Chapter 3
Osteology of Bony Fishes

In this chapter we introduce you to the structure of the skeleton in bony fishes, a subject of utmost importance for several reasons. First, you cannot understand how a fish swims, maneuvers, feeds, and breathes without knowing the mechanics of associated bones and muscles. Second, the adaptive variation in bone form among species is reflected in important taxonomic characters for identifying and classifying fish, whether at the species, family, or higher levels. Some of the skeletal characteristics of bony fishes help explain the impressive and rapid adaptive radiation of these fishes, enabling them to occupy an amazing variety of habitats in all aquatic environments. These characteristics include vertebral number, structure of the jaws, pelvic fin formula (relative number of soft and spiny rays), position of the pectoral and pelvic fins, and caudal fin composition.

To facilitate understanding, we treat the skeleton of fishes much as one would a mechanical device such as an automobile, bicycle, or watch. In order to comprehend their functions, one must thoroughly understand the basic structure of the bones and how they are arranged. To gain this familiarity with fish osteology, you must first actually dismantle a fish piece by piece, while carefully studying the construction, and then, after cleaning all the parts, reassemble the skeleton so that all of the bones are properly connected. Though this is painstaking work, it demonstrates essential relationships between structure and function, and it makes comprehension easier in the long run. During the dismantling process, you must carefully note the separate parts and their relative positions. Drawing and labeling individual parts make reassembly easier.

Therefore our objectives in this section are:

1. To describe the major steps in dismantling a fish, that is, how to separate the skull from the axial skeleton, remove the pectoral and pelvic girdles, and remove the various remaining portions of the head (operculum, hyoid arch, hyomandibular series, and gills) from the neurocranium. For additional instruction, refer to Konnerth (1965) and Hildebrand (1968).
2. To describe the preparation and cleaning of each major portion of the skeleton, giving explicit instructions and providing clear diagrams to follow. We will describe the osteology of bony fishes in a general way, so that biologists using different species will be able to recognize and note bones that vary somewhat in shape. Remember that although a bone's specific structure may be slightly different from species to species, its general structure, position, and function may remain the same. For this reason, a detailed diagram showing the osteology of a generalized teleost will serve well to describe most bony fishes.
3. To provide an annotated list of the names of bones, following the classic reviews by Regan (1910), Goodrich (1930), Starks (1930), Gregory (1933), Stokely (1952), Harrington (1955), Norden (1963), and Mujib (1967). This list includes what we feel to be the most commonly used recent names for particular bones (see also Lagler et al. 1977 and Bond 1979).
4. To provide additional directions for osteological study of specimens by clearing and staining, whereby the bones are differentially stained and the muscle tissues are made transparent.

Then in chapter 4, we describe how the major muscles interact with these bones, especially in the head region, the fins, and along the lateral musculature.

HOW TO DISMANTLE A FISH: THE START

First, remove the head from the body. Several junctions need severing in order to do this. The top of the **pectoral girdle** on both sides attaches to *the skull by the* **post-temporal** bone (see figs. 3.1, 3.4, 3.5, 3.8). Clear away the skin and muscle from the dorsal part of the pectoral girdle where it meets the skull. Once this area is relatively clean, move the pectoral girdle left to right and back in order to find the location of its connection with the skull. Be careful not to cut them, but carefully move the two bones and cut between them. Repeat this process on the opposite side.

The ventral attachment of the pectoral girdle is where the two **cleithra** meet the **urohyal**, a single bone lying medially between the two opercular series of bones (fig.

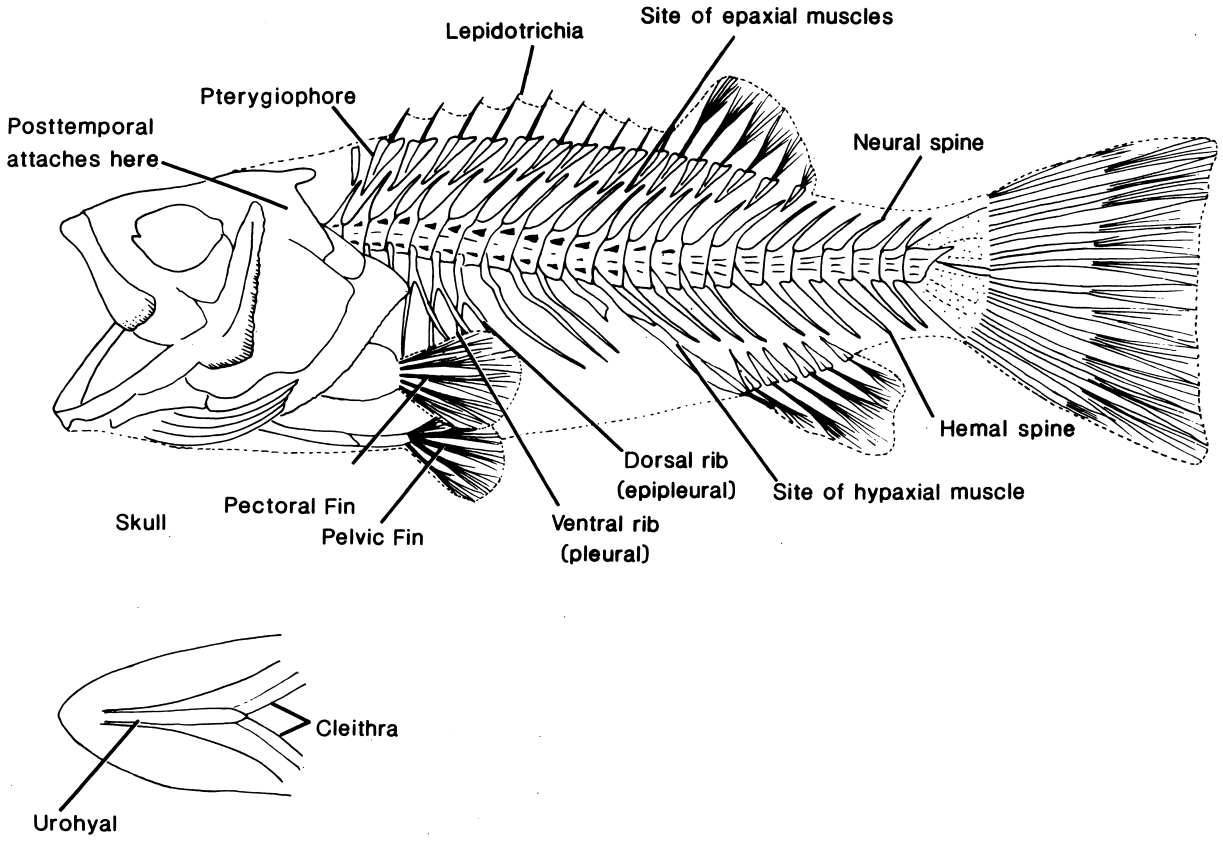

Figure 3.1 Diagram of the lateral view of the entire skeleton of a bony fish, showing its organization. Note the location of the posttemporal bone, one site of attachment of the pectoral girdle to the skull. Also note, in the lower left-hand insert, where the lower portion of the pectoral girdle, the cleithra, attaches to the urohyal.

