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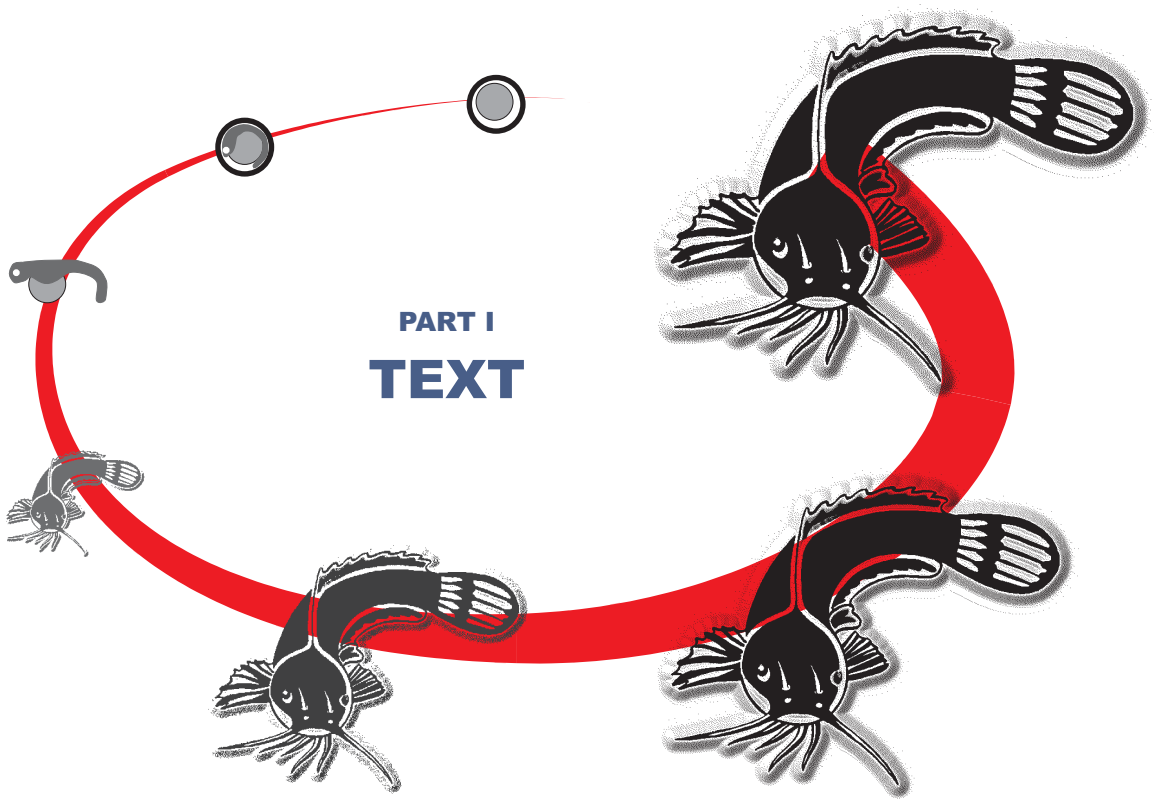
On how a larva becomes an adult catfish

a functional morphological approach to the cranial ontogeny
of the African catfish, *Clarias gariepinus* (Siluriformes, Clariidae)

Van larvale tot adulte katvis

een functioneel-morfologische benadering van de craniale ontogenie
van de Afrikaanse katvis, *Clarias gariepinus* (Siluriformes, Clariidae)



PART I

TEXT

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Chapter I.1 – History and aims

I.1.1 - History

The study presented in this thesis was performed at the Research Group of Vertebrate Morphology in Ghent, at which a vast background on cranial ontogeny, both functional and developmental morphology of fishes has been established over the years. Research had been done on salmons, cichlids, gobies, zebrafish, but not yet on catfishes. One problem, however, with research on the ontogeny is that ontogenetic series have to be available. Thanks to the Laboratory of Aquaculture and Ecology (KULeuven), such a series could be provided of the African catfish, *Clarias gariepinus*. This species was especially interesting as a study object for several reasons, both practically and scientifically:

1. material could be provided *ad libitum*;
2. the rearing of eggs and fry encompasses fairly little difficulties;
3. the size range between larval and juvenile specimens is substantial;
4. growth and development occur fast;
5. *C. gariepinus* is a well studied species, of which much information is available.

This study was part of an "F.K.F.O."-project, which comprised the study of *Clarias gariepinus*, as well as other clariids, from different scientific fields. Research was done on the genetic and enzymatic variability of different populations of *Clarias gariepinus* (G.G. Teugels - KMMA; F. Volckaert, F. Ollevier - KULeuven), as well as on the effect of domestication (P. Galbusera - KULeuven). Systematic research by means of allozymes and caryological evidence was compared to biometrical studies (G.G. Teugels - KMMA). Ontogenetic studies involved the shift in the protein composition of muscle tissue (B. Focant - ULg), as well as the ontogeny of the skull of species like *Heterobranchus longifilis* (P. Vandewalle, M. Chardon - ULg). The latter species was also used for ethological studies (P. Poncin - ULg), as well for studying growth and cultivation (G.G. Teugels - KMMA; J.-C. Philippart, ULg).

A morphological study on the cranial morphology had already been done by several authors, although mostly quite superficially [with exception of Nawar (1954)]. The nomenclature of the cranial structures in these papers demonstrated a high degree of inconsistencies, which required an initial priority of this study: to provide a conclusive nomenclature, which allowed comparison with other taxa. The ontogeny of the skull could provide substantial information concerning the origin of bones, whereas the study of the cranial lateral-line system could give data on the true nature of canal bones.

The study of the cranial ontogeny had already been initiated by Surlemont (ULg) and co-workers in 1983. Consequently, the purpose of this study was to provide additional data on the ontogeny, in order to obtain a more complete insight in the ontogeny of the skull of *Clarias gariepinus*. The studies of Surlemont and co-workers were especially focussed on the very early development of the skull, which proved to be very useful for the present study. However, data on later ontogeny were still lacking, and more details on both the skeletal and muscular ontogeny were needed for allowing further functional interpretations. The kinematical observations of Surlemont and co-workers were also very useful for testing some hypotheses that were suggested, based on morphological evidence.

I.1.2 - AIMS

The present study encompasses a constructional-morphological approach (**Fig. I.1- 1**) of the ontogeny of the cranial 'Bauplan[1]' of the African catfish *Clarias gariepinus* BURCHELL (1822). Aspects of form *s.l.*[2] are related to function *s.l.*[3], as they change during ontogeny. Causal and constructional relations during ontogeny were to be tested by means of several experiments. The ontogeny of the cranial 'Bauplan' was restricted to the musculo-skeletal system of the skull, whereas interactions of any kind with surrounding other organs were incorporated were possible. The myology of the branchial basket, as well as the ontogeny of the eye muscles, has not been dealt with in this thesis, because of time restriction. The study focussed on the process of cranial differentiation during normal ontogeny, which subsequently had to be extended in the study of abnormal development. As non-random anomalies were required for this study, the effect of several teratogens were tested on developing *C. gariepinus* larvae. Teratogens had to be used of which the teratogenic action is well known, as well as this action had to be very specific in order to distinguish between direct and indirect effects on cranial ontogeny.

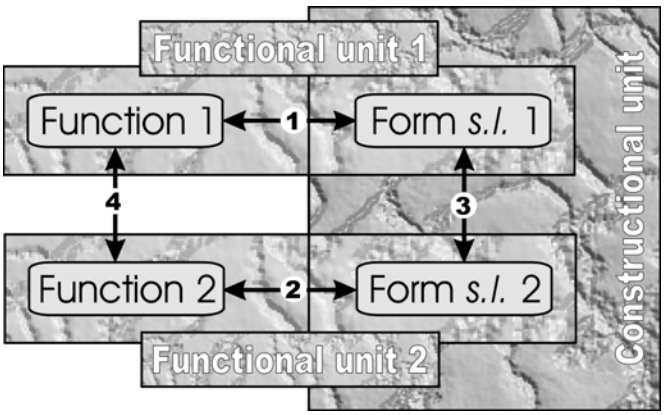


Fig. I.1- 1: Scheme of the concept of constructional morphology: relation between functions (4) is predicted from relations between constructional units (*i.e.*, 'Bauplan'-elements) (3), as well as function-form relations within a functional unit (1 and 2) [Modified from BAREL (1984)]

This thesis can largely be subdivided in two parts: (1) a descriptive part, and (2) a functional-morphological part. It has been opted not to separate both parts, as this is hardly possible (see V.1). In part II and III, both the description of normal and abnormal ontogeny is given, together with some functional interpretations. Part IV comprises a functional-morphological evaluation and discussion of the data of the different chapters combined.

The following fundamental questions describe the aims of the descriptive part of this study:

1. How does the normal form *s.l.* of the cranial skeleton (*i.e.*, the chondrocranium and osteocranium) becomes established during ontogeny?
2. How does the normal form *s.l.* of the cranial soft tissues (especially muscles) becomes established during ontogeny?
3. How is the fully developed cranial 'Bauplan' constituted, in comparison with other teleosts and especially other catfishes?

[1] According to VERRAES (1981), "The *Bauplan of the individuum* is the spatial and chronological set of all morphological features from conception to death".
[2] Form *s.l.* is defined by (1) presence, (2) shape (form *s.s.*), (3) size, (4) position, and (5) structure (BAREL *et al.*, 1976b; VERRAES, 1981).
[3] Function is generally related to actions or movements. However, in the present study, function is considered in the broad sense: immovable structures perform a function as well (reinforcement, attachment site, lever action, …).

The functional aspect of this study tries to give some conclusive ideas on the following questions:

4. How can the relation between 'Bauplan' elements during normal development be described, from a constructional-morphological point of view?
5. How does a developing larva cope with the changing functional demands it experiences during ontogeny?
6. Are there any developmental novelties, and how can they be functionally explained?

The answers to these questions may consequently contribute to the better understanding of abnormal ontogeny, as well as the opposite is true. The aims of the study of the abnormal ontogeny can be expressed by the following questions:

7. What is the effect of teratogens on the ontogeny of the cranial 'Bauplan'?
8. How can malformations be related to a direct effect of the teratogens or an indirect effect because of altered spatial constraints?
9. What is the effect of artificially induced dwarfism on the growth of *Clarias gariepinus*?
10. What is the effect of artificially induced dwarfism on the topography and constructional morphology of the cranial musculo-skeletal system?
11. What are the causal and functional relations between two or more developing structures?

Chapter I.2 - Material and methods

The studied specimens of the African catfish, *Clarias gariepinus* BURCHELL (1822) were obtained from the Laboratory of Ecology and Aquaculture at the Catholic University of Leuven (Prof. Dr. F. VOLCKAERT and Prof. Dr. F. OLLEVIER). Several batches of newly fertilised eggs were obtained and transported to an aquarium set-up at the lab, for further raising. Fertilised eggs were transferred onto a fine, synthetic mesh. Due to the activation of the adhesive organ of the eggs, they were attached to that mesh rather firmly. The mesh was then transferred into a thermally isolated tank of about 20 litres for transportation.

Different batches of eggs were used for building up a series of different ontogenetic stages, which were then used for clearing, sectioning, dissections and teratological experiments. For the study of the skeletal ontogeny, a batch of partially genetically identical eggs was used. Gynogenetic eggs were obtained by applying a combination of pressure shocks and temperature rises, in the Laboratory of Ecology and Aquaculture (Catholic University Leuven) (VOLCKAERT *et al.*, 1994).

I.2.1 - ONTOGENETIC SERIES OF *CLARIAS GARIEPINUS*

In order to provide an environment with good water quality for the developing embryos and larvae[1], different set-ups were applied and improved. Initially, the eggs were transferred into small tanks (1 litre) by cutting the mesh into small pieces. Methylene blue [*3,7-bis(dimethylamino)-phenazathionium chloride*] was added for protection against fungi, as saprolegniosis is very common in tanks with decaying eggs (FRANCIS-FLOYD & NOGA, 1994). Methylene blue has a wide range of effects on various diseases, and is less cumulative and carcinogenous than for example malachite green (CARRINGTON, 1985; ROYBALL *et al.*, 1995). The concentration applied is 3 ml per 10 ml of a 1% solution (TERVER, 1989). The temperature was kept constant at 25°C by floating the tanks in a larger tank which was heated. However, this method appeared to be too much time consuming, because of frequent water renewal, and appeared only useful if very small batches of eggs were kept.

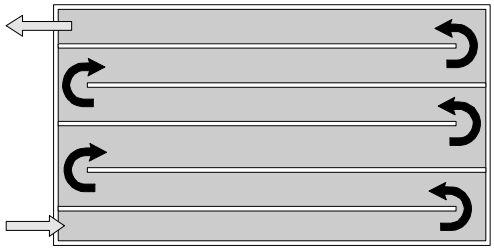


Fig. I.2- 1: Scheme of 'race way' aquarium for raising larval *Clarias gariepinus*, showing the direction of water flow (dorsal view)

Consequently, another method was tried out, in order to introduce a flow-through in the tanks where the eggs were kept. Therefore an aquarium was constructed for raising the eggs and larvae in small separate, perforated tanks which were suspended in a kind of race way tank (**Fig. I.2- 1**). The small containers were suspended in a water level of about 9 cm, at a distance of about 5 cm from the tank floor (**Fig. I.2- 2**). The inlet and outlet of the filter pump was placed in such a way that the water mass going through the tank had to go through all the 'race ways', thus providing all containers with an equal rate of water renewal. However, due to the very small size of the eggs (± 2 mm), the perforations had to be made so small that no sufficient water flow-through occurred in the containers, in order to expel the excess of waste material of decaying eggs, larvae and food. The flow rate was too low as well (± 360 litres/hour).

[1] Concerning the terminologies: 'embryo', 'larva, 'juvenile' and 'adult', some definitions and considerations are given in I.4.2.

Finally, a closed circulation system was built, onto which several tanks could be connected to a single, large biological filter. The total capacity of this system is about 400 litres, spread over four tanks (**Table I.2- 1**). The 'race way' tank was also connected to this system, as it appeared to be useful for raising small groups of larval fish, used in the teratological experiments (see I.2.3.a). The circulation was provided by a powerful, submergible water pump (EHEIM 1060) at a rate of ±2300 litres/hour. The flow rate of each tank could then be regulated independently. In order to prevent bacterial and fungal proliferation, an ultra-violet lamp was enclosed into the system. Denitrificating bacteria were introduced onto the filter medium ('bio balls'). Mechanical filtering occurred through a thick layer of filter cotton.

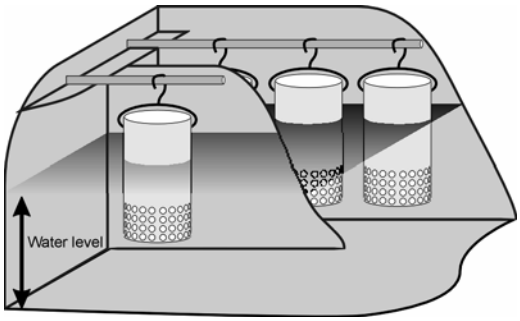


Fig. I.2- 2: Scheme of containers used for suspending the eggs and larvae of *Clarias gariepinus* in the 'race way' tank

Table I.2- 1: Capacity of used tanks

Tank	Length (cm)	Width (cm)	Height (cm)	number	Litres /tank
Filter	100	45	50	1	225
Race way tank	100	30	9	1	27
Large tank	60	30	30	2	54
Small tank	55	40	30	1	44

The eggs of *Clarias gariepinus* were submerged in the large tanks, while still attached to the mesh. The flow of water could be concentrated by putting the mesh in a large tube, open at both sides, which was placed under the inlet. Freshly hatched larvae could then leave at the bottom of the tube. This system provided a sufficient filtering of the water, as large batches of eggs could be raised, without any abnormally high mortality rate after hatching.

Hatching of the larvae started at about 24 hours after fertilisation, at a temperature of 25°C. Although temperature preference of *C. gariepinus* was found to be 30°C, high growth rates were observed at temperatures ranging from 25 to 33°C (BRITZ & HECHT, 1987; VERRETH & VAN TONGEREN, 1989). At 30°C, the incubation time is found to be around 20 hours (VIVEEN *et al.*, 1985). Embryogenesis and larval development in *C. gariepinus* occur very fast, which would make it difficult to distinguish ontogenetic events. Consequently, by raising the eggs at a lower temperature, the incubation time can be slightly extended, without affecting survival rate. This would allow a better discrimination between these ontogenetic events.

At hatching, the larvae of *C. gariepinus* are provided with a large yolk sac, which allows endogenous feeding for up to three days. At the fourth day posthatching, they start feeding actively (HECHT & APPELBAUM, 1987). Although larval *C. gariepinus* can be fed dry food very soon [from 7.8 days posthatching (VERRETH & VAN TONGEREN, 1989)], it is advised that their initial food should consists of hatched nauplii of *Artemia*, or decapsulated cysts (VAN DAMME *et al.*, 1990). After about three weeks, the larvae were gradually given dry food (commercially obtained), which consisted of ground flakes, as well as fine Trouvit 000 (at about 20 mm body length). This prolonged *Artemia*-feeding period is favourable, in order to decrease the mortality due to cannibalism, as was shown by VANDAMME *et al.* (1990). As the larvae grew, the size of Trouvit 000 particles was increased.

At different time intervals, larvae and juveniles were anaesthetised, using an MS-222 (methanosulfate salt, *3-aminobenzoic acid ethyl ester*) overdose, and fixed. Fixation was done using different fixatives. Small individuals were fixed in a mixture of paraformaldehyde and glutaraldehyde, dissolved in a cacodylate buffer (pH 7.4) for two hours at room temperature (**Table I.2- 2**) (SIRE, 1985). This fixative is known to reduce the effects of

shrinkage (DELEON *et al.*, 1989; REESE *et al.*, 1989). It also preserves the natural structures of different tissues, which is of great importance for the serial sections. Decalcification occurred in EDTA (0.1 M), added to the fixative, or Decalc. Postfixation with osmium tetroxide (OsO_4) was done for some of the larvae. However, as no transmission electron microscopy was performed, this postfixation was not essential. Bouin fixation was done on both small and larger specimens (**Table I.2- 3**).

Table I.2- 2: Composition of the paraformaldehyde-glutaraldehyde fixative

Product	Ratio
Paraformaldehyde (15%)	50 ml
Glutaraldehyde (25%)	6 ml
Sodium cacodylate buffer (0.2 M)	50 ml
$CaCl_2$ (0.5%)	0.2 ml
Aqua dest.	100 ml

Table I.2- 3: Composition of the Bouin fixative

Product	Ratio
Picrinic acid	1g
Glacial acetic acid	5 ml
Formaldehyde (40%)	25 ml
Aqua dest.	75 ml

Due to the acidic nature of the latter fixative, no decalcification had to be done, which implies that these specimens could be used for serial sectioning only. Larger specimens were also fixed in a 4% formaldehyde solution at neutral pH (**Table I.2- 4**).

Table I.2- 4: Composition of the buffered formalin fixative

Product	Ratio
Formaldehyde (40%)	400 ml
$NaH_2PO_4.H_2O$	16g
Na_2HPO_4.anhydride	26g
Aqua dest.	3600 ml

As the raising of larvae up to the juvenile/adult stage was too time consuming, and rather impossible in the set-up used, 30 specimens of commercially raised fish, at about 100 days posthatching, were obtained from Mr. Fleure (Someren, The Netherlands).

For this study, a total of 62 different specimens were used, ranging between 4.1 and 281.0 mm standard length (**Table I.2- 5**). Of these, 15 were used for serial sectioning, 41 for *in toto* clearing and four for dissections.

Table I.2- 5: Specimens of *Clarias gariepinus* used for the ontogenetic study (*Abbreviations:* AB = alcian blue 8GX, AMC = adductor mandibulae complex study, ARS = alizarine red S, CC = chondrocranium study, CLL = cranial lateral-line study, IT = improved trichrome, HM = hyoid muscles study, OC = osteocranium study, PAL = preanal length (see I.4.1), SL = standard length (see I.4.1), SOM = suspensorial and opercular muscles study, T = toluidin blue, TL = total length (see I.4.1), TM = tentacular muscles study)

Nr.	SL (mm)	TL (mm)	PAL (mm)	Days posthatching	Procedure	Gynogenetic	Staining	CC	OC	CLL	AMC	TM	HM	SOM
1	4.1	4.4	2.3	1	clearing	no	ARS+AB	1	1	1				
2	5.6	5.8	2.9	1	sectioning	no	T	1	1	1				
3	5.6	5.9	2.9	1	sectioning	no	T	1						
4	5.8	6.8	3.5	2	clearing	no	ARS+AB	1						
5	5.9	6.4	3.3	2	sectioning	no	T	1	1	1				
6	6.0	6.4	2.7	1	clearing	no	ARS+AB	1	1	1				
7	6.0	6.8	3.5	2	clearing	no	ARS+AB	1						
8	6.6	7.0	3.2	2	clearing	no	ARS+AB	1	1	1				
9	6.8	7.3	3.6	5	sectioning	no	T	1	1	1				
10	6.8	7.4	3.1	3	clearing	yes	ARS+AB	1	1					
11	6.9	7.5	3.4	3	clearing	no	ARS+AB	1						
12	6.9	7.5	3.6	7	clearing	yes	ARS+AB	1						
13	7.0	7.5	3.2	3	clearing	no	ARS+AB	1						
14	7.1	8.0	3.5	10	clearing	yes	ARS+AB	1						

15	7.1	8.7	3.7	9	clearing	no	ARS+AB	1						
16	7.2	7.8	3.8	7	sectioning	no	T	1	1	1	1	1	1	1
17	7.3	7.9	3.3	7	clearing	no	ARS		1					
18	7.4	8.1	3.6	13	clearing	yes	ARS+AB	1						
19	7.6	8.3	3.7	5	sectioning	no	T	1	1	1				
20	7.6	8.4	3.7	5	sectioning	no	T	1	1	1				
21	7.7	8.2	4.1	17	clearing	yes	ARS+AB	1						
22	7.7	8.7	3.7	9	clearing	no	AB		1	1				
23	7.8	8.4	4.1	17	clearing	yes	ARS+AB	1						
24	7.9	8.3	3.5	5	sectioning	no	T	1	1	1				
25	8.1	9.1	4.2	13	sectioning	yes	T	1						
26	8.2	8.6	4.1	17	clearing	yes	ARS+AB	1	1					
27	8.3	9.2	4.4	10	clearing	no	ARS		1					
28	8.4	9.1	4.2	13	sectioning	yes	T	1	1	1				
29	9.3	10.7	4.9	21	clearing	yes	ARS+AB	1	1					
30	9.5	10.1	4.8	21	clearing	yes	ARS+AB	1						
31	9.7	10.6	4.9	21	clearing	yes	ARS		1	1				
32	10.0	11.3	5.0	21	clearing	yes	ARS+AB	1	1	1				
33	10.7	12.3	4.4	21	clearing	yes	ARS		1	1				
34	10.8	11.9	5.3	21	clearing	yes	ARS+AB	1	1					
35	11.1	12.4	5.5	21	sectioning	yes	T		1					
36	11.6	13.0	5.0	26	clearing	yes	ARS+AB	1	1	1				
37	12.0	13.5	6.2	26	clearing	yes	ARS	1	1					
38	12.3	13.5	6.7	26	clearing	yes	ARS		1	1				
39	12.7	15.0	6.1	26	clearing	yes	ARS+AB	1	1	1				
40	13.0	14.7	6.4	31	sectioning	yes	T		1					
41	13.7	15.0	6.6	31	clearing	yes	ARS+AB	1	1	1				
42	14.0	15.2	7.4	31	clearing	yes	ARS		1	1				
43	14.8	16.4	8.1	31	clearing	yes	ARS	1	1					
44	15.2	16.9	8.0	26	sectioning	yes	T	1	1	1				
45	15.3	17.1	7.0	31	clearing	yes	ARS+AB	1	1					
46	15.4	16.7	7.7	31	clearing	yes	ARS		1					
47	15.5	17.8	8.3	31	clearing	yes	ARS+AB		1					
48	17.3	18.8	10.3	31	clearing	yes	ARS		1					
49	18.2	20.1	10.1	31	clearing	yes	ARS			1				
50	18.7	20.9	9.2	31	sectioning	yes	T		1					
51	19.0	21.1	11.1	31	clearing	yes	ARS+AB	1	1					
52	21.5	24.7	11.7	31	clearing	yes	ARS+AB	1	1					
53	46.8	50.2	23.9	119	sectioning	no	IT		1	1	1	1	1	1
54	125.5	144.9	67.1	100	clearing	no	ARS+AB		1	1	1	1	1	1
55	127.0	143.6	65.5	100	clearing	no	ARS+AB	1	1					
56	132.5	149.9	68.2	100	dissection	no	-				1	1	1	1
57	136.2	154.1	71.3	100	dissection	no	-			1	1	1	1	1
58	140.1	158.4	72.4	100	clearing	no	ARS		1					
59	142.8	163.5	75.7	100	dissection	no	-				1	1	1	1
60	144.5	166.9	78.3	100	dissection	no	-				1	1	1	1
61	174.5	187.5	90.9	?	clearing	no	ARS+AB		1					
62	281.0	318.5	150.9	?	-	no	-			1				

I.2.2 - MORPHOLOGICAL DESCRIPTIONS

As already mentioned, different procedures were used for studying the detailed morphology of the developing skull of *Clarias gariepinus*. The ontogeny of the chondrocranium and osteocranium could easily be followed by using *in toto* cleared material, however, serial sections proved to be very useful for checking internal structures, cellular structure, indistinct ossifications, attachments of ligaments or connection between bones, passages of nerves or blood vessels, etc. The study of the cranial musculature in larval *C. gariepinus* required serial sections, which were reconstructed three-dimensionally. Time restrictions only allowed the reconstruction of the musculature in three stages, explaining the large gap between 7.2 mm SL and 46.8 mm SL. However, these stages proved to be sufficient for the description of the ontogeny, as: (1) substantial transformations occur early during ontogeny, (2) early stages have already been described by SURLEMONT (and co-workers), (3) larger specimens could be studied through dissections.

I.2.2.a - *In toto* clearing and staining

The procedure, according to HANKEN & WASSERSUG (1981) for *in toto* clearing and staining of cartilage and bone appeared to give satisfying results for studying the skeletal ontogeny of *Clarias gariepinus*. In this protocol, trypsine was used as the clearing agent. The clearing activity of trypsine, however, was very slow, as it took weeks to clear even small individuals. As a result, different concentrations of the more aggressive potassium hydroxide (KOH) were tested on different sizes of *C. gariepinus*. It appeared that quicker, but equally good, results could be obtained by using a 1% KOH solution for small individuals (SL < 50mm) and up to 10% for larger ones (SL > 50mm). Staining was done using alizarine red S for bone and alcian blue for cartilage (**Table I.2- 6**). In the protocol of HANKEN & WASSERSUG (1981), the cartilage staining precedes that of bone. However, the alcian blue 8GX has to be dissolved in ethanol and glacial acetic acid. The latter is very acidic, which thus induces decalcification of the bone. As alizarine red S actually binds onto calcified matrix of bone, true ossifications may thus be masked after being decalcified during the cartilage staining. To avoid this problem, several stages of a size comparable to those which were stained with both alizarine red S and alcian blue 8GX, were stained with alizarine red S only (**Table I.2- 5**).

Table I.2- 6: Composition of the bone and cartilage stainings

Product	Ratio
BONE STAINING	
Alizarine red S	15mg
KOH	0.5g
Aqua dest.	100ml
CARTILAGE STAINING	
Ethanol (96%)	40ml
Glacial acetic acid	10 ml
Alcian blue 8GX	7.5 mg

Cleared specimens were studied using a WILD M5 stereoscopic microscope. Drawings were made using a camera lucida.

I.2.2.b - Serial sections

The use of serial sections was crucial for studying the detailed morphology of larval stages, as well as for discriminating exact insertion sites of muscles, pathways of nerves and blood vessels, positions of tendons and ligaments, types of ossifications, etc. in later stages.

For this study, different embedding materials and stainings were used. Small specimens were embedded in a two-component Epon polymer. Serial, semi-thin sections of 2 μm were cut using glass knives on a LKB pyramitome. Staining was done with toluidin blue (**Table I.2-7**). Larger specimens (> 30mm) were embedded in Technovit. Sections of 5 μm were cut using a Reichert "Polycut" microtome, whereas staining was done with toluidin blue as well. One specimen (46.8 mm SL) was embedded in Paraplast and cut with a Jung "Autocut" microtome. The 5 μm sections were stained using an improved trichrome staining according to MANGAKIS *et al.* (1964). This staining provides a larger colour discrimination between different tissues.

Table I.2- 7: Composition of toluidin blue staining

Product	Ratio
SOLUTION A	
Toluidin blue	1g
Aqua dest.	100ml
SOLUTION B	
Borax ($NaClO_4$)	1g
Aqua dest.	100ml
Solution A + B	1/1

The sections were all mounted on glass slides and covered. They were studied using a Leitz Diaplan light microscope. Drawings were made using a camera lucida.

I.2.2.c - Three-dimensional reconstructions

Several series of serial sections were used for generating graphical, three-dimensional reconstructions. The reconstructions involved the entire skull, as well as specific regions or structures of interest. A commercial software package (PC3D, Jandel Scientific) was implemented on an IBM compatible computer (80486 processor). The input of serial sections was done by tracing the different structures on the drawings of the sections. Prior to the tracing, a reference point and reference axis were introduced by means of superposition. Tracing was done using a digitising tablet (HIPAD Plus, Houston Instruments). Different reconstructions were made, from different angles and with different combinations of structures, in order to obtain a clear view of the morphology of the skull. These views were eventually plotted (7475A Plotter, Hewlett Packard). These plots were redrawn, thus making the reconstructions more clear.

I.2.2.d - Dissections

The osteology, myology and cranial lateral-line system of the larger specimen of *Clarias gariepinus* were studied by means of dissections, using a WILD M5 stereoscopic microscope. Osteological data was obtained both from unstained, stained and uncleared, and stained and cleared material. The discrimination of fibre orientation and insertion of the musculature was improved by using a iodium solution, which was sprayed over the muscles until fibres became apparent (BOCK & SHEAR, 1972) (**Table I.2- 8**).

Table I.2- 8: Composition of muscle fibre staining

Product	Ratio
Iodine	1.0g
KI	2.0g
Aqua dest.	100 ml

The course of the cranial lateral-line system was visualised in fixed, unstained and uncleared specimens by injecting a saturated alizarine red S solution into the canal openings. Canal openings and pit-lines were observed by changing the angle of the light source.

I.2.3 - Abnormal Development

Two strategies for inducing deformities in the developing skull of *Clarias gariepinus* were used. One is based on the teratogenic effect of certain chemical compounds, whereas the other involves the effect of environmental constraints.

I.2.3.a - Teratogens

A number of different chemical compounds were tested for their effect on the ontogeny of the skull in *Clarias gariepinus*. The choice of which teratogens to test was based on several factors: (1) the detailed teratogenic activity of the compound had to be known (preferably in fishes), and (2) the effect of the teratogen had to be targeted to a certain group of tissues. The latter implies that in a deformed skull, to a certain degree a distinction can be made between deformities directly induced by the teratogen, and deformities which are the result of altered spatial interactions because of the former deformities. A complete distinction, however, is hardly possible. In the following part, some background information on the tested teratogens is given, in order to point out the action of the chemical, as well as to support the choice of these teratogens.

The testing of the effect of the different teratogens was done following the same procedure (with exception of malathion, see later). Different concentrations, depending on the teratogen, were dissolved in a Holtfreter buffer (**Table I.2- 9**). Next, five or ten larvae of different age were transferred into a petridish, containing 10 ml of a given concentration (concentrations were expressed in molarity) (**Table I.2- 10**). For every experiment, one blanco was used, containing only Holtfreter buffer. During the experiment, the dishes were checked regularly and the mortality was noted. After treatment, the larvae were transferred into the tanks again. The purpose of these initial experiments was to determine which concentrations were lethal and which produced deformities, so that the most useful concentration could then be applied to a larger amount of individuals. Due to time and practical restrictions, attention was paid only to those deformities which arose during the experiment or within the two weeks after.

Table I.2- 9: Composition of the Holtfreter buffer

Product	Ratio
NaCl	7g
KCl	0.1g
$CaCl_2.H_2O$	0.298g
$NaHCO_3$	0.4g
Aqua dest.	1000 ml

The following teratogens were tested:

1. *2.4-Dinitrophenol*
2. *Colchicine*
3. *Diazo-oxo-nor-leucine*
4. *β-amino-propio-nitril*
5. *Retinoic acid*
6. *Malathion*

It has to be noted that the purpose of these experiments was not to determine the teratogenic effects of several compounds on the developing larvae of *Clarias gariepinus*, as this would require much more replica's.

The teratogens were merely a means to obtain deformed larvae, in a controlled manner, meaning that the background of the deformities is known.

1. 2,4-DINITROPHENOL

This phenolic compound (**Fig. I.2- 3**) is highly toxic, it induces a marked increase in metabolism, resulting in many symptoms, even resulting to death. The impact on the metabolism and oxygen consumption has been observed in several vertebrates. In the medaka (*Oryzias latipes*, Cyprinodontidae), 2,4-dinitrophenol appeared to be highly toxic, with lethal concentrations of 0.01% (WATERMAN, 1939). When exposed to this, or higher concentrations, any development is inhibited after a few cleavages. Several factors were observed to influence the toxicity of the 2,4-dinitrophenol concentrations (duration of exposure, embryological stage at exposure, temperature). In the medaka, deformations in the circulatory system were observed at concentrations from 0.0008%, resulting from the decrease of hart rate and amplitude. Effects on the neural tube were distinct as well.

The effect of 2,4-dinitrophenol (Sigma) on the metabolism and oxygen consumption may influence the activity of muscles, when administered at low concentrations. This was the reason why the effect of this chemical on the developing *Clarias gariepinus* was tested. A total of five different concentrations were tested, together with the control (**Table I.2- 10**). Larvae of three days posthatching were used, with an exposure period of up to 235 hours.

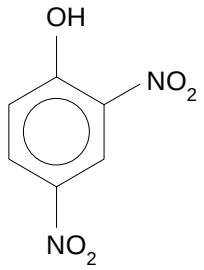


Fig. I.2- 3: Structural formula of 2,4-dinitrophenol

Survival was observed after that period in the control and three of the 2,4-dinitrophenol concentrations (1.0 10^{-4}M, 5.0 10^{-5}M and 2.5 10^{-5}M). Two more tests were then performed using only these three concentrations. However, no substantial deformations were observed in these larvae, and thus the experiments were no longer

Table I.2- 10: Molecular weight and concentrations used of the different teratogens tested (for data on malathion, see text)

	2,4-Dinitrophenol	Colchicine	Diazo-oxo-nor-leucine	β-Amino-propio-nitril	Retinoic acid
Mol. weight	184.1 g	399.4 g	171.2 g	256.3 g	300.4 g
Conc. 1	1.5 10^{-4}M	2.5 10^{-3}M	1.0 10^{-4}M	1.0 10^{-3}M	5.0 10^{-4}M
Conc. 2	1.0 10^{-4}M	1.0 10^{-3}M	1.0 10^{-5}M	8.0 10^{-4}M	1.0 10^{-4}M
Conc. 3	7.5 10^{-5}M	5.0 10^{-4}M	1.0 10^{-6}M	6.0 10^{-4}M	5.0 10^{-5}M
Conc. 4	5.0 10^{-5}M	2.5 10^{-4}M	1.0 10^{-7}M	4.0 10^{-4}M	1.0 10^{-5}M
Conc. 5	2.5 10^{-5}M	1.2 10^{-4}M	-	2.0 10^{-4}M	5.0 10^{-6}M
Conc. 6	-	2.5 10^{-6}M	-	1.0 10^{-4}M	1.0 10^{-6}M

continued.

2. COLCHICINE

Colchicine, which is a major alkaloid of *Colchicum autumnale* (Liliaceae) (**Fig. I.2- 4**), is known for its mitosis-blocking effect. It does so by inhibiting the polymerisation of the tubuline dimeres (α and β), which results in the absence of microtubuli that form the spindles during the metaphase (ALBERTS *et al.*, 1989). Exposure of embryo's and larval fishes to colchicine has resulted in major retardations in growth and development, depending on the concentration, moment of exposure, duration of the exposure (WATERMAN, 1940).

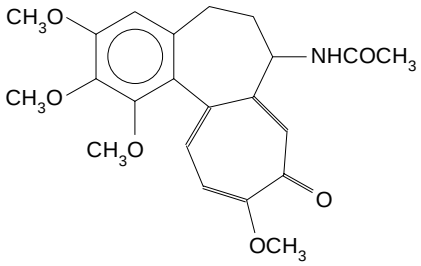


Fig. I.2- 4: Structural formula of colchicine

The reason why colchicine was used for inducing deformations in *Clarias gariepinus*, was because of the cyclopia-inducing potential of the chemical. Although the eyes are small in *C. gariepinus*, eye size is relatively much larger in larval specimens. In *Oryzias latipes*, cyclopia was induced at concentrations of 0.1%, with an exposure period of two to three and a half hours (WATERMAN, 1940). According to this author, cyclopia is believed to be the result of the failure of the evagination of the secondary telencephalic vesicles (presumably referring to the mesencephalic optic vesicles) and the lack of the cleavage of the prosencephalon (LANG *et al.*, 1976). Cyclopia comprises a whole series of deformations, intermediate between the normal situation of two eyes with two optical nerves, and a single eye, which may even be absent in the extreme situation (BALINSKY, 1975).

The initial believe that cyclopia resulted from the fusion between two fully formed eyes, was overruled by SPEMANN (1904), who claimed that the fusion occurs at the level of the optic 'Anlage' during embryology. STOCKARD (1907) demonstrated that in *Fundulus heteroclitus* (Cyprinodontidae), this was only correct in some cases, whereas he found that in other cases, two distinct optic nerves passed into a single optic cup. One year later, though, he withdrew this view, stating that cyclopia was the result of an 'indivision' of a normally single primordium (STOCKARD, 1908). According to ROGERS (1952), the presence of such a single primordium had not been proved, however. The latter author provided support, based on experimental work, to the hypothesis that "a single median eye in cyclopia may arise by fusion and regulation of the parts of two eye primordia". He also indicated that "the presence of a well-formed optic stalk is not essential to the growth of optic nerve fibres from the retina to the diencephalon". More recent studies, based on zebrafish mutants, showed that cyclopia is induced due to the absence of the floor plate of the diencephalon (HATTA *et al.*, 1994). Cyclopia is known to be induced by several teratogenic factors, both from genetical and chemical nature. A single eye was for example found in a trisomy 18 case (LANG *et al.*, 1976), whereas chemicals like magnesium chloride (STOCKARD, 1907; ROGERS, 1952; 1956), ethylalcohol (ROGERS, 1952; 1956), colchicine (WATERMAN, 1940) and lithium chloride (ROGERS, 1952) were used to induce cyclopia experimentally.

Several examples can be given which indicate the crucial role of the eye in the formation of the skull. First, there is the fact that a platybasic skull can be found in some fishes that have small eyes, like Siluriformes and Mormyriformes, whereas most, large-eyed fishes possess a tropibasic skull (DAGET, 1964; ADRIAENS & VERRAES, 1997e). Large eyes also require a very narrow interorbital septum, implicating different muscle architecture as well (ALEXANDER, 1965; ADRIAENS & VERRAES, 1997e). On the other hand, it has been observed in flatfishes that eye migration has a substantial influence on the skull morphology (BRILL, 1988; PODOSKINA, 1993). The spatial interactions between the developing eye and the surrounding skull becomes even more pronounced when an anomaly occurs during the development of the eye. CORSIN (1961) demonstrated that in salmons the trabecular bars do not fuse into a single trabecula communis when the eyes fail to develop. The effect of an asymmetrical difference in the eye size induced substantial distortions in both the neurocranial floor, roof and even lower jaw in salmons (VERRAES, 1974a). More extreme deformations of the cranial skeleton were observed in cyclopean salmons, where for example the neurocranial roof failed to become fused to the floor in the ethmoid region (BOLKER & THOMSON, 1992) (**Fig. I.2- 5**).

Induction of cyclopia in *Clarias gariepinus* could thus reveal some evidence on the spatial constraints that occur in the head during ontogeny. It could provide some evidence of the epigenetic control of skull

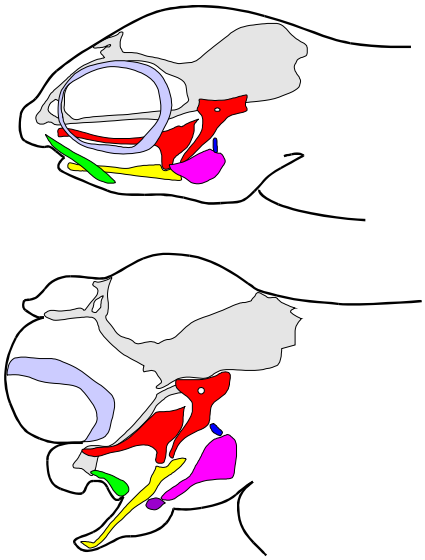


Fig. I.2- 5: Normal (top) and cyclopic (bottom) *Salmo* (lateral view) [after BOLKER & THOMSON (1992)]

development, as a deformed eye early during ontogeny can demonstrate: (1) the spatial interactions between the (non-skeletal) eyes and the (skeletal) chondrocranium, and consequently, (2) the interactions between the altered chondrocranium and the osteocranium, myology, nerves, and so on.

A total of six different concentrations (Sigma) were tested on larvae of three days posthatching (**Table I.2- 10**). Exposure lasted for 235 hours, resulting in an overall mortality already after 25 hours. This, however, was not the case for the 2.5 10^{-6}M concentration. In a second experiment, this concentration was tested on other larvae. After both experiments, however, no substantial deformities in the surviving larvae could be detected.

As 2,4-dinitrophenol and colchicine still have a more overall impact on the skull, it was opted not to proceed with inducing anomalies by using these chemicals. Chemicals with a more specific teratogenic effect were consequently focussed on. Four of them were selected: (1) diazo-oxo-nor-leucine, (2) β-amino-propio-nitril, (3) retinoic acid and (4) malathion.

3. DIAZO-OXO-NOR-LEUCINE

6-Diazo-5-oxo-L-norleucine (DON) is an analogue of the amino-acid glutamine (**Fig. I.2- 6**), produced by a *Streptomyces* and used as an antitumor antibiotic. Its teratogenic effect has been demonstrated in rats, where it inhibits the elevation of the cartilaginous palatal shelves on day 15 of gestation. These shelves rotate from a vertical position alongside the tongue, to a horizontal position, consequently fusing with the contralateral one and thus forming the secondary palate (DIEWERT & PRATT, 1979). This rotation coincides with an increased level of acid glycosaminoglycans in these shelves, especially hyaluronic acid (PRATT *et al.*, 1973). The teratogenic effect of DON is demonstrated to inhibit the synthesis of these glycosaminoglycans (GAGs) (= mucopolysaccharids) (PRATT *et al.*, 1973), as well as that of glycoproteins, by "inhibiting the glutamine-dependent conversion of fructose 6-phosphate to glucosamine 6-phosphate, thereby blocking the formation of UDP-activated (Uridine DiPhosphate[2]) sugars derived from glucosamine" (PRATT & GREENE, 1976). These components, the GAGs and the glycoproteins, play an important role in cartilage formation, as they are part of the extracellular matrix (GILBERT, 1988; ALBERTS *et al.*, 1989).

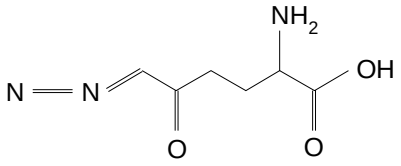


Fig. I.2- 6: Structural formula of 6-diazo-5-oxo-L-norleucine

Growth of cartilage, in general, can occur by cell division, cell volume increase or matrix volume increase. The matrix volume increase can result from secretion of extracellular matrix, consisting largely of GAGs and fibrous proteins (ALBERTS *et al.*, 1989). However, the hydrophilic nature of the GAGs can participate in swelling as well, as the high density of negative charges of the carboxyl groups attracts cations, as for example Na^+, which in its turn creates an osmotic underpressure, resulting in the sucking in of large amounts of water (ALBERTS *et al.*, 1989). Not only does this result in swelling of cartilage, it also plays a

[2] Uridine DiPhosphate participates in the conversion from sucrose phosphate to fructose 6-phosphate, thereby forming as well Uridine DiPhosphate Glucose (DEVLIN & WITHAM, 1983).

crucial role in creating an internal pressure, providing mechanical support and elasticity in for example articulatory facets.

Because of this targeted effect, DON appeared to be a very useful teratogen for testing on *Clarias gariepinus*. The fact that its action is concentrated on cartilage growth (by matrix production) and the mechanical properties of articulatory facets, makes it possible to discriminate between directly (chemically) induced deformations and indirectly (epigenetically) induced deformations. The change in cartilage growth could provide some insight in the spatial relation between growing cartilage and the surrounding bones, as there seems to be a close causal and functional relation between them (VERRAES, 1974b; HUYSSEUNE, 1985).

Four different concentrations of DON (Sigma) were tested on three groups of *C. gariepinus* larvae of different ages (6, 11 and 13 days posthatching) (**Table I.2- 10**). These experiments clearly showed that the sensitivity of the larvae to DON decreases as they grow older. Surprisingly, in all three cases, the larvae experienced a greater mortality in the 1.0 10^{-5}M concentration. After the experiment, the surviving larvae were transferred into large tanks (**Table I.2- 1**). After one month, they were anaesthetised and fixed. By means of a stereomicroscope, however, no deformations could be detected.

4. β-AMINO-PROPIO-NITRIL

β-3-Amino-propio-nitril (BAPN) (also referred to as 3-amino-propane-nitril) is a lathyrogenic compound, with a comparable teratogenic effect as DON (see above) (**Fig. I.2- 7**). BAPN administered orally to rats and hamsters, induced the failure of the elevation and fusion of the palatal shelves, although effects on several other skeletal structures have been observed as well. Some deformations even involved exencephaly, subcutane haemorrhage, ectocardia and gastroschisis[3] (BARROW & STEFFEK, 1974; WILEY & JONEJA, 1976; DIEWERT, 1981). Comparable deformations have been noted in amphibians and birds as well (HALL, 1972; BARROW & STEFFEK, 1974). The palatal cleft in rodents was initially believed not to be a direct result of BAPN teratogenic activity, but rather the result of a spatial constraint due to the failure of the lowering of the tongue, which in its turn was the result of a deformed lower jaw (DIEWERT, 1981). In that case, the palatal shelves remained lateral to the tongue and were prevented to rotate upwardly. However, earlier, cases had been observed where a cleft palate was the only observed deformation, after an oral administration of 600 mg/kg body weight to rats during gestation (PRATT & KING, 1972).

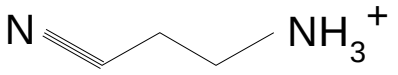


Fig. I.2- 7: Structural formula of β-amino-propio-nitril

BAPN is known to affect the formation of collagen and elastin, more especially, the formation of cross-links between collagen molecules. Collagen fibrils consist of packed collagen molecules, connected to each other through many cross-links. Each collagen molecule, in its turn, consists of three polypeptide chains that are linked together in a helix (ALBERTS *et al.*, 1989). The cross-links are established by aldehydes (*i.e.,* α-amino-adipic δ-semi-aldehyde), which are formed by the oxidative deamination of the ε-amino end of the lysyl residues in the polypeptides (PRATT & KING, 1972). This conversion is enzymatically catalysed by lysyl oxidase (SIEGEL & MARTIN, 1970). The effect of BAPN then involves the blocking of this lysyl oxidase enzyme, consequently preventing its normal catalysing activity (**Fig. I.2- 8**). The inhibition of collagen cross-linking formation has as a consequence that the collagen becomes more soluble (HALL, 1972). On the other hand, administration of BAPN to chicken embryo's demonstrated a possible effect on the non-sulfated, acid GAGs as well, as could be demonstrated by the staining pattern of alcian blue in cartilage (HALL, 1972). WILK *et al.* (1972) stated that this is

due to the effect on the GAGs metabolism, including and especially the sulphated chondroitine-4-sulphate and chondroitine-6-sulphate. The effect on GAGs, however, would only be in cases of high doses, whereas the effect on collagen formation is the most crucial teratogenic activity. Whether or not BAPN is the active teratogenic compound, or one of its metabolites, was demonstrated by WILK *et al.* (1972). They showed that cyano-acetic acid, nor β-alanine, nor cyano-ethanol, which are all possible metabolites, have any teratogenic effect on developing rodents. Also, BAPN does not become accumulated, although high concentrations could be observed in the brain and in cartilage (WILK *et al.*, 1972; DIEWERT, 1981).

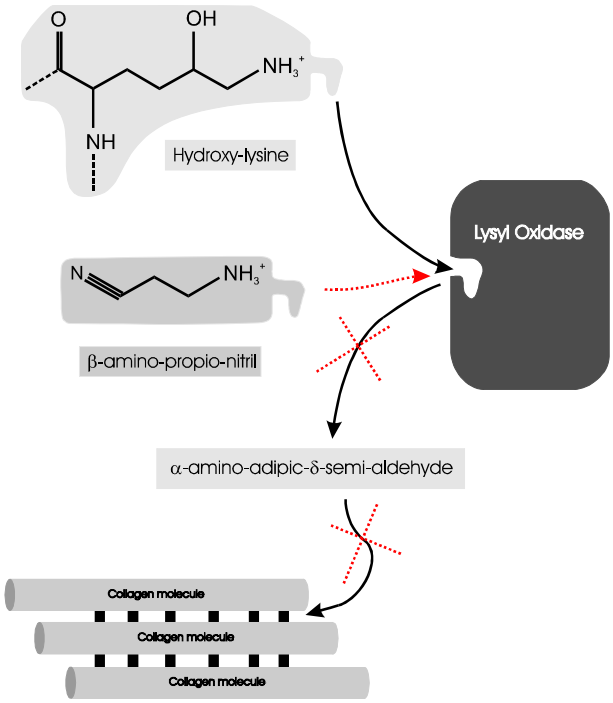


Fig. I.2- 8: The inhibition of hydroxylysine deamination in collagen molecules by the blocking of β-amino-propio-nitril, resulting in the inability to form cross-links between different collagen molecules (for more explanation, see text)

The targeted effect of BAPN on the formation of collagen, which forms a major component of the extracellular matrix in cartilage, has lead to the testing of its effect on larval *Clarias gariepinus* (ALBERTS *et al.*, 1989). Although the effect of BAPN in rats seemed to involve cartilaginous abnormalities, it has to be noted that effect of BAPN on bone collagen cannot be neglected, because bone collagen type I is known to have many cross links (MARKS & POPOFF, 1988). The direct effect of BAPN on bone could be eliminated when exposing larvae before ossification has started. BAPN was administered as a monofumarate salt (Sigma), resulting in a molecular weight of 256.3 g (in comparison of 70.1 of BAPN) (**Table I.2-10**) (**Fig. I.2- 9**). Fumaric acid had already been demonstrated to have no teratogenic effect on rodents (WILK *et al.*, 1972). This, however, implicated that for each monofumarate molecule, two BAPN molecules were present. In a first experiment, a total of five concentrations were tested on larval *C. gariepinus*: 10^{-1}M, 10^{-2}M, 10^{-3}M, 10^{-4}M and 10^{-5}M. Larvae of three different ages were exposed to BAPN (5, 13 and 15 days posthatching) during 40 hours. As was the case for DON, the older the larvae at the moment of exposure, the higher the survival rate. Concentrations of 10^{-2}M and higher were lethal to each group. Based on this, a second experiment was done using six different concentrations between 10^{-3}M and 10^{-4}M, tested on larvae of three days posthatching (**Table I.2- 10**). In a third experiment, 1 day and 14 days posthatching larvae were exposed to three of those concentrations (4 10^{-4}M, 2 10^{-4}M and 1 10^{-4}M).

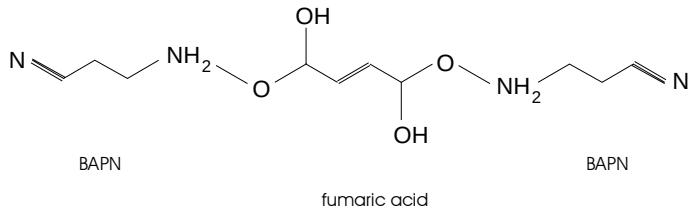


Fig. I.2- 9: Structural formula of the monofumarate salt of β-amino-propio-nitril

[3] Gastroschisis describes an anomaly involving a ruptured stomach wall.

Despite the little useful results of the previously tested teratogens, BAPN proved to be more useful. A general trend involved a reduction and deformation of the oral barbels. These were strongly shortened and in some cases heavily twisted (**Fig. I.2- 10**). Presumably, BAPN has affected the elastin molecules, which are abundant in catfish oral barbels (GHIOT & BOUCHEZ, 1980). More interesting deformations were obtained in the third experiment.

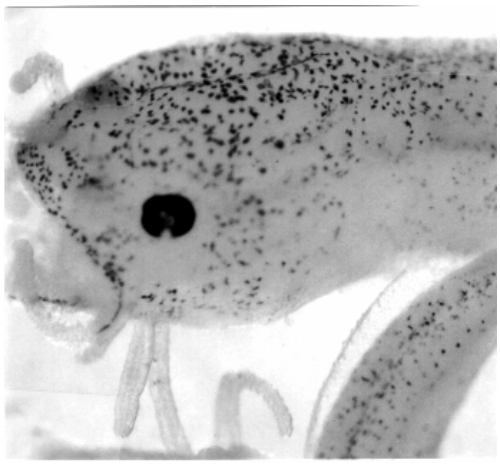


Fig. I.2- 10: Deformed oral barbels in larval *Clarias gariepinus* exposed to BAPN

In one specimen (exposed to 2 10^{-4}M), an ophthalmic asymmetry could be observed (**Fig. I.2- 11**). In this case, the left eye was less developed, compared to the right one. A direct effect of BAPN on the developing eye has not been noted in literature yet. Two hypotheses can thus be proposed. (1) The deformation is a result of spatial constraints that the left eye experienced during development, because of structural transformations of the cartilaginous skull surrounding it. These transformations could then be the direct effect of BAPN. (2) The occurrence of spontaneous anomalies in the developing organs, as for example the eye, cannot be ruled out.

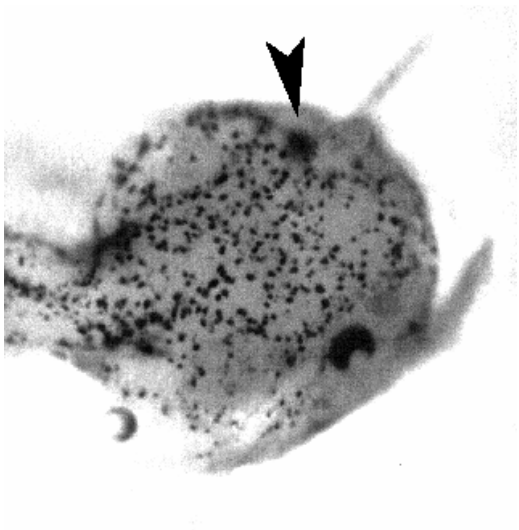


Fig. I.2- 11: Asymmetric ophthalmia in larval *Clarias gariepinus* exposed to BAPN (arrow indicates smaller left eye)

In another specimen (exposed to 1 10^{-4}M), the effect of BAPN appeared to be more clear. In this specimen, Meckel's cartilage is lacking almost completely (**Fig. I.2- 12**). The mouth opening is completely circular, positioned obliquely. At the level of the articulation of the mandibula with the suspensorium, a small process can be distinguished (arrow), corresponding to the remaining part of Meckel's cartilage. Both these deformed specimens were fixed and serially sectioned. Sections of the agnathian larva could then be used to rule out the possibility that this anomaly was due to a non-teratogenic factor, for example as a result of aggressive behaviour between larvae. However, due to time restrictions, a detailed study of this specimen could not yet be performed.

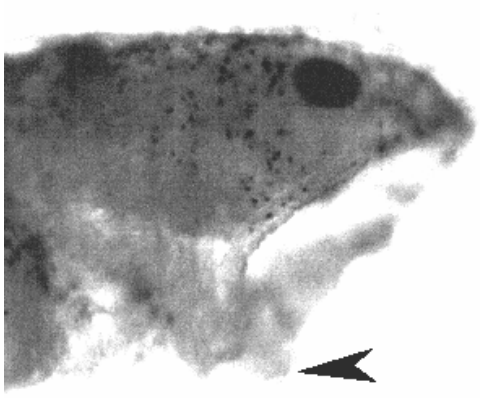


Fig. I.2- 12: Agnathia in larval *Clarias gariepinus* exposed to BAPN (arrow indicates remnant of Meckel's cartilage)

5. RETINOIC ACID

Retinoic acid is a carboxyl analogue of vitamin A. This vitamin is derived from β-carotene, which is bio-assimilated in plants, were it performs many important

roles (DEVLIN & WITHAM, 1983). Although not assimilated in animals, retinoic acid is long known to be a morphogen which plays a crucial role during organogenesis. Embryos, which obtain retinoic acid through the maternal vitamin A, produce a concentration gradient of *all-trans*-retinoic acid (**Fig. I.2- 13**) in their limb buds (GILBERT, 1988; ALBERTS *et al.*, 1989; MORRISS-KAY, 1992). This gradient, with the highest concentration anteriorly at the Zone of Polarising Activity (ZPA), is believed to determine the anterior-posterior polarity in the limbs. *All-trans*-retinoic acid is also known to affect the migration of crista neuralis cells, by interfering with the corresponding gene expression, as well as the process of apoptosis or cell death (MORRISS-KAY, 1992; OSUMI-YAMASHITA *et al.*, 1992). Programmed Cell Death (PCD) is known to play an important role in the formation of structures, as for example digits (MORRISS-KAY, 1992; KIMURA & SHIOTA, 1996). It involves the activity of transglutaminase, which is induced by retinoic acid (MORRISS-KAY, 1992).

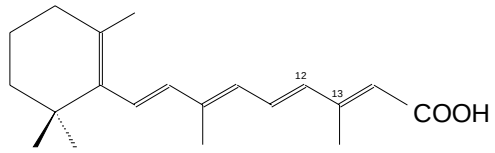


Fig. I.2- 13: Structural formula of *all-trans*-retinoic acid

The teratogenic effect of retinoic acid depends on the presence of the so-called CRBP's, CRABP's, RXR's and RAR's (MORRISS-KAY, 1992; 1993). CRBP's, or Cytoplasmatic Retinol Binding Proteins are important in embryos, as they are the binding site for maternal retinol, or vitamin A. The natural production of retinoic acid occurs through the conversion of this retinol. Of the two types of CRBP's, only CRBP I is present in embryo's[4]. The CRABP's are the Cytoplasmatic Retinoic Acid Binding Proteins. Of these extra-nuclear receptors, a spatio-temporal distribution difference is noted between the two types. CRABP I has been observed in the hind brain and cranial neural crest cells migrating to the heart. These receptors inhibit the access of retinoic acid to the nuclear receptor proteins and even mediate its degradation. CRABP II are more abundant though less expressive than the previous ones. Unlike the CRABP I, the CRABP II cannot be induced by retinoic acid. Once the CRABP I becomes saturated with retinoic acid, the retinoic acid can enter the nucleus were they can bind to the intra-nuclear receptors.

The RXR's or Retinoid 'X' Receptors are involved in the retinoic acid-bound gene expression. Activation of the RXR's, however, does require an isomerisation of the *all-trans*-retinoic acid to the 9-*cis*-retinoic acid. Of the RAR's, or Retinoic Acid Receptors, three groups have been discovered in mammals already (RAR-α[5], RAR-β[6] and RAR-γ[7]). These receptors are inactive, unless they are bound to retinoic acid. In that case, the complex attaches itself to a Retinoic Acid Response Element (RARE), which is situated in the promotor region of the target genes. In this way, the teratogenic effect of retinoic acid involves the alterations of gene expression, especially those that play an important role in the migration of the cranial neural crest cells and the segmentation of the rhombomeres in the hind brain. Apart from *all-trans*-retinoic acid, teratogenic effect of 13-*cis*-retinoic acid[8] has been observed also (**Fig. I.2- 14**). The deformations are concentrated on craniofacial development, presumably due to the isomerisation of this 13-*cis* form to the *all-trans* form (MORRISS-KAY, 1993).

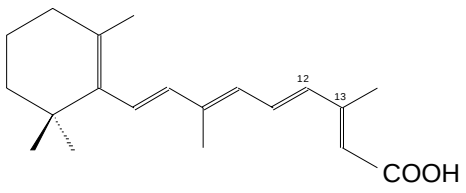


Fig. I.2- 14: Structural formula of 13-*cis* retinoic acid

[4] CRBP II is only present in non-foetal vertebrates, situated in the mucosa cells of the functional digestive system. There it enables the uptake of retinol from the digested food.
[5] The RAR-α has an overall distribution in the embryonic tissue. One of its seven isoforms, the RAR-α_2 is decoded by genes that are induced by retinoic acid itself (MORRISS-KAY,1992).
[6] All the genes decoding for the RAR-β isoforms are induced by retinoic acid. RAR-β receptors are synthesised in the interdigital mesenchym and the hind brain (MORRISS-KAY,1992; OSUMI-YAMASHITA *et al.*, 1992).
[7] The RAR-γ receptors are associated with condensations of the pre-cartilage mesenchym and cartilage itself. The receptor is shut down, once the ossification of cartilage has started (MORRISS-KAY,1992).
[8] 13-*cis*-Retinoic acid is also commercially available since 1982 as Accutane, which is used to cure severe cystic acne (GILBERT, 1988).

Craniofacial anomalies, due to retinoic acid, are very common deformations, as a result of its teratogenic effect (OSUMI-YAMASHITA, 1992; MORRISS-KAY, 1993). High levels of RAR-γ transcripts were observed in primordia of frontonasal, maxillary and mandibular prominences in embryonic mouse (OSUMI-YAMASHITA, 1992). The craniofacial abnormalities that have been observed in several vertebrates involve micrognathia, cleft palate, mouth-to-ear cleft, abnormal external ears, exencephaly, microcephaly, microphthalmia, anophthalmia, abnormal nares (SHENEFELT, 1971; FANTEL *et al.*, 1976; MORRISS & STEELE, 1976; ECKHERT & HURLEY, 1979; OSUMI-YAMASHITA, 1992). These may be the result of the inhibition of the segmentation of the hind brain, the disturbance of the migration of the cranial neural crest cells or an excessive cell death. Both these anomalies presumably involve the effect of retinoic acid on the expression of Homeobox genes (MORRISS-KAY, 1993; PENDLETON *et al.*, 1993). It has, for example been demonstrated in lidgap mutations in mice, which normally fail to develop a foetal eyelid over the eye during embryogenesis, this eyelid formation can be induced by exposing them to retinoic acid. It is also known that forebrain neural crest cells might contribute to structures associated with the eye (JURILOFF & HARRIS, 1993). MORRISS & STEELE (1976) revealed that in rat embryos, retinoic acid reduced the development of the pharyngeal arches, as did MORRISS-KAY (1993). A gradient could be observed from a normally developed Meckel's cartilage to a completely absent one (**Fig. I.2- 15**). The pharyngeal arches, or visceral arches, have a separate origin from the neurocranium: the visceral arches are exclusively derived from the migrated cranial neural crest cells, whereas the neurocranium is not (JOHNSTON, 1966; GRAVESON, 1993; SCHILLING & KIMMEL, 1994; AHLBERG, 1997). The neurocranium, however, appears to have a triple origin: (1) cartilage derived from the cranial neural crest, (2) cartilage of cephalic mesodermal origin, and (3) cartilage of somitic origin (COULY *et al.*, 1993; GRAVESON, 1993; WEBB & NODEN, 1993). Also, the neural crest cells seem to contribute to the formation of branchial muscle connective tissue, but not to the muscles themselves. This differentiation is such that each connective tissue, derived of a certain rhombomere, is attached to that skeletal element, which is derived from the same rhombomere (KÖNTGES & LUMSDEN, 1996).

Fig. I.2- 15: Reduction of Meckel's cartilage (arrow) in rat embryos, exposed to retinoic acid [*Abbreviations:* B = brain, O = otic capsule, M = maxillary) (After MORRISS-KAY (1993)]

The targeted action of retinoic acid on the migration of the cranial neural crest cells, which normally differentiate into the cartilage of the mandibular, hyoid and branchial arches, and part of the neurocranium, and the attachment connective tissue of muscles, could provide some interesting deformations. Deformations that would then be observed in those neural crest derived structures could be attributed to the direct action of retinoic acid, whereas abnormalities in surrounding structures could be the result of the spatial alterations. That is why retinoic acid was tested on *Clarias gariepinus*. *All-trans*-retinoic acid (Sigma), however, could not be dissolved in water-based solutions, like Holtfreter buffer, but has to be dissolved in fat-based solvents. However, as exposure of larvae to retinoic acid involved a water medium, this solvent had to be soluble in water. Consequently, the frequently used peanut, corn, cottonseed or olive oil for other vertebrates could not be used (FANTEL *et al.*, 1976; KOCHHAR, 1976; OSUMI-YAMASHITA *et al.*, 1992; JURILOFF & HARRIS, 1993). On the other hand, administration of retinoic acid, dissolved in those oils, could not be done through feeding either. Due to the fact

that the purpose of this experiment involved the induction of deformations by the effect of retinoic acid on the neural crest migration, the exposure had to be done very early during ontogeny. Exposure of *C. gariepinus* thus occurred at the egg stage, at which obviously no feeding occurred. As a result, the experiment initially consisted of testing the solubility of *all-trans*-retinoic acid in different of these fat-based solvents, as well as the teratogenic effect of these solvents on the developing larvae. A total of three solvents were used: (1) glycerine, (2) Tween 80 (= poly-ethylene-sorbitan-mono-oleate), and (3) DMSO (= dimethyl-sulfoxide). Glycerine proved to be harmless for the catfish larvae, however, the solubility of retinoic acid was insufficient (eight concentrations were tested, ranging from 1% to 0.005%). Tween 80, as used by SHENEFELT (1971), seemed to have a negative effect on the survival at concentrations of 0.08% and higher. However, lower concentrations of Tween 80 dissolved the retinoic acid insufficiently (identical concentrations as for glycerine were tested). Finally, the effect of DMSO was tested on the survival of *C. gariepinus* (RODRIGUEZ-TÉBAR & ROHRER, 1991). A 1% solution of DMSO proved to be harmless to egg stages of *C. gariepinus*. In total, three experiments were performed. Each consisted of three groups of eggs of different ages, exposed to six different concentrations of *all-trans* retinoic acid, dissolved in 1% DMSO (in turn dissolved in a 1% DMSO solution in Holtfreter), together with two blancos (one with Holtfreter, another one with 1% DMSO in Holtfreter) (**Table I.2- 10**). In total, eggs were exposed at six different moments during embryogenesis: 2, 3, 6, 22, 23 and 30 hours postfertilisation, in order to increase the possibility that retinoic acid is present in the embryo at the moment of cranial neural crest migration. The mortality rate after 24 hours of exposure was highest in the 23 hour postfertilisation eggs, and was lowest in the eggs of six hours postfertilisation.

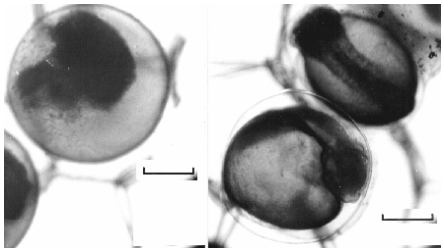


Fig. I.2- 16: Abnormal embryonic development in eggs of *Clarias gariepinus* exposed to *all-trans*-retinoic acid (bar = 0.5 mm)

Retinoic acid had a substantial effect on the embryogenesis in *C. gariepinus*. Serious deformations could already be observed at the egg stage, where embryos failed to develop their normal habitus (**Fig. I.2- 16**). These anomalies were, however, lethal and thus of no use for this study. It appeared that the use of retinoic acid, as a targeted teratogen, required additional experiments, in order to standardise and fine-tune the induction of deformities. As this was expected to be very much time consuming, the use of retinoic acid was no longer tested.

