THE EVOLUTION OF THE HYOBRANCHIAL APPARATUS

IN THE LARVAE OF AMPHIBIA

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INTRODUCTION

The present work is a part of the investigation being conducted in the laboratory of Evolutionary Morphology in the Zoological Academy of Sciences, in the Academy of Science of the USSR. It deals with the problem of the origin of terrestial vertebrates which "pertains to the most complex and interesting divisions of phylogeny and relates to many general questions of evolution" (Schmalhausen 1964). In these investigations by methods of comparative embryology we studied a series of systems of organs with the goal of explaining the transformation which took place in the course of the evolution of vertebrates from the ancestral Tetrapoda to contemporary Amphibia. The morphobiologic and the functional analysis (with the addition of data from paleontology) permits us to understand the adaptive significance of the transformations in organization, and thus to point out and explain the basic directions of evolution in the lower Tetrapoda. The latter point is particularly significant since there are now two mutually exclusive theories on the origins of the Tetrapoda. According to the theory of monophyletic origin of the terrestrial vertebrates, the Tetrapoda arose as a single branch from the rhipidistian crossopterygian fishes. All of the variations in extinct and extant forms is based upon their adaptive radiation into the terrestrial and aerial sphere of life. On the other hand, the majority of advocates of the theory of polyphyletic origin of the Tetrapoda, which was worked out primarily by the Swedish paleontological school, consider that those amphibians which have tails and legs arose from one of the branches of Rhipidistia, i.e., the Porolepiformes, and the tailless amphibian, Gymnophiona, and all the amniotes arose from another branch, the Osteolepiformes.

Our present work deals with the evolution of the hyobranchial apparatus in the larvae of Amphibia and investigates the transformation which took place in the course of development of the lower Tetrapods. By the term hyobranchial apparatus, we mean that complex of morphologic structures which occupies the area from the hyoid to the last gill arch inclusive, and all the muscles connected therewith. Ontogenesis of this morphofunctional system is concluded at the stage at which the larval hyobranchial apparatus is functioning and its morphogenesis ceases before metamorphosis. The evolution of the hyobranchial apparatus in the larvae of Amphibia as a single unit in connection with its function, which has not been investigated by anyone prior to this time, should be interesting for two reasons: first, from the time the vertebrates came up to dry land the basic direction in the evolution of adult Amphibia has consisted of their adaptation to the terrestrial and aerial spheres. The larvae of most Amphibia have continued to live in water. The study of the hyobranchial apparatus in the larvae hence gives us material which is more favorable for explaining the origin of this

system in amphibia. Second, such investigation can shed light not only on the origin of the hyobranchial apparatus in the larvae of the extant Urodela and Anura but also on those processes which led to the structures seen in Recent forms.

The problem in the present dissertation is the explanation of the following questions: 1. What function or functions does the hyobranchial apparatus fill in the larvae of Amphibia? 2. How is this function manifested, in other words, what is the mechanism of action of the hyobranchial apparatus? 3. How did the evolution of the hyobranchial apparatus take place in connection with its functions? 4. What was the origin of the mechanism of action for this system?

The resolution of the principal questions was facilitated by the fact that in the literature the functions and in part the mechanism of action for the hyobranchial apparatus of fish, primarily Teleostei, have been described. The construction of the hyobranchial apparatus is known for both fishes and the larvae of amphibians to the degree that homology of the majority of its elements is no longer in doubt. Also, the basic features of the phylogenetic connections of the lower tetrapods have also been explained. In addition, the mechanism of the expulsion phase of the buccal pump has been reconstructed by M. M. Voskoboenikov, (1932) and I. I. Schmalhausen, (1964). The mechanism for the suction phase in the respiration of crossopterygian fish is facilitated by the movements of the hyobranchial apparatus, the structure of which was described by Jarvik (1954, 1963, 1967).

MATERIALS AND METHODS

Larvae of four families of Urodela and six families of Anura were investigated. Following is a list of species (Ranodon sibiricus, Hynobius keyserlingii, Ambystoma sp., Tritutus cristatus, T. bulgaris, Pleurodeles waltlii, Salamandra maculosa, Necturus sp., Proteus anguineus, Xenopus laevis, Bombina bombina, B. orientalis, Pelobates fuscus, Rana temporaria, R. esculenta, Bufo bufo, B. viridis, Hyla arborea, H. japonica). The structure and development of the hyobranchial apparatus was studied in slide preparations and in a series of sections from which were made microphotographs, sketches, graphic and plastic reconstructions. Four hundred and twenty-three larvae at various stages of development were examined with morphologic methods. Analysis of functions and of the mechanism of action of hyobranchial apparatus was carried out on the basis of obser-