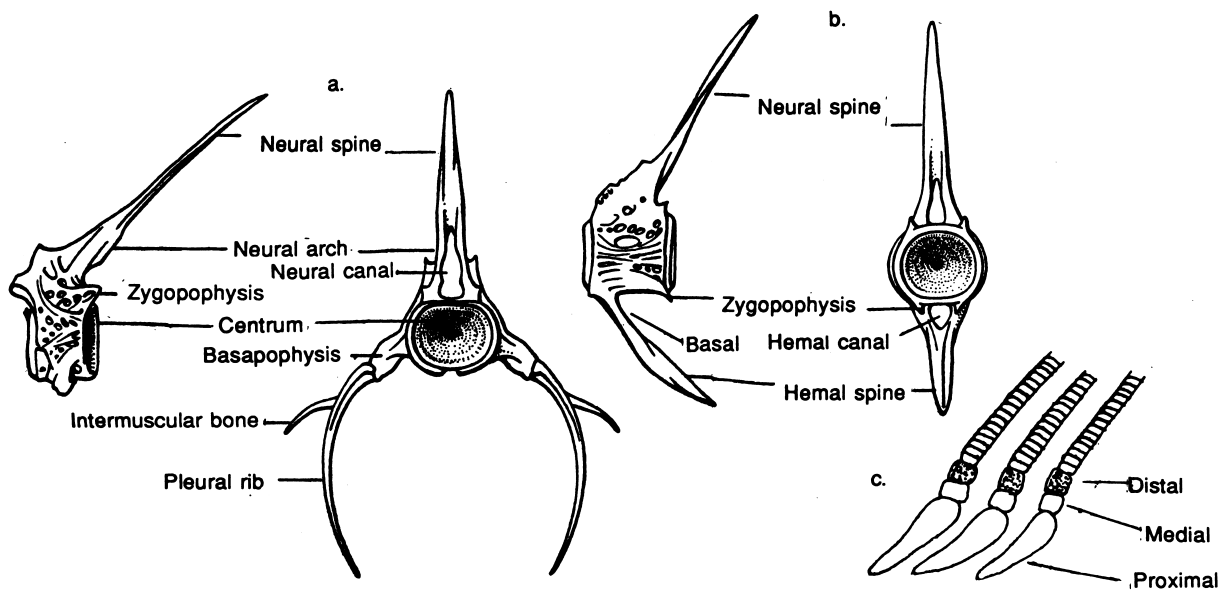

Figure 3.2 The two kinds of vertebrae found in teleosts: (a) abdominal; (b) caudal. (c) A lateral view of the pterygiophores of the median fins. Note that the three portions are shown separately; in most teleosts they are fused.
(a and b: Bond 1979, p. 72)

3.1). Again, move the two parts in order to find this junction and sever it with a sharp scalpel. To detach the posterior skeleton, cut between two of the most anterior vertebrae or in front of the first one, the **atlas vertebra**, and remove the remaining three sections of the fish: (1) the vertebral column, (2) median fins (dorsal, anal, and caudal), and (3) the **appendicular** skeleton (the pectoral and the pelvic girdles) (fig. 3.1). In some fishes, the pelvic girdle is attached at the ventral symphysis of the pectoral girdle ventrally (thoracic position), while in others it is located well behind (abdominal position). In either case, this girdle can be easily removed by severing tissue in front of the joined girdle bones, collectively called the **basipterygia** (fig. 3.4).

From here it is best to begin disarticulating relatively simple parts of the skeleton and then to proceed to the more complex and layered. Therefore, the following sections will cover in order: (1) the **axial skeleton**, excluding the skull but including median fins; (2) the **appendicular skeleton**; and (3) the skull and branchial arches, including the **suspensorium**, hyoid arch, branchial arches, and finally the braincase or **neurocranium**. The neurocranium supports the jaws and suspensorium, collectively called the **splanchocranium**. Diagrams that can be made into overlays best represent the layering of the head bones and reveal the spatial relations between various bones and their neighbors (see fig. 3.8 repeatedly during this dismantling and reconstructing exercise).

THE AXIAL SKELETON (WITHOUT THE SKULL)

Similarly structured elements are repeated along the main trunk or axis of the fish. First, remove the tissue surrounding the skeleton, primarily the **epaxial** (dorsolateral) and **hypaxial** (ventrolateral) muscle masses (see fig. 3.1). By repeatedly dipping the fish body into hot or warm water, you will soften the tissue surrounding the skeleton just enough to remove it easily. This saves considerable time and effort during the entire dismantling process. However, keep in mind that too long in the hot dip results in a mysterious pile of bones, a hopeless assembly problem for any beginner.

Be careful to preserve the many ribs and intermuscular bones extending sideways from the vertebral column. Reconstructing the whole fish skeleton takes considerable time but creates a valuable model for later study. If you do not reconstruct the whole fish, make sure you seek out, draw, and carefully study the construction and location of the structures mentioned in the following paragraphs.

The teleostean axial skeleton has two kinds of vertebrae. In the abdominal region, **precaudal vertebrae** bear ribs and intermuscular bones; they have neural spines but no haemal spines (fig. 3.2a). More posteriorly in the caudal or trunk region, **caudal vertebrae** bear no ribs and few, if any, intermuscular bones; they have prominent neural spines and have added haemal spines below (fig. 3.2b). **Pterygiophores** form the base of support for the dorsal and anal fin rays (fig. 3.2c). They are embedded in the dorsal musculature and are usually comprised of three fused bones, called the proximal, medial, and distal pterygiophores. On top of each pterygiophore, a small hemisphere, the **basal**, forms part of the fin-ray support. Several delicate muscles along the axis of the pterygiophores move fin rays laterally as well as longitudinally, providing considerable flexibility of fin orientation.

The caudal skeleton of teleosts, an important character for study of relationships and phylogeny, has become quite specialized in many species. It is made up of modified preterminal and terminal vertebrae, which support and strengthen the caudal fin for use in propulsion (fig. 3.3). Modified neural spines and neural arches form splints and bony plates called **epurals** and **uroneurals**, respectively. In advanced teleosts, the last two caudal vertebrae (the first ural and first preural centra, ural number two being lost) are fused into a single element called the **urostyle**. Below the urostyle four to six haemal arches are usually modified into platelike supports called **hypurals**. The terminology of the most posterior vertebrae can be confusing, but usually the last vertebra is the **ultimate** or **preural vertebra**, while the one immediately preceding is the **penultimate** or **preural II vertebra**. The term **antepenultimate** refers to the vertebra anterior to the penultimate.

THE APPENDICULAR SKELETON

The pectoral girdle and its associated fins are basically a series of linearly connected bones forming a circle around

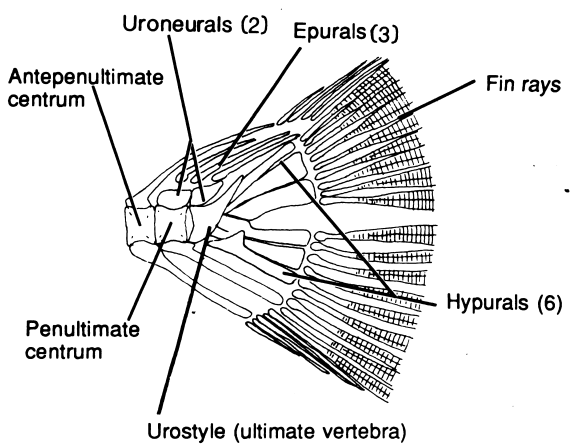

Figure 3.3 Generalized diagram of the caudal skeleton of teleosts. In more advanced teleosts, especially those that swim continually, fusion of various elements, such as the hypurals and preural vertebrae, occurs often.

