)	preuralcentrum 2, 3

No.	English Names
67 (III-7, Fig. 4)	urostyle (= preural centrum 1 + ural vertebra)
68 (III-7, Fig. 4)	hypural
69 (III-7, Fig. 4)	uroneural
70 (III-7, Fig. 4)	epural
71 (III-7, Fig. 4)	Parhypural
72 (III-7, Fig. 3)	neural spine
73 (III-7, Fig. 3; III-7, Fig. 4)	neural arch
74 (III-7, Fig. 3)	neural zygapophysis
75 (III-7, Fig. 3)	centrum
76 (III-7, Fig. 3)	hemal zygapophysis
77 (III-7, Fig. 3)	hemal arch
78 (III-7, Fig. 3)	parapophysis (= transverse process)
79 (III-7, Fig. 3; III-7, Fig. 4)	hemal spine
80 (III-7, Fig. 3)	neural canal
81 (III-7, Fig. 3)	hemal canal
82 (III-7, Fig. 3)	pleural rib (= rib)
83 (–)	epipleural
84 (–)	epineural
85 (III-7, Fig. 3)	epicentral
86 (–)	myorhabdoi (= myorabdoi)
87–96	**pectoral girdle**
87 (III-5, Fig. 1)	supratemporal (= extrascapular)
88 (III-5, Fig. 1)	posttemporal
89 (III-5, Fig. 1)	supracleithrum
90 (III-5, Fig. 1)	cleithrum
91 (III-5, Fig. 1)	scapula
92 (–)	mesocoracoid
93 (III-5, Fig. 1)	coracoid
94 (III-5, Fig. 1)	postcleithrum
95 (–)	clavicle
96 (III-5, Fig. 1)	actinost
97	**pelvic girdle**
97 (III-5, Fig. 1)	basipterygium
98–104	**pterygiophore**
98 (III-7, Fig. 1)	free interneural spine (= predorsal bone)
99 (III-7, Fig. 1; III-7, Fig. 2)	interneural spine
99, 102 (III-7, Fig. 1; III-7, Fig. 2)	proximal pterygiophore
100, 103 (III-7, Fig. 2)	median pterygiophore
101, 104 (III-7, Fig. 2)	distal pterygiophore
102 (III-7, Fig. 1; III-7, Fig. 2)	interhemal spine
105–112	**exoskeleton**
105 (III-7, Fig. 1)	dorsal spine
106 (III-7, Fig. 1; III-7, Fig. 2)	dorsal soft ray
107 (III-7, Fig. 1)	anal spine
108 (III-7, Fig. 1; III-7, Fig. 2)	anal soft ray
109 (III-7, Fig. 1; III-7, Fig. 4)	caudal soft ray
110 (III-5, Fig. 1)	pelvic spine
111 (III-5, Fig. 1)	pelvic soft ray
112 (III-5, Fig. 1)	pectoral soft ray

III 4 How to observe skeletons

1. **Studies on skeletons** are called osteology. Osteology is peculiar to Vertebrata and is essential for studies on their phylogenic evolution and species identification. This is because osteology has the following advantages.
   1) Highly objective data can be obtained because a stable and consistent result can be obtained regardless of when or who observes and measures a skeletal trait, due to its very low deformation in long-term storage.
   2) Because bones, in particular, are likely to be preserved as fossils among skeletons, osteology allows us to directly compare and examine historical taxa. In other words, in terms of the traceability of evolutionary history, this is an advantage over studies on muscles, visceral organs, chromosomes, genes, proteins, etc. from which no data can be obtained unless the specimen is contemporary, due to the difficulty in preservation.
2. **How to study skeletons of contemporary fish**
   1) X-ray photography: This has the advantage that individual variation in the counted values, including the number of the vertebrae and the number of the pterygiophores, can be reduced because many specimens can be relatively easily examined non-destructively if the specimens are unpaired skeletons (vertebral column, vertical fins, etc.). However, disadvantages include that the outline of cartilages and bone pieces with a thin periphery are not clear, and that it is impossible to understand the shape of paired skeletons due to overlap in the image.
   2) Separation of skeletons: Separate the skeleton from the body by boiling the raw or salted specimen or letting insects (Dermestidae, etc.) eat the fleshy parts. Preservation by dehydration is convenient for studies on the shape of individual bones and using the skeletons as educational materials due to ease of handling, but cartilages can be so deformed that their original shape is not preserved, and even bones will be a little deformed. Preservation of picked skeletons in alcohol can preserve the shape, but it has the disadvantage that the linkage between bones is not preserved because the bones will eventually disassemble.
   3) Preparation of double-stained transparent specimens: Stain the bones of specimens in red with alizarin red and their cartilages in blue by alcian blue, make the skin and the muscles of the specimens transparent using potassium hydroxide and trypsin, and observe the bones and cartilages. This method allows us to observe the best condition because the shape of the fish body is maintained unchanged, preserving the linkages between the bones and causing no deformation of bones and cartilages. However, it takes days to prepare a specimen, and the protocol requires skill. In addition, the handling of specimens is a little troublesome because they are preserved in glycerin. I have omitted this preparation method in this book because many informative books that have been recently published discuss this. (References: Osamu Fukuhara, Masaru Tanaka. 1987. Techniques for studies on the early life history of marine fish 1 - How to stain hard tissues of larvae and juveniles. Aquabiology, 9 (2): 97-99; Koichi Kawamura, Kazumi Hosoya. 1991. A modified double staining technique for making a transparent fish-skeletal specimen. Bulletin of Natural Research Institute of Aquaculture, (20): 11-18)
3. **Experimental method according to 2) above**
   1) Wrap the specimen with a sheet of gauze to prevent the loss of small bones and cartilages.
   2) Boil the specimen in dilute potassium hydroxide (KOH, below 0.1%) aqueous solution. Stop

the boiling at the point when a little of the redness of blood remains in the skeleton when you peel off the muscle in the vicinity of the most anterior part of the vertebral column. Excessive boiling can cause the neurocranial members to be dislocated at the joints. Once dislocated, they can never be restored. Although KOH makes it easy to remove flesh, excessive use is harmful because bones, particularly soft rays, will be fragile.

3) Collect the external skeletons in the vicinity except scales (I postpone this to another occasion) in the following order in general. Because most of the bones except for the axial skeletal are in a right-to-left pair, preserve those on the left side, and put those on the right side aside for supplementation.
4) Find the name of each bone, and note the mutual relationship through a joint by sketching the bones in a way that indicates their top and back as well as four quarters.
5) Draw an outline of the fish on another Kent paper prepared beforehand, and temporarily place the picked bones on a place equivalent to their *in vivo* location. Avoid paying too much attention to cleaning in this step and complete the procedure soon. Because the skeletons are arranged three-dimensionally, you need to be creative in arranging the bones two-dimensionally.
6) First, pick up the nasal bone (No. 7), the circumorbital [there are the lacrimal bone (No. 25), the infraorbital bone (No. 26), and, depending on the species, the supraorbital bone (No. 24) and the subinfraorbital bone (No. 27)], and the supratemporal bone (No. 87). Because the supratemporal bone is very likely to be lost, be sure to pick it up in this step. (A→B in the foldout figure.)
7) Remove the muscles for moving the jaws and the muscles at the pectoral fin base, and then pick up the bones forming the upper jaw [there are the premaxillary (No. 28), the maxillary (No. 29), and, depending on the species, the supramaxillary (No. 30)]. (B→C in the foldout figure)
8) After picking up the dentary (No. 31) and the angular bone (No. 32) forming the lower jaw, pick up the palatine arch (Nos. 32–43) and the opercular arch (Nos. 44–47) together and disassemble them later. (C→D in the foldout figure)
9) Pick up the basihyal (No. 48, only one at the center) from the position equivalent to the human tongue, and then the following hyoid arches (Nos. 48–53) together, and disassemble them later. (D→E in the foldout figure)
10) After picking up the urohyal (No. 55, only one at the center) which was between the bilateral hyoid arches, pick up all the branchial arches from both the right and left sides together, and disassemble them later. (No foldout figure. See the branchial arches of Japanese sea *Lateolabrax japonicus* in Figure 2 in III-2)
11) Disassemble and pick up the pectoral girdle (Nos. 88–96) and the pelvic girdle (No. 97) inferiorly in order. At this time, combine the soft rays together because they are fragile.
12) Remove the upper part of the dorsal muscle, and then pick up the dorsal-fin pterygiophores (Nos. 99–101) and rays (Nos. 105–106) by recording the positional relationships with the neural spine (No. 72). (See Figure 1 on p. 58.)
13) Collapse the contact plane (horizontal septum) between the myomeres of the dorsal and ventral muscles, record the position of the joints between the vertebra (Nos. 64–81) and the rib (No. 82) as well as the intermuscular bone (Nos. 83–86), and pick up the set except the vertebra. Repeat this for the following sets of myomeres.
14) Remove the lower half of the ventral muscle, and then pick up the anal-fin pterygiophores (Nos. 102–104) and rays (Nos. 107–108) by recording the positional relationships with the hemal spine (No. 79). (See Figure 1 on p. 58.)
15) Finish this procedure by picking up the neural cranium (Nos. 1–23) and the vertebral column (Nos. 64–81) together.

16) Carefully remove muscles, blood vessels, bone marrow, etc. from each of the picked skeletal elements. Then, clean the element and arrange and tape it on thick paper or in a wooden box, or preserve it in 70% ethyl alcohol solution.
17) You need to be extremely careful not to disassemble the caudal skeleton at the terminus of the vertebral column.

III 5

Observation of the splanchnocranium, pectoral girdle, and pelvic girdle

The major skeletal arranged in the axial part of a fish body is called the axial skeleton. The axial skeleton is composed of the cranium or the skull (neurocranium and splanchnocranium) followed by the notochord and the vertebral column to which the pleural ribs are attached, and a variety of intermuscular bones.

Because many fish crania are exposed medially and/or laterally, their morphological characteristics are often seen in descriptions of fish species. Therefore, to avoid missing bones that can be examined in species identification, it is important to know the site and morphological characteristics of individual bones.

It is not the case that all of the bones presented in the table in III-3 are present in a species used as the experimental material, and that the figures on p. 45–62 show all bones. In addition, you should pay close attention to the collection of individual bones because bones may have become soft and cartilages may have become hard.