6. MALATHION

Malathion, or S-[1,2-*bis*(ethoxycarbonyl)-ethyl]-0,0-dimethyl-phosphoro-dithioate), is considered to be one of the safest insecticides, used all over the world (MATSUMURA, 1985). It is a dithiophosphate insecticide, bound to a succinic acid ester (**Fig. I.2- 17**). The success of malathion is due to the selective toxicity of this pesticide, based on the differences in the enzymatic constitution of different organisms. Malathion itself is rather weakly active as a toxicant, however, once it is transformed into malaoxon (**Fig. I.2- 18**), the toxicity becomes more radical. In insects, malaoxon is formed rapidly through oxidation. The hydrolysis of malaoxon, which would result in a detoxification, is too slow, thus making malathion highly toxic to them. In mammals, the detoxificating hydrolysis occurs prior to oxidation, due to the presence of the malathion carboxyl-esterase enzyme[9] (HASSAL, 1990;

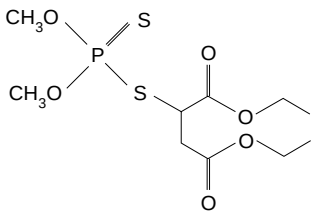
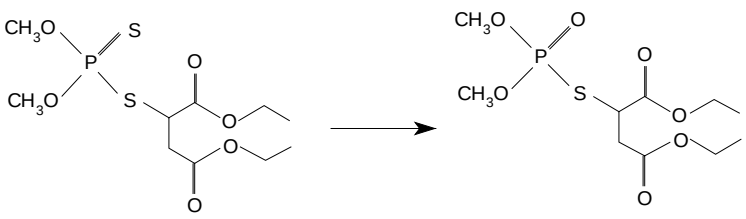


Fig. I.2- 17: Structural formula of malathion

[9] A malathion carboxyl-esterase has also been found in some populations of certain insects, which makes them resistant to this insecticide (ZAMBURLINI & BELLANTONE, 1993; WYHARD *et al.*, 1994).



Malathion Malaoxon

Fig. I.2- 18: Conversion of the low active malathion to the extreme active malaoxon, which occurs through fast oxidation in insects

RICHMONDS & DUTTA, 1992; DUTTA *et al.*, 1994; WYHARD & WALKER, 1994; WYHARD *et al.*, 1994). Consequently, the LC_{50} values of malathion are much higher in mammals than in insects (VERSCHUEREN, 1983).

As a result, malathion has extensively been applied to control several insect pests all over the world [*e.g.,* grasshoppers (BOUAICHI *et al.*, 1994); human lice (CHOSIDOW *et al.*, 1994); mosquitos (ZAMBURLINI & BELLANTONE, 1993); sheep blowfly (WYHARD & WALKER, 1994)]. However, low LC_{50} levels are observed in vertebrates, especially in fishes, where 96 hours LC_{50} values may range between 51 µg/l in *Cyprinodon variegatus* (Cyprinodontidae) and 12.9 mg/l in *Ictalurus nebulosus* (Ictaluridae) (VERSCHUEREN, 1983). Increasingly, researchers are beginning to focus on the side-effects of malathion on fish species, especially those that are commercially important (AREECHON & PLUMB, 1990; RANI *et al.*, 1990; DUTTA *et al.*, 1992; 1994).

The toxic effects of malathion result from the ability to inhibit the enzyme acetylcholinesterase (AChE) (RICHMONDS & DUTTA, 1992; KUHN & STREIT, 1994; DUTTA *et al.*, 1995; EHRICH *et al.*, 1995). Acetylcholinesterase plays a crucial role in the regulation of neural impulse transmission at the level of synapses, as it regulates the degradation of acetylcholine (ALBERTS *et al.*, 1989). Acetylcholine is one of the major groups of neurotransmitters, which is released into the synaptic cleft through the dumping of synaptic vesicles at the synapse of the axon (**Fig. I.2- 19**). Neurotransmitters function as chemical signals, which can be transformed into electrical signals. As the acetylcholine becomes attached to the specific receptor molecules[10] in the membrane of the postsynaptic neuron, an influx of positive ions is induced[11]. After each neurotransmission, the excess of acetylcholine has to be removed from the synaptic cleft. This occurs through the activity of acetylcholinesterase, which catalyses the breakdown of acetylcholine into acetic acid and choline. The blocking of this enzyme by malathion will consequently disable the breakdown of the acetylcholine excess, which will result in uncontrolled neurotransmissions.

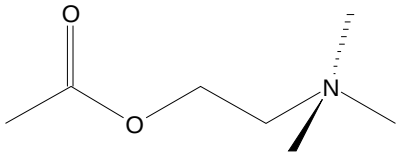


Fig. I.2- 19: Structural formula of acetylcholine

It can thus be expected that the major effect of malathion is on the neural control of muscular contractions (both at the level of interneuron contact, as well as neuron-muscle contact). Erratic muscular contractions and even paralysis have been observed (SHARMA *et al.*, 1983; AREECHON & PLUMB, 1990; SRIVASTAVA & SRIVASTAVA, 1990; DUTTA *et al.*, 1992; NGUYEN, 1997; NGUYEN *et al.*, 1997). Inactivity of fishes exposed to malathion is also believed to be the result of the accumulation of lactic acid (RANI *et al.*, 1990). The erratic muscular contractions are believed to lie at the basis of the frequently observed skeletal abnormalities in fish exposed to malathion (AREECHON & PLUMB, 1990; SRIVASTAVA & SRIVASTAVA, 1990). However, the more direct effect on bone formation may be due to the decreased activity of carbonic anhydrase[12] after exposure of organophosphate

[10] Two types of acetylcholine receptors can be distinguished: (1) nicotine ACh-receptors in skeletal muscles, and (2) muscarine ACh-receptors in heart muscle cells (ALBERTS *et al.*, 1989).
[11] Acetylcholine may also induce contractions in smooth muscle cells, as well as it induces the production of insulin in the pancreas (ALBERTS *et al.*, 1989).
[12] Carbonic anhydrase is part of the enzymatic repertoire of osteoclasts. It is bound to the cell membrane, where it takes part in the process of bone resorption (MARKS & POPOFF, 1988).

pesticides (SRIVASTAVA & SRIVASTAVA, 1990). However, this has not been reported for malathion itself. VIDEIRA *et al*. (1994) on the other hand noted that malathion affects the Ca^{2+} pump activity of sarcoplasmatic reticulum by interacting with the boundary lipids of the pump. Apart from the effect on muscular activity, effects have been observed for example on plasma glucose level, haematocrit and haemoglobin levels, brain acetylcholinesterase levels, necrosis of gill epithelia (AREECHON & PLUMB, 1990; RANI *et al.*, 1990; RICHMONDS & DUTTA, 1992; DUTTA *et al.*, 1994, 1995; NGUYEN, 1997; NGUYEN *et al.*, 1997).

Since malathion mainly affects the muscular activity, it is a highly useful teratogen for testing epigenetic interactions between the developing skeleton and musculature. The possible direct effect of malathion on osteogenesis can be ruled out when larvae are used in which no ossification has occurred yet. As a result of the erratic contractions of the developing muscles, an abnormal mechanical load will be exerted on the developing, cartilaginous skeletal elements. The effect of malathion on developing *Clarias gariepinus* larvae was tested in co-operation with the Laboratory for Biological Research in Aquatic Pollution (UG), with Dr. L. Nguyen and Dr. C. Janssen. The research of Dr. L. Nguyen involved the testing of *Clarias gariepinus* as a potential organism for performing early life stage toxicity tests (NGUYEN, 1997). The exposure of eggs and larvae of *C. gariepinus* was done by Dr. Nguyen. Anomalous larvae of those tests could then be obtained. In the tests of Dr. Nguyen, malathion was dissolved in a 0.01% acetone solution. Different stages (2-4 cell stage, late blastula stage and newly hatched larvae) were exposed to five concentrations, as well as a control concentration (0.3 mg/l - 0.63 mg/l - 1.25 mg/l - 2.5 mg/l - 5 mg/l). Survival increased with decreasing concentration and increasing age at exposure (NGUYEN, 1997: Fig. 2.27). Abnormalities in larvae, exposed at the 2-4 cell stage, significantly increased in the 1.25, 2.5 and 5.0 mg/l concentrations.

Larvae exposed at hatching showed a significantly increased number of abnormalities in the 2.5 and 5.0 mg/l concentrations, compared to the control concentration (NGUYEN, 1997: Fig. 2.28). The most frequently observed abnormalities involved spinal column deformities and pericardial oedema. Spinal deformities could already be observed prior to vertebrae formation, in which a strongly bent notochord could be observed (**Fig. I.2- 20**). It appeared that spinal column deformities in fishes, exposed to malathion, may be the result of the teratogenic effect of malathion, early during ontogeny[13]. In that case, erratic muscular contractions induced a deformed notochord, which in turn results in a deformed neural tube. As the neural tube is known to play an important inductive and topographic role in the ontogeny of the neural arches, it will affect the formation of the latter elements (HOLTZER, 1952; HOLTZER & DETWILER, 1953; WATTERSON, 1952; FLINT, 1977). Also, the deformed notochord most probably will interfere with the normal development of the vertebrae as well, as the development of the bony vertebral centra occurs around the notochordal sheath (BOLK *et al*., 1936; ADAMS & EDDY, 1949).

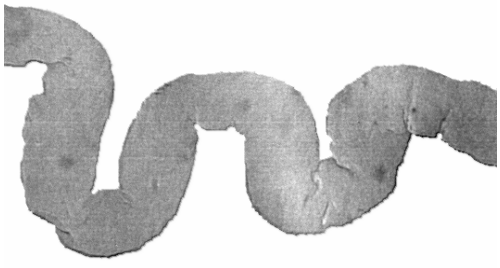


Fig. I.2- 20: Deformed chorda dorsalis in larval *Clarias gariepinus* after exposure to malathion

[13] The results of this study have already been published (NGUYEN *et al.*, 1997).

As the oldest stages of *C. gariepinus*, used in the above mentioned experiments (newly hatched larvae) possessed little or no functional muscles in the skull (as could be derived from the already performed ontogenetic study, see II.2), a separate experiment was done. Larvae of four days posthatching were exposed to a control solution (0.05% acetone) and five different concentrations of malathion (0.3M - 0.625M - 1.25 M - 2.5M - 5.0M). In order to reduce genetic variability, gynogenetic material was used, obtained from the Laboratory of Aquaculture and Ecology (Catholic University Leuven). Malathion was dissolved in a 0.05% acetone solution. The set-up of this experiment follows the procedure of the previous experiments (see above). Three days after exposure, some larvae were transferred into fresh water, as mortality was increasing substantially. Seven days after this transfer, these larvae were fixed and cleared *in toto*.

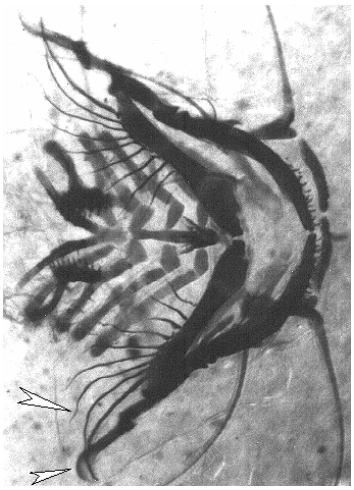


Fig. I.2- 21: Deformed branchiostegal rays and opercular bone in larval *C. gariepinus*, exposed to malathion (arrows indicate curvature of the bones) (ventral view)

By means of a stereoscopic microscope, some deformations could be observed in the skull. In two specimens, a deformed opercular bone and deformed branchiostegal rays were present (**Fig. I.2- 21**). These structures were curved at their distal ends, an anomaly which was not observed in any non-exposed specimen. However, a causal relation between altered muscular activity and this deformation does not seem obvious, at first sight. Possibly, the disturbed contraction of the opercular muscles, as well as those inserting onto the branchiostegal rays, may have induced this curving at their distal ends, *i.e.,* those parts of these bones which experience the largest excursions during movements. In another specimen, an abnormal caudal fin skeleton was present. Ventral to the notochord, an amorphous mass of cartilage is observed, whereas in the normal situation, the separate, cartilaginous hypurals can clearly be distinguished. Possibly, the erratic contractions of the body musculature resulted in abnormal loading of the tail region, which may have influenced the differentiation of skeletal elements.

I.2.3.b - Induction of dwarfism

The effect of spatial constraints on developing larval fish was tested on *Clarias gariepinus*. This was done by raising one half of a batch of eggs in a small tank, whereas the other half was raised in a large tank. Once the eggs had hatched and the larvae became larger, the specimens of this first half were transported into separate, although small, perforated containers (**Fig. I.2- 2**). Both groups of larvae were fed equally (*ad libidum*), had the same water quality, equal water temperature and equal light regime. The only factor that differed was their surrounding space. Both groups were raised and fixed at 38 days posthatching. Those specimens were then used for serial sectioning and *in toto* clearing (**Table I.2- 11**).

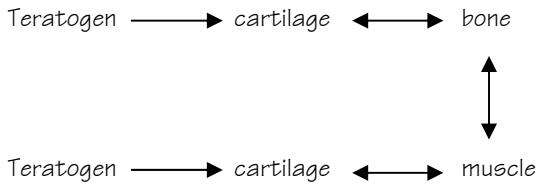
Table I.2- 11: Specimens used for dwarfism (for abbreviations see Table I.2- 5, p. 6)

Nr.	SL (mm)	TL (mm)	PAL (mm)	Procedure	Staining		SL (mm)	TL (mm)	PAL (mm)	Procedure	Staining
	NORMAL						DWARFS				
1	36.2	41.6	19.0	Clearing	ARS		20.8	24.8	10.0	Clearing	ARS
2	37.3	43.0	17.7	Clearing	ARS		23.3	28.5	12.3	Clearing	ARS+AB
3	40.8	47.3	18.5	Clearing	ARS		23.9	28.6	12.7	Clearing	ARS+AB
4	41.9	47.8	22.1	Clearing	ARS+AB		24.9	29.2	13.9	Clearing	ARS
5	44.1	50.8	22.7	Clearing	ARS+AB		25.1	30.1	11.8	Clearing	ARS
6	48.5	56.4	25.9	Sectioning	T		25.8	29.9	13.3	Clearing	ARS
7	50.0	58.1	28.2	Clearing	ARS+AB		27.5	32.5	12.3	Sectioning	T
Average						*Average*					
	43.8	49.3	22.0				24.5	29.1	12.3		

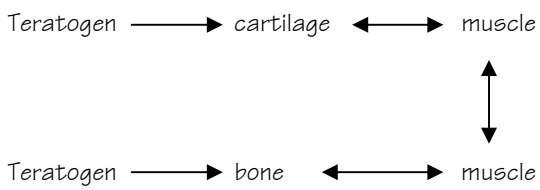
I.2.3.c - Evidence of epigenetic control of skull ontogeny

To conclude, the following overview can be given of the relation between the different teratogens tested and the interactions that are the main subject of this research:

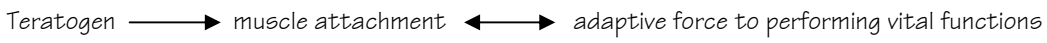
1 - *Diazo-oxo-nor-leucine* influences the formation of cartilage but does not affect muscle or bone. Consequently, deformations induced by them may provide information on the causal, spatial relation between developing cartilage and the surrounding muscular and osteological structures.



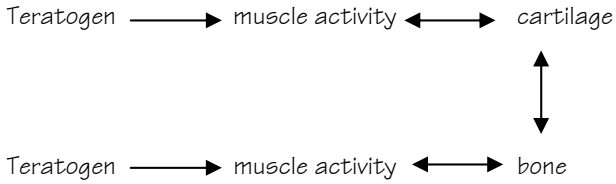
2 - *β-Amino-propio-nitril* influences the formation of cartilage and bone but does not affect muscle. Consequently, deformations induced by them may provide information on the causal, spatial and functional relation between developing cartilage/bone and the surrounding muscles. The effect of BAPN during the pre-ossification phase can also give information on the previous relation (as DON).



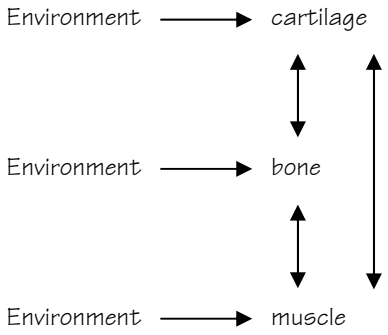
3 - *Retinoic acid* is involved in the gene expression of migrating neural crest cells, which give rise to most skeletal elements of the skull, especially the viscerocranium, and the attachment tissue for the muscles. Consequently, deformations induced by retinoic acid may provide information on the adaptive process of muscle development in response to altered insertion sites, in order to meet the functional demands in a developing larva that has to respirate, feed, …



4 - *Malathion* inhibits the activity of acetylcholinesterase, which disrupts the actions of normal muscular contractions in a developing larva (contraction force, contraction frequency, contraction speed, …). Consequently, deformations induced by malathion can provide some evidence of the influence of muscle contraction onto the developing skeletal elements.



5 - *Dwarfism* can give an idea of the spatial interactions between the environmental conditions and the ontogenetic processes. It can provide evidence of to what degree the ontogeny is epigenetically controlled by external factors, for example the amount of space surrounding the growing larva. It can give an indication of to what degree the ontogeny is a trade-off between tissue differentiation and functional demands.



As the study of all these interactions was too much within the time frame of this thesis, only the dwarfism was used for a preliminary further investigation (see Part III). It was, however, essential that the normal development of *Clarias gariepinus* was known in detail, before any assumptions concerning the abnormal development could be made. Consequently, most of the attention is paid to the different aspects of normal ontogeny in *C. gariepinus* (see Part II).

Chapter I.3 – Catfishes, a group of specialised Ostariophysi

I.3.1 - OSTARIOPHYSI

The Ostariophysi comprise a group of five orders, 59 families, 960 genera and about 6 507 species. They contain monotypic families, as well as one of the largest families of all, the Cyprinidae (± 2010 species). About 64% of all freshwater fishes can be grouped within the Ostariophysi, where they can be found world wide, with exception of Antarctica, Greenland and New Zealand (NELSON, 1994).

I.3.1.a - The taxon 'Ostariophysi'

REGAN (1911a, b) considered the Ostariophysi to be a group of species of very divergent forms and appearances, but which shared the common feature of the Weberian apparatus (**Fig. I.3- 1**). The author already mentioned that the Ostariophysi "are allied to generalized Clupeoids such as the Elopidae" (REGAN, 1911b). The Ostariophysi, according to REGAN (1911a, b) comprised the suborders Cyprinoidea and the Siluroidea (**Plate I.3-1A**). The Characiformes, belonging to the Cyprinoidea, were regarded as the least specialised group. Both groups show the typical malacopterygian features: a ventral pectoral fin, with the pelvic fins placed well behind them, and a ductus pneumaticus connecting the swimbladder to the gut. Other malacopterygian features, like the absence of fin spines and the presence of cycloid scales, have undergone secondary changes in some groups, especially catfishes (see below).

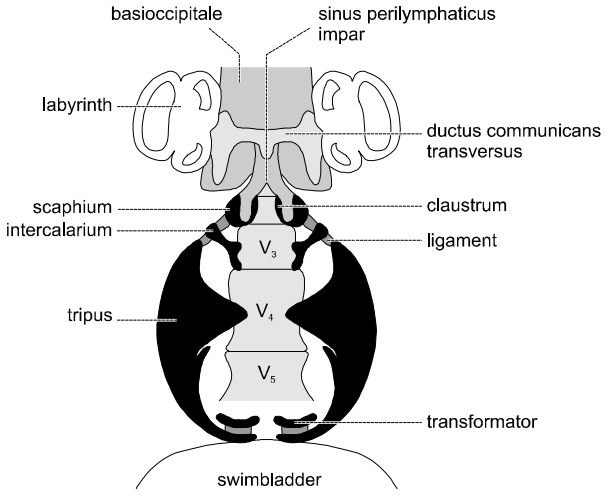


Fig. I.3- 1: Scheme of the Weberian apparatus [modified from STARCK (1979)]

In their paper on teleostean phylogeny, GREENWOOD *et al.* (1979) could not ascertain ostariophysan affinities with other major groups, although they already insinuated the possible common ancestry of their 'ostariophysans' and the Gonorhynchiformes. According to them, starting from the pholidophoroid lineage, one of three divisions gave rise to a salmonoid stock, from which evolved the Gonorhynchiformes. The latter were classified as an order of the protacanthopterygians (**Plate I.3-1B**). During that latter phase, the Ostariophysi might have split off. The clupeomorphs, however, showed no affinity with the ostariophysans, although they were not able to give a plausible position of that group. In correspondence with REGAN (1911a, b), the Ostariophysi comprised two groups, now raised at ordo level (Cypriniformes and Siluriformes) (**Plate I.3-1B**).

A close relationship between the group, until then referred to as the Ostariophysi, and the Gonorhynchiformes, was demonstrated by ROSEN & GREENWOOD (1970), based on evidence of the caudal

skeleton, the presence of a fright reaction mechanism, swimbladder morphology, presence of nuptial tubercles and a striking similarity in the mouth opening mechanism in *Phractolaemus ansorgii* (Gonorhynchiformes, Phractolaemidae) and *Bivibranchia* (Characiformes, Hemiodontidae). The fact, however, that in Gonorhynchiformes no "real sign of vertebral differentiation that would suggest a condition paralleling the development of Weberian ossicles", could be observed that convinced them of similar functionality, in a broad sense, as the Weberian apparatus, resulted in the subdivision of the Ostariophysi in the Anotophysi (Gonorhynchiformes) and the Otophysi (Ostariophysi *s.s.*) (ROSEN & GREENWOOD, 1970) (**Plate I.3-1C**).

Based on the comparison of adult morphology, GOSLINE (1971) grouped the Gonorhynchiformes (his Gonorhynchoidei) within the 'Clupeiformes', as a reflection of the affinities with the 'Elopoidei' and the 'Clupeoidei' (**Plate I.3-1D**). Caudal fin anatomy, however, again suggested a relation between the Gonorhynchiformes (his Gonorhynchoidei) and the Ostariophysi (his Cypriniformes), as well as with the Clupeiformes (his Clupeoidei), *i.e.,* the presence of a pleurostyl (see below). On the other hand, TAVERNE (1974b) suggested that the two features used by ROSEN & GREENWOOD (1970), claiming the Gonorhynchiformes as the primitive sister group of the Ostariophysi, were invalid (*i.e.,* a primitive state of the Weberian apparatus and the caudal skeleton in Gonorhynchiformes). It was again suggested that the Gonorhynchiformes were derived from the Protacanthopterygii, more specifically some primitive Pattersonellidae.

A first more detailed survey of the ostariophysan interrelationships was given by ROBERTS (1973). He stated that "evidence from caudal skeleton morphology supports relationships between Clupeomorpha and Ostariophysi, and between Clupeomorpha and Gonorhynchiformes, as well as between Gonorhynchiformes and Ostariophysi." He objected against the taxonomic value of the parallelism of the protrusion mechanism between *Phractolaemus* and *Bivibranchia*, because they do demonstrate both morphological and functional differences. Additionally, he opposed against the notion that characins and cyprinids would be more closely related to each other than to the catfishes, consequently raising the latter to the same level as the former two (**Plate I.3-1E**). His order of Cypriniformes than corresponds with the Otophysi of ROSEN & GREENWOOD (1970), containing the three suborders: (1) Characoidei, (2) Cyprinoidei and (3) Siluroidei.

A more extensive, detailed comparison of ostariophysan groups has lead to the classification of ostariophysans as used today. FINK & FINK (1981), in their first description of ostariophysan interrelationships, came to state five hypotheses, resulting in a new cladistic shift (**Plate I.3-1F**):

1. all five ostariophysan groups represent a monophyletic lineage: (1) Gonorhynchiformes, (2) Cypriniformes, (3) Characiformes, (4) Siluroidei and (5) Gymnotoidei;
2. the Siluroidei (catfishes) and Gymnotoidei (knife fishes) constitute a monophyletic group: the Siluriformes;
3. the Siluriformes are the sister group of the Characiformes, which comprise the Characiphysi;
4. the Characiphysi and Cypriniformes are sister groups and are referred to as the Otophysi;
5. the Gonorhynchiformes form the sister group of the Otophysi, constituting the Ostariophysi.

Recently, a revision of the characters described in that paper has been made (FINK & FINK, 1996), resulting in raising the Siluroidei and Gymnotoidei to the ordo level, both being grouped within the Siluriphysi (**Plate I.3-1G**). A survey of the classification, up to subfamilia level, is given in **Plate I.3-2**. The raising of the catfishes and knife fishes to the ordo level had also been proposed by GRANDE [1987: in TEUGELS (1996)].

As mentioned above, affinities have been found between ostariophysans and clupeomorphs. In general, ostariophysans are considered as "clupeocephalans but not neognaths" (LECOINTRE & NELSON, 1996). The same can be said about the Clupeomorpha. In one classification, the Euteleostei are considered as the sister group of the Clupeomorpha. It is regarded to comprise the Ostariophysi and the Neognathi, whereas the latter is subdivided into the Protacanthopterygii and the Neoteleostei (all other neognathans) (**Fig. I.3- 2**) (LECOINTRE & NELSON, 1996). In a more recent work, ARRATIA (1997) proposed a new classification of Teleostei, in which the affinities between Ostariophysi and Clupeomorpha are even more accentuated, as both are grouped in a subcohort Ostarioclupemorpha (**Plate I.3-3**). Common features involve early ossification of the palatine during ontogeny and the fusion of one hypural to the first ural centrum in the primitive condition. The Euteleostei distinguish themselves from these Ostarioclupemorpha by the facts that (1) the parhypural of the caudal skeleton is not fused with its centrum (= preural centrum 1), (2) the latter centrum lacks a neural spine and (3) a stegural is present (ARRATIA, 1997). Following this author, the hierarchical classification is then as follows:

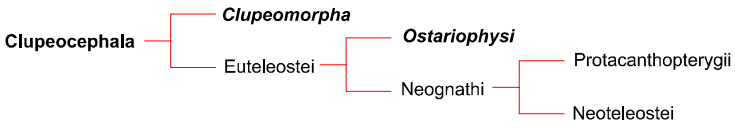


Fig. I.3- 2: Cladogram of the Clupeocephala, as proposed by ROSEN (1982) [in: LECOINTRE & NELSON (1996)]

Supercohort **Elopomorpha**
- *Ordo* **Elopiformes**
- *Ordo* **Albuliformes**
- *Ordo* **Anguilliformes**

Supercohort **Osteoglossocephala** new
- *Cohort* **Osteoglossomorpha**
  - *Ordo* **Osteoglossoidei**
- *Cohort* **Clupeocephala** new usage
  - *Subcohort* **Ostarioclupeomorpha** new
    - *Superordo* **Clupeomorpha**
      - *Ordo* **Ellimmichthyiformes** †
      - *Ordo* **Clupeiformes**
    - *Superordo* **Ostariophysi**
      - *Ordo* **Gonorhynchiformes**
      - *Ordo* **Cypriniformes**
      - *Ordo* **Characiformes**
      - *Ordo* **Siluriformes**
      - *Ordo* **Gymnotiformes**
  - *Subcohort* **Euteleostei** new usage

I.3.1.b - Ostariophysan interrelationships

This is a topic which has already been discussed to a great extent. At least three papers can be mentioned, entitled "Interrelationships of ostariophysans" (ROBERTS, 1973; FINK & FINK, 1981; 1996). Consequently, it is redundant to discuss all the characters which are recognised as ostariophysan synapomorphies. I therefore refer to REGAN (1911a, b), GREENWOOD *et al.* (1979), ROBERTS (1973), FINK & FINK (1981; 1996) and GAYET (1986; 1993). However, I will only refer to some more general features, frequently characterised as typical ostariophysan: (1) the Weberian apparatus, (2) the pleurostylar caudal skeleton, (3) the fright reaction and (4) the nuptial tubercles.

As mentioned above, Ostariophysi (etymology: *osteon* = bone, and *fysis* = bladder) posses a specialised set of anterior vertebrae, which in combination with the swim bladder have formed a sensory organ for the perception of sound or differences in pressure, *i.e.,* the Weberian apparatus (**Fig. I.3- 1**). In the Anotophysi, the differentiation is much less than in the Otophysi, where a true chain of ossicles interconnects the swimbladder with the labyrinth organ (GAYET & CHARDON, 1987). Much debate has been going on, in order to reveal the homology of these ossicles. Based on studies of BAMFORD (1948), RADERMAKER *et al.* (1989), FINK & FINK (1981; 1996), VANDEWALLE *et al.* (1990) and COBURN & FUTEY (1996), the following homologies can be proposed: (1) the *claustrum* represents a paired supraneural 1, (2) the *scaphium* represents the basidorsale 1, (3) the *intercalarium* represents the basidorsale II, (4) the *tripus* is a fusion of the rib and the parapophysis of the third vertebra, whereas (5) the same elements of the fourth vertebra form the *suspensorium*. The *transformator* represents a rostral process of the tripus. The scaphium brings about the connection between the apparatus and the labyrinth (at the level of the atria sinus imparis of the sinus perilymphaticus impar), whereas the connection with the swimbladder (at the level of the camera aerea weberiana) occurs through the transformator (CHARDON, 1967a). In relation to this Weberian apparatus connection, the swimbladder is subdivided, in generalised ostariophysans, in an anterior part (this camera aerea weberiana) and a posterior part (the functional buoyancy organ). This specialisation, for the perception of vibrations, is believed to be one of the major adaptive characters at the base of the their wide ecological and evolutionary diversity (GOSLINE, 1971; ROBERTS, 1975).

Another feature, frequently mentioned as typical ostariophysan involves the morphology of the caudal skeleton. Ostariophysans, together with Clupeomorpha, have a so-called pleurostylar type of caudal skeleton, instead of a so-called stegural type of most higher teleosts (GOSLINE, 1971). The major difference involves what part of the ural vertebrae takes part in the formation of the supportive structure for most of the hypurals. In the hypothesised ancestral situation [largely corresponding to the situation in *Amia calva* (Amiidae) (JARVIK, 1980)], a polyural skeleton consists of six separated urocentra (U_{1-6}) (= vertebral centra), each bearing dorsal uroneurals (UN_{1-6}) (neural arches) and ventral hypurals (HU_{1-5}) (= haemal arches) (**Fig. I.3- 3A**). In the course of evolution (or ontogeny), a fusion between urocentra and a reduction of urocentra has occurred (or occurs). Consequently, a diural caudal skeleton is formed in Teleostei (or juvenile Teleostei) (DE PINNA, 1996). In the stegural fin type, the first urocentrum actually represents the fused U_1 and U_2, whereas the others, if not reduced, fuse to a single urostyl (**Fig. I.3- 3B**). The latter thus supports HU_{3-6} (ARRATIA, 1997). The first urocentrum then supports HU_{1-2}. In the pleurostylar type, however, a fusion occurs between PU_1 (= preural centrum 1), U_1 and U_2, whereas the other urocentra have disappeared (**Fig. I.3- 3C**). Consequently, this 'urocentrum' supports the parhypurale (= haemal arch of PU_1) and HU_1 and HU_2. The remaining hypurals, however, are now supported by a central rod formed by the fused uroneurals (UN_{1-2}), instead of urocentra. In some ostariophysans, additional, separate uroneurals can

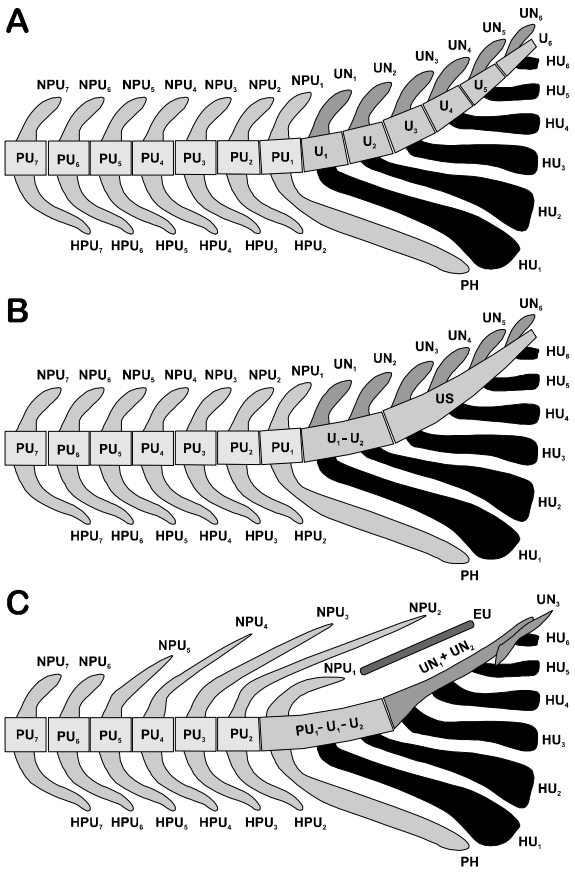


Fig. I.3- 3: Scheme of the caudal skeleton: A. hypothesised ancestral situation, B. stegural type, C. pleurostylar type (*Legends:* EU = epurale, HPU = haemal arch of preural vertebrae, HU = hypurale, NPU = neural arch of preural vertebra, PH = parhypurale, PU = preural centrum, U = urocentrum, UN = uroneurale, US = urostyl)

be observed (EASTMAN, 1980; FINK & FINK, 1981; ARRATIA, 1983). The thus formed gap between the pleurostyl and the neural arches of the preural centrum is partially filled with so-called epurals.

Another ostariophysan feature is the presence of a certain alarm substance or 'Schreckstoff' (GOSLINE, 1971; NELSON, 1994). This substance is a kind of pheromone, which induces a wide range of fright reactions in ostariophysans, when released in the water (PFEIFFER, 1977). The reaction may be as well intraspecifically as interspecifically. This fright reaction mechanism involves two crucial components: (1) the production of 'Schreckstoff' in alarm substance cells and (2) the behavioural response to the detection of such a substance. PFEIFFER (1977) gave a complete survey of both factors within most fishes, indicating that the presence of one of these components was strictly confined to ostariophysans (with some exceptions in Cyprinodontidae and Poeciliidae, as well as Percidae) (PFEIFFER, 1977; WALDMAN, 1982). Within the Ostariophysi, three groups could be distinguished: (1) those that possessed both the alarm substance cells and exhibited a fright reaction, (2) those that did posses the necessary cells, both showed no response to exposure, and (3) those that lacked both. WALDMAN (1982) demonstrated that both factors are not correlated chronologically during ontogeny, but that the fright reaction was observed to occur later than the production of alarm substance.

The presence of 'Schreckstoff' in both the Gonorhynchiformes and other ostariophysans, supports the hypothesis of sister group relation. It has even been demonstrated that their chemical nature may be very similar or even equal (PFEIFFER, 1977). Gonorhynchiformes have shown to react when exposed to alarm substance of otophysans and the other way around.

The presence of nuptial tubercles has been observed in some Characiformes (Parodontinae, Lebiasinidae and Characidae), in most Cypriniformes and some Siluriformes (Mochokidae, Sisoridae and Astroblepidae) (ROBERTS, 1973). They are absent in the Gymnotiformes (FINK & FINK, 1981). The tubercles may range from simple aggregations of non-keratinised epidermal cells to large structures, consisting of several layers of fully keratinised cells. In *Phoxinus* (Cyprinidae), up to nine different morphotypes of nuptial tubercles could be recognised (CHEN & ARRATIA, 1996). The so-called unculi refer to those types of tubercles which bear unicellular, horny projections. The distribution of tubercles is frequently found on top of the skull, but may occur at the ventro-lateral side or ventral side of the prepectoral part of the skull, as well as above the scales (CHEN & ARRATIA, 1996).

I.3.1.c - Ostariophysan zoogeography

Several hypotheses have been proposed concerning the evolutionary distribution pattern of ostariophysan fishes. As for today, not all groups of ostariophysans show an identical distribution (**Table I.3- 1**, **Fig. I.3- 4**). The Siluriformes show a cosmopolitan distribution, whereas the Gymnotiformes are confined to South America (NELSON, 1994). The majority of the ostariophysans can be categorised as primary freshwater fishes. Secondary fresh water fishes can be found in Cypriniformes (Cyprinidae), Characiformes and Siluriformes (*e.g.,* Clariidae, Siluridae, Claroteidae, Pangasiidae, Loricariidae), whereas peripheral species have been observed in Gonorhynchiformes (Chanidae, Gonorhynchidae) and Siluriformes (Aspredinidae, Ariidae and Plotosidae) only (ROBERTS, 1975; NELSON, 1994; TEUGELS, 1996).

Table I.3- 1: Distribution of ostariophysan groups [* in the Cypriniformes, only one genus (*Tribolodon*) (NOVACEK & MARSHALL, 1976), ** in Characiformes, three genera (CHARDON, 1967b), * some characiforms can be found in Southwest Texas (NELSON, 1994)]**

	Europe	*Asia*	*Africa*	*South America*	*Central America*	*North America*	*Marine*
Gonorhynchiformes							
Cypriniformes							*
Characiformes						***	**
Siluriformes							
Gymnotiformes							

The basal question, from which has to be started to reconstruct ostariophysan evolutionary dispersal, concerns the origin of the ostariophysans. It was generally agreed that they do not have an early Gondwanaland origin, as they are absent in Madagascar and the freshwaters of Australia (BRIGGS, 1979). According to DARLINGTON (1957), early ostariophysans emerged in Asia during the Mesozoicum, giving rise to the ancestral characiforms and siluriforms (**Plate I.3-4A**). Although later on, a South American origin was proposed (CHARDON, 1967b; NOVACEK & MARSHALL, 1976), the Asian origin was again defended by BRIGGS (1979) (**Plate I.3-4**). In that scenario, gonorhynchiform ancestors gave rise to an ancestral characiform group, from which the ancestral, and thus Asian, cypriniforms and siluriforms were derived (**Plate I.3-4D**). Subsequent dispersal occurred to Europe, through which they entered Africa, and through the Behring street, up to North America. A possible route of Siluriformes to North America through Europe and Greenland, during the Upper Jurassic, has been proposed as well (BRIGGS, 1979). Two hypotheses have been proposed to explain the absence of the Characiformes in North America: (1) they were outcompeted by the Cypriniformes, first in Asia and then in Europe, before they could disperse, or (2) the Behring street formed a temperature barrier for their direct dispersal (BRIGGS, 1979). The absence of the Cypriniformes in South America would then be explained by the fact that by the time they had reached Africa, the continent was already separated completely from South America (around the Middle Cretaceous, 90 million years ago).

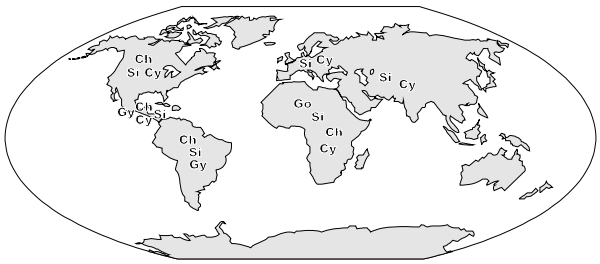


Fig. I.3- 4: Recent distribution of the ostariophysan orders (Ch = Characiformes, Cy = Cypriniformes, Go = Gonorhynchiformes, Gy = Gymnotiformes, Si = Siluriformes) (The marine distribution of some Siluriformes, is not shown here)

In this hypothesis of BRIGGS (1979) the Characiformes are believed to be the primitive otophysan group, from which Cypriniformes and Siluriformes have been derived. This is, however, in contrast with recent studies, indicating that the Cypriniformes are supposed to be the sister group of all other otophysans (FINK & FINK, 1981; 1996). It seems improbable that the hypothesis of BRIGGS (1979) could consequently be retained by just shifting the sequence, thus: ancestral gonorhynchiforms giving rise to a cypriniform group, which differentiated into a characiform and siluriform group. It would seem illogic that at first the characiforms would become derived from the cypriniforms, but would subsequently become outcompeted by the same group. In contrast to the opinions of CHARDON (1967b) and NOVACEK & MARSHALL (1976), that the Gymnotiformes are derived from the Characiformes, the theory of BRIGGS (1979) could support the origin of the Gymnotiformes from an ancestral, South American siluriform group. Without giving a proper scenario of dispersal, FINK & FINK (1981) suggested a Gondwana origin of the Ostariophysi, with the centre of dispersal in South America. As the amount of data on ostariophysan interrelationships, fossils and distribution is increasingly becoming available, a more valid hypothesis of ostariophysan zoogeography may be forthcoming.

I.3.2 - SILURIFORMES

Presently, 34 different families are recognised within the group of catfishes or Siluriformes, of which two are known from fossils only[1] (DE PINNA & VARI, 1995; ARRATIA & GAYET, 1995; TEUGELS, 1996) (**Plate I.3-2b**). They comprise a total of 416 genera and 2 584 species (TEUGELS, 1996). I will not go into detail for each of these families, as a splendid survey is given by BURGESS (1989) and TEUGELS (1996). I will only give some background information, indicating the wide morphological diversity and specialisations that have formed in catfishes. Some details of the cranial morphology of certain catfish families, are discussed in the following chapters.

I.3.2.a - Some general specialisations in catfishes

A survey of morphological specialisation in some catfishes has been given by ALEXANDER (1965). In many cases, catfishes are adapted to a benthic and nocturnal life style. Many morphological specialisations can consequently be related to adaptations to such a life style, as will frequently be demonstrated in this thesis. In general, catfishes possess broad, dorso-ventrally flattened skulls with small eyes, coupled to a reorganisation of cranial structures (see IV.2). Small eyes require only short eye muscles, which consequently makes the space to house them, *i.e.,* the myodomes, superfluous. The reduction in optical sensorial input is compensated by input through oral barbels and the Weberian apparatus.

In catfishes, the Weberian apparatus is more specialised then in other ostariophysans. The swim bladder only consists of one compartment anymore, *i.e.,* the camera aerea weberiana, which has become almost completely enclosed by the extended parapophyses of the fourth and fifth vertebrae (CHARDON, 1967a). The swim bladder remains uncovered laterally, where it comes to lie close to the surface of the body, in an area with little body musculature. Consequently, this region of the body wall functions as a kind of tympanum, transporting vibrations through the swim bladder and the Weberian apparatus to the inner ear. The reduction of the swim bladder, however, implicates the loss of buoyancy. The benthic behaviour may then be considered as one of the consequences of the specialisation of the Weberian apparatus, or the reduction of the swim bladder may

[1] ARRATIA & GAYET (1995) also described the fossil genus *Hoffstetterichthys* and *Incaichthys* as not yet assignable to a family.

be seen as a logic consequence of a benthic behaviour, thereby enabling the specialisation of the Weberian apparatus. Another specialisation in the catfish Weberian apparatus involves the increased fusion between vertebral centra, resulting in the formation of the vertebral complex.

The loss of the buoyancy function of the swimbladder has provided another functional shift in some catfishes. At least two different sound producing mechanisms, involving the swim bladder, have been found (in Pimelodidae, Ariidae, Doradidae, Mochokidae, Malapteruridae and Pangasiidae)[2]. A more simple method for sound production involves the pectoral spines. Most catfishes posses pectoral spines, which can be locked in the pectoral girdle, as a defence mechanism (see below). The base of the spine is frequently thickened, bearing several ridges of different sizes (TILAK, 1963d; GOEL, 1966; KAATZ, 1997). The movements, based on the muscular control for pectoral spine locking, enable the production of sound by stridulation of the spine base in the pectoral socket. In catfishes, this stridulation is believed to have evolved as a result of predation pressure (disturbance stridulation), to intraspecific communication in, for example, Callichthyidae (courtship stridulation) (KAATZ, 1997). The effectiveness of sound production, as an intraspecific communication, can easily be related to the presence of a highly specialised sound perception apparatus: the Weberian apparatus. In Sisoridae, stridulation may even occur with the dorsal spine (ALEXANDER, 1965).

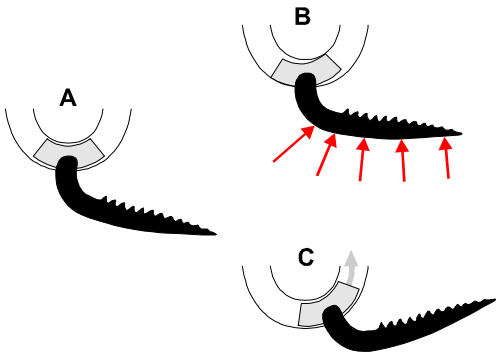


Fig. I.3- 5: Pectoral spine locking mechanism: A. unlocked position, B. locking, C. retraction [After ALEXANDER (1975a)]

Defence mechanisms in catfishes show some diversity as well. As mentioned, pectoral spines, as well as dorsal fin spines, can be erected and locked, making it extremely difficult for predators to manipulate and swallow them. The locking mechanisms are based on a friction lock, as explained by ALEXANDER (1965) (**Fig. I.3- 5**). In the dorsal spine locking mechanism, a reduced anterior fin ray enables the locking of the spine, which corresponds to the enlarged second fin ray (**Fig. I.3- 6**). In order to withstand large forces of a biting predator, not only does the mechanism have to hold, also the strength of the pectoral spine, the pectoral girdle and the attachment of the girdle to the skull are determining factors. All of them show adaptations to increased strength (TILAK, 1963d; SCHAEFER, 1984). In some lineages, the effectiveness of the spines as an anti-predator mechanism has been improved: (1) large scutes, covering the body, enhance the fortification of the dorsal and pectoral fin spines (as comparable to the situation in the stickle back), or (2) the integumentary sheath surrounding the spine produces toxic

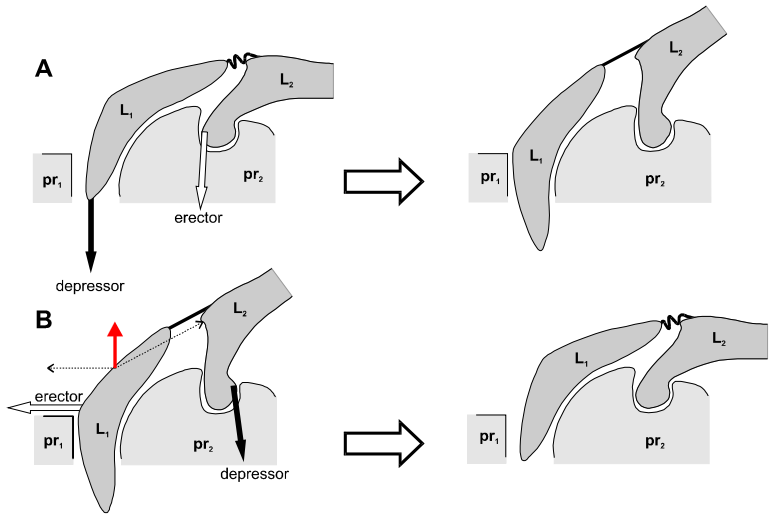


Fig. I.3- 6: Dorsal fin spine locking mechanism through dorsal fin erector and depressor muscles: A. spine erection, B. spine adduction (Legends: $L_{1\text{-}2}$ = dorsal fin lepidotrichs 1 and 2, $pr_{1\text{-}2}$ = proximal radials 1 and 2) [After ALEXANDER (1965)]

[2] Such sound producing mechanisms have also been found in Serrasalmidae (ALEXANDER, 1965).

substances (*e.g.,* in Ictaluridae, Ariidae, Heteropneustidae, Plotosidae) (BIRKHEAD, 1972; BURGESS, 1989).

Although the reduced eyes and the fact that catfishes are frequently nocturnal, prey detection is improved by specialised sensory apparatuses. As will be discussed in Chapter II.2.2, the oral barbels play an important role in prey detection, as well as obstacle detection. A specialised palatine-maxillary mechanism has enabled the application of the maxillary oral barbels as active probes. Electroreception has been considered as one of the features suggesting Siluriphysi monophyly (FINK & FINK, 1996). Gymnotiformes are characterised by the potential of creating weak electric fields for prey detection and communication, or even prey inactivation and predator repulsion (up to 600 V in *Electrophorus electricus*, Electrophoridae) (ALVES-GOMES *et al.*, 1995). In Gymnotiformes, for the reception, specialised tuberous electroreceptors can be distinguished, whereas Siluriformes have ampullary organs (HOPKINS, 1983; ARRATIA & HUAQUIN, 1995). Ampullary organs have been found in Clariidae, Heteropneustidae, Bagridae, Ictaluridae, Plotosidae and Pimelodidae (ARRATIA & HUAQUIN, 1995). The presence of an organ, generating electric pulses for predation and predator avoidance, has been observed in one genus of catfishes, *i.e.,* in *Malapterurus* (Malapteruridae). Here, the electric organ is believed to have a myoblastic origin, corresponding to the superficial part of the obliquus inferioris muscle of the hypaxials (HOWES, 1985).

Another adaptive feature, which facilitated the great dispersal and diversity in catfishes, involves the potential to perform aerial respiration (ROBERTS, 1975). Air breathing has been observed in other ostariophysans as well, but catfishes are the only ostariophysans which developed specialised accessory breathing organs. Several existing structures have become involved in air breathing (*e.g.,* the stomach in Loricariidae and Trichomycteridae, the intestine of Callichthyidae, the swimbladder of Pangasiidae) (BROWMAN & KRAMER, 1985), as well as *de novo* differentiations of existing structures have occurred (*e.g.,* paired diverticula of the branchial chamber in Heteropneustidae, transformations of branchial structures to an arborescent breathing organ in Clariidae) (OLSON *et al.*, 1990).

I.3.2.b - Affinities between catfishes

Although the subject of the present dissertation does not involve taxonomic nor phylogenetic approaches, it may be of interest to give a short overview of what is known at the moment concerning the phylogenetic relationships of catfishes. An overview of this has recently been published by TEUGELS (1996), from which it can be derived that a lot has been done, but still a lot has to be done. The monophyly of some families has been suggested (Diplomystidae, Siluridae, Austroglanididae, Ictaluridae, Ariidae, Mochokidae, Auchenipteridae, Akysidae, Cetopsidae, Hypophthalmidae, Trichomycteridae, Astroblepidae, Loricariidae and Scoloplacidae) (ARRATIA, 1987; HOWES, 1983a; SCHAEFER, 1987; 1990; BORNBUSCH, 1991b; DE PINNA & VARI, 1995; TEUGELS, 1996), whereas others are defined as paraphyletic (Pimelodidae, Schilbeidae and Callichthyidae) (HOWES, 1985; STRAUSS, 1985; TEUGELS, 1996) (**Plate I.3-5**).

It is generally accepted that the Diplomystidae represent the most plesiomorphic condition (**Plate I.3-5aA**). They are even considered as the sister group of all other catfishes (TEUGELS, 1996), and are believed to have even more plesiomorphic features than the Hypsidoridae, a fossil catfish family. ARRATIA (1987) gave a detailed description of the morphology of that family. Several features could be distinguished which were shared with primitive teleosts (toothed, non-reduced maxillary bone and the lapillus smaller or equally sized than the asteriscus), and which were plesiomorphic catfish features (caudal fin ray number 9/9 and presence of maxillary barbels only). Some features were shared with other generalised catfishes (*e.g.,* body scaleless, pectoral and

dorsal fin spines present, adipose fin present, fifth vertebra fused to the vertebral complex of the Weberian apparatus), whereas others were autapomorfies (*e.g.,* skin completely covered with papillae, hyomandibular articulation with the pteroticum, prooticum, sphenoticum and pterosphenoid, more than one row of maxillary teeth, double-headed palatine, …) (ARRATIA, 1987; ARRATIA & HUAQUIN, 1995).

Based on the morphology of the Weberian apparatus, CHARDON (1968) grouped the siluriforms into seven suborders: (1) Diplomystoidei, (2) Siluroidei, (3) Malapteruroidei, (4) Bagroidei, (5) Cetopsoidei, (6) Hypophthalmoidei, and (7) Loricaroidei. Recently, relationships between several families have been demonstrated, which do not correspond to the classification of CHARDON (1968) anymore: Siluridae and Schilbeidae are believed to be more closely related to the Malapteruridae than to other families (HOWES, 1985), and the former Helogenidae are now considered to be a subfamily of the Cetopsidae, thus being the sister group of the former Cetopsidae (DE PINNA & VARI, 1995). The lumping of genera in families of older literature has indicated some affinities between different families, supported by other evidence. Claroteidae, Bagridae and Austroglanididae are closely related, as previously, their genera comprised a single family, the Bagridae (MO, 1991). The latter author also presented a phylogenetic relationship of catfishes, which, however, was heavily criticised (DE PINNA & FERRARIS, 1992). Genera of Auchenipteridae and Doradidae, both families now considered as sister groups, were ranked into the Doradidae by REGAN (1991a) (TEUGELS, 1996). *Helicophagus*, previously ranked under the Schilbeidae (REGAN, 1911a) and now under Pangasiidae (ROBERTS & VIDTHAYANON, 1991), supports the affinity, as suggested by MO (1991) and TEUGELS (1996). *Heteropneustes*, previously known as *Saccobranchus*, was a genus of the Clariidae (REGAN, 1911a; DAVID, 1935) but is now considered to form the monotypic family, Heteropneustidae, which is closely related to the Clariidae (TILAK, 1963d). Characters, shared in common between families, have been demonstrated between the Cranoglanididae and the Bagridae plus the Pangasiidae (TEUGELS, 1996), and between the Amblycipitidae and the Schilbeidae plus the Bagridae (TILAK, 1967). The Chacidae share features with the Siluridae, Plotosidae, Bagridae, Pimelodidae and Aspredinidae, but because of lack of more evidence, a true relationship is not known (TILAK, 1971; BROWN & FERRARIS, 1988). Convergent features between allopatric families are present as well. The external morphology of the Old World, armoured Amphillidae, like *Belonoglanis* and *Trachyglanis*, closely resembles that of Neotropical Loricariidae, like *Loricaria* and *Rhineloricaria* (**Fig. I.3- 7**).

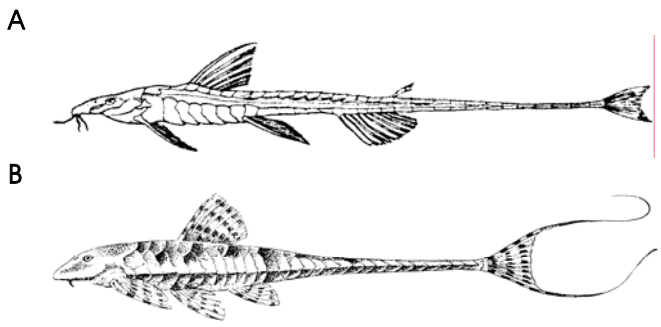


Fig. I.3- 7: Convergence between Old World catfishes and Neotropical catfishes: A. *Belonoglanis tenuis* (Amphiliidae), B. *Rhineloricaria lanceolata* (Loricariidae) [From BURGESS (1989) and STERBA (1990)]

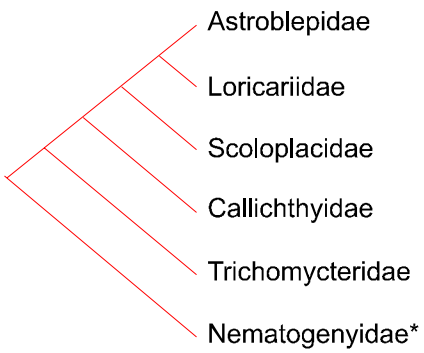


Fig. I.3- 8: Phylogeny of the Loricaroidea [According to SCHAEFER (1990)] (*Nematogenyidae are frequently considered as a subfamily of the Trichomycteridae)

The most specialised catfishes comprise what is grouped under the Loricaroidea (SCHAEFER, 1987). From that group, a congruent phylogeny has already been proposed (**Fig. I.3- 8**). The Neotropical family Loricariidae is an extremely divers group, showing adaptations of stream-living, algae

grazers. The mouth parts have been modified to a functional suction apparatus, for obtaining a firm grip against substrate, whereas lower jaw and upper jaw bear specialised teeth for algae scraping (MULLER & WEBER, 1992). As was already observed for other algae grazing fishes, especially Cypriniformes, a striking convergence of the teeth of algae grazing Loricariidae exists with other, invertebrate, algae grazers (ARENS, 1994). This type of feeding has implicated that other mechanisms have formed, enabling respiration while remaining attached to substrate (HOWES, 1983b; VANDEWALLE *et al.*, 1986). A ventrally positioned sucker-like mouth has been observed in other catfishes as well (*e.g.,* Ictaluridae, Sisoridae and Mochokidae) (LUNDBERG, 1982).

I.3.3 - CLARIIDAE

The Clariidae comprise a group of specialised catfishes (TILAK, 1963d). They inhabit freshwater rivers and lakes of Africa, Asia, Syria and the Philippines, although they have been observed to enter brackish water as well (ROBERTS, 1975; BURGESS, 1989). The family comprises 12 African genera with 74 species, and two endemic Asian genera with six species, as well as about 12 Asian *Clarias* species (SKELTON & TEUGELS, 1991; TEUGELS, 1996). The Clariidae are believed to have an Asian origin, from where they dispersed over the African continent (TEUGELS, *pers. comm.*). The oldest remains of clariids comes from the Pliocene of the Siwalik hills of India, followed by more recent fossils of Egypt (DAVID, 1935).

Generalised clariids can be recognised by the heavy, dorso-ventrally flattened head, bearing four pairs of oral barbels. The eyes are very small. The upper and lower jaws bear patches of small, villiform teeth. The posterior part of the branchial cavity is housed by the suprabranchial organ, *i.e.,* the arborescent, air breathing organ (**Fig. I.3- 9**). Their ability to perform aerial respiration enables them to migrate through deoxygenated swamps and pools, as well as migration over land. This lies at the base of the vast distribution of certain species, as for example the African species *Clarias gariepinus*, which has almost a Pan-African distribution (TEUGELS, 1986). Terrestrial migration is facilitated by the specialised locomotor pattern, in which pectoral spines and undulatory body movements are used. Consequently, *Clarias* species are sometimes called "walking catfish" (LONG & HUANG, 1995). In the generalised species, the body is elongated, bearing a long dorsal and anal fin. Both pectoral and pelvic fins are present and well developed. The supposedly more primitive genus *Heterobranchus*, has an adipose fin, of equal size as the dorsal one (**Plate I.3-6A**). In *Dinotopterus*, the adipose fin is much smaller (**Plate I.3-6B**). Reduced adipose fins can also be observed in some *Clarias* species [*i.e.,* the subgenus *Clarias (Dinotopteroides)*] (TEUGELS, 1986). In all these genera, these adipose fins are supported by elongated neural spines (TEUGELS, 1983). The general morphology of the skull involves a highly ossified configuration, bordered laterally by enlarged, plate-like bones. The suprabranchial organ is well developed.

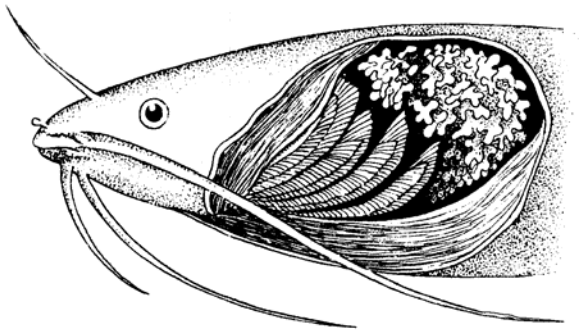


Fig. I.3- 9: Suprabranchial organ in Clariidae [From STERBA (1990)]

Within the Clariidae, an evolutionary trend has persisted in body form and fin morphology. As was demonstrated by PELLEGRIN (1927), clariids have become more and more anguilliform, coupled to a reduction of the adipose fin and paired fins, as well as the elongation of the dorsal and anal fins. In *Heterobranchus*, the

body is elongated but hardly anguilliform, and the short dorsal and anal fin are clearly separated from the caudal fin (TEUGELS *et al.*, 1990) (**Plate I.3-6A**). Roughly speaking, the evolutionary trend then persists through *Dinotopterus* to *Clarias (Dinotopteroides)* in the reduction of the adipose fin and the consequent elongation of the dorsal fin (**Plate I.3-6B**). In other *Clarias* species, the adipose fin is completely lost, its space being taken by the dorsal fin (TEUGELS, 1986) (**Plate I.3-6C**). In some *Clariallabes*, the body becomes more elongated, which is coupled to the elongations of median fins (**Plate I.3-6F**). In *C. platyprosopos*, the median fins are still separated from the caudal fin, whereas in *C. petricola*, they have already fused (GREENWOOD, 1956; JUBB, 1964; SKELTON & TEUGELS, 1991). Elongation of the body continuous through *Platyclarias, Gymnallabes*, reaching its extreme in *Channallabes* and *Dolichallabes* (**Plate I.3-6**) (BOULENGER, 1907; POLL, 1942; 1957; 1977; GREENWOOD, 1956; 1961). In the latter two monotypic genera, the median fins have become completely confluent with the caudal fin. The caudal fin itself becomes smaller, whereas its skeletal elements (*i.e.,* the hypurals) have progressively fused to each other (POLL, 1977). Pelvic fins are absent occasionally in *Clariallabes*, but persistently in *Channallabes* and *Dolichallabes* (**Plate I.3-6J-K**). In the extreme anguilliform *Channallabes*, even the pectoral fins may have disappeared (POLL, 1942) (**Plate I.3-6K**). An almost similar sequence can be found in the regression of the suprabranchial organ, although exceptions occur (BOULENGER, 1907; POLL, 1942a; 1957; 1977; GREENWOOD, 1956; 1961; JUBB, 1964).

The evolutionary trend within clariids, however, does not seem to be restricted to the elongation of the body and the reduction of the paired fins (and suprabranchial organ) only. The early descriptions of the anguilliform clariids showed a gradual reduction of cranial ossifications. As mentioned above, genera like *Heterobranchus* and *Clarias* possess a heavily ossified skull, bordered with large canal bones (see II.1.2). In *Clarias dussumerilii* and *Dinotopterus*, a gap between these canal bones reveals the first signs of reduced ossifications (DAVID, 1935). The trend becomes more pronounced in *Clariallabes*, where the canal bones become strongly reduced. In *Channallabes*, the canal bones are vestigial and separated by a large gap (DAVID, 1935). Coupled to this gap formation, the skull becomes increasingly smaller, especially in the postorbital and temporal region.

However, there seems to be no complete uniform correlation between these trends: (1) the gap between the canal bones in *Dinotopterus* is more pronounced than in most *Clarias* species, although the latter have no adipose fin anymore, and (2) the skull of *Channallabes* is broader than in *Gymnallabes*, although canal bones are more reduced in the former (CABUY, 1997). This led to the idea that this series in clariids is not an orthogenic series and the anguilliform clariids may represent a paraphyletic group.

Several species of the Clariidae have proven to be of great economic value, because of their high tolerance and rapid growth. Aquacultural exploitation of clariids is prominent in both Asia and Africa. Asian species involve *Clarias macrocephalus* (ALI, 1990) and *Clarias batrachus* (AREERAT, 1987; VIVEEN *et al.*, 1990). African species involve *Heterobranchus longifilis* (FAGBENRO *et al.*, 1979; LEGENDRE *et al.*, 1992; OTÉMÉ & SYLVAIN, 1995; NGUENGA *et al.*, 1996), *Clarias agboyiensis* (FAGBENRO & SYDENHAM, 1988), *C. anguillaris* (TEUGELS, 1984) and especially *Clarias gariepinus* (BABIKER, 1984; BOK & JONGBLOED, 1984; TEUGELS, 1984; CERONIO *et al.*, 1995; DE GRAAF *et al.*, 1995). The aquacultural potentials of these species has already resulted in allopatric exploitation and consequent colonisation of water systems (*e.g.,* The Netherlands, USA) (LOFTUS, 1979). The nutritional value of clariids has been appreciated since prehistoric ages. Pectoral spines of clariids have been found abundantly in archaeological sites of at least six to seven centuries before present (VAN NEER, 1993).

I.3.4 - *CLARIAS GARIEPINUS* Burchell (1822)

Clarias gariepinus (**Plate I.3-5bB**) is one of the most widespread and well known species of the African freshwater fishes, especially as a result of its economic value for aquaculture (see above). The original description of the species was done by BURCHELL (1822), which obtained the holotype from the "Ky-Gariep" river (SKELTON & TEUGELS, 1992). He described the holotype as *Silurus (Heterobranchus) gariepinus* and presumably, afterwards, ate it. The etymology of this species refers to the presence of the suprabranchial organ (*hetero*, different, and *branchia*, gill), as well as the place where it was caught: the "Gariep" river (hottentot name for the Orange River in South Africa) (TEUGELS, 1986). One of the earliest descriptions of an African clariid was already given in 1655, whereas LINNAEUS (1758) introduced the first binominal term: *Silurus anguillaris*. It was only in 1986 that the species, being a synonym of *Clarias anguillaris*, was redesignated as type species of the genus *Clarias* SCOPOLI, 1777 (TEUGELS & ROBERTS, 1987). Consequently, the (lost) holotype of BURCHELL (1822) was recognised as *Clarias gariepinus*. The extensive synonymy of *Clarias gariepinus* has been revised by TEUGELS (1982). These synonyms reflected different geographical populations of this species: (1) *C. lazera* of the Nilo-Sudan and Upper and Lower Guinea ichthyological provinces, (2) *C. mossambicus* of the Nilo-Sudan (East part), the Zaire, the Abyssinian highlands, the Nogal and the East Coast provinces, (3) *C. gariepinus* of the Zaire, the Quanza, the Zambesi and the north part of the Cape provinces, and (4) *C. capensis* from the southern part of the Cape province[3] (DAVID, 1935; ROBERTS, 1975). A neotype description of *Clarias gariepinus* was given by SKELTON & TEUGELS (1992).

Within the African species of the genus *Clarias*, six different subgenera are currently recognised: (1) *Clarias (Dinotopteroides)*, (2) *Clarias (Clarias)*, (3) *Clarias (Platycephaloides)*, (4) *Clarias (Brevicephaloides)*, (5) *Clarias (Clarioides)*, and (6) *Clarias (Anguilloclarias)* (TEUGELS, 1982). The species *C. gariepinus* belongs to the *Clarias* subgenus, together with the closely related and closely resembling species *C. anguillaris* (BENECH *et al.*, 1993). *Clarias gariepinus* is characterised by the number of gill rakers on the first branchial arch (24-110), which is the highest number within the African *Clarias* species (TEUGELS, 1986). The overall morphology reflects the generalised position, as described above. Four pair of oral barbels are present: (1) mandibular, (2) nasal, (3) internal mandibular, and (4) external mandibular ones. The suprabranchial organ is well developed (GREENWOOD, 1961). The coloration may range from uniformly greyish-greenish black to a marbled pattern, dorsal to the pectoral fins, whereas the belly is generally whitish. For additional biometrical data on this species, I refer to TEUGELS (1982; 1986) and SKELTON & TEUGELS (1992).

[3] The type locality of the former *Clarias capensis* is believed to be erroneously, as the Southern most limit of the distribution of *Clarias* is the South of the Orange river system (TEUGELS, 1986).

Chapter I.4 – Some terminologies used

I.4.1 – Biometric Data

In the course of this study, it appeared that body size was an important reference mark for comparing ontogenetic stages, instead of age (see III.1). Therefore, for each stage used, three measurements were taken (**Fig. I.4- 1**):

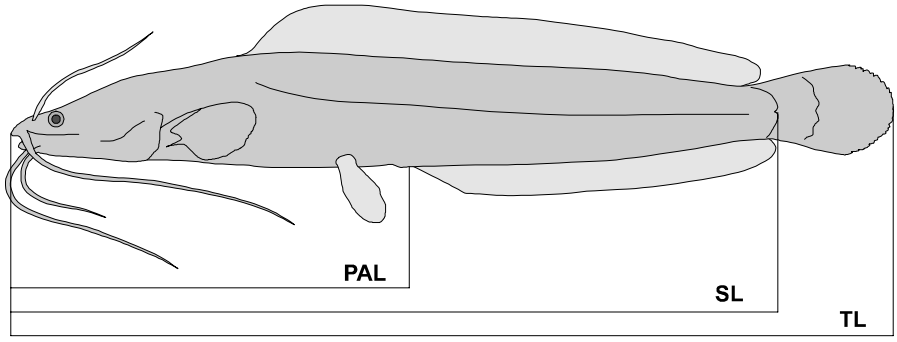


Fig. I.4- 1: Length measurements used in this study: TL = total length, SL = standard length, PAL = preanal length

1 - *Total Length* - TL → distance between rostral tip of the snout and the caudal margin of the tail fin.