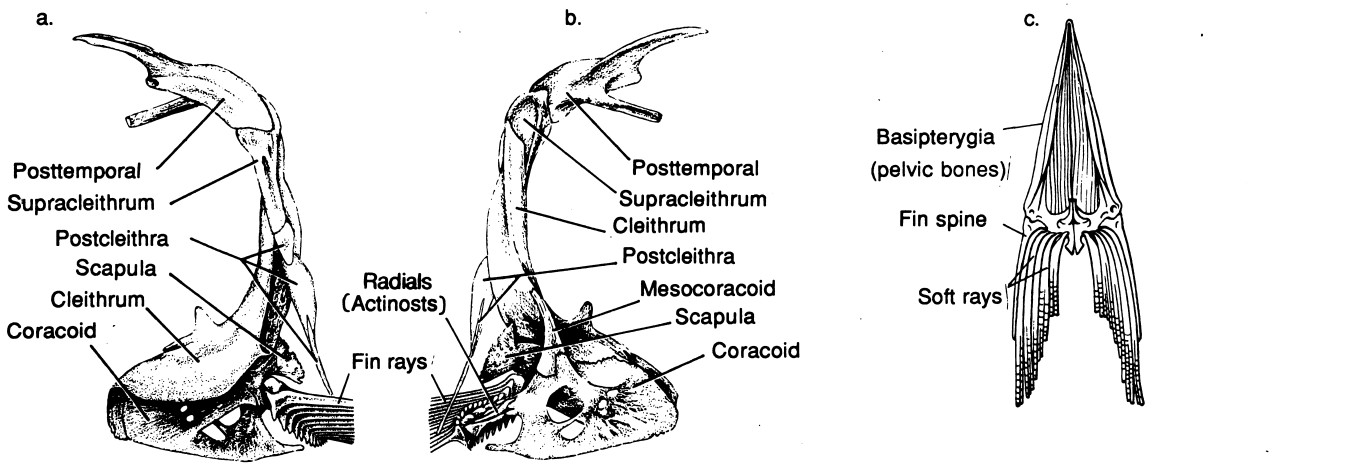

Figure 3.4 Diagram of the appendicular skeleton of the teleost *Pomolobus mediocris:* (a) outer view of left pectoral girdle; (b) inner view of left pectoral girdle; (c) ventral view of the pelvic girdle of *R. saxatilis.* (Parts a and b drawn by Margaret G. Bradbury, from Mead and Bradbury 1963; part c from Bond 1979)

the trunk of the fish just behind the opercular opening (fig. 3.4). The bones of this sequence begin with the posttemporal, which connects the pectoral girdle and neurocranium at attachment points on the **epiotic** and **opisthotic** bones (see figs. 3.5, 3.7, 3.8). Following the posttemporal, the girdle has, in order, the **supracleithrum, postcleithrum,** and **cleithrum** bones. Attached to the base of this semicircular series are the **scapula** and **coracoid,** which support the pectoral fin rays via a series of smaller bones called **radials** or **actinosts.** In more primitive fishes, like the salmonids, there is also a bone called the **mesocoracoid,** which acts as a brace between the cleithra (above) and the coracoids and scapulae (below). This series of bones, along with the muscles that move them, has made advanced teleosts more maneuverable in spatially complex environments, such as reef areas.

The simpler pelvic girdle has also increased bony fish maneuverability by enlarging and moving forward to insert under the pectoral fins. It consists primarily of a paired set of plates called **basipterygia,** to which the fin rays are directly attached (fig. 3.4). In most advanced teleostean fishes, the pelvic fin consists of a spine followed by several soft rays.

THE SKULL AND ITS ASSOCIATED BONES

Once you have prepared the posterior axial and appendicular skeletons, prepare to disassemble the skull and its associated bones. Before beginning, you should learn a few general techniques (see Konnerth 1965). Do not disarticulate the head bones too quickly or thoroughly since the main benefit *is step-by-step* observation of how the parts fit together and function as a whole. Use hot water sparingly to help soften tissues, but do not immerse the head so *it* disintegrates, leaving a confused pile of bones that cannot be easily reassembled. It is also best to dissect only one side of the head at a time. As you remove bones from one side, those on the other side remain intact as a guide for reassembly. For the same reason, it is best to keep the jaw complex with its suspension intact, as well as the hyoid with branchial apparatus. A good way to do this is to wrap sections in cheesecloth or rags and dip each carefully.

In a strict sense, the skull is part of the axial system, but here you will prepare the skeleton of the head region as a separate unit. Start with the superficial bones—the circumorbital series and the part of the branchiocranium associated with the opercular covering. Then prepare the splanchnocranium (oromandibular region), which includes the upper and lower jaws and suspensorium, consisting of the **palatine,** pterygoid series, **symplectic, quadrate,** and **hyomandibular** bones. Continue posteriorly with the bones of the hyoid apparatus and those partially supporting the branchial bones. After this second layer is removed, cleaned, and diagramed (fig. 3.5), carefully dissect out the tiny numerous bones comprising the branchial region and supporting the gills (fig. 3.6b,c). Once these superficial bones are removed from the head, you will find the bones that comprise the neurocranium, which is classified into **endodermal** (ethmoid, orbital, otic series) and **dermal** (nasal, frontal, parietal, and so on) parts.

GUIDELINES FOR PREPARING STUDY SKULLS

This section roughly follows Nelson (1963) and Konnerth (1965).