Splanchnocranium; Nos. 24–63 (= visceral skeleton): This is present in the periphery of the neurocranium and composed of many of the very slender bones. When picking these up, refer to the foldout figure to determine from where to pick them up. Temporarily place the removed bones on a sheet of paper by determining their arrangement based on Figure 1 below, and enter the name. Clean

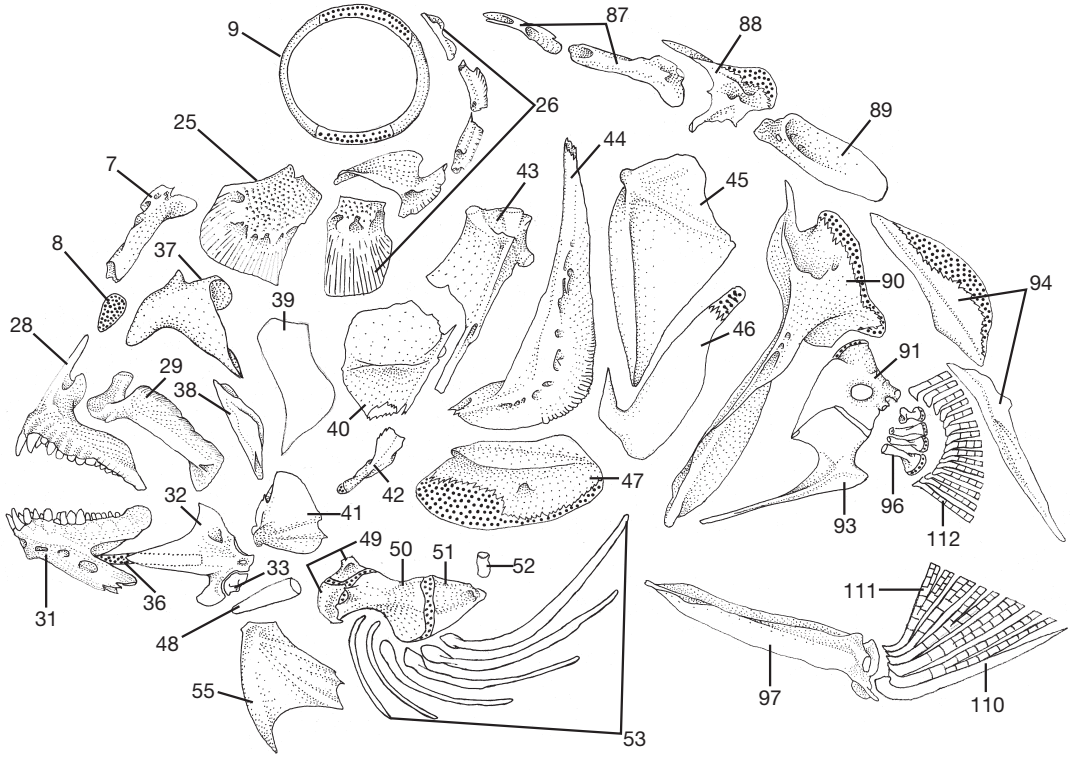


Fig. 1 Splanchnocranium of Red Sea Bream *Pagrus major*.
Large dots indicate cartilages. See table of III-3 for bone names and numbers.

the bones individually after observation and sketching, tape them to their original position, and make a clean copy of the names. Be careful to avoid an error in the anterior–posterior, top–bottom, and top–back orientations of each bone. Also, pay attention to the bones through which the lateral line canals on the head run (lacrimal bone; No. 25, infraorbital bone; No. 26, dentary; No. 31, premaxillary; No. 44, etc.) and the route, opening, and arrangement of tunnels through which vessels or nerves run. In addition, because there are not only the two jawbones but also the bones on which teeth are present (palatine: No. 37, etc.), follow the procedures below with careful observation.

Circumorbital; Nos. 24-27: The orbit of some splanchnocrania is constituted of a large lacrimal bone (No. 25) anterior to the eye and a series of infraorbital bones (No. 26) surrounding the posterior margin of the eye from immediately posterior to the lacrimal bone via the infraorbital part. The splanchnocranium of ancient fish has a supraorbital bone (No. 24) fringing the upper margin of the eye in some cases. Naturally, these are in a left-to-right pair. The inner margin contacting the eye is thick because sensory canals pass through it, and it has scattered openings for branched canals on the outer surface. In fish belonging to Perciformes, etc., the first and second anterior infraorbital bones have an overhang facing medially like it is supporting the eyeball, which is called the suborbital shelf. The position and number of the suborbital shelf are a clue for investigating relationships. Scorpaeniformes fish show a common peculiar specific trait that the posterior margin of the second infraorbital bone extends to form a joint with the preopercle (No. 44) on the surface. [In the anterior margin of the snout, there is a pair of thin plate-like nasal bones (No. 7) inside the right and left nostrils. This is free from the neurocranium, but considered a part of neurocranium instead of the splanchnocranium.]

Upper jaw; Nos. 28–30: Inside the nasal bone, there is an ascending process of the premaxillary (No. 28), and inferior (posterior) to the processes of the right and left bones, there is an oval or rice-ball-shaped rostoral bone (No. 8) (unpaired). After taking out the bones forming the upper jaw in conjunction with the premaxillary, the bone adjacent superiorly is the maxillary (No. 29), and, depending on the species, 1–2 slender supramaxillary bones (No. 30) run in parallel in its posterior dorsal part. On the inferior margin of the premaxillary, there are a variety of small to large teeth depending on the species. In ancient fish including Clupeidae, the premaxillary is small and the oral margin is formed by the maxillary, etc., where some species such as Amia, gars, and deep-sea fish in Argentiniformes and Stomiidae have teeth. In many of the advanced fish groups, such as Perciformes and Scorpaeniformes, the mouth is formed by only the premaxillary extending highly to the posterior direction, and the maxillary is recessive and does not face the oral margin. The developmental level of the ascending processes in the anterior superior part of the premaxillary varies remarkably depending on the species, from those in which it moves like it is rotating using the short rostoral bone (No. 8) immediately inferior as a fulcrum to the species in which the upper jaw can greatly protrude by sliding the long process.

After removing the upper jaw, removal of the muscle, etc. of the cheek allows you to observe a series of bones linked from the lower jaw to the posterior terminus of the operculum. Cut these bones, keeping the joints where possible off the hyoid arch inside (below of observation angle) them, cut the left-to-right jointing part of the lower jaw and temporarily place the whole piece longitudinally. After such preparation, disassemble and clean them step-by-step as follows.

Lower jaw, Nos. 31–36: The dentary (No. 31) located at the most anterior part of the selected bones is the only bone constituting the oral margin of the lower jaw. Because the dentary and teeth on the premaxillary have various morphologies associated with their feeding habit, you need to observe them sufficiently. The arrowhead-like bone sticking into the deep notch of this dentary anteriorly is called the angular bone (No. 32). Inside the dentary and angular, there is the slender Meckel's cartilage (No. 36) connecting the two bones. Because this will accompany either one when the bones are pulled apart, do not further disassemble it. The minute bone called the coronomeckelian (No. 35) at the

base of this cartilage on the angular side has been gaining phylogenic attention recently. Do not cut off a small bone called the retroarticular bone (No. 33), either, which is seen at the lateral side of the posterior inferior part of the angular.

Suspensorium, Nos. 37–47: These are a series of bones suspending the mandibular arch from the neurocranium, and composed of the opercular arch (Nos. 44–47), commonly called the gill cover, and the palatine arch (Nos. 37–43) constituting the inner wall of the oral cavity. The opercular arch is the main organ at the time of gill ventilation for gas exchange. In addition, because the four types of membrane bones constituting the arch can be observed from the surface, these are also valued as taxonomic characters. The large membrane bone at the upper right is called the opercle (No. 45). This forms a joint with the hyomandibular bone (mentioned below) at the anterior supra corner, and the presence or absence of any spine at the posterior margin is an important characteristic. The V-shaped thin subopercle (No. 46) contacts the opercle such that it supports it from the bottom. There is a large semilunar preopercle (No. 44) widely contacting the anterior margin of the opercle. The interopercle (No. 47) contacts the inferior side of both the preopercle and subopercle. The anterior margin of the preopercle forms a joint with the palatine arch. It is particularly frequent that the shape of the lateral margin of the preopercle is used as a taxonomic character.

The palatine arch is little exposed to the outside. However, if it has teeth, the teeth are exposed to the inner wall of the oral cavity, and are used as a taxonomic character. The somewhat T-shaped firm bone forming a joint with the upper part of the preopercle posteriorly is called the hyomandibular bone (No. 43). The hyomandibular forms a joint with the neurocranium at the upper condyle, and also with the opercle at the posterior condyle. Between the inferior terminus of the hyomandibular and the quadrate mentioned below is the slender rod-like symplectic (No. 42). There is a very thin, plate-like metapterygoid (No. 40) forming a joint with the anterior inferior part of the hyomandibular bone and the endopterygoid (No. 39) anterior to it. The fan-shaped quadrate (No. 41) forms a joint with the inferior part of the metapterygoid, endopterygoid, and symplectic. The quadrate forms a joint with the angular of the lower jaw at the condyle on the part equivalent to the fulcrum of a fan. At the anterior margin of the angular and the endopterygoid, there is the ectopterygoid (No. 38). There is the palatine arch (No. 37), the anterior tip of which is like a hook, attaching to the anterior half of this ectopterygoid and the endopterygoid. The hook-like part is exposed in the form of an inverted V beside the prevomer, the anterior terminus of the neurocranium, on the ceiling of the oral cavity. The presence of any tooth there and the shape of the tooth are important taxonomic characters.

Hyoid arch; Nos. 48–55: There are several curved, sword-like slender branchiostegals (No. 53) inside the lower part of the opercular arch. The bones supporting them are composed of the relatively wide bone, the ceratohyal (No. 50), with a large hole at the center or in a saddle shape; the epihyal (No. 51) sutured to the ceratohyal anteriorly; and the hypohyal (No. 49; composed of top and bottom ones) sutured to the ceratohyal posteriorly. These three types of four bones cannot be restored once disassembled. Because the number and shape of the branchiostegals and the arrangement of the attachment to the inferior margin of the ceratohyal and the epihyal vary greatly among fish species, these are important taxonomic characters and powerful factors for considering phylogenic relationships. There is a small, rod-shaped interhyal (No. 52) superior to the posterior terminus of the epihyal, which helps to form a joint with the symplectic in the palatine arch from the inner surface of the operculum. All of the above are left-to-right paired. A sheet of the triangular urohyal (No. 55) inferior to the most anterior basibranchial bone (No. 56 in the branchial arch below) sandwiched by the bilateral hypohyals, and the unpaired baseball-bat-shaped basihyal (No. 48) forming a joint with the anterior terminus of the basibranchial bone are the hyoid arch. The basihyal is the part equivalent to the human tongue. Some fish have teeth here as well.

Branchial arch; Nos. 56–63 (see Figure 2 in III-2): After removal of the upper and lower jaws,

the suspensorium, and the hyoid arch, the branchial arch appears. Pick these all up together, then remove the arch individually from the lateral side in order on both the right and left sides, clean them, and place them in a row symmetrically on the paper. In the arrangement, I advise you to extend them in a shape that is formed in a widely opened mouth if seen posteriorly. Lastly, arrange the multiple basibranchial bones (No. 56) in the middle of the lower branches of the branchial arch. The structure of each branchial arch is mentioned in detail in "III-2. Observation of gills".

Appendicular skeleton: This refers to the skeletons supporting the rays of the dorsal fin, anal fin, and paired fins from inside of the body. Here we can observe the pectoral girdle supporting the pectoral fin and the pelvic girdle supporting the pelvic fin, both of which are paired fins.

Pectoral girdle; No. 87–96: Removal of the skin and the muscle from the lateral surface of the occipital region to the start point of the pectoral fin will let you find one or two slender supratemporal bones (No. 87) in some species but none in other species. This bone has a groove where the lateral line runs, which in the posterior part branches in a Y-shape. In the fish in Sparidae, this bone looks like a rain gutter and is easily lost because it is smaller than a scale. Depending on the fish species, it is treated as a part of the neurocranium because it firmly fastens itself to the cranium. Following the supratemporal bone, the crab-claw-like posttemporal bone (No. 88) is seen. An oval supracleithrum (No. 89) forms a joint with the inferior part of the posttemporal bone from inside. After collecting these, remove them up to the most anterior cleithrum (No. 90) with the accompanying soft rays of the pectoral fin (No. 112) in a group without collapse. Consequently, the two relatively thin postcleithra (No. 94) remain at the most anterior part of the ventral muscle.