vations of living larvae as well as with the help of electrical excitation of individual muscles (in large axolotls). To observe the movements of the hyobranchial apparatus living larvae were placed in a chamber with a mirror bottom. A binocular microscope was focused on the reflection of the base of the oropharyngeal cavity through which the contours of the cartilage and muscle were visible through the skin. Through a large number of observations, a clear picture of the movements of the hyobranchial apparatus was compiled. In addition, observations were made on the movement of the hyobranchial apparatus in the fry of pike and in addition three series of sections from the larvae of _Neoceratodus_ and _Protopterus_ were studied as well as preparations of skulls of neotenic stegocephalid (_Dvinosaurus primus_ Amalitzki No. 39, 40, 41 and _Tungussogyrinus bergi_) from the collection of the Paleontological Museum of the Academy of Sciences of the USSR.

CHAPTER 1

THE EVOLUTION OF THE HYOBRANCHIAL APPARATUS IN THE LARVAE OF URODELA.

All Urodelan larvae which are "waiting predators" generally feed on zooplankton. The prey is captured at a distance with the aid of a stream of water which is induced by depression of the base of the oro-pharyngeal cavity and opening of the mouth. Upon entry of the prey the gill arches are closed by the gill flaps. The depression of the floor of the oro-pharyngeal cavity occurs by the action of the hyobranchial apparatus. The constriction of M. sternohyoideus elicits a retraction of the copula. The hyoid, which is suspended from the skull on two ligaments, then turns due to the presence of the free moving hyocopulatory articulation with its anterior or forward end downward. The copula is lowered simultaneously without changing its horizontal position. The forward portion of the base of the oro-pharyngeal cavity is consequently depressed. At the same time the M. ceratohyoideus exterior, a muscle which at this moment has a point of support on the hyoidal arch and is fixed by the contraction of M. sternohyoideus and by position of the hyoid ligaments, is contracted. The posterior, (i.e., dorsal) end of the ceratobranchiale 1 extends ventrally but its action extends to all of the gill arches so that the ventral and dorsal ends of ceratobranchiale are united by cartilaginous ridges -- iuncturae proximales and comissurae terminales. During the action of the M. ceratohyoideus exterior the back portion of the floor of the oro-pharyngeal cavity is depressed. A similar action is seen in all of the forms investigated, except for _Proteus anguineus_, in which M. sternohyoideus, because of its greater development and more extensive area of attach-

ment to the mucilaginous floor of the oro-pharyngeal cavity, may act both on the forward portion of the floor of the oro-pharyngeal cavity, by means of the turn of the hyoid, and on the floor of the gill region; thus it sharply strengthens the whole action of depression. After the prey has entered the mouth the jaws close. During the action of the hyobranchial apparatus the floor of the oro-pharyngeal cavity begins to rise and pushes almost all of the water which entered the mouth during the capture of the prey through the opening gill slit. This lifting movement takes place more slowly than the movement of depression. The M. intermandibularis posterior and the Mm. interhyoidei anterior and posterior play a primary role in this movement. During their contraction the floor of the oro-pharyngeal cavity swells and at the same time rises. The M. intermandibularis posterior has a point of attachment on the lower jaw, the M. interhyoideus anterior on the posterior end of the ceratohyale and through the above mentioned ligaments onto the skull, and the M. interhyoideus posterior is connected on the end of ceratobranchiale 1 which is connected with the skull only by means of the M. levator arcus branchialis 1. In the motion of lifting the Mm. levatores arcuum branchialum also play a role and they extend upward to the dorsal ends of the gill arches and the Mm. subarcuales obl. II, III facilitate a protraction of the ceratobranchiale. A significant role in the described movement is played by M. geniohyoideus, which in pulling the copula defines the movement of the hyoidal arch to its original position. The protraction of the copula during the action of the M. geniohyoideus is conditioned by the fact that it rests on the symphysis of the lower jaw, which is fixed at this moment as a consequence of the contraction of M. intermandibularis anterior.

The ingestion of food takes place at the end of the upward motion of the floor of the oro-pharyngeal cavity. At this time the gill arches close under the action of M. transversus ventralis IV. The pressure of the water remaining in the oro-pharyngeal cavity has increased due to the lifting of the floor, and the contained food goes to the region of lower pressure, that is, to the esophagus whence it is swallowed. The M. subarcualis rect. 1 serves as a sinergist in this motion, and this muscle in fixing the gill arches to the hyoidal creates a point of support for the M. transversus ventralis IV.