2 - *Standard Length* - SL → distance between the rostral tip of the snout and the tail root, represented by the base of the tail lepidotrichia and corresponding to the caudal border of the hypuralia of the caudal skeleton. It has to be noted, however, that the standard length of larval fish is not completely identical to standard length of juvenile specimens. In larval specimens, no tail lepidotrichia, nor hypuralia are present yet, so the distal tip of the notochord was taken.

3 - *PreAnal Length* - PAL → distance between the rostral tip of the snout and the anus. Although standard length and total length are most commonly used to express body size in fish, I also referred to preanal length as it gives an indication of the ratio of the tail to the torso.

I.4.2 – ONTOGENETIC PERIODS

Many terminologies have been proposed for categorising the ontogenetic periods of fishes. A most simplified distinction can be made between five different periods, with their corresponding terminology for designating the phenotype of the period:

1 - egg period ..... egg + embryo
2 - larval period ..... larva
3 - juvenile period ..... juvenile
4 - adult period ..... adult
5 - senescence period..... senescent

The embryonic period then spans between fertilisation until hatching, followed by the larval period which lasts until metamorphosis. The consequent juvenile period terminates at the moment of maturation. The corresponding adult period ends with the reproductive inactivation, whereas senescence follows until death (HEFFMAN *et al.*, 1997).

However, not all is said by this. Problems arise when defining critical events demarcating these periods. In general, the egg period thus ends at the moment of hatching. BALON (1975) on the other hand defines an embryonic period, starting at fertilisation but ending at the transition of endogenous feeding to exogenous feeding. Although, it is evident that some arbitrary event has to be used for standardising ontogenetic periods,

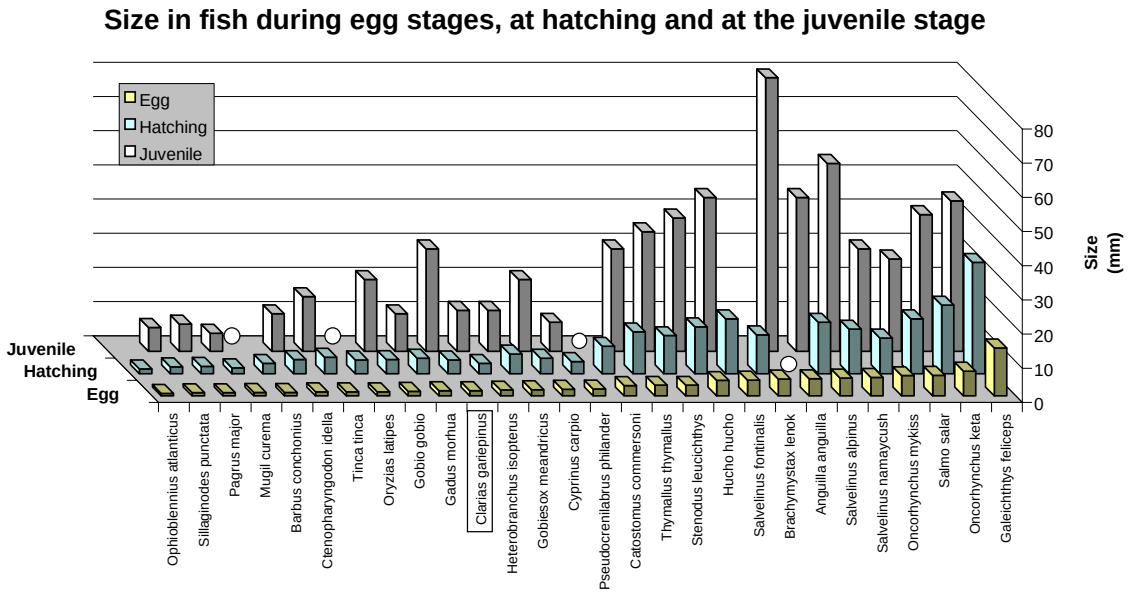


Fig. I.4- 2: Graph showing the relation between egg size, body size (TL) at hatching and size (TL) at the onset of the juvenile period in different fish species (species are ordered according to increasing egg size) (white dots indicate no data available) [data obtained from GREENWOOD (1955); BALON (1975); MCELMAN & BALON (1980); ALLEN (1983); LABELLE & NURSALL (1985); TILNEY & HECHT (1993); HOLDEN & BRUTON (1994); IWAMATSU (1994); BRUCE (1995); OSSE & VAN DEN BOOGAART (1995); DA COSTA *et al*., 1996; HUNT VON HERBING *et al*., 1996a; GORODILOV (1996)]

these two end points can hardly be compared between species as a reference point of ontogenetic differentiation. First, when looking at different early life histories of fish, it becomes clear that hardly all fishes are equally developed at the moment of hatching. This is both the case if development is expressed as size (**Fig. I.4-2**), or as ontogenetic differentiation. For example, in *Betta splendens* (Perciformes, Belontidae), no cartilage can be observed until 24 hours after hatching (MABEE & TRENDLER, 1996), whereas in *Poecilia reticulata* (Atheriniformes, Poeciliidae) and *Galeichthys feliceps* (Ostariophysi, Ariidae), almost all cranial bones have formed by the time hatching takes place (WEISEL, 1967; TILNEY & HECHT, 1993). The transition of endogenous to exogenous feeding depicts a developmental stage, which is better comparable between species, as active feeding requires a functional feeding apparatus, which is reflected in the ossification of related bones. One problem, however, is that this transition phase is not a sharply demarcated period. The period between initial exogenous feeding and complete yolk sac resorption may last very differently (*e.g.,* in *Clarias gariepinus*, three days, whereas in *Galeichthys feliceps* up to 40 days). However, OSSE & VAN DEN BOOGAART (1995) mentioned that "there are firm biological arguments for distinguishing the periods of egg, larva and juvenile", in contrast to embryo, larva and juvenile. This may be so for categorising major biological events in life history, related to environmental interactions, but cannot, to my opinion, be used to designate ontogenetic mile stones.

Metamorphosis is another problematic criterion, as a true metamorphosis, involving a substantial, accelerated, overall transformation at a specific moment during development, can only be observed in some teleosts (*e.g.,* elopomorphs, flatfishes, lanternfishes, holocentrids, ephippids…) (HEFFMAN *et al.*, 1997). Most teleosts, however, undergo a more direct development, where hardly any strict form transformation can be noted. Consequently, some characters have been introduced to demarcate the end of the larval period in this group by the complete differentiation of the median fin fold into a true dorsal and anal fin, supported by lepidotrichia (BALON, 1975). Other characters may involve the initiation (or termination) of squamation, vertebrae ossification or pigmentation (HOLDEN & BRUTON, 1994; HEFFMAN *et al.*, 1997), whereas allometric changes in body proportions may indicate a differential, sudden change in growth rate (OSSE & VAN DEN BOOGAART, 1995; STRAUSS, 1985). Growth is expected to stabilise, once the remodelling has stopped (COPP & KOVÁČ, 1996). Consequently, the transition from the larval phase to the juvenile phase is not always clearly defined (COPP & KOVÁČ, 1996). Although some exceptions occur (*e.g.,* male *Micrometrus minimus* mature before hatching), the end of the juvenile period is well demarcated by the onset of maturation. Senescence is manifested when growth reaches its asymptote (growth is not supposed to stop completely in fish) and gametogenesis has stopped (BALON, 1975). Again, substantial differences in time span of such a period is observed in fishes (from several days in salmons to many years in sturgeons), but it has no substantial effect on major ontogenetic differentiations (unless some degradation).

Attempts have already been done to subdivide the life history of *Clarias gariepinus* in different periods. HAYLOR (1992) noted the indirect development of *C. gariepinus*, in contrast to the also economically important catfish genus, *Ictalurus* (Ictaluridae), which "exhibits a direct development from 'sac-fry' (or eleutheroembryonic phase[1]) to the alevin stage[2]". The onset of the larval period in *C. gariepinus* is then characterised by the initiation of exogenous feeding. The involved transformations comprise "morphological and physiological changes to the digestive system, changes in body composition, the establishments of definite body proportions and the development of functional arborescent organs". The digestive system becomes completely functional, indicating the physiological end of the larval period, at 11.5 mm. The morphological end, reflected by the

[1] According to the terminology of BALON (1975), this eleutheroembryonic phase starts at hatching, until exogenous feeding starts.
[2] According to the terminology of BALON (1975), the alevin stage exists in those species lacking any larval period. It follows upon the eleutheroembryonic phase and lasts until squamation or fin ray formation is complete.

complete replacement of the median fin fold by fin rays, and the disappearance of fin fold rudiments around the caudal fin, occur at 30 mm (HECHT & APPELBAUM, 1987). The primordia of the arborescent organs appear at 30 and 50 mm length, at the level of the fourth and second branchial arch, respectively (GREENWOOD, 1961). In observations done during this studies, a primordium on the fourth arch could already be found at 21.5 mm SL (**Plate III.1-5A**), which confirms with the results of HAYLOR & OYEGUNWA (1993). The behaviour, related to the atmospherical respiration is already observed at 20 mm length (HAYLOR & OYEGUNWA, 1993). Consequently, for practical reasons (in fish farms), HAYLOR (1992) designated the initiation of exogenous feeding and the initiation of atmospherical respiration as the starting and end points, respectively, of the larval period in *C. gariepinus*, especially because both represent critical periods in survival.

However, as stated by COPP & KOVÁČ (1996) that "the process of remodelling terminates the larval period", and the main subject of this thesis involves ontogenetic processes and remodelling, I found it opportune to couple life history stages to ontogenetic demarcations. I will only briefly argument some of the criteria used to demarcate five different periods:

1 - EGG PERIOD---------- Evidently, this period corresponds to the period between fertilisation and hatching. This period equals to what I would call the embryonic period, characterised by the major embryonic processes like activation, cleavages, morula stage, blastula stage, gastrula stage, neurula stage and somite formation (coupled to the differentiation of the several organ systems). This does not imply that after hatching, no further somite formation will occur, but I consider them part of the larval period.

2 - LARVAL PERIOD ---------- At hatching, *Clarias gariepinus* hardly has all the structural elements of what might be expected of a true 'larva' (GREENWOOD, 1955; SURLEMONT & VANDEWALLE, 1991, *personal observations*) (**Fig. I.4- 3**). However as newly hatched *C. gariepinus* are able to swim actively, even demonstrating a photophobic behaviour, I find it not illogic to refer the them not as embryo's, but as larvae, as did GREENWOOD (1955). This view is supported by the definitions of embryo and larva according to OSSE *et al.* (1993): a larva distinguishes itself from an embryo by the fact that it can sustain self-maintenance. An opposing argument that can be suggested is that the initial ontogenetic processes after hatching in *C. gariepinus* involve embryonic processes, which thus cannot be designated to a larval period. However, the question can be raised whether a strict line can be drawn between embryonic processes and larval remodelling processes. If embryonic

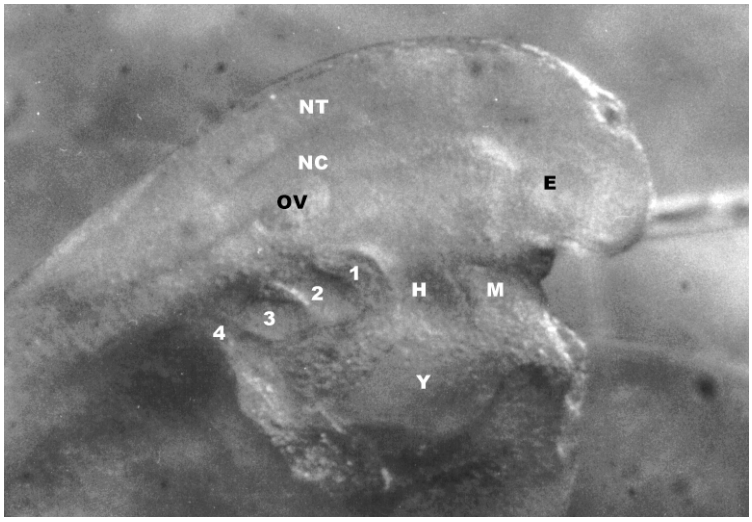


Fig. I.4- 3: Newly hatched larva of *Clarias gariepinus* (*Abbreviations:* H = hyoid arch, M = mandibular arch, NC = notochord, NT = neural tube, OV = otic vesicle, E = eye primordium, Y = yolk sac, 1-4 = branchial arches I-IV)

processes would involve cellular differentiation from precursor or progenitor cells, than embryogenesis would last until death (*e.g.,* differentiation of osteoblasts, osteocytes and osteoclasts).

3 - JUVENILE PERIOD ---------- Following COPP & KOVÁČ (1996), the larval period ends at the moment larval remodelling processes have stopped. These processes can be restricted to fin differentiation or squamation, but in the context of this thesis, I opted for cranial remodelling processes as a critic feature. Consequently, the end of the larval period coincides with the moment that the skull morphology resembles that of the adult situation, although size is reduced. This evidently involves especially the presence of all structures, as well as the state of ossification, muscle shape and muscle attachments. Based on the studied material, the end of the larval phase would be much later than what is proposed according to other criteria. It is even so, that strictly speaking, the material used in this study only allows to narrow down the transition phase from larval to juvenile period somewhere between 51 and 125 mm SL. More material in between these two should have to be studied to pin-point the exact starting point of the juvenile phase.

4 - ADULT PERIOD ---------- The definition of the starting point of this period corresponds to the one generally accepted, *i.e.,* the onset of gametogenesis. The effect on ontogenetic processes was not part of this study. Based on literature, however, no indication could be found that maturation related metamorphosis, as for example observed in salmons, is present in *Clarias gariepinus*.

5 - SENESCENCE PERIOD ------- The maximum size attained in *Clarias gariepinus* ranges somewhere around 1.4 m SL (BURGESS, 1989; SKELTON, 1993). However, no data could be obtained on the growth rate changes during late adult phase.

Based on these definitions of ontogenetic periods, it has to be noted that the title of this thesis is not completely correct. Strictly speaking, it should have been: "On how a larva becomes a juvenile catfish". Despite this, for didactical reasons, it was opted to use "adult catfish".

I.4.3 – SCIENTIFIC TERMINOLOGY OF ANATOMICAL STRUCTURES

The fact that scientific terminologies of animal structures and organs is not bound to an international code, has resulted in excessive introductions of new terms, referring to homologous structures. This, however, is a hotbed for confusions and homology errors. The latest trend in Anglicising of scientific language is a major contributor to such term proliferations (*e.g.,* dental, dentary or lacrymal, lachrymal, lacrimal). In an ontogenetic context, this gives even more problems, *e.g.,* dentary is sometimes used when referring to both the (1) os dentale, (2) the

fused complex between the os mentomeckelium and the os dentale, or (3) the fused complex between the latter two and the os spleniale. It is, however, justifiable that excessive use of latinised terminology aggravates fluent reading.

In this thesis, it is opted for to use a terminology as consistent as possible, without producing text with unnecessary redundant latinised terminologies. In the labels (and legends) of the figures, the latinised terminology is used more consistently, thereby providing a reference for comparison with terminologies of other work. In general, the prefix *cartilago* is not used for most cartilaginous structures, with some exceptions. The distinction between cartilaginous elements and their ossifications is made obvious by adding the prefix *os* for bony structures, or by explicitly adding 'bone' [*e.g.,* ceratobranchiale and os ceratobranchiale (or ceratobranchial bone)]. Complexes, formed by different bones during ontogeny, are designated a complex name, composed of all the constituting parts (*e.g.,* os dento-splenio-mentomeckelium refers to the complex formed by the dental bone, the splenial bone and the mentomeckelian bone). The myology is described using latinised terms, followed by 'muscle'. Some terms are Anglicised or 'muscle' is omitted, when no confusion is possible. In the labels (and legends), the prefix *musculus* is used. The same can be said about artery and *arteria*, vein and *vena*, nerve and *nervus*, commissure and *commissura*, ligament and *ligamentum*, bridge and *pons*, and process and *processus*.

One new scientific terminology has been introduced, which only could be found being expressed in a native language: the 'polar cartilages', 'cartilages polaires' or 'Polknorpel'. As a consistent, universal, and thus latinised nomenclature is appropriate, *cartilago polaris* is introduced (see II.1-1).

I.4.4 – Bone Histology

The purpose of this chapter, or this thesis, is far from giving an extensively detailed overview of the skeletal histology of *Clarias gariepinus*. It is assumed, however, that a brief summary of some features of cartilage and bone histology would be in place, in order to give at least some histological background. As each element of the '*Bauplan*' can be described by a set of parameters, *i.e.,* size, shape, structure, material composition and position (Verraes, 1981), it is obvious that functional adaptations are reflected at the structural level as well. To follow the definition of Verraes (1981), "the structure of an element of the Bauplan is the topographic set of all material cellular, intra-cellular and inter-cellular parts which fill up the internal space of the element, on the microscopic or the ultra-microscopic level". In this section, some observations at light microscopic level are given.

I.4.4.a - Cartilage

In *Clarias gariepinus*, six different types of cartilage could be distinguished: (1) cell rich hyaline cartilage (CRHC), (2) matrix rich hyaline cartilage (MRHC), (3) fibro/cell-rich cartilage (FCRC), (4) elastic/cell rich cartilage (ECRC), (5) 'Zellknorpel', and (6) scleral cartilage [terminologies according to Benjamin (1989; 1990)].

1. CELL RICH HYALINE CARTILAGE + MATRIX RICH HYALINE CARTILAGE

Cell rich hyaline cartilage distinguishes itself from matrix rich hyaline cartilage, by the fact that cells occupy more than 50% of the cartilage volume (BENJAMIN, 1990). During ontogeny, the ratio of cell volume to matrix volume changes. Early during ontogeny, all hyaline cartilage is cell rich, where very little matrix is present (**Plate I.4-1A**). Later on, the matrix contents increases, in relation to cell contents, but not substantially in most structures (**Plate I.4-1B-D**). In the ethmoid region, matrix contents appeared to be larger, where the cartilage might be considered as the transition from cell rich hyaline cartilage to matrix rich hyaline cartilage (**Plate I.4-5A**).

Early during ontogeny, the chondrocytes are approximately equally sized and randomly shaped, bearing many straight margins (**Plate I.4-1A**). Later on, the cells become more rounded (**Plate I.4-1B**). Once ossification is persistent, a difference in cell shape can be observed in some structures, indicating differential cell activity (**Plate I.4-2**). In between two bones, a growth zone can frequently be distinguished, containing proliferating, flattened and densely packed cells (**Plate I.4-2B, D**). At the margin of this growth zone, maturing chondrocytes can be distinguished (**Plate I.4-2B, D**) from the more distally situated resting chondrocytes, constituting the resting hyaline cartilage (**Plate I.4-2B-C**). Mineralisation of cartilage could initially be observed at the level of perichondral ossifications (see below) (**Plates I.4-5A, 6C, 7C**). The amount of mineralised cartilage, however, was low. Later during ontogeny, a more substantial mineralised hyaline cartilage could be observed at the level of the articulation between the neurocranium and the suspensorium. At the base of the articulatory rim of the neurocranium, a mineralised zone was observed, transversing the complete rim (**Plate I.4-3B**). Cartilage resorption, through chondroclastic activity was persistent in larger larval specimens (*e.g.,* 27.5 mm SL, dwarf specimen) (**Plate I.4-3C**). Substantial cartilage resorptions could be observed in the neurocranial cartilage (**Plate I.4-7B**), Meckel's cartilage, hyoid bar (**Plate I.4-8A**), suspensorium and branchial arches (**Plate I.4-8B**).

2. FIBRO/CELL RICH CARTILAGE + ELASTIC/CELL RICH CARTILAGE

Articular surfaces require a tissue with special mechanical properties. In *Clarias gariepinus*, two types of tissues were observed at articulatory surfaces: (1) chondroid bone (see below) and (2) a cartilage-like tissue, consisting of densely packed cells. BENJAMIN (1990) observed such a cartilage in corresponding regions in *Clarias batrachus*, referring to the tissue as fibro/cell rich cartilage (FCRC). This FCRC is a typical articular tissue. In *C. gariepinus*, such a tissue could be observed in between the hypohyal cartilages (**Plate I.4-3A**), in between the hypobranchials and the copulae, as well as at the articulation between the neurocranium and the suspensorium (**Plate I.4-3B**). Such cartilage lacks a discrete perichondrium, although a superficial layer of extremely flattened cells is observed (**Plate I.4-3A**).

Already early during ontogeny, the oral barbels become apparent (**Plate II.1-2A**). At 6.0 mm SL, a distinct central rod is present, which stains with alcian blue, indicating the presence of acid glycosaminoglycans (**Plate II.1-3**). Observations of GHIOT & BOUCHEZ (1980) demonstrated that the supporting rods of *Ictalurus* (Ictaluridae) and *Pimelodus* (Pimelodidae) did not contain any cartilage, whereas elastin formed a supporting network. In contrast to these observations, a true cellular composition in *Clarias gariepinus* suggests that true cartilage is present (**Plate I.4-4**). The cartilage of the barbel rods can easily be recognised by the closely packed cells, surrounded by little matrix. Toluidin staining results in a colour difference with the skeletal cartilage, as barbel cartilage is highly chromophylic (turning purple) (**Plate I.4-4D**). In his earlier paper, BENJAMIN (1989) mentioned the presence of hyaline chondroid cartilage (HCC) in the oral barbels of *Heteropneustes fossilis*

(Heteropneustidae), which is closely related to the Clariidae. He also mentioned that "the most characteristic cartilage of barbels is Schaffer's (1930) *Zellknorpel*". This HCC can be distinguished from 'Zellknorpel', especially by the fact that in the latter, the chondrocytes have shrunk in their lacunae and have no hyaline appearance. In *Clarias gariepinus*, the latter two features are observed in the oral barbel cartilage (**Plate I.4-4A**). An additional, typical feature of 'Zellknorpel' involves the unicellular arrangement in structures like hemibranchial supporting rods. This configuration could for example be observed in the basal plates of the mandibular barbels (**Plate I.4-4A-B**). Based on these observations, it would be evident to classify the oral barbel cartilage in *C. gariepinus* as 'Zellknorpel'. However, in a later paper, BENJAMIN (1990) wrote that 'ECRC (referring to elastic/cell rich cartilage) is characteristic of the barbels and maxillary oral valves of catfish". He also mentioned that "in *Leiocassis siamensis, Clarias batrachus* and *Pimelodus pictus*, the cartilage in the mandibular barbels is continuous with a complex root piece to which the fibres of the protractor hyoidei are attached". Consequently, the oral barbel cartilage of *C. gariepinus* should be classified as elastic/cell rich cartilage. Until some further research can give some conclusive results, the oral barbel cartilage in *C. gariepinus* will be regarded as ECRC. Such results could for example be obtained by performing differential staining for elastin.

3. 'ZELLKNORPEL' + SCLERAL CARTILAGE

'Zellknorpel', if not present in the oral barbels, could be observed in the gill filaments (**Plate I.4-8B**). A stream of mono-layered chondrocytes was present, supporting the primary filaments of the gills, as is a common feature in teleosts (BENJAMIN, 1990).

BENJAMIN (1990) considered the scleral cartilage, supporting the eye ball and serving as reinforced attachments for the eye musculature, as a separate category of cartilage. In *Clarias gariepinus*, the sclera consisted of a thin, mono-layered cartilage.

I.4.4.b - Bone

The terminology of bony tissue, as discussed in this thesis, follows that of PATTERSON (1977), MEUNIER (1982) and HUYSSEUNE (1986). PATTERSON (1977) distinguished three main categories of bones: (1) cartilage bone, (2) dermal bone, and (3) membrane bone. A fourth type, which could not be grouped in one of them, involves the so-called chondroid bone (HUYSSEUNE, 1986). Of all four types of bone, separate representatives have been observed in *Clarias gariepinus*. Additionally, fusions occurred between the different types of bone (see II.1.2):

1 - perichondral bone	⇔	membranous bone
2 - perichondral bone	⇔	dermal bone
3 - perichondral bone	⇔	chondroid bone
4 - dermal bone	⇔	dermal bone

1. CARTILAGE BONE

The larval skeleton of vertebrates is constituted of cartilaginous structures. During ontogeny, as mechanical loading increases, perichondral osteoprogenitor cells differentiate into osteoblasts (MEUNIER, 1982). As they surround the cartilaginous structure, they initiate perichondral ossification, which is the primal phase of

ossification in vertebrates. By producing a thin layer of osteoid[3], the osteoblast may or may not become enclosed in this matrix, which may subsequently become mineralised by hydroxy-apatite crystals (**Plate I.4-6C**). Whether osteoblasts become enclosed, consequently differentiating into osteocytes, or remain at the surface of the continuously added bone matrix, results in the presence of cellular or acellular bone. Cellular bone is a typical feature of some primitive teleost lineages (with some exceptions), whereas acellular bone is most frequently observed in higher taxa (again with some exceptions) (MEUNIER & HUYSSEUNE, 1992). At a later stage, cartilage has been observed to become mineralised as well, at the close connection with the perichondral bone (**Plates I.4-5A, 6C, 7C**). Resorption of this cartilage, generally is preceded by perichondral ossification (HUYSSEUNE, *pers. comm.*) (**Plate I.4-3C**). Such chondroclastic resorptions may be accompanied by the invasion of a blood vessel into the cartilage, consequently supplying new osteoblasts. The thus formed intracartilaginous canal may become lined with bone. Even more, the cavity formed within the perichondral bone, as a result of extensive cartilage resorption, may be filled with bony trabeculae. Such an intracranial ossification is then referred to as en(do)chondral ossification. Another phenomenon observed in fishes, in relation to substantial cartilage resorption, is the filling of the thus formed cavity with fat tissue.

In *Clarias gariepinus*, the first bones to develop involve dermal bones (see below, see II.1.2). Perichondral ossification can be observed from the 6.0 mm SL specimen on (**Table II.1-3**). At 7.2 mm SL several cartilage structures are already covered by a thin, perichondral bone (**Plate I.4-6E**). Initially, perichondral bone consists of a thin, ossified layer, following the surface of the cartilaginous template (**Plate I.4-5A**), however, later on, small tubercles can be observed, to form at a perpendicular angle (see below) (**Plate I.4-10A**). Young bone appears to be acellular, however, at later stages enclosed osteocytes can clearly be distinguished, both in perichondral and dermal bone (**Plate I.4-5B**). Canaliculi, interconnecting these osteocytes, cannot be observed at light microscopic level. Perichondral ossification is frequently accompanied by bony nodules in the cartilage (**Plate I.4-7A-C**). These are considered as globuli ossei, which arise as the perichondral bone invades the lacuna of peripheral, degenerated chondrocytes (MEUNIER, 1982). In the specimens used for this study, endochondral ossification could only be observed in the largest specimen, in the hyoid bar only (**Plate I.4-8A**). In this specimen, the invasion of the cartilage by a blood vessel could clearly be distinguished. The consequently formed short, intracartilaginous cavity is completely surrounded with bone. Fat tissue formation in perichondral bone, after cartilage has been resorbed, cannot be observed in *C. gariepinus* (**Plate I.4-8B**).

Apart from perichondral bones (and endochondral bones), the so-called parachondral bones can be observed in *Clarias gariepinus* as well. Although lying closely packed against cartilage or perichondral bone, it is still separated from it by at least a layer of perichondrium and periosteum, or two layers of periosteum, respectively (**Plate I.4-5A**).

2. DERMAL BONE

Dermal bones thus lack any cartilaginous prophase, neither ontogenetically nor phylogenetically (PATTERSON, 1977). Dermal bones may enclose a canal of the cranial lateral-line system or may not (see II.1.3). Ossification in *Clarias gariepinus* starts with the formation of dermal bones, as mentioned above. Early bones all are simple and plate-like (**Plate I.4-1A**). Growth and bone deposition appears to occur especially at the margins of these plates, reflected by the concentration of osteoblasts (**Plate I.4-6A**). Later on, dermal bones start to form apolamellae as well, perpendicular to its surface (**Plate I.4-1C**). Again, bone growth appears to be concentrated

[3] Osteoid refers to the unmineralised bone matrix, which major components involve: (1) *collagen* (especially type I), (2) *osteonectin* (most abundant non-collagenous protein), (3) *osteocalcin* (also a non-collagenous protein), (4) *glycosaminoglycans*, (5) *proteoglycans*, (6) *glycoproteins*, and (7) *lipids* (MEUNIER, 1982; MARKS & POPOFF, 1988).

at the margins of those apolamellae (**Plate I.4-6B**). Eventually, almost all dermal bones in the skull of *C. gariepinus* become extremely cancellous, which is considered to be an adaptation to rapid growth (DE RICQLÈS, *pers. comm.*; SIRE *et al.*, 1993). Prior to the formation of such apolamellae, fusions may already have occurred between dermal bones (**Plate I.4-1C**). Bone growth initially occurs rather parallel to the bone surface, as can be derived from the cement lines[4], and more specifically the resting lines (**Plate I.4-5B**). Later on, osteoclastic bone resorption and secondary bone deposition can be derived from the more irregular cement lines, *i.e.,* the reversal lines (**Plate I.4-5B**). Osteoclastic activity can be observed both at the external surface of the bone, as well as in the spaces formed by the trabecular apolamellae (**Plate I.4-5B**) (SIRE *et al.*, 1990).

3. MEMBRANE BONE

Membrane bones are those bones that ossify "in membrane deep in the mesoderm, with no ontogenetic or phylogenetic connection with the ectoderm" (PATTERSON, 1977). These bones involve those that are homologous with cartilage bones in more primitive vertebrates, as well as sesamoid bones and outgrowths of cartilage bones (PATTERSON, 1977). Examples of the latter two cases can be observed in *Clarias gariepinus*. Sesamoid bones involve the entopterygoid bone and part of the parurohyal bone (see II.1.2). Apolamellae are observed on all perichondral bones. Not only do they substantially increase the outer surface of many bones (*e.g.,* the ceratohyal bones) (**Plate I.4-6D**), they also form large, plate-like outgrowths (*e.g.,* the suspensorial bones) (**Plate II.1-34B-D**). The complexity of the membranous apolamellae does not differ from that of the dermal trabeculae, as in both the bony bars, perpendicular to the perichondral or dermal bone surface, are interconnected by transverse bars. The question can be raised whether a true difference exists between the mechanism responsible for the trabeculae formation in dermal bones and the apolamellae formation in perichondral bone. A discussion dealing with this question, however, is beyond the scope of this thesis.

4. CHONDROID BONE

At many articulations, an unusual kind of tissue, closely resembling cartilage invaded by bone, can be observed. These articulations involve those between (1) the maxillary bone and the autopalatine bone (**Plates I.4-9, 10D**), (2) the autopalatine bone and the lateral ethmoid bone (**Plates I.4-10A-B, 11**), (3) the autopalatine bone and the antorbital bone (**Plates I.4-7D, 9D**), and (4) the lower jaw and the suspensorium (**Plate I.4-10C**). According to the terminology of BERESFORD (1981; 1993), two types of chondroid bone can be distinguished: (1) type I, which "may arise "*de novo*" from a blastema by a particular mode of differentiation of skeletal precursor cells", and (2) type II, which "forms by mineralization and persistence of pre-existing cartilage" (HUYSSEUNE, 1986). In cichlids, chondroid bone of type I can be observed at the articulation between the upper pharyngeal jaw and the neurocranium. Several aspects of such chondroid bone have already been studied extensively: development (HUYSSEUNE, 1986) and adult morphology (HUYSSEUNE & VERRAES, 1986), histochemistry of the different layers in chondroid bone (HUYSSEUNE & VERRAES, 1990) and ultrastructural morphology (HUYSSEUNE & SIRE, 1990). Consequently, the differentiation of undifferentiated fibroblasts into osteoblast-like cells, secreting chondroid bone matrix, could be revealed (HUYSSEUNE & SIRE, 1990). In the chondroid bone, different layers can be distinguished: (1) an unmineralised layer, (2) a mineralisation front, (3) the mineralised layer and (4) a resorption front (HUYSSEUNE & VERRAES, 1990).

[4] Cement lines are characterised by the fact that they are slightly hypermineralised, and less fibrillary, in comparison with the adjacent bone matrix (CASTANET, 1981; MEUNIER, 1982).

In the specimens of *Clarias gariepinus*, used for this study, chondroid bone formation cannot be observed together with cartilage and bone resorption at its base. Chondroid bone formation seems to involve true cartilage, suggesting type II chondroid bone, according to BERESFORD (1981). An example can be given: early during ontogeny (7.2 mm SL), the articulation of a rudimentary maxillary bone with the palatine is intermediated by a small, cartilaginous nodule, the submaxillary cartilage (see II.2.2) (**Plate II.2-13**). This submaxillary cartilage lies closely against the ventrolateral face of the rostral tip of the palatine, and is covered laterally by the maxillary bone (**Plate I.4-9A**). Later during ontogeny, large cells have become enclosed by what appears to be a bony matrix (**Plate I.4-9B**). During further ontogeny, more and more large cells become enclosed in the thus formed articulatory facet of the maxillary bone. At that stage, different layers can be distinguished as well in this chondroid bone (**Plate I.4-11**). Consequently, several arguments can be given which may suggest that this chondroid bone involves the metaplasy of chondrocytes to bone producing cells: (1) the submaxillary cartilage lies at the exact position, both in relation to the maxillary bone and the palatine, as the chondroid bone, (2) no resorption of the submaxillary cartilage, followed by a secondary formation of chondroid bone can be observed at this level, and (3) although not undisputed, such a metaplasy has been observed in osteichthyans (MEUNIER, 1982). However, it would be premature to state this, as this level of discrimination cannot rule out the possibility that the chondrocytes of the submaxillary cartilage do not indeed become replaced by bone producing cells, instead of transforming into them.

The occurrence of type I chondroid bone formation in *C. gariepinus* cannot be ruled out either, as for example comparable chondroid bone can be observed at the dorsal articulatory facet of the maxillary bone, whereas no cartilaginous prophase was observed. However, a more detailed study would be required: (1) to give a complete overview of all chondroid bone formations, (2) to give developmental information of these bones, in order to distinguish between type I and II, and (3) to elucidate whether a true metaplasy occurs or not.

Chapter II.1 – The cranial skeleton

II.1.1 - The Chondrocranium[1]

The ontogeny of the chondrocranium of 31 different stages of the African catfish, *Clarias gariepinus* (Siluriformes: Clariidae) is studied, both from cleared and stained, and sectioned material. The examined material ranges from 4.1 mm SL (one day post-hatching) to 127.0 mm SL (100 days post-hatching). The chondrocranium of *C. gariepinus* appears to correspond to the general adaptive trends in siluriforms, especially in relation to the reduction of the eye size and the dorsoventral flattening of the skull. The platybasic neurocranium involves several modifications related to the trabecular bars, the hypophyseal fenestra, the ethmoid region and even the olfactory nerves. Certain reductions are present, which are observed in all siluriforms (*e.g.,* absence of the pila lateralis, the commissura lateralis, the myodomes) or are part of a variable trend within siluriforms (*e.g.,* reduction of the taenia marginalis anterior and the tectum synoticum). Compared with some other siluriform species, the neurocranium of *C. gariepinus* is well developed, for example in the otic region. The same is observed in the splanchnocranium where some general siluriform trends persist (*e.g.,* isolation of palatine from pterygoquadrate, presence of 'hyo-symplectic-pterygoquadrate' plate). Some trends, as observed in other siluriforms, are also present (*e.g.,* interhyal continuous with suspensorium and ceratohyal, Meckel's cartilage initially continuous with the suspensorium). The branchial basket is well developed as all expected elements are present (basibranchials I-V, hypobranchials I-IV, ceratobranchials I-V, epibranchials I-IV). Concerning the infrapharyngobranchials, a wide range of variation has been reported in literature. Based on the observed ontogeny of *C. gariepinus* and data from the literature, a hypothesis is formulated which indicates the presence of a general reductional trend within siluriforms. In *C. gariepinus* all four (I-IV) infrapharyngobranchials develop, although the anterior two are much reduced and have fused with each other.

INTRODUCTION

The study of the skull of vertebrates in general, and of osteichthyans in particular, is in most cases restricted to the adult bony part. Those studies dealing with the ontogeny of the skull frequently focus on the development of the osteological components of the fish skull, with little or no attention paid to the relation with the chondrocranium (*e.g.,* Kobayakawa, 1992; Vandewalle *et al.*, 1995). A compilation of studies, done on the ontogeny of the cartilaginous skull of fishes, has been given by de Beer (1937) and Daget (1964). Quite some attention has been

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paid to the chondrocranium of siluriforms: Ictaluridae (KINDRED, 1919; ARRATIA, 1992), Callichthyidae (HOEDEMAN, 1960a; HOWES & TEUGELS, 1989), Ariidae (BAMFORD, 1948; SRINIVASACHAR, 1958a), Plotosidae (SRINIVASACHAR, 1958a), Schilbeidae (SRINIVASACHAR, 1957a), Bagridae (SRINIVASACHAR, 1957b); Clariidae (SURLEMONT *et al*., 1989; SURLEMONT & VANDEWALLE, 1991; ADRIAENS & VERRAES, 1994), Heteropneustidae (SRINIVASACHAR, 1959).

Concerning the general morphology of the siluriform chondrocranium, some important transformations have been noticed, compared to the more primitive teleosteans. The major part of these changes can in some way be related to the benthic and nocturnal life style of many catfishes. Most striking are the consequences of the reduction in eye-size (ADRIAENS & VERRAES, 1997e). The consequently decreased spatial constraints allows the contralateral trabecular bars to remain separated along the greater part of their length, thus forming a large hypophyseal fenestra. This type of skull construction belongs to the platybasic (= platytrabecular) type, in contrast to the tropibasic (= tropidotrabecular, tropitrabecular, tropidobasic) type which is found in most teleosts (DAGET, 1964). A reduction of the eye diameter also implies a reduction in the bulk and length of the extrinsic eye muscles, and no, or rudimentary, myodomes to house them are needed (DAGET, 1964; ALEXANDER, 1965). The platybasic skull of siluriforms generally becomes dorsoventrally depressed, which is believed to be an adaptation for stability when lying on the bottom, as well as to reduce the drag in currents (ALEXANDER, 1965). Another adaptation for this benthic behaviour may be the loss of the interhyal during ontogeny in different catfishes (ADRIAENS & VERRAES, 1994). A reduction in visual input is probably compensated for in catfishes by the presence of a set of oral barbels, equipped with taste and touch sensory organs (ALEXANDER, 1965; GHIOT & BOUCHEZ, 1980). One pair of these barbels, the maxillary barbels, is part of a palatine-maxillary mechanism, which enables a controlled mobility. The development of this mechanism is partially enabled by the separation of both skeletal and muscular elements of the palatine part of the palatoquadrate [GOSLINE, 1975a; GHIOT *et al*., 1984; FINK & FINK, 1981 (character 24); ARRATIA, 1992 (character 2)].

In the present chapter, a detailed description is given of the ontogeny of the chondrocranium in the African catfish, *Clarias gariepinus* BURCHELL (1822) [synonymised with *C. lazera* and *C. mossambicus* (TEUGELS, 1982)]. Several ontogenetic stages are used to describe the morphological transformations that occur, resulting in the cartilaginous template for the bony skull. Seven of these stages are described here. The description of the developing osteocranium is given in the next chapter (see II.1.2). Special attention is paid to the development of the orbito-nasal region, as well as to the formation of the foramina of the otic region. In the splanchnocranium, special attention is paid to the development of the infrapharyngobranchials, where an attempt is made to distinguish a trend within the Siluriformes. Based on these data and data from the literature, some considerations are made concerning the comparative morphology of siluriform chondrocrania and some trends during its ontogeny. The ontogeny of the postcranial Weberian apparatus and vertebrae is not described. For these structures we refer to RADERMAKER *et al*. (1989).

RESULTS

The applied nomenclature for the anatomical structures basically follows the nomenclature used by DAGET (1964). Arguments for some divergent nomenclature are given in the discussion.

4.1 MM SL SPECIMEN (**Plate . II.1-1**)

Neurocranium - Already one day after hatching, the major part of the skull base is formed. The chondrification appears to be concentrated in close contact with the notochord. The anterior part of the notochord is bordered by two lateral cartilaginous strips, which are interconnected anteriorly. Based on their position, the lateral elements must correspond to the parachordal cartilages. The cartilage abutting against the anterior margin of the notochord then represents the acrochordal cartilage. Anterolaterally, an anterior basicapsular commissura connects the parachordals to the anterior otic cartilage. At one side, in this specimen, the posterior otic cartilage can already be distinguished, bearing a small ventral extension. No asymmetry, however, could be observed in any other stage. This extension covers the posterolateral part of the otic vesicle. In general, the basicapsular commissures connect the otic cartilages to the parachordal plate (or basal plate). The latter corresponds to the fused parachordal cartilage and the basiotic lamina. This lamina, however, can hardly be distinguished as a separate element in *C. gariepinus*. The fusion with the parachordal cartilages may already have occurred at the level of blastemes or the basiotic lamina may be lost. The partially enclosed, large foramen, surrounded by the otic cartilages, the anterior basicapsular commissure and the parachordal plates, will be penetrated by both the glossopharyngeus (IX) and vagus (X) nerves. Consequently, this foramen corresponds to the metotic fissure (**Plate II.1-9A**).

The interorbital region is already supported, as the paired cartilaginous trabecular bars (= trabeculae cranii) are extended from the anterolateral margin of the acrochordal cartilage. Close to this connection, each trabecular bar is slightly curved outwards, whereas the larger rostral part runs parallel to the median plan of the skull. Most probably, the caudal small part corresponds to the polar cartilage (= cartilago polaris), whereas the rostral part corresponds to the trabecula (*s.s.*). The position of the fissure, through which passes the internal carotid artery, and which is clearly observed in later stages, supports this hypothesis (see Discussion). Anteriorly, the trabecular bars are still separated from each other, thereby bordering a relatively large interorbital space, thus representing a typical platybasic skull type.

Splanchnocranium - The cartilaginous dorsal and ventral elements of the mandibular and hyoid arch are already present, whereas the premandibular palatine is not. All mandibular and hyoid parts are fused to each other from the moment they arise, thus forming a single cartilaginous piece. The distinction between the pterygoquadrate (dorsal part of the mandibular arch) and the hyosymplectic (dorsal part of the hyoid arch) cannot be made. This part of the fused complex has been referred to as the 'hyo-symplectic-pterygoquadrate plate' (ARRATIA, 1992). The hyosymplectic bears a distinct posterior opercular process, for the articulation with the opercular bone, which has already started to differentiate at this stage (see II.1.2). The foramen for the passage of the hyomandibular nerve trunk (VII) is already formed. Dorsally the hyosymplectic articulates with the neurocranium at the level of the anterior otic cartilage.

Surprisingly, Meckel's cartilage is fused to the pars quadrata of the pterygoquadrate, whereas the ceratohyal is fused to the hyosymplectic. The latter fusion occurs through a small cartilaginous bar which corresponds to the interhyal, as can be derived from later stages. Left and right lower jaws have fused rostrally. The ceratohyals are also fused together, via a cartilaginous mass interconnecting their rostral tips. The latter cartilaginous mass has the position of the basihyal although presumably does not correspond to it (see Discussion). Posterior to and continuous with this alleged basihyal, lies a forked chondrification. The anterior part corresponds to the first basibranchial cartilage, whereas the two branches correspond to the future

hypobranchials I and ceratobranchials I. Both elements, however, cannot yet be distinguished at this stage. No epi- or infrapharyngobranchials nor other branchial arches could be observed.

5.6 MM SL SPECIMEN (**Plate II.1-2**)

Neurocranium - At this stage, both posterior and anterior otic cartilages have formed at both sides. Caudally, the posterior otic cartilages become extended, as they grow in the direction of a lateral process of the parachordal plates. Serial sections revealed the true nature of this process, as the path of the glossopharyngeus (IX) and vagus (X) nerves could be followed. The glossopharyngeus nerve passes through the skull floor, anterior to this process, whereas the vagus nerve passes it posteriorly. This process thus corresponds to the posterior basicapsular commissure and not to the occipital arch, which lies posterior to the nervus vagus. Consequently, the large foramen is bordered by (1) the anterior basicapsular commissure rostrally, (2) the parachordal plates medially, (3) the otic cartilages laterally and (4) the posterior basicapsular commissure caudally. As the vagus nerve no longer passes through the skull floor through this foramen, the foramen does no longer correspond to the fissura metotica but to the basicapsular fenestra. Anterodorsally the anterior otic cartilage bears a small process, which most probably corresponds to the primordia of the taenia marginalis posterior. Anteriorly, the trabecular bars have become elongated and start to curve medially, but still remain separated from each other in the ethmoid region. Anteriorly, the trabecular bars have formed a lateral plate-like extension, the solum nasi.

Splanchnocranium - For the first time, the palatine is discernible, already articulating with the solum nasi of the ethmoid plate. From this level on, the cartilaginous connection between Meckel's cartilage and the pars quadrata of the pterygoquadrate is lost, and an articulation is formed. The serial sections showed that an articulation was present but not yet pronounced, which indicates that the articulation may just have been formed at a SL of about 5.6 mm. Rostrally, both mandibular bars are still fused together. The ceratohyals are still continuous with the hyosymplectic, through the interhyal, as well as being connected to each other rostrally. The cartilage interconnecting the hyoid bars is elongated posteriorly, as it is fused to the basibranchial element of the first branchial arch. The hyosymplectic itself has undergone no major transformations, except becoming more substantial. Rudiments from the first three branchial arches can now be distinguished. The anterior one consists of the ceratobranchials which are continuous with the basibranchials. The differentiation of the hypobranchial I, which arises from the cartilage anterior to the ceratobranchial, could not be made yet. The following two arches are represented by rudimentary ceratobranchials only: the primordium of the ceratobranchial III being shorter than that of ceratobranchial II. Still no epi- or infrapharyngobranchials could be discerned. Sections showed that these three branchial arches may already be functional, as hemibranchial structures were present at their caudal side.

6.0 MM SL SPECIMEN (**Plate II.1-3**)

Neurocranium - At this level, the ethmoid region becomes differentiated. The trabecular bars have reached each other anteriorly, where they have fused. Based on the shape of the thus formed ethmoid plate (= cartilago ethmoideum), it appears that the fusion has occurred through a medial extension of the trabecular bars and is not only the result of the mediad curvature of the rostral tips. Laterally, the ethmoid plate has small

processes corresponding to preethmoid cornua. This trabecular fusion occurs earlier as it is already observed in a 5.8 mm SL larva, thus forming a large hypophyseal fenestra. Rostrally, the ethmoid plate bears a dorsal process, which will fuse with the skull roof later and which eventually will contribute to the formation of the precerebral lamina. In most teleosts a medial and single process is formed, referred to as the septum internasale. In *Clarias gariepinus*, however, two lateral processes are formed. To avoid confusion and misinterpretation, these processes in *C. gariepinus* will be referred to as the precerebral processes. At the lateral face of the ethmoid plate, the solum nasi has become more pronounced and has formed a dorsal process as well: the orbito-nasal lamina, *sensu strictu*. This lamina is situated at the level of the articulation between the solum nasi and the palatine. The taeniae marginales posteriores now have become extended anteriorly, where they become branched at the level of the orbito-nasal lamina, *sensu strictu*. Three branches are formed: (1) medially, the rudiments of the epiphysial bridge (= pons epiphysialis) arise, (2) rostrally, a sphenoseptal commissure reaches up to the precerebral process of the ethmoid plate and (3) laterally, a spheno-ethmoidal commissure is directed to the orbito-nasal lamina, *sensu strictu*. The fact that the lamina orbito-nasalis, *sensu strictu,* is situated at the same level as the epiphysial bridge, suggests that the anterior taenia marginalis is strongly reduced in *C. gariepinus*. Prior to the fusion between the spheno-ethmoidal commissure and the orbito-nasal lamina, *sensu strictu*, a foramen for the ophthalmic ramus is formed (**Plate II.1-8A**). Serial sections of a 5.9 mm SL stage revealed, however, that this foramen arises by the secondary formation of a cartilaginous bridge between the spheno-ethmoidal commissure and the sphenoseptal one, ventrally to the ophthalmic ramus (**Plate II.1-4**). The fused spheno-ethmoidal commissure and orbito-nasal lamina, *sensu strictu* give rise to the first reinforcement between the skull roof and the skull floor, *i.e.,* the orbito-nasal lamina, *sensu latu*. The now partially enclosed sphenoid fissure, surrounded by the taenia marginalis posterior, the trabecular bar and the anterior basicapsular commissure, is penetrated by several cranial nerves: the olfactory nerve (I) (= fila olfactoria), the optic nerve (II) (= fasciculus opticus), the oculomotorius nerve (III), the trochlearis nerve (IV), the trigeminus nerve (V), the abducens nerve (VI) and the facialis nerve (VII). At the level of their fusion with the otic capsules, the taeniae marginales posteriores have become broader. This differentiation is referred to as the postorbital process, which bears, at its ventrolateral face, the anterior part of the hyosymplectic articulation.

The trabecular bar bears a dorsal process, medial to the orbito-nasal lamina, which will give rise to the preorbital base (= preorbital root) (**Plate II.1-8B**). In general, however, the preorbital base arises as a ventral outgrowth of the taenia marginalis anterior, and not as a dorsal extension of the trabecular bars (DAGET, 1964). Posteriorly, the trabecular bars show a small, medial excavation for the housing of the internal carotid artery. In the otic region, the formation of a cartilaginous roof covering the brain has been initiated, as the otic cartilages start to grow medially. At this stage, both otic cartilages are connected through cartilaginous bars to the parachordal plate, leaving two foramina. From the position of the foramina and the penetration of certain cranial nerves, as well as observations of further stages, the possible true nature of these structures could be revealed (**Plate II.1-9**). The basicapsular fenestra of the previous stage (5.6 mm SL) has now become subdivided into two small foramina by the formation of the basivestibular commissure. The anterior basicapsular fenestra is enclosed by: (1) the anterior basicapsular commissure anteriorly, (2) the otic cartilages laterally, (3) the parachordal plates medially and (4) the basivestibular commissure posteriorly. Presumably, no nerve or blood vessel passes through this fenestra as it disappears later during ontogeny. The other foramen, the posterior basicapsular fenestra is then surrounded by: (1) the basivestibular commissure anteriorly, (2) the posterior otic cartilage laterally, (3) the parachordal plate medially and (4) the posterior basicapsular commissure posteriorly. This fenestra corresponds to the foramen of the glossopharyngeal nerve since that nerve runs through it. Caudally to the posterior basicapsular commissure, the occipital arch is formed, which is already fused with the

parachordal plates. These paired pilae occipitales grow dorsally, as they border the notochord, but have a laterodorsal extension as well, toward of the otic capsules.

Splanchnocranium - The major changes that occurred in this stage are related to the further differentiations of the gill arches, whereas several bones have started their development. In relation to the ossification of the maxillary bone, a submaxillary cartilage has formed at the rostral tip of each palatine. This cartilage facilitates the articulation between the palatine and the maxillary bone, as part of the palatine-maxillary mechanism. At the posterior tip of Meckel's cartilage a more pronounced retroarticular process is formed, whereas a distinct articulation with the quadrate is now present. At the anterodorsal border of the quadrate, a small pterygoid process is present. The ceratohyals are rather flattened structures, directed in an oblique, vertical plane. They are still interconnected rostrally, but the connection with the basibranchial is lost. At their rostral tips, the hyoid bars now show a small incision at the anterior face, where the hyoid artery passes. The first signs of the differentiation of the hypohyals thus have appeared. No sign of a basihyal is observed. The hyosymplectic articulates with the ventrolateral wall of the neurocranium, in front and partially below the anterior semicircular canal.

The ceratobranchials II and III now have become extended, apparently both ventrally and dorsally. The ceratobranchials IV and V have developed as well at this stage. The medial tips of ceratobranchials I and II are broadened, which corresponds to hypobranchials I and II. The hypobranchials have not yet become separated from the ceratobranchials. In between the left and right hypobranchials I and II, a single cartilaginous copula is present. At this stage, it consists of the fused basibranchials I, II and III, corresponding to the anterior copula. All epibranchials (I-IV), as observed in adults, are present now, as well as all infrapharyngobranchials (I-IV). No uncinate process could be observed on epibranchial III. All epibranchials are rod-like, whereas the last one, epibranchial IV, appears to be more substantial than the other ones. The infrapharyngobranchials are differently built: I and II are strongly reduced and continuous with the epibranchials and have fused to each other and also to the third, whereas III and IV are separate, solid elements articulating with the epibranchials. The latter two lie perpendicular to the corresponding epibranchials. The difference in shape is related to the fact that the infrapharyngobranchials III and IV play a supportive role for the upper pharyngeal jaws, whereas the anterior two do not. These pharyngeal jaws *s.s*[2], which are dermal bone plates bearing teeth, are already observed in this stage.

6.6 MM SL SPECIMEN (**Plate II.1-5**)

Neurocranium - As most structures were formed in the previous stage, further development is for the greater part characterised by fusion and enlargement of those existing structures. In the ethmoid region, two additional reinforcements between skull roof and skull floor have occurred: (1) the precerebral process fuses with the sphenoseptal commissure, and (2) the preorbital base fuses with the taenia marginalis posterior. As a result, the sphenoid fissure of the previous stage now becomes completely closed and divided. The foramen, anterior to the preorbital base, is penetrated by the fila olfactoria. The foramen, posterior to this base, is then penetrated by the other nerves which previously penetrated the sphenoid fissure (II - VII). The latter foramen is referred to as the sphenoid fenestra. The fila olfactoria only penetrate the olfactory foramen and do not run through the orbito-nasal foramen. The latter foramen, bordered by the orbito-nasal lamina (dorsally and laterally), the solum nasi

[2] The terminology adopted here is the following: (1) pharyngeal jaws *s.l.*, when referring to the complete structure involving related cartilage and bones; and (2) pharyngeal jaws (*s.s.*), when referring to the dermal tooth plates only. As in *Clarias gariepinus* the tooth plates remain separated in the upper pharyngeal jaws *s.l.*, the term 'pharyngeal jaws' used here refers to 'pharyngeal jaws *s.l.*.

(ventrally) and the preorbital base (medially), is penetrated by the orbito-nasal artery. The medial processes of the taeniae marginales posteriores have become extended, but have not reached each other yet. The otic cartilages have become extended medially as well, where they have fused with the laterodorsal part of the pilae occipitales. Consequently, the foramen for the vagus nerve is bordered completely. Left and right pilae remain separated from each other. Posterolaterally, the otic capsule bears a distinct process, here referred to as the postotic process.

Splanchnocranium - The palatine now has developed a double-headed, rostral tip, for the articulation with the maxillary bone (ADRIAENS & VERRAES, 1997a) (see II.2.2). This double-headed rostral tip does not show a double articulation with the maxillary bone, which is the case in Diplomystidae (ARRATIA, 1987), but forms a slit into which the latter bone fits and articulates. Meckel's cartilage has hardly changed, whereas the pterygoid process has enlarged. The quadrate has become extended, compared to the previous stage. This trend is noted in the later stages as well. The ceratohyal is still continuous with the hyosymplectic through the interhyal. Rostrally, left and right hypohyals have become separated from each other. The incision for the hyoid artery can clearly be distinguished now, as well as a posterior process.

The branchial basket is now almost completely formed. Only the fifth basibranchial element appears to be missing. The fourth basibranchial lies in front of the base of the fourth branchial arch. The hypobranchial cartilage of branchial arches I to III now have become isolated from the ceratobranchials, thus articulating with them from this stage on. Hypobranchial IV cannot be distinguished yet, but as the medial tip of the ceratobranchial IV bears a large, unossified region later during ontogeny, it can be suggested that the hypobranchial cartilage does not become separated from that of the ceratobranchial IV during ontogeny (see II.1.2). No sign of hypobranchial V is apparent.

7.1 MM SL SPECIMEN (**Plate II.1-6**)

Neurocranium - The cartilage between the precerebral processes becomes elevated now, thus forming a transverse precerebral lamina. The olfactory foramen becomes smaller as the preorbital base becomes expanded in an anteroposterior direction (**Plate II.1-8C**). The previously cylindrical orbito-nasal lamina now has become plate-like, transversely positioned on the solum nasi. It is pierced by the ophthalmic foramen, dorsally, and the orbito-nasal foramen, ventrally. In between these foramina, the nasal barbel originates. The epiphysial bridge is now completed, as the left and right processes have fused. This narrow bridge consequently divides the large opening in the skull roof into a prepineal and a postpineal fontanella. The medial incision of the trabecular bar for the internal carotid artery becomes extended laterally, creating a rather narrow connection in the skull floor. Few changes have occurred in the shape of the otic capsules, except for the occipital arches which have become extended. Left and right pilae occipitales have now fused above the neural tube, forming the tectum posterius, thus enclosing the foramen magnum. The posterior position of this left and right fusion suggests that it is the pilae occipitales which have fused, and not the otic capsules, which would result in a tectum synoticum. During further ontogeny, the otic capsules do not fuse with each other, when they become reinforced by several ossifications. Posterior to the tectum posterius, the supraneurals situated in front of the second and third vertebrae have fused, forming a solid cartilaginous plate overlying the basidorsals 2 and 3 (RADERMAKER *et al.*, 1989). The foramina of the glossopharyngeus and vagus nerves have decreased their

diameter, whereas the anterior basicapsular fenestra has disappeared. This must occur between 6.9 mm SL and 7.1 mm SL (**Plate II.1-9C-D**).

Splanchnocranium - Meckel's cartilage, which has now developed a dorso-lateral coronoid process, is no longer fused to the contralateral one. The retroarticular process has become even more elongated posteriorly. The pterygoid process is well-developed, as it grows in the direction of the palatine, but never contacts it. The quadrate has a well developed facet for the articulation with the mandible[3]. Still no distinction can be made between the pterygoquadrate and the hyosymplectic. The hypohyals can be distinguished even better from the ceratohyals as the rostral incision and the caudal process are more pronounced.

The anterior copula, which is the result of the fusion between the anterior three basibranchials, now fits with its anterior tip in between the caudal processes of the hypohyals. The fourth basibranchial, which was observed in the previous stage, is now apparently elongated, its lateral margin abutting against the base of the fourth branchial arch, its posterior margin lying in front of the fifth ceratobranchials. Apparently the fifth basibranchial has formed and is fused to the fourth, thus forming the copula posterior. The hypobranchials of the first and second branchial arches are situated laterally to the copula, articulating with it. The third hypobranchials articulate with the posterior tip of the anterior copula, and also contact each other. The first signs of the fourth hypobranchials are evident, as the medial tip of the ceratobranchial is broadened and bears a small incision. The third epibranchial bears a caudally directed uncinate process. The fourth epibranchial is still more stout than the other ones, possibly acting as the support of the fourth infrapharyngobranchial, which is also more substantial.

10.0 MM SL SPECIMEN (**Plate II.1-7**)

Neurocranium - At this stage, some regions in the cartilaginous skull have become more solid. The ethmoid plate has become extended posteriorly, whereas the olfactory foramen and the sphenoid fenestra have become smaller, due to the expansion of the preorbital base. This base now runs from well in front of the orbito-nasal lamina up to about half-way to the eye. No preorbital foramen was observed penetrating the preorbital base. The sphenoseptal commissures are more solid, as well as the precerebral lamina interconnecting the left and right commissures. The epiphysial bridge has also thickened, whereas a median posterior widening is noted, corresponding to the taenia tecti medialis posterior. In the skull floor, the trabecular bars have become even narrower at the level of the fissure for the internal carotid artery. This weakening is, however, compensated by the broadening of the skull roof at that level, as well as osteological reinforcements (see II.1.2). The posterior taeniae marginales have broadened as well, as they become more confluent with the otic capsules. The broadening of the taeniae is coupled to the formation of a foramen, penetrated by the ramus oticus of the nervus facialis (SURLEMONT, 1983). Different stages showed that this foramen arises by the initial formation of a medial incision in the taenia marginalis (7.1 mm SL, **Plate II.1-6**), which later gets closed off medially by a minute cartilaginous strip (in between 7.4 and 7.7 mm SL, **Plate II.1-8D**). No commissura lateralis, nor a pila lateralis is formed. The otic capsules still have not fused with each other, whereas the tectum posterius now appears to have fused with the previously mentioned supraneurals.

[3] 'Mandible' in this thesis refers to one half of the functional lower jaw: early during ontogeny this corresponds to Meckel's cartilage, whereas later on, the combination of this cartilage with the surrounding bones is meant.

Splanchnocranium - No significant changes have occurred, compared to the previous stage. The foramen for the truncus hyomandibularis has enlarged, whereas the opercular process is more pronounced. The interhyal is still continuous with both the ceratohyal and the hyosymplectic, although a constriction can be seen at the fusion with the ceratohyal. Later during ontogeny, in the 21.4 mm stage, this fusion is lost, whereas the former is still continuous with the hyosymplectic (VANDEWALLE *et al.*, 1985).

At its medial tip, the fourth ceratobranchial element has become differentiated, as it has become broader and has developed a small caudal process. This part most probably corresponds to the fourth hypobranchial, which remains continuous with the cartilage of the ceratobranchial.

19.0 MM SL SPECIMEN (**Plate II.1-10**)

This stage is only mentioned to give the relation between the fully developed chondrocranium and the initial osteocranium. However, some changes have occurred in the chondrocranium, compared to the previous situation. The ethmoid plate has become even further extended posteriorly. It appears that the taenia marginalis anterior does form in *Clarias gariepinus*, though late in ontogeny (see III.1). The foramen for the internal carotid artery now has cut its way completely through the trabecular bars, resulting in the confluence of the hypophyseal fenestra and the sphenoid fenestra. At this stage, this loss in reinforcement is compensated by the ossifications of the skull floor and skull roof (see II.1.2). Each of the cartilaginous parts of the neurocranium have become enclosed, covered or partially replaced by bones, with exceptions of some smaller regions in between the bones.

DISCUSSION

Published data generally suggest that the chondrification of the neurocranium starts simultaneously with that of the splanchnocranium (DE BEER, 1937). However, asynchronies have been observed where the neurocranial skeleton arises prior to the visceral one [*e.g., Catostomus commersoni* (Cypriniformes, Catostomidae) (MCELMAN & BALON, 1980); and *Heteropneustes fossilis* (Siluriformes, Heteropneustidae) (SRINIVASACHAR, 1959)], or afterwards as in *Heterobranchus longifilis* (Siluriformes, Clariidae) (VANDEWALLE *et al.*, 1997). In the latter species, primordia of the mandibular and hyoid arch are formed prior to neurocranial elements. In *Clarias gariepinus* both neurocranium and splanchnocranium were observed in the first stage, however, as earlier stages of *C. gariepinus* (smaller than 4.1 mm SL) were not available, it cannot be excluded that the situation may be comparable to that in the closely related *H. longifilis*.

It has to be noted that when comparing with data from the ontogenetic study of *C. gariepinus* done by SURLEMONT *et al.* (1989) and SURLEMONT & VANDEWALLE (1991), some ontogenetical evidence suggests that the 5.2 mm TL stage of SURLEMONT *et al.* (1989) is more developed than the presently observed 5.6 mm SL (5.9 mm TL) specimen.

NEUROCRANIUM

PLATYBASIC SKULL - As already mentioned, the adaptation of most catfishes to a benthic and nocturnal life style is reflected in several structural transformations, compared to generalised teleosts. These, however, are not restricted to the fully developed, bony skull but arise early during ontogeny. When, for example, compared to the Characiformes [closely related according to the phylogeny proposed by FINK & FINK (1981)], the general trend in overall siluriform head morphology appears to involve a reduction of eye size, as well as the dorsoventral flattening of the skull. This trend can, to a certain degree, be expressed as the ratio of the eye diameter to the interorbital distance (ADRIAENS & VERRAES, 1997e). In Characiformes this ratio may range from 0.3 (in *Citharinops distichodus*, Distichodontidae) (ROMAN, 1966) up to 2.2 (in *Hepsetus odoe*, Hepsetidae) (DAGET, 1962), whereas the average value is about 1.0 (based on biometrical data from literature of 29 species of four families) (BOULENGER, 1909-1916; GILTAY, 1929; POLL, 1945; 1954; 1967a; 1967b; DAGET, 1954; POLL & GOSSE, 1963; ROMAN, 1966; POLL & DAGET, 1968; MAHNERT & GÉRY, 1987; TEUGELS & THYS VAN DEN AUDENAERDE, 1990). In Siluriformes the ratio may range from 0.1 (in *Dinotopterus cunningtoni*, Clariidae) (TEUGELS, 1986) up to 1.2 (in *Cheirocerus eques*, Pimelodidae) (STEWART & PAVLIK, 1985) with an average value of approximately 0.4 (based on biometrical data from literature of 58 species of 17 families) (STEINDACHNER, 1914; BOULENGER, 1915; PELLEGRIN, 1928; SCHULTZ, 1942; CRASS, 1960; BLACHE, 1964; THYS VAN DEN AUDENAERDE, 1964; MYERS & WEITZMAN, 1954; 1966; ALFRED, 1966; ROMAN, 1966; POLL *et al.*, 1972; GLODEK, 1976; THYS VAN DEN AUDENAERDE & DE VOS, 1982; DE VOS & LEVEQUE, 1983; NIJSSEN & ISBRÜCKER, 1983; 1987; 1990; STEWART, 1985; 1986; STEWART & PAVLIK, 1985; TEUGELS, 1986; VARI & ORTEGA, 1986; RISCH, 1987; REIS & SCHAEFER, 1992; LUCENA *et al.*, 1992). Within the siluriforms this trend is even more extreme in Clariidae (TEUGELS, 1986) (**Plate IV.2-6**). As can be expected, the spatial impact of the eye size on the surrounding structures will be of more importance during the early development than in the adult situation. It is observed that in *Clarias gariepinus* the eye diameter of a 7.2 mm TL specimen is approximately 14.3% of the head length (SURLEMONT, 1983) whereas in adults of 685 mm TL (600 mm SL) it reaches only 6.1% (SKELTON & TEUGELS, 1992). As has been demonstrated by Corsin (1961), the development of the trabecula communis in tropibasic skulls is influenced by the spatial interactions with the developing eyes. When the eyes fail to develop properly in salmons, it is noted that the trabecular bars do not fuse with each other, and the growth of the taenia marginalis is influenced (VERRAES, 1974a). The reduced eye size in siluriforms thus explains the absence of a pronounced trabecula communis, and consequently the formation of a platybasic skull.

SKULL FLOOR - The neurocranial ontogenetic sequence generally starts with the parachordal cartilages on either side of the notochord, followed by the chondrification of the trabecular bars. Both fuse early in ontogeny, thus forming the initial neurocranial floor from the interorbital region up to the otic and occipital region. In general, the distinction can be made between the trabecula *s.s.* and the polar cartilages[4](SRINIVASACHAR, 1959). The position of the incision, into which passes the internal carotid artery, may indicate the anterior margin of the cartilago polaris (GOODRICH, 1958; BERTMAR, 1959). In relation to the platybasic skull configuration, the contralateral trabecular bars (= trabecula cranii) remain separated from each other by a broad fenestra hypophysea. This requires a broad ethmoid plate or medially curved trabecular bars. In most catfishes the ethmoid plate is broad, although the trabecular bars appear to be slightly curved medially as well, *e.g., Clarias gariepinus* (**Plates II.1-2, 3**). In Schilbeidae, which have laterally compressed skulls, the ethmoid is narrower, with the trabecular bars

[4] Concerning the anatomical nomenclature of the 'polar cartilage', no consistent scientific name has been introduced yet. In general these structures are referred to in a native language, as for example 'polar cartilage' (DE BEER, 1937; GOODRICH, 1958; JARVIK, 1980), 'cartilages polaires' (DEVILLERS, 1958; DAGET, 1964) or 'Polknorpel' (MARINELLI, 1936). In order to introduce a single, consistent and scientific terminology to avoid confusion, we introduce the term 'cartilago polaris'.

curved medially (SRINIVASACHAR, 1957a). In general the trabecular bars fuse with the caudal border of the ethmoid plate, although in callichthyids it may fuse with its dorsal face (HOEDEMAN, 1960a). The trabecular bars become broadened as well in both a lateral (for the support of the nasal sac), and a medial direction (for the support of the forebrain). The lateral expansion results in a solum nasi, which may be very much pronounced (*e.g., Silonia*, Schilbeidae) (SRINIVASACHAR, 1957a). It is well developed in clariids as well (**Plate II.1-7**) (SRINIVASACHAR, 1959). The medial expansion results in a fusion between the anterior part of the left and right trabecular bars. Consequently, the platybasic siluriforms do have, although only slightly, a trabecula communis (SRINIVASACHAR, 1958a). In many siluriforms, rostrolateral processes of the ethmoid plate are formed. These preethmoid cornua are distinct in Ariidae (SRINIVASACHAR, 1958a), whereas Plotosidae (SRINIVASACHAR, 1958a) and Schilbeidae (SRINIVASACHAR, 1957a) do not have them. In Clariidae and the closely related Heteropneustidae, as well as in Pangasiidae, the preethmoid cornua are rudimentary (**Plate II.1-7**) (SRINIVASACHAR, 1957a; 1959).