Next, remove the muscle at the base of the picked pectoral fin and remove all rays of the pectoral fin (No. 112) from the attachment base in a group. In the remaining attachment base, four hand-drum-shaped actinosts (No. 96) are generally seen, but these are easily lost because they are very small in some species. The actinosts are supported by two thin, plate-like bones. The one occupying the upper half is the scapula (No. 91), with a hole near the center, whereas the lower half is occupied by the hammer-shaped coracoid (No. 93). Both bones form a joint with the posterior margin of the cleithrum (No. 90), a very large and rigid bone covering the entire area from the upper to the lower terminus of the posterior margin of the gill slit. In the ancient fish groups, such as Clupeiformes and Cypriniformes, there is a special bone connecting the cleithrum and the coracoid called the mesocoracoid (No. 92).

Pelvic girdle; No. 97: Pick up the basopterygium (No. 97) supporting the pelvic fin in conjunction with the pelvic fin rays (No. 111 and 112). The pelvic girdle is composed of only a pair of basopterygia in many of the advanced fish, and directly supports 1 spine and 5 soft rays. However, in the ancient fish groups such as Salmoniformes, it only supports many soft rays through the radials.

III 6

Observation of the neurocranium

Head of Agnatha is only a cluster of several cartilages. The neurocranium of extant advanced Chondrichthyes is called the chondrocranium and is composed of a mass of cube-type cartilages. The neurocranium of Teleostei is composed primarily of many cartilaginous bones and membranous bones (= membrane bones) sutured together, and a minor cartilaginous part. Such a neurocranium is called an osteocranium. The neurocranium protects the brain, the most anterior part of the spinal cord.

At the time of the observation, remove the left half of the splanchnocranium, pectoral and pelvic girdles, etc. in the vicinity of the head you observed earlier, cutting the remaining neurocranium (called the skull in some cases) off the joint with the first vertebra to pick it up. If possible, I advise you to use two individuals of the same species in the observation of the neurocranium to disassemble one and sketch the other in parallel. Once it is disassembled, it can never be restored.

As the arrangement of bones constituting the neurocranium is three-dimensional and complicated, it is difficult to represent all parts unless you sketch the observation results not only from the lateral sides but also from five directions, including the lateral, dorsal, ventral, anterior, and posterior directions.

At the center of the apex of the neurocranium is a prevomer (No. 6), the apex of which has a horseshoe shape in its inferior surface. This horseshoe-like part is located immediately posterior to the joint part of the right and left premaxillaries (No. 28) among the already observed splanchnocranium, and can be identified without anatomy. The presence or absence of teeth in this part and, if there are teeth, the number and shape are important taxonomic characters and helpful for predicting the feeding habit. You can see that, from both sides, the palatine arch (No. 37) among the splanchnocranium connects to the prevomer anteriorly. There is a slender, plate-like parasphenoid (No. 23) covering nearly all of the central part of the cranial base following the prevomer. This is located at the center of the ceiling of the oral cavity. Ancient fish groups are also equipped with teeth here in some cases. The basioccipital (No. 22) at the posterior terminus of the cranial base forms a joint with the vertebral column. Being surrounded by four bones, basioccipital, exoccipitals (No. 18) dorsal bilateral to the basioccipital, and dorsal supraoccipital (No. 19), the foramen magnum is formed at the posterior terminus of the cranium through which the central nerve runs. This foramen magnum connects to the neural canal of the vertebra.

An ethmoid (No. 1) is sutured to the posterior dorsal part of the prevomer. The posterior terminus of the ethmoid, which is like a cartilage and penetrates the inter-orbital part, is called the ethmoidal cartilage (No. 2). A rostoral bone (No. 8) forms a joint with it posteriorly, and a pair of slice-like nasal bones (No. 7) forms a joint with the rostoral posteriorly. Because these are free from the main body, they are picked up at the time of observation of the splanchnocranium. A pair of lateral ethmoids (No. 3) overhangs on both lateral sides of the ethmoid. On the dorsal surface, a pair of longitudinally long plate-like frontals (No. 10) hangs on the dorsal surface of the binocular part. In fish species with large eyes, this bone is highly indented from the lateral surface to be narrow. Between the right and left frontals, a supraoccipital (No. 19) is inserted anteriorly, and the mid-dorsal line bulges like a plate. On the lateral sides of the supraoccipital, the parietal (No. 20), the epiotic (No. 16), and the exoccipital (No. 18) lie on a line in this order posteriorly, and are sutured to the supraoccipital.

The largely overhanging autopterotic (No. 15) is sutured to the lateral side of each parietal, and the autosphenoid (No. 14) is sutured to the anterior. [Because the supratemporal bone (No. 87) forms a joint with or is adjacent in the vicinity of the posterior part of the autopterotic, the supratemporal bone is treated as a part of the neurocranium in some cases. However, in this book, it was mentioned above

as a part of the pectoral girdle.]

Between the posterior lateral side of the parasphenoid and the exoccipital, the prootic (No. 17) is sutured, and the auditory and static organs, including the otolith, are protected inside. There is a small opisthotic (No. 21) posterior superior to the prootic and superior to the exoccipital, but its presence cannot be shown unless you sketch it from the posterior surface.

There is a basisphenoid (No. 12) that is sandwiched by the right and left prootics and vertically protruding to the posterior inferior part between the eyes. On the superior part, the foramen magnum sandwiched by the right and left autosphenoids on the anterior surface of the cerebral ventricle is the route of the optic nerve and eye-moving muscle. There is a pterosphenoid (No. 11) dorsal to the basisphenoid.

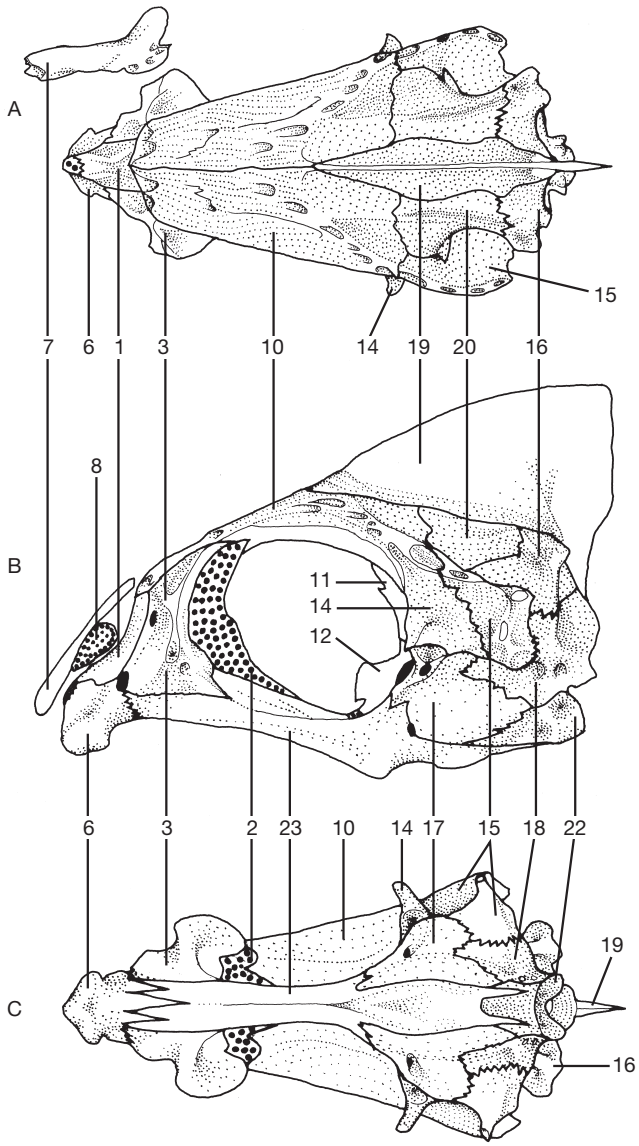


Fig. 1 Neurocranium of Red Sea Bream *Pagrus major*. A, dorsal view; B, left lateral view; C, ventral view. Large dots indicate cartilages. See table of III-3 for bone names and numbers.

III 7

Observation of the vertebral column and vertical fins

In the axial skeleton of Agnatha, the notochord is developed in the median, being accompanied by a very small number of cartilage pieces on the dorsal side, and several clusters of cartilages that only gather in the head. In extant advanced Chondrichthyes and Teleostei, the notochord in the embryonic stage similar to that of Agnatha is subsequently replaced by the vertebra that has developed from the circumference, and consequently remains rudimentarily at the joint part of each vertebra as the fish grows. A series of vertebrae that are consecutive in a segmental manner from immediately posterior to the cranium, called the vertebral column (Nos. 64–86), is attached by a variety of intermuscular bones, including ribs and myorhabdoids.

For observation, after the lifting of the pectoral and pelvic girdles, remove the left lateral muscle to expose the vertebral column. Individual detachment of the sarcomeres from the anterior will let you find curved, sword-like intermuscular bones running along the horizontal septum separating the lateral muscle top and bottom. These are called the supraneural bones (No. 85). The one at the anterior terminus attaches to the transverse process (No. 78) of the first centrum (No. 75). The more posterior, the lower the attachment position becomes, such as near the rib base, or, depending on the species, the lateral side of the inferior part of the centrum or even the hemal arch (No. 77). The sword-like firm bone that forms a joint with the transverse process of the abdominal vertebra (No. 64) in the vicinity of the third centrum or posterior and runs along the peritoneum as if wrapping the viscera is called the

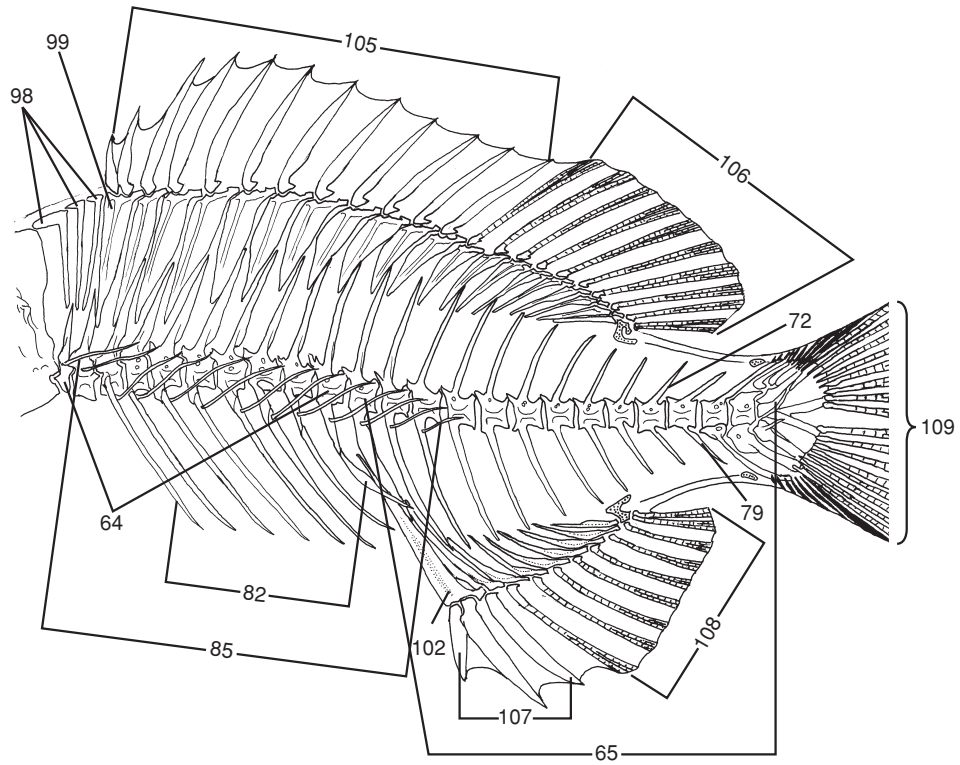


Fig. 1 Vertebrae and vertical fins of Red Sea Bream *Pagrus major*.
See table of III-3 for bone names and numbers.

rib (No. 82). In more ancient fish groups, apart from these, there are intermuscular bones including the epipleural ribs (No. 83) and the epicentrum (No. 84) running from the ribs and the centrum laterally. Moreover, the slender bones called the myorhabdoids (No. 86) are added to the inside of the diaphragm in some cases without attaching to the axial skeleton. When there are many types and a high number of bones like this, it is referred to as "bony".