This mechanism for the capture of food is used by the larvae of all Urodela investigated. We were not able to observe the movements of the hyobranchial apparatus which are especially connected with breathing. The external gills, organs for gas exchange in the larvae of Urodela, move independently of the hyobranchial apparatus; movements of the latter

2

assist the capture and swallowing of food but not breathing. The mechanism of action of this system is based on rotation of the hyoidal arch in the sagittal plane. We were able to show that the mechanism for depressing the floor of the oro-pharyngeal cavity derives from the buccal pump and this is also true for the mechanism of raising the floor of the oro-pharyngeal cavity. Consequently, the mechanism for respiratory motions in the ancestors of the terrestrial vertebrate was transformed into a mechanism for the capture of food by the larvae of Urodela. This conditioned a high degree of homology of the hyobranchial apparatus in fish and the larvae of tailed Amphibia. Nonetheless, it was mainly the loss of the breathing function that determined the major sequential transformations of the hyobranchial apparatus, as shown both in a comparison of the larvae of Urodela with fishes as well as in comparisons of the larvae of the investigated species one with another. Already in Rhipidistia the reduction of the dorsal regions of the gill arches (pharyngeal and epibranchial) is connected with the transition to pulmonary respiration (Schmalhausen, 1964) and this reduction in the larvae of Urodela is supplemented by a reduction of ventral elements. In all of them the fifth arch has completely disappeared (in the Proteidae, the IV arch as well) and the hypo-element of the IV arch also disappears, the hypo-elements of the III arch are reduced (a rudiment of the hypobranchiale III is found only in the larvae of Hynobiidae and Cryptobranchidae) and even II (in Proteidae). This process led to the disappearance of the posterior gill slits. In the larvae of Urodela there are three slits which are situated between I and II, II and III, III and IV ceratobranchialia (in the Proteidae two slits remain between I and II, and II and III). Thus the most posterior gill arch in the fish which is located between the IVth and Vth arches (in the Proteidae the preceeding one as well) and the most forward slits between the hyoid and the first gill arch are reduced. The position of this arch is occupied by the ceratohyoideus exterior as a homolog to the M. opercularis in fishes.

The reduction of the hyobranchial apparatus occurs together with several changes associated with the perfection of the mechanism for the capture of food. First of these was a modification of the movement most responsible for this process, namely the depression of the floor of the oro-pharyngeal cavity. The union of the urohyale and basibranchiale from independent elements of the hyobranchial skeleton in the Rhipidistia to a single copula in the larvae of Urodela facilitated a better transmission of the action of Mm. sterno- and geneohyoidei to the hyoidal arch. The transformation of the M. opercularis into the M. ceratohyoideus exterior and the associated appearance of commissurae terminalis in the larvae of Urodela created a possibility for depressing the posterior part of the floor of the oro-pharyngeal tract.

The mechanism for lifting the floor of the oro-pharyngeal cavity remained virtually unchanged; apparently the speed with which water is expelled through the gill slits does not have great biological significance, since the capture of food is not determined by this. The perfection of the mechanism for closing the gill slits during the capture of food is connected with the differentiation of M. subocularis rect. IV. In the larvae of Hynobiidae, this muscle has one concavity which extends from the fourth to the first gill arch and it is thus a common constrictor of the gill slits. In the larvae of Ambystomidae and Salamandridae there are two supplementary portions of the M. subocularis rect. IV which unite the IV arch directly with II and III. Crests on the ventral ends of ceratobranchialia serve to strengthen all three parts.

The reconstruction of the hyobranchial apparatus driven by the need for perfection of the prey captive mechanism is most clearly seen (among the investigated forms) in the Proteidae. Here a progressive development of the M. sternohyoideus and M. ceratohyoideus exterior facilitates a stronger depression of the floor of the oro-pharyngeal cavity than is possible in the larvae of other Urodela. The extension of the area of support of M. sternohyoideus on the soft floor of the oro-pharyngeal cavity was made possible by the high degree of reduction of the gill arches. The distinctiveness of the structure and mechanism of action of the hyobranchial apparatus of Proteidae is explained by the particular biology of this blind cave form which is required to feed on very mobile and quickly moving Crustacea. There is time for a proteid to make only one ingesting movement, at the moment the tip of the snout touches the prey, and this movement must be strong enough to promote a high probability of capture.

It is thus evident that differences in the construction of the hyobranchial apparatus are insignificant among the investigated forms. The larvae of higher families (Ambystomidae and Salamandridae) are only distinguished from the larvae of the primitive families (Hynobiidae) by the disappearance of the rudiment of hyobranchiale III and by the acquisition of a more highly differentiated apparatus for closing the gill slits. Only in the Proteidae was there a more significant reconstruction of the hyobranchial apparatus. Thus the stability in the construction of this system corresponds to the stability of the mechanism for the capture of food.

