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

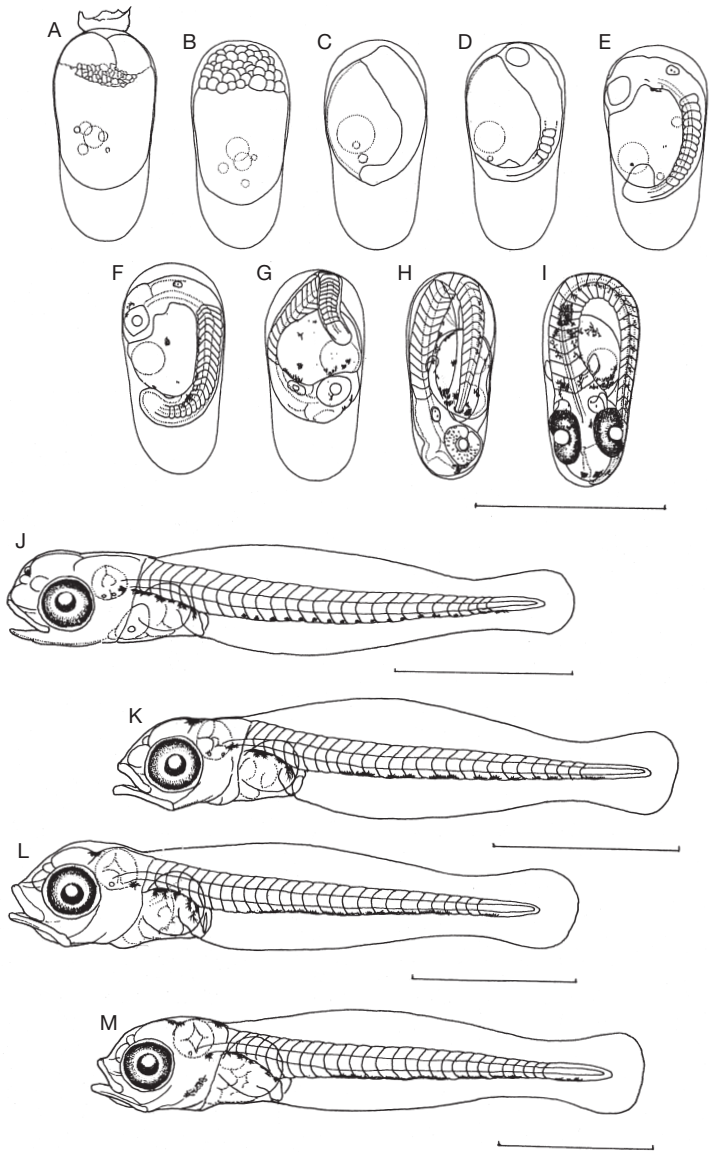


Fig. 1 Oogenesis and larvae of Nagasaki Damsel *Pomacentrus nagasakiensis*.
A, 2-cell stage, 39 minutes after fertilization; B, early morula stage, 5 hour 4 minutes after fertilization; C, organization of embryo, 16 hour 6 minutes after fertilization; D, 4-myomere stage, optic vesicle & Kupffer vesicle 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,