After recording the positional relationships above in a rough sketch, expose the vertebral column. At the same time, expose the pterygiophores (Figure 2) of the dorsal and anal fins as well, and sketch the positional relationships between these bones and the neural spines/hemal spines of vertebrae in particular by outline. Thereafter, pick up the free interneuron spine at the start of the dorsal fin (No. 98) from the posterior of the neurocranium existing to the base, then pick up the interneuron spines (Nos. 99–101) in order posteriorly in combination with the ray (No. 105 or 106) supported by the spine, and clean them. Similarly, pick up the interhemal spines (Nos. 102–104) in combination with a ray (No. 107 or 108) in the anal fin. Each of the interneuron/interhemal spines is composed of three parts, the proximal pterygiophore (Nos. 99 and 102), median pterygiophore (Nos. 100 and 103), and distal pterygiophore (Nos. 101 and 104), in order posteriorly. All proximal pterygiophores are ossified. The median pterygiophores are also ossified and distinct in the anterior, but the ones in the posterior are difficult to find because they are cartilaginous. The distal pterygiophores are even more difficult to find because they are all small cartilages. In addition, it is impossible to completely pick up the soft

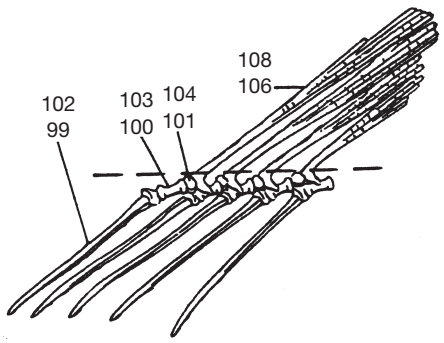


Fig. 2 Dorsal and anal fins and their supporting bones of teleost. From Bond (1979).

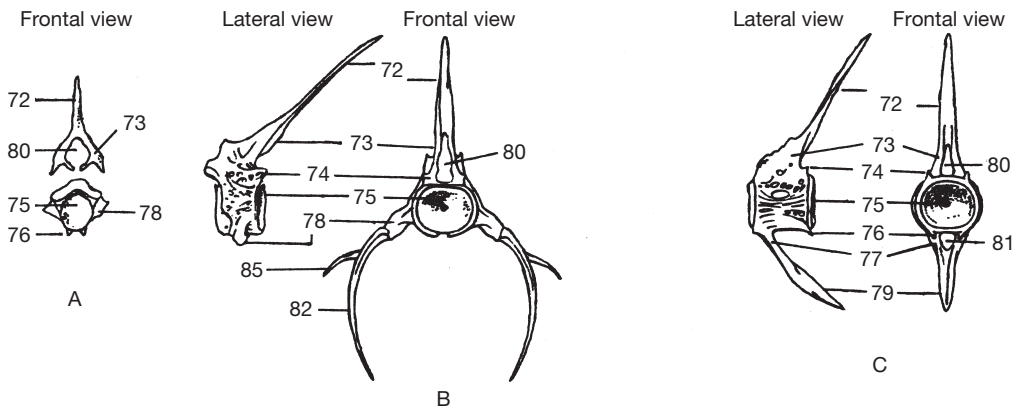


Fig. 3 Vertebrae of teleost.
A: 1st ventral vertebra. B: 2nd and posterior ventral vertebra. C: caudal vertebra. From Bond (1979).
See table of III-3 for bone names and numbers.

rays (Nos. 106 and 108) because they are slender and fragile. Do not disassemble the soft rays of the caudal fin (No. 109) because the rays form a direct joint with the terminus of the vertebral column.

Lastly, cut the vertebral column at the joint parts to observe the individual vertebrae (Figure 3).

1) The vertebral column is composed of a series of vertebrae, and broadly classified into the abdominal vertebrae (No. 64) in the trunk and the caudal vertebrae (No. 65) lining the tail.
2) The drum-shaped part forming the central part of each vertebra is called the centrum (No. 75). All centra have the neural spine (No. 72) (called neurapophysis in Chondrichthyes) on the dorsal side. The caudal vertebrae (No. 65) have the hemal spine (No. 79) (hemopophysis likewise) on the ventral side. In each basal part, the neural canal (No. 80) and the hemal canal (No. 81), holes through which the spinal cord or the blood vessel runs, are formed by the neural arch (No. 73) and the hemal arch (No. 77), respectively. Some of the anterior vertebrae sometimes form a joint with the centrum without fusion of their neural arch. The number is generally one in fish of Perciformes, but multiple in the ancient fish groups.
3) Each centrum has 8 zygapophyses (Nos. 74, 76) at a maximum paired between right and left on the anterior, posterior, dorsal, and ventral sides. However, missing items vary depending on the place and species.
4) The abdominal vertebrae (No. 64), except for several anterior ones, have the transverse process (No. 70) on both the right and left ventral sides, where the rib (No. 82) forms a joint. Each rib is attached by the supraneural bone (No. 85). The transverse processes of the anterior vertebrae protrude nearly laterally, whereas the more posterior the position is, the more inferiorly they

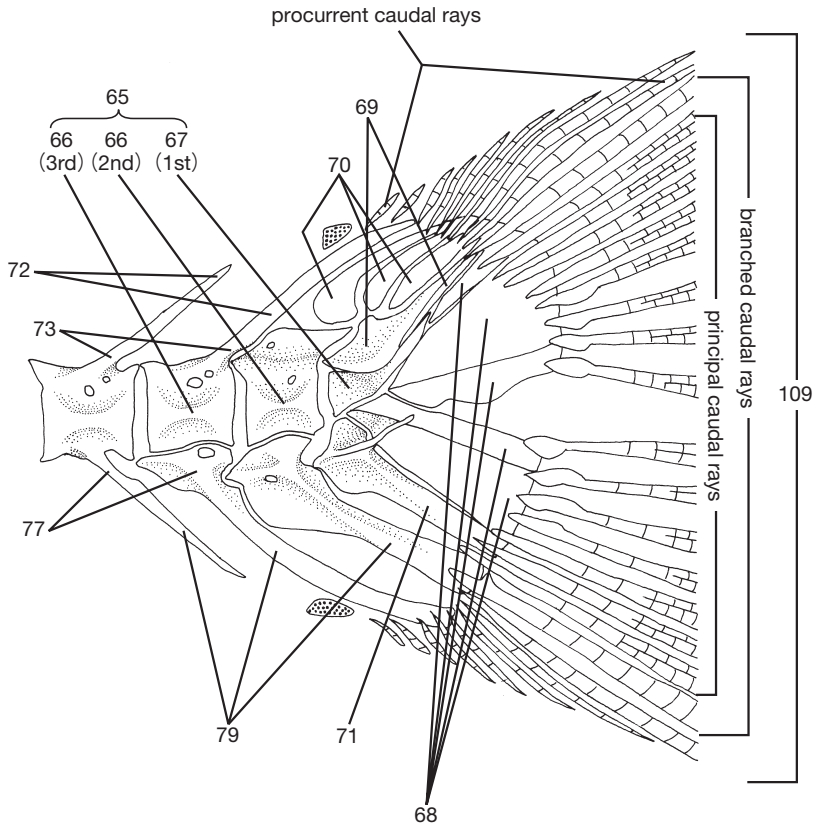


Fig. 4 Caudal skeletons of Red Sea Bream *Pagrus major*.
Large dots indicate cartilages. See table of III-3 for bone names and numbers.

protrude, and eventually the right and left transverse processes fuse, making a hollow. This fused skeletal part is the hemal arch (No. 77), and the hollow part is the hemal canal (No. 81). Even if there is a process in both sides inferior to the hemal canal or the processes are fused to form a short spine, it is still considered an abdominal vertebra (No. 64). However, the clearly elongated spine is judged to be a caudal vertebra (No. 65).

5) The caudal vertebrae near the posterior terminus are remarkably deformed/fused to support the caudal fin. The vertebrae in this vicinity are generically named the caudal skeleton (Figure 4). Because the difference in these fusing conditions varies depending on the evolutionary level of the species, it is valued for determining phylogenic relationships. Because the variation is remarkable even in extant Teleostei, I explain it by focusing on red seabream *Pagrus major* here.

   The centrum of the terminal vertebra is called the urostyle (No. 67), and it plays a central role in the caudal skeleton. This is a small triangle if seen laterally, but a thick round bone if seen posteriorly. Therefore, it can be distinguished from the surrounding plate-like bones. The plate-like bone that forms a joint with the bottom of the urostyle and is equipped with a wire-like process bilaterally on the base is the parhypural (No. 71). There are two hemal spines specialized to a similar thickness immediately anterior to it, but they are not fused with the centrum (No. 79). The hemal spines with a normal thickness located more anterior are fused with each centrum.

   A large and a small uroneural bone (No. 69) are seen to contact the dorsal side of the urostyle. These are left-to-right paired, distinguishing them from others. Between them and the last neural spine (No. 73), there is a boomerang-shaped thin bone followed by the 2 epurals, for a total of 3 epurals (No. 70). Because the epurals come off by adhering to the caudal fin rays when lifted, removal of the rays requires care.

   Posterior to the urostyle, there are 5 flat bones named hypurals (No. 68) that are extended in a fan shape. This is composed of several fused hemal spines. Those in red sea bream exhibit typical morphology of fish in Perciformes. The hypurals are further fused with each other or with other bones in the vicinity depending on the species.

   All of the branched caudal rays are supported by the parhypural and 5 hypurals (3 in the upper half and 2 in the lower half). Superior to them, there is an unbranched soft ray that nearly reaches the posterior terminus of the caudal fin and is supported by the fifth hypural. Also inferior to the rays, there is an unbranched soft ray that reaches nearly to the posterior terminus of the caudal fin and is supported by the parhypural. These two unbranched soft rays and all branched soft rays are collectively called the principal caudal rays. The rays located more anterior (superior and inferior) to the principal caudal rays are not included in the number of caudal fin rays in usual counting because the anterior ones are embedded beneath the skin and become small rudimentarily (see the counting method in II-5). However, these are valued in phylogenic studies, and counted using transparent specimens in which the skeletons are double-stained.

6) The number of fish vertebrae is an important taxonomic character expressed by the formula: total number of vertebrae (V) = number of abdominal vertebrae (AV) + number of caudal vertebrae (CV). It is counted by estimating the urostyle as the last caudal vertebra. When discussing the shape of vertebrae, it is occasionally convenient if we count the bones anteriorly, like preural centrum 1, 2, …, n, in addition to expressing the bones by the posterior ordinary number of the abdominal vertebrae and caudal vertebrae separately. At this time, the urostyle is estimated as the first preural centrum. This is based on the concept that the ural vertebra and the first preural centrum separately seen in the primitive fish are fused in the advanced fish groups.