Comparative embryological investigation of the hyobranchial apparatus of Urodelan larvae permitted us to estimate its evolutionary development, i.e., the establishment of the basic qualities of organization which define the above mentioned mechanism of action.

3

The mesenchymal layers of the arch of the hyo-branchial skeleton in the larvae of all the investigated forms initially rests in a transverse position. Then, to the degree that there is relocation of the stomadeum from the ventral side of the embryo to the end of the snout (in actively feeding larvae the mouth is terminal), the hyoid and the gill arches change their position. As a result they are situated obliquely with relationship to the longitudinal axis of the body (the dorsal end of each arch is located significantly more caudal than the lower). This creates the possibility of turning of the hyoid arch in the sagittal plane and at the same time the whole mechanism of action of the hyobranchial apparatus.

Parallel with the change in position of the arches, the hyobranchial skeleton differentiates and the muscles which control it develop further. The copula forms in the medial part of the floor of the oro-pharyngeal cavity. In the majority of the forms investigated it arose immediately in the form of a single element. Only in Ranodon sibiricus at one of the stages of development were we able to discover a branchial segmental-like formation of the copula, which is encountered only in Acanthodii (Watson, 1937). The branchiale basalia already fuse in the following stage. This shows that the evolution of the basalia elements of the hyobranchial skeleton involved fusion rather than reduction. This, as well as the fusion of the basalbranchialia and urohyale during the transition from fishes to tetrapods, facilitated a better transmission of the action of the M. sternohyoideus to the hyoid arch.

In the initially continuous mesenchyme formation of the hyoid and gill arches independent centers of cartilage are differentiated and these correspond to the later division of the arches into hypo- and carato- elements. During the early stages of development of hynobiid larvae the hypohyalia lie under the edge of the ceratohyalia. This bend disappears as in the Rhipidistia (Jarvik, 1954, 1963), and the hyoid assumes a definite form for the larvae of Urodela. In the larvae of Ambystomidae and Salamandridae the hyoid is formed directly without the above mentioned curve. Commissurae terminales which unite the posterior ends of the ceratobranchiali arise as outgrowths from these after the gill arches have been completely formed, that is, their development occurs by means of positive anabolism, extensions of the final stages of development of their ceratobranchiali. These elements of the hyobranchial skeleton are distinctive only for the larvae of Amphibia; the elements are new formations, and hence not homologous to the epibranchia of fish as suggested by Fox (1954). By means of a positive anabolism the supplementary portions of M. subcularis rect. IV and of the crest which serve for their strengthening have also developed in the larvae of the higher Urodela. The reduced parts of the hyobranchial apparatus (4th and 5th gill slits, hyobranchiale III) evolved through negative anabolism. Conse-

quently, the evolutionary transformation of the hyobranchial apparatus in the larvae of Urodela which are connected with the loss of the breathing function took place by means of a negative anabolism and the transformation which conditioned the perfection of the mechanism for food capture, by means of the positive mode of phyloembryogenesis.

CHAPTER II

THE HYOBRANCHIAL APPARATUS OF THE STEGOCEPHALIDS

The study of the structure of the hyobranchial skeleton in the stegocephalids was restricted to representatives of Labyrinthodontia (groups which are considered to be the ancestors of Anura). In the literature there is information on the structure of the hyobranchial skeleton of representatives of one family of Lepospondyli (Lysorophus coctinus) and four families of Labyrinthodontia (Dvinosaurus, Platyceps, Gerrothorax, Trimerorhachis, Micropholis, Branchiosaurus). All of these stegocephalids are neotenic forms and possess larval structures of the hyobranchial apparatus.

The number, form, and correspondence of elements of the hyobranchial apparatus (with the exception of the well-developed hyobranchiale III in series of representatives from both groups) were the same as in the larvae of Urodela. The arches of the hyobranchial skeleton were always situated obliquely with respect to the longitudinal axis of the body. In Dvinosaurus the hyoid was suspended by ligaments from the skeleton (Bystrov, 1938, 1939). Epibranchials were absent in all of the above mentioned stegocephalids. There were three gill slits, the same number as in the larvae of extant Urodela (the first and the last gill slits of the ancestral tetrapods were absent). In addition, evidence of external gills was found in some stegocephalids. Dvinosaurus was also noted to have external gills (Bystrov, 1938). The similarity of the structure of the hyobranchial skeleton in both the larvae of stegocephalids and the larvae of extant Urodela presumes a similarity in their mechanism of action. The presence of external gills indicates that the hyobranchial apparatus of stegocephalids as well as of the larvae of urodelans serves for the capture of food. Thus, we were able to explain that despite variations among the several representatives, the hyobranchial apparatus of the larvae of Labyrinthodontia and the larvae of Lepospondyli was constructed and functional on the same principle as that of the larvae of the extant Urodela.