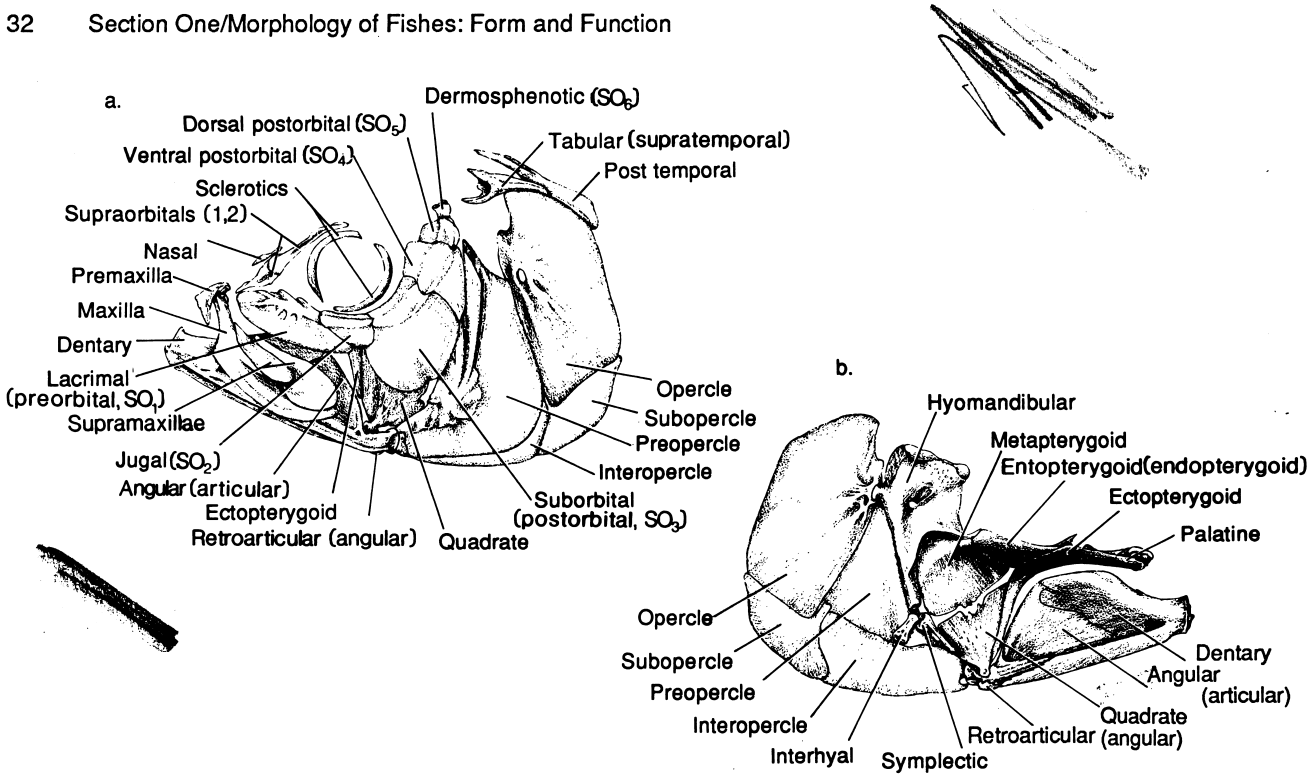

Figure 3.5 Superficial face bones and suspensorium of *Pomolobus mediocris*: (a) lateral view of the left side of the head, underlying bones of the neurocranium not shown; (b) inner view of the left suspensorium, lower jaw, and opercular series. (Drawn by Margaret G. Bradbury, from Mead and Bradbury 1963)

1. Remove the skin and as much tissue as possible from the head.
2. Remove both eyes and their associated muscular and nervous tissue.
3. Locate the **circumorbital series** and remove the left side by inserting a sharp scalpel underneath it and undercutting in a circular fashion. Should you have a scorpaeniform fish as a specimen, cut carefully in the posterioventral region about the retrorse suborbital stay. Keep the circumorbital series connected until you are ready to completely dissect out all tissue. Once each small bone is removed, mark it or tape it in its natural position onto a nearby piece of paper to use as a guide for reassembly.
4. Slice between the right and left halves of the upper and lower jaws. This will allow you to separate both sides of the head anteriorly.
5. Disarticulate the jaw assembly from the rest of the head bones by cutting between the lower jaw at the symplectic or hyomandibular bone and the small bone to the gill apparatus, the **interhyal**. Then swing the entire jaw apparatus laterally away from the skull while noting where it articulates. At the point where the hyomandibular attaches dorsally at the sphenotic bone, slice through connective tissue and remove the entire side of the jaw (see figs. 3.5, 3.7, and 3.8).
6. To remove the hyoid and branchial complex from the remaining head region, disarticulate the interhyal from inside the hyomandibular series on the opposite side and cut around the bones of the gills at the roof of the mouth (the upper pharyngeal bones) that are usually fused and closely attached to the base of skull (see figs. 3.5, 3.6, and 3.8). Then, to further clean the bones of the gill arches and their supporting bones, proceed carefully to tease and pick at the tissue holding the bones together. Disassembly of the anteriolateral bones, the hyoid series—from the glossohyal to interhyal—does not require special care because the linkages are fairly easy to reconstruct. However, the branchial apparatus is difficult to clean and reassemble completely. You may want to keep it intact while simply locating the position of each tiny component under the connective tissue. Simply dry the entire branchial apparatus for several days before returning it to its proper *anatomical* place. Unfortunately, it often shrivels up or twists so that it may not fit properly.
7. Now return to the splanchnocranium and remove the most exterior series of bones, which are the opercular bones, from the jaw assembly (figs. 3.5, 3.8). The opercular series can be separated on each side from the jaws by carefully cutting between the interopercle and the preopercle bones from the posterior ends of the preopercle forward. Next detach the anterior end of the interopercle from the mandible and clean the four remaining bones.
8. Clean the suspensorium and the jaw bones (figs. 3.5,

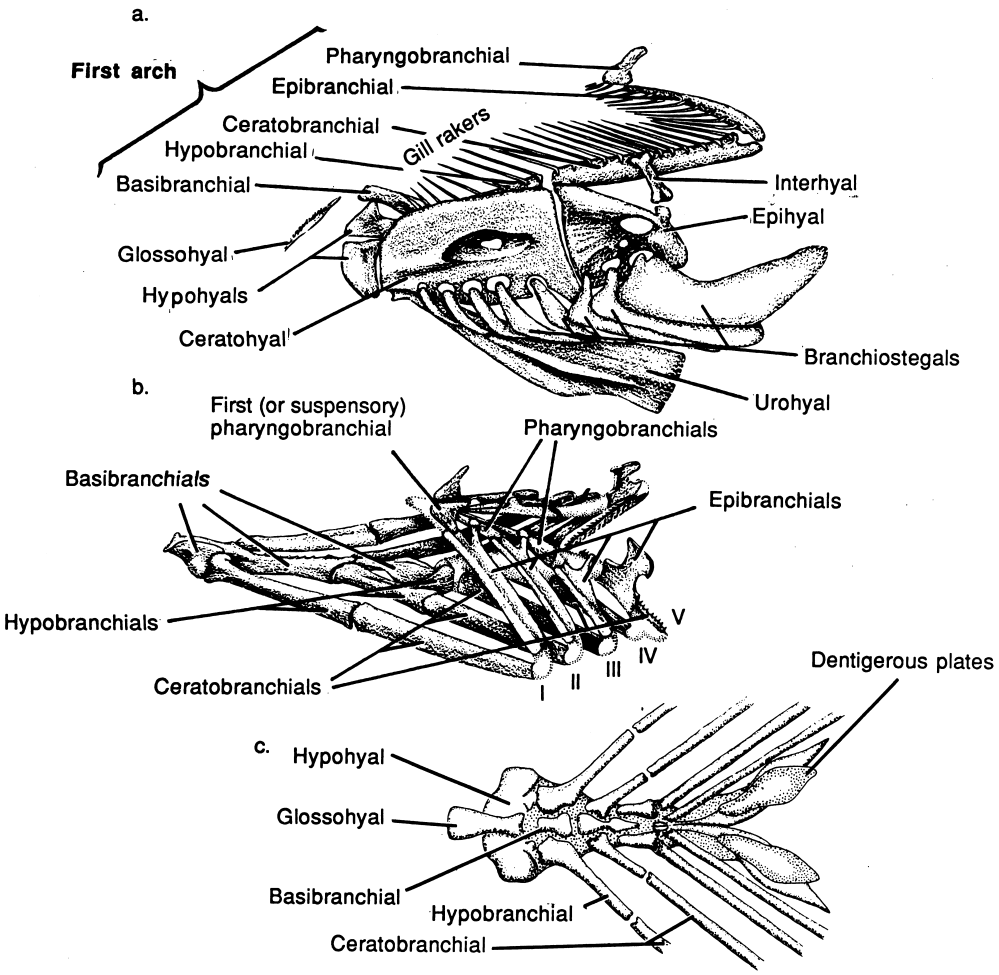

Figure 3.6 Hyoid arch in a branchial basket of *Pomolobus mediocris:* (a) lateral view of left hyoid arch with associated branchiostegal rays and first branchial arch; (b) three-quarter view of branchial arches; (c) dorsal view of the base of the same structure for the rockfish *Sebastes.* (Parts a and b drawn by Margaret G. Bradbury, from Mead and Bradbury 1963; part c after Morganroth and Morganroth 1969)

3.8). Retain the connections among these bones for orientation; limit the cleaning process to dipping them into hot water for short periods, then scraping and picking with scalpel and forceps.