III 8

Observation of the muscular system

The muscular system of fish controls their various life activities, including the movement of various organs, swimming, respiration, and the ingestion and digestion of food. The muscles are broadly classified into striated muscle and smooth muscle based on their cellular morphology. Another classification is based on the organ where the muscles are arranged, which broadly divides the muscles into three types. The striated muscle constituting the heart wall is called the cardiac muscle. The striated muscles moving fins and bones are skeletal muscles. The smooth muscles constituting the visceral organs are called visceral muscles. Moreover, there are also physiological categories of involuntary muscle and voluntary muscle. The cardiac muscle and the visceral muscle are involuntary muscles that are unconsciously governed by the autonomic nervous system to act at all times. The skeletal muscle is the voluntary muscle governed by the brain to consciously adjust activity. The skeletal muscles are described below.

The cells constituting the skeletal muscle are called muscle fibers because they have a slender shape. These are multinucleated cells in which a large number of myofibrils are assembled.

Lateral muscle: The lateral muscle is an assembly of muscle fascicles arranged regularly on both sides of the trunk and tail. Extension and shrinkage of the lateral muscle give the fish the power to swim. In many cases, the lateral muscle is composed of W-shaped myomeres (Figure 1-7, 8) subcutaneously lining the lateral side. The anterior and posterior myomeres are separated by the diaphragm (Figure 1-6). In addition, in Chondrichthyes and Teleostei, each myomere is divided into a right and left

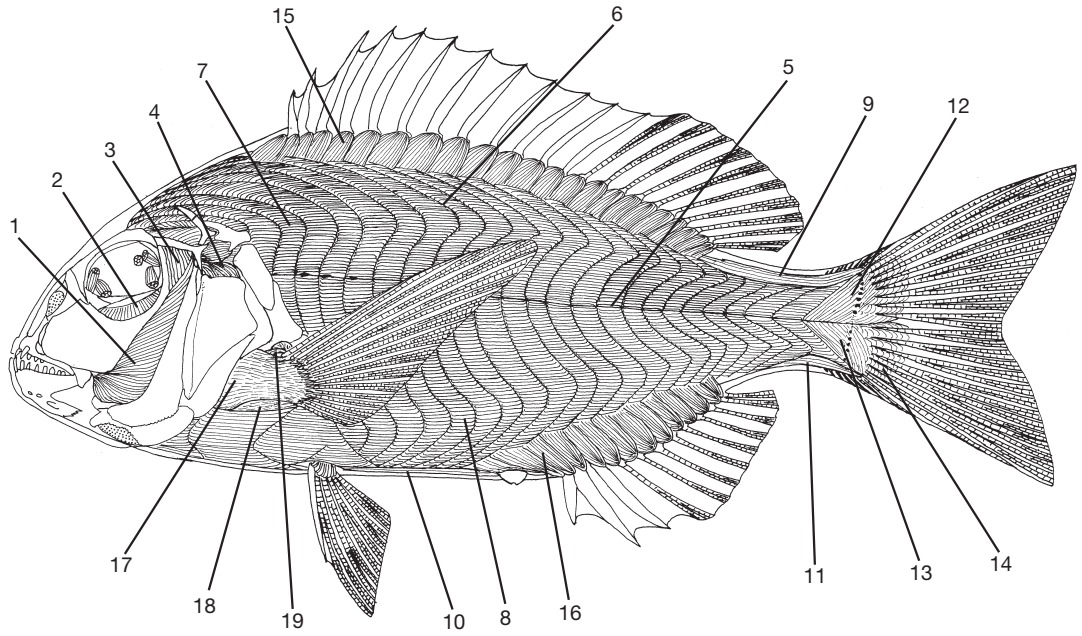


Fig. 1 Muscle system of Red Sea Bream *Pagrus major*.
1. adductor mandibulae; 2. adductor arcus palatine; 3. levator arcus palatine; 4. levator operculi; 5. horizontal septum; 6. myoseptum; 7. epaxialia; 8. hypaxialis; 9. supracarinalis posterior; 10. infracarinalis medium; 11. infracarinalis posterior; 12. flexor dorsalis; 13. flexor ventralis; 14. intermedialis; 15. inclinator dorsales; 16. inclinator anales; 17. abductor superficialis; 18. aductor profundus; 19. arrector ventralis

side by the medium septum or the vertical septum, composed of a tendon-like membrane connecting vertebra, and by the abdominal cavity, and divided into the dorsal muscle (Figure 1-7) and the ventral muscle (Figure 1-8) by the horizontal septum (Figure 1-5) lying between the vertebrae and the body surface. No horizontal septum is seen in Agnatha.

When observing the lateral muscle of Chondrichthyes and Teleostei, in many cases, each myomere is W-shaped on the body surface but has complicated morphology in the deep layer because it is equipped with two anterior muscle cones protruding anteriorly (Figure 2-5) and a posterior muscle cone protruding posteriorly (Figure 2-4), separately on the top and bottom of the centra. In sharks, the W-shaped myomeres are bent even more posteriorly in the dorsal part, and another anterior muscle cone develops in the deep layer of this part. In Agnatha, the shape of each myomere has a dull bending part, relatively simple and arched, and lacks the anterior muscle cone and the posterior muscle cone.

In the surface of the lateral muscle, muscular fascicles have a reddish brown color due to the abundant myoglobin and dense distribution of capillary vessels running longitudinally in a zonal manner along the horizontal septum (Figure 2-6). This is called the surface dark muscle (Figure 2-1), and its developmental level varies considerably depending on the species. The surface dark muscle is generally well developed in surface swimmers such as Japanese sardine *Sardinops melanostictus*, flying fish, Pacific saury *Cololabis saira*, mackerel, and Japanese Spanish mackerel *Scomberomorus niphonius*, and poorly developed in bottom-dwellers including wrasse, sargassum fish *Histrio histrio*, lizardfish *Saurida* sp., Platycephalidae, and sea robin *Chelidonichthys spinosus*.

Moreover, highly migratory surface swimmers such as skipjack/tuna, shortfin mako shark *Isurus oxyrinchus*, and salmon shark *Lamna ditropis* have paired red muscle fascicles running bilaterally along the vertebral column. This is called the true dark muscle (Figure 3-4). The true dark muscle is the part medial to the anterior muscle cone of the lateral muscle, and in the shortfin mako shark *Isurus oxyrinchus* it is apart from the vertebrae and embedded in the lateral muscle. In frigate/bullet tuna *Auxis* (Figure 3) and skipjack *Katsuwonus pelamis*, the dark muscle is remarkably developed and accounts for around 1/5 of the ordinary muscles.

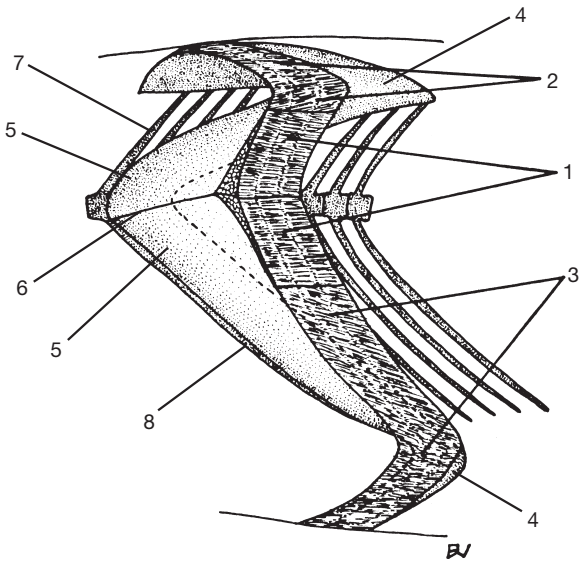


Fig. 2 One myoseptum of lateral muscle of a salmonid fish, King Salmon *Oncorhynchus tschawytscha*.
1, superficial dark muscle or lateralis superficialis; 2, epaxial; 3, hypaxialis; 4, posterior cone; 5, anterior cone; 6, horizontal septum; 7, neural spine & medium septum; 8, hemal spine & medium septum. From Winterbottom (1974).

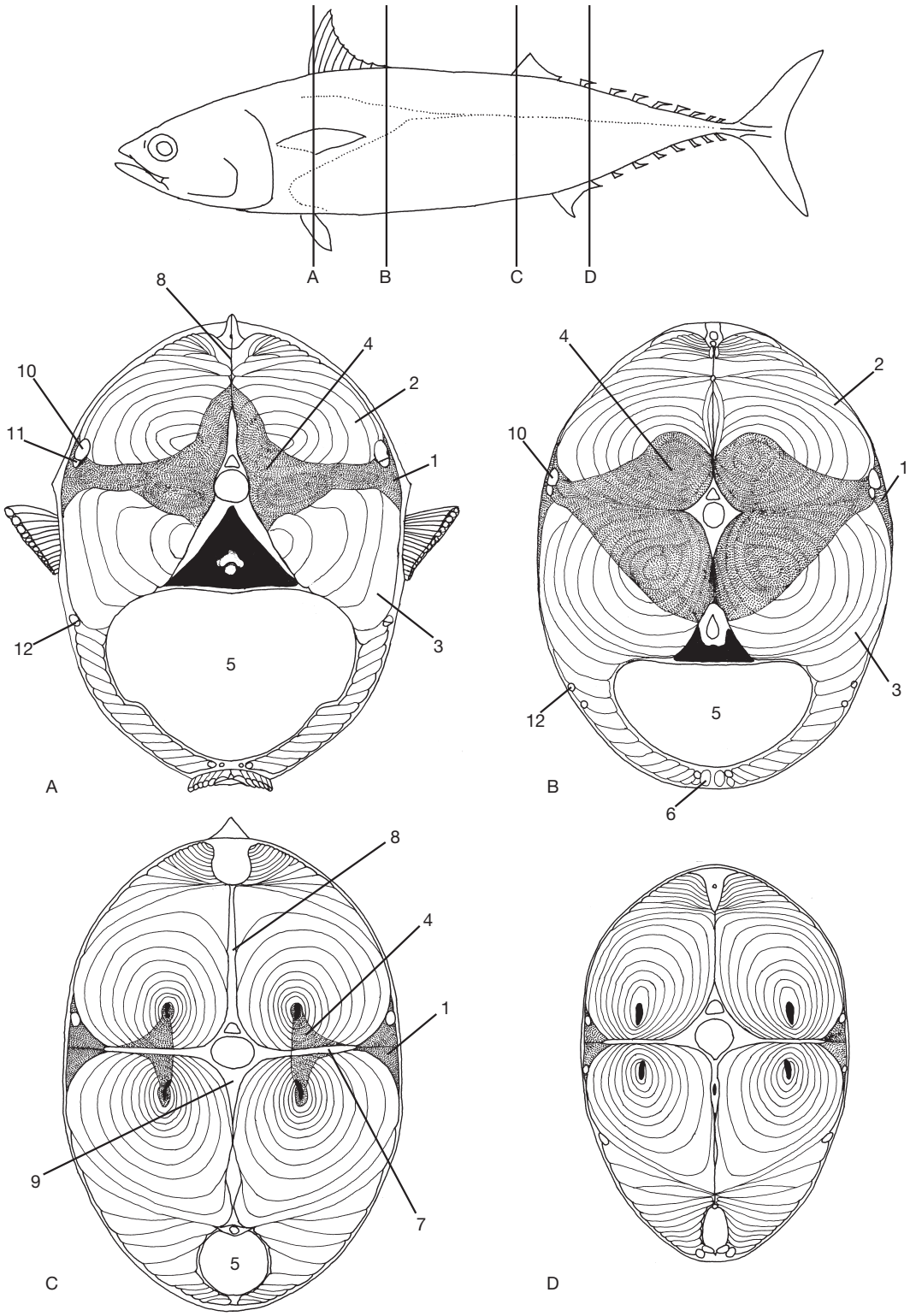


Fig. 3 Cross section of muscle of Frigate Tuna *Auxis thazard*. A-D on upper figure indicate section place of lower figures. 1, superficial dark muscle; 2, epaxial; 3, hypaxialis; 4, true dark muscle; 5, abdominal cavity; 6, infracarinalis medius; 7, horizontal septum & epineural; 8, medium (vertical) septum & neural spine; 9,medium septum & hemal spine; 10, lateral line canal; 11, dorsal cutaneous vessel; 12, ventral cutaneous vessel.