CHAPTER III

THE EVOLUTION OF THE HYOBRANCHIAL APPARATUS IN THE LARVAE OF ANURA

I. I. Schmalhausen (1964) has suggested that the hyobranchial apparatus of tadpoles was formed under conditions of rapid flow of mountain streams

4

poor in zooplankton. The following associated characteristics appeared in the larvae of Anura: an apparatus of lip cartilage which serves for the capture and chewing of the growing plants that serve as basic food, a long convoluted intestine which is suitable for the digestion of this food, and an external gill concavity which facilitates the acquisition of a streamlined body form. The origin of the last item caused loss of the external gills and the development of hidden homologous gills situated on the ceratobranchialia as in fishes. We were not able to observe the motion which specially serves for breathing. For this reason the mechanism for the transport of food, even though there are peculiarities imposed by the development of concealed gills

Such an interpretation of the function of the hyobranchial apparatus in the tadpoles is confirmed by the absence of gills in the larvae of Xenopus laevis and by the observation that the motion of the hyobranchial apparatus serves only for the transport of food.

The large food masses entering the mouth in dispersed condition require a change in the motion pattern of the hyobranchial apparatus. In tadpoles, as distinct from the larvae of Urodela, ingestion occurs by means of frequent oscillations which are not of great amplitude. These oscillations create a weak constant stream of water which facilitates the transport of food into the interior of the oro-pharyngeal cavity. Food is not captured at a distance. In none of the investigated forms do the oscillations of the hyobranchial apparatus occur in the sagittal plane as in the larvae of Urodela, but rather in the transverse plane.

The depression of the floor of the oro-pharyngeal cavity takes place during the motion of the ceratohyalia and the gill chambers (ceratobranchialia) with the medial edges downward. The M. orbitohyoideus and M. suspensoriohyoideus draw the lateral ends of the hyoid upward. Ceratohyalia turn in the geoquadrate articulation which serves as the only connection of the hyobranchial apparatus of tadpoles with the skull. During this turning, the medial ends of the ceratohyals, which are spread and thickened, are depressed; together with the copula I pars reuniens and copula II which are situated along the medial line of the body. The Mm. levatores arcuum branchialium also contract, drawing upward the back and posterior regions of the gill chambers. As a result, the latter turn and the hyobranchial disc which is furthermore connected immoveably with copula II is depressed. The forward region of the ventral wall of the esophagus in the tadpoles of Phaneroglossa is connected to the posterior region of the hypobranchial disc. For this reason, the esophagus opens when the hypobranchial disc is depressed. As a result of the complex of described motions, the entire median region of the floor of the oro-pharyngeal cavity curves upward and the water which has poured-in through the oral opening

carries the elements of food from the lip cartilage into the depths of the cavity. During the depression of the oro-pharyngeal cavity the gill arches close due to the contraction of Mm. constrictores branchiales, i.e., the oro-pharyngeal cavity is isolated from the external gill chamber. The latter chamber is isolated from the outside by a dropping of the walls of its efferent canal and this wall plays the role of a valve. The return movement of the ceratohyalia with the medial ends upward takes place during the action of M. interhyoideus anterior. This muscle contracts and at the same time lowers the lateral ends of the hyoid. Together with the medial ends of the ceratohyalia, the pars reuniens and both copulae are raised. However, the M. interhyoideus anterior draws the ends of the hyoid not only ventrally but caudally as well attempting to join them horizontally.

Copula I is hindered by this movement and it supports the medial ends of the ceratohyals. Simultaneously with the contraction of the M. interhyoideus anterior, the Mm. subarcuales recti and obliqui contract and turn the gill chambers with a movement which is counter to the movement of depression. The simultaneous contraction of the above mentioned muscles raises the median portion of the floor of the oro-pharyngeal cavity. With this a stream of water carrying food material changes its direction and enters the gill slits while the particles of food are retained due to the presence of a special filter apparatus.

In the larvae of Phaneroglossa the filtered water exits through the gill slits into the external gill chamber, sweeping over the gill branches in the process. During the next intake pulse the retained food material is drawn into the esophagus, while the next lifting of the buccal floor causes the water to be filtered again. After entering the external gill chamber the last batch of water is expelled. Thus each successive drop of the floor of the oro-pharyngeal cavity will cause the water that enters into the esophagus to be further enriched by the food particles which remained in the oro-pharyngeal cavity during the previous elevation of its floor.