9. Finally you can clean the neurocranium by more severe soaking and picking since most of the skull bones are securely sutured together. Cleaning and degreasing the skull and all associated bones (and softening the connective tissues) can be accomplished by any of several procedures and chemical treatments. Restricted dipping in hot water to soften tissues and then scraping or picking the tissues away are the best techniques when you want to keep structures intact. However, once cleaned, most bones must still be degreased and perhaps bleached. Simply soaking in hot water removes small amounts of oil, but detergents that contain enzymes or "presoakers" (Ossian 1970) are particularly effective; for stubborn jobs, soak in 5 percent bleach for 15 to 60 minutes. Acetone is a less effective substitute for detergents. Degrease your specimen with care, however; it would be a shame to disarticulate your hard work at this late stage by too strong a final cleaning. A technique that works quickly on both fresh-frozen and preserved specimens using trypsin or pancreatin is described by Mayden and Wiley (1984).

10. Once your skull or skeleton has been dissected, cleaned, and degreased, assemble the various bones by gluing them back together with common model airplane glue (or, even better, a glue gun). To keep certain bones oriented correctly during the drying phase, use a malleable clay to temporarily secure joints until the glue dries. The skull or entire skeleton can then be mounted on a stand that holds the specimen through the **foramen magnum** of the skull.

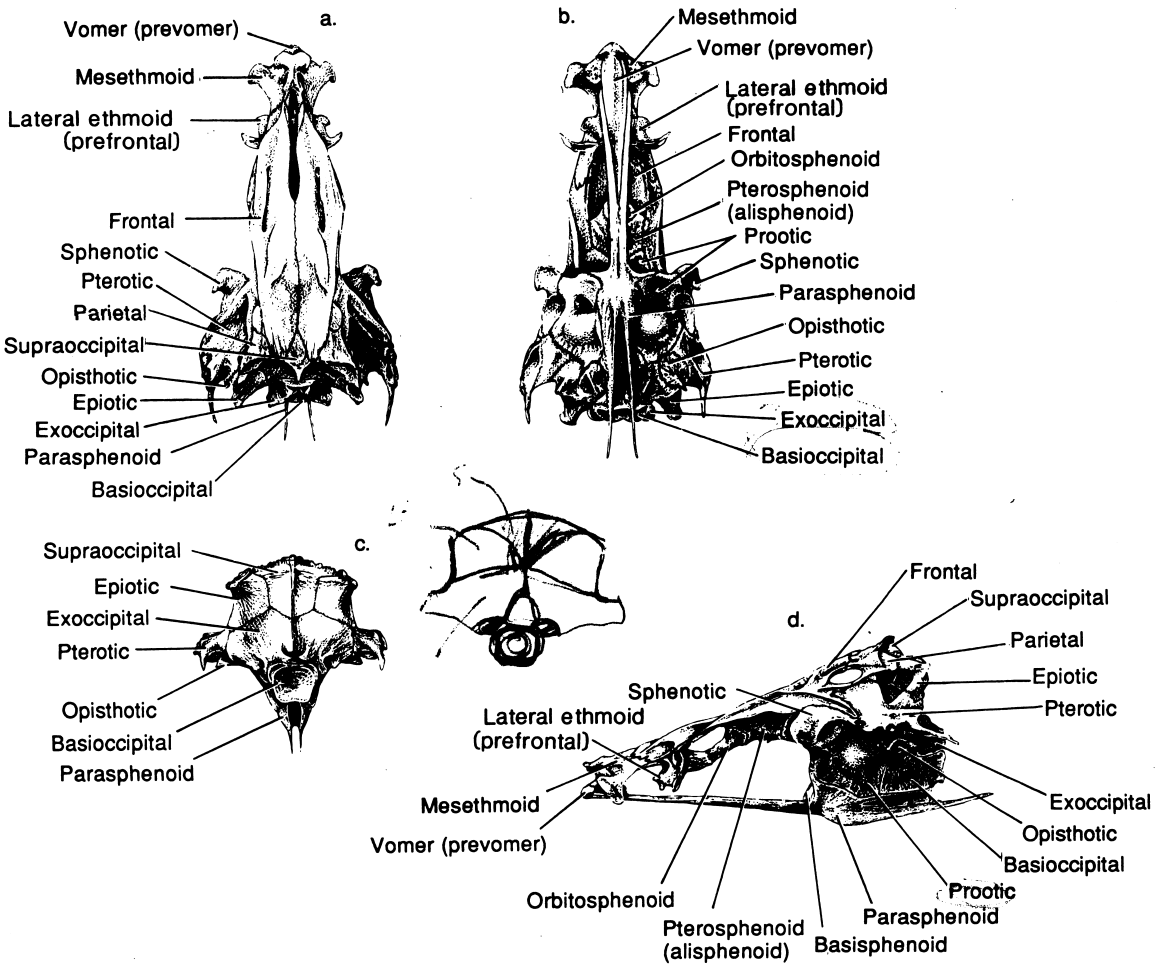

Figure 3.7 Neurocranium of *Pomolobus mediocris:* (a) dorsal view; (b) ventral view; (c) posterior view; (d) lateral view. (Drawn by Margaret G. Bradbury, from Mead and Bradbury 1963)

Now you can study the completed skeleton. You should carefully study the following figures (previously referred to in this guide) with specimen in hand. Note how your fish skeleton differs from the figures of generalized configurations of bones. Continuously ask yourself what functional reasons explain the differences in your specimen from those pictured.

ANNOTATED AND DESCRIPTIVE LIST OF TELEOST BONES

The following complete list of teleostean bones is extracted from several review references on fish osteology (Harrington 1955, Norden 1963, Mujib 1967, and Bond 1979). This is not intended to be the final word on bone terminology, but rather it provides synonyms for bone names taken from different authors so that researchers who know the same bones by different names can communicate. The latest or most widely accepted name precedes its synonyms.

I. Axial skeleton
  A. Skull
    1. Neurocranium
      a. Olfactory region

Mesethmoid (Ethmoid, Hypethmoid): a medial bone lying between nasal capsules.

Lateral Ethmoids (Prefrontals): a pair of bones in the ethmoid region, which separate the olfactory capsule from the orbit.