Jaw muscles and opercular muscles: The most remarkable muscle among the muscles for closing the mouth is the adductor mandibulae (Figure 1-1). Normally, this muscle is broadly divided into three muscles that arise from the palatine arch centering the quadrate and connect the medial and posterior surfaces of the dentary and the upper jaw, and the muscle fascicles lying in the inner cavity of the lower jaw, a total of four groups. The developmental status of the muscle fascicles of these parts varies depending on the species. The major muscles to open the lower jaw include the protractor hyoidei. This is a pair of slender muscle fascicles that arise from the medial surface near the joint between the right and left dentaries and reaches nearly all of the hyoid arch (excluding the urophyal and the interhyal) including the branchiostega.

Dorsal posterior to the adductor mandibulae, there is the dilator operculus connecting vertically toward the posterior part of the orbit of the neurocranium and the dorsal surface of the anterior part of the opercle. Posterior to this is the levator operculi (Figure 1-4) connecting vertically toward the dorsal surface of the posterior part of the opercle. In addition, there is the adductor operculi connecting horizontally the medial lateral wall of the neurocranium and the medial surface of the dorsal part of the opercle. All of these control opercular movement.

Muscles to move fins: The muscles for moving the dorsal fin and the anal fin among vertical fins are symmetrically arranged and share a basic structure, whereas the muscles for moving the caudal fin are very distinct.

Dorsal fin: The inclinator dorsalis (Figure 4-1) arises bilaterally from each ray base toward the medial surface of the skin. This acts to inclinate the ray right and left. In puffers, this muscle is remarkably developed and wraps the lateral muscle to act not only as an inclinator but also as a flexor and extensor for the body. This appears to have been developed in relation to the fact that a puffer

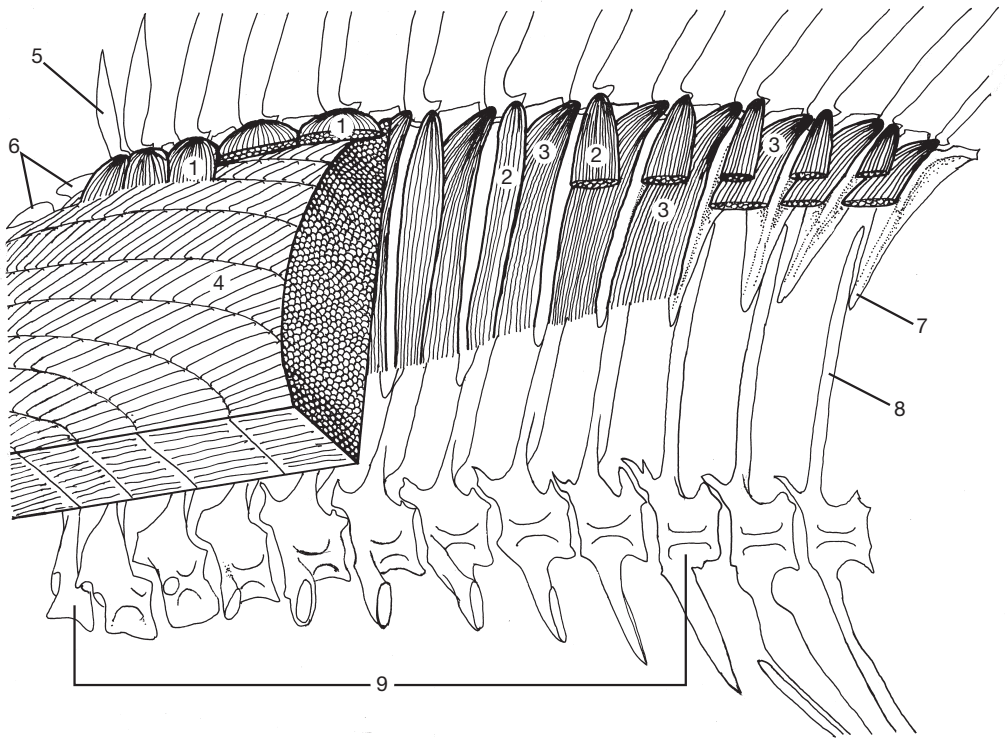


Fig. 4 Muscles to move dorsal fin and related bones of Red Sea Bream *Pagrus major*.
1, incinator dorsales; 2, depressor dorsales; 3, erector dorsales; 4, epaxialis; 5, dorsal fin spine; 6, free neural spine; 7, proximal pterigiophores; 8, neural spine; 9, abdominal vertebrae.

moves forward by the movement of the dorsal and anal fins. Inside the lateral muscle (Figure 4-4) are the erector dorsalis (Figure 4-3), running from the anterior terminus of the base of each ray (Figure 4-5) toward the distal pterygiophore (Figure 4-7) immediately anterior; the neural spine (Figure 4-8), or the medium septum; and the suppressor dorsalis (Figure 4-2), running from the lateral surface of the posterior terminus of the base toward the distal pterygiophore immediately inferior, the neural spine, or the medium septum. The former functions to erect the ray and the latter functions to fold the ray posteriorly, and both are left-to-right paired. In addition, these are fixed posteriorly and anteriorly by the supracarinalis anterior connecting the neurocranium and the most anterior pterygiophore and the supracarinalis posterior connecting the last neural spine and the last pterygiophore.

Caudal fin (see the foldout figure): The posterior terminus of the vertebral column has complicated muscles involved in the movement of the caudal fin. The major ones include the flexor dorsalis and the flexor ventralis, which arise from a wide range of the lateral surface of the caudal skeletal and are arranged radically mainly at the base of the branched soft rays of the caudal fin, the adductor caudalis connecting the lower central soft rays of the caudal fin and the urostyle as well as the hypurals, and the interradialis caudalis between the caudal rays. Among them, the muscles with the most important function are the flexor dorsalis and the flexor ventralis, which are separately composed of the muscle fascicles that are further divided into, again, top and bottom.

Pectoral fin (see the foldout figure): The muscles for moving the pectoral fin also include many types of muscles that contract medially, laterally, superiorly, or inferiorly. Major ones include the triangle abductor superficialis that lies on the posterior and ventral surfaces of the cleithrum and attaches to the anterior surface of the lateral side of the ray base through its posterior terminus, and the abductor profundus that lies on the medial lateral surface of the coracoid and attaches to the posterior inferior process of the base of all soft rays.

Pelvic fin: In the pelvic fin, the major muscles are the abductor profundus pelvicus, which is located on the ventral surface of the pelvic girdle and is associated with the pelvic fin ray through its posterior terminus, the arrector ventralis pelvicus, which arises from the ventral surface of the lateral side of the pelvic girdle to connect to the lateral surface of the first pelvic ray, and the adductor profundus pelvicus, which lies on the dorsal side of the pelvic girdle and adheres to the dorsal surface of the pelvic ray base.

A detailed explanatory review of fish muscle that is highly recommended is: Winterbottom, R. (1974) A descriptive synonymy of the striated muscles of the Teleostei. Proc. Acad. Nat. Sci. Philad., 125 (12): 225-317.

IV

Related field of experimental ichthyology

IV 1 Sketching eggs, larvae and juveniles

The observation and description of the shape of fish eggs, egg development, and morphological changes of newly hatched larvae accompanying growth not only clarify relationships by similarity and dissimilarity but also are important in egg and fry production for healthy fry growth, and are essential for promoting these studies. Sketching these materials by means of sharp lines and dots uses nearly the same basic technique as that of adult fish. However, because the subjects are small and eggs and fish in the larval stage are transparent and easily damaged, the equipment and materials used are diverse and the procedure is also distinct.

1. Equipment and materials used

These include microscopes, drawing apparatuses, microscope photography equipment, and profile projectors. These tools have developed recently as represented by popularly used digital cameras and digital photography equipment. The microscopes include the binocular stereomicroscope and the trinocular biological microscope in addition to the monocular biological microscope. Choose a proper model according to the purpose. Briefly, in the case of sketching by observing through a microscope, use a monocular biological microscope or a binocular stereomicroscope. For drawing equipment, use a monocular microscope (this can project clearer images than binocular microscopes) compatible to the equipment. For photography, use a binocular or a trinocular microscope with a cylinder compatible with a camera. In addition, to measure the size of the subject, one of the binocular or trinocular cylinders should be equipped with a micrometer. The microscopic observation of eggs or larvae requires a glass slide on which to place these materials. Such glass slides include excavated slides with a cavity. In addition, glass slides specifically for larvae and juveniles (with a pool-like water-retainable well where organisms can be accommodated) have recently become available. Regarding illumination, in addition to the transmission illumination accompanying the microscopes, the use of a fiber type light source of incident light provides brighter images (Be very careful to avoid the evaporation of moisture and dryness of the sample from the heat generated by the illumination. Cool light illuminators that avoid heat generation are on the market.). The sketching should be done with an H-2H pencil, but use a pen in the case of article submission, etc.

2. Sketching egg development

First, collect 5–10 eggs for sketching from the rearing tank and keep them in an approximately 1-liter beaker already filled with the rearing water for all observations. Float the beaker in water in a 5–10 liter container with a thermometer (water-bath method). Place an airstone in the beaker for weak aeration.