The mechanism of action of the tadpole hyobranchial apparatus is characteristic for all the investigated forms. It is remarkable that one cannot detect elements of the breathing movement of fish in it. The external gill concavity thus does not participate in the pumping of the water. In contrast to the condition in fishes the water is not sucked through the gill slits but passes them passively. The two-cycle pump of the larvae of Anura thus works as a one cycle pump in the larvae of Urodela. Investigation of the ontogenesis of the higher hyobranchial apparatus in anuran larvae permits us to explain the particular features of its construction and function which are connected with the change in the plane of oscillation. As in the urodelan larvae the mesenchymal formations of the arches of the hyobranchial skeleton are initially positioned in the transverse plane. The same complement of elements of

the hyobranchial skeleton develops in tadpoles as in urodelan larvae. The single new element is _copula_ I. Shortly after the arches are laid down the formation of the long convoluted intestine begins. The latter literally forces the heart into the region of the hyobranchial apparatus, changing its configuration and the entire course of its further development. The hyobranchial skeleton moves somewhat rostrally, consequently the hyoid comes in contact with the quadrate cartilages. At this spot the quadrate articulation develops. _Copula_ II (we succeeded in showing the homologue of the copula in urodelan larvae) having lost the possibility for growing caudally remains arrested as a short cartilage throughout the entire larval life of Anura, of a size equivalent to that seen in the early stages of larval development in the Urodela.

Hyobranchialia I and II approach one another and fuse. In the larvae of Phaneroglossa the mouth remains ventral until metamorphosis. Consequently the arches of the hyobranchial skeleton retain their initial transverse position. The change of configuration and topography of the hyobranchial skeleton observed in the anuran larvae must then have arisen as a result of a deviation from the course of development characteristic for the urodelan larvae. This deviation apparently results from interactions in forming the intestine and the heart and the lack of any tendency by the mouth to translocate. In tadpoles the hyobranchial apparatus then acts differently as the plane of its oscillation changes (since the arches lie in a transverse position rotation of the hyoid in the sagittal plane cannot lower the floor of the oro-pharyngeal cavity). It must be emphasized that this deviation arose by means of a changed construction in correlated mutual relationships (co-adaptation) of the hyobranchial apparatus with the organs surrounding it. The transformation of the hyobranchial apparatus took place not as a result of direct adaptation to the changed character of the food but indirectly, because of a change in other organs.

The deviation described above determined all further evolution of the hyobranchial apparatus. _Hypohyalia_ noted in the beginning stages of development of the hyoid under the edge by the _ceratohyalia_ (as in the hynobiid larvae) do not change their position, but spread out and fuse forming the _pars reuniens_, which is not cartilaginous but remains prochondrial for the entire larval life. Consequently the _pars reuniens_ (which arises as the result of deviation in the development of _hypohyalia_ particular to the larvae of Urodela) is the homologue of these elements of the hyobranchial skeletons and is not basal as believed by the majority of investigators. The _pars reuniens_ serves as an elastic connection between the _ceratohyalia_ when these move in the transversal plane. _Copula_ I which possesses the most primitive construction in pelobatid tadpoles (it is lacking in the tadpoles of _Xenopus laevis_) arose in the process of evolution of the larvae of Anura of arcolaxis and

has no homologue among the ancestors of the tailless amphibians. The remaining elements of the hyobranchial skeleton in the larvae of Anura are changed because of a deviation evolved by means of positive anabolism. The transport of dispersed food material required an increase in the amount of water circulated through the oro-pharyngeal cavity. With this a proportional increase of the medial parts of the _ceratohyalia_ occurred, leading to the restructuring of the _ceratobranchialia_ of the gill chambers and the fusion of _hyobranchialia_ into a broad disk. Among the forms investigated, the tadpoles of Discoglossidae and Pelobatidae are more primitive in several structures of the hyobranchial system than are the tadpoles of the Ranidae, Bufonidae and Hylidae. Maximal spreading of the hyoid and the gill chambers is noted in the tadpoles of _X. laevis;_ the latter characteristically feed on decaying matter and phytoplankton and hence require increased amounts of water to pass through the oro-pharyngeal tract.

The transformation of the hyobranchial skeleton is paralleled by the transformation of its musculature. The _M. ceratohyoideus exterior,_ which lowers the _ceratobranchialia_ in the larvae of Urodela, disappears. New muscles develop, namely the _Mm. orbita_ and _suspensoric hyoidea_. The _Mm. levatores arcuum branchialium_ expand and in the tadpoles of _X. laevis_ may even fuse into a single muscle layer. The _Mm. constrictores branchiales_ develop in anuran larvae as a result of the development of the concealed gills and the reduction of the gill flaps (which in the larvae of Urodela close the gill slits during the intake of water into the oro-pharyngeal tract cavity). The presence of these muscles in the tadpoles of _X. laevis_ attests to the fact that gill breathing has in this species disappeared for a second time. The development of _Mm. constrictores branchiales,_ which close the gill slits, is the single characteristic that conditioned the unification of the functions of breathing and food transport in the hyobranchial apparatus of tadpoles.