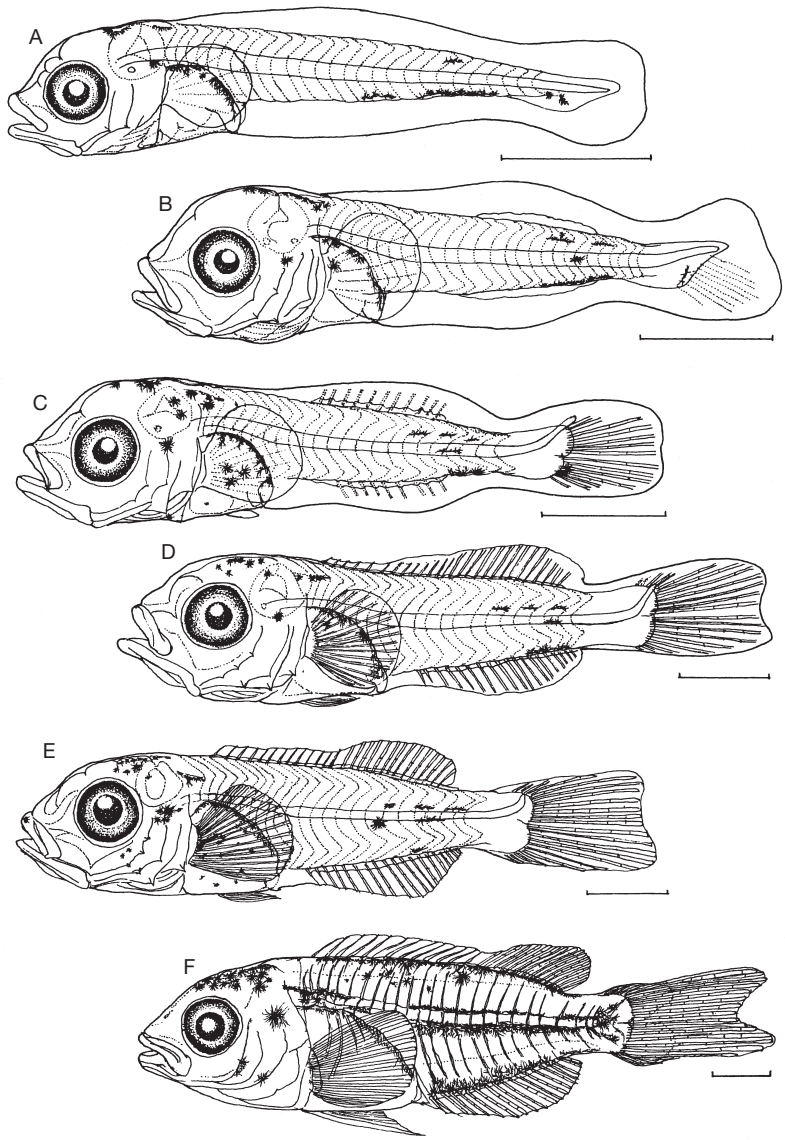


Fig. 2 Larvae and juvenile of Nagasaki Damsel *Pomacentrus nagasakiensis*.
A, postlarva, 8 days after hatching, 2.42 mm TL; B, 9 days after hatching, 5.02 mm TL; C, 12 days after hatching, 5.26 mm TL; D, 15 days after hatching, 7.19 mm TL; E, juvenile stage, 16 days after hatching, 8.01 mm TL; F, 19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

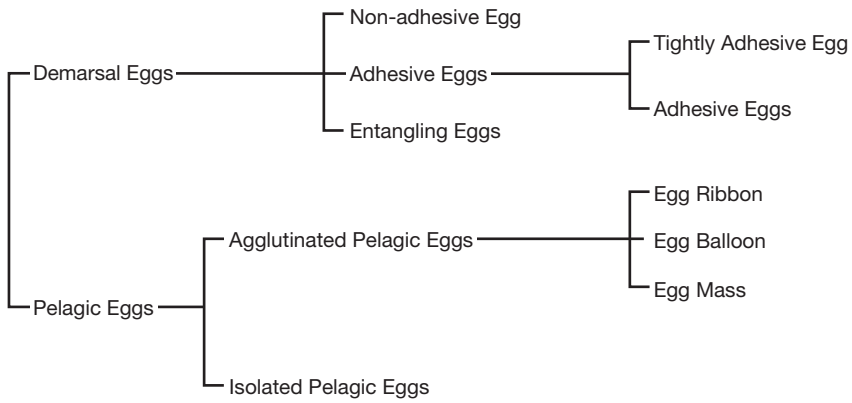


Fig. 1 Types of teleosts eggs. Modified from Ikeda & Mito (1988).

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.