As a consequence of the broad ethmoid plate, a transverse precerebral lamina is formed instead of a longitudinal septum internasale (**Plate II.1-7A**). This, together with the posterior position of the lamina orbito-nasalis, enables the fila olfactoria to lie in a transverse direction of the olfactory lobes, instead of an anteroposterior one. This implies that they can be rather short as the lobi olfactorii come to lie medial to the nasal sacs, instead of posteromedially (SURLEMONT, 1983). In *Ictalurus nebulosus* it is noted that "... the olfactory lobe protrudes through this foramen ...", the latter referring to the olfactory foramen (KINDRED, 1919). In some cases, the precerebral lamina becomes extended posteriorly in a 'nasal septum' (*e.g., Arius jella* and *Ailia coila*) (SRINIVASACHAR, 1957a; 1958a). According to DAGET (1964) this precerebral lamina and the internasal septum are homologous.

SKULL ROOF - The chondrocranial roof in teleosts is generally formed by the taenia marginalis (frequently referred to as the 'orbital cartilages'). They arise as separate cartilaginous elements which become elongated in an anterior and posterior direction (DE BEER, 1937; DAGET, 1964). The taenia marginalis can be subdivided in a taenia marginalis anterior (anterior to the epiphysial bridge) and a taenia marginalis posterior (posterior to the epiphysial bridge). The anterior one normally bifurcates at its rostral tip: an anteriorly directed commissura sphenoseptalis and a laterally directed commissura spheno-ethmoidalis are consequently formed (BERTMAR, 1959; DAGET, 1964). In siluriforms, however, the taenia marginalis posterior appears to arise as a rostral extension of the anterior otic cartilage, at the processus postorbitalis, instead of being isolated from it (**Plate II.1-2B**) (SRINIVASACHAR, 1959: 399; HOEDEMAN, 1960a: 76). The taenia marginalis anterior becomes reduced or is almost completely absent, since in most siluriforms the epiphysial bridge lies at the same level as the lamina orbito-nasalis. A distinct taenia marginalis anterior is present in *Arius* (Ariidae) and *Plotosus* (Plotosidae) (SRINIVASACHAR, 1958a) but is short in *Silonia* and *Ailia* (Schilbeidae) (SRINIVASACHAR, 1957a). In species like *Heteropneustes* (Heteropneustidae) (SRINIVASACHAR, 1959), *Pangasius* (Pangasiidae) (SRINIVASACHAR, 1957a), *Ictalurus* (Ictaluridae) (KINDRED, 1919), *Callichthys* (Callichthyidae) and *Clarias* (**Plate II.1-5**) (SRINIVASACHAR, 1959) the taenia marginalis anterior is completely reduced, whereas its bifurcation is situated at the level of the epiphysial bridge (**Plate II.1-8A**). In siluriforms the sphenoseptal and the spheno-ethmoidal commissures fuse with two dorsal processes of the chondrocranial floor: (1) the precerebral process of the ethmoid plate and (2) the lamina orbito-nasalis, *s.s.*, of the solum nasi, respectively (**Plate II.1-8**). The latter fusion generally precedes the former one during ontogeny. The sphenoseptal commissure then becomes elongated rostrally and fuses with a small precerebral process. Surprisingly, in *H.*

longifilis this commissure fails to develop; here the precerebral process becomes elongated in a caudal direction, until it fuses with the taenia marginalis (VANDEWALLE *et al.*, 1997).

A pons epiphysialis is present in most siluriforms, although in *Heteropneustes fossilis* it fails to develop (SRINIVASACHAR, 1959). A small taenia tecti medialis posterior is present in *Clarias gariepinus* (**Plate II.1-7E**), *Arius jella*, *Plotosus canius* (SRINIVASACHAR, 1958a), *Ailia coila, Silonia silondia* and *Pangasius pangasius* (SRINIVASACHAR, 1957a), whereas it is not distinct in *Ictalurus nebulosus* (KINDRED, 1919), *Callichthys callichthys* (HOEDEMAN, 1960a) and *Heterobranchus longifilis* (VANDEWALLE *et al*., 1997).

SKULL WALL - At the same level of the lamina orbito-nasalis, a preorbital base is generally present in siluriforms. This structure arises as a ventral process of the taenia marginalis which eventually fuses with the underlying trabecula cranii. In *Clarias gariepinus*, however, a small process is found on the trabecula cranii which suggests that the preorbital base arises on the trabecula cranii in this species (**Plate II.1-8B**). In siluriforms this base becomes broad, thus separating the olfactory and sphenoid foramina, and may be perforated by a preorbital foramen (= 'preoptic foramen') [*e.g.,* Pangasiidae (SRINIVASACHAR, 1957a), Ariidae, Plotosidae (SRINIVASACHAR, 1958a), Ictaluridae (KINDRED, 1919)]. Such a foramen is absent in Clariidae (**Plate II.1-8D**) (SRINIVASACHAR, 1959; VANDEWALLE *et al.,* 1997), Heteropneustidae (SRINIVASACHAR, 1959) and Schilbeidae (SRINIVASACHAR, 1957a). Surprisingly, it can be observed in the dwarf specimens of *Clarias gariepinus* (see III.1).

OTIC CAPSULES - Although some changes in position and shape of the involved structures are observed, in respect to the generalised teleostean situation, the otic region is comparable in most siluriforms. According to DAGET (1964) the otic capsule arises as two consecutive elements (cartilago oticalis anterior and posterior). The anterior one becomes connected to the cartilago parachordalis through a commissura basicapsularis anterior, whereas the posterior one is attached through a commissura basivestibularis (rostrally) and a commissura basicapsularis posterior (caudally). The identification of these structures is based on their relation with the nervus glossopharyngeus and the nervus vagus. In *Clarias gariepinus*, and in siluriforms in general, the primordia of the otic region comprise a cartilago oticalis anterior, connected to the cartilago parachordalis through a commissura basicapsularis anterior (**Plate II.1-9A**) (SRINIVASACHAR, 1957a; 1959). Posterior to this commissure the glossopharyngeus and vagus nerves pass through a fissura metotica. At the 6.0 mm SL stage of *C. gariepinus* two foramina are present, penetrating the floor of the otic capsules (**Plate II.1-9B**). Serial sections (of a 5.9 mm SL stage) revealed that the glossopharyngeus nerve passes through the posterior one. The vagus nerve, on the other hand, lies caudally in a fissure bordered by the pila occipitalis (medially) and the cartilago oticalis posterior (laterally). Eventually, this fissure becomes closed as the pila occipitalis and the otic capsule fuse dorsally. The anterior foramen becomes reduced and eventually disappears (**Plate II.1-9C-D**). In a generalised teleostean situation the foramina of these two cranial nerves are separated from each other by the commissura basicapsularis posterior, whereas the glossopharyngeus nerve passes through the fenestra basicapsularis posterior. Consequently, in the case of *C. gariepinus* the cartilaginous bar in between the two foramina in the otic floor, corresponding to the fenestra basicapsularis anterior and posterior, represents the commissura basivestibularis (**Plate II.1-9B**). In the fully developed chondrocranium the posterior basicapsular fenestra (= foramen nervus glossopharyngeus) and the foramen for the vagus nerve remain present (**Plate II.1-9D**). A comparable situation can be found in *Heteropneustes fossilis* (SRINIVASACHAR, 1959) and *Plotosus canius*

(SRINIVASACHAR, 1958a). In *Arius jella* the fenestra basicapsularis anterior remains present and is rather large, whereas the glossopharyngeus and vagus nerves still pass through the fissura metotica (SRINIVASACHAR, 1958a). The same construction is found in the schilbeid *Ailia coila*, but foramina are absent in *Silonia silondia*.

In the occipital region, a fusion between the left and right part of the braincase is present in siluriforms. This fusion can be spread over the otic capsules and the occipital arches. However, in several catfishes only the pilae occipitales appear to fuse, thus forming a tectum posterius. The fusion between the otic cartilages, resulting in a tectum synoticum is absent (*e.g.,* Clariidae, Callichthyidae) (**Plate II.1-7A**) (HOEDEMAN, 1960a) or greatly reduced (*e.g.,* Ariidae, Plotosidae, Pangasiidae) (SRINIVASACHAR, 1957a; 1958a). In *Ailia coila* it is rather pronounced (SRINIVASACHAR, 1957a). In many cases, however, the distinction between the two tecta is rather difficult to determine. An additional fusion observed in the nuchal area of *C. gariepinus* occurs between this tectum posterius and the first supraneural cartilages (**Plates II.1-6A, 7A**) (RADERMAKER *et al.*, 1989). A close contact between the third neural arch and the tectum posterius is observed in *Ictalurus nebulosus* (Ictaluridae) (KINDRED, 1919). Based on the relation between the supraneurals and the basidorsals in *C. gariepinus*, it appears that supraneurals of vertebrae two and three are involved (**Plate II.1-6C**). This might explain the absence of the anterior three supraneurals in adult Characiphysi, which is considered a synapomorphic feature by FINK & FINK (1981: characters 58, 59, 61). Apparently, only the anterior one is absent (= synapomorphy of Ostariophysi) (FINK & FINK, 1981: character 58), whereas supraneural two and three have fused with the neurocranium. Evidence from ontogeny even suggests that the anterior supraneural forms the claustrum of the Weberian apparatus (COBURN & FUTEY, 1996).

SPLANCHNOCRANIUM

PREMANDIBULAR ARCH - A synapomorphic feature of Siluriformes is the discontinuity of the palatine with the pterygoquadrate (FINK & FINK, 1981; ARRATIA & SCHULTZE, 1990; ARRATIA, 1992). This decoupling has enabled the development of a highly specialised palatine-maxillary mechanism for the controlled movements of the maxillary barbel (ALEXANDER, 1965; GOSLINE, 1975a; ADRIAENS & VERRAES, 1997a). Exceptionally, a secondary fusion between the palatine and the pterygoquadrate is present in Ariidae (BAMFORD, 1948: 380; SRINIVASACHAR, 1958a: 993). SRINIVASACHAR (1957a; 1958a; 1959), rather confusingly, referred to the palatine as 'pterygoid process', which he distinguished from the 'processus pterygoideus' of the pterygoquadrate. The palatine, which is considered to be homologous with the epipremandibular element (DAGET, 1964; JARVIK, 1980), articulates with the lamina orbito-nasalis by means of an 'ethmopalatine process' (**Plate II.1-8D**) (SRINIVASACHAR, 1958a). Presumably this articulation corresponds to the ethmopalatine articulation of other teleosts. The question could be raised whether this isolation of the palatine in siluriforms is a secondary separation between the premandibular and mandibular arch, which would support the hypothesis that the palatine really is derived from the premandibular arch. Supposedly, as an adaptation to the mechanical stress, generated in the palatine-maxillary mechanism, a submaxillary cartilage assists in the articulation of the maxillary bone with the palatine (**Plate II.1-8D**).

MANDIBULAR ARCH - In Siluriformes, in general, a single cartilaginous 'hyo-symplectic-pterygoquadrate plate' is formed, which even may be continuous with Meckel's cartilage (ARRATIA, 1990). In some siluriforms, however, this fusion is a secondary process. In *Galeichthys felis* (Ariidae) four separate elements are formed (pterygoquadrate,

hyosymplectic, interhyal and ceratohyal with hypohyal) whereas the pterygoquadrate eventually fuses with the hyosymplectic (BAMFORD, 1948). In other catfishes a trend is observed for additional fusions of the 'hyo-symplectic-pterygoquadrate plate' with the interhyal [*e.g.,* Ictaluridae (KINDRED, 1919; ARRATIA, 1992), Schilbeidae, Pangasiidae (SRINIVASACHAR, 1957a), Heteropneustidae (SRINIVASACHAR, 1959), Callichthyidae (HOEDEMAN, 1960a; HOWES & TEUGELS, 1989), Trichomycteridae (ARRATIA, 1990)] and even with the neurocranium [*e.g.*, Callichthyidae (HOEDEMAN, 1960a; HOWES & TEUGELS, 1989), Trichomycteridae (ARRATIA, 1990)]. This trend (*i.e.,* the fusion with the interhyal) is persistent in the Clariidae as well, as is shown in the present study (**Plate II.1-7C**) as well as in the literature (SRINIVASACHAR, 1959; SURLEMONT *et al.*, 1989; SURLEMONT & VANDEWALLE, 1991; ADRIAENS & VERRAES, 1994; VANDEWALLE *et al.*, 1997). In some of these siluriforms the interhyal is continuous with the ceratohyal as well, whereas in others an articulation is found. An interhyal, which initially is fused to the hyosymplectic has been observed in other ostariophysans as well, for example *Chanos chanos* (Gonorhynchiformes, Chanidae) (ARRATIA, 1990) and *Hepsetus odoe* (Characiformes, Hepsetidae) (BERTMAR, 1959).

In most siluriforms, Meckel's cartilages of both sides are fused to each other (Ariidae, Plotosidae, Ictaluridae, Heteropneustidae and Clariidae) (KINDRED, 1919; SRINIVASACHAR, 1958a; 1959), which is not the case in Callichthyidae (HOEDEMAN, 1960a). A processus coronoideus is present, although generally it is not pronounced. However, in later stages of *Clarias gariepinus*, once cartilage resorption and ossifications have started, the coronoid process becomes more distinct (**Plate II.1-11**). The continuity of Meckel's cartilage with the pars quadrata in the early stages is believed to play an important role for a passive mouth opening (SURLEMONT *et al.*, 1989). The retroarticular process is present but small in *C. gariepinus*, which is the general trend in siluriforms. However, in some species it may be reduced almost completely (SRINIVASACHAR, 1958a).

HYOID ARCH - The hyosymplectic generally bears a foramen for the passage of the truncus hyomandibularis of the nervus facialis. This foramen is generally observed in siluriforms, although in several species the nerve passes in front of the hyosymplectic, through an anterior slit (SRINIVASACHAR, 1957a; 1958a). Apparently the general ontogenetic sequence involves a passage of the truncus in front of the hyosymplectic which becomes enclosed by the anteriorly extending hyosymplectic cartilage, until a complete foramen is formed. In *Galeichthys felis* (Ariidae) the nerve initially passes through a slit at the posterior margin of the hyosymplectic, where it appears to cut its way through until it passes in front of the latter (BAMFORD, 1948). Although the symplecticum is considered to be absent in siluriforms, some evidence suggests the presence of a rudimentary, non-ossified symplectic region in the suspensorial cartilage. In *Diplomystes camposensis* (Diplomystidae), which represents the primitive catfish configuration, a rudimentary symplectic bone is present (ARRATIA, 1992: fig. 14). The absence of the symplectic bone in the majority of siluriforms may be a consequence of the cartilaginous link between the hyosymplectic and the pterygoquadrate (ARRATIA, 1990: 210). In a 21 mm TL stage of *Clarias gariepinus* HOWES & TEUGELS (1989) observed a gap where the margin between the pars quadrata and the symplecticum could be expected. Such a gap, however, was not observed in the examined specimens of the present study.

The hyoid bars of siluriforms are well developed. As already mentioned the ceratohyals are continuous with the interhyal in some siluriforms, whereas they are continuous with the hypohyals in all species. In general the latter bear an anterior groove for the passage of the arteria mandibularis [nomenclature according to VERRAES (1973)], although a true foramen is present in *Arius jella* (SRINIVASACHAR, 1958a). In *Clarias gariepinus* the hypohyals

initially are fused to each other as well as to the median cartilage of the branchial basket (**Plates II.1-1C, 2C**). The branchial cartilage becomes separated from the hypohyals, prior to the separation of the contralateral hypohyals (**Plates II.1-3C, 5C**). Suggestions have been made that the anterior part of the median branchial cartilage, which is in close relation with the hypohyals, would be a fusion between the basihyal and the first basibranchial (SRINIVASACHAR, 1959: 382). However, the position and number of basibranchials indicate that this anterior part is more likely to correspond to the first basibranchial only (see below). A basihyal would be expected anterior to the hypohyals, instead of posterior to them (NELSON, 1969). It is therefore generally accepted that the basihyal is absent in siluriforms (ARRATIA & SCHULTZE, 1990).

BRANCHIAL ARCHES - In the branchial basket of siluriforms, the following structures are generally distinguished: (1) an anterior and a posterior copula, (2) the hypobranchials I to III, (3) the ceratobranchials I to V, (4) the epibranchials I to IV. Concerning the infrapharyngobranchials, some variation is observed (see below). The ontogenetic sequence, observed in siluriforms, involves a differentiation from anterior to posterior. For each arch, the sequence starts with the chondrification of the ceratobranchials, followed by the hypobranchials and basibranchials, and eventually the epi- and infrapharyngobranchials (SRINIVASACHAR, 1959; VANDEWALLE *et al.,* 1997).

Based on both chondrocranial and osteocranial evidence, it appears that in siluriforms the anterior copula comprises the anterior three basibranchials, whereas only basibranchial II and III will ossify (NAWAR, 1954; SRINIVASACHAR, 1958b; LUNDBERG, 1982; DE PINNA & VARI, 1995) (see II.1.2). Some authors considered these as the ossa basibranchialia I and II, instead of II and III (TEUGELS *et al.*, 1991; SRINIVASA RAO & LAKSHMI, 1984). However, based on the fact that "for teleostomes, established usage dictates that a basibranchial be given the name or number of the paired arch-element behind it ..." (NELSON, 1969: 480) suggests that the basibranchial bones represent those of the second and third branchial arch. In Diplomystidae, the first basibranchial element becomes reduced, whereas the ossified second and third ones become separated from each other (ARRATIA, 1987: fig. 17). In a 127.0 mm SL stage of *Clarias gariepinus*, however, basibranchials I to III remain fused to each other, and basibranchial IV remains fused to the fifth one, thus forming an anterior and a posterior copula (**Plate II.1-7B**). These copulae correspond to the mistakenly named basibranchial I and II of *H. longifilis* (VANDEWALLE *et al.*, 1997: fig. 8B).

The siluriform hypobranchials of the first three branchial arches become separated from the ceratobranchial elements during ontogeny (**Plate II.1-5C**) (SRINIVASACHAR, 1959; VANDEWALLE *et al.,* 1997). In most siluriforms, the hypobranchials I and II articulate with the lateral margin of the anterior copula, whereas the third one lies in between the anterior and posterior copulae. Occasionally, the left and right hypobranchials III fuse (*e.g., Pangasius pangasius*, *Plotosus canius*) (SRINIVASACHAR, 1957a; 1958a). In some other species they come into close contact with each other (*e.g., Clarias gariepinus*) (**Plate II.1-20C**) or remain separated as the two copulae overlap in that region (*e.g., Arius jella*) (SRINIVASACHAR, 1958a). Hypobranchial IV has never been observed as a separate element in catfishes. However, the differentiation of the medial tip of ceratobranchial IV and the reduced ossification of that part suggests that hypobranchial IV is present but remains fused to the ceratobranchial IV (**Plates II.1-7B, 36D**).

Concerning the epibranchial configuration within siluriforms, a consistent trend is present. Epibranchials I to IV are always developed, whereas the fifth one, which frequently occurs in the closely related Characiformes, does not develop (BERTMAR, 1959; DAGET, 1964). An uncinate process of epibranchial III is present in *Clarias gariepinus* (**Plates II.1-7D, 13**), although it was not observed in several other siluriform species (SRINIVASACHAR, 1957a; 1958a; 1959). Although the form and number of epibranchials is rather constant in siluriforms, the way they are connected to the infrapharyngobranchials is very variable, as a result of the different infrapharyngobranchial constructions.

The developmental status of the infrapharyngobranchials in siluriforms, on the other hand, has led to varied nomenclature (**Table II.1- 1**, left part). According to the literature, the number of infrapharyngobranchials may range between two and four. Processes of reductions and fusions lie at the base of the high degree of assumed variation. In the primitive diplomystid situation, all four infrapharyngobranchials are present and even become separately ossified (NELSON, 1969: fig. 5B; ARRATIA, 1987: fig. 17). The anterior two infrapharyngobranchials are slender and lie parallel to the epibranchials, whereas the third and fourth ones are more solid and lie transversally against the epibranchials. An important feature is the presence of a cartilaginous mass interconnecting the medial bases of the infrapharyngobranchials I and II with epibranchials I and II, as well as with the anterior tip of infrapharyngobranchial III. Based on the interpretation of this cartilaginous mass, two possible hypotheses can be proposed for siluriforms (**Plate II.1-12**): (1) the cartilage is an additional cartilage which becomes reduced in most catfishes but remains present in *Clarias gariepinus*, or (2) no additional cartilage is present in diplomystids, but the cartilaginous mass in question represents the fused bases of the anterior two, partially reduced, infrapharyngobranchials with the epibranchials, which may even or may not be fused with the proximal tip of the third infrapharyngobranchial. A more detailed study of the ontogeny of the epi- and infrapharyngobranchial elements in *C. gariepinus*, however, reveals that the cartilaginous mass, at the medial tip of the anterior two epibranchials, is confluent with them, as well as with the third infrapharyngobranchial from the moment they arise (**Plate II.1-13A**: 9.1 mm SL stage). This configuration remains established until the connection with the latter one is lost (**Plate II.1-13D**: 21.5 mm SL stage). The position and the relation of this cartilaginous mass to the epibranchials during ontogeny, as well as its presence form the onset of epibranchial elements formation, suggests that indeed this mass represents the remains of infrapharyngobranchials I and II, instead of being an addition. If this hypothesis be applied for the configuration of other taxa described, a clear trend within siluriforms can be proposed (**Table II.1- 1**, right part). In descriptions of the siluriform chondrocranium, four infrapharyngobranchials are recognised, except in the Clariidae and closely related Heteropneustidae. Presumably, as the remnants are very indistinct (in *C. gariepinus*), they may have become lost completely or they may have been overlooked. In the descriptions of the osteocranium, all four infrapharyngobranchials are ossified, and consequently observed, in only a few species. In some cases, a fusion is proposed between the bony third and fourth infrapharyngobranchial (TILAK, 1961; 1963b; 1965a; 1967). However, when comparing this fused complex with the configuration in *C. gariepinus*, it is more likely to correspond with the fourth infrapharyngobranchial bone only, whereas TILAK's second one then corresponds with the third one in *C. gariepinus*. The ontogenetic evidence in *C. gariepinus* reveals no fusion between two ossification centra, which suggests that the complex infrapharyngobranchial might be a misinterpretation. Due to the fact that the strongly reduced first and second infrapharyngobranchials remain cartilaginous, they are consequently not recognised as such in osteological descriptions, which explains the assumed reductions (NAWAR, 1954; LUNDBERG, 1982). The fact that the anterior two infrapharyngobranchials become reduced in siluriforms, may have led to the interpretation of the single-unit remnant of them, as the second

Table II.1- 1: Status of the infrapharyngobranchials in some Otophysi, based on data from literature (*# IPB* = number of infrapharyngobranchials observed; *reduced* = serial number of the infrapharyngobranchials that are reduced; *fusion* = serial number of the infrapharyngobranchials that have fused with each other; *os-IPB* = serial number of the infrapharyngobranchials that are ossified) (Taxonomy according to FINK & FINK, 1981)

			According to the literature				*According to the hypothesis*				
Ordo-Subordo	*Familia*	*Species*	*# IPB*	*Reduced*	*Fusion*	*os-IPB*	*# IPB*	*reduced*	*fusion*	*os-IPB*	*Reference*
Cypriniformes	Cyprinidae	*Rasbora daniconius*	4	-	1-2-3-4	?	4	-	1-2-3-4	?	TEWARI, 1971
		Notropis bifrenatus	4	-	2-3	1-(2-3)	4	-	2-3	1-(2-3)	HARRINGTON, 1955
		Cyprinus carpio	4	-	1-2-3	2-3	4	-	1-2-3	2-3	NELSON, 1969
	Homalopteridae	*Protomyzon griswoldi*	4	-	1-2-3	1-2-3-4[5]	4	-	1-2-3	1-2-3-4	NELSON, 1969
		Homaloptera caldwelli	4	-	1-2-3-4	2-3	4	-	1-2-3-4	2-3	NELSON, 1969
	Catostomidae	*Catostomus macrocheilus*	3	4	-	1-2-3	3	1[6]	-	2-3-4	WEISEL, 1960
Characiformes	Hepsetidae	*Hepsetus odoe*	4	-	-	1-2-3	4	-	-	1-2-3	FINK & FINK, 1981
			4	-	-	?					BERTMAR, 1959
	Hemiodontidae	*Argonectes longiceps*	4	-	?	1-2-3-4	4	-	?	1-2-3-4	ROBERTS, 1974
		Hemiodus semitaeniatus	3	4	?	1-2-3	3	4	?	1-2-3	ROBERTS, 1974
		Bivibranchia protractila	3	4	?	1-2-3	3	4	?	1-2-3	ROBERTS, 1974
		Anodus melanopogon	3	4	?	1-2-3	3	4	?	1-2-3	ROBERTS, 1974
	Parodontidae	*Saccodon wagneri*	3	4	?	1-2-3	3	4	?	1-2-3	ROBERTS, 1974
	Characidae	*Brycon meeki*	3	4	?	1-2-3	3	4	?	1-2-3	WEITZMAN, 1962
Siluriformes	Diplomystidae	*Diplomystes chilensis*	4	-	?	1-2-3-4	4	-	1-2-3	1-2-3-4	ARRATIA, 1987
			3	4	1-2-3	1-2-3					NELSON, 1969
	Ictaluridae	*Trogloglanis pattersoni*	2(?)	1-2(?)	3-4	3-4	4	-	1-2-3-4	3-4	LUNDBERG, 1982
	Pangasiidae	*Pangasius pangasius*	4	-	3-4	?	4	-	3-4	?	SRINIVASACHAR, 1957a
	Plotosidae	*Plotosus canius*	4	-	2-3-4	?	4	-	2-3-4	1-3	SRINIVASACHAR, 1958a
			2	3-4	-	1-2					TILAK, 1963a
	Schilbeidae	*Silonia silondia*	4	-	3-4	?	4	-	3-4	?	SRINIVASACHAR, 1957a
		Ailia coila	4	-	3-4	?	4	-	3-4	?	SRINIVASACHAR, 1957a
		Clupisoma garua	4	-	-	1-2-3-4	4	-	1-2	?	RASTOGI, 1963
		Schilbe sp.	2	2-4[7]	-	1-3	4	-	1-2	1-3-4	NELSON, 1969
		Eutropiichthys vacha	4	-	3-4	1-2-(3-4)	4	-	1-2/3-4	1-3-4	TILAK, 1961
	Ariidae	*Arius jella*	4	-	3-4	?	4	-	3-4	?	SRINIVASACHAR, 1958a
		Osteogeniosus militaris	4	-	1-2-3-4	1-2-(3-4)	4	-	1-2-3-4	1-3-4	TILAK, 1965a
		Arius tenuispinis	3	1	-	3-4	4	-	1-2	3-4	SRINIVASA RAO & LAKSHMI, 1984
	Siluridae	*Wallago attu*	3	1	3-4	2-(3-4)	4	-	3-4	2-3-4	TILAK, 1963a
		Callichrous bimaculatus	3	1	3-4	2-(3-4)	4	-	3-4	2-3-4	TILAK, 1963a
	Amblycipitidae	*Amblyceps mangois*	3	1	3-4	2-(3-4)	4	-	1-2/3-4	3-4	TILAK, 1967
	Claroteidae	*Parauchenoglanis guttatus*	3	1	-	3-4	4	-	1-2	3-4	FINK & FINK, 1981
	Cetopsidae	*Helogenes marmoratus*	3(?)	1(?)	-	3-4	4	-	1-2	3-4	DE PINNA & VARI, 1995
		Paracetopsis bleekeri	3(?)	1(?)	-	3-4	4	-	1-2	3-4	DE PINNA & VARI, 1995
	Clariidae	*Clarias gariepinus*	2	1-2	3-4	3-4	4	-	1-2/3-4	3-4	NAWAR, 1954
		Heterobranchus longifilis	2	1-2	3-4	3-4	4	-	1-2/3-4	3-4	VANDEWALLE *et al.*, 1997
	Heteropneustidae	*Heteropneustes fossilis*	2	1-2	3-4	?	4	-	1-2/3-4	3-4	SRINIVASACHAR, 1959
			2	1-2	?	3-4					SRINIVASACHAR, 1958b
Gymnotiformesi	Sternopygidae	*Eigenmannia*	4	-	-	1-2-3	4	-	-	1-2-3	FINK & FINK, 1981
		Sternopygus macrurus	4	-	-	1-2-3	4	-	-	1-2-3	NELSON, 1969
	Gymnotidae	*Gymnotus carapo*	4	-	-	2-3	4	-	-	2-3	NELSON, 1969
	Electrophoridae	*Electrophorus electricus*	4	-	3-4	1-2-3	4	-	3-4	1-2-3	NELSON, 1969

[5] In *Protomyzon* two small ossifications are present, one in front of the second and one behind the third infrapharyngobranchial.
[6] Based on fig.7 of WEISEL (1960) and the description in the text, it appears more likely that infrapharyngobranchial I is absent.
[7] According to NELSON (1969) the infrapharyngobranchial IV is considered as an upper pharyngeal jaw, although it is a perichondral bone.

infrapharyngobranchial only, since it is positioned in front of the third (TILAK, 1963a; 1967; FINK & FINK, 1981; SRINIVASA RAO & LAKSHMI, 1984). Apart from the fusion between infrapharyngobranchials I and II, a general trend in siluriforms appears to involve a fusion between infrapharyngobranchials III and IV (**Table II.1- 1**). It is, however, important to mention that an extensive study of the ontogeny of the infrapharyngobranchial elements of different catfish species is required to confirm this hypothesis.

CONCLUSIONS

The overall chondrocranial ontogeny of *Clarias gariepinus* follows the general trend observed in siluriforms. Structures like the pila lateralis and commissura lateralis are absent, as well as a myodome. The platybasic skull type, which is present in all currently known siluriforms, involves a large fenestra hypophysea and a precerebral lamina instead of an internasal septum. The notochord does not reach the latter fenestra, as the cartilago acrochordalis is present. The epiphysial bridge is well developed, as well as the preorbital base and the lamina orbito-nasalis. No foramen is observed in the preorbital base. The taenia marginalis anterior appears to be reduced completely in larval *Clarias*, as is the case for some other siluriforms. The otic capsule is completely developed, except for the tectum synoticum. The tectum posterius becomes fused to the supraneural cartilages of the second and third vertebrae. The fenestra basicapsularis anterior arises initially, but disappears later during ontogeny. The glossopharyngeus and vagus nerves pass through separate foramina.

The splanchnocranium corresponds to the general siluriform situation. The palatine is separated from the pterygoquadrate from the moment it is formed and no secondary fusion occurs. The pterygoquadrate is fused to the hyosymplectic, thus forming a 'hyo-symplectic-pterygoquadrate plate', penetrated by a foramen truncus hyomandibularis. This plate initially is continuous with Meckel's cartilage, at the level of the pars quadrata, and the hyoid arch through the interhyal. The former connection is lost prior to the latter one. A well defined opercular process is present, as well as a distinct pterygoid process. The ceratohyals are fused to the hypohyals, whereas left and right hypohyals initially fuse in the middle. All basibranchials (I-V) develop, although they could not always be distinguished as separate elements. Hypobranchials I to III become isolated from the ceratobranchials, which is not the case for the fourth one. All four epibranchials are present, as well as all four infrapharyngobranchials. Ontogenetic evidence indicates that the developmental status of the latter structures may have been misinterpreted by several authors. A hypothesis, which suggests that the anterior two infrapharyngobranchials in many siluriforms may be reduced to a single cartilaginous mass, may demonstrate after all that a general trend in infrapharyngobranchial morphology is present within siluriforms.

II.1.2 - THE OSTEOCRANIUM[8]

The ontogeny of the bony skull of the African catfish, *Clarias gariepinus*, is studied from initial ossification until a complete skull is formed. The ossification sequence in *C. gariepinus* appears to be related to the functional demands that arise in a developing larva. Early ossification of the opercular bone coincides with the initiation of opercular skin movements. Early ossifications involve several dentigerous bones, formed shortly before the transition phase from endogenous to exogenous feeding. The enlarging branchiostegal membrane becomes supported by the gradual adding of branchiostegal rays. Parasphenoid ossification may be related to the protection of the brain during prey transport, while the several hyoid bones, including the parurohyal, are formed in relation to the increasing loads exerted onto the tendons of the sternohyoideus, and consequently onto the hyoid bar. Overall skull reinforcement occurs almost simultaneously, with a whole set of perichondral bones arising especially at places of high mechanical load. The suspensorium becomes protected against dislocation in an anteroposterior direction through a ligamentous connection, which even becomes partially ossified, forming the sesamoid entopterygoid. Later, the cranial lateral-line system becomes enclosed by a set of gutters, which close, frequently becoming plate-like later in ontogeny. The brain also becomes covered dorsally. Additional dentition (prevomeral tooth plates) formation appears to coincide with the formation of the opercular four-bar system, as well as with the time when the digestive system becomes completely functional. Eventually, unossified regions between the bones become closed off, fortifying and completely covering the skull.

INTRODUCTION

An extensive literature has been produced on the descriptive cranial osteology of siluriform fishes, mostly on adult forms (*e.g.*, MCMURRICH, 1884; BHIMACHAR, 1933; MERRIMAN, 1940; HUBBS & MILLER, 1960; TILAK, 1961; 1963a, b; 1964; 1965a, b; 1967; 1971; RASTOGI, 1963; GAUBA, 1966; 1970; TAVERNE & ALOULOU-TRIKI, 1974; LUNDBERG, 1975; 1982; SRINIVASA RAO & LAKSHMI, 1984; VIGNES & GARCIA, 1987; DE PINNA, 1988; HOWES & FUMIHITO, 1991; TEUGELS *et al.*, 1991; KOBAYAKAWA, 1992; CHEN & LUNDBERG, 1995; DE PINNA & VARI, 1995; FERRARIS, 1996). Special attention has been paid to some clariid species (NAWAR, 1954; GREENWOOD, 1956; POLL, 1957; 1977; TILAK, 1963c). Much confusion concerning homologies is being cleared up, as data from several studies and several catfish groups are put together (FINK & FINK, 1981; 1996; ARRATIA & GAYET, 1995; ARRATIA & HUAQUIN, 1995).

The main purposes of the present paper are, first, to describe the ontogeny of the cranial bones, in order to observe the presence or absence of cranial bones. Additionally, ontogenetic stages provide information concerning fusions or reductions of certain skeletal elements, of which no trace can be seen in

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adults. Further, an attempt is made to consider the ontogeny of the skeletal elements from a functional morphological point of view: why do certain bones develop at a certain moment and why do some develop simultaneously while others don't? Such attempts (WEISEL, 1967; VERRAES, 1974b; 1975; 1977; VERRAES & ISMAIL, 1980; HUNT VON HERBING *et al.*, 1996b; MABEE & TRENDLER, 1996) can yield important information on the adaptations of structures present at a certain stage. Additionally, as was indicated by ARRATIA (1987), "detailed ontogenetic studies of different structures in different groups are needed as base for a future phylogenetic interpretation of the relationships of the families of Siluroidei."

ARRATIA & SCHULTZE (1990) noted that "the sequence of appearance of bones is maintained intraspecifically and interspecifically within extant neopterygians despite age differences." An epigenetic control of bone formation has been demonstrated by several experiments, from which it could be derived that once a certain bone is formed (genetic differentiation), its growth is regulated to a certain extent through the mechanical load (and other factors as hormones and metabolic factors) (epigenetic differentiation) [for references, see HERRING (1993) and CARTER *et al.* (1996)] (see III.1). The rate of development of other structures like the suprabranchial organ, and consequently the coupled behaviour of air breathing, depends on the water temperature during ontogeny (HAYLOR & OYEGUNWA, 1993). The fact that the embryogenesis and early larval transformations of *Clarias gariepinus* occur extremely fast, in which the uptake of yolk resources occurs in a very efficient way (KAMLER *et al.*, 1994), implies that skull formation must be fast also. However, a developing larva has to deal with a varying set of demands in order to survive, with most of these demands related to body size (OSSE, 1990; OSSE & VAN DEN BOOGAART, 1995). Consequently, skull formation may be expected to be coupled to the developmental needs that arise, where those apparatuses, which can satisfy those needs, have to develop prior to their corresponding needs.

RESULTS

In the following part, a description is given of the osteocranium in different stages, based on the cleared and sectioned material. It should, however, be noted that some cleared specimens, especially the smallest ones, the staining was insufficient to make a clear distinction between certain bones and their margins. Serial microscopic sections, however, revealed clearly the presence or absence of bones. The drawings were based on cleared material, so drawings of certain stages may not show the presence of a certain bone, although that bone was already observed in serial sections of a smaller specimen. Despite this, the drawings do give a reliable representation of the morphology, as well as the chronology of ossification.

For anatomical references of the chondrocranium, we refer to ADRIAENS & VERRAES (1997c) (see II.1.1), whereas the relation of the cranial bones with the cranial lateral-line system is dealt with separately (see II.1.3) (ADRIAENS *et al.*, 1997). The initiation of ossification of the cranial bones, grouped per region, is given in (**Table II.1-3, p. 88**). The ontogeny of the Weberian apparatus is given in the figures, but is not discussed here. For that part we refer to RADERMAKER *et al.,* (1989). For a general review of bone nomenclatural synonymies, we also refer to HARRINGTON (1955).

A - 4.1 mm - 6.0 mm SL stage (**Plate II.1-14**)

Neurocranium - No signs of any ossification.

Splanchnocranium - The first signs of bony elements involve the opercular bone. At 4.1 mm SL, where the chondrocranium is still in a primordial phase (ADRIAENS & VERRAES, 1997c), a condensation of what appears to be a bony matrix (although still unstained in the cleared specimen), at the level of the opercular process of the hyosymplectic could be observed. In the 5.9 mm SL serial sections, a condensation of cells could be observed at that spot, although no bony matrix could be distinguished. Presumably, this condensation concerns the initiation of opercular bone formation by osteoblasts. At 5.9 mm SL, dentition is present in the lower jaw, borne by a dentary. The latter is a dermal plate covering the lateral face of Meckel's cartilage. The rostral tips of that cartilage, however, are still unossified at that stage. They will ossify perichondrally, forming the mentomeckelian bone (between 5.9 and 6.0 mm SL). This ossification appears to initiate at the anterior tip of the dermal dentary, which is fused to it from the moment it arises. This bony lower jaw at this stage is referred to as the os dento-mentomeckelium (**Plate II.1-15B**). No signs of other ossifications could be discerned.

B - 6.0 mm - 6.6 mm SL stage (**Plate II.1-15**)

Neurocranium - Several bones are present at this stage. Rostrally a paired, rudimentary premaxillary bone lies at the ventral surface of the ethmoid plate. Although rudimentary, they already bear several teeth (**Plate II.1-15B**). The two premaxillaries do not yet meet in the midline (**Plate II.1-15A**). At the rostral tip of the palatine a maxillary bone is formed, already enclosing the base of the maxillary barbel. The articulation of the maxillary bone with the palatine is improved by an additional cartilaginous element, the submaxillary cartilage.

Splanchnocranium - The opercular bone has clearly ossified now. It consists of a horizontal rod bearing a ventral, triangular membranous plate. The articular facet is more differentiated. Along the caudoventral margin of the posterior part of the ceratohyal, four branchiostegal rays have developed. Based on their position and the further development of these rays, it is clear that those four involve the posterior four branchiostegal rays of adults (radii branchiostegi VII - X). During further ontogeny they become enlarged whereas other branchiostegal rays are added in front of them. At the ventral face of the fourth infrapharyngobranchial element, a tooth plate is formed. This plate corresponds to the tooth bearing part of the upper pharyngeal jaws.

C - 6.6 mm - 7.7 mm SL stage (**Plates II.1-16, 17A**)

Neurocranium - Whereas in the previous stages, the neurocranial floor is reinforced by cartilaginous elements only, a bony support is present at 6.6 mm SL. The parasphenoid arises as what appears to be a U-shaped bone, following the curvature of the trabecular bars and the acrochordal cartilage. Although the hypophyseal fenestra is open almost completely, the foramen for the internal carotid artery is already cut off (**Plate II.1-16A**). The serial sections of the 5.9 mm SL stage reveal no sign of a parasphenoid whereas the 6.8 mm SL stage already has a parasphenoid closing off the hypophyseal fenestra almost completely. At that stage, the parasphenoid is no longer U-shaped. The premaxillary bones have become more elongated. At 7.2 mm SL, ossification at the

anterior tip of the notochord has started, corresponding to the basioccipital. Exoccipitals arise as perichondral ossifications of the inner side of the cranial walls surrounding the lagena and the sacculus.

Splanchnocranium - At 6.6 mm SL, the dento-mentomeckelian complex reaches up to the coronoid process of Meckel's cartilage. In the middle, the left and right mentomeckelian parts of the complexes are separated from each other by the fused tips of the Meckelian cartilages. At the caudal face of the hypohyals, two ossicles have formed, separated from each other by and ventral to the anterior copula (**Plates II.1-16C, 17A**). These paired elements correspond to the sesamoid, unfused parts of the future, median parurohyal bone. Two more branchiostegal rays have been added, as six of them border the ceratohyal. At 7.2 mm SL, most of the splanchnocranial perichondral bones have formed. The hyoid bar bears an anterior ceratohyal bone, as well as a ventral hypohyal one. The cartilaginous hyosymplectic-pterygoquadrate plate bears an ossification at its articular facets with the mandibula and the opercular bone, *i.e.*, the quadrate and opercular process of the hyomandibular bones, respectively. The ventral plate of the opercular bone now reaches the level of the interhyal, whereas the horizontal rod is extended posteriorly. The bone is still triangularly shaped. Serial sections of a 7.2 mm SL specimen indicate the differentiation of a dorsal process at the lateral face of the articulatory facet of the opercular bone with the hyosymplecticum. At this stage, a very indistinct tendon of the dilatator operculi inserts onto this process (see II.2.4). The branchial basket bears most of its ossifications at this 7.2 mm SL stage. All five ceratobranchials and all four epibranchials are present. Ossification appears to start at the anterior half of the cartilaginous elements. The ceratobranchials V, however, do not appear to possess any teeth yet.

D - 7.7 mm - 10.0 mm SL stage (**Plates II.1-18, 19A, 20A**)

Neurocranium - At 7.7 mm SL the parasphenoid now completely covers the hypophyseal fenestra, leaving only a paired foramen for the entrance of the internal carotid artery (**Plates II.1-18A, 19A**). Posteriorly it becomes extended and almost contacts the basioccipital, which now has an extracranial ossified part (**Plate II.1-19A**). The exoccipital bones cover the bases of the pilae occipitales (**Plate II.1-18A**). Posterior to the chondrocranium, two small ossicles are formed which lie between the caudal margin of the otic capsules and the parapophysis of the fourth vertebra (**Plates II.1-18, 19A**). Based on its position, in relation to the cleithral bone, as well as on evidence from further ontogeny, this bone must correspond to the dorsal process of the posttemporal part of the future posttemporo-supracleithral bone complex. The premaxillaries now almost meet in the midline (**Plate II.1-18C**).

Splanchnocranium - Both processes, *i.e.*, the lateral and ventral processes, of the future dento-splenio-mentomeckelian complex can already be distinguished at 7.7 mm SL. A total of nine branchiostegal rays is present. The left and right parurohyal parts have become forked at their caudal tip, whereas both are connected with their rostral tip to the ventral hypohyals through a ligament (**Plate II.1-17B**). The upper pharyngeal tooth plate is enlarged and heavily toothed, but is still supported by the fourth infrapharyngobranchial element only (**Plates II.1-18C, 20A**). At 8.4 mm SL a gutter has formed, lying lateral to the posterior part of the dental bone. This gutter corresponds to the dental splenial, but whether or not one or more of these splenial bones are involved could not be discerned. As expected, the initiation of this gutter formation occurs at the level of a superficial neuromast, which has started to sink in. More anteriorly, the neuromasts have also started to invaginate, although not so deeply, and are not yet supported by a gutter. At this stage, a perichondral

ossification, surrounding the articulation between Meckel's cartilage and the pars quadrata of the pterygoquadratum, indicates the presence of the articular bone (**Plate II.1-21B**). Observations of several stages indicate that the perichondral articular and retroarticular bones, and the dermal angular bone arise at about the same moment (ADRIAENS *et al.*, 1997). The retroarticular bone is formed as an ossification of the ventral face of the cartilaginous retroarticular process of Meckel's cartilage. The angular bone is plate-like, bordering the posterior part of Meckel's cartilage ventrolaterally. At the ventromedial face of the, still cartilaginous, palatine a thin, plate-like bone is formed. At this stage (8.4 mm SL), this bone appears to be surrounded by ligamentous tissue. Based on its morphology, position and data from further ontogeny, this bone must correspond to the sesamoid "entopterygoid" type 4 (ARRATIA, 1992). The hyoid bar now bears a posterior ceratohyal ossification (frequently referred to as the epihyal). The paired, sesamoid part of the parurohyal bone has become fused to what appears to be a perichondral ossification of the ventral face of the anterior basibranchial element. The complex nature of this bone, which has the position and function of the pure dermal urohyal bone of teleosts, led to the designation of the name "parurohyal" for that bone in Siluriformes (see Discussion) (ARRATIA & SCHULTZE, 1990). Teeth were observed at this stage on the dorsal face of the fifth ceratobranchial bone. Both pharyngeal jaws are thus present from 8.4 mm SL onwards.

E - 10.0 mm - 11.6 mm SL stage (**Plates II.1-19B, 20B, 21**)

Neurocranium - The ossification of the skull roof has started. The ossification centres of the frontal bones are situated at the branching point of the taenia marginalis posterior in the epiphysial bridge, the lamina orbitonasalis and the commissura sphenoseptalis. Apparently, the neurodermal component of the frontal bone is formed initially, as a gutter-like bone follows the taenia marginalis posterior and the commissura sphenoseptalis. At the level of the epiphysial bridge, a medial opening of that gutter is present, corresponding to the future epiphysial branch of the supraorbital canal (ADRIAENS *et al.*, 1997). Posterior to the frontal bone, two paired and consecutive gutter-like bones border the otic capsules laterally (**Plate II.1-21A**). The anterior one represents the neurodermal component of the dermosphenotic bone, whereas the posterior one corresponds to the neurodermal component of the dermopterotic bone. Apparently the supraorbital canal is already elongated into the otic and temporal canals at this stage. Both neurodermal components are still separated from each other, although the dermopterotic bone already reaches the posttemporo-supracleithral complex. Based on its morphology, the latter complex is represented by both the posttemporal and supracleithral parts at this stage. Three processes can be distinguished: (1) a dorsal one, which reaches the dorsocaudal margin of the neurocranium, medial to the pterotic bone, (2) a ventral one, which runs to the lateroventral face of the pterotic bone, and (3) a ventromedial process, which runs towards the parapophysis of the fourth vertebra. Most probably, the two anteriorly directed processes represent the dorsal and ventral processes of the posttemporal bone, and the ventromedial one corresponds to the transscapular ligament of the supracleithral bone, which has become ossified (see Discussion). In the skull floor, the parasphenoid has reached the basioccipital, and even overlaps it (**Plate II.1-19B**). The foramina for the internal carotid arteries have shifted laterally, coupled to the ongoing excavation of the trabecular bars. At that level the parasphenoid has formed two conspicuous lateral wings that reach up to the posterior margin of the sphenoid fenestra (**Plate II.1-19B**). At the anteromedial face of the otic capsules, the initiation of the prootic bone covers both the ventral and dorsal surface of the cartilaginous floor (**Plates II.1-19B, 21A**). At this stage the bone is rather slender, and broadens at its posterior half. The basioccipital now covers the exposed notochord completely. The exoccipital expands along the dorsal

face of the pila occipitalis (**Plate II.1-21A**). At 11.1 mm SL, additional skull roof bones are formed. In the ethmoid region, two dermal plates come to cover the preethmoid cornua and the ethmoid cartilage, which have already started to become ossified perichondrally, both ventrally and dorsally. At the dorsal face, however, this bone bears a distinct lateral, plate-like extension, which indicates a possible dermal origin, although a membranous origin cannot be excluded. The ventral and dorsal perichondral ossification must correspond to the hypo- and supraethmoid bones, respectively. If the bones, showing a paired nature, in fact do represent dermal bones, they probably represent the laterodermethmoid bones (TAVERNE, *pers. comm.*) (see II.1.3). Consequently, this bony complex is referred to as the mesethmoid (ADRIAENS *et al.*, 1997). The posterior part of the skull roof has also started to ossify. As is the case for the ethmoid region, the roof of the occipital region appears to consist of two dermal plates covering an unpaired, perichondral bone. This perichondral supraoccipital covers the tectum posterius, as well as the roof of the posterior part of the otic capsules. The dermal plates overlap with the whole supraoccipital bone, but are extended anteriorly, up to the anterior part of the otic capsule. Apparently, what appears to correspond to the parietals becomes fused to the supraoccipital at the moment they are formed, as has been observed in several siluriform fishes (see Discussion). This complex is further referred to as the parieto-supraoccipital bone. However, what part of this complex exactly corresponds to the supraoccipital, would require more ontogenetic stages, as well as observations at an ultrastructural level. At the level of the dermopterotic bone, the otic cartilage has begun to ossify perichondrally, corresponding to the autopterotic bone. A corresponding ossification of the autosphenotic bone, however, is still lacking.

Splanchnocranium - Suspensorial ossifications, like the quadrate and hyomandibular bone, can be discerned on the cleared specimens of 10.0 mm SL, whereas no sign of a symplectic bone is present (**Plate II.1-21B**). The latter bone does not develop at all in *Clarias gariepinus*, which is a general feature for Siluriformes (FINK & FINK, 1981; 1996). The hyomandibular bone encloses the foramen for the truncus hyomandibularis of the nervus facialis (VII), as well as the base of the opercular process (**Plate II.1-21B**). Between the hyomandibular and quadrate bones, a gutter-like preopercular bone follows the ventrolateral border of the suspensorium, thereby partially covering the cartilaginous interhyal. The anterior ceratohyal bone is now apparent in the cleared specimen as well. The forked branches of the parurohyal bones have become extended, both in an anterior and posterior direction. Their medial tips have fused now with each other posteriorly (**Plate II.1-17C**). Ceratobranchial ossifications are observed in the cleared specimen. Both their articular facets, with the hypobranchials and epibranchials, remain unossified. Apart from an increase in the number of teeth, no striking difference can be observed in the upper pharyngeal tooth plate (**Plate II.1-20B**). No more branchiostegal rays have been added since the previous stage; thus, nine are present. At 11.1 mm SL, the angular bone bears a lateral gutter, which indicates the fusion with at least one splenial bone. Consequently, a complex angulo-splenio-articulo-retroarticular bone is formed.

F - 11.6 mm - 12.7 mm SL stage (**Plates II.1-17D, 19C, 20C, 22**)

Neurocranium - Ventral to the ethmoid plate, the premaxillaries become more and more plate-like, as a posterior extension is noted. The membranodermal component of the frontal bone can now be clearly distinguished from the neurodermal, gutter-like one, as it has started to cover the postpineal foramen and, consequently, the brain. Posterolaterally, the frontal bone gutter has reached that of the dermosphenotic bone. The latter bone, as well as the dermopterotic bone, have started to form the membranodermal component as

well. Additionally, a perichondral ossification covers the taenia marginalis posterior at the level of these dermosphenotics, especially at the ventral side (**Plate II.1-19C**). Apparently, the autosphenotic has formed also. It is directly fused to the dermal counterpart, as is the case for the dermopterotic and autopterotic bones. This fused bony complex of double origin will consequently be referred to as the sphenotic bone (= dermosphenotic and autosphenotic bones) and the pterotic bone (dermopterotic and autopterotic bones). At the posterior corners of the chondrocranium, contacting the pterotics, lies the posttemporo-supracleithral bone, bearing a foramen for the temporal canal (**Plate II.1-22B**). The latter feature is an additional argument for stating that the posttemporal is present and part of the bony complex. From this stage on, the parasphenoid plays an important role in the reinforcement of the skull floor, as the trabecular bars have become split in two by the continued lateral expansion of the foramen for the internal carotid artery (**Plate II.1-22A**). At this stage, the parasphenoid consequently establishes the ventral connection between the anterior part of the neurocranium (anterior to the sphenoid fenestra) and the posterior part (posterior to the fenestra). The previously slender, lateral wings of the parasphenoid have consequently become broader. The basioccipital has formed two longitudinal ridges between which the posterior extension of the parasphenoid comes to lie (**Plate II.1-19C**). At this stage, the basioccipital supports the otoliths of the lagena and the sacculus, *i.e.*, the asteriscus and the sagitta, respectively. Lateral to the basioccipital, the exoccipitals have enclosed the foramen of the nervus vagus (X). The latter bones form the lateral margins of the foramen magnum, thus enclosing the pilae occipitales, until they contact the parieto-supraoccipital bone dorsally. The prootics have become enlarged and ovally shaped, and take part in the ossification of the border of the sphenoid fenestra (**Plate II.1-19C**). They support the otolith of the utriculus, *i.e.*, the lapillus. Anteriorly, behind the premaxillary bones, two small, splinter-like bones arise on the ventral face of the ethmoid plate (**Plate II.1-19C**). As can be derived from further ontogeny, these bones correspond to the prevomeral tooth plates that are formed prior to the median prevomeral bone itself. Real teeth, however, could not yet be discerned (tooth germs are present though). At this stage, the first signs of the dermal bones, bordering the rigid skull laterally, are present. At the level of the palatine, two small ossicles have formed, enclosing the anterior part of the infraorbital canal. These ossicles correspond to the neurodermal component of the antorbital bone and lacrimal bone (= os infraorbitale I) (**Plate II.1-22B**).

Splanchnocranium - The previously cartilaginous lower jaw has now become almost completely enclosed by bone. An anterolateral process of the angulo-splenio-articulo-retroarticular complex fits between the ventral and lateral processes of the dento-splenio-mentomeckelian complex. At this interdigitating surface, the coronoid process, as well as almost the complete medial face of Meckel's cartilage is still exposed (**Plate II.1-20C**). At the level of its articulation with the orbito-nasal lamina, the palatine has become ossified perichondrally, thus forming the autopalatine bone (**Plate II.1-19B**). The articular facet of the autopalatine itself is still cartilaginous, although surrounded by bone. The entopterygoid is now a small, triangular bone, bordering the autopalatine ventrally (**Plates II.1-19C, 20C, 22B**). The anterior tip of the pterygoid process of the suspensorium has also started to ossify, forming the metapterygoid bone. This perichondral bone, however, is still separated from the quadrate bone. The latter has become expanded dorsally and caudally, compared with the previous stage. Dorsally, the initiation of the membranous outgrowth of the quadrate has started. Although still separated from the quadrate, the hyomandibular bone has formed the corresponding membranous outgrowths as well, at its anterior border. The preopercular bone has become tube-like now, as the gutter has closed. This bone still follows the ventrocaudal margin of the hyosymplectic, with its anterior part lying horizontally and its posterior part vertically. At the level of the articulation with the neurocranium, the hyomandibular bone bears a cartilaginous rim. Compared to the previous stage, the opercular bone has extended ventrally even more. In this cleared and

stained specimen, three ossifications of the hyoid bar are discernible: the anterior ceratohyal in the middle, and the newly formed posterior ceratohyal and ventral hypohyal (**Plates II.1-20C, 22B**). The latter bone is penetrated by a foramen, through which passes the arteria mandibularis (**Plate II.1-17D**). The articulation of the nine branchiostegal rays occurs with the anterior ceratohyal bone and the cartilaginous part separating the latter from the posterior ceratohyal bone. The parurohyal bone now clearly forms one single unit, as a horizontal bony lamella has formed between the median and the two lateral processes (**Plate II.1-17D**). The ligamentous connection of the parurohyal with the hyoid bar occurs at the level of the ventral hypohyals. The anterior copula bears two ossified rings at the position of the second and third basibranchial bones. At their anterolateral tips, the hypobranchials I and II have started to ossify as well (**Plate II.1-20C**). All the epibranchial bones are observed in the cleared specimen of 11.6 mm SL. The upper pharyngeal tooth plate has enlarged rather isometrically, but is still supported mainly by the unossified, fourth infrapharyngobranchial element. At 12.0 mm SL all mandibular ossifications are present. The last to develop is the coronomeckelian bone, which is formed at the medial face of the coronoid process, between the dento-splenio-mentomeckelian and the angulo-splenio-articulo-retroarticular bone complexes (**Plate II.1-23A**). At this stage, the splenial gutter of the dental bone is still open anteriorly, but gradually becomes closed off and incorporated in the bony complex (**Plate II.1-23**).

G - 12.7 mm - 21.5 mm SL stage (**Plates II.1-17E, 24, 25A**)

Neurocranium - The bones of the skull roof have started to close up the unossified regions, as they make contact with the surrounding bones. The laterodermethmoids follow the commissura sphenoseptalis as they grow posteriorly. Serial sections of a 13.0 mm SL specimen show that the laterodermethmoids are fused to the perichondral supraethmoid, up to the level of the precerebral lamina. Posterior to that level, the supraethmoid is missing, whereas the dermal bones have extended above the posterior part of this lamina and the sphenoseptal commissures, separated from it by connective tissue. This configuration supports a possible dermal origin of these bones. However, separated dermal bones could not be discerned in the ontogenetic stages used for this study. The plate-like part of the frontal bone becomes extended anteriorly, medially and posteriorly. Posteriorly, the frontals have contacted the parietals (these are fused to the supraoccipital bone), and the initiation of an interdigitation can be observed. Medially, the frontals have continued to cover the pre- and postpineal fenestra, as well as enclosing the cartilaginous epiphysial bridge (**Plate II.1-24A**). Anterior to the entrance of the supraorbital canal into the frontal bone, a small, gutter-like nasal bone has differentiated. Laterocaudally, the bony skull margin is formed by the sphenotic and pterotic bones, covering the posterior part of the taenia marginalis posterior and the otic capsule. The sphenotic bone is restricted to the taenia marginalis and the anterior part of the otic capsule, whereas the pterotic bone covers about two thirds of that capsule. The perichondral part of both these bones has expanded over the otic capsules, ventrally and dorsally. Posteriorly, the two anterior processes of the posttemporo-supracleithral bone have expanded anteriorly, as the fork between them fits onto the posterior margin of the pterotic bone (**Plate II.1-24B**). The ossified transscapular ligament is a stout process, contacting the laterodorsal margin of the parapophysis of the fourth vertebra. In the skull floor, two bones have been added. Ossification of the lateral ethmoid has started at the level of the articular facet of the lamina orbito-nasalis, for the articulation with the autopalatine (**Plate II.1-25A**). It borders the articular facet laterally, and has covered the ventral part of the preorbital base. At the anterodorsal border, the pterosphenoid bones have appeared at the ventral side of the taenia marginalis posterior (**Plate II.1-25A**). The prevomeral tooth plates have expanded, and the first teeth can be distinguished. The parasphenoid becomes

extended anteriorly, touching the prevomeral tooth plates. Posteriorly, the parasphenoid narrows and terminates in a slender process lying between the longitudinal ridges of the basioccipital. This kind of overlap allows the long and slender interdigitation, which can be observed in later stages. The basioccipital has enlarged, especially in an anterior direction, as it now forms the complete floor for the sacculus, enclosing the sagitta (**Plate II.1-25A**). The exoccipitals now border the foramen for the glossopharyngeus nerve posteriorly. At the lateral face of the skull, a second infraorbital bone has been added, posterior to the lacrimal bone. As is still the case for the anterior two canal bones, this infraorbital bone is tubular. It borders the eye ball anteroventrally (**Plate II.1-24B**). Ventral to the preopercular bone, a triangular interopercular bone is formed, close to the anterior margin of the opercular ventral tip. Serial sections of a 15.2 mm SL specimen indicate that the lateral ethmoid consists of a perichondral ossification, bearing a plate-like extension. The lamina orbito-nasalis ossifies perichondrally, which becomes laterally and dorsally enlarged by a bony plate. However, a separate ossification of the plate-like part could not be discerned. Primordia of the infraorbital bones III and IV can be distinguished now. At 18.7 mm SL, the prevomeral tooth plates are interconnected and have fused to the median prevomeral bone. This bone already interdigitates with the parasphenoid. Three dermal canal bones are added at this stage, covering the skull laterally: the third and fourth infraorbital bones, and the suprapreopercular one. All of them are still tubular or gutter-like. The infraorbital series is completed in this stage. The onset of perichondral ossification of the orbitosphenoid could also be discerned, covering the cartilaginous floor of the ethmo-orbital region.

Splanchnocranium - The bones of the hyoid arch have expanded, especially the anterior ceratohyal. It is, however, still separated from both the ventral hypohyal and posterior ceratohyal by cartilage (**Plate II.1-24B**). The interhyal is still cartilaginous and is continuous with both the hyoid arch and the hyosymplectic cartilage. As is the case for the previous stage, all branchiostegal rays articulate with the anterior ceratohyal and the cartilaginous part between the latter and the posterior ceratohyal. All suspensorial bones have expanded, covering the cartilaginous parts between them. The membranous outgrowths of the quadrate and hyomandibula are more pronounced, and a distinct articular ridge between the latter and the neurocranium is present (**Plate II.1-24B**). The articulation between the suspensorium and the neurocranium is situated at the level of the posterior part of the sphenotic and the anterior part of the pterotic. Evidence of a paired origin of the parurohyal is now lost, as the anterior tips have fused as well. Consequently, a foramen is present, through which passes the parurohyal artery, as can be derived from serial sections of a 46.8 mm SL specimen (**Plates II.1-17E, 26**). At 18.7 mm SL, the third infrapharyngobranchial becomes ossified too.

H - 21.5 mm - 127.0 mm SL stage (**Plates II.1-17F, 25B, 27**)

Neurocranium - All bones are present. Most of them have started to close off the unossified parts almost completely. In the 21.5 mm SL specimen, the premaxillaries have expanded posteriorly, thus supporting the nasal sacs. The ethmoid plate and precerebral lamina are almost completely ossified, as the contralateral laterodermethmoid bones have fused medially (or are interconnected through the supraethmoid bone). The nasal bones have become tubular, in which the branching of the supraorbital canal can already be distinguished (ADRIAENS *et al.*, 1997). In the cleared specimen, the lateral ethmoid ossification is now observed on the dorsal face of the skull (**Plate II.1-27A**). The frontal bones have expanded substantially in all directions. Anterolaterally, they interdigitate with the lateral ethmoid, anteriorly with the mesethmoid, posterolaterally with the sphenotics and the pterotics, caudally with the parieto-supraoccipital complex and medially the two frontals

meet at the level of the epiphysial bridge (**Plate II.1-27A**). Like the frontals, the parieto-supraoccipital bone has started to close off the postpineal fenestra. Posteriorly, the former bone complex bears a distinct, pointed supraoccipital process, which reaches up to the fourth vertebra. Rudimentary contact between the parieto-supraoccipital bone and the pterotic bone is already established. The anterior expansion of the pterotic bone has deprived the sphenotic bone from any contact with the parieto-supraoccipital bone complex. Medial expansion of the sphenotic bone, at the ventral side of the skull, has resulted in the ossification of the lateral border of the sphenoid fenestra. At its posterior margin, the posttemporo-supracleithral bone has become plate-like and borders the pterotic bone both caudally and laterocaudally. The lateral-line canal, which leaves this bone complex, is enclosed by a pair of small ossicles. In the skull floor, the orbitosphenoid is clearly visible (**Plate II.1-25B**). It arises as a paired ossification of the ethmoid plate and the preorbital base, lateral to the anterior part of the parasphenoid. The orbitosphenoids are still separated from the surrounding bones and border the sphenoid fenestra anteriorly. The lateral wings of the parasphenoid have expanded extensively, as they contact the ventral extension of the pterosphenoid bones. Consequently, this connection subdivides the sphenoid fenestra into an anterior foramen for the optic nerve and a posterior trigemino-facial foramen. The prootics have come into close contact with the parasphenoid, sphenotics, pterotics, basioccipital and exoccipital bones, but no true interdigitation has occurred (**Plate II.1-25B**). Basioccipital and parasphenoid, however, already strongly interdigitate. The second infraorbital bone has extended caudally, as it now borders the eye ventrally as well (**Plate II.1-27B**). The third infraorbital bone lies at the posterior margin of the eye. A gap is still present between the third and the second infraorbital bone. All these infraorbital bones are still tubular, apart from the fourth one, which lies along the lateral margin of the skull, between the frontal and sphenotic bones. Apparently, the fourth infraorbital bone arises last, although it consequently becomes the largest one of the series, as can be derived from the later stages. The suprapreopercular bone has become plate-like, where the neurodermal and membranodermal components can already be clearly distinguished. In the 46.8 mm SL specimen, a plate-like, nasal, triangular in cross-section, could be observed, indicating the presence of the membranodermal component. An articulation is present between the small antorbital bone and a small cartilaginous protuberance of the anterior articular facet of the autopalatine (**Plates I.4-9D, III.1-8A**). The antorbital bone partially encloses the base of the nasal barbel.

Splanchnocranium - The mandibula has become more heavily ossified, as the dento-splenio-mentomeckelian complex becomes connected more solidly to the angulo-splenio-articulo-retroarticular complex through an extensive interdigitation (**Plates II.1-23B-C, 27B**). The splenial bones have closed their gutters completely, leaving only the pores where the mandibular canal leaves the bone (ADRIAENS *et al.*, 1997) (**Plate II.1-45**). At its medial face, the coronoid process gradually becomes enclosed as well (**Plate II.1-23C**). A well developed articular facet for the articulation with the quadrate is present, bearing a substantial retroarticular process. The autopalatine is almost completely ossified, except for its rostral and caudal tips (**Plate II.1-25B**). Anteriorly, the tip establishes an articular facet for the double-headed maxillary bone, enclosing the maxillary barbel. Although no true articulation is present with the caudal tip of the autopalatine, an ossification is lacking (maybe for growth purposes). The sesamoid entopterygoid bone connects to the metapterygoid bone and the prevomeral bone through a ligamentous strap. The metapterygoid bone has started to form its membranous outgrowths, as was already the case for the quadrate and the hyomandibular bone. Although more extensively ossified, the latter two bones are still separated from each other by cartilage (**Plate II.1-27B**). The preopercular bone has initiated the formation of the membranodermal component, and the branching of the preopercular canal can be recognised in the neurodermal part. The opercular bone is still triangular, being ligamentously connected to the

interopercular bone at its ventral tip. The latter bone has become broader compared to the previous stage. The anterior ceratohyal bone now reaches the posterior one. Interdigitation between these two bones has started only at the dorsal side of the hyoid bar, whereas cartilage separates them ventrally (**Plate II.1-27B**). Anteriorly, the anterior ceratohyal bone has reached the ventral hypohyal bone, where the first signs of interdigitation are apparent (**Plate II.1-17F**). The articulation between the hyoid bar and the branchiostegal rays is still comparable to that in the previous stage, *i.e.*, at the level of the anterior ceratohyal bone and the cartilaginous region posterior to it. At this stage, one branchiostegal ray is added, comprising a total of ten. The parurohyal bone bears a small but distinct foramen, and the three caudal processes have become elongated (**Plate II.1-17F**). Anteriorly, the double ligamentous connection to the ventral hypohyals is still present. The bones of the branchial basket have not changed substantially compared to the previous stage. Basibranchials of the second and third branchial arches are separated by cartilage on the anterior copula. Very small hypobranchials are present on the first and second branchial arch. All ceratobranchials are well ossified. Their articular facets, with the hypo- and epibranchials, however, remain unossified. Although no fifth epibranchial has developed, the corresponding ceratobranchial element bears an unossified head at both ends. The third epibranchial element bears a well developed, medially directed uncinate process at its caudal margin. At 46.8 mm SL, the splanchnocranium is complete. A small, gutter-like splenial bone is present lateral to the articulation between the mandibula and the suspensorium. This splenial embraces the sensory canal, at the transition from the mandibular to the preopercular canal. Dorsomedially, the angulo-splenio-articulo-retroarticular complex has become extended rostrally, coming into contact with the dento-splenio-mentomeckelian bone. As a result, the cartilaginous coronoid process is enclosed by bone laterally and medially. At its dorsomedial face, the hypohyal cartilage bears a small, but distinct process, which becomes ossified at this stage. Lying at the dorsal face of the ventral hypohyal, this bone corresponds to the dorsal hypohyal. The fourth infrapharyngobranchial element also starts to ossify, bordering the cartilage dorsally, medially and ventrally.