The muscles which participate in the raising of the floor of the oro-pharyngeal cavity have been subjected to just as great a transformation. The _M. interhyoideus posterior_ is well expressed in the larvae of Pelobatidae, but absent in a series of investigated forms. The _M. interhyoideus anterior_ is wide in the larvae of Urodela; but appears as a narrow strip in connection with the transverse location of the hyoid in the larvae of all the Phaneroglossa. In the larvae of _X. laevis_ it is again broad. The _M. intermandibularis anterior_ and _M. geniohyoideus_ which determine the protraction of the hyoid and copula in the larvae of Urodela are in tadpoles kept from this function by the hyobranchial apparatus, and instead participate in the mechanism of action for the lip cartilage. The _Mm. subarcuales obl._ II and III are independent in the larvae of Urodela. In tadpoles (with the exception of those _Hyla_) they are fused into a single muscle and in _X. laevis_ they furthermore lose their connection with the copula. The evolutionary transformations of

the musculature of the hyobranchial apparatus of
tadpoles occurred primarily through positive ana-
bolism which could be clearly traced in a whole
series of instances. The transformation of the
M. intermandibularis posterior and M. sternohyoid-
eus occupies a particular place. These muscles
play a leading role in the mechanism of action
for the hyobranchial apparatus in the larvae of
Urodela, but do not function in tadpoles; for
this reason they are always incompletely developed
although they have not disappeared completely in
any of the investigated forms. The preservation
of the primordia for M. intermandibularis post-
erior and M. sternohyoideus is explained by the
fact that they induce movements of the hyo-
branchial apparatus in adult Anura (A. S.
Severtzov, 1961). Consequently one observes
not a reduction but a retardation in anuran
larvae, a temporary restraint in the develop-
ment of these muscles which is elicited by
their exclusion from the mechanism of action
of the larval hyobranchial apparatus and by
the necessity of preserving them for future
function. Speaking more generally, retarda-
tion may begin when any component is excluded
from the functioning of the provisional or
temporary system even though the component is
preserved for functioning at subsequent stages
of ontogenesis. The offered conception to a
certain degree explains the adaptive signifi-
cance of regulated retardation in ontogenesis -
a very little studied class of heterondria.
Thus in the larvae of Anura a mechanism of
action of the hyobranchial apparatus is developed.
The evolution of the latter proceeded primarily
by means of positive modes of phyloembryogene-
sis.

CHAPTER 4

HYOBRANCHIAL APPARATUS OF RHIPIDISTIA

In the investigation of mechanism of action
of the hyobranchial apparatus in the larvae of
Amphibia, the elaborate descriptions and recon-
structions of the structure of the hyobranchial
apparatus of Rhipidistia (Jarvik, 1954, 1963,
1967) give us the possibility to reconstruct the
mechanism of action for this system in the
crossopterygian fishes and also to weigh the
arguments in favor of the theory of polyphyle-
tic origins of the tetrapods advanced by Jarvik.
The structure and location of the pulmonary discs
in Glyptolepis and Holoptychius (Porolepiformes)
and in Eusthenopteron (Osteolepiformes) is such·
that they condition the mobility of the floor of
the oro-pharyngeal cavity and hence the rising
and falling movements that assure the oscilla-
tion of the hyobranchial appa·ə·u·. T'·ə structure
of the hyobranchial skeleton of Eusthenopteron
and in particular of Glyptolepis is similar to
the structure of this system in the larvae both
of the stegocephalids and of the extant Urodela
(the most important shared characteristic is
the oblique or slanting location of the hyoid
and gill arches which shows that the oscillations