In the case of recording the developmental process of eggs, sketches are often made from the viewpoint of the top of the blastodisc to help understand the manner of cleavage. However, after embryonic formation, it is preferable to view it from the right side so that the embryo is positioned right or left of the yolk sac (sketching embryos from the back, front, or halfway makes the whole appearance unclear). However, in the case of a pelagic egg, it is difficult to fix it in the intended orientation on a glass slide retaining water because it rotates. In this case, accommodate multiple eggs so that they stick to each other, making it difficult for them to move, and then rotate the egg using a

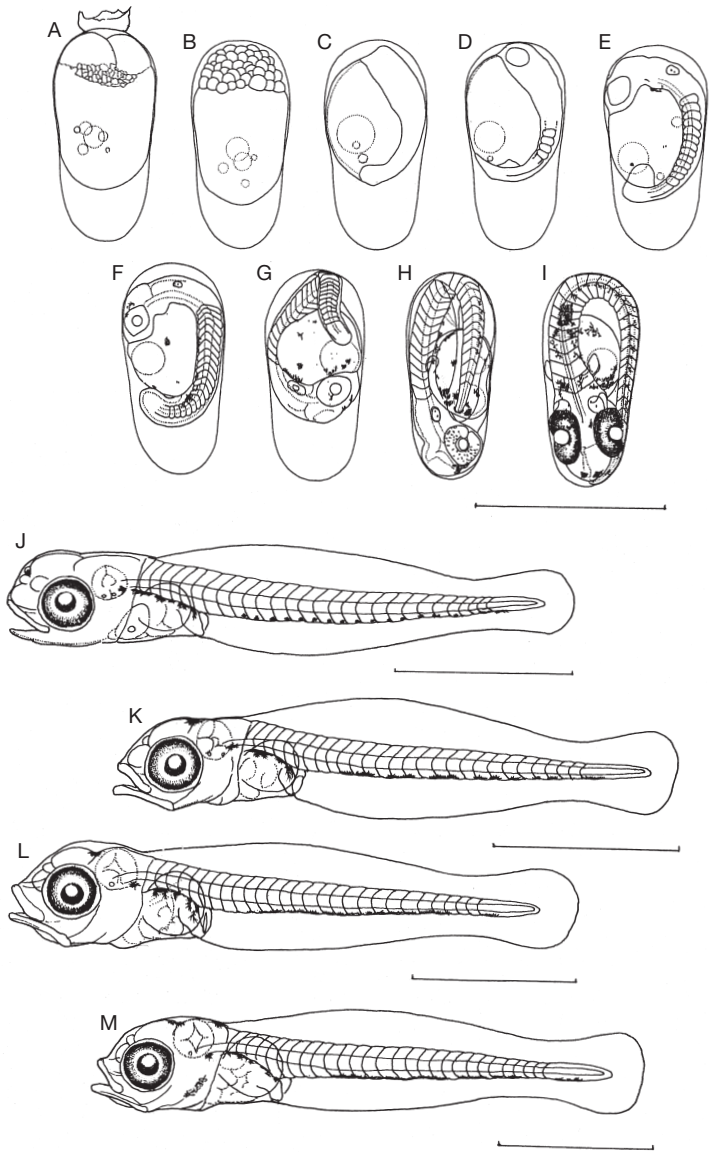


Fig. 1 Oogenesis and larvae of Nagasaki Damsel *Pomacentrus nagasakiensis*.
A, 2-cell stage, 39 minutes after fertilization; B, early morula stage, 5 hour 4 minutes after fertilization; C, organization of embryo, 16 hour 6 minutes after fertilization; D, 4-myomere stage, optic vesicle & Kupffer vesicle are organized, 19 hour 16 minutes; E, 13-myomere stage, otic vesicle (otosyst) is organized, melanophores appear on yolk sac, 24 hour 36 minutes; F, 20-myomere stage, lens is organized, 31 hours; G, 25-myomere stage, tail is detached from surface of yolk sac, 31 hour 33 minutes; H, 27-myomere stage, granule melanophores appear on eye, 47 hours 15 minutes; I, just before hatch, 112 hour 46 minutes; J, hatched larva, 3.08 mm TL; K, prolarva, 24 hour after hatching, 3.08 mm TL; L, postlarva, 3 days after hatching, 3.59 mm TL; M, 5 days after hatching, 3.68 mm TL. Scales indicate 1 mm.

mounted needle to set it in the intended orientation for observation. On the other hand, a demersal egg is relatively easy to fix in an intended orientation because it does not rotate. Figures 1 and 2 show an example of a sketch of the manner of egg development and larval/juvenile growth of Nagasaki damsel *Pomacentrus nagasakiensis*. The developmental stages of the egg in the sketch are after blastodisc bulging, the 2-cell stage, the first cleavage, and immediately before hatch, each of which is a time point when a characteristic change is seen. The size measurement should be the diameter in the case of round eggs, and the major and minor axes in other cases, as well as the oil globule diameter (large, medium,

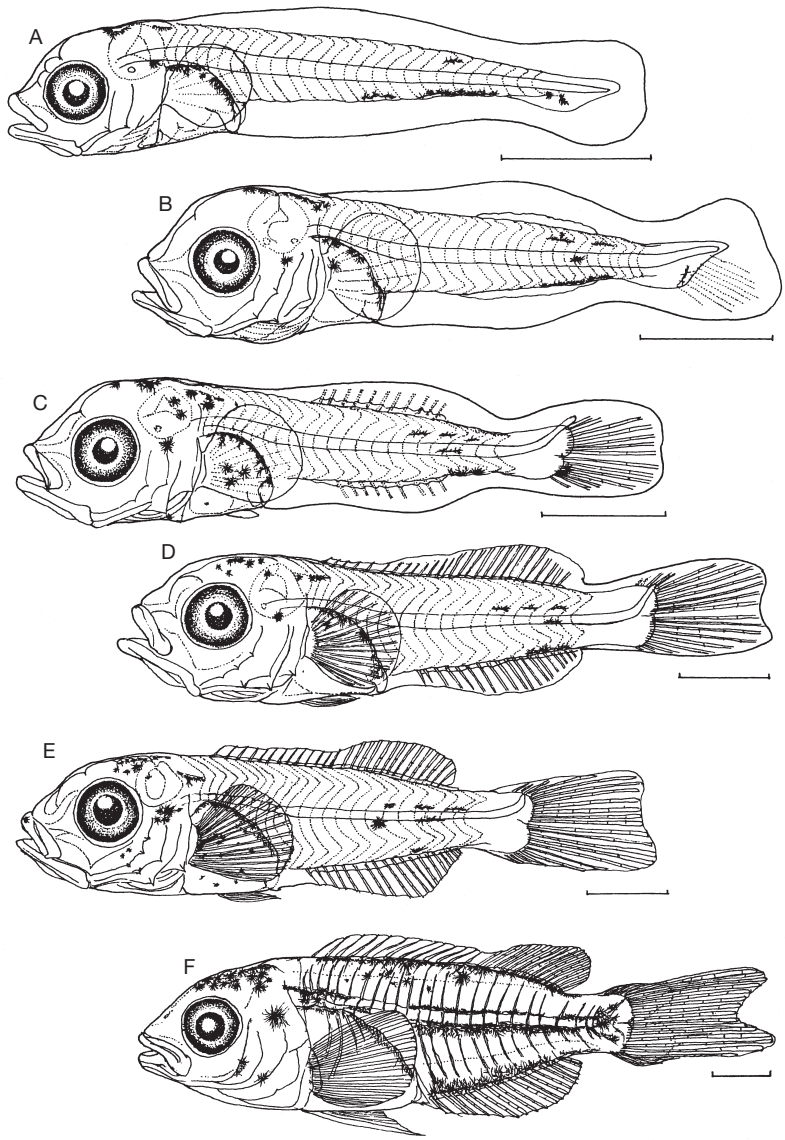


Fig. 2 Larvae and juvenile of Nagasaki Damsel *Pomacentrus nagasakiensis*.
A, postlarva, 8 days after hatching, 2.42 mm TL; B, 9 days after hatching, 5.02 mm TL; C, 12 days after hatching, 5.26 mm TL; D, 15 days after hatching, 7.19 mm TL; E, juvenile stage, 16 days after hatching, 8.01 mm TL; F, 19 days after hatching, 12.0 mm TL. Scales indicate 1 mm.

and small separately if multiple globules of different sizes exist).

Because egg development is quick in the case of pelagic eggs, and hatching happens mostly within 24 hours, continuous observation is relatively easy. In the case of demersal eggs, which are slow to hatch, work can be done with pauses in the observation and the occasional check of the developmental stage after embryonic formation.

3. Sketching larvae and juveniles

To sketch a newly hatched larva, collect around 5 larvae from the rearing tank and accommodate them in a beaker in the same way as the case of sketching egg development, and use them for

observation. Collect a larva together with rearing water using a dropping pipette, place it on a glass slide dedicated for larval observation, and repeat. Then, arrest the motion using an anesthetic for cold-blooded animals. At this time, dissolve a small amount of the anesthetic in a stepwise manner in water until you confirm the arrest because concentrated anesthetic can shrink or kill the sample. In addition, observe the sample promptly after the arrest, and replace it with a new one when the transparent body starts becoming white. Because in many hatched larvae, pelvic fins and vertical fins (dorsal, anal, caudal fins) are not differentiated and are all like thin membranous fins, be very careful to avoid damage or folding. Usually during sketching, the sample is placed so that the head is on the left and the left lateral side faces up. At this point, you need to correct the body using a mounted needle so that it has no body twist and folding of the fins/membranes and is perfectly sideways. Regarding the magnification in the microscope, first, set it low so that you can observe the whole body and sketch the basic morphology including the outline. After this, increase the magnification and observe the parts in detail for additional or new description. As for the light source, for basic sketching, illumination solely with transmission light usually works. However, even in that case, adjust the light level to allow clear observation. In addition, combination with incident light from above allows even more detailed observation because it provides shadows. During the early larval stage from immediately after hatch to complete absorption of yolk sac, measure the total length (from the snout tip to the posterior terminus of the caudal fin membrane), body length (from the snout tip to the terminus of the notochord), yolk length (major and minor axes), oil globule diameter (major and minor axes in the case of the size of elongated eggs), and preanus length (from the snout tip to the anus), and count the number of myomeres (the number in the ventral and tail parts bordering the anus, and the total number). From the late larval stage after yolk sac absorption to immediately before the juvenile stage when the number of rays of each fin reaches the fixed number of the species, measure the total length, body length, and preanus length, and count the number of spines and soft rays and record the emergence and change in mottles and spots (Recording by a camera is convenient for body color/mottles and spots. I advise adding the details of mottles and spots, etc. to the sketch based on this record).

1. Classification of fish eggs

Fish reproduction is broadly classified into two types, viviparity and oviparity. First, in the case of *in vivo* fertilization by copulation (sexual intercourse) between males and females, females of some fish spawn large eggs wrapped in a hard sheath (Japanese bullhead shark *Heterodontus japonicus*, skate), or give birth to larvae and juveniles (Scorpaenidae, *Sebastes*) or juveniles that have grown to the same morphology as that of the parent fish (surfperch *Ditrema temmincki*, banded houndshark *Triakis scyllium*, red stingray *Dasyatis akajei*). Among them, those that spawn eggs are called "oviparous," and others are called "viviparous" (all of those using *in vivo* fertilization are called viviparous in some cases). On the other hand, as seen in many fish species, the type of *ex vivo* fertilization in which unfertilized eggs released by a female are mixed with sperm released by a male(s) is called "viviparity." The group of sharks and rays includes both "oviparous" species that spawn eggs and "viviparous" species that give birth to juveniles/fry.