I - 127.0 mm SL stage (**Plates II.1-17G, 23D, 25C, 28 - 37**)

Neurocranium - This juvenile stage is to some degree a copy of the adult configuration, although reduced in size. The mesethmoid is relatively broad, bearing two well developed preethmoid processes, which correspond to the well ossified preethmoid cornua (ADRIAENS & VERRAES, 1997c). Interdigitation occurs with the lateral ethmoids, laterally, and the frontals, posteriorly. Together with the lateral ethmoid, the laterally curved preethmoid processes enclose the nasal bone. The lateral ethmoid has become plate-like, enclosing the cartilaginous orbito-nasal lamina. At its ventral face, the initial articular facet between this lamina and the palatine can still be distinguished, as an ossification is lacking (**Plate II.1-30A**). Medially the lateral ethmoid bears a funnel through which the olfactory lobes pass. Laterally, it bears a distinct process for the articulation with the dorsal process of the second infraorbital bone (**Plate II.1-28A**). Posteriorly, the lateral ethmoid interdigitates with the frontals, but no overlapping could be observed. At its lateral margin, the lateral ethmoid is connected through a connective tissue sheet to the dorsal margin of the anterior part of the fourth infraorbital bone. The frontals are the largest, paired skull bones, and border the anterior fontanella. Compared to the previous stage, both frontals are sutured to each other posteriorly, thus subdividing the postpineal fenestra into a postepiphysial part of the anterior fontanella and the posterior fontanella (**Plate II.1-28A**). In small specimens, the anterior fontanella is bordered anteriorly by the mesethmoid. A second region of interdigitation between the contralateral frontals occurs at the level of the epiphysial bridge, which has become completely enclosed by a tubular outgrowth of

the dermal frontals (**Plate II.1-30B**). In the skull roof, the frontals connect through sutures to the sphenotics (posterolaterally), the pterotics (posteriorly) and the parieto-supraoccipital bone complex (posteromedially). Laterally, they connect through connective tissue to the central part of the fourth infraorbital bone. Ventrally, the frontals connect rigidly to the orbitosphenoids and the pterosphenoids (**Plate II.1-25C**). The sphenotics have become plate-like as well, enclosing the anterolateral part of the otic capsule (**Plate II.1-25C**). They are bordered by the frontals and pterotics, to which they are strongly sutured. They have lost contact with the parieto-supraoccipital bone (**Plate II.1-28A**). Ventrally, they connect to the prootic and pterosphenoid bones. At their ventrolateral margin, they enclose the anterior part of the articular facet for the suspensorium, which is preceded by a stout lateroventral process (**Plates II.1-30C, 34E**). The pterotics are large bones, enclosing the posterolateral part of the otic capsule, and bearing large lateral plate-like extensions. Medially, they are sutured to the parieto-supraoccipital bone. The connection with the posttemporo-supracleithral bone is restricted to the posterolateral part. Consequently, the pterotics form part of the posterior margin of the juvenile skull (**Plate II.1-28A**). Ventrally, they connect to the prootics and exoccipitals (**Plates II.1-25C, 30D**), and form the posterior part of the articular facet for articulation with the suspensorium (**Plate II.1-34E**). In a specimen of 140.1 mm SL, however, the pterotic appeared to be separated partially from the parieto-supraoccipital and exoccipital bones (which may be due to insufficient staining), where a very small ossicle was found covering that region (**Plate II.1-29)**. Based on its position, as well as the fact that it appears to be a perichondral bone, at the level of the posterior semicircular canal, it must correspond to the epiotic, which could not be observed in any previous stage. It is, however, possible that because of its reduced size and insufficient staining, it is indistinguishable in the other specimens. The bone was even overlooked in adults by NAWAR (1954). The parieto-supraoccipital bone complex is large, with its previously paired anterior parts now fused to each other medially. Apparently, a true fusion has occurred, in contrast with the posterior part of the frontals, as no signs of a suture can be observed anymore (**Plate II.1-30E**). The left-right fusion is not complete, as a posterior fontanella remains. Observations of larger specimens indicate a slow, but progressive closure of both the anterior and posterior fontanella. At its ventral face, the complex bears a median ridge, which is formed in the mediosagittal septum. Caudolaterally, the bone interdigitates with the exoccipitals (**Plates II.1-29, 30E**). The posttemporo-supracleithral bone is a plate-like complex, bordering the pterotics laterally and laterocaudally. Ventrally, it bears a mediocaudally directed process for the articulation with the parapophysis of the fourth vertebra, as well as an articular facet for the articulation with the cleithral bone (**Plate II.1-30F**). It does not bear any connection with the basioccipital, which is the case in many siluriform fishes (**Plate II.1-25C**). Most presumably, this process corresponds to the ossification of the transscapular ligament (see Discussion).

Anteriorly, two plate-like premaxillaries articulate with the ventral face of the preethmoid processes of the mesethmoid. Premaxillaries are completely covered with teeth, with the exception of their posterior margin. They lack any differentiations like an ascending process or maxillary process (**Plate II.1-31A**). No true articular facet, with secondary cartilage, is present between the premaxillary bone and the mesethmoid. Both connect only through connective tissue. An articulation of the premaxillary with the maxillary bone is also absent. Only a distinct string of ligamentous tissue connects the two. The maxillary is typically advanced siluriform, as it is socket-like, enclosing the base of the maxillary barbel. It bears a double-headed articular facet for the articulation with the rostral tip of the palatine (**Plate II.1-31B**). As in most siluriform fishes, the maxillary bone has been modified to take part in the palatine-maxillary mechanism (ADRIAENS & VERRAES, 1997a) (see II.2.2). The antorbital bone is still small and overlies the rostral tip of the palatine. Compared to the previous stage, most infraorbital bones have undergone a major transformation in shape, as they are no longer tubular. A distinct plate-like bone underlies the tubular part. The lacrimal bone bears a serrated ventral edge, whereas the second infraorbital bone

possesses a massive part for the articulation with the lateral process of the lateral ethmoid (**Plates II.1-28A, 44**). Consequently, both these bones form the anterior border of the orbita, whereas the remaining part of the second infraorbital bone borders the orbita anteroventrally. The posteroventral and posterior border is demarcated by the third infraorbital bone (**Plate II.1-28B**). Dorsally, the slender supraorbital process of the fourth infraorbital bone forms the dorsal margin, it is, however, elongated and much broader posterior to the eye. In *Clarias gariepinus*, the fourth infraorbital bone reaches up to the suprapreopercular bone. The latter bone has become plate-like as well, filling up the gap between the fourth infraorbital bone and the posttemporo-supracleithral bone (**Plate II.1-28B**).

The skull floor consists of a central, longitudinal and narrow bridge, which becomes broader posteriorly, as it forms the base of the braincase. Anteriorly, the hypoethmoid part of the mesethmoid interdigitates with the prevomeral bone. The latter has become arrow-like, bearing two, well developed tooth plates, to which it is fused. Posteriorly, the prevomeral bone interdigitates through a very slender, forked suture with the parasphenoid (**Plate II.1-32A**). Dorsally, the prevomeral bone is connected to the lateral ethmoid, more exactly to the part enclosing the olfactory lobes (**Plate II.1-25C**). The parasphenoid consists of a small anterior part, which is expanded laterally at the level of the earlier formed lateral wings, which contact the pterosphenoid bones. Posterior to these wings, the parasphenoid becomes narrow again, finally interdigitating posteriorly with the basioccipitals. The interdigitation with the latter occurs through many more sutures than is the case for the prevomeral bone (**Plate II.1-32B**). At its dorsal face, the parasphenoid connects rigidly to the orbitosphenoid (in front of the lateral wings), the pterosphenoids (at the lateral wings), and the prootics (posterior to the lateral wings) (**Plates II.1-25C, 32B**). The basioccipital no longer forms an articular surface with the first vertebra, but now interdigitates with the complex of vertebrae, formed in relation to the Weberian apparatus (**Plates II.1-29, 32C**). Lateroventrally, the basioccipital connects to the exoccipitals, posteriorly, and to the prootics, anteriorly, both through short sutures. At its dorsal face, the basioccipital bears a bowl-like structure housing the sinus impar perilymphaticus of the Weberian complex (**Plate II.1-29**) (CHARDON, 1967a; RADERMAKER *et al.*, 1989). The orbitosphenoids connect to the lateral ethmoids anteriorly, the frontals dorsally, and the parasphenoid posteriorly. They are separated from the pterosphenoids by the foramen for the optic nerve (**Plate II.1-25C**). Although the orbitosphenoids are formed as paired ossifications, they have fused into a single, gutter-like bone in the juvenile stage (**Plate II.1-32D**). The pterosphenoids, on the other hand, remain paired ossifications, which connect to the orbitosphenoids, the parasphenoid, and the sphenotics (**Plates II.1-25C, 32E**). The prootics form the anterolateral floor of the brain cavity and enclose the utriculus with its otolith (the lapillus) (**Plate II.1-32F**). The perichondral prootics are sutured to a whole set of bones: the pterosphenoid, the parasphenoid, the sphenotics, the pterotics, the exoccipitals, the basioccipital and the parieto-supraoccipital. They take part in the bordering of the trigemino-facial foramen (**Plate II.1-25C**). Finally, the exoccipitals form the posterolateral ossifications of the brain cavity floor, and also enclose the otoliths of the sacculus and the lagena (*i.e.*, the sagitta and asteriscus, respectively) (**Plate II.1-32G**). These bones interdigitate with the basioccipitals, prootics and the parieto-supraoccipital bone, whereas a synchondrosis appears to be present with the pterotics. The exoccipital bone encloses the foramina for the glossopharyngeus, vagus, and hypoglossus nerves (**Plates II.1-25C, 29, 32G**).

Splanchnocranium - The mandibula is fully ossified, although Meckel's cartilage remains partially exposed (**Plate II.1-23D**). The coronoid process is well protected by bone, with only its dorsal tip remaining uncovered. Caudally, the retroarticular process is rather short and bears two processes, both for the attachment of ligaments: a ligament running to the interopercular bone and one to the hyoid bar (**Plate II.1-33A**). The dento-splenio-mentomeckelian bone complex is provided with a large patch of teeth. Two types of teeth are observed:

villiform teeth at the outer margin, and conical teeth at the inner margin (**Plate II.1-33B**). The surface for dentition appears to be expanded, as a kind of rostral extension of the bone has formed. At its medial face, this bone complex bears a socket that encloses the anterior part of Meckel's cartilage. Caudally, a well developed ventral and lateral process can be distinguished, between which fits the lateroventral process of the angulo-splenio-articulo-retroarticular complex. At the level of the coronoid process, a distinct coronomeckelian bone covers Meckel's cartilage dorsally, medial to the coronoid process (**Plate II.1-23D**). The autopalatine is a rod-shaped bone, bearing three unossified regions: (1) the anterior tip that enables the articulation with the maxillary bone, (2) the slender and elongated articular facet for the articulation with the lateral ethmoid, and (3) the posterior tip, which does not articulate with any bone (**Plate II.1-33C**). Between the mandibula and the suspensorium, the number of isolated splenial bones has increased to two, whereas in larger specimens three of them were present (**Plate II.1-28B**) (ADRIAENS *et al.*, 1997).

The quadrate bone bears a solid articular facet with the mandibula and is provided with a substantial dorsal, membranous outgrowth (**Plate II.1-34C**). The quadrate connects both synchondrally and through sutures with the metapterygoid bone, anteriorly, and with the hyomandibula, posteriorly (**Plate II.1-34A**). The hyomandibula also has a membranous plate, which connects to a similar outgrowth of the quadrate (**Plate II.1-34A**). Dorsally, a cartilaginous strip is present for the articulation with the neurocranium. Anterior to this strip, the bone has a distinct dorsal process, which fits into a cavity in the sphenotic bone, additionally, a ventral process of the sphenotic fits into a cavity posterior to the hyomandibular process (**Plate II.1-34E**). Due to such a connection, the suspensorium and neurocranium are interlocked to a certain degree (see Discussion). At its posterior margin, the hyomandibula bears a substantial opercular process with a cartilaginous articular facet (**Plate II.1-34B**). At its medial face, the hyomandibula bears two foramina: (1) a small foramen, just above the insertion of the ligament running to the hyoid bar, and (2) a larger one posterior and dorsal to it. This larger foramen is penetrated by the truncus hyomandibularis (**Plate II.1-34A**), whereas through the smaller foramen a blood vessel enters, presumably contributing to the supply of chondroclast for cartilage resorption of the hyosymplectic cartilage. Compared to the situation in previous stages, the foramen through which the truncus leaves, has shifted ventrally, due to the fact that a bony crest of the hyomandibular bone has grown ventrally, thereby covering the foramen of the hyosymplectic cartilage. At this stage, the latter foramen is bordered by both the hyomandibula and the preopercular. Medially, the hyomandibula forms a ridge for the attachment of a stout ligament that runs to the hyoid bar (**Plates II.1-34A, IV.2-3**) (ADRIAENS & VERRAES, 1994). The unossified part, separating the hyomandibula from the quadrate, corresponds to that part which in previous stages was connected to the interhyale, and which may be considered as an unossified symplectic. At its ventral margin, the hyomandibula interdigitates with the preopercular bone (**Plates II.1-28B, 34A**). This bone does not bear a distinct vertical and horizontal limb, as is the case in many catfishes (**Plate II.1-37A**). Its caudal margin consists of the neurodermal part enclosing the preopercular canal with two of its branches (ADRIAENS *et al.*, 1997). At its anterodorsal side, it bears a plate-like extension for the interdigitation with the hyomandibula and the quadrate (**Plate II.1-37A**). The plate-like expansion has persisted in the metapterygoid and entopterygoid bones as well (**Plate II.1-34D**). As could be derived from the previous stages, the ossification of the metapterygoid initiates from a perichondral ossification of the pterygoid process, which can still be distinguished in this stage. In this stage, both ventrally and dorsally, this central rod bears plate-like extensions. The ventral extension corresponds to the ectopterygoid process described by ARRATIA (1992). Considerable discussion concerns the homology of the pterygoid bones in catfishes, and even in fishes in general (HOWES & TEUGELS, 1989; ARRATIA, 1990; 1992). The metapterygoid is strongly connected to the quadrate through the ventral synchondrosis, but more strongly through the extensive suturation along the dorsal plates (**Plate II.1-34A**). Consequently, the metapterygoid no

longer contacts the hyomandibula. Rostrally, the metapterygoid has a straight margin, connected to the entopterygoid through a ligamentous strip. The entopterygoid in *Clarias gariepinus* is ligamentously connected to a whole set of surrounding bones: (1) the metapterygoid, posteriorly, (2) the autopalatine, dorsally, (3) the prevomeral bone, anteriorly, and (4) the lateral ethmoid, anterolaterally. Caudally, the entopterygoid bone bears a distinct process (**Plate II.1-34D**).

The hyoid bar is almost completely ossified, although cartilaginous strips appear to persist between all bones (**Plates II.1-28C, 35A**). At the medial face of the ventral hypohyal, the dorsal hypohyal remains separated by cartilage from both the ventral hypohyal and the anterior ceratohyal (**Plate II.1-35B**). The ventral hypohyal is much larger than the dorsal. Ventromedially, the latter bone forms an interdigitation with the anterior ceratohyal bone, whereas the rest is interconnected through a synchondrosis. Medioventral to these sutures, the ventral hypohyal is attached through a stout ligament that is connected to the anterior tips of the parurohyal bone (**Plate II.1-17G**). A basihyal bone is absent. The anterior ceratohyal bone is the largest of the hyoid bar (**Plate II.1-35C**). At its medial face, it bears a shelf against which the first six branchiostegal rays articulate (**Plate II.1-35A**). The following two rays articulate with the cartilaginous strip, which separates the anterior ceratohyal bone from the posterior one ventrally. The posterior two branchiostegal rays articulate with the ventral margin of the posterior ceratohyal bone. The posterior ceratohyal bone tapers caudally, and terminates as a solid cone (**Plate II.1-35D**). At the dorsal face of this cone, attaches a stout ligament that connects the hyoid bar to the hyomandibula (**Plate II.1-34A**). Another large ligament connects the lateral face with the medial process of the mandibular retroarticular process (**Plate II.1-33A**). At this stage, the interhyal is completely reduced (ADRIAENS & VERRAES, 1994). The parurohyal bone has become more solidly ossified, with three distinct, caudal processes. Although the median process is the result of a fusion of two processes, it is more slender than the lateral processes (**Plate II.1-17G**). The two heads of the paired sternohyoideus muscle fit nicely into the fork of the parurohyal bone (ADRIAENS & VERRAES, 1997d). The length of the ligaments, in relation to the size of the parurohyal, appears to have become reduced substantially, as the rostral tips of the parurohyal now partially fit into a small gap at the medial face of the ventral hypohyals. The main body of the parurohyal still bears a foramen, penetrated by a ventral branch of the ventral aorta (**Plate II.1-26**). The latter artery is referred to as the parurohyal artery. It enters the parurohyal dorsally, where it splits into two arteries before it leaves the bone ventrally. Between the two rostral processes of the parurohyal, a vein (here referred to as the vena parurohyalis) branches off from the vena jugularis inferior and curves caudally, against the ventral side of the bone and between the two branches of the artery (**Plate II.1-26A**). The branchiostegal rays differ slightly in shape, depending on their position. The anterior ray is a slender rod, with a broader articular part, whereas the posterior ray has a distinct articular facet, penetrated by a foramen (**Plate II.1-35E**), with a distal, elongated, plate-like part (**Plate II.1-35A**).

Ossification of the branchial basket appears to have been completed, although several cartilaginous elements lack any perichondral bone (**Plate II.1-28C**). The anterior copula still bears two ossifications, corresponding to the second and third basibranchial bones (**Plate II.1-36B-C**). No signs of the first basibranchial could be discerned at this stage (**Plate II.1-36A**). The posterior copula also lacks ossifications of the fourth and fifth basibranchials too (**Plate II.1-28C**). Hypobranchial bones I and II of the previous stage have expanded, as they now cover almost half of the surface of the corresponding cartilaginous elements (**Plates II.1-28C, 36A-B**). The third hypobranchial element is still unossified (**Plate II.1-36C**). The cartilaginous fourth hypobranchial is believed to remain fused to the ceratobranchial cartilage, and is unossified as well (**Plate II.1-36D**) (ADRIAENS & VERRAES, 1997c). All five ceratobranchials are well ossified. They consist of the central, perichondral rod, which bears two caudal ridges for attachment of the gill filaments, as well as for the housing of blood vessels and nerves. The tooth plate, covering the dorsal face of the fifth ceratobranchial bone, has become broader, especially at its anterior half

(**Plate II.1-36E**). The epibranchials are more elongated, compared with the previous stage, especially epibranchials I and II (**Plates II.1-13E, 28C**). This elongation appears to be coupled to the elongation of the third infrapharyngobranchial bone. Medially, these bones converge and attach to each other through cartilage, which most probably corresponds to the unossified remains of infrapharyngobranchials I and II (ADRIAENS & VERRAES, 1997c). This cartilage connects these epibranchials to the anterior tip of the third infrapharyngobranchial bone (**Plate II.1-28C**). Epibranchial bone III has a well developed uncinate process at its caudal margin (**Plate II.1-36C**). Medially, this epibranchial articulates with the posterior tip of the third infrapharyngobranchial and the anterior margin of the fourth one (**Plate II.1-28C**). The fourth epibranchial bone bears a horizontal, plate-like extension at its middle portion, for the support of the uncinate process (**Plate II.1-36D**). Medially, this epibranchial articulates with the posterolateral margin of the fourth infrapharyngobranchial element. As mentioned, only the third and fourth infrapharyngobranchials ossify in *Clarias gariepinus* (**Plate II.1-28C**). The third is rod-like, with both its articular tips non-ossified (**Plate II.1-36C**). The fourth one is rather triangular, with its lateral margin non-ossified (**Plate II.1-36D**). The upper pharyngeal tooth plate is enlarged, and is supported dorsally by the fourth infrapharyngobranchial bone, as well as the medial part of epibranchials III and IV (**Plate II.1-28C**).

The opercular series in juvenile *Clarias gariepinus* consists of the opercular bone, the preopercular, and interopercular bones (**Plate II.1-28B**). The subopercular bone is missing, as is the case in most siluriform fishes (see Discussion). The preopercular bone attaches to the suspensorium, as mentioned above. The opercular bone is triangular and articulates with the opercular process of the hyomandibula (**Plate II.1-28B**). Consequently, at its anterior margin, the opercular bone bears a well developed articular facet, which is in contact with a medial crest for the insertion of the levator operculi muscle and the opercular part of the hyohyoidei adductor muscles (**Plate II.1-37B**) (ADRIAENS & VERRAES, 1997b). The ventral tip of the opercular bone attaches to the interopercular bone through a ligamentous strip (**Plate II.1-28B**). This interopercular bone remains triangular, as it tapers rostrally to become elongated in a stout ligament that is attached to the lateral process of the mandibular retroarticular process (**Plates II.1-33A, 37C**). The interopercular bone also attaches through connective tissue to the lateral face of the posterior ceratohyal bone.

DISCUSSION

According to the terminology of PATTERSON (1977), all three bone types in fishes are formed in *Clarias gariepinus*: cartilage bones, dermal bones and membrane bones. In *C. gariepinus* all cartilage bones ossify perichondrally. True enchondral ossification was not observed until later stages (see I.4.4.b). Bony elements are compact ossifications early in ontogeny, whereas most of them become spongious in the later larval stage. The cartilage bones, lining the cartilaginous structures, are surrounded with membranous apolamellae, which frequently are formed in a radiating manner (see I.4.4). Most dermal bones are extremely trabecular, with the exception of the prevomeral and parasphenoid bones. Dermal tooth plates become spongy as well. At least one type of membrane bone is present: the sesamoid bones (coronomeckelian and entopterygoid bones). Whether or not the plate-like extension of the perichondral lateral ethmoid bone corresponds to the

Table II.1- 2: Overview of the bones constituting the fully formed skull in *Clarias gariepinus*

Bone type	*Paired*	*Unpaired*	*Total*
Dermal anamestic bone	16	2	34
Dermal canal bone	16	0	26
Perichondral bone	32	3	67
Sesamoid bone	2	0	4
Tooth plate	4	0	8
Compound bone	1	3	5
Total	68	8	144

membranous prefrontal can, however, not be ascertained. The fully developed skull of *C. gariepinus* consists of 68 different paired and 8 different unpaired bones, which can be subdivided into six categories (**Table II.1- 2**): (1) 18 anamestic, dermal bones, of which two are unpaired, (2) 13 dermal canal bones, all paired, (3) 35 perichondral bones, of which three are unpaired, (4) two paired sesamoid bones, (5) four paired tooth plates, and (6) four bones of a compound nature, of which three are unpaired.

Ostariophysans, in general, together with some other primitive teleostean lineages (MEUNIER & HUYSSEUNE, 1992), are characterised by the presence of cellular bone. Bone in *Clarias gariepinus* is cellular, although the number of osteocytes is low (**Plate I.4-5B**).

SILURIFORM RELATIONSHIPS OF THE OSTEOCRANIUM OF CLARIAS GARIEPINUS

Although a comparative osteology is beyond the scope of this paper, some of the features, characterising the cranial osteology of *Clarias gariepinus*, will be briefly discussed.

The paired origin of the prevomeral bone has been observed in most teleost fishes (DE BEER, 1937; VERRAES, 1973). This bone is considered non-homologous with the vomeral bone of tetrapods, although it is frequently referred to as "vomeral bone" in teleosts (DE BEER, 1937; HARRINGTON, 1955; DAGET, 1964). In *Clarias gariepinus*, it appears that the prevomeral bone itself is unpaired (whether or not a fusion occurs between paired blastemata or not could not be discerned) and becomes fused to paired tooth plates. In the closely related heteropneustid species, *Heteropneustes fossilis*, an unpaired prevomeral bone arises, prior to its dentition (SRINIVASACHAR, 1958b). There is a general consensus now that the toothless prevomeral bone is an unpaired, dermal bone that becomes fused to paired tooth plates, whereas the latter may be homologous with pharyngeal tooth plates of the premandibular arch (DAGET, 1964; SRINIVASA RAO & LAKSHMI, 1984; ARRATIA, 1987). The prevomeral bone in siluriform fishes is generally T-shaped, bearing two patches of teeth at its lateral wings and interdigitates with the parasphenoid through very long sutures (BHIMACHAR, 1933; MERRIMAN, 1940; TILAK, 1963a; 1965a; LUNDBERG, 1982; SRINIVASA RAO & LAKSHMI, 1984; HOWES & FUMIHITO, 1991). Both tooth patches may be fused to each other (MERRIMAN, 1940; TILAK, 1961; 1963a, c; RASTOGI, 1963; ARRATIA, 1987; HOWES & FUMIHITO, 1991) or may even be absent (TILAK, 1965a; 1967; GAUBA, 1970; LUNDBERG, 1982; VIGNES & GARCIA, 1987; FERRARIS, 1996). Fusion between both tooth plates in adult Clariidae appears to be the general trend (TILAK, 1963c; TEUGELS, 1982). In case dentition, and thus paired tooth plates are lacking, an unpaired origin of the prevomeral bone has been observed (KINDRED, 1919), with its lateral wings sometimes being absent (LUNDBERG, 1982; HOWES, 1983a). Heavily dentigerous prevomeral tooth plates are present in Pangasiidae and Siluridae, where these plates become closely aligned with autogenous palatine tooth plates, or may even fuse to them (TILAK, 1961; ROBERTS & VIDTHAYANON, 1991). In Chacidae, the prevomeral bone is absent as an independent ossification (TILAK, 1971; BROWN & FERRARIS, 1988).

For a discussion and in- and outgroup comparison of the parietal and supraoccipital bone complex, we refer to FINK & FINK (1981; 1996) and ARRATIA & GAYET (1995). The presence of a separate parietal ossification, which has been observed in several siluriform taxa, is now generally accepted to explain the compound nature of this bone (ARRATIA, 1987; FINK & FINK, 1996).

For a discussion on the homology of the posttemporo-supracleithral bone in Siluriformes, we refer to LUNDBERG (1975), ARRATIA & GAYET (1995) and FINK & FINK (1981; 1996). Concerning the transscapular ligament,

several constructions appear to be present in Siluriformes. In many catfishes, the posttemporo-supracleithral bone connects to the lateral face of the basioccipital through a bony strut (BHIMACHAR, 1933; MERRIMAN, 1940; HUBBS & MILLER, 1960; TILAK, 1961; 1963a; 1965a; 1967; 1971; RASTOGI, 1963; LUNDBERG, 1975; POLL, 1977; SRINIVASA RAO & LAKSHMI, 1984; HOWES & FUMIHITO, 1991). In others, no such a connection is observed. In such cases, a medial strut generally connects to the anterior vertebrae, at the level of the vertebral centre (TILAK, 1963a) or contacts the parapophyses (SRINIVASACHAR, 1958b; NAWAR, 1954; TILAK, 1963c; GAUBA, 1970; HOWES, 1983a; present study). REGAN (1911a) mentioned that a lower process of the posttemporal (posttemporo-supracleithral bone of present study) to the basioccipital is absent in Clariidae, Loricariidae and Callichthyidae. This author also mentioned that the posttemporal is absent in Clariidae, whereas the supracleithral bone is connected "with a posterior process firmly united to the air-bladder capsule." In Diplomystidae, ARRATIA (1987) observed a ventromedial process of the posttemporo-supracleithral bone, which is bifurcated and which is attached to the cranium (at the level of the basioccipital) and to the parapophyses through ligaments. The question raised here is whether the process of this bone complex, attaching to the basioccipital in most catfishes, corresponds to the one connecting it to the Weberian apparatus in others. The connection to the basioccipital is believed to be the ossified Baudelot's ligament, which generally runs from the supracleithral bone to the basioccipital in other teleosts. LUNDBERG (1975) considered the connection to the parapophyses of the vertebrae as a differentiation (aventral process) of the transscapular ligament of his supracleithrum (= posttemporo-supracleithrum of present study). A double connection of the posttemporo-supracleithral bone to the basioccipital and Weberian apparatus, as observed by ARRATIA (1987) in Diplomystidae, is considered to be a feature of the Siluriphysi (present Siluriformes and Gymnotiformes) (FINK & FINK, 1981: character 98). Although such a bifurcation is absent in *Clarias gariepinus*, it could indicate the homology of the ventromedial process of the posttemporo-supracleithral bone with a part of the transscapular ligament in other catfish groups.

A dermopalatine is considered to be absent in ostariophysan fishes. Tooth plates at the level of the autopalatine are then regarded as neomorphic, autogenous tooth plates (FINK & FINK, 1981; 1996; ARRATIA & SCHULTZE, 1991; ARRATIA, 1992). The dermopalatine described by VANDEWALLE *et al.,* (1993) in *Clarias gariepinus*, corresponds to the sesamoid entopterygoid bone of the present study. The "thin dermal plate" covering the anterior end of the cartilaginous pterygoid process probably may have been confused with the perichondral metapterygoid, which bears a dorsal and ventral membranous apolamella. In contrast to VANDEWALLE *et al.,* (1993), the cartilaginous connection between the metapterygoid and the entopterygoid could not be observed. The dermopalatine in *Galeichthys felis* (Ariidae), reported by BAMFORD (1948: Fig. 7B), appears to correspond to the prevomeral tooth plates, as they attach to the lateral ethmoids at the same spot as is the case in most siluriforms.

The homology of the urohyal bone in osteichthyan fishes has been extensively reviewed by ARRATIA & SCHULTZE (1990). In most teleost fishes the urohyal bone is an unpaired ossification of the aponeurosis, between the contralateral sternohyoideus heads, bearing two rostral processes for the ligamentous connection with the ventral hypohyals, and a ventral, horizontal plate (DE BEER, 1937; PATTERSON, 1977; ARRATIA & SCHULTZE, 1990). In siluriform fishes, however, a perichondral ossification participates in the formation of that bone, together with a paired bone of apparent sesamoid origin. This was also observed in the present study, in which development of the parurohyal bone is very similar to that observed in *Trichomycterus areolatus* (Trichomycteridae) (ARRATIA & SCHULTZE, 1990). In *Clarias gariepinus* a perichondral bone appeared at the ventral margin of the cartilaginous basibranchial I (8.4 mm SL), after the paired elements were observed (6.6 mm SL). At 8.4 mm SL, the

perichondral part was already fused to the sesamoid one. The chondroid bone in the parurohyal, as observed in some catfishes could, however, not be observed in *C. gariepinus* (ARRATIA & SCHULTZE, 1990).

Three hypotheses can be put forward: (1) the perichondral part corresponds to the endoskeletal urohyal of sarcopterygians, which becomes fused to dermal bones, the latter are homologous with the sarcopterygian interclavicles (PATTERSON, 1977), or (2) as PATTERSON (1977) in a way predicted, the perichondral part corresponds to the ventral part of the anterior basibranchial bone, which becomes fused with the paired tendon bones. A final possibility is (3) the secondary invasion of the ossification centres of the tendons of the sternohyoideus muscle into the basibranchial cartilage, which thus become perichondral, as was already proposed by ARRATIA & SCHULZE (1990). However, they mentioned that "the parurohyal of siluroids is a novelty within teleosts in its combination of paired tendon bones with the addition of secondary cartilage or chondroid bone." In *Clarias gariepinus*, no such secondary cartilage, nor chondroid bone could be observed. The cartilage involved was the basibranchial I. An ossified basibranchial I is absent in presently studied siluriforms, but could be observed in some other ostariophysans, *e.g.*, Cyprinidae (HARRINGTON, 1955; CUBBAGE & MABEE, 1996), Catostomidae (Cypriniformes) (WEISEL, 1960), Characidae (WEITZMAN, 1962), Hemiodontidae (Characiformes) (ROBERTS, 1974). Although the parurohyal of Siluriformes is considered to be homologous with the urohyal of other ostariophysans and other teleosts (ARRATIA & SCHULTZE, 1990), a detailed ontogenetic study, at an ultrastructural level, of the parurohyal and basibranchial cartilage might yield a conclusive result on the true nature of the parurohyal in siluriform fishes.

Another feature related to the parurohyal bone, in contrast to the teleostean urohyal bone, is the penetration of it by an artery. ARRATIA & SCHULTZE (1990) noted the passage of the hypobranchial artery, which runs anteriorly and enters the parurohyal bone dorsally in *Trichomycterus areolatus* and *Noturus flavus* (Ictaluridae). It penetrates the bone, leaving it ventrally. In *T. areolatus* the artery runs anteriorly, splitting into three branches at the anterior margin of the hypohyals. In *N. flavus*, however, the artery splits into two, immediately after it leaves the parurohyal (ARRATIA & SCHULTZE, 1990: Fig. 19). Each branch then gives off a lateral branch at the anterior margin of the hypohyals. The situation in *Clarias gariepinus* appears to differ even more (**Plate II.1-26**): a ventral branch of the ventral aorta runs to the dorsal face of the parurohyal bone and penetrates it through the foramen. Ventrally, it immediately splits off two branches, as in *N. flavus*, but they run posteriorly instead of anteriorly. The terminology "hypobranchial artery" is not adopted in the present study, as it appears that the artery penetrating the parurohyal is not homologous with the hypobranchial artery observed in other teleosts. In teleosts, the hypobranchial artery always splits off from an efferent branchial artery, in most cases the second one (BERTIN, 1958a; GOODRICH, 1958). In *C. gariepinus* the hypobranchial artery splits off from the fourth efferent branchial artery (NAWAR, 1955b). As this is not the case for the artery penetrating the parurohyal bone in *C. gariepinus*, it cannot be referred to as the hypobranchial artery. Consequently, the term "arteria parurohyalis" is applied.

The absence of the subopercular bone is a synapomorphic feature of Siluriformes (GOSLINE, 1973; FINK & FINK, 1981; 1996). In *Clarias gariepinus*, none of the ontogenetic stages revealed the presence of any subopercular primordium either. Suprisingly, a "small, rudimentary suboperculum" was observed in *Plotosus canius* (Plotosidae) and *Osteogeniosus militaris* (Ariidae) (BHIMACHAR, 1933).

SEQUENCE OF BONE FORMATION AS A RESPONSE TO FUNCTIONAL DEMANDS

The sequence of bone formation appears to be related to functional demands that arise in developing larvae and juveniles. These demands tend to vary to a large extent during the transformation from larva to adult, with several vulnerable moments at which the functional demands, and structural adaptations to support these demands barely compensate for each other (GALIS *et al.*, 1994).

The initiation of cranial ossification during ontogeny in teleost fishes appears to be coupled to respiratory and feeding requirements. In *Clarias gariepinus* the opercular bone is the first one to be formed (4.1 mm SL), supporting the opercular skin fold (**Plate II.1-38**). Opercular ossification starts at the articular facet of the opercular process of the hyosymplectic, as could be observed in other teleosts (VERRAES, 1973; HUYSSEUNE, 1985). From there on, the bone becomes a posteriorly extended rod, bearing a ventral plate (**Plate II.1-15B**). Myological data indicate that some of the muscles acting on the opercular bone arise between 5.2 and 6.8 mm SL. Although the adductor and levator operculi are present at that stage, it is only the levator muscle that already inserts onto the opercular bone (ADRIAENS & VERRAES, 1997b). However, prior to a possible muscular displacement of the opercular bone, the opercular skin fold is displaced by active respiratory movements, which already appear to occur at the 5.2 mm SL stage (SURLEMONT *et al.*, 1989). At this stage, opening and closing of the mouth is observed, coupled to ad- and abduction of the cheek region. A similar correlation between the onset of opercular ossification and the initiation of active respiratory movements was observed in *Galeichthys feliceps* (Siluriformes, Ariidae) (TILNEY & HECHT, 1993), *Catostomus commersoni* (Cypriniformes, Catostomidae) (MCELMAN & BALON, 1980) and *Gadus morhua* (Gadiformes, Gadidae) (HUNT VON HERBING *et al.*, 1996a). In *Poecilia reticulata* (Cyprinodontiformes, Poeciliidae) ossification already starts while still lying within the egg membrane. Although no true active respiration, by means of gills, is performed, respiratory movements were already observed (WEISEL, 1967). It appears that the alternating mechanical load onto the opercular skin fold, early during ontogeny, may be related to the early ossification of the opercular bone, prior to mechanical load exerted by muscular contraction of the opercular muscles (HERRING, 1993). It may be even possible that the causal, inductive effect of the hyosymplectic, in the onset of opercular ossification (HUYSSEUNE, 1985), is triggered by the alternating mechanical loads, but this is purely hypothetical and could not be supported with evidence. The formation of a substantial, horizontal rod in the opercular bone in *C. gariepinus* may well be related to the insertion of the levator operculi since, in the adult situation, this rod corresponds to the levator operculi crest (**Plate II.1-37B**) (ADRIAENS & VERRAES, 1997b).

Other ossifications related to the onset of respiratory movements are the branchiostegal rays, as they support the branchiostegal membrane in a similar way as the opercular bone supports the opercular skin fold (**Plate II.1-38**). The initiation of branchiostegal ray ossification generally follows that of the opercular bone rather rapidly, *e.g.*, in *Clarias gariepinus*, *Heterobranchus longifilis* (VANDEWALLE *et al.*, 1997), *Galeichthys feliceps* (TILNEY & HECHT, 1993), *Chrysichthys auratus* (VANDEWALLE *et al.*, 1995), *Barbus barbus* (VANDEWALLE *et al.*, 1992), *Danio rerio* (CUBBAGE & MABEE, 1996), *Catostomus macrocheilus* (WEISEL, 1967), *Oncorhynchus mykiss* (VERRAES, 1973), *Poecilia reticulata* (WEISEL, 1967), *Oryzias latipes* (LANGILLE & HALL, 1987). In *Gadus morhua*, however, a time lapse is present between the two ossifications (HUNT VON HERBING *et al.*, 1996a). The gradually adding of branchiostegal rays consequently provides the support of the gradually increasing branchiostegal membrane. The early differentiation of a dorsal process of the opercular bone in *C. gariepinus* appears to be related to the insertion of the dilatator operculi muscle. At 7.2 mm SL the insertion of a very indistinct tendon of the dilatator onto the

opercular bone was misinterpreted in a previous study (ADRIAENS & VERRAES, 1997b), as in the serial sections, the tendon appeared to be the anterior ossification of the opercular bone.

Early ossifications involve most, but not all, dentigerous bones. In *Clarias gariepinus* the premaxillaries, dental bones and upper pharyngeal tooth plates are present from the 6.0 mm SL stage on, whereas the initiation of tooth formation could already be observed in the 5.6 mm SL specimen. The other toothed bones, the lower pharyngeal and prevomeral tooth plates arise at the 8.4 mm and 11.6 mm SL stage, respectively. (**Table II.1- 3**, **Plate II.1-38**). Mandibular elevation occurs from the 5.2 mm SL stage on, as a well developed adductor mandibulae becomes functional (SURLEMONT *et al.*, 1989; ADRIAENS & VERRAES, 1996). At the transition from endogenous to exogenous feeding, at about 7 mm SL, most tooth-bearing bones are thus present (**Plate II.1-38**). A rapid increase in food uptake occurs in the first few days of exogenous feeding, whereas once the feeding process has stabilised, the gastric content remains fairly constant (close to 21% of the body weight) (HAYLOR, 1993). Early ossification of the dentigerous bones could also be found in the closely related *Heterobranchus longifilis* (VANDEWALLE *et al.,* 1997). In *Chrysichthys auratus*, the dentary appears prior to the premaxillaries and pharyngeal tooth plates, although their dentitions arise synchronously (VANDEWALLE *et al.*, 1995). In *Galeichthys feliceps* the dentaries also appear earlier than the premaxillaries (13.9 mm and 18.8 mm TL, respectively) (TILNEY & HECHT, 1993). In cypriniform fishes, which lack any dentition on either the dentary or premaxillary [homoplastic feature of Gonorhynchiformes and Cypriniformes (FINK & FINK, 1981; 1996; TAVERNE, *pers. comm.*)] the lower pharyngeal jaw, which is toothed, appears as one of the first ossifications, *e.g.*, *Danio rerio* (CUBBAGE & MABEE, 1996), or at least prior to the premaxillaries, *e.g.*, *Barbus barbus* (VANDEWALLE *et al.*, 1992), *Catostomus macrocheilus* (WEISEL, 1967) and *C. commersoni* (MCELMAN & BALON, 1980). This shift in sequence is an additional support for the supposition that the sequence of cranial ossification is a result of an evolutionary adaptation to accommodate the arising functional demands for the active uptake and manipulation of food (VANDEWALLE *et al.*, 1994). Additionally, it was observed in *Lates calcarifer* (Centropomidae, Perciformes) that dentigerous bones appeared only after a whole set of toothless bones (both dermal and perichondral). This appears to be related to an ontogenetic shift in feeding method as a response to prey size alteration during growth. In *Lates*, bones related to suction feeding appear first, followed by dentigerous bones at the moment prey grasping is performed (KOHNO *et al.*, 1996a). In *Chanos chanos* (Chanidae, Gonorhynchiformes) a dentition was not observed, not even 150 hours after mouth opening. At this moment, feeding appeared to be not of the suction/grasping type, but rather of the "straining" type (KOHNO *et al.*, 1996b). It is believed that the morphogenesis of certain bones is, in a way, influenced by the presence or absence of teeth (HUYSSEUNE, 1989). In *C. gariepinus*, the mentomeckelian bone is the first perichondral bone to appear (**Plate II.1-38**). Presumably, it may assist in reinforcing the dentary plate to the cartilaginous lower jaw, in order to be able to resist pressure forces more adequately.

The transition from endogenous feeding to exogenous feeding implies that solid food particles have to be taken in and consequently transported from the oro-branchial cavity into the oesophagus. Along their course, these particles pass along the hypophyseal fenestra, which is very large in platybasic skulls. It is thus not surprising that from that moment on, the overlying brain has to be protected more strongly from objects passing below than by the epithelial lining alone. A parasphenoid is formed preventing possible damage to the brain, a little prior to the transition phase (**Plate II.1-38**). The role of the parasphenoid, as a reinforcement of the skull base, does not appear to be as critical as could be supposed at this moment, as the trabecular bars are still intact, unlike salmonids (VERRAES, 1974b). The resorption of the middle part of these trabecular bars occurs

Table II.1- 3: Ossification sequence and type of the cranial bones in *Clarias gariepinus*, grouped per region (Legend: *D* = dermal anamestic bone; *DC* = dermal canal bone; *M* = membrane bone; *PC* = perichondral bone; *S* = sesamoid bone; *TP* = tooth plate)

Region	Bone	Initiation (mm SL)	Bone type
NEUROCRANIUM			
Ethmoid region	mesethmoid	11.1 mm	PC+D
	nasal	12.7 mm	DC
	lateral ethmoid	12.7 mm	PC+M
	prevomeral tooth plate	11.6 mm	TP
	prevomeral bone	18.7 mm	D
Orbito-temporal region	frontal	10.0 mm	DC
	orbitosphenoid	18.7 mm	PC
	pterosphenoid	12.7 mm	PC
	parasphenoid	6.6 mm	D
	antorbital	11.6 mm	DC
	lacrimal	11.6 mm	DC
	infraorbitale II	12.7 mm	DC
	infraorbitale III	15.2 mm	DC
	infraorbitale IV	15.2 mm	DC
Otic region	dermosphenotic	10.0 mm	DC
	autosphenotic	11.6 mm	PC
	prootic	10.0 mm	PC
	dermopterotic	10.0 mm	DC
	autopterotic	11.1 mm	PC
	posttemporo-supracleithrum	7.7 mm	DC+D
Occipital region	parieto-supraoccipital	11.1 mm	D+PC
	Epiotic	? 140.1 mm	PC
	Exoccipital	7.2 mm	PC
	Basioccipital	7.2 mm	PC
Maxillary bones	Premaxillary	6.0 mm	TP
	Maxillary	6.0 mm	D

Region	Bone	Initiation (mm SL)	Bone type
SPLANCHNOCRANIUM			
Premandibular arch	autopalatine	11.6 mm	PC
Mandibular arch	dentary	5.9 mm	D
	mentomeckelium	6.0 mm	PC
	splenial (dentary)	8.4 mm	DC
	angular	8.4 mm	D
	articular	8.4 mm	PC
	retroarticular	8.4 mm	PC
	splenial (angular)	11.1 mm	DC
	splenials (inter-articular)	46.8 mm	DC
	quadrate	7.2 mm	PC
	metapterygoid	11.6 mm	PC
	entopterygoid	8.4 mm	S
	coronomeckelium	12.0 mm	S
Hyoid arch	ventral hypohyal	7.2 mm	PC
	dorsal hypohyal	46.8 mm	PC
	anterior ceratohyal	7.2 mm	PC
	posterior ceratohyal	8.4 mm	PC
	interhyal	-	-
	hyomandibula	7.2 mm	PC
	symplectic	-	-
	parurohyal[9]	6.6 mm	S+PC
Branchial arches	basibranchials II - III	11.6 mm	PC
	hypobranchials I - II	11.6 mm	PC
	hypobranchials III-IV	-	-
	ceratobranchials I - V	7.2 mm	PC
	epibranchials I - IV	7.2 mm	PC
	infrapharyngobranchial III	18.7 mm	PC
	infrapharyngobranchial IV	46.8 mm	PC
	lower pharyngeal tooth plate	8.4 mm	TP
	upper pharyngeal tooth plate	6.0 mm	TP
Opercular bones	opercular	4.1 mm	D
	preopercular	10.0 mm	DC
	interopercular	11.6 mm	D
	suprapreopercular	18.7 mm	DC
	branchiostegal rays 7-10	6.0 mm	D
	branchiostegal rays 5 - 6	6.6 mm	D
	branchiostegal rays 2 - 4	7.7 mm	D
	branchiostegal rays 1	21.5 mm	D

[9] Although the parurohyal is not part of the hyoid arch, it is mentioned here because of its close relation with the hyoid bar.

approximately at 11.6 mm SL. At that stage, the parasphenoid has become broader at its lateral wings, thus interconnecting the interrupted bars. However, its role in supporting the central axis of the skull, together with the basioccipital, may not be ruled out completely (WEISEL, 1967). Early ossification of the parasphenoid is a general feature in teleost fishes, but when comparing its appearance in relation to the standard length, it is present in clariid species much earlier than, for example, in some silurids (KOBAYAKAWA, 1992) and cyprinids (VANDEWALLE *et al.*, 1992). In some cases the parasphenoid develops even earlier, at lower standard length values (*e.g.*, some Cyprinodontiformes) (WEISEL, 1967; LANGILLE & HALL, 1987). In the guppy, *Poecilia reticulata*, the parasphenoid and several other bones are present long before hatching starts, and consequently long before active food uptake (WEISEL, 1967), while in the cyprinodont *Oryzias latipes*, the parasphenoid is formed a little prior to hatching (LANGILLE & HALL, 1987).

Exogenous feeding requires a functional apparatus too, which enables active uptake of food particles. The sternohyoideus muscle is found to insert on the hyoid bar at the 6.8 mm SL stage (SURLEMONT & VANDEWALLE, 1991). The paired, sesamoid parts of the parurohyal bone can already be observed a little earlier (6.6 mm SL). This ossification of the tendons of the sternohyoideus is an adaptation to mechanical stress resistance, indicating a possible activity of that muscle. As this is the case (SURLEMONT & VANDEWALLE, 1991), a functional mouth opening mechanism is present from this stage on: contraction of the sternohyoideus results in the depression of the hyoid bar, which is coupled to the depression of the lower jaw through the protractor hyoidei muscle and/or the ligament between the lower jaw and the hyoid bar (OSSE, 1969; WINTERBOTTOM, 1974; VERRAES, 1977; LAUDER, 1980c; LAUDER & LIEM, 1980; AERTS, 1991) (see IV.1).

Once the primary functional demands are dealt with, the overall skull must become reinforced as it gradually becomes larger. Quite simultaneously, a whole set of perichondral bones are formed, which enable such a reinforcement (**Plate II.1-38**). These ossifications include both neurocranial elements, as well as splanchnocranial parts. The first neurocranial perichondral ossifications involve the reinforcement of the attachment of the skull to the notochord (basi- and exoccipital bones). Suspensorial ossifications appear around the articular facets, which already have become functional: the quadrate for the mandibular articulation and the hyomandibula for the neurocranial and the opercular articulations. All these articulations, which thus become reinforced, become increasingly important in feeding and respiratory movements. The ossification of the ventral hypohyal may be related to the ligamentous insertion, which connects the hyoid bar to the parurohyal, as well as the protective role of that bone for the hyoid artery [arteria mandibularis according to VERRAES (1975)]. As mentioned before, tension forces are already exerted onto this ligament from the 6.8 mm SL onwards; a reinforcement may thus be required. The anterior ceratohyal ossification may have a supportive function for the elongated cartilaginous hyoid bar, especially at the insertion site of muscles, which are already present and inserting onto it (ADRIAENS & VERRAES, 1997d). It may also support the increasing number of articulating branchiostegal rays. A similar relation has been observed in other teleosts (VERRAES, 1975). A similar interpretation holds for the long, branchial skeletal elements: the cerato- and epibranchials. An asynchrony in epibranchial ossification in *Heterobranchus longifilis* is related to the formation of a suprabranchial organ (VANDEWALLE *et al.*, 1997). Such an asynchrony does not occur in *Clarias gariepinus*, which indicates that the asynchrony may be absent or that it is spread over a much shorter time span.

Apart from overall reinforcement, the perichondral ossification of the above mentioned structures may also be coupled to a change in feeding behaviour. It has been observed that *Clarias gariepinus* performs cannibalism extensively. Two types of cannibalistic behaviour can be distinguished, based on body length and the way prey items are swallowed (HECHT & APPELBAUM, 1987). Type I cannibalism starts at about 8 mm total length

(corresponds to about 7.5 mm SL) until approximately 45 mm TL, and is characterised by capturing the prey by the tail, swallowing it up to the head, followed by biting off the head. This implies that large forces have to be produced by the adductor mandibulae complex, and consequently that a high mechanical stress is exerted onto the oral jaws. A well developed adductor mandibulae complex is present at 7.2 mm SL (ADRIAENS & VERRAES, 1996). The ossification of the quadrate may thus be an adaptation to resist large pressure forces during biting.

Once exogenous feeding has become obligatory and cannibalism has started, mouth opening becomes more and more important. The mouth opening mechanism, mentioned before, involves the retraction of the hyoid bar through the contraction of the sternohyoideus (see IV.1). This muscle interconnects the hyoid bar with the pectoral girdle, at the level of the cleithral bone. During contraction of the sternohyoideus, the cleithrum is held in position, or even retracted through the hypaxials (OSSE, 1969; LAUDER, 1980c; LAUDER & LIEM, 1980; MULLER, 1987). The connection of the cleithral bone to the neurocranium, through an articulation, can be advantageous for this kind of mechanism. The formation of the posttemporo-supracleithral bone can consequently assist in the reinforcement of the hyoid mouth opening mechanism.

The gradual strengthening of the mandibular articulation follows the onset of the cannibalistic behaviour (**Plate II.1-38**). Rather simultaneously, the angular, articular and retroarticular bones are formed. The articular facet of the initial bony mandibula is thus reinforced, in order to withstand the increasing pressure during the biting off of catfish heads. The transport (or fragmentation) of catfish bodies, on the other hand, is also improved, as the lower pharyngeal tooth plate is present. As the muscles acting onto them are present as well, both pharyngeal jaws can now become functional. This will play a crucial role in food fragmentation and/or transport from the orobranchial chamber into the oesophagus (VANDEWALLE *et al.*, 1994). This transport also requires a subsequent suction activity, in which the hyoid bar plays an important role (LIEM, 1990; AERTS, 1991). The posterior ossification of the hyoid bar consequently contributes to its increasing reinforcement, especially for the attachment of ligaments (ligamentum angulo-ceratohyale and ligamentum hyomandibulo-ceratohyale).

The ossification of the entopterygoid bone in *Clarias gariepinus*, and perhaps other siluriform fishes, may be related to increasing tensions that are exerted onto the ligament, interconnecting the suspensorium with the ethmoid region. As is proposed by ARRATIA (1992), this entopterygoid is of sesamoid origin, being the ossification of the middle part of the formerly mentioned ligament. Mineralisation is presumably induced by mechanical stress, whereas bone formation is induced at sites that experience an alternating mechanical load; *e.g.*, bending, pressure, tension, torsion (HERRING, 1993). The increasing use of mouth opening and mouth closure, as well as orobranchial expansions for feeding and respiratory purposes, will increasingly load the suspensorium. Powerful biting will exert forces onto the suspensorium in an anteroposterior direction. As the articular facet of the suspensorium with the neurocranium is not yet secured, this ligament may contribute in preventing the suspensorium from being pulled backward, especially since in Siluriformes, in general, the direct connection between suspensorium and the ethmoid region, through the palatine, is absent (ALEXANDER, 1965; GOSLINE, 1975a; FINK & FINK, 1981; 1996; ARRATIA, 1992). In most other teleosts, the entopterygoid appears prior to the metapterygoid (WEISEL, 1967; VERRAES, 1973; VANDEWALLE *et al.*, 1992; CUBBAGE & MABEE, 1996), although the former is considered not to be of sesamoid origin (ARRATIA, 1992).

The following bones that are formed involve canal bones of the skull roof. A little earlier, canal bones of the mandibular sensory canal were already formed, possibly coupled to the early ossification of the other mandibular bones. Apparently, the skull roof bones arise in close contact with the underlying cartilaginous skull, although they are of dermal origin (*cfr.* "parachondral bone") (VERRAES, 1974b; VERRAES & ISMAIL, 1980). The chondrocranium becomes bordered almost along its whole length: the frontal bone in the orbito-temporal

region, posterior to it the dermosphenotic, and even more posterior the dermopterotic, which closes up the space between the dermosphenotic and the posttemporo-supracleithral bone. Consequently, the main sensory canal is completely enclosed and protected. Such a close synchrony between the ossification of these bones is found in many other teleosts (KOBAYAKAWA, 1992; VANDEWALLE *et al.*, 1992; 1995; CUBBAGE & MABEE, 1996; VANDEWALLE *et al.*, 1997). In *Oncorhynchus mykiss*, the frontal appears well in front of the dermosphenotic and dermopterotic bones (VERRAES, 1973). The preopercular part of the preoperculo-mandibular canal is protected too at that moment, since it is enclosed by the preopercular bone.

Shortly after, two unpaired, bony complexes are formed, covering the left and right sides of the chondrocranium in the ethmoid region and the occipital region, respectively. In this way, the paired bones, formed before, are now closed off anteriorly and posteriorly by the mesethmoid and the parieto-supraoccipital bones, respectively. Again, the dermal parts of these bones arise in close contact with the cartilage, which has become perichondrally ossified. At this moment, all skull roof bones are present, and from now on, they can start to enlarge medially and laterally to close off the still unprotected part of the brain. Additionally, the increased contraction load on the insertion sites of several muscles onto these bones, must play an important role in ongoing ossification (*e.g.*, levator arcus palatini, dilatator operculi, levator operculi).

The development of the prevomeral tooth plate coincides with the formation of the opercular four bar-system, which is formed at 11.1 mm SL. This bar-system plays a crucial role in mouth opening in many adult teleosts (LIEM, 1970; AERTS & VERRAES, 1984; VERRAES, 1977; WESTNEAT, 1990) (see IV.1). A possible functional relation between an improved mouth opening for food uptake and the ossification of additional tooth plates is supported by the fact that at about this length (11.5 mm SL) the digestive system becomes completely functional (**Plate II.1-38**) (HECHT & APPELBAUM, 1987; HAYLOR, 1992). This allows improvement of the carnivorous behaviour of *Clarias gariepinus*, as is demonstrated by the already ongoing cannibalism (at about 8 mm TL) (HECHT & APPELBAUM, 1987). Apart from a required, improved mouth opening, the increase in prey size involves an elevated load onto several elements, like the lower jaw, and the suspensorial and branchial elements. Increased tension applied to the tendon of the adductor mandibulae, attached to the lower jaw, can be related to the induction of its ossification at that stage, *i.e.,* the coronomeckelium. The suspensorium and the branchial basket elements enable orobranchial expansion, as well as they aid in prey transportation to the pharyngeal jaws. Reinforcement of the pterygoid process, at the level where the ligament running to the ethmoid region is attached, also occurs through ossification of the metapterygoid. Manipulation of larger prey requires that larger forces be exerted by those elements that enable orobranchial transport. Increased muscular activity onto the branchial floor may be related to ossification of the basibranchials and hypobranchials at the level where muscles insert onto them.

Although it is able to perform filter feeding (GROENEWALD, 1964; TEUGELS, 1986),, the active hunting behaviour of *Clarias gariepinus* involves the use of well developed barbels for prey location, as well as for the sampling of potential food (ALEXANDER, 1965; 1966; GOSLINE, 1973). Although the muscular control of most of these barbels is restricted (GHIOT, 1978; ADRIAENS & VERRAES, 1997d), the maxillary barbel can be moved extensively, due to the palatine-maxillary mechanism (ALEXANDER, 1965; GOSLINE, 1975a; ADRIAENS & VERRAES, 1997a). In *C. gariepinus* the maxillary barbel is rather large [up to 174 % of the head length (TEUGELS, 1986)]. The protraction and retraction will entail a substantial drag. This implies that forces, needed for movements of the barbel, will have to increase correspondingly, which involves increasing load onto the skeletal elements of the palatine-maxillary mechanism. The maxillary bone, enclosing the base of the barbel, bears two well developed articular facets, for the articulation with the anterior tip of the palatine (ADRIAENS & VERRAES, 1997a). The palatine itself becomes rod-like, articulating medially with the ethmoid region (at the level of the orbito-nasal lamina).

Posterior to this articulation, the extensor tentaculi muscle connects the palatine to the neurocranium. It can thus be expected that autopalatine ossification provides: (1) a reinforced insertion site for the extensor tentaculi muscle, and (2) support for the rigidity of the palatine, in order to withstand bending forces, which would inactivate movement of the barbel in a rotating type of palatine-maxillary mechanism.

The last sensory canal to become enclosed and protected by bones is the infraorbital canal, as the antorbital and lacrimal bones are present at 11.6 mm SL. Late development of these bones is probably related to the late development of the infraorbital canal itself, in relation to the other canals, as occurs in other ostariophysan fishes (LEKANDER, 1949). However, very early ossification of the antorbital was observed in *Galeichthys felis* (Siluriformes, Ariidae) (BAMFORD, 1948). Possibly, the infraorbital canal is more protected between the eye and the mandibula than are the other canals. Ossification of the infraorbital canal bones proceeds anterocaudally. In other ostariophysans, the antorbital bone develops earlier than the infraorbitals, which appears to be the case in *Clarias gariepinus* as well, although not so distinct (LEKANDER, 1949). In relation to the supraorbital canal, the nasal bone is the last to develop (ADRIAENS *et al.*, 1997). The remaining exposed part of the preoperculo-mandibular sensory canal also becomes enclosed, as the suprapreopercular bone arises between the preopercular and the pterotic bones.

As the chondrocranium enlarges, the unossified space of the orbito-temporal region of the skull floor expands. Fortification through ossification may consequently become necessary, as is demonstrated by the development of the pterosphenoids, the lateral ethmoids, and later, the orbitosphenoids. Additional bridge formation between the pterosphenoid and the parasphenoid contributes to the reinforcement of the sphenoid fenestra, which consequently becomes subdivided into two. Such a reinforcement is present in most catfishes, as mentioned above. In the siluriform chondrocranium, the commissura lateralis, which forms the lateral wall of the trigemino-facial chamber and subdivides the sphenoid fenestra, is absent (DE BEER, 1937; DAGET, 1964; ALEXANDER, 1965). The absence of such a support between the skull roof and skull floor is compensated for by this secondary, bony bridge.

The late ossifications of the third and fourth infrapharyngobranchials can be coupled to the increasing load exerted onto these elements. They play a crucial role in the support of the upper pharyngeal jaws. Additionally, muscles needed for the manipulation of these jaws insert on them. The dorsal hypohyals do not appear to have any direct mechanical loading, as no muscles or ligaments insert on them, as well as the articulation with a basihyal does not occur in siluriform fishes [the latter is absent in Siluriformes (ARRATIA & SCHULTZE, 1990)]. Whether or not this plays a part in the late ossification cannot be confirmed.

The formation of the last canal bones, the splenials, is presumably related to the increasing length of an unprotected part of the preoperculo-mandibar canal, with increasing body length. At 46.8 mm SL, one of these bones could be found, whereas the number reached three in larger specimens. In that way, the sensory canal becomes enclosed and protected in a manner that still allows flexibility at that point, close to the mandibular joint.

Once the essential ossifications are present, the increasing mechanical load onto the body, as a result of increasing body length and muscle size, contraction strength, prey size, *etc.*, can be supported by thickening of the bones. In juveniles, the bones become rather thick through spongy bone formation. The interdigitation becomes extremely complex, reinforcing the connection between separate bones. The skull roof becomes gradually closed off completely, as a decrease of fontanella size has been observed in larger specimens. A splendid example of additional reinforcement against mechanical load is the locking device of the suspensorium. Most ostariophysans, *i.e.*, Characiformes, Siluriformes and Gymnotiformes, have an articular ridge,

instead of two articular condyles, for the articulation with the neurocranium (ARRATIA, 1992). Two articular condyles are believed to withstand re- and protraction forces to a greater extent (BAREL, *pers. comm.*; ADRIAENS & VERRAES, 1977b). In *Clarias gariepinus*, however, the articular ridge is reinforced through the formation of an interlocking between the hyomandibular bone and the neurocranial bones, thus allowing lateral swinging of the suspensorium during respiration and feeding (ADRIAENS & VERRAES, 1997b), but simultaneously preventing anteroposterior displacement during biting. An even more extreme interdigitation can be found in some anguilliform clariids, which also possess an enormous adductor mandibulae complex (CABUY, 1997).

VARIATION IN OSSIFICATION SEQUENCE: A RESULT OF VARIATION IN FUNCTIONAL DEMANDS?

In their paper, MABEE & TRENDLER (1996) extensively described the ossification sequence of *Betta splendens* (Belontiidae), which they compared with data from literature of *Oryzias latipes* and *Barbus barbus*. It appears that the ossification sequence in teleosts comprises sequences that are conserved during evolution, whereas other sequences have altered. The same kind of study was done in some poeciliids (STRAUSS, 1990b). Consequently, different conserved sequence pairs, meaning a fixed sequence of two bones within all the teleosts studied, were present. As they mentioned that ossification sequence statistics do not reflect a strict taxonomic coherence, other factors must be responsible. One of the possible determinants of ossification sequence can be the difference in functional demands of the different taxa. Differences in early life histories of fishes, reflected, for example, by the difference in feeding type and food type, can play an inductive role in the differentiation of a certain set of bones (KOHNO *et al.*, 1996a, b). For suction feeding fishes, other bones will of importance during initial feeding than those for performing suction-grasping feeding. Mechanical loads will act on different bones if feeding involves oral manipulation, compared with feeding by pharyngeal jaw manipulation. Undoubtedly, the ossification sequence has a genetic basis. However, this basis is subjected to interspecific, as well as intraspecific variation (MABEE & TRENDLER, 1996). Intraspecific variation may in turn be seen as a response to intraspecific variation in functional demands of developing larvae.

CONCLUSIONS

To conclude, some suggestions can be made concerning the relation of the ossification sequence and the presence of certain functional demands. It appears to be clear that early ossifications can be related to primordial respiration, as well as the formation of a primordial feeding apparatus during the transition phase from endogenous to exogenous feeding. As larvae grow, the overall mechanical load, related to skull support, muscle insertion, prey size, *etc.*, will also increase. Such demands are coped with in the growing *Clarias gariepinus* larvae as fortifications arise, especially at the level of those articulations related to the feeding apparatus. Protection of the brain from the outside initiates at the skull floor, prior to a dorsal protection, which may be related to initial food transport. Protection of the brain roof follows the enclosure of the cranial lateral line system of the skull roof. Optimisation of mouth opening coincides with the completion of the development of digestive system functionality. Prey capture is also improved at this stage, as the prevomeral tooth plates are formed. Prey detection is improved, due to the ossification of the palatine-maxillary mechanism.

II.1.3 - The Cranial Lateral-Line System[10]

The cranial lateral-line system, as well as the canal bones are well developed in the African clariid catfish *Clarias gariepinus*. A generalised cranial lateral-line pattern is present (supraorbital, infraorbital, preoperculo-mandibular, otic, postotic and temporal canals). The supratemporal commissure, however, is missing, although a supraorbital commissure is present (formed through the fusion of the epiphysial branches). In addition to canals, some pit-lines are present which cover both canal regions and non-canal regions (vertical, horizontal, oral, anterior, middle and posterior pit-lines). In this paper, several ontogenetic stages of the canal related bones in *C. gariepinus* are studied. A description of the canal bones, as well as some considerations concerning their nomenclature are given. All canal bones develop, whereas the parietal bone appears to have fused to the supraoccipital bone during ontogeny, as has been observed in some siluriforms. The extrascapulars (= supratemporals) are missing in *C. gariepinus*, as is the case in many siluriforms. The posttemporal and supracleithral bones have fused as well. Surprisingly, some separate splenial bones, enclosing the distal part of the mandibular canal are present. Some secondary modifications indicate the apomorphic features of the Clariidae. The circumorbital bones, from which the antorbital bone has lost the antorbital commissure, and the suprapreopercular bone are enlarged, plate-like bones. The nasal bone has undergone some secondary, plate-like extensions as well.

INTRODUCTION

The lateral-line system in fishes and in most larval and aquatic amphibians, plays an important role in the detection of water movements, related to prey or predator localisation or schooling behaviour (Lannoo, 1987a; 1987b; Partridge & Pitcher, 1980; Webb, 1989a). During ontogeny, superficial, primary neuromasts, after sinking in, become interconnected through the formation of canals in the epidermis (Bamford, 1948; Lekander, 1949; Dijkgraaf, 1962; Webb, 1989b). The general pattern of lateral-lines comprises one pair of trunk lateral-line canals, seven pairs of cranial lateral-line canals, two cranial lateral-line commissures and several lines of superficial, sensory organs. In generalised teleosts, the cranial lateral-line canal system comprises: (1) a supraorbital canal, (2) an infraorbital canal, (3) an otic canal, (4) a preopercular canal, (5) a mandibular canal, (6) a post-otic canal, (7) a temporal canal, (8) an ethmoid commissure and (9) a supratemporal or occipital commissure (**Plate II.1-39A**) (Webb, 1989a). However, during evolution, several canals have been reduced or have been added to this generalised pattern, or even have fused with each other in a different way (Lekander, 1949; Harrington, 1955; Verraes, 1973; Gosline, 1975b; Jarvik, 1980; Webb, 1989a; Arratia & Huaquin, 1995).

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Several studies indicated a rather firm relation between the embedded neuromasts and the development of cranial dermal bones enclosing the lateral-line canals. As a result, the cranial lateral-line pattern has been a basis for several taxonomic and phylogenetic studies (ILLICK, 1956; ROSEN & MENDELSON, 1960; BRANSON & MOORE, 1962; GOSLINE, 1974; 1975b; PARIN & ASTAKHOV, 1982; TAVERNE, 1986; WEBB, 1989a; ARRATIA & GAYET, 1995; ARRATIA & HUAQUIN, 1995). In early osteichthyans the cranium consisted of a mosaic of small, canal bearing, dermal bones, in between which non-canal bearing or anamestic bones were situated (PARRINGTON, 1949). DE BEER (1937) stated that the lateral-line system would only determine the position of the ossification centre. Experiments of MOY-THOMAS (1941) and PINGANAUD-PERRIN (1973) demonstrated the formation of canal-lacking frontal bones after extirpation of the lateral-line primordia and even the ossification primordia. According to PARRINGTON (1949) the control between canal bone and neuromasts should be interpreted as the other way around: "... the precursors of dermal ossifications influence the courses of the lateral-lines than by the current belief that the lateral-lines influence the position of the dermal bones." This hypothesis could explain the presence of several dermal canal bones, which have lost the connection with a lateral-line canal. Ontogenetic evidence, however, revealed the double nature of an adult canal bone (DEVILLERS, 1947; LEKANDER, 1949; DAGET, 1964; PINGANAUD-PERRIN, 1973): (1) a neurodermal component surrounding the sensory organs, and (2) a membranodermal, plate-like bone supporting the latter component. Both components eventually fuse during ontogeny, thus forming the adult canal bones. This can account for the hypothesis, generally accepted now, that the interactions between sensory canals and canal bones is present only with the neurodermal part of the canal bone, whereas the membranodermal element develops rather independently of the latter canals. Such hypothesis can consequently provide an explanation for the presence of bones, known to be associated with canals, in case the sensory canal is absent.