Vomer (Prevomer): a median bone, usually bearing teeth, at the anterior extremity of the roof of the mouth.

Preethmoids: paired bones in the floor of the nasal capsules.

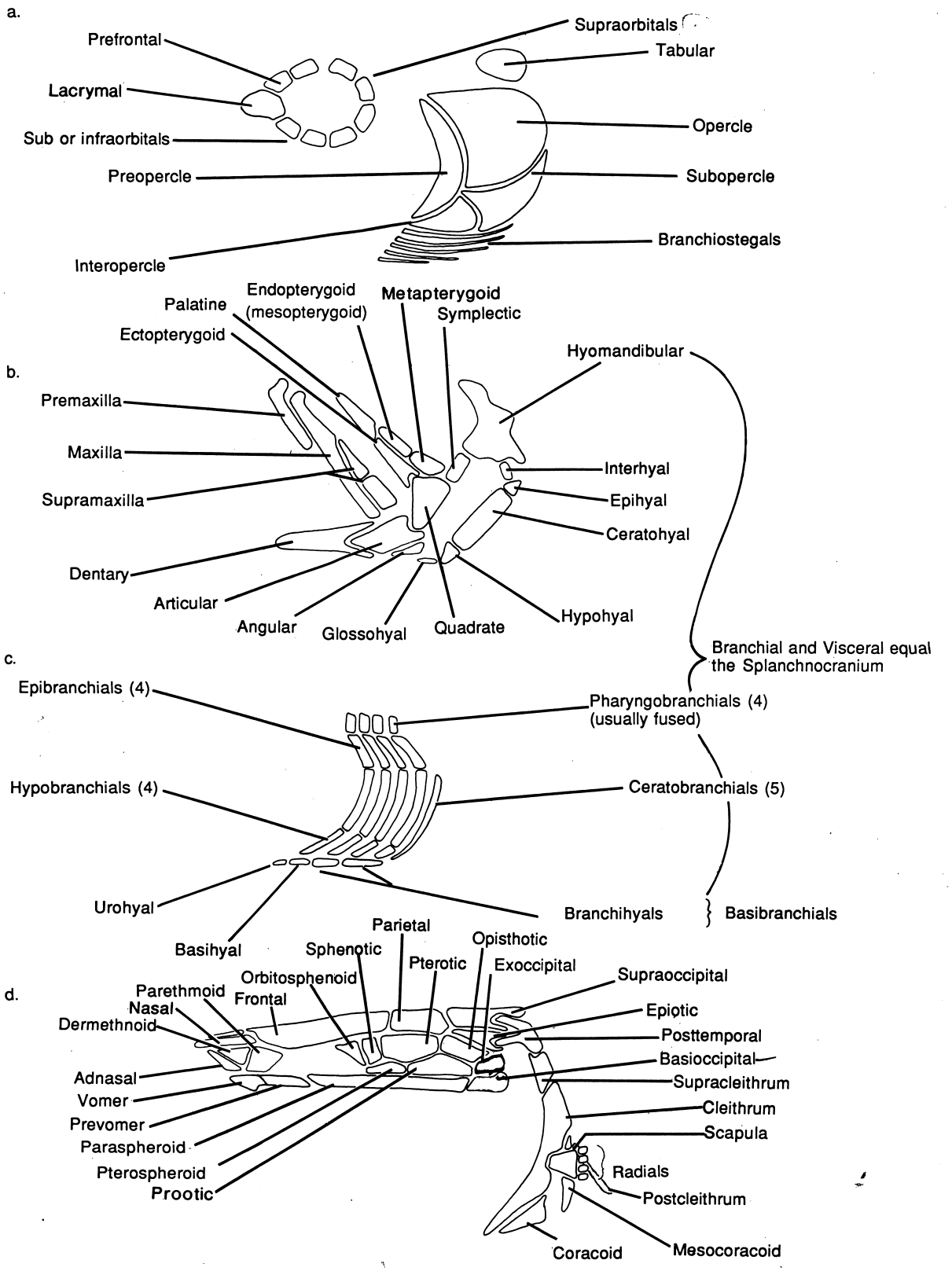

Figure 3.8 Diagrammatic layering showing the bones of the teleost skull: (a) the superficial suspensorium; (b) the facial suspensorium; (c) the internal branchial apparatus on the hyoid arch; (d) the neurocranium and the pectoral girdle. Superimpose these layers in order to visualize which systems are interconnected. If you have access to transparency copiers, copy the three superficial layers and superimpose them on each other, layer by layer. (*Modified from Harder 1975*)

Supraethmoids (Mesethmoids): paired bones dorsal to the mesethmoid (ethmoid).
Kinethmoid (Rostral): median unpaired bone in cyprinoid fishes.
Nasals: a pair of small tubular bones lying on the sides of the anterior tips of the frontals.

b. Orbital region
Frontals: a pair of bones that form most of the dorsal surface of the cranium, covering the orbitosphenoid and pterosphenoids.
Orbitosphenoid: median bone between orbits, forming the floor and walls of the anterior end of cranial cavity (in *Salmo* and *Cyprinus*).
Pterosphenoids (Alisphenoids): paired lateral bones joining the orbitosphenoid in front and the sphenotics (autosphenotics) and prootics behind (in *Salmo*).
Sclerotics (Sclerotic cartilage): pair of hemispherical cartilages surrounding the eyeballs.

"Suborbitals": a series of paired bones around the margin of the orbit; usually six or less; bear the suborbital lateral line canal.
Lachrymal (Preorbitals, SO_1): the most anterior bone in suborbital series; often the largest.
Jugal (SO_2)
Postorbital (SO_3)
Fourth Suborbital (SO_4)
Fifth Suborbital (SO_5)
Dermosphenotic (SO_6): the last four are also called the postorbitals.

"Supraorbitals": paired bones along the upper margin of the orbit; not traversed by the lateral line.
Supraorbitals (number 1 and number 2)

c. Otic region
Sphenotics (Autosphenotics): paired bones lying beneath the dermosphenotics and forming the lateral processes behind the orbit.
Pterotics (Autopterotics, Autopalatines): paired bones each enclosing the horizontal semicircular canal of the inner ear; join the sphenotic (autosphenotic) in front, the prootic below, and the epiotic and exoccipital behind; covered by posttemporal.
Prootics: paired bones each forming the base of the otic capsule and enclosing the utriculus in a ventrolateral bulla; join pterosphenoid in front, sphenotic and pterotic above, and exoccipital and basioccipital behind.
Epiotics (Epioccipitals): paired bones each enclosing the posterior semicircular canal; join supraoccipital above, pterotic in front, and exoccipital beneath and behind.
Opisthotics (Intercalary): paired bones, often excluded from otic capsule, lying beneath and behind pterotic; cover junction of pterotic, epiotic, and supraoccipitals, and perhaps the sphenotic.
Exoccipitals: paired bones at back of skull; form the sides of the foramen magnum, with condyles articulating with the first vertebra.
Supraoccipital: median bone forming posterior roof of skull; often bears a crest.
Supratemporals (Tabulars, Extrascapulars, Scalebones): paired bones covering the pterotic; contain part of lateral line and articulate with the posttemporal bone of the pectoral girdle.
Parietals: paired bones on roof of skull behind the frontals and partly or wholly separated by supraoccipital.
Basioccipital: bone forming posterior base of skull, articulating with centrum of the first vertebra.
Basisphenoid: small, median, Y-shaped bone in rear of orbit.
Parasphenoid: long, unpaired bone running midline below the orbits; between prevomer and basioccipital.
Foramen Magnum: posterior opening in cranium through which the spinal cord passes as it leaves the brain.