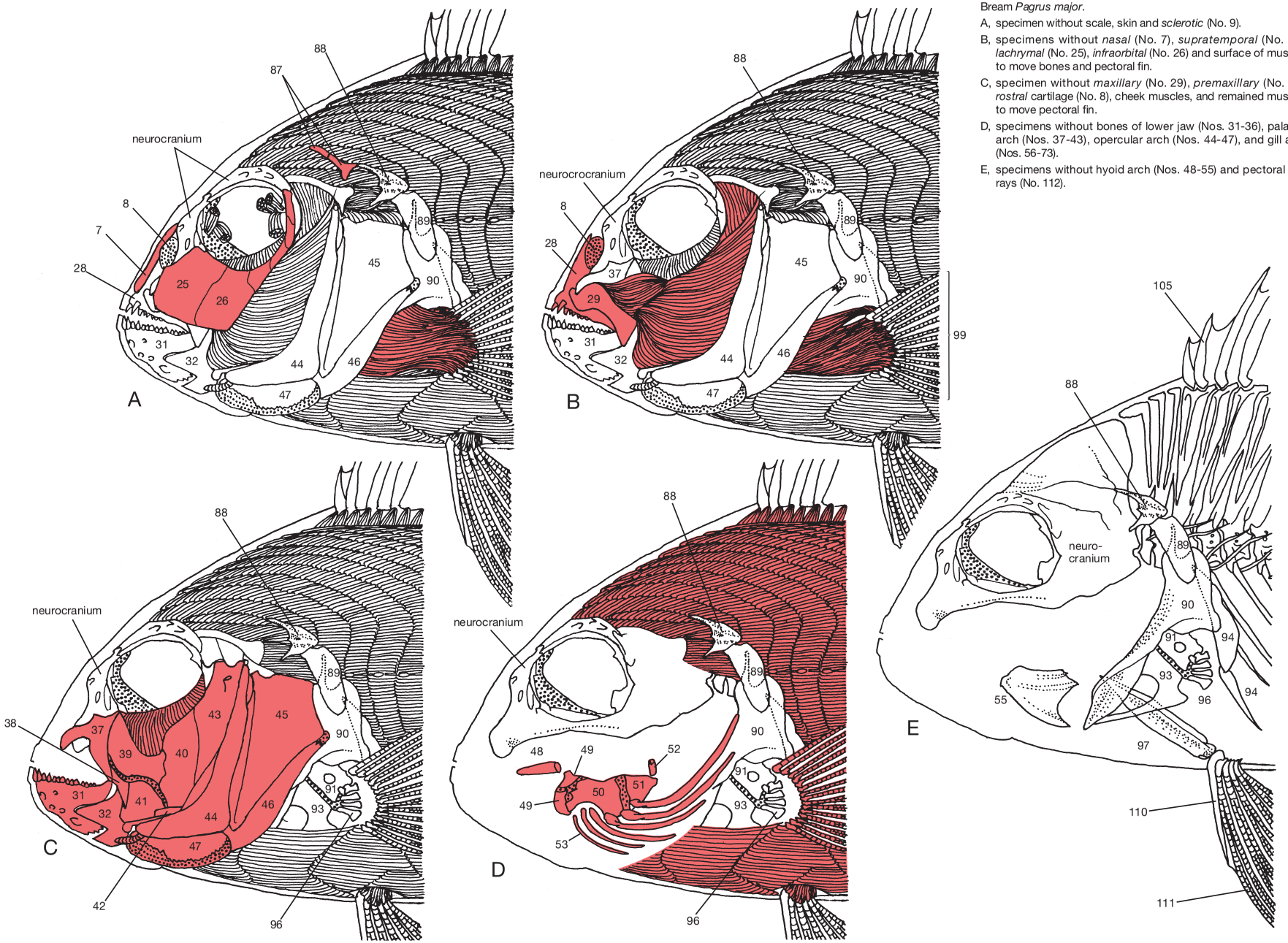
Young stage: The morphological characteristics are very similar to those of adult fish. However, fish are in the middle of development because body color, mottles, or spots peculiar to the species have not yet appeared. This is equivalent to the stage in which the growth is most active. The generally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