Although not always consistent, the cranial lateral-line pattern can aid in the identification of the cranial bones, together with evidence from ontogeny and the position of the dermal bones in relation to the underlying, cartilaginous skull with its perichondral ossifications (LEKANDER, 1949; PARRINGTON, 1967). In this chapter, the cranial lateral-line system of *Clarias gariepinus* BURCHELL (1822) is described and discussed, together with some ontogenetic evidence and evidence from literature. Some considerations are given for a proper nomenclature of those bones, which previously have been given different names. The nomenclature adopted follows the one proposed by HARRINGTON (1955). As was stated by WEITZMAN (1962), the nomenclature adopted in his paper did not necessarily imply homologies between other fishes and tetrapods, but only for the sake of usage of conventional terminology. The same can be applied for the current chapter. Concerning the terminology referring to parts of the lateral-line system itself, see **Plate II.1-39**.

RESULTS

The skull of adult *Clarias gariepinus* has a rigid roof, consisting of strongly interdigitating bones, both of dermal and perichondral origin (see II.1.2). They enclose the nasal cavity and border the dorsal margin of the orbit (**Plate II.1-40**). The dorsal surface of the juvenile and adult skull roof is ornamented. Lateral to this roof, some dermal bones (the infraorbital and suprapreopercular bones) are attached through connective tissue, thus enabling some mobility in a mediolateral direction. The cranial lateral-line canal pattern in *C. gariepinus* corresponds to that of the generalised siluriform situation (ARRATIA & HUAQUIN, 1995). The latero-sensory canals are almost completely embedded in the dermal bones, except for some canal pores and primary superficial tubules (WEBB, 1989a). These narrow and simple tubules are branches of the existing canals which overlie the bones and skin,

and which bear terminal pores (**Plate II.1-39B-C**). The position of the tubules onto the bones is marked by grooves. In addition to canals, some superficial neuromasts, arranged in pit-lines can be distinguished. These lines overlie regions of existing canals or cover non-canal regions. The number of pit organs, or superficial neuromasts, of each line is variable, as has been observed in several specimens.

In the observed specimens of *C. gariepinus* the four main canals (supraorbital, infraorbital, preoperculo-mandibular and temporal canal), together with the related canal bones, are described, as well as the pit-lines observed in the juvenile situation. Some considerations concerning the applied nomenclature of the canal bones, will be dealt with in the discussion.

SUPRAORBITAL CANAL (**Plates II.1-41A, 42A, 43**)

The supraorbital canal in *Clarias gariepinus* runs from the anterior nostril to the anterior fontanella, caudally fusing with the infraorbital canal. At the level of the fontanella, a medial branch is formed which meets the contralateral one medially. These epiphysial branches may or may not fuse in the middle of the fontanella. In some specimens a fused canal with a single tubule and pore (S_4) was found (**Plates II.1-41A, III.1-11A**), whereas in other specimens the branches, each bearing one terminal pore, did not fuse. When not fused, the tubules can be directed in the same or the opposite way. Most frequently, however, both tubules have fused and are directed posteriorly. The thus formed commissure has been referred to as the supraorbital commissure (BAMFORD, 1948; GOSLINE, 1974), the 'pseudocommissure supraorbitaire' (DAGET, 1964), the orbital branch of the supraorbital canal (LUNDBERG, 1982), the epiphysial branch of the supraorbital canal [as it covers the anterior fontanella at the level of the ossified part of the frontal bone, enclosing the epiphysial bridge (see II.1.2)] (WEITZMAN, 1962; ARRATIA & HUAQUIN, 1995) or canal 11 (LUNDBERG, 1975). Anteriorly, the supraorbital canal has three pores: two nearby the anterior nostril (S_1 and S_2) and one at the level of the anterior margin of the anterior fontanella (S_3). The epiphysial branches terminate in one or two pores, as mentioned above (S_4) [= epiphysial pore according to BORNBUSCH (1991a)]. Posterior to the anastomosis wit the infraorbital canal, an otic canal runs posteriorly. Anterior to the anastomosis the supraorbital canal bears a parietal branch, ending in a superficial tubule with a single, terminal pore (S_5) [= parietal pore according to BORNBUSCH (1991a)]. This branch, generally related to the parietal bone, has been observed in other teleosts as well, where it has been referred to as the parietal branch (WEITZMAN, 1962; ARRATIA & GAYET, 1995; ARRATIA & HUAQUIN, 1995), 'canal antérieur' (DEVILLERS, 1947; LUNDBERG, 1982) or canal 9 (LUNDBERG, 1975).

In *C. gariepinus* five bones, canal as well as non-canal bones, are associated with the supraorbital canal: the nasal, the lateral ethmoid, the mesethmoid, the frontal and the sphenotic bones. The latter bone will be discussed in the section on the infraorbital canal. An anterior pit-line can be distinguished, superficially on the frontal and sphenotic bones, medial to the parietal branch (see below).

Os nasale (**Plates II.1-40, 42A, 43**) - In *Clarias*, this canal bone is enclosed in a space formed by the mesethmoid bone and the lateral ethmoid bone (**Plate II.1-40**). Anteriorly, the nasal bone is bordered by the tubulous anterior nostril (**Plate II.1-42A**). The nasal is connected to the surrounding bones by connective tissue, thus allowing some dorsal expansion of the nasal sac. In juveniles, the nasal bone is composed of both the neurodermal and the membranodermal components. The nasal bone appears in the 12.7 mm SL larvae (**Table II.1- 3, p. 88**). Here, a small gutter-like bone lies lateral to the sphenoseptal commissure of the chondrocranium. Still in the 18.2 mm SL

stage, only the neurodermal component appears to be present, whereas in the 46.8 mm SL specimen, the canal bearing part is already enclosed in a plate-like bony structure.

The supraorbital canal becomes divided in the nasal bone, as a medial branch is formed (S_1). In *C. gariepinus*, the two branches leave the nasal bone through separate openings, terminating into a superficial tube with a single pore: the medial one anteromedial to the anterior nostril (S_1), the lateral one posteromedial to it (S_2) (**Plate II.1-41A**).

Os mesethmoideum and *os lateroethmoideum* (**Plates II.1-40, 42A-B, 43**) - Although these two bones are not canal bones, they support the anterior portion of the supraorbital canal. The first one is a compound bone as a result of a fusion between an anamestic dermal bone and a perichondro-enchondral one. In the 11.1 mm SL *Clarias gariepinus*, a paired dermal ossification, overlying the dorsal face of the ethmoid cartilage and the sphenoseptal commissures, is observed. These membranous bones most probably correspond to the paired dermal laterodermethmoid bone found in pholidophorid fishes (PATTERSON, 1975). The perichondral ossifications of the ethmoid cartilage are also observed, both dorsally and ventrally. In the 46.8 mm SL, a clear distinction can still be made between these perichondro-endochondral supra- and hypoethmoid bones and the dermal unpaired bone which results from the fusion of the two laterodermethmoids of younger specimens.

The laterally situated and paired *lateral ethmoid bone* in the 11.6 mm SL stage showed a perichondral ossification at the level of the articulation between the lamina orbito-nasalis and the palatine. Sections of the 46.8 mm SL specimen, however, revealed a substantial, lateral, membranous outgrowth of the perichondral bone. This lateral ethmoid bone bears a lateral process, which articulates with the dorsal face of the second infraorbital bone (**Plates II.1-40, 43, 44**). An enchondral ossification was absent. In the discussion, some comment on all the parts of the ethmoid complex is given.

Before it enters the frontal bone, the supraorbital canal runs through a furrow between the lateral ethmoid and the mesethmoid bone. This furrow is situated onto the dorsal surface of the lateral ethmoid bone, lateral to the suture with the mesethmoid bone. Before the canal continues into the frontal bone, a third superficial tubule is formed, which runs lateroposteriorly to the canal (S_3).

Os frontale (**Plates II.1-40, 42, 43**) - This largest canal bone of the skull of *Clarias gariepinus* interdigitates with the mesethmoid and the lateral ethmoid bones. Posterolaterally it is attached to the sphenotic bones, whereas posteriorly, it sutures with the parieto-supraoccipital bone (**Plates II.1-40, 42**). In the mid-line, the contralateral frontal bones are connected to each other posteriorly, whereas at the anterior three quarters of their length, they are separated by the anterior fontanella. Somewhere in the middle, the frontals bear a medial process, enclosing the cartilaginous epiphysial bridge, which are sutured to each other (**Plate II.1-43**). The first signs of a frontal bone ossification is observed in the 10.0 mm SL stage (**Table II.1- 3, p. 88**). A gutter-like bone covers the dorsal face of the sphenoseptal commissure and the taenia marginalis, whereas a small medial projection along the epiphysial bridge has already developed. In the 11.6 mm SL stage, a plate-like extension of the frontal bones, corresponding to the membranodermal component, is discernible. Consequently, the bone becomes extended in all directions. The canal enclosing the parietal branch can already be distinguished from the main supraorbital canal in the 12.7 mm SL stage. In the 15.2 mm SL larva both frontal bones meet in the midline at the level of the epiphysial bridge, where an interdigitation is established.

In the juvenile *C. gariepinus*, both the epiphysial branch and the parietal branch are enclosed by bone. In one specimen, the parietal branch is enclosed in both the frontal bone and the sphenotic bone (**Plate**

II.1-42A). The epiphysial branch is enclosed by that part of the frontal bone, which overlies the process enclosing the epiphysial bridge.

INFRAORBITAL CANAL (**Plates II.1-41A-B, 42, 44**)

In *Clarias gariepinus*, the infraorbital canal is enclosed completely by a series of circumorbital bones. The canal runs from the anterior nostril, along the ventral and posterior side of the orbit, up to the anastomosis with the supraorbital canal (**Plates II.1-41A-B, 42**). The canal bears six canal openings: four of them as simple pores, two of them through a superficial tube ending in a terminal pore (**Plate II.1-41A-B**). The anteriormost pore (I_1) is the elongation of the infraorbital canal in the antorbital bone (**Plate III.1-11A**). The second pore (I_2) however, leaves the lacrimal bone (= first infraorbital bone), at its junction with the antorbital, and runs along the ventrolateral margin of the latter bone before it penetrates the epidermis (46.8 mm SL specimen). The close relation between the second tubule and the antorbital bone may give the impression in cleared material that the latter bone bears a split infraorbital canal. The third tubule is situated at the junction between the lacrimal and second infraorbital bone, its simple pore lying anterior to the eye (I_3). In between the second and the third infraorbital bones, lies I_4. The third and fourth infraorbital bone each bear a superficial tubule, situated at their dorsal margin (I_5 and I_6, resp.).

In *C. gariepinus* five circumorbital bones (= antorbital + infraorbital bones), loosely attached to the lateral border of the rigid skull, enclose the infraorbital canal. The dermosphenotic bone (= fifth infraorbital bone) has become incorporated in the rigid skull, as ontogenetic evidence shows (see II.1.2). The latter bone bears the anastomosis between the infraorbital canal and the supraorbital one. The variation in morphology and number of the circumorbital bones has frequently resulted in a nomenclatural chaos. In general, the nomenclature is based on the position of the bone in relation to the other circumorbitals, on the canalisation condition in the bone or on some typical morphological features (SMITH & BAILEY, 1962). As WEITZMAN (1962) already stated, the nomenclature applied for the circumorbital series, and especially for the first two bones, is in a rather chaotic state. He proposed a simple, arbitrary system of numbered infraorbital bones, posterior to an antorbital bone in characins. The first infraorbital is then considered as homologous with the lacrimal bone. In literature, the anteriormost circumorbital bone in catfishes has generally been referred to as the lacrimal bone as well (KINDRED, 1919; BHIMACHAR, 1933; BAMFORD, 1948; EATON, 1948; TILAK, 1961; 1963a; 1965a; 1967; RASTOGI, 1963; TAVERNE & ALOULOU-TRIKI, 1974; LUNDBERG, 1982; LUNDBERG *et al.*, 1991; SRINIVASA RAO & LAKSHMI, 1984; TEUGELS *et al.*, 1991), although some different nomenclature has been applied (NAWAR, 1954; SRINIVASACHAR, 1958b; TILAK, 1963c; GAUBA, 1966). However, evidence from diplomystids, which are considered to be the most primitive siluriforms (ARRATIA, 1987), shows that the anteriormost circumorbital bone most probably corresponds to the antorbital bone, as it still bears the branched rostral part of the infraorbital canal (see Discussion) (ARRATIA & HUAQUIN, 1995). As a result, in the current paper, the first circumorbital bone will be referred to as the antorbital bone. The first infraorbital bone will be referred to as lacrimal whereas the fifth one is called 'dermosphenotic bone'.

Os antorbitale (**Plates II.1-40, 42, 44**) - This anteriormost circumorbital bone is the smallest in the series, and is situated at the rostral tip of the palatine, close to the articulation between the latter and the maxillary bone (**Plate II.1-40**). Although the difference is not as striking as for the infraorbital bones, the neurodermal part can be distinguished from the membranodermal one, the latter being rather small (**Plate II.1-44**). A small tubulous antorbital bone could be distinguished from the 11.6 mm SL stage on (**Table II.1- 3, P. 88**). At 12.7 mm SL it

overlies the articulation between the palatine and the maxillary bone. Although still tubulous in the 18.2 mm SL larva, it is plate-like in the 46.8 mm SL specimen. Surprisingly, the antorbital is connected to the rostral tip of the palatine. The latter bears a dorsal, cartilaginous process which differentiates into chondroid bone (**Plate I.4-7D**). A cross section at this level shows that the antorbital partially encloses the base of the nasal barbel as it overlies a lateral wall of the nasal sac.

The infraorbital canal leaves the antorbital bone anteriorly, and opens through a pore at the posterolateral face of the anterior nostril (I_1). Posteriorly, the canal runs into the lacrimal bone, after a first branch has been split off, terminating in a pore, posterior to the antorbital one (I_2).

Os lacrimale (**Plates II.1-40, 42, 44**) - This first infraorbital bone abuts against the posterior margin of the antorbital bone (**Plate II.1-44**). As is the case for all infraorbital bones, the lacrimal is directed in an anteroposterior way. It is plate-like, possessing a more distinct membranodermal component, compared to the antorbital bone, with a serrated ventral margin (**Plate II.1-44**). The lacrimal is observed for the first time in the 11.6 mm SL specimen as a small tube posterior to the almost equally sized antorbital bone (**Plate II.1-22**). In the 12.7 m SL specimen the lacrimal has become larger than the antorbital (**Plate II.1-24**). The bone remains tubulous in the 18.2 mm SL stage, but has become plate-like in the 46.8 mm SL specimen.

The infraorbital canal enters the lacrimal anteriorly, posterior to its second branch. The latter branch is elongated into a tubule which follows the ventral margin of the antorbital bone (**Plates II.1-41A, 44**). Posteriorly, the lacrimal abuts against the lower half of the infraorbital bone II (**Plate II.1-44**).

Os infraorbitale II (**Plates II.1-40, 42, 44**) - This infraorbital bone is the most substantial one of the series. Dorsocaudally the bone is excavated to fit the eye ball, which it borders anteriorly and ventrally (**Plate II.1-44**). Anterodorsally, the infraorbital bone articulates with a lateral process of the lateral ethmoid bone (**Plates II.1-40, 42A**). This articulation, together with the connective tissue between the infraorbital bone IV and the rigid skull, can function as a hinge for the infraorbital series, thus enabling a lateral displacement during respiration and allowing a lateral expansion for the adductor mandibulae complex during its contraction. Medially a subocular shelf is present for the support of the eye ball. The tubulous infraorbital bone II appears at 12.7 mm SL (**Table II.1-3, p. 88**). At 18.2 mm SL it has extended posteriorly, following the curvature of the eye ball. Still the bone is tube-like, apparently without any articulation with the lateral ethmoid bone yet.

Between the infraorbital bone I and the infraorbital bone II, the infraorbital canal has a branch which ends in a simple pore, laterally to the posterior nostril (I_3) (**Plate II.1-41A-B**). The infraorbital canal leaves the infraorbital bone II at the ventral face of the eye ball (I_4) (**Plate II.1-41B**).

Os infraorbitale III (**Plates II.1-40, 42, 44**) - In *Clarias gariepinus*, the posterior margin of the eye ball is bordered by the third and fourth infraorbital bone (**Plate II.1-42B**). The former one is triangular in shape and bears a groove which houses a superficial tubule of the infraorbital canal (**Plate II.1-44**). The posterior, plate-like extension covers the adductor mandibulae complex rostrally (**Plates II.1-42B, II.2-1**) (ADRIAENS & VERRAES, 1996). The associated groove is situated at the posterodorsal border of the infraorbital bone. The infraorbital bone III cannot yet be observed at 12.7 mm SL but primordia appear to be present in the 15.2 mm SL larva. No plate-like extension, however, can be observed yet.

The infraorbital canal enters the bone rostrally, at the connection with the infraorbital bone II. Posteriorly, a fifth tubule is formed which is directed rather horizontally, although variation has been observed (I_5) (**Plate II.1-**

41A-B). Dorsal to the branching point, the infraorbital canal is enclosed only partially in the infraorbital bone III, as it lies in a gutter onto the latter bone, before it enters the infraorbital bone IV (**Plate II.1-44**).

Os infraorbitale IV (**Plates II.1-40, 42, 44**) - The largest in the series, the infraorbital bone IV, forms the posterior and dorsal margin of the orbit. It also covers the lateral face of the adductor mandibulae complex in *Clarias gariepinus*, which inserts onto it (**Plate II.1-42B**) (ADRIAENS & VERRAES, 1996) (see II.2.2). Anterodorsally, the bone bears a large process covering the major dorsal part of the eye ball. This supraorbital process runs up to the articulation between the infraorbital bone II and the lateral ethmoid bone (**Plate II.1-40**). As can be observed in the third infraorbital bone, this bone bears a caudal plate-like extension as well, marked with a groove at its dorsocaudal margin (**Plate II.1-44**). The infraorbital bone IV primordium was observed for the first time at 15.2 mm SL (**Table II.1- 3, p. 88**). It is gutter-like, lying against the ventral border of the rigid neurocranium. As a result, a gap is present between the infraorbital bone III and IV. In the 46.8 mm SL, however, both infraorbitals have become plate-like and the gap has been closed.

The infraorbital canal enters the infraorbital bone IV at its anteroventral face. The superficial tubule is split off at its posterodorsal border, as it lies horizontally onto the bone (l_6). As is the case for the infraorbital bone III, the dorsally extended infraorbital canal is partially enclosed in a gutter (**Plate II.1-44**). From there on, the canal enters the rigid skull, into the sphenotic bone.

Os sphenoticum (**Plates II.1-40, 42, 43**) - In general, the last bone in the infraorbital series, enclosing the postorbital anastomosis between the supraorbital canal and the infraorbital one, is called the dermosphenotic bone, although many exceptions are known (see Discussion) (BAMFORD, 1948; DAGET, 1964; GOSLINE, 1975b; JARVIK, 1980). In adult *Clarias gariepinus*, the dermosphenotic bone is not present as a separate canal bone, but has fused with an underlying, perichondral autosphenotic bone. This bony complex is referred to as the sphenotic bone. At 10.0 mm SL a small dermosphenotic bone can be observed at the lateral margin of the posterior part of the taenia marginalis (**Plate II.1-21**). The bone is gutter-like, thus only comprising the neurodermal component. In this stage, it is still completely isolated from the surrounding bones. In the 11.6 mm SL specimen, a perichondral ossification has started along the ventral face of the taenia marginalis, corresponding to the autosphenotic bone (**Plate II.1-19C**). Laterally, a plate-like process is present, corresponding to the membranodermal part of the dermosphenotic bone. The neurodermal part is still gutter-like. In the 12.7 mm SL specimen however, the gutter has closed and a canal is formed, which covers the lateral margin of the taenia marginalis (**Plate II.1-24A**). At this stage, the sphenotic bone has come into contact with the pterotic bone posteriorly, and comes to lie in close contact with the frontal bone anteromedially. At 18.2 mm SL, the close connection between the sphenotic canal and the one in the frontal and pterotic bones has formed. At the lateral face of the sphenotic bone, the fourth infraorbital bone abuts, its dorsal canal opening straight in front of the lateral canal opening of the sphenotic bone. At this level, the anastomosis of the infraorbital and supraorbital canal can clearly be distinguished in the sphenotic bone. In juvenile *C. gariepinus* the sphenotic bone bears a groove which houses the superficial tubule of the parietal branch. The parietal branch is embedded in the frontal bone, with the superficial tubule covering the sphenotic bone, its position marked by a groove (**Plate II.1-42A**). Apparently some variation appears to occur as in another specimen, this groove covered the pterotic bone (**Plate II.1-40**).

The infraorbital canal enters the sphenotic bone at the anterior half of its lateral margin, whereas the supraorbital canal enters at its mediorostral margin (**Plate II.1-43**). The anastomosis of both canals is situated medially. The otic canal leaves the sphenotic bone at its posterior margin, consequently penetrating the pterotic bone.

PREOPERCULO-MANDIBULAR CANAL (**Plates II.1-41, 42, 45, 46**)

The otic canal runs from the postorbital anastomosis of the infraorbital and supraorbital canal, up to the anastomosis between the postotic and preoperculo-mandibular canal. The preoperculo-mandibular canal can be divided into a preopercular part (with three pores) and a mandibular part (with six pores) (**Plate II.1-41**). In *Clarias gariepinus* the preopercular canal is enclosed by both neurocranial bones and suspensorial bones. The mandibular part is enclosed by the bones constituting the lower jaw. In *C. gariepinus* the canal starts rostrally at the mandibula, close to the symphysis (**Plates II.1-41C, 45**). The contralateral mandibular canals are not connected to each other, even though they do approach each other closely (**Plate II.1-41C**). Rostrally the mandibular canal bears two branches, each terminating in a simple pore. Both these pores (PM_1 and PM_2) are situated medially to the bases of the internal mandibular barbel (**Plate II.1-41C**). Anterolaterally a third branch is present, its pore lying in between the internal and external mandibular barbel (PM_3). The pore of a fourth branch lies posteriorly against the base of the external mandibular barbel (PM_4). The fifth branch terminates at the lateral side of the lower jaw (PM_5). The sixth pore is situated at the level of the articulation between the lower jaw and the suspensorium (PM_6), whereas the two following tubules border the preopercular bone (PM_7 and PM_8) (**Plate II.1-41**). The latter two branches are part of the preopercular canal. The third branch of the latter canal splits off above the opercular slit (PM_9), ventrally to its fusion with the otic canal.

Concerning the nomenclature for the bones constituting the lower jaw, different opinions have been proposed whether the dentary and the angular bone have fused with the splenial bones and the perichondral ossifications of Meckel's cartilage, or not. The nomenclature applied in this paper follows the strategy of LEKANDER (1949) and JARVIK (1980), *i.e.*, a nomenclature which represents the complexity of the bony structure: the os dento-splenio-mentomeckelium and the os angulo-splenio-articulo-retroarticulare. Surprisingly, apart from the latter two bones, the mandibular canal is enclosed distally in several splenial bones, before it continues into the preopercular canal (**Plates II.1-42B, 45**). The latter canal is enclosed by the preopercular and suprapreopercular bones, before it fuses with the otic canal in the pterotic bone.

Os dento-splenio-mentomeckelium (**Plates II.1-33B, 42B, 45, 46**) - This bone complex surrounds the anterior part of Meckel's cartilage, whereas its posterior part covers it laterally (**Plate II.1-45B**). The first signs of the dento-splenio-mentomeckelian bone could be observed at 5.9 mm SL where a lamellar bone lies against the rostrolateral face of Meckel's cartilage (**Table II.1- 3, p. 88**). This bone most probably corresponds to the dentary. No other ossifications are present. In the 6.0 mm SL *Clarias gariepinus*, the anterior half of the cartilaginous lower jaw is covered by a bony element. The rostral tip of Meckel's cartilage is perichondrally ossified, as can be derived from serial sections of a 7.2 mm SL specimen. However, the bone covering the rostrolateral face of Meckel's cartilage, although fused to the perichondral bone, corresponds to the above mentioned dermal, lamellar bone. It can thus be accepted that the rostral tip corresponds to the mentomeckelian bone, with the dermal bone corresponding to the dentary (HARRINGTON, 1955). Apparently the dentary in *C. gariepinus* initially arises independently from the mentomeckelian bone, the latter ossifying later but early in ontogeny. No signs of any separate splenial bones could be observed yet, nor is the mandibular canal enclosed in the dentary. At 8.4 mm SL, however, a gutter-like bone has formed against the ventral face of the dento-mentomeckelian complex. The single gutter lies approximately along the whole length, except for the rostralmost part. During further ontogeny, the gutter gradually becomes closed , until it encloses the mandibular canal completely (15.2 mm SL) (**Plate II.1-23**). In the 46.8 mm SL specimen of *C. gariepinus*, no distinction could be made anymore between the splenial bones and the dento-mentomeckelian complex. The thus formed dento-splenio-mentomeckelian bone still

forms the major part of the lower jaw in the juvenile specimens (**Plate II.1-46**). The complex bears a ventral and a lateral process, which interdigitate with the angulo-splenio-articulo-retroarticular bone.

A three-dimensional reconstruction of the lower jaw of the 46.8 mm SL *C. gariepinus* shows the path and branching of the canal in the dento-splenio-mentomeckelian bone (**Plate II.1-45**). The two anteriormost branches leave the bony complex at its anteroventral face, which is slightly curved inwards (PM_1 and PM_2). The third opening is situated at the lateral face, at the level where the curvature of the lower jaw starts (PM_3). The fourth opening lies right below the coronoid process of Meckel's cartilage (PM_4). The following opening lies in between the interdigitation between dento-splenio-mentomeckelian bone and the angulo-splenio-articulo-retroarticular bone (PM_5).

Os angulo-splenio-articulo-retroarticulare (**Plates II.1-42B, 45, 46**) - The posterior part of the adult lower jaw consists of a bony complex, somehow analogous to the dento-splenio-mentomeckelium: it is constituted by an anamestic dermal bone, two perichondral bones and a splenial bone. This angulo-splenio-articulo-retroarticular complex arises around the articulatory facet of the lower jaw with the suspensorium. During ontogeny this bone does not develop simultaneously with the dento-splenio-mentomeckelian bone. At 8.4 mm SL no signs of an angular, nor splenial bones were observed. However, a perichondral ossification was found at the level of the articulatory facet. Posterior to this articular bone, and separated from it, the perichondral retroarticular bone could be distinguished, at the ventral face of the retroarticular process. Surprisingly, the dermal angular bone appeared to be present in the 8.3 mm SL specimen, whereas no sign of any perichondral ossification could be noted. This angular bone is a small lamellar bone, lying against the ventral face of Meckel's cartilage at the level of the articulation between the lower jaw and the suspensorium. Apparently, both dermal and perichondral ossifications of the angulo-splenio-articulo-retroarticular develop rather simultaneously. At 10.0 mm SL a gutter-like bone can be distinguished, lying in the extension of the gutter of the dental splenial bone. Apparently the angular splenial bone has formed. The angular bone now covers the lateral face of the coronoid process of Meckel's cartilage. Although not clearly visible on the poorly stained specimen, it appeared to cover the complete lateral face of the mandibula at that level and to have fused to the splenial bone. In the 11.6 mm SL specimen all ossifications have fused clearly now. The angulo-splenial part can, however, still be recognised by the two bony rims forming a gutter. The thus formed angulo-splenio-articulo-retroarticular bone has reached the dento-splenio-mentomeckelian bone, at the level of the coronoid process. In the 15.2 mm SL specimen the gutter is almost completely closed, leaving two openings for the mandibular canal and its branch. The juvenile angulo-splenio-articulo-retroarticular bone does not completely surround Meckel's cartilage, as the latter lies unprotected against the medial face of the bone complex (**Plate II.1-33A**). Only the posterior tip is enclosed in the articular part of the bone complex.

The mandibular canal enters the angulo-splenio-articulo-retroarticular bone as it leaves the dento-splenio-mentomeckelian bone (**Plate II.1-45**). At this spot the canal bears a lateral branch (PM_5). Posteriorly, the canal leaves the angulo-splenio-articulo-retroarticular bone, close to the mandibular articulation, at its lateral face.

Ossa splenialia (**Plates II.1-42B, 45**) - Surprisingly, some isolated canal ossicles enclose the posterior part of the mandibular canal in late larval and juvenile *Clarias gariepinus*. In the 46.8 mm SL specimen, a single ossicle is observed, whereas in larger specimens (136.2 mm SL) up to three of them were present. Apparently some additional splenial bones have formed to enclose the exposed part of the sensory canal in between the mandibula and the preopercular bone. More bones are formed as the length of the not enclosed part of the

preoperculo-mandibular canal increases, suggesting that the splenial bones protect the latter part. As the latter part of the sensory canal undergoes flexions during jaw movements, a single canal bone would not be suitable. Several small splenial bones, however, still allow such flexions. Such ossa splenialia have also been described and figured in some diplomystid (ARRATIA, 1987: fig. 15) and sisorid catfishes (TILAK, 1963b: fig. 21; MAHAJAN, 1966: fig. 2). The latter author referred to them as the infrapreopercular ossicles.

That part of the mandibular canal enclosed in these splenial bones bears a branch with a superficial tubule. The latter is directed caudally and terminates in a single pore (PM_6) (**Plate II.1-45**).

Os praeoperculare (**Plates II.1-42B, 46**) - The preopercular canal bone has become part of the suspensorium in *Clarias gariepinus*, as is the case for most Actinopterygii [from the semionotiform level on (GARDINER, 1967)]. In *C. gariepinus* the bone interdigitates with the ventrocaudal margin of the hyomandibular bone. At 10.0 mm SL a gutter-like bone lies against the lateral face of the ventrocaudal margin of the cartilaginous suspensorium (**Plate II.1-21B**). It stretches along the posterior part of the quadrate and the anterior part of the hyosymplectic. At 11.6 mm SL, however, the gutter has closed and has become extended, its anterior tip lying against the ventrolateral border of the quadrate bone, close to the mandibular articulation. Posteriorly, the preopercular bone covers the base of the opercular process of the ossifying hyomandibular bone, at its lateral face. The preopercular bone thus follows the curvature of the suspensorial ventral border. At 18.2 mm SL the pores for the branches of the preopercular canal can be discerned clearly. Two small canal bearing processes are present, a dorsal one directed posteriorly and a ventral one directed anteriorly. In the 46.8 mm SL specimen, the membranodermal component is present, sutured to the ventrocaudal margin of the hyomandibular bone. In the juvenile preopercular bone, both branches can still clearly be distinguished as a continuous gutter is present at the medial face (**Plate II.1-46**). The lateral face of the preopercular bone bears a small rim for the insertion of the adductor mandibulae complex (**Plate II.1-42B**).

As the mandibular canal leaves the splenial bones, it continues in the preopercular canal enclosed anteriorly by the preopercular bone. The canal enters the bone at its rostroventral tip. Along its course, this part of the canal bears two preopercular branches (PM_7 and PM_8). Dorsocaudally the canal runs into the suprapreopercular bone, entering it after a last tubule is split off (PM_9).

Os suprapraeoperculare (**Plates II.1-40, 42, 46**) - This plate-like bony is loosely attached to the rigid skull in *Clarias gariepinus*. Within the siluriforms the presence of such a bone appears to be rather variable (see Discussion, **Table II.1- 4, p. 110**). In the cleared specimens the suprapreopercular bone could be noted from the 18.7 mm SL stage on (**Table II.1- 3, p. 88**). Both membranodermal and neurodermal components are present and appear to have fused already. The suprapreopercular bone fits in between the lateral border of the pterotic and the dorsal tip of the preopercular bone (**Plate II.1-42**). Caudally the bone partially covers the levator operculi (**Plates II.1-42, II.2-35**).

The preopercular canal enters the suprapreopercular bone at its ventral margin, after a last branch is split off (PM_9) (**Plates II.1-41, 42, 46**). Dorsally the canal leaves the bone as it is directly continued into the pterotic bone.

Os pteroticum (**Plates II.1-40, 42, 43**) - As is the case for the sphenotic bone, the pterotic one has a complex origin (see Discussion). At 10.0 mm SL a gutter-like bone is present at the level of the otic region (**Table II.1- 3, p. 88**) (**Plate II.1-21**). The latter covers the dorsal face of the lateral neurocranial rim, starting half way the otic capsule up to the postotic process of the chondrocranium. Apparently, this gutter-like bone represents the

neurodermal part of the dermopterotic bone. The dermopterotic bone is already in close contact with the posttemporo-supracleithral bone, however, it is still separated from the sphenotic bone. At 12.7 mm SL this contact is present also (**Plate II.1-24**). The pterotic gutter has not yet closed, whereas half way the gutter, a ventral pore is present, through which enters the preopercular canal. Dorsomedially and ventromedially to the dermopterotic gutter, a perichondral ossification of the lateral part of the otic capsule has initiated, thus forming the autopterotic bone. Apparently the dermopterotic and autopterotic bones have already fused, giving rise to the complex pterotic bone. During further ontogeny the gutter is closing, coupled to a medially progressing ossification of the otic capsule. In the 18.2 mm SL specimen the canals have closed completely, whereas the perichondral part of the complex has reached the lateral border of the parieto-supraoccipital bone. Although they have fused, the distinction between the autopterotic part and the dermopterotic part can still be made: the horizontal plate-like part, enclosing the sensory canals represents the dermal component, whereas the ventral part enclosing the lapillus otolith corresponds to the autopterotic part (**Plates II.1-30D, 43**).

The pterotic bone encloses two anastomoses: one between the otic canal, the preoperculo-mandibular canal and the postotic canal, and another between the postotic canal and the temporal canal with its pterotic branch [the latter branch is referred to as the pterotic branch of the otic canal by ARRATIA & GAYET (1995)]. A clear distinction, however, cannot be made between the latter sensory canals, as the supratemporal commissure is missing. In general the postotic canal runs between the anastomosis of the preoperculo-mandibular canal and the otic canal and the anastomosis of the supratemporal commissure and the temporal canal (WEBB, 1989b). In *C. gariepinus*, however, a pit-line can be observed covering the site where a supratemporal commissure could be expected. The latter crosses the postotic canal, right in front of the branching point of the temporal canal.

TEMPORAL CANAL (**Plates II.1-41A-B, 42, 43**)

The smallest cranial lateral-line canal, the temporal one, forms the transition from the cranial lateral-line system to the trunk lateral-line system (**Plates II.1-41, 42, 43**). As mentioned, the canal splits off a lateral branch, *i.e.,* the pterotic branch, which terminates in a superficial tubule overlying the posttemporo-supracleithral bone (T_1). As the canal leaves the posttemporo-supracleithral bone caudally, a second branch with superficial tubule is split off (T_2).

Os posttemporo-supracleithrum (**Plates II.1-40, 42, 43**) - Quite some discussions have been made concerning the homology of the posteriormost canal-bearing bone in the catfish skull roof (see Discussion). In *Clarias gariepinus* the dermal posttemporo-supracleithral bone has become part of the rigid skull roof, thus being excluded from the pectoral girdle. In *C. gariepinus* the posttemporo-supracleithral bone appeared between 7.2 and 7.7 mm SL (**Table II.1- 3, p. 88**). It is small and plate-like, lying in between the dorsal part of the cleithral bone and the posterior part of the otic capsule. Sections of the 8.3 mm SL specimen revealed that the small lamellar posttemporo-supracleithral bone was covered by a, not yet invaginated, neuromast. Apparently, the membranodermal part of the latter bone develops first. In the 10.0 mm SL specimen the posttemporo-supracleithral bone has formed several processes, one running up to the parapophysis of the fourth vertebra, and two running rostrally to the pterotic bone (**Plate II.1-21B**). From this stage on the posttemporo-supracleithral bone is in close contact with the posterior margin of the pterotic bone. No clear distinction of a canal bearing part could be observed, however. The posttemporo-supracleithral bone of the 11.6 mm SL specimen, on the

contrary, does show a pore through which, most presumably, the temporal canal enters (**Plate II.1-22B**). During further ontogeny, the processes become extended, as does the dorsal plate-like part. Eventually (18.2 mm SL) the posttemporo-supracleithral bone interdigitates with the posterolateral margin of the pterotic bone, as is still the case in the juvenile situation (**Plates II.1-40, 43**).

The temporal canal enters the posttemporo-supracleithral bone at its anteromedial side (**Plates II.1-42, 43**). At the caudal margin of the bone, a groove is present, marking the second branch of the temporal canal (T_2), the sensory canal itself being continued in the body lateral-line.

PIT-LINES (**Plate II.1-41**)

In *Clarias gariepinus* four separate cranial pit-lines are present, three of them are lying on the dorsolateral face of the head (**Plate II.1-41**). The number of neuromasts in each line varied between specimens. (1) The pit-line situated at the lateral face of the head is rather long and is constituted of two or three lines which have been observed in other Halecostomi (LEKANDER, 1949; JARVIK, 1980; ARRATIA & GAYET, 1995; ARRATIA & HUAQUIN, 1995). The *horizontal pit-line* runs from the preopercular canal, at the base of the last superficial tubule (PM_9), horizontally in the direction of the infraorbital canal. Before it reaches the latter it bends ventrally to the posterior part of the mandibular canal, at the level of the PM_6 pore. This ventral part of the pit-line corresponds to the *vertical pit-line*. In larger specimens (281 mm SL specimen), however, the pit-line is extended even more as it bends again in a rostral direction, running horizontally along the lateral face of the lower jaw, where it eventually curves up again at the level of the infraorbital pore I_4. This pit-line presumably corresponds to the *oral pit-line* of cyprinids (LEKANDER, 1949). (2) Posterior to the parietal branch of the supraorbital canal, a short pit-line is present. This *anterior pit-line* is slightly bent. (3) Rather perpendicular to the latter pit-line lies one which covers the area where a supratemporal (or occipital) commissure would be expected. This *middle pit-line* runs from the posterodorsal face of the pore PM_9 up to the posterior fontanel where it bends anteriorly. (4) The posteriormost pit-line follows the path of the temporal canal. Anteriorly, this *posterior pit-line* is slightly bent medially.

DISCUSSION

NOMENCLATURE OF THE CANAL BONES AND CANAL RELATED BONES

For a general revision of the nomenclature of the cranial bones, with their synonyms, we refer to HARRINGTON (1955). Ethmoid ossifications in teleost skulls have frequently been referred to as the mesethmoid bone, although this name should only be applied to the mammalian skull. Other names (dermethmoid, rostral, supraethmoid, hypoethmoid, *etc.*) have been used as well. The understanding of the teleost mesethmoid complex became often confused because of different names that were applied to the same bone or different bones were called by the same name. Fortunately, PATTERSON's (1975) monograph on pholidophorid and leptolepid braincases has greatly contributed to clarify the situation. Solid bases exist now to discuss the problem and a general consensus slowly grew to standardise the names of the bones and to define their meanings (**Plate II.1-47**).

The name dermethmoid or rostral should be reserved to the dermal, median and unpaired bone covering the mesethmoid complex, which in pholidophorids, leptolepids and in some elopomorphs and

clupeomorphs bears an ethmoid sensory commissure. The names supraethmoid and hypoethmoid are now frequently used for a dorsal and a ventral unpaired ossification, respectively, appearing in the embryonic cartilaginous mesethmoid. The supraethmoid and hypoethmoid could be perichondral bones, endochondral bones or both at the same time. In the past, the names supraethmoid and hypoethmoid were often misused for the dermethmoid (= rostral) and the true supraethmoid (NORDEN, 1961: pl. 3A, 4; DAGET, 1964: fig. 25; TAVERNE, 1977a: fig. 43, 47, 48; *etc.*). Frequently, fusions occur between the supraethmoid and the dermethmoid (TAVERNE, 1977a: fig. 76), and between the hypoethmoid and the dorsal face of the dermal prevomer. The latter fusion then causes a thickening on the anterior portion of the prevomer, when endochondrally ossified, or becomes an anterodorsal lamellar process of the prevomer, when perichondrally ossified (PATTERSON, 1975: fig. 132a, b; TAVERNE, 1977b: fig. 1). Cases also exist where the cartilaginous ethmoid ossifies directly as one piece, like in *Hiodon* (TAVERNE, 1977a: fig. 7), without a division between a supraethmoid and a hypoethmoid. In some teleosts, like the mormyrids, the dermethmoid, supraethmoid and hypoethmoid are fused (TAVERNE, *pers. comm.*). In others, like the elopids, the small endochondrally ossified supraethmoid is fused with the prevomer, as is the perichondrally ossified hypoethmoid (TAVERNE, 1974a: fig. 9 where the supraethmoid is erroneously called hypoethmoid). The name mesethmoid frequently points out the fused dermethmoid and supraethmoid but sometimes also the fused dermethmoid, supraethmoid and hypoethmoid, or the fused supraethmoid and hypoethmoid, or the supraethmoid alone when the dermethmoid disappears. So, it is always important to see in each case which bone or group of bones the name mesethmoid is covering.

Apart from the dermethmoid (= rostral), a pair of lateral dermal bones develop in the mesethmoid region of pholidophorid fishes, partially underlying the dermethmoid and partially covering the nasal septum and the bottom of the nasal cavity (PATTERSON, 1975). They were called the laterodermethmoids. In teleost evolution, these laterodermethmoids fuse with the median dermethmoid, forming a lateral wing eventually covering a lateral process of the endochondral mesethmoid. The fusion could be only partial and a sutural line could persist in some leptolepids (WENZ, 1967; PATTERSON, 1975) or could be complete as in clupeomorphs (PATTERSON, 1975: fig. 132a; TAVERNE, 1977b: fig. 1, 2). In many teleosts, the laterodermethmoids are completely integrated in the dermethmoid and could not be identified anymore. In some teleost lineages, like the osmerids or the esocids, there is a pair of dermal bones covering the mesethmoid (STARKS, 1926: fig. 3, 19, 20; REMBISZEWSKI, 1964: fig. 1A; *etc.*). They are sometimes named proethmoids. These bones are most probably laterodermethmoids free from a disappeared dermethmoid. The laterodermethmoid appears to be the old rhinal bone of the holosteans which fuses with the premaxillary to form its ascending process covering the bottom of the nasal fossa and is pierced by the olfactory nerve (I) foramen (BJERRING, 1972: fig. 1).

In pholidophorids and some primitive teleosts, the supraethmoid could extend downwards on each side of the internasal septum as a perichondrally ossified wing (PATTERSON, 1975; TAVERNE, 1977b: fig. 1, 2 where that bone is erroneously called the rhinal). In hiodontids, osmerids and some Stomiiformes, that pair of osseous wings become free from the supraethmoid, forming then a pair of capsular ethmoids (WEITZMAN, 1967: fig. 2; TAVERNE, 1977a: fig. 3, 7; *etc.*).

In some archaic teleosts, another endochondral bone could appear, ventrally on each side of the hypoethmoid. This bone is often fused with the hypoethmoid and the prevomer as in osteoglossomorphs (TAVERNE, 1978: fig. 84 where it is called rhinal), but it could remain free as in Ichthyodectiformes (TAVERNE, 1977c: fig. 6, 7, 8 where it is called also rhinal). This pair of bones have been named in various ways: latero-basal ethmoids in osteoglossomorphs (TAVERNE, 1979), ethmo-palatine bones in ichthyodectiforms (PATTERSON & ROSEN, 1977) and proethmoid or preethmoid bones in cyprinids (HARRINGTON, 1955: fig. 3, 4, 5). These bones appear to

be homologous with the pair of endochondral bones which develops in the ventral region of the cartilaginous mesethmoid of *Amia calva* (JARVIK, 1980: fig. 15B, C; TAVERNE, *pers. comm.*).

The posterior portion of the ethmoid complex is inhabited by a pair of lateral ethmoid bones, each of them perichondrally and endochondrally ossified. Sometimes the bone is only formed by the perichondral ossification or even it is completely missing, leaving the region totally cartilaginous. In some cases, the perichondral and endochondral ossifications do not fuse and a small cartilaginous area separates them. Then, the perichondral ossification has been often misunderstood and interpreted as a dermal prefrontal or parethmoid associated with the lateral ethmoid (DAGET, 1964: 240; TAVERNE, 1974a: fig. 10; *etc.*), but it appears to be clear now that a dermal bone never exists at the lateral ethmoid level. Although in general the lateral ethmoids are not associated with the sensory canals, they assist in the support of the supraorbital canal of *Clarias gariepinus*. They even form a distinct groove to house this canal. In *Parapimelodus* (Pimelodidae) the supraorbital canal becomes highly branched, some of these branches even covering the lateral ethmoid bones without leaving distinct grooves (ARRATIA & GAYET, 1995).

In the pholidophorids the anterior myodome becomes perichondrally ossified, forming a small unpaired median osseous cone (PATTERSON, 1975) which sometimes is called the ethmomyodomial bone. It disappears in practically all teleosts except in some osmerids and stomiiforms (WEITZMAN, 1967: fig. 1, 7, 12; *etc.*).

In catfishes, the adult mesethmoid forms a massive and well ossified rostrum with all the bony elements fused together. The dermal components appear very soon in the ontogeny as a pair of small bones dorsal to the sphenoseptal commissures of the chondrocranium (BAMFORD, 1948: fig. 4B, 7A; own observations in *C. gariepinus)*. They quickly extend backwards until they suture with the frontals (KINDRED, 1919: pl. III, fig. 3) and fuse, both with each other and with the underlying bones of the mesethmoid complex. These two dermal bones probably are laterodermethmoids. In order to be consequent in the suggested bone nomenclature, this bone complex in *C. gariepinus* should be referred to as the os supraethmo-hypoethmo-laterodermethmoideum, but for convenience, the terminology mesethmoideum is applied here. It appears that a true dermethmoid (= rostral) component is absent in the siluriform mesethmoid. In some catfishes, the dermal components of the mesethmoid completely disappear (SRINIVASACHAR, 1958b: 167), whereas the mesethmoid only becomes perichondrally and endochondrally ossified. The ossification of the cartilaginous mesethmoid begins perichondrally and soon surrounds the complex dorsally and ventrally, whereas the endochondral ossification begins a little later in only one piece, without a distinction between a dorsal supraethmoid and a ventral hypoethmoid bone (KINDRED, 1919: pl. IV, fig. 7; MAHAJAN, 1966: fig. 3). The perichondral and endochondral ossifications fuse. This complex also fuses with the dermal component when it exists in the adult mesethmoid, as previously said. However, in exceptional cases, the dermal bone could stay distinct from the perichondro-endochondral one in the silurid mesethmoid (KOSCHKHAROFF, 1905-1907). In the studied specimens of *C. gariepinus*, no endochondral ossifications could be observed.

Concerning the nomenclature of the circumorbital bones, several discussions have been made (WEITZMAN, 1962). Evidence from ostariophysan phylogeny can assist in resolving the nomenclatural problems for the infraorbitals in catfishes. An antorbital bone is present in Gonorhynchiformes, although without any trace of a sensory canal (TAVERNE, 1986). In *Chanos* the antorbital bone is bordered ventrally by a larger lacrimal bone, enclosing the rostral part of the infraorbital canal, followed by a series of four infraorbital bones (FINK & FINK, 1981: fig. 7). Within the Otophysi, the circumorbital series is complete in cypriniform and characiform fishes, generally including a supraorbital, an antorbital and six infraorbital bones (ROBERTS, 1973: characteristic feature 10). The antorbital of cyprinids encloses the rostral part of the infraorbital canal, where the latter has disappeared in the

antorbital of characins (LEKANDER, 1949; WEITZMAN, 1962; FINK & FINK, 1981; ANDRÉ, 1987; MAHNERT & GÉRY, 1987). In siluriforms an antorbital bone is still present in the primitive Diplomystidae, as well as in the loricarioid *Nematogenys* (Nematogenyidae) (ARRATIA, 1992; ARRATIA & HUAQUIN, 1995). In diplomystids (ARRATIA & HUAQUIN, 1995) and ictalurids (KINDRED, 1919; EATON, 1948, LUNDBERG, 1982), the antorbital encloses a forked anterior part of the infraorbital canal, most probably corresponding to a dorsal antorbital commissure and a ventral ethmoid commissure, a situation observed in lower osteichthyan and teleostean fishes (JARVIK, 1980; TAVERNE, 1986). In most catfishes, the bone enclosing the rostral part of the infraorbital canal, frequently referred to as the lacrimal bone, bears a dorsal, plate-like extension in many cases (KINDRED, 1919; BHIMACHAR, 1933; TILAK, 1961; 1963a; 1965a; 1967; RASTOGI, 1963; LUNDBERG, 1982; SRINIVASA RAO & LAKSHMI, 1984; TEUGELS *et al.*, 1991). According to several authors, the latter dorsal extension does not bear a dorsal antorbital commissure, whereas the infraorbital canal only penetrates the bone along its ventral part [*e.g.*, *Rita buchanani* (Bagridae) (BHIMACHAR, 1933)]. In other catfishes, however, the dorsal extension of the antorbital bone has disappeared (*e.g.*, *Callichrous bimaculatus* (Siluridae) (TILAK, 1963a); *Glyptothorax cavia* (Sisoridae) (GAUBA, 1966); *Cochliodon cochliodon* (Loricariidae) (LÓPEZ & MIQUELARENA, 1991). The same is the case for *Clarias gariepinus*. Although some have hypothesised that the antorbital might have fused with the lacrimal (KINDRED, 1919; BAMFORD, 1948), it could be suggested that both remain separate, whereas within siluriforms a trend is present of a reduction of the antorbital commissure in the antorbital bone, followed by the loss of the membranodermal part underlying the latter commissure. NAWAR (1954) erroneously considered the antorbital bone of *C. lazera* (= *C. gariepinus*) (TEUGELS, 1982) as a fragmentation of the maxillary bone. TILAK's (1963c) figures of the Asian clariids, *C. batrachus* and *C. dussumieri* show a small bone in front of the lacrimal bone but do not indicate any nomenclature. Concerning the term 'lacrimal' bone, some variations in writing have been used. HARRINGTON (1955) proposed 'lachrymal' instead of 'lachrimal', 'lacrimal' or 'lacrymal', whereas VERRAES (1973) stated that the only correct etymological term should be 'lacrimal' bone, derived from the Latin 'lacrima' or tear. The following infraorbital bones have sometimes been subdivided in suborbital and postorbital bones. Suborbitals, however, preferably should be used for those anamestic bones derived from the fragmented anterior part of the preopercular bone in some Palaeonisciformes (GARDINER, 1967; MOY-THOMAS & Miles, 1971). In *C. gariepinus* the infraorbital bone III and IV border the eye ventrocaudally and dorsocaudally, respectively. This probably resulted in the nomenclature used by NAWAR (1954): the suborbital bone and supraorbital bone, respectively. Supraorbitals, however, are anamestic bones which have been lost in siluriforms (FINK & FINK, 1981). The presence of the infraorbital canal in this 'supraorbital' of NAWAR (1954) made ALEXANDER (1965) conclude that it had to be the dermosphenotic bone. In *C. gariepinus*, however, the dermosphenotic bone encloses the postorbital anastomosis between the infraorbital and supraorbital canals. During ontogeny, it fuses with the underlying autosphenotic bone, thus forming the sphenotic bone complex. The same is observed in *Heteropneustes fossilis* (Heteropneustidae) (SRINIVASACHAR, 1958b). BAMFORD (1948) and KINDRED (1919) refer to a postfrontal bone enclosing the anastomosis, but as stated by HARRINGTON (1955) the latter nomenclature is wrongly considered synonymous with the dermosphenotic. The fact that the number of infraorbital bones in catfishes may differ interspecifically (see below), it is hard to trace any homologies between them. It may thus be convenient to refer to the anteriormost infraorbital as the lacrimal, whereas the other infraorbitals are numbered. The last infraorbital bone, generally enclosing the postorbital anastomosis between the infraorbital and supraorbital canal, should then be referred to as the dermosphenotic bone, or when fused with the perichondral autosphenotic bone, as the sphenotic bone. However, other relations have to be considered, as for example, in Ictaluridae the frontal bone encloses the fusion of the infraorbital and supraorbital canals. The latter rostral shift of the anastomosis is considered to be coupled to an extension of the cranial musculature up to the skull roof (LUNDBERG, 1982).

HARRINGTON (1955) used the term 'dentary' or 'dental bone' to indicate the anterior part of the adult lower jaw. As he stated, the latter consists of a complex formed by the dentary, the mentomeckelian bone and the splenial bones. In descriptions of ontogenetic development of the cranial bones, it is, however, unpractical to use one and the same terminology when speaking of both a dermal bone and a complex formed of three bones. In this thesis it is thus advocated to use 'dentary' only when referring to the dermal, anamestic bone. The complex should then be referred to as the composition of the names of the bones constituting it. The same can be applied to the 'angular bone'. Another point is the recognition of the dentary (and angular bone) as a canal bone, whereas the splenial bones are considered as the neurodermal components of the latter. In *Clarias gariepinus*, however, some separate splenial bones are observed, posterior to the lower jaw. Apparently not all splenial bones are coupled to a mandibular bone (if of course they are homologous with true splenial bones). This, and the fact of a generally used nomenclature, resulted in the applied nomenclature for both mandibular bones in this paper.

The suprapreopercular bone has been designated different names as well, as its presence in ostariophysans appears to be rather erratic (**Table II.1- 4, p. 110**). Its presence in many species is mentioned as an ostariophysan character by GREENWOOD *et al.* (1979), as well as a character of protacanthopterygians. In general the suprapreopercular bone or bones are present in the Gonorhynchiformes, Characiformes and Siluriformes. In most Cypriniformes the latter is absent, as well as in some Characiformes. The absence of the bone in the latter taxa, however, is considered to be a derived feature (VARI, 1995). NAWAR (1954) and TILAK (1963c) considered the suprapreopercular bone as the dermosphenotic bone. The latter, however, can not enclose the dorsal part of the preopercular canal (ALEXANDER, 1965). Frequently the suprapreopercular bones are referred to as the 'subtemporals' (BHIMACHAR, 1933; TILAK, 1963a), or even 'intertemporal' bone (SRINIVASACHAR, 1958b). Intertemporals, however, generally are related to the otic canal, where it is believed to have fused with the 'membranopterotic', the 'supratemporal' and the 'autopterotic' bones, thus forming the pterotic bone (BAMFORD, 1948). According to LEKANDER (1949) the perichondral component of the latter complex is the opisthotic bone, instead of the autopterotic one. No ontogenetic evidence in the cleared material of *Clarias gariepinus* points to a possible fusion between those elements (this would however have to be confirmed on a basis of a detailed study of serial sections).

The homology of the temporal bones in catfishes have been a subject of several discussions, for example the supratemporal-extrascapular problem. According to LUNDBERG (1975), the extrascapular bones have disappeared in Siluriformes, whereas bones that previously had been considered as extrascapulars correspond to the separate components (membranodermal and neurodermal) of the posttemporal bone. Opposition to this interpretation was already given by FINK & FINK (1981), but the more extensive comparative work of ARRATIA & GAYET (1995) revealed the true nature of the extrascapular bones within catfishes. Although the general trend appears to correspond to a loss of the extrascapulars in siluriforms, they are still present in both fossil (*e.g.,* Andinichthyidae †, Hypsidoridae †, Ictaluridae) and extant species (*e.g.,* Diplomystidae, Pimelodidae, Ictaluridae, Aspredinidae) (ARRATIA & GAYET, 1995). In *Clarias gariepinus* no supratemporal commissure is present, nor are extrascapular bones. In gymnotiforms the extrascapular bones are present (CHARDON & DE LA HOZ, 1974; VISCHER, 1989; ARRATIA & GAYET, 1995).

Table II.1- 4: Distribution of the suprapreopercular bones in some ostariophysan fishes, based on data from literature

Suprapreopercularis absent			Suprapreopercularis present			
Familia	**Species**	**Reference**	**Familia**	**Species**	**Number**	**Reference**
CYPRINIFORMES			GONORHYNCHIFORMES			
Catostomidae	*Catostomus macrocheilus*	WEISEL, 1960	Chanidae	*Chanos chanos*	1	FINK & FINK, 1981
Cyprinidae	*Abramis blicca*	LEKANDER, 1949				
	Aspius aspius	GOSLINE, 1975b	CYPRINIFORMES			
	Cyprinus carpio	GREGORY, 1933	Cyprinidae	*Alburnus alburnus*	1[11]	LEKANDER, 1949
	Leuciscus rutilus	LEKANDER, 1949				
	Notropis bifrenatus	HARRINGTON, 1955	CHARACIFORMES			
	Opsariichthys uncirostris	FINK & FINK, 1981	Anostomidae	*Anostomus ternetzi*	1[12]	WINTERBOTTOM, 1980
	Phoxinus phoxinus	LEKANDER, 1949		*Anostomus spiloclistron*	1	WINTERBOTTOM, 1980
	Salmostoma bacaila	GOSLINE, 1975b		*Synaptolaemus cingulatus*	1	WINTERBOTTOM, 1980
	Rasbora dusonensis	GOSLINE, 1975b		*Abramites sp.*	1	VARI, 1995
			Characidae	*Xenocharax spilurus*	3	FINK & FINK, 1981
CHARACIFORMES			Erythrinidae		1	ROBERTS, 1969
Anostomidae	*Gnathodolus sp.*	WINTERBOTTOM, 1980	Hepsetidae		1	ROBERTS, 1969
	Anostomus plicatus	WINTERBOTTOM, 1980				
	Pseudanos trimaculatus	WINTERBOTTOM, 1980	SILURIFORMES			
Characidae	*Brycon guatemalensis*	GOSLINE, 1975b	Amblycipitidae	*Amblyceps mangois*	2	TILAK, 1967
	Brycon meeki	WEITZMAN, 1962	Bagridae	*Rita buchanani*	2	BHIMACHAR, 1933
Ctenoluciidae		VARI, 1995	Clariidae	*Clarias batrachus*	1	TILAK, 1963c
Curimatidae	*Gasterotomus latior*	ROBERTS, 1974		*Clarias dussumieri*	1	TILAK, 1963c
Hemiodontidae	*Anodus melanopogon*	ROBERTS, 1974		*Heterobranchus longifilis*	1	GREGORY, 1933
	Bivibranchia protractila	ROBERTS, 1974	Diplomystidae	*Diplomystes*	2-3	ARRATIA, 1987
	Argonectes longiceps	ROBERTS, 1974		*Olivaichthys*	2-3	ARRATIA, 1987
	Hemiodus semitaeniatus	ROBERTS, 1974	Heteropneustidae	*Heteropneustes fossilis*	1	SRINIVASACHAR, 1958b
	Hemiodus quadrimaculatus	ROBERTS, 1974	Ictaluridae	*Ameiurus sp.*	2	KINDRED, 1919
Parodontidae	*Parodon guyanensis*	ROBERTS, 1974		*Ictalurus serracanthus*	1	LUNDBERG, 1982
	Saccodon wagneri	ROBERTS, 1974		*Noturus insignis*	3	LUNDBERG, 1982
			Plotosidae	*Plotosus arab*	1	TILAK, 1963a
SILURIFORMES				*Plotosus canius*	1	TILAK, 1963a
Ariidae	*Arius tenuispinis*	SRINIVASA RAO & LAKSHMI, 1984	Siluridae	*Callichrous bimaculatus*	1	TILAK, 1963a
	Galeichthys felis	BAMFORD, 1948	Sisoridae	*Glyptothorax cavia*	1	GAUBA, 1966
Bagridae	*Auchenoglanis sp.*	TEUGELS *ET AL.*, 1991				
	Parauchenoglanis sp.	TEUGELS *ET AL.*, 1991				
Schilbeidae	*Eutropiichthys murius*	TILAK, 1961				
	Eutropiichthys vacha	TILAK, 1961				
GYMNOTIFORMESI						
Rhamphichthyidae	*Sternopygus macrurus*	FINK & FINK, 1981				
Rhamphichthyidae	*Eigenmannia virescens*	CHARDON & DE LA HOZ, 1974				

Another problem is whether the posttemporal or supracleithral bones have been incorporated in the skull in some catfishes. REGAN (1911a) stated that "the posttemporal, when present, is a small plate rigidly attached to the skull,...". Apparently, the author has the small extrascapular (= supratemporal) bone preserved in some siluriforms mistaken as the posttemporal one, as did LUNDBERG (1975). REGAN (1911a) also mentioned that in Clariidae "the posttemporal is absent" and that the "supracleithrum is a plate rigidly attached to pterotic, without lower limb, with a posterior process firmly united to air-bladder capsule". Once again, LUNDBERG (1975) concluded in the same way that the posttemporal bone has disappeared in some siluriform families, including the Clariidae, and that it was in this case the supracleithrum which was providing the articulation between the skull and the pectoral girdle. However, in catfishes, this bone generally offers an anteroventral process connecting it with the pterotic or the intercalar bone, and a dorsal oblique process attaching it to the epiotic (=

[11] In some cyprinids the suprapreopercular canal bone has fused with the opercular bone.

epioccipital) bone (see for instance LUNDBERG, 1975: fig. 2A, B, C). That, however, is the normal pattern of a posttemporal and not of a supracleithrum. But the bone also shows a notch in which the tip of the cleithrum articulates, and a large ventromedial process joining the basioccipital, generally identified as an ossified Baudelot's ligament (FINK & FINK, 1981). These two structures recall thus a supracleithrum. Although the double nature of this complex could not be clarified on account of the ontogenetic evidence, as was also mentioned by ARRATIA & GAYET (1995: 502), the wisest solution appears to consider this bone as a compound posttemporo-supracleithrum, as did some other authors (FINK & FINK, 1981; ARRATIA, 1987; ARRATIA & GAYET, 1995) (see II.1.2). In the closely related Gymnotiformes, such a fusion has been observed during ontogeny (*e.g., Hypopomus*, Hypopomidae), but whether this situation can be compared to the siluriform condition remains to be studied (ARRATIA & GAYET, 1995).

CANAL BONES IN CATFISHES

The presence and shape of the various canal bearing bones appears to have undergone some changes in several catfishes. In most catfishes the canals, with the related bones, are well developed, although some reductions have occurred. Canals are lacking in *Corydoras* (Callichthyidae), resulting in the absence of a nasal bone and infraorbitals (LEKANDER, 1949). The extreme reduction of the cranial lateral-line system in *Listrura* (Trichomycteridae) resulted in the absence of canals in the sphenotic and frontal bones, which is considered to be an apomorphic feature, uniquely derived in the latter genus (DE PINNA, 1988). In the mochokid *Synodontis caudalis* the sphenotic bone has lost its otic canal, as the postorbital anastomosis of the infraorbital and supraorbital canal are separated from the postotic canal (TAVERNE & ALOULOU-TRIKI, 1974). In *Clarias gariepinus* the cranial lateral-line system is well developed, with most of the associated canal bones present, with exception of the extrascapular bones.

Some of the canal bones remain tubulous in most catfishes. In general this is the case for the nasal bone, the infraorbital bones and the suprapreopercular bones. Tubulous nasals are present in both fossil and recent siluriforms (ARRATIA, 1992). In recent groups, it has been observed in the primitive Diplomystidae (ARRATIA, 1987; ARRATIA & HUAQUIN, 1995), as well as in Amblycipitidae (TILAK, 1967), Ariidae (SRINIVASA RAO & LAKSHMI, 1984), Bagridae (BHIMACHAR, 1933; TEUGELS *et al.*, 1991), Helogenidae (LUNDBERG, 1975), Ictaluridae (KINDRED, 1919; EATON, 1948; LUNDBERG, 1982), Mochokidae (TAVERNE & ALOULOU-TRIKI, 1974), Nematogenyidae (ARRATIA, 1992), Plotosidae (TILAK, 1963a), Schilbeidae (TILAK, 1961; RASTOGI, 1963), Siluridae (TILAK, 1963a; KOBAYAKAWA, 1992), Sisoridae (GAUBA, 1966), and Ariidae (TILAK, 1965a). In Clariidae, the nasal has become plate-like, thus covering the nasal sacs to a greater extent (NAWAR, 1954; TILAK, 1963c) (see II.1.2). Ontogenetic evidence from *Clarias gariepinus* clearly shows the development of an initially tubulous nasal bone, which later becomes enclosed in a lamellar part. Surprisingly, the latter apomorphic feature has not been observed in the closely related Heteropneustidae, where the nasal bone remains tubulous in the adult situation (SRINIVASACHAR, 1958b).

Tubulous circumorbital bones have been observed in those genera having tubulous nasals, although some exceptions exist. In the schilbeid *Clupisoma garua* the infraorbital bone, posterior to the lacrimal bone, has become triangular, as it is attached to the lateral margin of the lateral ethmoid bone (RASTOGI, 1963). In *Heteropneustes fossilis* (Heteropneustidae) the infraorbital bone IV (by SRINIVASACHAR (1958b) referred to as the

[12] In some Anostominae the suprapreopercular canal bone has fused with the posterior infraorbital bone.

supraorbital bone) is plate-like. In *Clarias gariepinus* none of the circumorbital bones are purely tubulous (**Plate II.1-44**). Although small, the antorbital bone is not circular in cross section, but clearly shows a lamellar part, enclosing a canal. As a result of its connection with the anterior tip of the base of the nasal barbel, the connection between the palatine and the antorbital may have a double function: (1) assisting in generating changes in volume of the nasal sacs during ab- and adduction of the rostral tip of the palatine (ALEXANDER, 1965), and (2) coupling of the ab- and adduction of the palatine to a manipulation of the nasal barbels. GHIOT *et al.* (1984) mentioned that in *Bagrus bayad* (Siluriformes : Bagridae) and *Ameiurus (= Ictalurus) nebulosus* (Siluriformes : Ictaluridae) no muscular manipulation of the nasal barbels is possible. However, they did mention that the bases of the latter barbels 'sont unis aux pièces osseuses voisines (c'est-à-dire le nasal, le lacrimal et l'ethmoïde latéral) par des fibres courtes', the latter presumably referring to collagenous fibres. The lacrimal bone of *C. gariepinus* bears a ventral plate with serrated edge, whereas the second infraorbital bone shows some structural specialisations. Its robust anterior tip supports the mechanical stress in the articulatory facet with the lateral ethmoid bone. In the middle the bone bears a medial, plate-like projection which supports the eye ball, although this differentiated subocular shelf is generally absent in malacopterygians (SMITH & BAILEY, 1962). The infraorbital bone III and IV in *C. gariepinus* have become plate-like to a high degree, especially the fourth one. The development of the plate-like circumorbitals, instead of tubulous bone, is a derived feature observed in some specialised siluriforms (FINK & FINK, 1981). Next to morphological differences of the infraorbital bones, a variation in the number is found in catfishes as well. Eight tubulous infraorbitals, loosely attached to the skull, have been observed in *Diplomystes nahuelbutaensis* (the anterior one is considering as the antorbital bone and thus not counted) (FINK & FINK, 1981; ARRATIA, 1987). The number can range from four (*e.g.*, *Tachysurus serratus*, Ariidae) (TILAK, 1965a) up to ten (*e.g.*, *Plotosus arab*, Plotosidae) (TILAK, 1963a), or they may be lacking completely (*e.g.*, *Corydoras*, Callichthyidae) (LEKANDER, 1949).

Apart from variations in canal bones, some variation in the position of the grooves, which mark the superficial tubules, has been observed as well. Little variation appears to occur in the opening of the epiphysial branch at the anterior fontanella, leaving no mark on the non-ornamented part of the frontal bone (ARRATIA & GAYET, 1995). These authors observed a general trend of the groove, lying in the extension of the parietal branch, to be elongated from the pterotic bone to the parieto-supraoccipital complex. In some cases this groove is extremely long, ranging from half way the frontal bone up to one third of the parieto-supraoccipital bone (*e.g., Hoffstetterichthys pucai* †). In extant catfishes this groove is much shorter (*e.g., Arius felis*, Ariidae) or even absent (*e.g.,* Diplomystidae) (ARRATIA & GAYET, 1995). In *Clarias gariepinus* this groove is still present and short. Surprisingly it is not situated onto the parieto-supraoccipital bone but covers the pterotic bone (**Plate II.1-40**) or sphenotic bone (**Plate II.1-42A**).