2. Branchiocranium
   a. Oromandibular region (the Splanchnocranium equals the jaws and palate)
      (1) Upper jaw
      Premaxillaries (Premaxilla): paired bones forming front of gape; toothed.
      Maxillaries (Maxilla): paired bones behind or above the premaxillaries.
      Supramaxillaries (Supramaxilla): one or two pairs of bones above the maxil-laries; in primitive teleosts only.

(2) Lower jaw
Angulars (Anguloarticulars with next bones in more advanced teleosts): paired bones occupying part of posterior end of lower jaw.
Retroarticulars (Angulars, Retroangulars): paired bones each at the lower posterior corner of the angular.
Dentaries: large paired bones forming the front of the lower jaw and fused at the front with the ossified tip of the sesamoid angular (Meckel's cartilage).
Sesamoid Angular (Meckel's cartilage, Coronomeckelian): paired bones each inside of angular bone of lower jaw and involved in attachment of mandibular adductor muscle.
(3) Suspensorium (Palatines, Pterygoids, Hyomandibulars)
Palatines (Autopalatines): paired bones forming the most anterior component of the pterygoquadrate arch.
Entopterygoids (Endopterygoids): paired, articulating with palatine in front and joining metapterygoid behind.
Metapterygoids: paired bones, each joining the entopterygoid in front and articulating with hyomandibular behind.
Ectopterygoids (Pterygoids): paired bones, each joining the entopterygoid above and the quadrate behind; between palatine and quadrate.
Quadrates: paired bones, each joining the ectopterygoid in front, the entopterygoid above, and articulating beneath with the angular of the lower jaw.
Hyomandibulars: paired bones each articulating above with the otic capsule and the symplectic below.
Symplectics: small paired bones each at the lower tip of the hyomandibular.
4. Opercular series (Operculum)
Opercles (Operculars): flat paired bones comprising most of the gill cover.
Preopercles (Preopercular): paired bones ahead of opercle, partially cover hyomandibular and carry a branch of lateral line canal; lie just behind suspensorium of lower jaw.
Interopercles (Interoperculars): paired bones lying below preopercles, separating them from the subopercles.
Subopercles (Suboperculars): paired bones lying below opercles and overlapping the branchiostegal rays.
Subtemporals (Suprapreopercles): one or more small paired dermal tube bones carrying the lateral line canal across the gap between the preopercle and supratemporal (tabulars); often absent.
b. Hyoid region
Interhyals: small paired cartilage bones, each connecting the epihyal beneath to the symplectic or hyomandibular above.
Epihyals: paired bones each joining the ceratohyal beneath; bear three pairs of branchiostegal rays.
Ceratohyals: paired bones joining the hypohyals in front and bearing branchiostegal rays below.
Hypohyals (upper and lower): paired set of bones joining ceratohyal behind and joining with the glossohyal medially.
Glossohyal (Basihyal): unpaired bone lying just above the junction of the lower hypohyals with front end free; forms base of tongue.
Urohyal: unpaired bone behind and beneath the glossohyal; it arises in a septum between the longitudinal throat muscles.
Branchiostegal Rays (Branchiostegals): from 6 to 34 pairs of rays arising from the ceratohyal or epihyal and forming the floor of the branchial chamber.
c. Branchial region
Pharyngobranchials (Dorsal Pharyngeal Plate, Infrapharyngobranchials): paired bones forming upper members of the first (usually) four branchial arches; the fourth sometimes bear teeth.
Pharyngeal Plate: three pairs of small, toothed dermal plates that are found in gills; the first pair born on the third pharyngobranchials, the second pair on the fourth pharyngobranchials, and the third pair on the fifth pharyngobranchials.
Epibranchials: paired cartilage bones beneath pharyngobranchials of the first (usually) four arches; articulate with ceratobranchials below.
Ceratobranchials: paired bones beneath epibranchials on (usually) all five arches; the fifth sometimes bears the lower

pharyngobranchial teeth; articulate below with hypobranchials.

Hypobranchials: paired bones beneath ceratobranchials of the (usually) first four arches; sometimes covered dorsally by tooth-bearing plates; in salmonids, they are on each of the first three gill arches.

Basibranchials: usually three unpaired bones lying in midline, articulating end-to-end with each other and with the glossohyal in front and grasped by the paired hypobranchials.

B. Vertebral column
1. Vertebrae
Abdominal Vertebrae (Precaudal Vertebrae)
Caudal Vertebrae
Atlas Vertebra
Ultimate Vertebra (Preural Vertebra)
Penultimate Vertebra (Preural II Vertebra)
Epineurals

II. Appendicular skeleton
A. Median fins
1. Caudal fin
Epurals: two or three median bones that lie dorsal of uroneurals; detached; believed to be modified neural spines.
Uroneurals: three pairs of bony plates that lie over the last three upturned vertebrae and the urostyle; believed to be remnants of neural arches; the larger, more anterior pair is sometimes called the caudal bony plate.
Urostyle (Ural Vertebra): the cartilaginous termination of the vertebral column; in salmonids it curves dorsad behind the last three upturned vertebrae.
Hypurals: series of (usually) six expanded haemal spines that lie ventrally of the three upturned vertebrae and the urostyle; often fused in advanced fishes.
Parhypural: the last haemal spine (see Bond 1979).
2. Dorsal fin and anal fins
Pterygiophores: a series of three endoskeletal rods that support the dorsal and anal fins; have three segments, including (1) round distal bone, (2) short, horizontal middle bone, and (3) long, pointed proximal bone.
Fin rays: dermal bony rays of two kinds: (1) soft and articulated fin rays, and (2) spiny, nonarticulated fin rays.
Interneurals: a series of median supporting rods that lie in the muscle, anterior of dorsal fin, between the bifurcated neural spines.
Interhaemals: similar series of median supporting rods as interneurals, but lie in the muscle, anterior of anal fin between the bifurcated haemal spines.