of the hyobranchial apparatus must have taken place
in the sagittal plane during the action of the M.
sternohyoideus). Consequently the mechanism of
action of the hyobranchial apparatus in Rhipidis-
tia, which served primarily for breathing, was
similar to such mechanisms in the larvae of the
stegocephalids and·the larvae of the extant Urodela,
but not in the larvae of Anura. One of the argu-
ments used to substantiate the theory of the
polyphyletic origin of the tetrapods advanced by
Jarvik (1963, 1967) is based on his homology of
a series of elements of the hyobranchial skeleton
of Eusthenopteron and the larvae of Anura. In
his opinion the sublingual pivot is homologous
to copula I, the basibranchiale I to copula II
and the basibranchiale II to the median fissure
of the hyobranchial disc. The cited homologous
features allowed Jarvik to come to the conclusion
that the hyobranchial apparatus of the larvae of
Anura developed on the basis of the structure of
this system in Eusthenopteron. We succeeded in
showing that of the homologous features advanced
by Jarvik only the comparison of basibranchiale ·
I in Eusthenopteron and of the basal elements of
the hyobranchial skeleton of the tadpoles copula
II was justified. Copula I is a new formation
which is characteristic only of the larvae of
Anura (see chapter 3) and the medial fissure of
the hyobranchial disc arose during the process of
the proliferation and fusion of the hypobranchia-
lia and does not have anything in common with
the basal elements. Nor is it possible, as
presumed by Jarvik, to reconstruct the muscula-
ture of the hyobranchial apparatus of Eusthenopteron
using the correspondences to analogous muscles
in the larvae of Anura; all that is further em-
phasized by this is the similarity of the forms
compared. The muscles of the hyobranchial appara-
tus of the tadpoles are highly changed as a result
of the origin of a new mechanism of action. The
presence of a sublingual pivot (which is not
known in any of the extinct or contemporary forms)
and a series of other characteristics in the con-
struction of the hyobranchial skeleton attest to
the specialization of Eusthenopteron. Thus the
structure of its hyobranchial apparatus cannot
be considered as a prototype for the structure of
this system in the larvae of Anura and it is not
possible to confirm or establish the theory of
the polyphyletic origins of the tetrapods by
means of such comparison.

CONCLUSIONS

In summary we must characterize the basic
factors which determine the transformation of the
hyobranchial apparatus and its evolutionary trends
in the larvae of Amphibia. The evolution of the
hyobranchial apparatus in the larvae of stego-
cephalids and the larvae of the extant Urodela
is characterized by the loss of its function
in breathing and its perfection as a mechanism
for prey capture. The loss has permitted the re-
duction in a series of elements of the hyo-
branchial skeleton and the disappearance of
several gill openings, as well as several

progressive transformations of the hyobranchial apparatus. The high evolutionary stability of this apparatus is explained by the fact that its action permits the animal to capture prey at a distance; it thus represents the perfection of the basic function of feeding. Both reduction and progressive transformation of the hyobranchial apparatus are most noticeable in the Proteidae, in which only two pairs of gill openings are preserved. Theoretically reduction might be expected to have proceeded further in the urodelan larvae, since only the single anterior pair of gill openings is necessary for expulsion of water from the oro-pharyngeal cavity. However, the hyobranchial apparatus cannot be reduced further. The complete loss of gill openings, observed in some adult Amphibia, would if carried out in all stages mean a basic transformation of the mechanism of hyoid action. Thus the tendency for a reduction of the hyobranchial apparatus in the evolution of urodelan larvae is offset by the need to perfect its action in other functions. The hyobranchial apparatus in the larvae of Anura arose by modification of the hyobranchial apparatus in the larvae of stegocephalids and was formed according to the same principle as in the larvae of extant Urodela. The appearance of the breathing function in the hyobranchial apparatus of the tadpoles seems to have had no effect on its evolution. The transformation of this system took place as a result of a change in the correlated connections of the hyobranchial apparatus and the organs surrounding it in the ontogenesis of anuran larvae. The deviation which is defined or determined by a change in the area of oscillation for the tadpole hyobranchial apparatus is a most important factor which determined its further evolution. It seems to have led to the perfection of a new mechanism for action, that is to a proliferation of cartilage and a corresponding reconstruction of the musculature. The most extreme expression of this tendency among the forms is the construction of the hyobranchial apparatus in the tadpoles of the species Xenopus laevis. In all of our work, the data obtained on the ontogenesis and the morphology of the hyobranchial apparatus in amphibian larvae and the comparison with the structure of the hyobranchial apparatus of stegocephalids and crossopterygian fishes confirm the theory of monophylétic origin of the Tetrapoda.

In our investigation of the evolution of the hyobranchial apparatus in amphibian larvae we examined the evolution of the caenogenetic system, i.e., the complex provisional or temporary adaptations which serve only in the course of the larval period of life. At the time of metamorphosis, the hyobranchial apparatus becomes subject to the basic morpho-functional reconstruction. The mechanism for the evolutionary transformation of temporary traits has not previously been investigated. The proofs gathered from our work that embryonic adaptation evolved by means of phyloembryogenesis. They also let us extend the boundaries of the theory of phyloembryogenesis. Now it can be said that not only the characteristics or traits of definitive organization but also of embryonic adaptation are subject to the ordered manner of development which was determined by A. N. Severtov.