Eggs of Teleostei are divided into "demersal eggs", which have high specific gravity and sink in water, and "pelagic eggs", which float in water (Figure 1). Among them, demersal eggs are divided into "non-adhesive eggs", "adhesive eggs", and "entangling eggs" based on the adhesion manner of the surface of the egg membrane. Among these three types, non-adhesive eggs such as the eggs of salmon/trout and Japanese eel catfish *Plotosus japonicus* have no adhesion apparatus and adhesiveness on the surface of the egg membrane and therefore are spawned in a scattered manner on the bottom of rivers, lakes, or the sea. "Adhesive eggs" have adhesive structures such as attachment filaments and an adhesion apparatus, and/or adhesiveness on the egg surface, and adhere to other eggs or objects. They are further divided into "adhesive eggs" with adhesive structures, such as the eggs of goby, pearl-spot chromis *Chromis notata*, ayu *Plecoglossus altivelis*, and *Spirinchus lanceolatus* and "tightly adhesive eggs" with tight adhesiveness on the egg membrane surface, such as the eggs of Cyprinidae, puffer, sculpin, etc. "Entangling eggs" of the Pacific saury *Cololabis saira*, flying fish, and medaka *Oryzias latipes* have long filamentous structures on both poles of the egg that entangle the egg on seaweeds, water plants, etc. The pelagic eggs are divided into "agglutinated pelagic eggs", where there are many

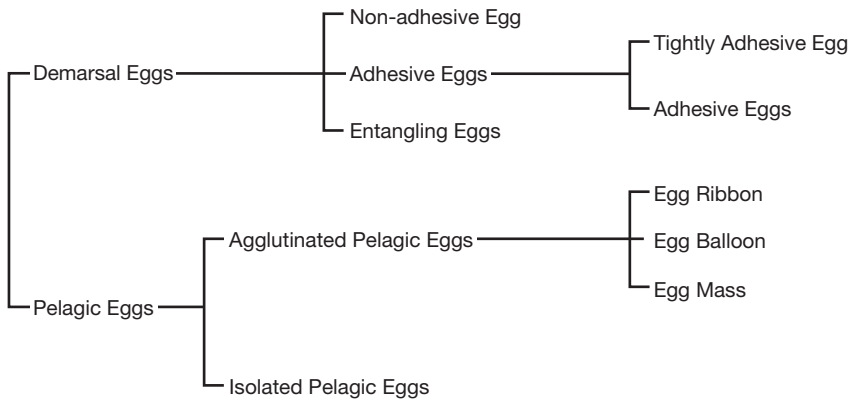


Fig. 1 Types of teleosts eggs. Modified from Ikeda & Mito (1988).

eggs contained in a gelatin-like substance, and "isolated pelagic eggs" that separately float in water. Moreover, "agglutinated pelagic eggs" are divided based on the shape of the sack wrapping the egg into a ribbon-like "egg ribbon", including eggs of frogfish and monkfish; a sack-like "egg balloon", including eggs of luna lionfish *Pterois lunulata* and Carapidae; and a bulky "egg mass", including eggs of mangrove dragonet *Callionymus enneactis*.

Isolated pelagic eggs are classified based on the number of oil globules, structure of the egg membrane, breadth of the perivitelline space, and the presence or absence of any segment on the yolk, etc.

The number and size of eggs/larvae and juveniles produced by a female fish differ depending on the manner of reproduction. Briefly, the number tends to be lowest in viviparous cartilaginous fish that give birth to larvae and juveniles, and increases in the order cartilaginous fish spawning demersal eggs, teleost fish spawning demersal eggs, teleost fish of Scorpaeniformes giving birth to larvae, and teleost fish spawning pelagic eggs. On the other hand, the trend is size is opposite that of the number, from the smallest in fish spawning pelagic eggs to the largest in viviparous cartilaginous fish giving birth to larvae and juveniles. Generally, the relationship between the number and the size of eggs is that species spawning larger eggs produce a smaller number and species spawning smaller eggs produce a larger number. Therefore, it is called "a small number of large eggs and a large number of small eggs". In addition, the larvae at the point of hatching are large in viviparity and demersal eggs, and at in the stage in which organs, including the eyes, mouth, digestive organs, and fins, have developed. On the other hand, larvae from pelagic eggs are small and their organs are undeveloped. This is considered to be a possible "reproduction strategy" to leave as many offspring as possible. In other words, it is not problematic that the number of larvae of viviparity and larvae hatched from demersal eggs is small because they are large and their organs are in a developed stage, making the probability of subsequent survival higher. On the other hand, because larvae hatched from pelagic eggs are small and their organs are undeveloped in many cases, their survival rates are low.

2. Characteristics of Teleostei by growth stage

Many researchers have proposed distinct classifications of the developmental stages of Teleostei according to various criteria. Here, I divide the life cycle into egg, early larval, late larval, juvenile, young, immature, adult, and old stages according to Watanabe and Hattori (1971), and overview the morphological and ecological characteristics of these stages individually below.

Egg stage: This is the stage from fertilization to immediately before hatching, in which fish growth depends on the nutrition (yolk) inside the egg membrane provided by the parent. The growth rate is strongly influenced by the environmental water temperature, and varies depending on the spawning season in the habitat of parent fish and species. The time required for hatching is generally longer in demersal eggs and large eggs, and shorter in pelagic eggs and small eggs. In addition, it is longer at low water temperature and shorter at high water temperature.

Early larval stage: This is the stage from immediately after hatching to the complete absorption of yolk. In succession from the egg stage, fish growth depends on the nutrition provided by the parent fish. However, it different from the egg stage because fish are not protected by an egg membrane and grow by contacting the outer environment directly. For this reason, adaptation to the outer environment, including water temperature and flow, is more necessary than in the egg stage, and management by physiological changes influences survival. Generally, in fish species spawning pelagic eggs, larvae immediately after hatch have insufficient organogenesis, represented by not yet melanized eyes and an unopened mouth and anus. On the other hand, in fish species spawning demersal eggs, organs are more developed before hatching, as indicated by the fact that eyes are already melanized and the mouth and

anus are formed and opened inside the egg membrane during the egg development. Therefore, initial depletion is higher in fish spawning pelagic eggs than in fish spawning demersal eggs.

Late larval stage: This is the stage from after the absorption of yolk to the time the number of fin rays reaches that particular to the species. This stage shows the most remarkable changes both morphologically and physiologically. "Transformation" also occurs in this stage. Fins as swimming organs differentiate and their spines and soft rays are formed in this stage. Ecological changes due to increased swimming ability are seen. In larvae hatched from pelagic eggs, the eyes are melanized and functional, the mouth and anus are opened, and fish start consuming food from the external environment upon entering this stage. On the other hand, in larvae hatched from demersal eggs, the eyes are completely melanized and functional, and fish look for and eat food aggressively of their own will.

Juvenile stage: The spines and soft rays of fins are already in the number particular to the species, but not all of the body form elements that adult fish possess have been prepared, and the body form is in the early stage of development. Because the swimming ability is greatly improved, fish swim in a school and ingest food more actively than earlier. In bottom-dwellers and species inhabiting rocky shores, coral reefs, etc., fish settle themselves on the bottom in this stage, and, depending on the species, reach a stage of acquiring cannibalism and/or territory.

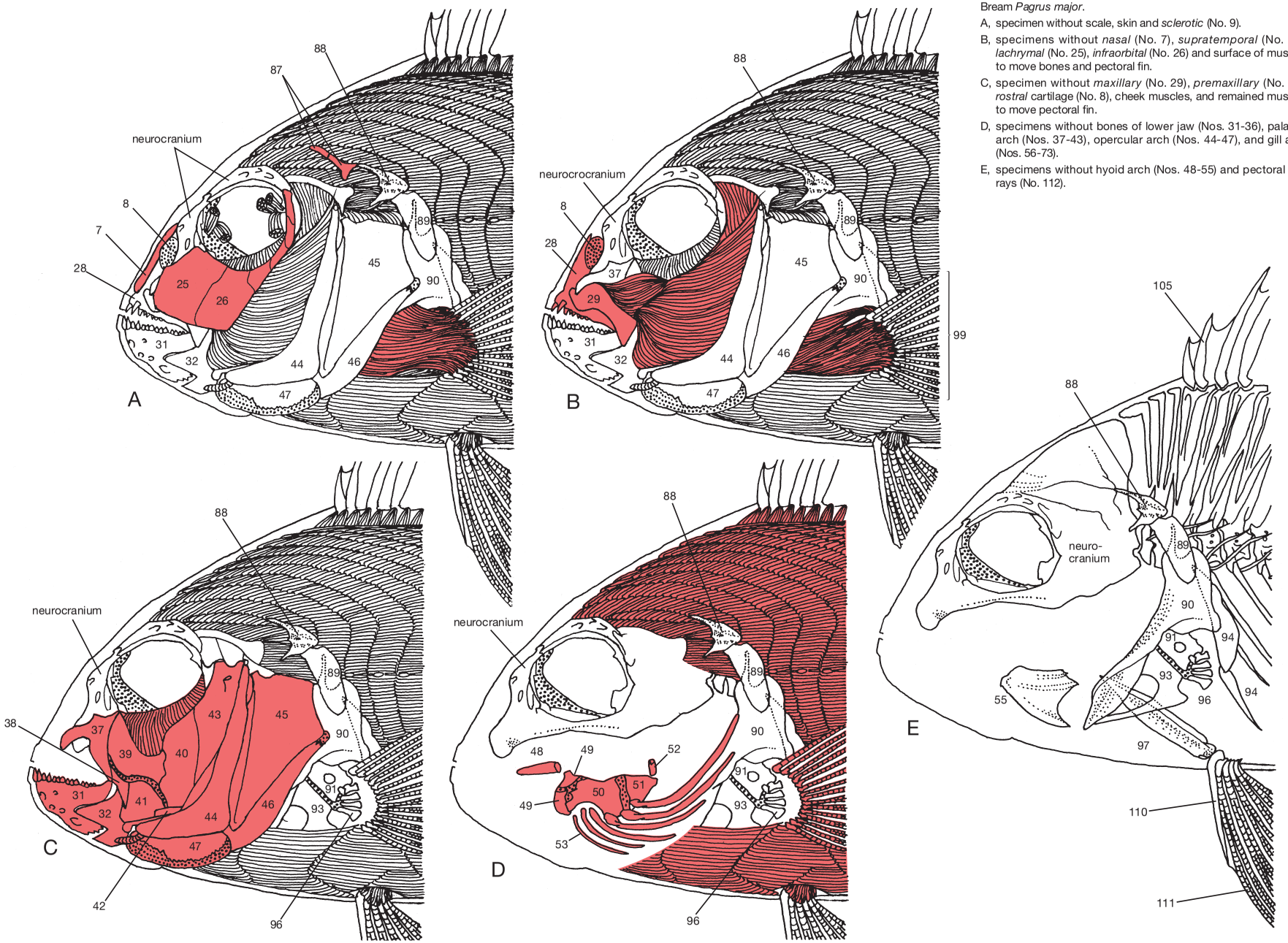
Young stage: The morphological characteristics are very similar to those of adult fish. However, fish are in the middle of development because body color, mottles, or spots peculiar to the species have not yet appeared. This is equivalent to the stage in which the growth is most active. The generally mentioned "fry stage" is equivalent to this stage.

Immature stage: Size, apparent morphology, body color, mottles, and spots are nearly similar to those of adult fish. However, fish are in a sexually immature stage.

Adult stage: This is the stage in which fish are sexually mature and functional. "Biological minimum form" is equivalent to the first stage of this period.

Old stage: This is the stage in which viability and fertility deteriorate.

Growth stages are classified as above in general. However, although the late larval stage and juvenile stage can be relatively clearly classified from appearance, it is difficult to clearly classify other stages.



Sequential dissections to show bone distributions of Red Sea Bream *Pagrus major*.

A, specimen without scale, skin and *sclerotic* (No. 9).

B, specimens without *nasal* (No. 7), *supratemporal* (No. 87), *lachrymal* (No. 25), *infraorbital* (No. 26) and surface of muscles to move bones and pectoral fin.

C, specimen without *maxillary* (No. 29), *premaxillary* (No. 28), *rostral* cartilage (No. 8), cheek muscles, and remained muscles to move pectoral fin.

D, specimens without bones of lower jaw (Nos. 31-36), palatine arch (Nos. 37-43), opercular arch (Nos. 44-47), and gill arch (Nos. 56-73).

E, specimens without hyoid arch (Nos. 48-55) and pectoral soft rays (No. 112).