As the canal bones in teleosts generally have a double origin, a certain sequence in formation of the components (neurodermal and membranodermal) might be present. In cyprinids a mixed developmental pattern is present (LEKANDER, 1949). When present the membranodermal component develops prior to the neurodermal one, otherwise, the bone arises as a tubulous one (*e.g.*, nasale). As the neurodermal part arises, it lies separated from the underlying membranodermal part or is fused to it from the moment it arises. The fact that the nasal bone in some teleosts does have a plate-like shape, might be the result of a secondary lamellar extension of the neurodermal part (DAGET, 1964). In *Alburnus alburnus* (Cyprinidae) it is even observed that the neurodermal part of a suprapreopercular bone is fused to the underlying opercular bone (LEKANDER, 1949). This sequence in development has been observed in other teleosts as well (*e.g.*, Salmonidae) (DEVILLERS, 1947). In

Clarias gariepinus some evidence shows that the same sequence is not followed by all canal bones. The prior formation of the lamellar bone, followed by formation of the tubulous bone could be observed in the lower jaw bones and the posttemporo-supracleithral bones, but not for the infraorbital bones. The same has been observed in ictalurids as well (KINDRED, 1919), indicating a possible, secondary extension.

Concerning the sequence of the development of the different canals, some regularity appears to be present in teleosts (LEKANDER, 1949; KAPOOR, 1961). In the present study, the development of the canals themselves could not be followed in the cleared material. However, some considerations concerning the developmental sequence of the bones related to the different canals can be given. Initially, the bones related with the canals covering the dorsal face of the skull are found: anteriorly the frontal bone, in the middle the sphenotic bone and posteriorly the pterotic and posttemporo-supracleithral bones (10.0 mm SL). At the same time, the primordia of the preoperculo-mandibular canal bones are present too: the dental splenial bone, the angular splenial bone and the preopercular bone. Later the first signs of the infraorbital canal arise (antorbital and lacrimal bones) (11.6 mm SL). Consequently the nasal bone appears, together with the second infraorbital bone (12.7 mm SL). The third and fourth infraorbital bones are the last of the infraorbital series to develop (15.2 mm SL). Later the suprapreopercular bone is present as well (18.2 mm SL). At last, the splenial bones at the mandibular articulation are formed (46.8 mm SL). This sequence corresponds, in a way, to the one observed in, for example, cyprinids (LEKANDER, 1949).

CONCLUSIONS

The cranial lateral-line system in *Clarias gariepinus* is well developed, as well as the canal related bones. Reductions in canals are observed in the absence of a supratemporal commissure, as is the case for most siluriforms. Instead, a supraorbital commissure, through the fusion of the epiphysial branches, is present. Apart from canals, five to six pit-lines are present. All canal bones are present, except for the separate parietal bone (fused to the supraoccipital bone in all siluriforms), separate posttemporal bone (fused to the supracleithral bone in all siluriforms) and the extrascapular bone (absent in most siluriforms). In *C. gariepinus* some features, related to cranial lateral-line system bones, appear to be apomorphic, compared to most other catfishes. (1) The nasal bone is no longer tubulous. Even in closely related Heteropneustidae, the latter is tube-like. (2) The antorbital bone bears no antorbital commissure nor any distinct dorsal lamellar process. (3) The infraorbital series are plate-like bones, instead of tubulous ones. (4) The number of infraorbital bones has been reduced to five (*Diplomystes* generally has eight, dermosphenotic included). (5) The number of suprapreopercular bones is reduced to one, whereas (6) the suprapreopercular bone has differentiated into a large, plate-like bone instead of small tube-like ossicles.

Chapter II.2 – The cranial myology

II.2.1 - The Adductor Mandibulae Complex[1]

The adductor mandibulae complex has been a subject of discussion and uncertainties due to a wide range of differentiations that have occurred in teleosts during evolution. In Siluriformes a specific modification of a part of the muscle complex has resulted in the formation of a retractor muscle of the maxillary barbel. The main part of the muscle complex, responsible for the closure of the mouth, has undergone some changes as well, which are at the base of the homology problems encountered by different authors. In this chapter the muscles have been studied in three ontogenetic stages of the siluriform *Clarias gariepinus* (Clariidae), two of them are described. Based on the ontogenetic evidence and the literature, the following muscles are recognised: (1) the very weakly differentiated adductor mandibulae A_2A_3', where only little distinction can be made between the A_2 and the A_3' muscle parts, and (2) the adductor mandibulae A_3''. Caudally, both muscles are separated from each other by the levator arcus palatini, but are fused together anteriorly, inserting onto the lower jaw. In juvenile *C. gariepinus*, a differentiation has occurred in the A_3'' muscle, thereby forming a distinct pars superficialis and a pars profunda. No A_1 nor an A_ω muscle is present.

INTRODUCTION

Ontogenetic descriptions of the cranial structures of teleosts generally are confined to the skeletal elements (Bamford, 1948; Srinivasachar, 1953, 1957a, 1958a, b; Verraes, 1973; Kobayakawa, 1992; Vandewalle *et al.*, 1995). Little attention has been paid to the development of the cranial muscles (Jarvik, 1980; Surlemont *et al.*, 1989; Surlemont & Vandewalle, 1991). This field of research, however, can yield a vast amount of information concerning modifications that arise during ontogeny. These modifications can be the result of changes in behaviour (*e.g.*, feeding, respiration, *etc.*) and growth, coupled to changes in functionality of different mechanisms and mechanical demands. The muscles of both larval and juvenile specimens of *Clarias gariepinus* are investigated and described in detail. Some considerations are made concerning homology, function, adaptation, *etc.*

According to Jarvik (1980), all of the cranial muscles originate from an ontogenetic primordium of the visceral musculature, with exception of the eye musculature and the sternohyoideus, which originate from the somatic musculature. Up till now, the extrinsic eye muscles are believed to be derived from the epaxial part of the anterior three head somitomeres (premandibular, mandibular and hyoid segments) (Hildebrand, 1995). The visceral musculature primordium can be divided into three parts, each of them innervated by a different nerve: (1) the mandibular muscle plate, innervated by the ramus mandibularis of the nervus trigeminus, (2) the hyoid

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muscle plate, innervated by the ramus hyomandibularis of the nervus facialis, and (3) the branchial muscle plates, innervated by the rami posttrematici of the nervus glossopharyngeus and the four portions of the nervus vagus. The mandibular muscle plate forms a dorsal masticatory plate and a ventral intermandibular muscle plate. A further subdivision of the masticatory part results in a dorsal constrictor dorsalis and an adductor part. The constrictor dorsalis gives rise to the levator arcus palatini and the dilatator operculi. The adductor part forms the palatomandibularis (the latter is presumed to have a premandibular origin), and the adductor mandibulae. The ventral intermandibular muscle plate forms the intermandibularis, which becomes subdivided in an anterior and a posterior muscle. The hyoid muscle plate can be subdivided in a constrictor hyoideus dorsalis, forming the adductor hyomandibulae and two opercular muscles (adductor operculi and levator operculi), and the constrictor hyoideus ventralis, from which the interhyoideus anterior and interhyoideus posterior originate. The latter muscle is usually referred to as the hyohyoideus. Each of the five branchial muscle plates, which will not be discussed here, become subdivided in a dorsal and ventral part, thus forming the different branchial arch muscles.

This part of the thesis is confined to the adductor mandibulae complex, which has been a subject of discussion for several fish species. In generalised teleosts this muscle is situated in the cheek area, generally covering the lateral surface of the suspensorium (**Plate II.2-1**). The muscle complex connects the latter with the lower jaw, thereby functioning as the mouth-closing muscle. During evolution some differentiation has led to a subdivision of the muscle into different parts, which may or may not have undergone a morphological and/or functional shift (WINTERBOTTOM, 1974). Generally four portions can be distinguished: (1) the dorsal and outer A_1-part, inserting onto the maxillary bone, (2) the ventrolateral A_2 part that inserts onto the dorsomedial face of the lower jaw, (3) the deeper A_3-part that is attached to the medial face of the lower jaw, and (4) the A_ω part that originates on the medial face of the lower jaw and runs up to a myocomma separating the muscle from the A_2 part. An example of evolutionary modification has occurred in siluriforms where a part of the A_3muscle, which in most cases connects the maxillary barbel with the suspensorium, frequently functions as a retractor of the barbel (GOSLINE, 1975a; GHIOT *et al.*, 1984) (see II.2.2). In some cases, parts of the muscle may as well have a barbel extension function (SINGH, 1967). This part of the adductor mandibulae complex will not be discussed in this chapter, but will be described in Chapter II.2.2.

RESULTS

7.2 MM SL SPECIMEN (**Plates II.2-2, 3, 4**)

In this stage, the adductor mandibulae complex is well developed and presumably already functional. The muscle complex originates from the lateral face of the cartilaginous suspensorium [a very thin hyomandibula can already be distinguished (**Table II.1-3, p. 88**)], which is continuous with the ceratohyal through the interhyal, the latter disappearing during ontogeny (ADRIAENS & VERRAES, 1994) (see IV.2). Anteriorly the muscle is attached to the lower jaw (**Plate II.2-2**). The functional lower jaw is still built up by the Meckel's cartilage, although lateral to it a dermal dentary bone is present (the latter bone has fused with the perichondral mentomeckelian bone) (see II.2.1). At its origin on the suspensorium the adductor mandibulae complex is already differentiated into a superficial and a deeper portion (**Plates II.2-3, 4**). Both portions are separated from each other by the levator arcus palatini. The latter runs from the neurocranium to the lateral surface of the hyosymplectic, where it inserts

more ventrally than the deeper part of the adductor mandibulae complex (see II.2.4). Anterior to the levator arcus palatini the two muscle parts become confluent. This single muscle part, passing medially along the processus coronoideus of the lower jaw, and inserts rostrally of the processus onto the dorsal face of Meckel's cartilage. No clear tendon could be distinguished yet.

Concerning the nomenclature of the different parts of the adductor mandibulae complex in siluriforms, no certainty seems to be provided in the literature. In this paper the nomenclature used is based on descriptions given by TAKAHASI (1925), WINTERBOTTOM (1974), and HOWES (1983a). Consequently, the following nomenclature is used: as the superficial part is a poorly differentiated muscle, consisting of the A_2 part and the superficial fibres of the A_3 part of the complex, it is referred to as the adductor mandibulae A_2A_3'. The deeper portion corresponds to the deeper fibres of the A_3, thus here referred to as the adductor mandibulae A_3''. No evidence of the presence of an adductor A_1 nor an A_ω is found. Argumentation concerning the use of this nomenclature is given in the Discussion.

Adductor mandibulae A_2A_3' – This part originates from the lateral face of the suspensorium, thereby covering the ventral part of the levator arcus palatini. The muscle is attached to the hyomandibula, posterior to the insertion of the levator arcus palatini, and to the quadrate, ventral to the insertion of the levator arcus palatini (**Plates II.2-3A-B, 4A-B**). The origin on the hyomandibula caudally reaches the base of the processus opercularis. Anteriorly the muscle fuses with the A_3'' part of the complex. The fusion of the dorsal fibres is situated more anteriorly than the one of the ventral fibres (**Plates II.2-3C, 4C**). These ventral fibres meet directly anterior to the levator arcus palatini. The fused muscle part then runs to the lateral side of the pterygoid process of the suspensorium, along the medial face of the coronoid processus, and inserts on the dorsal surface of the jaw (**Plates II.2-3A, 4**).

Adductor mandibulae A_3'' – This muscle lies immediately against the lateral face of the suspensorium, thereby covering the foramen truncus hyomandibularis. The muscle does not reach as far posteriorly as the levator arcus palatini, which inserts on the hyomandibula, caudal to the A_3'' muscle. The origin of the A_3'' muscle is spread over the lateral face of the anterior part of the hyomandibula and the dorsolateral face of the quadrate (**Plates II.2-3C, 4C**).

The path of the ramus mandibularis of the nervus trigeminus, which innervates the adductor mandibulae complex, is sometimes used as a determining character for the homology of the subdivisions of the muscle complex (WINTERBOTTOM, 1974). In the larval *Clarias gariepinus* the ramus runs ventrally against the lower jaw, up to the processus coronoideus, where it rises along the lateral side of the jaw. Consequently, it passes between the processus coronoideus and the lower jaw, between the processus and the adductor mandibulae complex, and runs along the lateral side of the muscle complex. From there the ramus runs up to the dorsal face of the muscle complex, at the level of the splitting of the dorsal fibres. Eventually it passes along the dorsomedial side of the adductor mandibulae A_3'' and the dorsolateral side of the retractor tentaculi, up to the trigemino-facial ganglion.

125.5 MM SL SPECIMEN (**Plates II.2-1, 5, 6, 7**)

During ontogeny a further differentiation in the adductor mandibulae complex is noted in *Clarias gariepinus*. As is the case for the 7.2 mm SL specimen, two major portions can be distinguished, which are separated by the

levator arcus palatini (**Plate II.2-5A-B**), and are connected to each other anteriorly. Again, no evidence of the adductor A_1 nor the A_ω is present.

Adductor mandibulae A_2A_3' - This superficial portion forms the largest part of the muscle complex. The muscle can be divided in a dorsal α-part and a ventral β-part, separated by a well-developed tendon. The dorsal part is more elongated posteriorly than the ventral one, as it covers the anterior opercular muscles (**Plate II.2-5A**). The origin of the dorsal part is spread on the dermal bones that are part of the neurocranium, whereas the origin of the ventral part is confined to the suspensorium. The $A_2A_3'\alpha$ originates from the medial surface of the posterior part of the infraorbital bone IV, from the medial face of the anterior part of the suprapreopercular bone, and from the lateral margin of the sphenotic bone (**Plates II.2-1, 5A**). The infraorbital bone III covers the muscle but does not bear an insertion of it. The ventral part, the $A_2A_3'\beta$, is attached to the lateral face of the posterior half of the hyomandibular bone, to the lateral face of the preopercular bone, and to the lateral face of the quadrate. Anteriorly the fibres insert, through a complex of tendons, on the dorsomedial surface of the angulo-splenio-articulo-retroarticular complex, and on a bony ridge dorsal to the non-ossified cartilage of Meckel, corresponding to the coronomeckelian bone (**Plate II.2-7C**). The medial tendons are orientated in a vertical plane, whereas the lateral tendon are orientated horizontally, thereby dividing the A_2A_3' in a dorsal and a ventral part (**Plate II.2-8**). This was already noted in the 46.8 mm SL late larval stage.

Adductor mandibulae A_3'' - This deeper part runs medially along the levator arcus palatini and is almost completely covered by it (**Plate II.2-5B**). The adductor muscle covers the posterior portion of the medially situated retractor tentaculi. The A_3'' is lodged into a cavity of the rostral part of the hyomandibular bone, and is attached to both the skull and the suspensorium (**Plate II.2-5C**). During ontogeny the muscle appears to differentiate to a further extent than the superficial A_2A_3', as in the former two separate portions can be distinguished (**Plate II.2-7**). The *pars superficialis*, which lies directly against the medial face of the levator arcus palatini, forms the largest portion of the A_3'' muscle. The muscle fibres are directed in such a way that they form the transition from the slightly obliquely directed fibres of the A_2A_3' muscle to the vertically directed fibres of the pars profunda of the A_3'' muscle. The fibres of the A_3'' pars superficialis originate on the skull rostrally: on the ventral face of the frontal bone, on the ventral face of the sphenotic bone, and on the lateroventral face of the pterosphenoid bone. Caudally they originate on the hyomandibular bone and the anterodorsal face of the quadrate. At its medial face, the hyomandibular bone forms an anteriorly extended membranous plate, which functions as the insertion site for the retractor tentaculi and the deeper part of the A_3'' muscle (**Plate II.2-6A**). Due to the medial position of this plate, the superficial part of the A_3'' muscle is bordered caudally by the anterior margin of the perichondral part of the hyomandibular bone, upon which the muscle originates as well (**Plates II.2-5C, 6**). Rostrally the muscle inserts tendinously on the dorsomedial face of the coronomeckelian bone, medial to the tendon complex of the A_2A_3' muscle. The tendon of the pars superficialis is the smallest of the adductor complex (**Plate II.2-7B**). The deeper part of A_3'', the adductor mandibulae A_3'' *pars profunda*, is completely separated from the pars superficialis. The fibres are directed in a rather dorsoventral way, instead of the oblique orientation observed in the other parts of the adductor complex. The pars profunda originates on the lateral face of the membranous outgrowth of the hyomandibular bone. The anterior part of this hyomandibular plate is covered by the insertion of the tentacular retractor muscle, while the posterior part bears the insertion of the A_3'' pars profunda muscle. The insertion of the pars profunda is rather aponeurotic onto the angulo-splenio-articular part of the bone complex and is situated medially but more posteriorly to the insertion site of the pars superficialis (**Plate II.2-7A**).

The path of the ramus mandibularis was studied in the 46.8 mm SL specimen. The ramus runs from the ventral margin of the lower jaw, now formed by the dento-splenio-mentomeckelium complex and the angulo-splenio-articulo-retroarticular complex, which partially enclose Meckel's cartilage. At the rostral tip of the processus coronoideus, the ramus passes between the angulo-splenio-articular part of the bone complex and Meckel's cartilage. At the insertion site of the adductor mandibulae complex on the lower jaw, the ramus passes lateral to the muscle complex and medial to the angulo-splenio-articulo-retroarticular bone. From there on, it lies against the lateral face of the retractor tentaculi and the mediodorsal face of the adductor mandibulae A_3", until it reaches the brain.

DISCUSSION

During evolution the adductor mandibulae has undergone changes in size, orientation, and structure. Configurations are found, going from a single muscle restricted to the palatoquadratum-maxillary chamber of the Palaeoniscoidei, to a highly developed muscle complex inserting onto the neurocranium, as can be found in several recent teleosts (SCHAEFFER & ROSEN, 1961). The fact that fusions and differentiations have occurred during evolution of the muscle complex, both in ostariophysan fishes and in non-ostariophysan fishes, has led to a variety of applied nomenclature and numerous uncertainties. In general, the ostariophysan adductor muscle complex becomes differentiated into four parts: (1) the A_1 muscle, which is assumed to be non-homologous with the A_1 muscle of higher euteleostean fishes (FINK & FINK, 1981), (2) the A_2 muscle, (3) the A_3 muscle, and (4) the A_ω muscle.

The *A_1 muscle* [= pars maxillaris (TAKAHASI, 1925; DATTA MUNSHI & SINGH, 1967; ANKER, 1974)], is the superficialmost subdivision, which in most cases inserts upon the maxillary bone (EATON, 1935; WINTERBOTTOM, 1974; GOSLINE, 1986). In Gonorhynchiformes, Cypriniformes, and some primitive Characiformes (Citharinidae) the insertion is direct upon the posterior or lateral margin of the maxillary bone. In higher Characiformes, however, the muscle is attached to the maxillary bone through the primordial ligament ["maxillomandibular" ligament of HOWES (1983a)], which runs from the mandibular processus coronoideus up to the maxillary bone (TAKAHASI, 1925; SCHAEFFER & ROSEN, 1961; FINK & FINK, 1981). In Gymnotiformes, which are closely related to the Siluriformes, the A_1 muscle inserts onto the lower jaw, instead of the upper jaw (FINK & FINK, 1981), although some lineages show some muscular attachment to a primordial ligament (*e.g., Gymnotus*) or to the maxillary bone (*e.g., Rhamphichthys*) (HOWES, 1983a). According to HOWES (1983a), the attachment of the A_1 muscle to the lower jaw is considered to be plesiomorphic although FINK & FINK (1981) assume that type of insertion to be a secondary modification of the primitive situation where A_1 inserts onto the maxillary bone. In Siluriformes, the A_1 muscle is presumed to be lost (FINK & FINK, 1981). This seems to be the case for some genera, as was observed in *Liobagrus* (Amblycipitidae) and *Pseudobagrus* (Bagridae) by TAKAHASI (1925). However, HOWES (1983a) described in *Hypophthalmus edentatus* (Hypophthalmidae), *Callophysys macropterus* (Pimelodidae), and *Tandanus tandanus* (Plotosidae) the presence of an A_1 muscle, the latter being fused posteriorly with the A_2 muscle. The same condition was found in the primitive *Diplomystes* (Diplomystidae), as well as in the more advanced siluriform superfamily Loricaroidea [*e.g., Nematogenys* (Nematogenyidae), *Trichomycterus* and *Henonemus* (Trichomycteridae)]. In those genera the muscle inserts onto a sheet of connective tissue, connecting the lower jaw with the maxillary bone (SCHAEFER & LAUDER, 1986). In those species possessing a distinguishable A_1 muscle, the

ramus mandibularis of the trigeminal nerve seems to run along the medial side of the A_1 muscle and lateral to the A_2 muscle (HOWES, 1983a; SCHAEFER & LAUDER, 1986). A special modification has occurred in some Callichthyidae where the A_1 muscle, partially fused to the A_2 muscle, inserts through a sheet of connective tissue to the lower jaw, as well as to the premaxillary bone (HOWES, 1983a; SCHAEFER & LAUDER, 1986).

In some cases the A_1 muscle can be subdivided into a dorsal $A_1\alpha$ part and a ventral $A_1\beta$ part (TAKAHASI, 1925). WINTERBOTTOM (1974) refers to an opposite nomenclature, *i.e.*, a dorsal $A_1\beta$ part and a ventral $A_1\alpha$ part. In the plesiomorphic situation, both parts are still confluent but are simply divided into a dorsal and ventral bundle by a strongly developed tendon, generally inserting onto the primordial ligament. In some derived situations, however, both muscle bundles have become separated whereas the dorsal part inserts onto the maxillary bone and the ventral part is attached to the lower jaw [*e.g., Halichoeres* (Perciformes : Labridae)] (GOSLINE, 1986). Even in ostariophysan fishes different configurations can be found (TAKAHASI, 1925). In the primitive cypriniform fishes *Opsariichthys* and *Zacco* (Cyprinidae) the A_1 muscle is still undivided (FINK & FINK, 1981). In derived genera, such as *Cyprinus* (Cyprinidae) and *Cobitis* (Cobitidae) a dorsal $A_1\alpha$ and a ventral $A_1\beta$ part, divided by a tendon, can be distinguished (TAKAHASI, 1925). TAKAHASI (1925) stated that in some siluriform genera, such as *Parasilurus* (Siluridae) the A_1 muscle is separated into a dorsal $A_1\alpha$ muscle, inserting onto the maxillary bone and thus functioning as a tentacular retractor muscle, and a ventral $A_1\beta$ muscle, which had become incorporated into the pars mandibularis, being the fused A_2 and A_3 muscle. This hypothesis of the homology between the $A_1\alpha$ muscle and the retractor tentaculi has been a topic of discussion (ALEXANDER, 1965; HOWES, 1983a; SCHAEFER & LAUDER, 1986) (see II.2.2).

In *Clarias gariepinus* however, no evidence was found in any of the three ontogenetic stages of an adductor mandibulae A_1, nor did SURLEMONT *et al.* (1989) and SURLEMONT & VANDEWALLE (1991) report one in other stages. Cross sections of the muscle complex in the 7.2 mm or the 46.8 mm SL specimen revealed no signs of a fusion of the A_1 muscle with the complex, formed by the A_2 muscle and the lateral portion of the A_3. An earlier description of the myology of adult *C. gariepinus* gave no indication of the presence of the A_1 muscle either (NAWAR, 1955a).

The *A_2 muscle*, generally constituting the major part of the complex, is situated in the ventrolateral region of the cheek. It is musculously attached to the suspensorium and inserts through a tendon onto the posterior margin of the processus coronoideus. It also inserts onto a myocomma of the A_ω, when present (EATON, 1935; DATTA MUNSHI & SINGH, 1967; WINTERBOTTOM, 1974; SUDA, 1996). As was the case for the A_1 muscle, the A_2 muscle has also undergone some further differentiations in certain taxa where a dorsal $A_2\alpha$ and a ventral $A_2\beta$ can be distinguished, separated by a conspicuous tendon (WINTERBOTTOM, 1974). In the basic configuration, the A_2 forms a separate muscle, lying lateral to the A_3 muscle (GOSLINE, 1986). In some modified adductor mandibulae complexes, however, a partial to complete fusion has occurred between the two muscles, as will be discussed below.

The *A_3 muscle* generally forms the medialmost part of the muscle complex and connects the lateral face of the suspensorium with the medial face of the dentary bone, although in some cases it may originate on the skull as well (WINTERBOTTOM, 1974). As stated, fusions have occurred in teleosts between the A_3 muscle and other muscles or muscle parts. In Acanthuridae (Perciformes) and Ostraciidae (Tetraodontiformes) a fusion with the $A_1\beta$ muscle has been observed (WINTERBOTTOM, 1974). More frequently, a fusion with the A_2 muscle is found [*e.g., Scomber* (Perciformes : Scombridae) (WINTERBOTTOM, 1974); some Siluriformes (TAKAHASI, 1925)]. This fused complex, referred to as the pars mandibularis (TAKAHASI, 1925; DATTA MUNSHI & SINGH, 1967) or the pars articularis

(ANKER, 1974), inserts on the mandibula. In Cypriniformes, these two muscles are still separated from each other. The A_3 muscle, caudally divided into two parts, lies medially against the A_2 muscle. The A_3' part lies against the lateral face of the levator arcus palatini, the A_3" part medial to it.

In Siluriformes, however, only two parts of the pars mandibularis can be distinguished: one portion lateral to the levator arcus palatini and one medial to it. Presumably a fusion has occurred between the A_2 muscle and the A_3' muscle. In *Parasilurus asotus* (Siluridae) this A_2A_3' muscle inserts onto the lower jaw through two separate tendons, which may correspond to the A_2 and A_3' tendons.

In *Clarias gariepinus* it was observed that the A_2A_3' inserts through a complex of tendons on the dorsal face of the lower jaw, at the level of the cartilaginous processus coronoideus. Such a fusion between the A_2 and the A_3' part has been found in several teleosts (OSSE, 1969; WINTERBOTTOM, 1974). Some evidence that supports such a fusion in *C. gariepinus* is the orientation of the tendons (**Plate II.2-8**). In the 46.8 mm SL late larval stage and the 125.5 mm SL juvenile it was observed that the aponeurotic tendon of the lateral part of the A_2A_3' is horizontally directed, thereby dividing the muscle in a dorsal α part and a ventral β part. On the other hand, the aponeurotic tendon complex of the medial fibres is directed in a rather vertical way. As already mentioned, the A_2 muscle in teleosts frequently can be subdivided in a dorsal $A_2\alpha$ part and a ventral $A_2\beta$ part, separated by the tendon of the muscle, which is elongated caudally into a somehow horizontal aponeurosis (WINTERBOTTOM, 1974; DECLEYRE *et al.*, 1990). This is also the case for *C. gariepinus* where the fibres of the $A_2\alpha$ originate more posteriorly than the $A_2\beta$ part (**Plate II.2-5A**). A comparable subdivision within the A_3 muscle does not occur frequently, as the flat tendons are orientated in the same vertical plane as the muscle.

The *A_ω muscle* [= pars mentalis (TAKAHASI, 1925); intramandibularis (DATTA MUNSHI & SINGH, 1967); pars dentalis (ANKER, 1974)] is the smallest portion, generally connecting the medial face of the dentary or articular bone with the A_2 muscle (EATON, 1935; DATTA MUNSHI & SINGH, 1967; WINTERBOTTOM, 1974; SUDA, 1996). Insertions on the A_3 have been observed also (TAKAHASI, 1925; DECLEYRE *et al.*, 1990). In some cases, however, the muscle is elongated and inserts onto the quadrate and the preopercular bone (WINTERBOTTOM, 1974). In Siluriformes, the A_ω only appears to be present in some species [*e.g., Parasilurus* (Siluridae), *Plotosus* (Plotosidae) (TAKAHASI, 1925)]. However, a general reduction or loss of the muscle is the rule for catfishes (ALEXANDER, 1965). This is also the case for *C. gariepinus* where no A_ω could be observed, neither in the larval stage nor the juvenile stage.

Further subdivisions of the adductor mandibulae complex are noted in some Loricaroidei: a retractor palatini (= a *de novo* formation) and a muscle 'C' (= medial subdivision of the adductor mandibulae complex) (HOWES, 1983b; SCHAEFER & LAUDER, 1986).

In a 4.0 mm SL stage [4.2 mm larva of SURLEMONT & VANDEWALLE (1991)], the adductor mandibulae complex is formed, but does not yet insert onto the mandibula (SURLEMONT *et al.*, 1989; SURLEMONT & VANDEWALLE, 1991). Surprisingly, the muscle complex is already subdivided into a lateral part (referred to by the authors as the "adducteur 2") and a medial part ("adducteur 3"), caudally separated by the developing levator arcus palatini ("muscle élévateur de l'hyomandibulaire"). In the 5.0 mm SL specimen [5.2 mm larva of SURLEMONT & VANDEWALLE (1991)] the muscle complex attaches onto Meckel's cartilage, the latter still being continuous with the pars quadrata of the suspensorium. From this moment, adduction of the lower jaw is possible, whereas abduction occurs as a passive recoil due to the elastic features of the cartilaginous connection with the suspensorium (see IV.1). In this stage, the elevation of the lower jaw during contraction of the adductor mandibulae complex is considered to be for respiratory reasons only, and not yet for feeding (SURLEMONT *et al.*, 1989). In the 7.2 mm specimen, however, mouth opening is enabled through the protractor hyoidei and the sternohyoideus muscles,

whereas the cartilaginous connection between the mandibula and the suspensorium is lost (**Plates II.2-3, 4**) (see IV.1). The developmental state of the adductor mandibulae complex in that specimen is comparable to the one in the 5.0 mm SL specimen. Later during ontogeny, in the 46.8 mm SL specimen, a tendon complex, which was not observed in the 7.2 mm SL specimen, is present connecting the A_2A_3' muscle to the posterior ossification of Meckel's cartilage, being the angulo-splenio-articulo-retroarticular complex. Additionally, a subdivision of the A_3" muscle into a pars superficialis and a pars profunda can be observed at its caudal part. The adductor mandibulae complex of the 125.5 mm SL juvenile corresponds to the adult situation (NAWAR, 1955a). However, some differences in descriptions are present: NAWAR (1955a) referred to a 'm. adductor mandibulae profundus' and a 'm. adductor mandibulae superficialis' that are distally separated from each other by the levator arcus palatini. The superficial muscle corresponds to the A_2A_3' muscle, where the deeper muscle corresponds to the A_3" muscle. However, NAWAR (1955a) did not recognise any subdivision of the deeper muscle, the adductor mandibulae A_3". An even more pronounced subdivision of the A_3" is present in *Gymnallabes typus*, which is a specialised clariid species with an enormous adductor mandibulae complex (CABUY, 1997).

CONCLUSIONS

The origin of the elements of the adductor mandibulae complex is doubtful, as has been mentioned by several authors (TAKAHASI, 1925; ALEXANDER, 1965; WINTERBOTTOM, 1974; HOWES, 1983a; SCHAEFER & LAUDER, 1986). This is due to fusions and subdivisions that have occurred through evolutionary modifications. Some evidence, however, is present, which reveals the possible true nature of the muscle parts: evidence from other ostariophysans (TAKAHASI, 1925) as well as evidence in the structure of the muscle parts and their tendons.

The adductor mandibulae complex of juvenile *Clarias gariepinus* is composed of two major muscles: (1) the adductor mandibulae A_2A_3' and (2) the adductor mandibulae A_3", both caudally separated from each other by the levator arcus palatini. Rostrally the two muscles have fused and insert upon the dorsomedial face of the lower jaw, upon the coronomeckelian bone and the complex of the articular bone, the retroarticular bone, and the angulo-splenial bone. The attachment of the muscles onto the lower jaw occurs through a complex of short tendons, which may be elongated, thereby subdividing the A_2A_3' muscle into a dorsal α part and a ventral β part. Ontogenetic evidence shows that already early in ontogeny the muscle complex is subdivided into a lateral and a medial part, separated by the levator arcus palatini. The adductor mandibulae A_1 and A_ω are absent in *C. gariepinus*.

II.2.2 - THE TENTACULAR MUSCLES[2]

Siluriforms are characterised by the presence of a palatine-maxillary mechanism, which enables a controlled mobility of the maxillary barbels. In *Clarias gariepinus* the ontogeny of this mechanism is studied and described, as well as those muscles related to the maxillary barbel. Two muscles are distinguished: (1) *retractor tentaculi*, connecting the maxillary to the suspensorium, and (2) *extensor tentaculi*, running from the ventrolateral face of the skull to the posterior half of the palatine. These typical catfish muscles are derived from muscles that are present in generalised teleost fishes. The retractor muscle is believed to be derived from the A_3 muscle of the adductor mandibulae complex. The extensor muscle is formed from the anterior fibres of the adductor arcus palatini. The palatine is rod-like in *C. gariepinus*, which initially articulates with the cartilaginous orbito-nasal lamina in larval specimens and with its ossification, the lateral ethmoid, later on. The articulation occurs via a long cartilaginous strip on the dorsal face of the autopalatine, thereby enabling both a rotation and a restricted sliding.

INTRODUCTION

The siluriform fishes, or catfishes, are all characterised by the presence of barbels surrounding the snout region. Depending on the taxon, the number of barbels varies from one to four pairs (ALEXANDER, 1965). In those species where only one pair occurs, it is the maxillary barbel, which is present, *e.g.,* in Diplomystidae, Loricariidae and Callichthyidae (GOSLINE, 1973; 1975a). A small maxillary barbel can also be distinguished in some cypriniforms [*e.g., Cyprinus*, *Gobio* (Cyprinidae)] (ALEXANDER, 1966; GOSLINE, 1973). In those catfishes carrying four pairs of barbels, the following types can be distinguished: one pair of maxillary, one pair of nasal, and two pairs of mandibular barbels (**Plate II.2-9**). The nasal barbels are situated between the anterior and posterior nostrils. The mandibular barbels, a medial and a lateral pair, lie at the ventral side of the lower jaw and are embedded at their bases into the hyoid protractor muscle (ADRIAENS & VERRAES, 1997d) (**Plate II.2-26A**) (see II.2.3). The maxillary barbel is situated on the rostro-lateral face of the snout. This barbel is usually the largest one, being as long as or even longer than the whole body length, *e.g.,* in *Pimelodina* (Pimelodidae) (Stewart, 1986). The barbels are supported by a central rod, comprising a dense network of elastin, with or without true cartilage (BENJAMIN, 1990). The surrounding skin is covered with mucus cells and taste buds (GHIOT & Bouchez, 1980). Generally, the barbels are simple but in some taxa they can bear side branches, as in some Mochokidae and all Doradidae (ALEXANDER, 1965).

The maxillary barbels are distinguished from the others by their connection to a mobile mechanism. This palatine-maxillary mechanism is present in all catfish (GOSLINE, 1975a); several types of these mechanisms have been described in different siluriform taxa (EATON, 1948; ALEXANDER, 1965; SINGH, 1967; GOSLINE, 1975a; GHIOT, 1978; GHIOT *et al.*, 1984; ARRATIA, 1992). Generally, this mechanism consists of a set of elements, which are part

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of the feeding apparatus in most non-siluriform teleosts (**Plate IV.2-8**). These elements are the palatine and the maxillary, to which a set of tentacular muscles is attached. The palatine has become isolated from the rest of the suspensorium in siluriforms, instead of being connected to the pterygoid region, as in most teleosts. It has a bar-like shape and articulates with the skull, in general at its middle. Its rostral tip is connected to the maxillary. This kind of articulation with the skull enables such movements of the rostral tip, which make the maxillary rotate. The toothless maxillary has been modified into a socket-like structure, enclosing the base of the central rod of the barbel (REGAN, 1911a; EATON, 1948). Consequently a rotation of the maxillary results in a displacement of the barbel. The mechanism is triggered by a set of muscles that have been derived from other muscles, which are present in non-siluriform fishes (TAKAHASI, 1925; WINTERBOTTOM, 1974). Different strategies have been developed in the muscular pattern of the palatine-maxillary mechanism. In general, a retractor muscle, connected to the maxillary, and an extensor muscle, connected to the palatine, can be distinguished. Other strategies have been described as well (SINGH, 1967; ALEXANDER, 1965; GOSLINE, 1975a).

The benthic life style of most siluriform fishes has affected the evolutionary development of certain elements of the 'Bauplan'. The reduction of the optical sensory organs, related to the very small eyes, is one of many adaptations to this life style (ALEXANDER, 1965). This reduction has affected the development of the cartilaginous skull, which in siluriforms is of the platybasic type, instead of the tropibasic type of most teleosts (DAGET, 1964; ADRIAENS & VERRAES, 1997e). In siluriforms the reduced visual sense is partially compensated by the strongly developed barbels that both have a taste and tactile function. The coupling of the sensory function of the barbels to a palatine-maxillary mechanism increases their importance as sensory organs. The mouth opening and closing mechanism is sometimes coupled to the palatine-maxillary mechanism in such a manner that, when opening the mouth, the maxillary barbel is projected in front of the mouth, which enables sampling of potential food (ALEXANDER, 1965; 1966; GOSLINE, 1973).

In those papers dealing with the palatine-maxillary mechanism, a comparative approach is usually made, which may reveal some evolutionary relationship and even historical geographical distribution (ALEXANDER, 1965; SINGH, 1967; GOSLINE, 1975a; GHIOT *et al.*, 1984). In this paper the results of an ontogenetic study of those muscles of the African catfish *Clarias gariepinus* (Clariidae), related to the maxillary barbel, are given. The muscles related to the movements of the mandibular barbels are dealt with in II.2.3. In the literature, varying nomenclature has been applied for the barbel muscles, as will be discussed briefly. In this chapter a terminology according to WINTERBOTTOM (1974) is used.

RESULTS

In *Clarias gariepinus* two muscles are found that take part in the palatine-maxillary mechanism: (1) a retractor tentaculi and (2) an extensor tentaculi.

7.2 MM SL SPECIMEN (**Plates II.2-10, 11, 12, 13, 14**)

Retractor tentaculi - Already in this larval stage, both maxillary barbel muscles can be distinguished (**Plate II.2-10**). The retractor tentaculi is almost completely covered laterally, by the adductor mandibulae complex (ADRIAENS & VERRAES, 1996). The muscle originates from the medial face of the suspensorium, at the anterior margin of the hyosymplectic (**Plates II.2-11, 12**). Ossification of the hyosymplectic is detected at this stage as a very thin lining of

perichondral bone. The muscle initially runs along the dorsal margin of the pterygoid process, eventually passing along its lateral face. At the level of this process, the muscle runs between the adductor mandibulae A_3" and the adductor arcus palatini (**Plates II.2-10, 11A, 12A**). Rostrally it inserts on the caudal face of the maxillary via a small tendon. The insertion of the tendon on the maxillary is situated as far as possible from the articulation of the bone with the palatine and the submaxillary cartilage, thereby maximising the power output during contraction (**Plates II.2-11, 13**). The maxillary is already well formed and articulates with a cartilaginous complex formed by the palatine and the submaxillary cartilage.

Extensor tentaculi - The extensor tentaculi is a relatively small muscle, covered by the eye and connecting the palatine to the neurocranium (**Plate II.2-14**). This muscle originates from the lateral face of the preorbital base and partially from the medial face of the orbito-nasal lamina of the cartilaginous skull, precisely posterior to its articulation with the palatine. The dorsoventrally directed fibres insert on the dorsal face of the posterior half of the cartilaginous palatine (**Plates II.2-11, 12, 13**). No ossification of the palatine is observed at this stage. (**Table II.1-3, p. 88**). The insertion is spread along the posterior half of the palatine, from exactly posterior to its articulatory facet up to its posterior tip (**Plates II.2-13B-C, 14**). The palatine articulates with the neurocranium at the orbito-nasal lamina (**Plate II.2-14**).

125.5 MM SL SPECIMEN (**Plates II.2-11, 15, 16**)

Retractor tentaculi - In both the 46.8 mm SL specimen and the 125.5 mm SL specimens the maxillary barbel musculature is comparable to the adult situation (NAWAR, 1955a). The retractor tentaculi has become a large muscle, originating from the ossified suspensorium and inserting via a long and stout tendon on the maxillary (**Plates II.2-15A-B**). The origin of the muscle is spread over the hyomandibular, the quadrate and the metapterygoid. From its medial face, the hyomandibula has a, rostrally directed, membranous outgrowth (**Plate II.2-6**) (see II.2.1). This plate-like structure increases the insertion surface for the retractor tentaculi, the latter being attached to the major part of the lateral face of this plate. The dorsal fibres of the muscle originate from just below the dorsal margin of the hyomandibula (**Plate II.2-15B**). The ventral fibres originate from the quadrate. The medial fibres originate from the posterolateral face of the metapterygoid. Posterior, the muscle lies against the medial face of the adductor mandibulae A_3". From this point, it runs anteriorly along the ventromedial side of the eye and lateral to the palatine and the extensor tentaculi muscle. Anterior to the eye the muscle is covered laterally by the infraorbitals I to III. Eventually the muscle inserts via a well-developed tendon to the posterior face of the maxillary. In both the 46.8 mm SL and 125.5 mm SL specimens the insertion on this bone is situated on the dorsal part of the posterior face (**Plate II.2-16**). Posteriorly the conspicuous tendon is continued into an aponeurosis, partially dividing the retractor in a dorsal and a ventral part.

The maxillary is well developed and articulates with the lateral face of the rostral tip of the ossified palatine, the autopalatine (**Plate II.2-16**). The maxillary bears two articulatory condyles at its proximal end that facilitate the previously mentioned articulation (**Plate II.1-31B**). The dorsal condyl bears a ligamentous connection with the autopalatine. At its distal end, the maxillary has a tube-like socket into which inserts the central rod of the maxillary barbel (**Plates II.1-31B, II.2-16**). Manipulation of the retractor tentaculi makes the maxillary rotate backward around the rostral tip of the autopalatine, resulting in an adduction of the maxillary barbel. The articulation of the two is held in place during rotation by a cap of connective tissue and a ligament (**Plate III.1-9**).

Extensor tentaculi - The less conspicuous extensor tentaculi lies between the skull and the dorsolateral face of the autopalatine (**Plates II.2-15C, 36C**). The muscle originates from the ventral side of the perichondral part of the lateral ethmoid, dorsal to the lateral tip of the prevomer. The lateral ethmoid is the ossification of the rostro-lateral part of the chondrocranium, thereby enclosing the orbito-nasal lamina (**Plate II.2-17**) (DAGET, 1964) (see II.1.2). The autopalatine articulates with the lateral ethmoid at this lamina. The insertion of the extensor tentaculi muscle is spread over the medial face of the ossified orbito-nasal lamina. Posterior, the origin is situated at the ventral and ventrolateral face of the lateral ethmoid. In the 46.8 mm SL the medial fibres also originate from the lateral face of the ptero- and orbitosphenoids. All the fibres insert on the posterior half of the autopalatine (**Plate II.2-16**). The insertion is muscular; however in the 46.8 mm SL a small tendon accents the anterior fibres. The insertion only occurs posterior to the articulatory facet, at the dorsolateral face of the autopalatine. Distally, the autopalatine forms a dorsolateral ridge on which the fibres insert (**Plate II.2-18**). From there on the insertion becomes spread over the dorsal face of the bone. A differentiation of the extensor tentaculi appears to have occurred as a partially distinct, medial part and lateral part are present. The lateral part originates from the neurocranium, inserting on the dorsolateral face of the autopalatine. The medial part does not appear to originate from the skull but from the lateral face of the entopterygoid, at the level of the ligamentous connection between the autopalatine and the entopterygoid. Posteriorly this medial part is not distinguishable, leaving only one muscle bundle running from the lateral side of the neurocranium (*i.e.*, from the orbitosphenoid and the pterosphenoid) to the cartilaginous posterior tip of the palatine.

At the articulation zone between the lateral ethmoid and the autopalatine, the former still bears a cartilaginous facet (**Plate II.2-17**, arrows). In the middle, the autopalatine bears a distinct articulatory facet on its dorsomedial face (**Plate II.2-16**, af-VII). This facet is narrow but is anteroposteriorly elongated. The structure of the two articulations suggests that not only a rotation but a restricted translation in an anteroposterior direction is also possible. Apart from this direct connection between the lateral ethmoid and the autopalatine, both are, as already mentioned, connected indirectly: the autopalatine is attached to the entopterygoid through a ligamentous strap. Additionally a ligamentous link is present between the entopterygoid and the lateral ethmoid. These connections indicate that the entopterygoid in *Clarias gariepinus* corresponds to the sesamoid 'entopterygoid' type four of ARRATIA (1992).

DISCUSSION

The structure and the origin of the elements related to the palatine-maxillary mechanism have been investigated to a great extent, however, much confusion remains. The homology of the retractor muscle of the maxillary barbel is one of these much discussed topics. Several hypotheses have been proposed concerning the homology, but the general agreement exists that the muscle is derived from a part of the adductor mandibulae complex, based on evidence from the innervation (see below). However, there is still discussion as to which part of the complex gave rise to the barbel retractor muscle. In general, two hypotheses are proposed: (1) the retractor tentaculi is derived from the A_1 muscle, and (2) it is derived from the A_3 muscle.

(1) The fact that the retractor tentaculi inserts on the maxillary has led to the idea that the retractor muscle would be homologous with that part of the adductor mandibulae complex, which normally inserts on the maxillary, *i.e.,* the A_1 part. In generalised ostariophysans, the A_1 muscle is the superficial part of the complex, which inserts on the posterior or lateral margin of the maxillary (WINTERBOTTOM, 1974); directly (as in Cypriniformes,

Gonorhynchiformes and some primitive Characiformes) or through a primordial ligament (as in other Characiformes) (FINK & FINK, 1981) (see II.2.1). The latter ligament generally connects the maxillary with the lower jaw. A contraction of this A_1 muscle will generate a backward rotation of the maxillary, coupled to an elevation of the lower jaw (MOTTA, 1984; GOSLINE, 1986). TAKAHASI (1925), who investigated the cranial muscles of different otophysans, found different configurations of the adductor mandibulae complex and the retractor muscle. The A_1 muscle (TAKAHASI's maxillaris part), is the most superficial part of the complex in cyprinoid fishes, as observed in *Opsariichthys* (Cyprinidae). In this species the muscle is well developed, consisting of a dorsal $A_1\alpha$ part and a ventral $A_1\beta$ part. Rostrally the fibres are attached to a primordial ligament, which runs from the articular and dentary to the maxillary. In siluriforms, however, different patterns of the maxillaris part are observed. Although in some cases the maxillaris part is reduced completely [*e.g., Liobagrus* : Amblycipitidae, *Pseudobagrus* : Bagridae and some Trichomycteridae (SCHAEFER & LAUDER, 1986)], it generally is present. Surprisingly, when present, the muscle, connected tendinously to the maxillary, lies medial to the mandibularis section, the latter corresponding to the A_2 and A_3 muscles. TAKAHASI (1925) hypothesised that the ventral part of the A_1 muscle, the $A_1\beta$, migrated ventrally along the primordial ligament, eventually inserting only on the lower jaw, thereby fusing with the mandibularis. The dorsal part however, the $A_1\alpha$, kept its insertion to the maxillary through the primordial ligament. The loss of the connection of the primordial ligament with the lower jaw results in a tendinous connection between the $A_1\alpha$ and the maxillary, and no connection between the latter and the lower jaw, as is the case for *Parasilurus* (Siluridae). TAKAHASI (1925) described a complete gradient of muscle patterns from which he concluded that the retractor tentaculi of Siluriformes is homologous with the $A_1\alpha$ of his Cyprinoidei (= Cypriniformes of the present study).

(2) The fact that the A_1 muscle normally is situated at the lateral position of the muscle complex and the fact that the retractor tentaculi generally lies medial to the complex, has led to the assumption that the retractor tentaculi would be derived from the deeper part of the complex, as was noted by EATON (1948). ALEXANDER (1965) thus stated that the adductor tentaculi, as he called the retractor tentaculi, was derived from the A_3 muscle instead of the A_1 one. HOWES (1983a) demonstrated the possible evolutionary shift from an undifferentiated A_3 muscle, inserting on the medial face of the lower jaw, to a differentiated medial part of the A_3 muscle, the retractor tentaculi, which formed a *de novo* insertion on the primordial ligament. In the primitive siluriform *Diplomystes* (Diplomystidae), an inner division of the adductor mandibulae complex is attached both to the lower jaw, via a tendon covering the dorsal face of the lower jaw, and to both the maxillary and the lower jaw by a sheet of connective tissue. In *Tandanus* (Plotosidae), the primordial ligament has lost its muscular attachments, thereby solely connecting the maxillary to the lower jaw (HOWES, 1983a). In this species both the A_1 and A_2 muscles insert directly on the lower jaw, which is considered to be the plesiomorphic condition by HOWES (1983a), although the opposite was stated by FINK & FINK (1981). In *Pimelodus* (Pimelodidae) and *Clarotes* (Claroteidae), a homologous ligament is present, connecting the maxillary with the coronoid process of the dentary, but an extension of the ligament, running up to the fascia of the medial division of the adductor mandibulae complex is present as well. In *Megalonema* (Pimelodidae) the primordial ligament is posteriorly branched; the medial branch attaching to the medial part of the adductor mandibulae complex, the lateral branch connecting to the lower jaw (HOWES, 1983a). In *Corydoras* (Callichthyidae), the primordial ligament is formed by a connective tissue sheet, which runs from the coronoid process of the lower jaw to both the maxillary and the premaxillary (SCHAEFER & LAUDER, 1986). Here, a shift of the dorsal extension of the primordial ligament has occurred from the maxillary to the highly mobile premaxillaries. The A_1 muscle inserts on the lateral face of the lower jaw, as well as on the connective tissue to which the retractor tentaculi muscle is connected also. An analogue division of the

medial part of the adductor complex, inserting on the maxillary has also been reported in non-siluriforms (*e.g., Aphredoderus*, Aphredoderidae, Perciformes) (EATON, 1935).

A detailed morphological study of the ontogeny of the adductor mandibulae complex in *Clarias gariepinus* suggests the absence of the A_1 muscle (ADRIAENS & VERRAES, 1996) (see II.2.1). The functional adductor mandibulae then comprises the complex of (1) the fused A_2 and A_3' muscle parts, and (2) a medial A_3" part. Both the A_3 parts (A_3' and A_3") are separated by the levator arcus palatini. It can thus be suggested that the A_1 muscle is lost in *C. gariepinus*, whereas the retractor tentaculi is derived from the A_3" part of the adductor mandibulae complex.

The origin of the extensor tentaculi seems to be less doubtful. It is generally accepted that the extensor muscle is derived from the adductor arcus palatini (TAKAHASI, 1925; ALEXANDER, 1965; WINTERBOTTOM, 1974; GOSLINE, 1975a; FINK & FINK, 1981), which originates from the lateral margin of the parasphenoid and inserts on the dorsal margin of the suspensorium. TAKAHASI (1925) distinguished three portions: (1) the hyomandibular, (2) the pterygoid and anteriorly (3) the palatine portion. Apparently, the decoupling of the palatine from the rest of the suspensorium in siluriforms has resulted in the isolation of those fibres inserting on it, from the rest of the adductor arcus palatini.

Different nomenclature has been applied to indicate the tentacular muscles, especially for the extensor tentaculi, which is frequently referred to as the 'abductor tentaculi' (NAWAR, 1955a; ALEXANDER, 1965). GOSLINE (1975a) refers to the muscle as the 'palatine part of the adductor arcus palatini'.

As mentioned above, the innervation of the tentacular muscles basically reveals their true origin. In larval *Clarias gariepinus* the ramus mandibularis of the trigeminal nerve runs anteroposteriorly from the ventral face of the cartilaginous lower jaw to the coronoid process where it rises along the lateral side of the jaw. It then passes medially to the process, between the latter and the adductor mandibulae complex to the lateral side of that muscle complex. From there on it rises to the dorsal side of the adductor mandibulae muscles and eventually above the retractor tentaculi muscle. Posterior to the eye a side branch penetrates between the adductor mandibulae complex and the retractor tentaculi muscle. In 46.8 mm SL specimen the ramus has a comparable path (ADRIAENS & VERRAES, 1996). Posteriorly it lies against the medial side of the adductor mandibulae A_3", dorsolaterally to the retractor tentaculi. During early ontogeny, it is the adductor part of the mandibular muscle plate that becomes innervated by the ramus mandibularis. This part will eventually form the adductor mandibulae complex (JARVIK, 1980). The extensor tentaculi, on the other hand, is formed by the constrictor dorsalis part of the hyoid arch musculature (DATTA MUNSHI & SINGH, 1967; WINTERBOTTOM, 1974; JARVIK, 1980). The innervation occurs through the ramus hyomandibularis of the nervus facialis, which also innervates the adductor arcus palatini (ALEXANDER, 1965).

Different evolutionary trends have been detected in the palatine-maxillary mechanism within the siluriforms. A basic mechanism, where only the maxillary is functional, is present both in siluriforms and non-siluriform ostariophysans. In some cyprinoid teleosts the maxillary bears a small barbel at its ventral margin [*e.g., Cyprinus*, *Gobio* (Cyprinidae)]. In these fishes the A_1 muscle is attached to the maxillary through the primordial ligament. Opening of the mouth will make the maxillary rotate forward, resulting in a forward and ventral displacement of the barbel. Contraction of the A_1 muscle will, together with other muscular activity for mouth closure, return the maxillary and barbel to their original position (MOTTA, 1984). In siluriforms, however, the displacement of the maxillary barbel is due to the mobility of the palatine (GOSLINE, 1973). A basic siluriform

construction occurs in the primitive catfish *Diplomystes* (Diplomystidae). In this species a toothed, non-modified maxillary is connected to the maxillary barbel only through surrounding tissue (GOSLINE, 1975a). During mouth opening the maxillary barbel is displaced in an anteroventral direction instead of an anterolateral direction, as is the case in more specialised catfish. In general, two major types of palatine-maxillary mechanisms can be distinguished: (1) the sliding type and (2) different rotating types (GOSLINE, 1975a). In the sliding types, the articulation between the palatine and the skull enables a translation of the palatine in an anteroposterior direction. During the posterior movement of the palatine, the proximal tip of the maxillary is retracted. Through the connection between the distal point of the maxillary and the premaxillary, via connective tissue or via a distinct ligament, the maxillary rotates in such a way that the maxillary barbel is abducted (**Plate II.2-19**). The opposite action is generated through the anterior translation of the palatine. In the rotating types the maxillary barbel is abducted through an exorotation of the rostral tip of the palatine, coupled to an endorotation of the caudal tip. Adduction results from the opposite action. The muscles responsible for these actions may differ between taxa as two functional units control the ab- and adduction of the maxillary barbel. First, the abduction of the barbel generally is generated through a muscular action on the palatine. Thereby muscles connect the caudal half of the palatine with the skull in such a way that when contracting, this part of the palatine is shifted backward (*e.g., Ictalurus nebulosus* : Ictaluridae) or rotates medially [*e.g., Clarias gariepinus* (Clariidae), *Bagrus bayad*, *Chrysichthys longibarbis* (Bagridae)] in the sliding type and the rotating type, respectively (GHIOT *et al.*, 1984). Exceptionally, the abduction of the barbel is generated through muscular activity on the maxillary itself. In *Callichrous pabda* (Siluridae) three slips of muscles are attached to the proximal tip of the maxillary in such a manner that when contracting, the maxillary barbel is abducted. In this species no muscle is attached to the posterior half of the palatine (SINGH, 1967). Secondly, the adduction of the barbel is generally accomplished through the contraction of a retractor muscle, connecting the maxillary to the suspensorium. In this situation the distal tip of the abducted maxillary is retracted. As a result the proximal tip of the maxillary is displaced in an anterior direction, thereby pulling the palatine anteriorly in the sliding type (*e.g., Ictalurus*), or rotating the rostral half of the palatine medially in the rotating type (*e.g., Clarias gariepinus*, *Bagrus bayad*, *Chrysichthys longibarbis*) (GHIOT *et al.*, 1984). Again, exceptions are noted where the palatine induces the retraction of the maxillary barbel and some, or all, fibres of the muscle inserting on the palatine, are directed anteroposteriorly in such a way that during contraction the palatine is shifted anteriorly [*e.g., Bagrus bayad* (Bagridae), *Pimelodus clarias* (Pimelodidae)] (ALEXANDER, 1965; GHIOT, 1978; GHIOT *et al.*, 1984). *Chrysichthys* is another exception where no muscle, responsible for the retraction of the barbel, is present. In this species the elasticity of the tissue surrounding the palatine induces the retraction of the barbel. In the different rotating types, the rotation of the palatine is probably associated with a restricted sliding, as can be deduced from the shape of its articulations.

Apart from a pure retraction and extension of the maxillary barbel, further specialisations of the mechanism have enabled some species to perform a depression and elevation of the barbels as well, as for example noted in the catfish *Pimelodus* by ALEXANDER (1965) and GHIOT (1978). In this species the extensor tentaculi can be subdivided into five different muscle parts: (1) a lateroanterior, (2) a lateromedial, (3) a lateroposterior, (4) a tentacular abductor and (5) a tentacular adductor part. Due to the spread insertion on the palatine, a rotation of the bone around its longitudinal axis is permitted. One of these parts, the tentacular adductor, inserts on the ventral side of the palatine. When contracting it will, apart from a forward sliding, also perform a rotation of the palatine, which results in a depression of the barbel. When combined with the contraction of the barbel abducting muscle, the barbel can be purely depressed. On the other hand, three of

these muscles are inserted on the dorsal side of the palatine (1-3), which enable an elevation of the barbel during contraction.

In *Clarias gariepinus* the palatine-maxillary mechanism is of the rotating type, but a restricted sliding probably occurs. This can be deduced from the shape of the elongated articulatory facet on the dorsal face of the autopalatine (**Plate II.2-16**, af-VII). A distinct ligamentous connection is found between the maxillary and the premaxillary (**Plate III.1-9C**). The fact that the extensor tentaculi is partially divided into lateral and medial parts, as was observed in the 46.8 mm specimen, might contribute to a restricted mobility for depression and elevation of the maxillary barbel.

In some species, the palatine-maxillary mechanism does not function independently. It may be that the mechanism is coupled to other mechanisms. As already mentioned, in the primitive situation it is coupled to the mouth opening and closing mechanism, which provides sampling of food. This is profitable for species with short maxillary barbels, which rotate ventrally instead of laterally. In most catfishes, however, the maxillary barbels are well developed and highly mobile in a lateral direction. In these, the barbels can be placed in front of the head, thereby functioning as a food or obstacle detector. It is observed that *Clarias gariepinus* frequently moves its maxillary barbels when swimming around.

In *Bagrus bayad* a ligamentous connection is present between the metapterygoid and the palatine (GHIOT *et al*., 1984). This connection may affect the position of the palatine when, during respiration, the branchial cavity becomes enlarged, partially through abduction of the suspensorium. In *C. gariepinus* a ligamentous connection between the suspensorium and the palatine is observed as well. The sesamoid 'entopterygoid' type four of ARRATIA (1992) is attached to the posterior part of the palatine via a ligament, which inserts on the medial face of the palatine and runs ventrally to the extensor tentaculi.

The anterior tip of the palatine lies against the lateral face of the olfactory sac. This sac bears several diverticula in *Clarias gariepinus*, as is noted in the 46.8 mm SL specimen. The rotation of the rostral tip of the palatine during ab- and adduction of the maxillary barbel may affect the volume of the nasal sac. During the retraction of the barbel, the rostral tip is displaced medially, resulting in a decrease in volume of the nasal cavity. The lateral displacement of the rostral tip during the abduction of the barbel will create a negative pressure in the cavity, which will then refill with water. This process was demonstrated in a *Clarias* species by ALEXANDER (1965) who used Indian ink to demonstrate a flow of fluids through the nasal sacs during ab- and adduction of the maxillary barbels.

In the 46.8 mm SL specimen of *C. gariepinus*, a connection is present between the dorsal side of the palatine rostral tip and the antorbital. The latter bone, however, is attached to the base of the nasal barbel. Consequently, the displacements of the palatine may generate some movement in the nasal barbels as well.

CONCLUSIONS

Already during early ontogeny, the palatine-maxillary mechanism can become functional. In a 7.2 mm SL *Clarias gariepinus* larva, both skeletal elements (palatine, maxillary bone, orbito-nasal lamina) and muscular elements (retractor tentaculi and extensor tentaculi) are present. Although no ossifications of the related cartilaginous elements are observed, the muscles insert on them. Morphological evidence, as well as data from

the literature, suggest that in *C. gariepinus*, the retractor tentaculi is derived from the adductor mandibulae A_3, whereas the A_1 is lost.

Later during ontogeny, the orbito-nasal lamina and the palatine ossify, thus forming the lateral ethmoid and the autopalatine, respectively. These bones articulate with each other via a well developed articulatory facet. The shape of this facet suggests that during ab- and adduction of the maxillary barbel, the palatine rotates but a restricted sliding is possible as well. The muscular configuration corresponds to the one found in many siluriforms: a retractor tentaculi running from the suspensorium to the posterior face of the maxillary bone, and an extensor tentaculi interconnecting the lateral face of the skull (at the level of the lateral ethmoid) to the posterior half of the palatine. Some differentiations in the extensor muscle suggest the possibility of a restricted depression and elevation of the maxillary barbel. Additionally, the palatine-maxillary mechanism appears to be coupled to nasal sac ventilation and nasal barbel manipulations.

II.2.3 - THE HYOID MUSCLES[3]

Three ontogenetic stages of the African catfish *Clarias gariepinus* have been used to describe and discuss the ontogeny of the hyoid musculature. Based on ontogenetic evidence, as well as the literature, an asynchrony in the development of the muscles is observed: the intermandibularis and the protractor hyoidei are the first to develop and to bear their insertions, followed by the hyohyoideus inferior and the sternohyoideus. The hyohyoideus adductor and abductor muscles are the last of the hyoid muscles to develop. In the juvenile stage (136.2 mm SL specimen), the intermandibularis is still present. The protractor hyoidei is well developed, as it may play an important role in the opening of the mouth, the elevation of the hyoid bars, and, as a typical catfish feature, the displacement of the mandibular barbels. The protractor hyoidei arises as three pairs of muscle bundles (a pars ventralis, a pars lateralis and a pars dorsalis), of which the pars ventralis and the pars lateralis become fused to each other. This fusion gives rise to four different fields of superficial fibres for the manipulation of the mandibular barbels. The pars dorsalis, with its tendinous insertion, may be of more importance for mouth opening and/or hyoid elevation. The hyohyoideus muscle is well differentiated into an inferior, abductor and adductor muscles, acting on the hyoid bars, the branchiostegal rays and the opercular bone.

INTRODUCTION

In actinopterygians the hyoid bars are well known to play an important role in mechanisms like respiration and suction feeding. The depression and elevation of the hyoid bar control the required changes in volume of the orobranchial cavity (SCHAEFFER & ROSEN, 1961; OSSE, 1969; ANKER, 1974; LAUDER, 1981; SCHAEFER & LAUDER, 1986; WESTNEAT & WAINWRIGHT, 1989; WESTNEAT, 1990; AERTS, 1991). An additional participation of the hyoid bars in the suction feeding mechanism is established through the hyoideo-mandibular connection (LIEM, 1970; VERRAES, 1977; LAUDER, 1980a; 1980b; LAUDER & LIEM, 1980). The morphology and the attachment of different muscles contribute to the great mobility and functional significance of these bars.

During teleostean evolution, the hyoid arch has become differentiated into two functional units: a dorsal hyosymplectic and a ventral hyoid bar, interconnected by an interhyal. In generalised teleosts, the hyosymplectic articulates with the posterolateral face of the neurocranium, at the level of the otic capsules. At its anterior and posterior face it bears a process, the pterygoid and the opercular process, respectively. In general, the hyosymplectic arises separately from the palatoquadrate of the mandibular arch, which during ontogeny become sutured to each other, thus forming the suspensorium. The ventral part consists of a hyoid bar, supporting several branchiostegal rays along the ventral margin. Both elements of the hyoid arch articulate with each other through an interhyal, which is believed to be a new element in the hyoid arch in teleostean

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fishes (DAGET, 1964; JARVIK, 1980), although it has been observed in crossopterygians as well (*e.g., Latimeria chalumnae*) (LAUDER, 1980b). The presence of the interhyal plays an important role for the efficiency of hyoid depression (ANKER, 1974; LAUDER, 1980a). In some cases, secondary reductions have occurred within both the dorsal part [*e.g.,* symplectic bone in catfishes (ALEXANDER, 1965; ROBERTS, 1973; FINK & FINK, 1981; ARRATIA, 1992)], the interhyal [*e.g.,* in catfishes (ARRATIA, 1990; 1992; ADRIAENS & VERRAES, 1994)] and the ventral part [*e.g.,* dorsal hypohyal in some catfishes or ventral hypohyal in most Osteoglossomorpha (ARRATIA & SCHULTZE, 1990)].

Ontogenetically three major muscle primordia can be distinguished, which play an important role in the formation of the hyoid musculature: (1) the intermandibularis, (2) the interhyoideus, and (3) the sternohyoideus (TAKAHASI, 1925; DATTA MUNSHI & SINGH, 1967; GREENWOOD, 1971; WINTERBOTTOM, 1974; JARVIK, 1980; SURLEMONT & VANDEWALLE, 1991). The intermandibularis is derived from the intermandibularis part of the mandibular muscle plate. Early during ontogeny, this muscle becomes subdivided into an intermandibularis anterior and posterior. As is the case for all the mandibular arch muscles, these muscles are innervated by the ramus mandibularis of the trigeminal nerve. The interhyoideus develops from the muscle plate of the hyoid arch. The ventral part of the hyoid muscle plate, the constrictor hyoideus ventralis becomes subdivided into an interhyoideus anterior and an interhyoideus posterior. All the muscles derived from the hyoid arch muscle plate are innervated by the ramus hyomandibularis of the nervus facialis [ramus hyohyoideus in GREENWOOD (1971)]. The third muscle, the sternohyoideus, is a differentiation of the hypobranchial muscle plate of the first spinal myomeres, which grows forward towards the hyoid bars. Innervation occurs through the branches of the occipito-spinal nerves. Further differentiations of these three primary muscles, both by fusions and additional subdivision, give rise to the formation of the functional muscles of the hyoid bars. Generally, six major muscles can be distinguished: (1) the intermandibularis, which is homologous to the early intermandibularis anterior (GREENWOOD, 1971; WINTERBOTTOM, 1974). This muscle becomes extended between the left and right mandibular ramus, close to the symphysis. The intermandibularis posterior, however, generally fuses with the interhyoideus anterior, thereby forming (2) the protractor hyoidei. This fusion has led to a wide range of forms of the protractor hyoidei, thereby sometimes creating some uncertainties concerning the nomenclature (GREENWOOD, 1971; WINTERBOTTOM, 1974). In most teleosts, this muscle, frequently and incorrectly referred to as the geniohyoideus[4], connects the lower jaw with the hyoid bars (ANKER, 1974; WINTERBOTTOM, 1974; ELSHOUD, 1978; LAUDER & LIEM, 1980; LAUDER, 1979; 1981; LIEM, 1984; WESTNEAT, 1990). However, some functional shifts have occurred in Ostariophysi, as will be discussed later. The interhyoideus posterior will give rise to the hyohyoideus, which generally becomes subdivided into three functional muscles. (3) The hyohyoideus inferior connects the ipsi-lateral hyoid bars, thereby inserting medially on an aponeurosis. (4) The hyohyoideus abductor connects the anteriormost branchiostegal ray to the rostral tip of the hyoid bar. (5) The larger hyohyoidei adductores are spread over and between the branchiostegal rays, up to the medial face of the opercular bone. (6) The sternohyoideus forms the muscular connection between the pectoral girdle and the hyoid bars, as will be discussed later. In siluriforms, certain specialisations of the hyoid musculature can be related to the presence of mandibular barbels (GREENWOOD, 1971).