B. Paired fins
1. Pelvic fins
Basipterygium (Pelvic Bone): a triangular endochondral bone that, in pairs, forms the pelvic girdle; collectively called basipterygia.
Pelvic fin rays
2. Pectoral fins
Posttemporals: a pair of forked dermal bones that connect the pectoral girdle to the epiotic and opisthotic bones of the cranium.
Supracleithrum (plural: Supracleithra): a pair of curved dermal bones located in the pectoral girdle that connect the cleithra with the posttemporals.
Postcleithrum (plural: Postcleithra): usually three pairs of small, scalelike dermal bones that lie in a vertical series posterior to the cleithra; occur in all salmonids; in some fishes these bones act as a brace for the pectoral girdle.
Cleithrum (plural: Cleithra): a pair of large, curved intramembranous bones that form most of the pectoral girdle.
Scapula (plural: Scapulae): a pair of flat bones located in the pectoral girdle; articulate anteriorly with coracoids and posteriorly with the cleithra.
Coracoid (plural: Coracoids): a pair of triangular bones of the pectoral girdle; articulates with the cleithra and the scapulae.
Mesocoracoid (plural: Mesocoracoids): a pair of curved bones that act as braces between cleithra (above) and coracoids and scapulae (below); in primitive fishes like salmonids.
Radials (Actinosts): small endochondral bones of each pectoral girdle that articulate proximally with scapulae and distally with the fin rays.
Pectoral fin rays

METHODS FOR CLEARING AND STAINING FISHES

Over the years, several techniques have been developed so that it is unnecessary, at least for small fish or small portions of large fish, to dissect out all the bones to study osteology. In these techniques, the general approach is to stain the entire fish with a noticeable dye (such as alizarin red) and then to clear the external tissue so that only the bones retain the stain. These specimens can then be studied

by a dissecting microscope to decipher their bony structures with the fish specimen intact. In more recent advances, both cartilage and bone are stained differentially on the same specimen (Dingerkus and Ohler 1977). The following guide to clearing and staining is a modified version of several published methods (Hollister 1934, Taylor 1967a and 1976b, Simons and Van Horn 1970/71 and 1971, Dingerkus and Ohler 1977, Dingerkus 1981, Brubaker and Angus 1984, and Mayden and Wiley 1984).

It has long been known that methylene blue and toluidine blue are good stains to use on cartilage, and alizarin red S is very good at staining bone. Usually potassium hydroxide is used for clearing the tissues so that the stained bones and cartilage can be seen. A paper by Simons and Van Horn (1970/71) proposed, in addition, alcian blue for staining cartilage, especially on fresh material. It produces superior results when compared to either methylene or toluidine blue. However, in general this dye produces poor results in fishes preserved in either formalin or alcohol, primarily due to difficulty in removing the excess alcian blue from the flesh. More recently, however, biologists have been using enzyme solutions to clear the flesh, either instead of potassium hydroxide or in conjunction with it; this enables the researcher to obtain results that are far more reliable and useful, in which the cartilage remains a deep blue color. The resulting specimens have intense blue cartilage, dark red bones, and clear, transparent flesh, without being torn apart by the procedures used. In addition, these specimens can be preserved, and they still produce high quality results, even those that have been in formalin or alcohol for many years.

The following materials are needed for this procedure:

1. Alizarin red S powder
2. Potassium hydroxide (KOH)
3. Distilled water
4. Borax or sodium borate powder
5. Glycerine
6. Trypsin or pancreatin powder
7. Thymol crystals
8. 40% formaldehyde solution
9. 3% hydrogen peroxide (H_2O_2)
10. Xylene
11. Alcohol, either 95% ethanol or 99% isopropyl
12. Alcian blue 8GN
13. Glacial acetic acid

(Note: Items 10 and 11 are used if the fish is fatty or you plan to embed it in plastic.)

The stock solutions are prepared as follows:

1. Stock potassium hydroxide: Mix 100 grams of potassium hydroxide in 900 ml of distilled water to make a 10% solution (100g/liter).
2. 2% potassium hydroxide: Mix two parts 10% KOH solution (solution #1) to eight parts distilled water to make a 2% solution.
3. 10% formalin: Mix one part 40% formaldehyde to three parts distilled water.
4. Bleaching solution: Mix two to three parts 3% hydrogen peroxide with seven to eight parts 2% potassium hydroxide (solution #2).
5. Saturated sodium borate solution: Mix sodium borate in distilled water to make a saturated solution; allow to settle until clear.
6. Enzyme buffer: Mix three parts saturated sodium borate (solution #5) with seven parts distilled water.
7. Stock stain solution:
   a. Mix the following:
      5 ml 50% glacial acetic acid
      10 ml glycerine
      60 ml 1% chloral hydrate
   b. Saturate with alizarin red S stain.
8. Stain solution: Mix 1 ml of stock stain solution (solution #7) in 100 ml 2% KOH (solution #2).
9. Alcian blue stain: Mix 10 mg alcian blue 8GN with 80 ml 95% ethyl alcohol and 20 ml glacial acetic acid (50%). This solution must be made fresh.
10. Glycerine solutions: Prepare the three solutions for use in sequence:
    a. 30% glycerine in 2% KOH
    b. 60% glycerine in 2% KOH
    c. 90% glycerine in 2% KOH

Methods for preparing, staining, and clearing fish specimens:

1. Fix specimens in 10% formalin (solution #3). Allow specimens to remain in this solution for three to four days. For specimens already in formalin or alcohol, ignore this step and proceed to step #2.
2. Soak specimens in water until the formalin is removed. If the specimen is not to be cleared immediately, store it in 40% isopropyl alcohol.
3. Scale and eviscerate the specimen as far as possible here. Remove the eyes. In delicate specimens, scaling should be done after the staining.
4. Bleach the specimen in a solution of 2% KOH and hydrogen peroxide (solution #4) until most dark pigments are gone. The specimens should appear yellowish. This step should take five to seven days.
5. Place the specimen directly into the alcian blue stain solution (solution #9) for 24 to 48 hours.
6. Transfer the specimen to distilled water for two to three hours, or until it sinks.
7. Place specimen in enzyme buffer (solution #6). Make the volume of this solution about 10–40 times the volume of the specimen. Mix 1/4 tsp. trypsin or pancreatin powder to each 400 ml of enzyme buffer. Change this solution once a week. Leave the fish in enzyme solution

until most of the vertebral column can be seen and the caudal peduncle is clear. For small specimens this process should take several days to a week; for large ones it may take several weeks or even months.

8. Place the fish in stain solution (solution #8) for several hours or until the fin rays are deep red. Be careful not to overstain the tissue.
9. *Place the specimen* in 2% KOH (solution #2) until the excess stain is leached out. Several changes of the solution may be needed. Small fish usually destain in one to two days, while larger specimens may take longer. If dark yellowish areas still remain in the tissue, return the fish to the enzyme solution until they disappear.
10. Place specimen in 30% glycerine in 2% KOH for 24 hours.
11. Place specimen in 60% glycerine in 2% KOH for 24 hours.
12. Place specimen in 90% glycerine in 2% KOH for 24 hours.
13. Store specimen in pure glycerine to which a few crystals of thymol have been added to prevent mold and bacteria from growing.

Some notes regarding potential problems and difficulties with this technique follow:

1. Fishes with especially thick connective tissue or excessive fat deposits will require special treatment such as longer soak times in the clearing solutions. You will have to experiment with various modifications in order to satisfy the requirements of your particular group of fishes.
2. Some fish, after clearing, will have a dark brownish substance along the haemal arch. Soaking the fish in 2% KOH for several days will usually remove this coloration.
3. The fin rays of certain fish, especially some flatfish, seem to be damaged by 2% KOH. The tip of the rays begins to split and fray after several days in this solution. If possible, watch your specimen carefully each day and remove it from the KOH as soon as you notice any damage. If excess stain still remains in the tissue, the specimen should be returned to the enzyme solution until destained. The bones of fish destained in enzyme solution will be lighter in color than those destained in 2% KOH. However, destaining is faster in the enzyme solution.
4. For extremely old specimens that have been stored in formalin for a long time, this technique might not be successful.