In this part of the thesis, three ontogenetic stages of the above mentioned hyoid muscles in *Clarias gariepinus* BURCHELL (1822) (Clariidae) are described. Some functional-morphological considerations are given concerning the structures of the muscles, as well as concerning some typical catfish adaptations of the protractor hyoidei. Discussion on the function of the different muscles is based on anatomical evidence only. No cinematographic or electromyographic analyses have been done. In this thesis, interpretations of possible functions of a certain muscle are based on morphological configurations: the earliest possible onset of muscle function is taken from the moment a certain muscle bears both its insertions.

RESULTS

The nomenclature used for the described muscles is according to WINTERBOTTOM (1974).

7.2 MM SL SPECIMEN (**Plates II.2-20, 21, 22, 23**)

In this larval stage, most of the muscles in question can be distinguished: (1) the intermandibularis, (2) the protractor hyoidei, (3) the hyohyoideus inferior, (4) the complex of the undifferentiated hyohyoideus ab- and adductor muscles and (5) the sternohyoideus (**Plate II.2-20**).

Intermandibularis – This muscle lies at the ventroposterior margin of the rostral tip of the lower jaw, close to its symphysis (**Plates II.2-21A-B, 22**). The mandibula in this larval stage is formed by Meckel's cartilage, which already bears a rostral perichondral ossification, the mentomeckelian bone, and the dermal dentary. Anteriorly, the muscle fibres are attached to the ventromedial face of the mentomeckelian, while the posterior fibres originate at the ventromedial margin of the dentary (**Plate II.2-23A**). The caudalmost fibres appear to be still continuous with the rostral fibres of the protractor hyoidei.

Protractor hyoidei – This is the largest of the hyoid muscles (**Plate II.2-20B**). It covers the ventral face of the anterior part of the ceratohyals, thereby surrounding the bases of the mandibular barbels (**Plates II.2-20B, 21**). Both the external and the internal mandibular barbel bases penetrate the muscle completely, as on the dorsal face of the protractor muscle, two pair of mandibular barbel extensions can be observed (**Plate II.2-22A-B**). The muscle originates on the ventrolateral face of the anterior ossification of the hyoid bar, the anterior ceratohyal. The site of origin is located caudally against the attachment site of the hyohyoideus inferior (**Plates II.2-20B, 21A-B**). From there on, the protractor muscle fibres run lateral against the hyohyoideus inferior, up to the bases of the mandibular barbels and the dentary, where they insert. Some of the ventral fibres do not reach the dentary but meet the ipsi-lateral fibres at a median aponeurosis (**Plates II.2-20, 21A-B**). The attachment of the fibres to the dentary is situated at the ventromedial margin, posterior against the caudal fibres of the intermandibularis.

Hyohyoideus inferior – The inferior hyohyoideus muscle is a small muscle bundle, which runs along the ventral surface of the ceratohyal (**Plates II.2-20B, 21A-B, 22A**). Caudally the fibres originate on the ventrolateral side of the ceratohyal, medial to the protractor hyoidei (**Plate II.2-23B**). This attachment site is situated caudally to the articulation between the second branchiostegal ray and the hyoid bar. From there, the fibres run anteriorly to a median aponeurosis, which is not attached to any skeletal element (**Plate II.2-21A-B**).

Hyohyoidei ab- and adductor - In the muscle complex, no distinction can be made yet between the abductor part and the adductor part. The rostral, conical part of the muscle lies at the medial face of the hyohyoideus inferior. It is attached through a very indistinct tendon to the hyoid bar. Caudally, the muscle becomes sheet-like, lying in the margin of the branchiostegal membrane. Although present, the muscle cannot be completely functional at this stage, as no insertion is observed on the branchiostegal rays.

[4] According to GREENWOOD (1971) "it is clear that on grounds of homology and ontogeny the muscle should not be called a geniohyoideus in teleost fishes". It has been shown that "the teleostean 'geniohyoideus' is in no way homologous to that of the tetrapods, from which the name has been taken" (WINTERBOTTOM, 1974).

Sternohyoideus – This muscle is already well developed. The slender muscle connects the pectoral girdle with the rostral tip of the hyoid bars (**Plates II.2-20B, 21A, 22A-B**). Caudally, all the fibres originate on a horizontal crest of the cleithrum, lateral to the attachment of the pharyngoclavicularis externus muscle (**Plate II.2-23C**). No fibres originate from a myoseptum, separating the sternohyoideus from the hypaxial obliquus inferioris. Caudally, the left and right muscle bundles are separated from each other, with the conus arteriosus in between them. The muscle runs between the ventrally situated ab- and adductor complex of the hyohyoideus muscle, and the dorsally lying branchial arches (**Plate II.2-20**). Anteriorly the ipsi-lateral bundles converge, until they meet, thereby enclosing the ventral aorta. Rostrally, the insertion of both muscles is separated and situated at the posterior surface of the hypophyal. The parurohyal cannot bet distinguished in this 7.2 mm SL specimen, although the two sesamoid ossicles are already observed in a 6.6 mm SL specimen.

46.8 MM SL SPECIMEN (**Plate II.2-24**)

Intermandibularis – This muscle is now completely separated from the protractor hyoidei, as an interconnection between the left and right internal mandibular barbel bases lies in between the two muscles (**Plate II.2-24A**). The fibres originate on the ventral side of the mandibula, on the dento-splenio-mentomeckelian bone (see II.1.2). The muscle connects left and right lower jaws, close to the symphysis.

Protractor hyoidei – In this stage, it has become well developed. Caudally the fibres originate on the ventrolateral surface of the anterior ceratohyal bone, lateral to the attachment of the hyohyoideus inferior. As the fibres run rostrally and pass the bases of the mandibular barbels, the fibres become subdivided into three distinct, ipsi-lateral pairs of muscle bundles (**Plate II.2-24B**). The ventral bundle pair (= protractor hyoidei pars ventralis) inserts on the medial face of the bases of the internal mandibular barbels, whereas the lateral pair (= protractor hyoidei pars lateralis) inserts on the medial face of the bases of the external mandibular barbels. The dorsal bundle pair (= protractor hyoidei pars dorsalis) is elongated rostrally into a tendon, which attaches to the ventral face of the dentary. This tendon originates from the laterodorsal fibres of this pars dorsalis. Caudally, where no subdivisions can be distinguished, the tendon lies on the dorsal surface of the protractor hyoidei, until it continues into muscle fibres. As already mentioned, the protractor hyoidei has become completely separated anteriorly from the intermandibularis (**Plate II.2-24A**).

Hyohyoideus inferior – This muscle has its origin along the ventral surface of the anterior ceratohyal and ventral hypohyal bones. In serial sections, the fibres of the muscle are directed in different trajectories (**Plate II.2-24C**). The ventral fibres run transversally between the left and right hyoid bars, meeting in a median aponeurosis. The dorsal fibres, however, are directed more longitudinally. In between the two muscle parts, two tendons can be distinguished, which are elongated into the adjacent hyohyoidei abductores (see below).

Hyohyoideus abductor – The abductor muscle has become isolated from the adductor one, connecting the first branchiostegal ray to the rostral tip of the hyoid bar. The muscle originates through a long and narrow tendon on the ventral surface of the ventral hypohyal bone. This tendon is elongated into a flat muscle sheet, which inserts on the first branchiostegal ray of the opposite side. Consequently a crossing over of the ipsi-lateral tendons is noted at the level of the parurohyal bone. Anterior to the crossing over, the tendons are covered both dorsally and ventrally by fibres of the hyohyoideus inferior (**Plate II.2-24C**). The insertion site of the muscle on the rostral

margin of the first branchiostegal ray is spread over the complete length of the ray, whereas the lateral margin of the muscle extends into the lateral margin of the branchiostegal membrane. The lateral fibres do not insert on the branchiostegal ray, but are attached to a myoseptum separating the muscle from the first hyohyoideus adductor.

Hyohyoidei adductores – These muscles now form a continuous muscle sheet between the first branchiostegal ray up to the medial face of the opercular bone, thereby enclosing all the rays lying in between. The muscles inserting on the branchiostegal rays attach to the latter along the whole length. The lateral fibres lie laterally to the rays, as they insert on the myocommata separating the different muscles.

Sternohyoideus - At this ontogenetic stage the sternohyoideus is enlarged caudally to a great extent, as can be seen in the 136.2 mm SL specimen as well (**Plate II.2-27C**). The muscle is attached to both the ventral and the dorsal surfaces of the cleithrum. Caudally, the fibres lying dorsal to the horizontally limb of the cleithrum are separated completely from the fibres attached to the ventral surface of the bone. More anteriorly, the lateral fibres of these muscle divisions are fused, thereby covering the laterorostral margin of the cleithral bone. The left and right muscle bundles are separated from each other by a vertical myoseptum. The hemibranchia in the branchial cavity cover the dorsal surface of these muscle bundles. Anteriorly, the fibres fit in between the three caudal processes of the parurohyal bone, on which they insert (**Plate II.1-17**). The medial process of the parurohyal bone lies in the same plane as the myoseptum separating the ipsi-lateral muscle bundles. At the level of these three processes, the fibres are concentrated into four distinct bundles: two of them are attached to the ventral surface of the medial process, the other two are attached to the dorsal surface (**Plate II.2-24D**). Both the ventral and the dorsal bundles insert on the medial face of the lateral processes of the parurohyal bone. A broad sheet of connective tissue can be distinguished, connecting the lateral processes and the ventral bundles to the underlying hyohyoideus inferior (**Plate II.2-24D**, arrow).

136.2 MM SL SPECIMEN (**Plates II.2-25, 26, 27, 28**)

Intermandibularis – At this stage, the intermandibularis muscle is comparable to that of the 46.8 mm SL specimen. In a ventral view, the separation of the muscle from the anterior fibres of the protractor hyoidei can be observed (**Plate II.2-25A**). The muscle connects the left and right lower jaws. The insertion is spread over the ventral face of the anterior part of the dento-splenio-mentomeckelian bone, close to the mandibular symphysis (**Plate II.2-25B**).

Protractor hyoidei – The protractor has become a solid and large muscle, connecting the hyoid bars to both the lower jaw and to the mandibular barbels (**Plate II.2-25**). The origin of the muscle is spread over the anterior ceratohyal, up to the anterior margin of the non-ossified area between the anterior and the posterior ceratohyal bones (**Plate II.2-25**). The origin of the protractor hyoidei lies lateral to the origin of the hyohyoideus inferior, which inserts on the anterior ceratohyal as well. From there on, the superficial fibres run anteriorly up to different insertion sites, resulting in four distinct superficial muscle fields (**Plate II.2-26**). The lateral fibres insert on the posterior and lateral margin of the bases of the external mandibular barbels (field P_1) [nomenclature of the fields following GHIOT *et al*. (1984)]. The medial fibres run up to a median aponeurosis, thereby interconnecting the left and right protractor muscles (field P_7). Superficial fibres originate from the bases of the mandibular barbels as well. Muscle

fibres are observed running from the medial face of the base of the external mandibular barbel, up to the lateral face of the base of the internal mandibular barbel (field P_3). Other fibres connect the medial face of the base of the internal mandibular barbel to the structure interconnecting the ipsi-lateral internal mandibular barbels (field P_4). These superficial fibres correspond to the pars ventralis and lateralis, as is observed in the 46.8 mm SL specimen (**Plate II.2-24B**). The tendon of the pars dorsalis, as is observed in the 46.8 mm SL *Clarias gariepinus*, originates from its ventral fibres and inserts on the ventral face of the dento-splenio-mentomeckelian bone. The dorsal fibres insert on the caudal margin of the lower jaw and run medially where they meet the ipsi-lateral fibres at a median aponeurosis (**Plate II.2-25B**).

Hyohyoideus inferior – This muscle is now a solid muscle covering the ventral face of the anterior ceratohyal and the ventral hypohyal (**Plates II.2-25, 27A**). The origin of the muscle is spread over the whole ventral surface of those two bones. All fibres insert on a medial aponeurosis. The orientation of the fibres changes from transverse (anterior fibres) to oblique (posterior fibres).

Hyohyoideus abductor - The morphology of the hyohyoideus abductor is also comparable to the situation in the 46.8 mm SL specimen. The crossing over of the tendons of the muscles can clearly be distinguished as they run from the ventral face of the parurohyal bone up to the ventral hypohyals (**Plate II.2-28**).

Hyohyoidei adductores – These muscles now form a strong muscular sheet between the first branchiostegal ray and the opercular bone (**Plate II.2-37B**), into which the other branchiostegal rays are embedded. The developmental state is comparable to the 46.8 mm specimen. The marginal muscle fibres correspond with the margin of the branchiostegal membrane, which covers the pectoral girdle.

Sternohyoideus – The sternohyoideus has become a very solid muscle, triangular in shape. The origin of the muscle is spread along the whole rostral margin of the ventral limb of the cleithral bone (**Plate II.2-27C**). The cleithrum bears a thin, rostral crest for the attachment of the sternohyoideus fibres, both at its ventral and its dorsal face. The fibres of the sternohyoideus are completely separated from the hypaxial muscles. Caudally, left and right sternohyoideus meet one another entirely in the midline. Rostrally the muscle fits into the fork formed by the caudal processes of the parurohyal bone. The parurohyal is connected to the ventral hypohyals through two stout ligaments (ligamenta parurohyalo-hypohyalia). The sternohyoideus consists of three myomeres, divided by two myocommata (**Plate II.2-27C**). The medial fibres of the middle myomere originate directly on the cleithral bone, whereas the major part of the fibres are attached to the caudal myocomma.

DISCUSSION

Not all the described hyoid muscles develop simultaneously in *Clarias gariepinus* (**Table II.2- 1**). The intermandibularis, the protractor hyoidei and the hyohyoideus inferior are present already in the 4.5 mm SL fry [4.7 mm larva of SURLEMONT & VANDEWALLE (1991)], although no insertion is observed (SURLEMONT *et al.*, 1989). In the 5.0 mm SL fry, the sternohyoideus is developed, lying at the ventral face of the branchial basket. The muscle reaches from the pectoral region to the posterior margin of the hyohyoideus inferior, but does not insert yet. The intermandibularis and protractor hyoidei, however, are already provided with both their insertions at this level (SURLEMONT & VANDEWALLE, 1991). It is only in the 6.2 mm SL larva [6.8 mm larva of SURLEMONT & VANDEWALLE (1991)]

that the hyohyoideus inferior and the sternohyoideus attach to the hyoid bars (SURLEMONT *et al.*, 1989). In the 7.2 mm SL larva, the hyohyoideus abductor and adductor muscles can be observed, inserting through an indistinct tendon onto the rostral part of the hyoid bar. These muscles, however, which cannot be distinguished from each other yet, do not yet insert on the branchiostegal rays. The insertion was observed in the 46.8 mm SL specimen. However, this presumably does not exclude some constriction and expansion possibilities, as the muscles lie embedded in the branchiostegal membrane.

Table II.2- 1: Data on the presence of the cranial muscles during ontogeny in *Clarias gariepinus* (- = not present yet, -/+ = present but no insertion, + = present and inserting) (* the insertion of the muscles is not on the opercular bone but on the opercular process of the hyosymplectic) [(1) following SURLEMONT *et al.* (1989); (2) following SURLEMONT & VANDEWALLE (1991)]

Muscle	*4.0 mm (2)*	*4.5 mm (2)*	*5.0 mm (1)*	*6.2 mm (2)*	*7.2 mm*	*46.8 mm*	*125.5 mm*
intermandibularis	-	-/+	+	+	+	+	+
protractor hyoidei	-	-/+	+	+	+	+	+
hyohyoideus inferior	-	-/+	-/+	+	+	+	+
hyohyoideus abductor	-	-	-	-	+	+	+
hyohyoidei adductores	-	-	-	-	-/+	+	+
sternohyoideus	-	-	-/+	+	+	+	+
adductor mandibulae	-	-/+	+	+	+	+	+
levator arcus palatini	-	-/+	+	+	+	+	+
adductor arcus palatini	-	-/+	+	+	+	+	+
extensor tentaculi	-	-	-	+	+	+	+
retractor tentaculi	-	-	-	+	+	+	+
levator operculi	-	-	-/+	+	+	+	+
adductor operculi	-	-	-/+*	-/+*	-/+*	+	+
dilatator operculi	-	-	-/+*	-/+	+	+	+

Looking at the ontogeny of the skeletal elements of the suspensorium, four major stages can be distinguished. Initially, the suspensorium, Meckel's cartilage and the hyoid bars form one single cartilaginous unit, as can be observed in the 4.5 mm and 5.0 mm SL larvae [4.7 mm and 5.2 mm larvae of SURLEMONT *et al.* (1989); SURLEMONT & VANDEWALLE (1991)]. Secondly, the cartilaginous connection between the lower jaw and the quadrate part of the suspensorium is lost, and a real articulation is formed in the 5.6 mm SL larva. Thirdly, the cartilaginous connection between the hyoid bars and the suspensorium, constituted by the cartilaginous interhyal is lost, as the interhyal becomes a separate structure articulating with both the suspensorium and the hyoid bars (at 21.4 mm) (VANDEWALLE *et al.*, 1985) (see IV.2). Fourthly, the interhyal becomes completely reduced and a ligamentous connection is present between the hyoid bars and the suspensorium (in the 136.2 mm SL specimen) (ADRIAENS & VERRAES, 1994).

As already mentioned, the *intermandibularis* in the juvenile *Clarias gariepinus* corresponds to the anterior portion of the primordium of this muscle, as the posterior part has fused with the interhyoideus anterior. This seems to be the case in most teleosts. However, in some Osteoglossomorpha (Notopteridae and Mormyridae), the two muscles (posterior intermandibularis and anterior interhyoideus) remain separated (GREENWOOD, 1971). In the latter paper the interhyoideus seems to correspond to the interhyoideus anterior. A reduction of the anterior intermandibularis is noted in those species where a fusion has occurred between the two mandibular rami, as the muscle normally contributes to the adduction of the two rami, and consequently

the adduction of the suspensoria (ANKER, 1974). Within the ostariophysan fishes, the muscle is well developed in Cobitidae (but not in the other Cypriniformes) and in Siluriformes.

The morphology of the *protractor hyoidei* frequently shows a complex muscle structure, in which the basic configuration of the subdivisions can only be discerned from ontogenetic studies. The protractor hyoidei is recognised only when a fusion between the posterior intermandibular and anterior interhyoideus muscle has occurred. Generally, this protractor then connects the medial face of the mandibula, close to the symphysis, with the hyoid bars at the level of the hypohyals, and the anterior and posterior ceratohyals (WINTERBOTTOM, 1974). Expansion of insertions have been observed in several teleosts, where the protractor becomes attached to the branchiostegal rays [*e.g.,* in Osteoglossidae, Pantodontidae (GREENWOOD, 1971), Cyprinidae (TAKAHASI, 1925)] (WINTERBOTTOM, 1974). The rostral attachment of the muscle to the dentary seems to be constant, although additional insertions on the angular bone occur (LIEM, 1967). In several cases, the protractor hyoidei has a typical 'X'-shape, bearing two separate bundles anteriorly and posteriorly, fused to each other in the middle (TAKAHASI, 1925; SAXENA & CHANDY, 1966; LAUDER, 1980a; 1981; LAUDER & LIEM, 1980), although other shapes are frequent [*e.g.,* Y-shape in *Epibulus insidiator* (Labridae)] (WESTNEAT & WAINWRIGHT, 1989). The secondary subdivision of the protractor muscle in a superficial part and a deeper part, as is observed in *Clarias gariepinus*, seems to be a general feature for siluriform fishes. TAKAHASI (1925) referred to a 'geniohyoideus inferior', corresponding to the protractor hyoidei pars ventralis and lateralis, and a 'geniohyoideus superior', corresponding to the protractor hyoidei pars dorsalis. In Mormyridae, where the posterior intermandibularis and the anterior interhyoideus remain separated from each other, the latter muscle consists of two pairs of muscle bundles: a medial (or inner) division and a lateral division (GREENWOOD, 1971). The position of the lateral division, and especially the fact that it inserts through a tendon onto the anterior half of the mandibula, may suggest a correspondence with the tendinously inserting protractor hyoidei pars dorsalis. The deeper fibres of this pars dorsalis, covering the tendinous part, may then correspond to the inner division (**Plate II.2-25B**). When comparing the position of the posterior intermandibularis in Mormyridae with that in *C. gariepinus*, this muscle may then correspond to the protractor hyoidei pars lateralis and ventralis, into which the mandibular barbels have become embedded. However, some additional ontogenetic evidence, as well as comparative material would be required to elucidate such homologies. Several authors refer to a 'geniohyoideus anterior' and a 'geniohyoideus posterior' (OSSE, 1969; ANKER, 1974; LAUDER & LIEM, 1980; WESTNEAT, 1990). It has been demonstrated that these two muscles contracted independently from each other (OSSE, 1969; LAUDER & LIEM, 1980). This anterior part presumably corresponds to the intermandibularis posterior, whereas the posterior part corresponds to the interhyoideus anterior. As these two muscles are innervated by two different nerves, an independent contraction of those two muscles could be explained (VERRAES, 1973). In those ostariophysan fishes where no subdivisions can be found, it is presumed to be the result of a secondary fusion between the superficial and the deeper part. Exceptionally, the protractor hyoidei can be reduced completely, as is observed in *Liobagrus* (Amblycipitidae) (TAKAHASI, 1925). The activity of the protractor hyoidei muscle can even differ interspecifically, as was observed in several characiforms (LAUDER, 1981).

The *hyohyoideus* is derived from the interhyoideus posterior (WINTERBOTTOM, 1974). An undifferentiated hyohyoideus muscle can still be found in some living teleosts, where the muscle runs from the medial face of the opercular bones, passing along the branchiostegal rays up to the medial face of the hyoid bar (*e.g.,* in Osteoglossomorpha, Salmonidae, Cobitidae) (TAKAHASI, 1925; GREENWOOD, 1971; VERRAES, 1973; LAUDER & LIEM, 1980). Although only one muscle can be observed in Cobitidae, two rostral insertion sites can be distinguished, corresponding to the insertion sites of two subdivisions of the hyohyoideus observed in most Cypriniformes and all Siluriformes (TAKAHASI, 1925). The latter author then refers to an 'inferior hyohyoideus muscle', inserting on the hyoid

bar, and a 'superior hyohyoideus muscle', attaching onto the branchiostegal rays. This inferior part corresponds to the hyohyoideus inferior used in this chapter, whereas the superior part corresponds to both the hyohyoideus abductor and adductor muscles (TAKAHASI, 1925; DATTA MUNSHI & SINGH, 1967; SINGH, 1967; OSSE, 1969; ANKER, 1974).

This *hyohyoideus inferior* generally covers the anterior, ventral portion of the hyoid bars, onto which it inserts. The muscle fibres run medially, as they insert on a median aponeurosis, thereby covering the parurohyal bone. In some cases, the muscle inserts on the latter bone as well, in which case no median aponeurosis is present (WINTERBOTTOM, 1974).

The *hyohyoideus abductor* is responsible for the expansion of the branchiostegal membrane. In general the muscle connects the first branchiostegal rays with the rostral tip of the hyoid bars. However, some morphological modifications concerning the insertions of the tendons have been noted, as the tendon of the abductor, attached to the branchiostegal ray on one side of the hyoid bar, runs up to the hyoid bar of the same side, of the opposite side or of both sides. In *C. gariepinus* it is observed that the tendons cross over each other, as they pass through the hyohyoideus inferior (**Plates II.2-28, III.1-9G**). In *Gasterosteus aculeatus* (Gasterosteidae), a single tendon, which is forked anteriorly, connects the muscle with the ipsi-lateral, rostral tips of the hyoid bar (ANKER, 1974). In those cases where the abductor muscle is absent, a caudal shift of the fibres of the hyohyoideus inferior or the protractor hyoidei can be observed, which consequently insert on the first branchiostegal rays (WINTERBOTTOM, 1974). As a result, the contraction will generate an abduction of the branchiostegal rays as well.

In general the *hyohyoidei adductores* form the, sometimes continuous, muscle sheet between the first branchiostegal ray and the opercular bone, although insertions are found on the subopercular or the interopercular bones as well (DATTA MUNSHI & SINGH, 1967; OSSE, 1969; WINTERBOTTOM, 1974). Contraction of the muscles results in the constriction of the branchiostegal membrane. In *C. gariepinus* the caudal insertion is restricted to the opercular bone (**Plate II.2-37B**). NAWAR (1955a) made a further nomenclatural subdivision as he referred to the 'interbranchiostegales', indicating the muscles interconnecting the branchiostegal rays, and the 'opercular branchiostegalis', being the muscle running from the last branchiostegal ray up to the medial face of the opercular bone.

The *sternohyoideus* configuration in teleosts is rather constant (GREENWOOD, 1971; WINTERBOTTOM, 1974). In most cases this muscle runs from the horizontal limb of the cleithrum up to the parurohyal bone, which is believed to be a complex of a perichondral bone and the paired sesamoid ossifications of the sternohyoideus tendons (DE BEER, 1937; DEVILLERS, 1958; ARRATIA & SCHULTZE, 1990). As in most teleosts, some fibres are connected to an aponeurosis with the inferior obliquus hypaxial muscle (*e.g.,* ADRIAENS *et al.*, 1993), it is not the case in *C. gariepinus*. The insertion of the sternohyoideus in the latter species is, however, spread over the plate-like horizontal limb, both along its dorsal and ventral margin. In most teleosts, the sternohyoideus plays a crucial role in mouth opening, suspensorial abductions and hyoid depressions (LAUDER, 1980c; LAUDER & LIEM, 1980; AERTS, 1991). In *C. gariepinus* the sternohyoideus is a solid but short muscle, which may influence the depression capacities during hyoid depression (ADRIAENS & VERRAES, 1994) (see IV.2).

The principal function of the hyoid bar is to act as a lever for the expansion of the orobranchial cavity (SCHAEFFER & ROSEN, 1961). Such expansions, however, are generated through a whole set of mechanisms in which the neurocranium, the lower jaw, the maxillary bones, the suspensorium, the branchiostegal rays, the opercular bones and the gill arches are involved (ALEXANDER, 1967a; 1969; GOSLINE, 1971; ANKER, 1974; LAUDER & LIEM, 1980; LAUDER, 1980a; 1980c; 1981; LIEM, 1984; MULLER, 1987; WESTNEAT, 1990; AERTS, 1991). The effect of

suspensorial abduction will be of greater importance in those teleosts with a laterally depressed skull, where the suspensoria are high, compared to dorsoventrally flattened skulls, with short suspensoria (see IV.2). In those teleosts having a dorsoventrally depressed head, like most catfishes do, the elevation of the skull and the depression of the hyoid bar will play a more important role (ALEXANDER, 1965; GOSLINE, 1973). In siluriforms, however, the neurocranial movements are believed to be obstructed by the strongly fused anterior vertebrae in the complex Weberian apparatus, but mainly in a lateral direction (GOSLINE, 1977). In teleosts with dorsoventrally flattened skulls, it can be suggested that an extensive hyoid depression can be unfavourable (ADRIAENS & VERRAES, 1994) (see IV.2). In general, the head of catfishes is dorsoventrally flattened, being an adaptation to a benthic life style (ALEXANDER, 1965). Consequently, the floor of the orobranchial cavity is relatively broad, which implies that only a slight depression of the hyoid bars will result in a substantial volume increase (see IV.2). Furthermore, HUGHES (1970) argued that in bottom-living fishes, the suction pump system, enabled through an abduction of the opercular bone, is far more important for aquatic respiration than the pressure pump system is, in which an elevation of the hyoid bar and an adduction of the suspensoria induce the water flow. In *Clarias gariepinus*, an adaptation to an improved opercular abduction is observed, as a shift of the insertion site of the adductor operculi resulted in an abduction action of the latter muscle, and thus a functional shift of the antagonist to the protagonist of the well developed dilatator operculi (ADRIAENS & VERRAES, 1997b) (**Plate II.2-37**) (see II.2.4).

One typical catfish specialisation in hyoid musculature, is related to the presence of the mandibular barbels. In several catfishes, the function of the protractor hyoidei is not restricted to jaw opening or hyoid elevation. As the bases of the mandibular barbels are embedded into the superficial fibres of the protractor muscle, contractions of the muscle will control the orientation of these barbels. In some catfishes a total of up to seven of these different fields could be recognised: (1) P_1 connecting the hyoid bar with the basal process of the external mandibular barbel, (2) P_2 between the plate, interconnecting the bases of the internal and external barbel, and the external mandibular barbel base, (3) P_3 between the bases of the internal and the external barbels, (4) P_4 between the internal barbel base and the median aponeurosis, (5) P_5 between the caudal tip of the external mandibular barbel base and the median aponeurosis, (6) P_6 between the base of the internal mandibular barbel and the hyoid bar and (7) P_7 running from the hyoid bar up to the median aponeurosis (GHIOT, 1978; GHIOT *et al.*, 1984). In *C. gariepinus*, four of these fields were observed (**Plate II.2-26**). The largest field, the P_7, connects the ipsi-lateral hyoid bars to each other. Even though the fibres of this field do not insert on the bases of mandibular barbels, they will probably have some effect on the displacement of the barbels. A medial displacement can presumably be generated for the internal and external barbels independently: contraction of the P_1 field may retract the base of the external mandibular barbel, which may result in the medial displacement of the distal part of the barbel, whereas a combined contraction of the P_1 and the P_3 field may have the same effect on the internal barbel. The opposite displacement, in a lateral direction, may be generated as follows: contraction of the P_4 for the internal barbels, P_3 and P_4 for the external barbels. SINGH (1967) used terms like 'retractor tentaculi' and 'protractor tentaculi' to indicate the differentiated protractor hyoidei, attached to the bases of the mandibular barbels.

Another specialisation within the Ostariophysi, as an adaptation to a benthic life style, has been observed in some hill stream cyprinids, like *Garra mullya* and *Crossocheilus latius punjabensis* (Cyprinidae) (SAXENA & CHANDY, 1966). These species have developed a mental suctorial disc, which enables them to attach themselves to the substrate in order to withstand strong currents. A vacuum is created and sustained below these discs through contraction of a modified intermandibularis and protractor hyoidei.

In the Clariidae, the hyoid bars play an additional important role in the respiratory mechanism, as the members of this catfish family are able to perform aerial respiration, due to the presence of a suprabranchial

organ (WILLEM, 1951; DATTA MUNSHI, 1961; GREENWOOD, 1961). During this kind of respiration, an air bubble is swallowed at the water surface, and transported from the oral cavity, along the branchial cavity into the suprabranchial cavity (HELLIN & CHARDON, 1981; VANDEWALLE & CHARDON, 1991). The air bubble is pushed from the oral cavity into the branchial cavity through the lifting of the hyoid bar, generated through the contraction of the protractor hyoidei and the adductor mandibulae. It was observed in an abnormal specimen, lacking any mobility in the hyoid bars, that the transport of the air bubble to the suprabranchial organ was impossible (VANDEWALLE & CHARDON, 1991).

CONCLUSIONS

In *Clarias gariepinus*, the hyoid muscles investigated, develop and attach themselves to skeletal elements in a non-synchronous way. Ontogenetically, the intermandibularis, the protractor hyoidei and the hyohyoideus inferior are the first to develop, followed by the sternohyoideus. The last of the six muscles to become functional are the hyohyoideus abductor and adductor muscles. This time difference in functionality, coupled to an ontogenetic asynchrony in the skeletal development, may result for example in a shift in mouth opening and respiratory mechanisms (see IV.1).

The myology of the juvenile *C. gariepinus* reveals some structural adaptations. The superficial fibres of the protractor hyoidei are arranged into four fields, based on the place of origin and insertion. Several of these fields may generate a displacement of the internal and external mandibular barbels. A detailed morphological study reveals a subdivision of this muscle in a pars lateralis, a pars ventralis and a pars dorsalis. The hyohyoideus muscle is well developed and differentiated in the inferior, the abductor and the several adductor muscles. The sternohyoideus is broad but rather short, probably related to the need of a restricted hyoid depression during respiration and feeding.

II.2.4 - The Suspensorial and Opercular Muscles[5]

Three ontogenetic stages of *Clarias gariepinus*, are studied and compared with data from the literature. The development of the suspensorial and opercular muscles is described and some functional aspects are discussed. Five muscles can be distinguished: (1) the adductor arcus palatini, (2) the levator arcus palatini, (3) the adductor operculi, (4) the dilatator operculi and (5) the levator operculi. Data from this study, as well as data from the literature indicate that these muscles do not develop synchronously, which is reflected in the presence of different mechanisms for aquatic respiration. It is observed that the apparatus needed for the pressure pump system, develops prior to that of the suction pump system. In juvenile *C. gariepinus*, however, the suction pump system appears to be more important for the aquatic respiration, as some adaptations to an improved dilatation of the gill cover are found. Apart from their role in aquatic respiration, the muscles are assumed to be functional during atmospheric respiration, in which the suprabranchial organs are used.

INTRODUCTION

The suspensorium and the opercular apparatus are known to play an important role in the respiratory mechanisms and suction feeding. During respiration a double pumping system (pressure pump and suction pump) creates a water flow through the gills, thus enabling the exchange of respiratory gasses (Hughes, 1970; Ballentijn, 1972). Initially, a negative pressure is generated in the orobranchial cavity, generating a water flow into the mouth. Three major mechanisms can be responsible for the volume increase of the orobranchial cavity: (1) the elevation of the mouth roof, (2) the depression of the mouth floor and (3) the lateral displacement of the mouth walls (Schaeffer & Rosen, 1961; Dalela *et al.*, 1974; Elshoud, 1978; Muller, 1987; Aerts, 1991). During the inspiration phase, water is prevented to enter the opercular slit through an opercular skin fold and the branchiostegal membrane (Gosline, 1973; Alexander, 1975a). The transport of the water volume from the orobranchial cavity through the gills, into the opercular cavity, is generated by a decrease in the volume of the orobranchial cavity (pressure pump) and the expansion of the opercular cavity (suction pump). During this phase, the water is prevented to flow back through the mouth opening by the presence of mouth valves. Eventually, the water is expelled from the opercular cavity, again prevented by the mouth valves to flow in the wrong direction.

The muscular activity, needed for a maximal volume increase of both the orobranchial and the opercular cavity, is provided by a whole set of cranial muscles (Ballentijn & Hughes, 1965; Alexander, 1975a; Osse, 1969; Elshoud, 1978; Liem, 1984). The elevation of the neurocranium occurs through the contraction of the epaxial muscles, attached to the supraoccipital region of the skull. The lowering of the mouth floor can be generated through a combined activity of the hypaxial muscles (retraction of the pectoral girdle), the

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sternohyoideus muscle (depression of the hyoid bars), the protractor hyoidei (lowering of the mandible) and the levator operculi (lowering of the mandible through the opercular four-bar system). Two suspensorial and two opercular muscles are responsible for the ab- and adduction of the side walls of the orobranchial and opercular cavities: (1) the levator arcus palatini, (2) the adductor arcus palatini, (3) the dilatator operculi and (4) the adductor operculi (GOSLINE, 1973; ANKER, 1974; ALEXANDER, 1975a; BAREL *et al.*, 1976a; OSSE, 1969; LAUDER & LIEM, 1980; AERTS & VERRAES, 1984).

The mechanism for extensive suction feeding can be considered as an extension of the respiratory mechanism, where the apparatus elements experience movements with a greater amplitude (ALEXANDER, 1975a). In this way, by creating a sudden and substantial volume increase in the orobranchial cavity, food particles in front of the mouth are sucked in (ALEXANDER, 1969; 1970). However, within teleosts several additional, structural modifications have occurred to improve the success of this type of feeding (*e.g.,* protrusion) (SCHAEFFER & ROSEN, 1961; OSSE, 1969; GOSLINE, 1973; ALEXANDER, 1967b; 1975a; MOTTA, 1984; WESTNEAT & WAINWRIGHT, 1989; WESTNEAT, 1990).

Ontogenetic evidence can play a crucial role in the understanding of these functional mechanisms, found in adult organisms, as it demonstrates the formation of the mechanism from a basic configuration. Ontogenetic shifts in mechanisms can be considered as a reflection of the shift in functional demands that occur in a developing fish larva. In this part of the thesis, the ontogeny of these muscles responsible for the movements of the suspensorium and the gill cover are studied in order to investigate whether some adaptations to a benthic life-style are present. Within both the suspensorial set and the opercular set, muscles are derived from both the mandibular muscle plate and the hyoid muscle plate (DATTA MUNSHI & SINGH, 1967; WINTERBOTTOM; 1974; JARVIK, 1980). The mandibular muscle plate forms a masticatory and an intermandibular part. The former differentiates into a constrictor dorsalis and an adductor part. The anterior part of this dorsal constrictor will give rise to the levator arcus palatini, whereas the posterior part will differentiate into the dilatator operculi. The hyoid muscle plate becomes subdivided into a constrictor hyoideus dorsalis and a constrictor hyoideus ventralis. From this constrictor dorsalis, the adductor arcus palatini becomes differentiated anteriorly, whereas the adductor operculi and levator operculi are formed posteriorly. During evolution, the levator operculi becomes differentiated as a part of the adductor operculi from the holostean level on (SCHAEFFER & ROSEN, 1961). In some cases, a further differentiation of the dorsal constrictor into the hyomandibular adductor muscle has been observed (WINTERBOTTOM, 1974).

RESULTS

The nomenclature used in this paper is according to WINTERBOTTOM (1974). In all three ontogenetic stages of *Clarias gariepinus* two suspensorial muscles and three opercular muscles are found (NAWAR, 1955a).

7.2 MM SL SPECIMEN (**Plates II.2-29, 30, 31, 32, 33**)

Levator arcus palatini – This solid muscle connects the lateroventral face of the cartilaginous skull to the suspensorium. The muscle originates posteriorly to the eyes, ventrally on the taeniae marginales, thereby partially covering the lateral face of the sphenoid fissure (**Plates II.2-29, 30A, 33C**). The muscle inserts laterally on the non-ossified part of the hyosymplectic, between the insertions of the adductor mandibulae A_2A_3' and the adductor

mandibulae A_3" (**Plate II.2-33B-C**). The fibres of the A_2A_3' muscle lie against the lateral face of the levator arcus palatini, its insertion both ventrally and caudally to that of the levator arcus palatini. The A_3" muscle, however, situated against the medial face of the levator muscle, inserts dorsally to the latter muscle (ADRIAENS & VERRAES, 1996) (**Plate II.2-3**). At both ends, the fibres insert directly on the cartilaginous elements, without any evidence of a tendon or aponeurosis. At the level of the insertion, no ossifications of either the taeniae marginales or the hyosymplectic were observed.

Adductor arcus palatini – The suspensorial adductor forms a muscular sheet between the dorsal margin of the suspensorium and the neurocranium (**Plates II.2-29, 30**). Anteriorly, the fibres originate on the cartilaginous trabecular bars. Ventrally to the latter, the parasphenoid bone is attached through connective tissue. This connective tissue appears to be continuous with the medial fibres of the adductor arcus palatini as well (**Plate II.2-33A**). Caudally, the origin of the muscle gets broader, as it becomes spread both over the lateral margin of the parasphenoid bone and the ventral part of the trabecular bars. Fibres originate on the trabecular bars, from the level of the rostral tip of the pterygoid process of the suspensorium, up to the level of the articulation between suspensorium and neurocranium. The muscle borders the mouth cavity dorsolaterally, as it descends up to the dorsal margin of the suspensorium (**Plate II.2-33A-C**). Anteriorly, the fibres insert on the rostral tip of the pterygoid process. From there on, the insertion is continuous along the dorsal margin of the whole pterygoid process, the pars quadrata of the pterygoquadrate and the anterior, non-articulating part of the hyosymplectic (**Plates II.2-29, 30**). No ossifications of the latter structures are observed at the insertions sites. The retractor tentaculi is attached to the hyosymplectic, lateral to the adductor arcus palatini (**Plate II.2-33B-C**).

Dilatator operculi - This anterior opercular muscle is already developed, lying in a somewhat anteroposterior direction against the ventrolateral face of the neurocranium base (**Plates II.2-29, 31, 32**). Anteriorly, the fibres originate on the lateral face of the taenia marginalis, at the level of the articulation with the suspensorium (**Plate II.2-31**). Rostrally the dilatator is covered laterally by the levator arcus palatini (**Plates II.2-29, 33C**). The distinction between the fibres of both muscles could not always be observed clearly, as the differentiation of the dorsal mandibular constrictor muscle plate probably is not yet completed. The dilatator operculi runs posteriorly, towards the base of the hyosymplectic processus opercularis. Caudally, the muscle lies against the dorsal face of the adductor mandibulae A_2A_3'. It inserts on the processus dorsalis of the opercular bone through a very indistinct tendon (**Plate II.2-32C**).

Adductor operculi – At this stage, the opercular adductor connects the posterior part of the neurocranium with the suspensorium, instead of inserting on the opercular bone. The origin of the muscle is situated at the ventrolateral margin of the neurocranium, at the level of the otic capsule (**Plates II.2-29, 31**). At this point, the fibres of the adductor muscle are covered both laterally and caudally by the levator operculi. From there on, the adductor operculi runs ventrally, towards the opercular process of the suspensorium. Opposed to what is to be expected, the muscle is not attached to the opercular bone in this larval stage, but it appears to insert on the opercular process of the hyosymplectic (**Plates II.2-29, 31A-B**). The hyomandibular ossification of the latter has already initiated, but not at the site of insertion.

Levator operculi - The third and caudal opercular muscle, the levator operculi, is a slender muscle sheet, interconnecting the dorsal part of the opercular bone with the neurocranium (**Plates II.2-29, 31**). It originates from the lateral surface of the neurocranium, at the otic capsules, where no ossification could be observed yet. As

mentioned, the muscle fibres originate laterally and caudally to the origin of the adductor operculi fibres (**Plates II.2-29, 31A-B, 32A-B**). The fibres are orientated dorsoventrally, as they run downwards up to the opercular bone. The insertion on the latter bone is situated on the dorsal part, at its lateral face (**Plates II.2-29, 31A, 33D**). An insertion ridge, which is observed in the later ontogenetic stages (see below), cannot be distinguished as yet.

46.8 MM SL SPECIMEN (**Plate II.2-34**)

Levator arcus palatini – The levator has become a relatively, very thin muscle sheet. The anterior muscle fibres originate on the lateral, membranous component of the lateral ethmoid. More posteriorly, the muscle originates mainly on the medial face of the infraorbital IV [supraorbital bone in NAWAR (1954)], whereas the medial fibres attach to the ventral surface of the frontal. The descending fibres separate the two parts of the adductor mandibulae complex: the A_2A_3' part lying laterally, and the A_3'' part lying medially. At this level, the anterior part of the muscle inserts through a sheet-like tendon on the lateral face of the quadrate and the hyomandibular bones. Apart from the ventral attachment site, this sheet is separated from the hyomandibular and quadrate bones by the A_3'' adductor mandibulae. The caudal fibres lie directly against the lateral surface of the hyomandibula and are attached to it musculously.

Adductor arcus palatini – This is the antagonist of the previous muscle, originating from the bones constituting the neurocranial floor. The fibres are attached musculously to the lateral border of the orbitosphenoid, the pterosphenoid [the presence of this bone is not mentioned by NAWAR (1954)] and the parasphenoid. The former two bones are ossifications of the trabecular bars and the taeniae marginales, respectively, whereas the parasphenoid has a dermal origin, covering the ventral face of the fenestra hypophysea (see II.1.1). The muscle has become much thicker, compared to the previous stage. Lateroventrally, the fibres attach to the ossifications of the suspensorium, spread over the dorsal rim the entopterygoid, the metapterygoid, the quadrate and the hyomandibular. Each of these bones bears a dorsal, membranous outgrowth to which the adductor arcus palatini fibres are attached. In the serial sections, a ligament connecting the sesamoid entopterygoid to the autopalatine was found.

Dilatator operculi – The dilatator originates far in front of the other opercular muscles. Anteriorly, the muscle still appears to be continuous with the levator arcus palatini. More caudally, the fibres of the dilatator operculi attach to the medial face of the fascia of the levator arcus palatini. The origin of the muscle is spread over the plate-like part of the lateral ethmoid, the ventral surface of the frontal and the anterior part of the sphenotic. Along its major length, the muscle runs against the medial face of the levator arcus palatini. Caudally, it covers the lateral surface of the hyomandibular, at the level of its articulation with the neurocranium. The fibres insert by means of a firm tendon, lying against the lateral face of the adductor operculi (**Plate II.2-34B-D**). The tendon appears to become confluent with the connective tissue, which attaches the adductor operculi to the opercular bone. This tendon complex inserts on the medial face of a processus dorsalis of the opercular bone, at the level of its articulation with the hyomandibula (**Plate II.2-34E-F**).

Adductor operculi – This muscle lies against the lateral surface of the hyomandibular, as it extends up to the opercular (**Plate II.2-34**). The origin of the anterior fibres is situated in a cavity formed by a lateral ridge on the hyomandibular. Caudally, the fibres originate on the lateral face of the hyomandibular, but also on the

connective tissue lining the dorsolateral wall of the branchial cavity. An insertion on the sphenotic, as suggested by NAWAR (1955a), is not observed. The muscle is covered laterally by the dilatator operculi and its tendon (**Plates II.2-34B-C**). The adductor operculi runs ventrocaudally as it covers the dorsolateral face of the opercular process of the hyomandibula (**Plates II.2-34C-D**). The fibres insert on the dorsal process of the opercular bone, medial to the insertion site of the tendon of the dilatator operculi (**Plate II.2-34E**). This tendon appears to become confluent with the connective tissue, which attaches the adductor operculi to the opercular (**Plate II.2-34E-F**).

Levator operculi – At this stage, the levator originates on the lateral ossification of the otic capsules, *i.e.,* the pterotic. The latter consists of a dermal, canal-bearing dermopterotic bone, and a perichondral autopterotic bone, which have fused (see II.1.1). The medial fibres are attached to the ventral surface of the pterotic bone. The lateral fibres, however, are covered by and originate on the dermal suprapreopercular [dermosphenotic bone in NAWAR (1954; 1955a)]. These fibres run ventrally, up to the medial face of the opercular bone. The lateral fibres insert on the dorsal margin of that bone, whereas the ventral fibres attach to a bony ridge on its medial face.

125.5 MM SL SPECIMEN (**Plates II.2-35, 36, 37**)

Levator arcus palatini – The suspensorial levator still forms a thin muscle sheet, connecting the lateral ethmoid, the infraorbital IV and the frontal to the suspensorium. The insertion on the suspensorium is restricted to the quadrate and the hyomandibular, through a conspicuous aponeurosis (**Plate II.2-35B**). As is the case for the 7.2 mm and 46.8 mm SL specimen, the muscle runs between the A_2A_3' and A_3" parts of the adductor mandibulae complex.

Adductor arcus palatini – The adductor now forms a firm muscular connection between the neurocranial floor and approximately the whole length of the suspensorium (**Plate II.2-36B-C**). The origin of the fibres is comparable to the situation found in the previous ontogenetic stage: anterior fibres originate from the lateral face of the orbitosphenoid and the pterosphenoid, whereas the major part of the fibres start from the lateral margin of the parasphenoid (**Plate II.2-36B-C**). The fibre direction is almost parallel. The fibres attach laterally to the dorsal rim of the suspensorial bones. The insertion is spread over the entopterygoid, metapterygoid, quadrate and hyomandibular bones (**Plate II.2-36**). Each of them dorsally bears a plate-like extension to which the fibres are attached. In between the muscle fibres, some elongated strings of the aponeurotic connective tissue are observed.

Dilatator operculi - The rostral tip of the dilatator operculi lies far in front of the other opercular muscles, as the rostral fibres originate on the lateral ethmoid, close to the eye (**Plate II.2-35C**). The muscle lies as a very narrow bundle between the dorsal part of the levator arcus palatini and the adductor mandibulae A_3" (**Plate II.2-35C**). The origin of the dilatator operculi is spread along the posterior part of the frontal and the anterior part of the sphenotic. From there on, the muscle's direction is ventrocaudally, inserting through a long tendon on the medial face of the opercular dorsal process (but lateral to the processus opercularis of the hyosymplectic). The tendon is V-shaped and lies against the lateral face of the adductor operculi (**Plates II.2-35, 37A**).

Adductor operculi – This muscle still originates on the lateral surface of the hyomandibular, as was observed in the 46.8 mm SL specimen (**Plate II.2-35**). The muscle is rather solid, compared to the dilatator operculi. The adductor muscle is partially covered by the tendon of the dilatator muscle (**Plate II.2-37**). The muscle inserts on the dorsal process of the opercular bone, lateral to the opercular process of the hyomandibular.

Levator operculi – This is the most solid one of the three opercular muscles. It originates from the pterotic and the suprapreopercular, as was observed in the 46.8 mm SL stage (**Plate II.2-35**). The fibres insert on the bony ridge on the medial surface of the opercular bone. This insertion covers the whole length of this ridge (**Plate II.2-37B-C**).

DISCUSSION

As observed in other cranial muscles of *Clarias gariepinus*, an ontogenetic asynchrony is present in the suspensorial and opercular muscles (ADRIAENS & VERRAES, 1996; 1997d) (**Table II.2- 1, p. 137**). The suspensorial muscles develop first; they are already observed in a larva of 4.5 mm SL [4.7 mm larva of SURLEMONT & VANDEWALLE (1991)]. At this stage, no insertion was found.

The insertions of both the levator and the adductor arcus palatini appear at 5.0 mm SL (SURLEMONT *et al.*, 1989). The three opercular muscles are observed for the first time in the 5.0 mm SL larva, but their insertions on the opercular are not yet present. According to SURLEMONT & VANDEWALLE (1991), in this larval stage, the dilatator operculi attaches to the opercular process of the hyosymplectic.

Later during ontogeny, in the 6.2 mm SL larva, the levator operculi has reached the dorsal margin of the rudimentary opercular (SURLEMONT & VANDEWALLE, 1991). The adductor operculi is still attached to (or lies against) the opercular process of the hyosymplectic, whereas the dilatator operculi has extended caudally. The latter muscle lies against the laterodorsal face of the opercular process.

A similar configuration is still observed in the 7.2 mm SL larva, although the dilatator operculi now has a tendinous insertion on the dorsal process of the opercular bone.

In the 46.8 mm SL specimen all the muscles are present with both origin and insertion, and thus, most probably, have become functional. The levator arcus palatini now has a ventral aponeurotic connection with the suspensorium, instead of a musculous one. At this level the suspensorium is completely ossified. The origin of the dilatator operculi has spread, both in an anterior and a posterior direction. The muscle inserts with a long tendon on the dorsal process of the opercular bone. The adductor operculi inserts onto the medial face of the same process, but surprisingly lateral to the processus opercularis of the hyomandibular, instead of medial to it. Such a situation has been described in other teleosts as well, *e.g.,* by OSSE (1969), DALELA *et al*. (1974) and WINTERBOTTOM (1974). The adductor operculi configuration in *C. gariepinus* makes it impossible for the muscle to adduct the gill cover, opposite to what has been stated by NAWAR (1955a). The muscle will, however, abduct the gill cover and consequently function as a protagonist to the dilatator operculi instead of the antagonist. Presumably, the adduction of the opercular bone is taken over by the levator operculi and the opercular part of the hyohyoideus adductor muscle (**Plate II.2-37B**).

The possibility that the adductor operculi of *C. gariepinus* arose as a secondary subdivision of the dilatator operculi can be overruled, based on neurological evidence. Being derived from the hyoid arch muscle plate, the adductor operculi is innervated by the ramus hyomandibularis of the facial nerve (VII), whereas the dilatator operculi is derived from the mandibular arch muscle plate, and thus is innervated by the ramus mandibularis of the trigeminal nerve (V) (DATTA MUNSHI & SINGH, 1967; WINTERBOTTOM, 1974; JARVIK, 1980). As a result, a

difference in innervation of these muscle plates can be used to determine homologies. In *C. gariepinus* the adductor operculi is innervated by a branch of the truncus hyomandibularis, just before the latter enters the foramen in the hyomandibula (**Plates II.2-34, III.1-10**). This branch most probably corresponds to the ramus opercularis profundus of the facial nerve (SAXENA, 1969; FREIHOFER, 1978). As a result, it can be argued that in *C. gariepinus* the adductor operculi has undergone a shift in insertion site, and thus a shift in function.

The asynchrony of the development of muscles is reflected in different respiration mechanisms during ontogeny (**Table II.2- 2**). Aquatic respiration results from the generation of a water flow through the orobranchial and opercular cavities, generally by enlarging and subsequently reducing the volume of these cavities. At the 4.0 and 4.5 mm SL larval stages, no respiration apparatus is present yet. At this stage respiration probably still occurs through diffusion. Some water replacement in the orobranchial cavity may occur through the undulating movements of the body, resulting in the left and right swinging of the mouth, but more important, the forward swimming with an opened mouth.

Table II.2- 2: Presence of the mechanisms needed for aquatic respiration during ontogeny in *Clarias gariepinus* (- = not possible yet, + = possible) [(1) SURLEMONT *et al.* (1989); (2) SURLEMONT & VANDEWALLE (1991)]

Action	*4.0 mm SL (2)*	*4.5 mm SL (2)*	*5.0 mm SL (1)*	*6.2 mm SL (2)*	*7.2 mm*	*46.8 mm*	*125.5 mm*
abduction of the suspensorium	-	-	+	+	+	+	+
adduction of the suspensorium	-	-	+	+	+	+	+
elevation of the opercular	-	-	-	+	+	+	+
adduction of the opercular	-	-	-	+	+	+	+
dilatation of the opercular	-	-	-	-	+	+	+
elevation of the neurocranium	-	-	+	+	+	+	+
retraction of the pectoral girdle	-	-	+	+	+	+	+
protraction of the pectoral girdle	-	-	-	+	+	+	+
depression of the hyoid bars	-	-	-	+	+	+	+
elevation of the hyoid bars	-	-	+	+	+	+	+
depression of the lower jaw	-	-	+	+	+	+	+
elevation of the lower jaw	-	-	+	+	+	+	+
expansion of the branchiostegal membrane	-	-	-	-	+	+	+
constriction of the branchiostegal membrane	-	-	-	-	+	+	+

Later, in the 5.0 mm SL larva, the lowering of the mandible and the displacements (both ab- and adduction) of the side walls of the orobranchial cavity structurally have become possible. At this stage, the uptake of water may occur through: (1) the depression of the lower jaw, (2) the elevation of the neurocranium and (3) the abduction of the suspensoria (**Table II.2- 2**). For the transport of this water through the gills, only the pressure pump system is functional (as the opercular muscles do not yet insert on the opercular bone): (1) elevation of the lower jaw through the contraction of the adductor mandibulae complex, (2) elevation of the hyoid bars through the combined contraction of the adductor mandibulae complex and the protractor hyoidei and (3) adduction of the suspensoria, both through the activity of the adductor arcus palatini and the intermandibularis (**Table II.2- 2**). The only possible depression of the hyoid bars at this level is the passive recovery after elevation, due to the elastic properties of the cartilaginous interhyal, which is continuous with both hyoid bar and suspensorium.

In the 6.2 mm SL larva, uptake of water is assisted through the depression of the hyoid bars, as the sternohyoideus is present with both its insertions (SURLEMONT & VANDEWALLE, 1991). Thus, at this stage, almost the

complete apparatus for the uptake of water into the orobranchial cavity is present and can be functional, as it is in the adult situation. The adduction of the opercular bone is structurally possible as well, through the contraction of the levator operculi. This adduction plays an important role in closing the opercular cavity with the opercular bone and the opercular skin fold, during the uptake of water.

The suction pump apparatus is observed to be functional (based on anatomical evidence) in the 46.8 mm SL specimen: (1) abduction of the opercular may occur through the contraction of the dilatator operculi and the adductor operculi and (2) the expansion of the branchiostegal membrane may occur through the contraction of the hyohyoideus abductor (ADRIAENS & VERRAES, 1997d) (see II.2.3). Return to the original position may be brought about by the hyohyoidei adductores, thereby assisting the levator operculi in the closing of the opercular cavity during water uptake.

The combined activity of the different muscles during orobranchial expansions, has been demonstrated extensively through electromyographic studies (OSSE, 1969; ELSHOUD, 1978; LAUDER & LIEM, 1980; LAUDER, 1981; LIEM, 1984). In *Channa* (LIEM, 1984), in *Perca fluviatilis* (OSSE, 1969) and in *Gasterosteus aculeatus* (ELSHOUD, 1978), a basic muscular sequence can be observed during aquatic respiration: (1) the expansion phase of the orobranchial cavity is marked by a rather synchronous contraction of the levator arcus palatini, the hyohyoideus inferior, the dilatator operculi and the levator operculi. These contractions result in the abduction of the suspensorium, the expansion of the branchiostegal membrane, the abduction of the gill cover and the depression of the lower jaw, respectively. (2) The compression phase is performed through an approximately synchronous contraction of the adductor mandibulae complex, the protractor hyoidei, the hyohyoidei adductores and eventually the adductor arcus palatini and the adductor operculi. As a result, the mouth is closed, the hyoid bars are elevated, the branchiostegal membrane is constricted, the suspensoria are adducted and at last, the opercular bone closes the opercular slit. From this point on, the cycle can start over again. Surprisingly, the activity of the sternohyoideus and the epaxials, for the retraction of the hyoid bars and the elevation of the neurocranium respectively, does not seem to be confined to separate, substantial phases of contraction during normal respiration. This is, however, the case during the air ventilation in *Channa* (LIEM, 1984) and during feeding in several teleosts (OSSE, 1969; LAUDER, 1981; GALIS *et al.*, 1994).

One of the mechanisms responsible for the expansion of the orobranchial cavity is the abduction of the suspensoria. Consequently, the suspensorium is attached to the neurocranium in such a manner that in general a lateral swinging is allowed. Several constructions and articulations of the suspensorium have evolved, improving this kind of orobranchial expansions. In palaeoniscoid fishes the palatoquadrate is still separated completely from the hyosymplectic, which is attached to the neurocranium through a set of three articulations (GARDINER, 1973). The obliquely directed hyosymplectic, however, restricted the lateral displacement of the suspensorium (SCHAEFFER & ROSEN, 1961). During subholostean and holostean evolution, the hyomandibula has become more closely associated with the palate, whereas in Holostei, the suspensorium becomes reinforced even more (metapterygoid becomes firmly attached to the hyomandibular). Articulation between the suspensorium and the neurocranium still occurs through an articulatory ridge.

In Actinistia and most Actinopterygii the hyomandibula bears a double-headed articulatory facet with the neurocranium (GARDINER, 1973) [*e.g., Elops saurus* (Elopidae); *Anguilla rostrata* (Anguillidae); *Denticeps clupeoides* (Denticipitidae); *Orestias ispi* (Cyprinodontidae) (ARRATIA & SCHULTZE, 1991); *Blennius pholis* (Blenniidae) (VANDEWALLE *et al.*, 1982); *Pomatoschistus* (Gobiidae) (MESTERMANN & ZANDER, 1984); *Serranus* (Serranidae) (BENMOUNA *et al.*, 1984); Scaridae (BELLWOOD & CHOAT, 1990); Cichlidae (BAREL *et al.*, 1976b)].

However, in most Ostariophysi, and thus in *Clarias gariepinus* as well, the hyomandibula hinges with the neurocranium through an articulatory ridge (FINK & FINK, 1981; ARRATIA, 1992). But as in catfish the pterygoquadrate has lost its connection with the palate, the only articulation between the suspensorium and the neurocranium occurs through this ridge (ALEXANDER, 1965). An articulatory ridge, instead of a pair of condyles, could enable the suspensorium to be shifted forth and backwards. In *C. gariepinus*, however, the hyomandibula bears a dorsal process interlocking with a ventral, sphenotic process, which prevents this kind of shifts (**Plate II.1-34E**). An articulatory ridge can assist in withstanding torque forces during suspensorial ab- and adduction, as the rostral articulation between suspensorium and neurocranium is lost in catfishes. In *C. gariepinus,* the lateral displacement of the suspensorium has a limited impact during aquatic respiration. Due to the dorsoventrally flattened head, the height of the suspensoria, and thus the volume increase through its abduction, is rather restricted (ADRIAENS & VERRAES, 1994) (see IV.2). HUGHES (1970) argued that in bottom-living fishes, the suction pump mechanism is far more important in aquatic respiration than the pressure pump system. For this suction pump mechanism, no extensive abduction of the suspensorium, no elevation of the neurocranium and no depression of the hyoid bars are needed. In this case, a negative pressure is generated in the opercular cavity mainly through the dilatation of the gill cover (abduction of suspensoria will probably assist here). Consequently, water is sucked in from the orobranchial cavity, through the gills, into the opercular cavity.

As a result, the activity of the dilatator operculi and the hyohyoideus abductor would be of greater importance for aquatic respiration in *C. gariepinus*. Both muscles are observed to be well developed in this species. The functional shift of the adductor operculi to an opercular dilating muscle may contribute to an optimisation of a strong suction pump system, and thus supports the hypotheses that a suction pump system is more important in *C. gariepinus* than the pressure pump system is.

During teleostean evolution, some adaptations occurred to enable a higher mobility of the opercular bone as well. The dorsad shift of the articulatory facet of the opercular bone with the hyomandibula, increased the activity possibilities of the dilatator operculi, as has been stated by SCHAEFFER & ROSEN (1961). This shift resulted in a more horizontally directed dilatator operculi, which reduces the force component in a vertical plane during contraction. This component results in an elevation (or depression) of the gill cover. The force component in a horizontal plane, resulting in the dilatation of the gill cover, will consequently increase. The loss of this elevation component of the dilatator operculi is then compensated by the differentiation of the adductor operculi into a separate levator operculi, as has been observed from the holostean level on.

In general, the adductor arcus palatini, has undergone some secondary modifications in catfish. In non-siluriform otophysan fishes, TAKAHASI (1925) distinguished three parts in the muscle, still continuous with each other: (1) the palatine part anteriorly, (2) the pterygoid part in the middle and (3) the hyomandibular part posteriorly. As can be deduced from the nomenclature, each muscle part inserts on the corresponding bone (or bones) of the suspensorium. As already mentioned, the catfish palatine has become isolated from the rest of the suspensorium as an adaptation to the development of a palatine-maxillary mechanism (EATON, 1948; ALEXANDER, 1965; SINGH, 1967; GOSLINE, 1975a; GHIOT, 1978; GHIOT *et al.*, 1984; ARRATIA, 1992; ADRIAENS & VERRAES, 1997a). This skeletal isolation gave rise to a muscular isolation as well, as the palatine part of the adductor arcus palatini muscle became modified into a separate muscle, referred to as the extensor tentaculi (see II.2.2). Apparently, this subdivision occurs in catfishes even before the muscles are formed. In *Clarias gariepinus*, no continuous adductor arcus palatini (including the palatine part) is observed, as both muscles are separated from each other from the moment they arise (6.2 mm SL larva) (see II.2.2).

A coupling of the suspensorial movements to the palatine-maxillary mechanism seems to be present in some catfishes. In *C. gariepinus*, a ligamentous connection is observed between the rostral tip of the sesamoid entopterygoid and the autopalatine. Due to this connection, lateral displacements of the suspensorium may affect the rotation or sliding of the autopalatine bone, and hence manipulating the ab- and adduction of the maxillary barbel. It can be expected that an abduction of the suspensorium will abduct the posterior tip of the palatine as well. This will make the maxillary, and thus the maxillary barbel, to be retracted. Consequently, the adduction of the suspensorium might facilitate the extension of the barbel. A similar connection between the metapterygoid bone and the palatine was observed in *Bagrus bayad* (Siluriformes, Bagridae) (GHIOT *et al.*, 1984), although it is suggested here that the ligament only functions as a restriction against an extensive protraction of the palatine. The fact, however, that in *C. gariepinus* the entopterygoid is connected to the prevomeral bone, anteriorly, may suggest that this ligament also prevents a dislocation of the suspensorium in a posterior direction.

Clariidae are known as well for the presence of an accessory breathing organ. In this family, parts of the branchial basket have been modified into a suprabranchial organ, enabling the uptake of atmospheric oxygen into the circulatory system. The suprabranchial organ consists of a set of fans, which are modified hemibranchia of all four epibranchials. These enclose two arborescent organs, derived from the second and the fourth epibranchials (DATTA MUNSHI, 1961; HELLIN & CHARDON, 1981). During aerial respiration, the gill cover plays an important role in *C. gariepinus*, as it assists in the transport of the inhaled air bubble into the suprabranchial cavity, but as well in the release of the exhaled air bubble (HELLIN & CHARDON, 1981; VANDEWALLE & CHARDON, 1991).

CONCLUSIONS

In *Clarias gariepinus* two suspensorial (adductor and levator arcus palatini) and three opercular (dilatator, adductor and levator operculi) muscles are developed. The asynchrony in the ontogeny of these muscles in *C. gariepinus* is reflected in the functionality of those apparatuses, responsible for respiration. From the 5.0 mm SL larva on, the apparatus related to the pressure pump system can generate a water current through the gills, whereas the suction pump apparatus may become functional at about 7.2 mm SL. This shift in respiratory systems is due to the fact that the opercular bones, together with its insertions of the dilatator and adductor operculi, only develop later. It has been suggested that in bottom-living species, this suction pump system is more important for respiration than the pressure pump system. Some morphological evidence is observed in *C. gariepinus*, which supports the hypotheses of the presence of an improved opercular dilatation during aquatic respiration. The adductor operculi, which has undergone a shift in insertion position, has become a protagonist to the dilatator operculi, instead of counteracting to it.

In catfish, the development of the palatine-maxillary mechanism has involved a secondary subdivision of the adductor arcus palatini. Hence, a distinct extensor tentaculi is formed, which is already separated from the adductor arcus palatini from the moment it arises.

Apart from their role during aquatic respiration, the suspensorial and opercular muscles take part in the process of atmospheric respiration in *C. gariepinus*.

Chapter III.1 - Induced dwarfism: an indication of epigenetic control of skull ontogeny

In order to investigate the question whether the ontogeny of teleosts should be related to body size or to age, as well as the question concerning the epigenetic impact of reduced growth onto the developing skull, an experiment is done involving artificially induced dwarfism in *Clarias gariepinus*. Growing of larvae in a spatially constrained environment induced specimens which are of substantially smaller body size than those reared in normal conditions. A morphological study also reveals that the development is retarded, and can even be compared to the situation of an equally sized, but younger specimen. Possible mechanisms, controlling the reduction in both size and developmental status could not be revealed, however, a suggestion is made concerning hormonal regulation due to a stress situation. Additionally, changes in functional demands may partially explain for the reduced ossification. Endocrine control could explain both for the reduction in size, as well as the retardation in ontogeny. Miniaturisation is a process of great importance in evolution, as well as it gives an indication of 'functional adaptations' in organisms to changing environments. Methodologically, the size-dependency of development, and thus not age-dependency, implies that special care has to be taken when interpreting data from both age determination, as well as data dealing with heterochronies.

INTRODUCTION

Fertilised eggs are confronted with a process of increase, which can be subdivided in: (1) a quantitative increase, known as growth, and (2) a qualitative increase, known as development (encompassing differentiation and morphogenesis) (BATT, 1980; GILBERT, 1988). Although not apparent, both these factors are not always coupled to each other. As teleostean eggs are protected by an egg shell, which after hardening can resist high loading (up to 3.5 kg in weight in trout) (RIEHL, 1996), the growth of the egg is restricted to the space within this shell (IWAMATSU, 1994). Consequently, egg development, by blastular cleavages, is confined within this shell, which constrains the volume increase of the developing egg. This problem is solved as "in most species, there is no net increase in embryonic volume during cleavage"[1] (GILBERT, 1988). On the other hand, isometrically growing organisms may lack any increase in developmental complexity. When studying development of organisms, it is therefore crucial to distinguish between growth and development.

Studying growth of organisms is one aspect of studying evolutionary diversity. The basis for growth (and growth rate) is determined genetically in each species [so-called within-species genetic differences according

[1] In the case of telolecithal eggs, this statement is correct if the egg is considered as the combination of the zygote and the yolk sac. It is of course a fact that the relative volume of the embryo and the yolk sac change during early embryogenesis: yolk material becomes converted into embryonic material.

to McDowall (1994)]. In that case, growth genes involve DNA that encodes for factors initiating and regulating growth. Many growth-regulating molecules have been discovered, of which most involve endocrine products (Jenkin, 1970; Batt, 1980; Babiker, 1984; Marks & Popoff, 1988; Simone, 1990; Gannam & Lovell, 1991; Lin, 1996, Takagi & Björnsson, 1996). The chromosome configuration is another genetic factor which is found to have an impact on growth. Influences of both number (to a lesser degree) (Henken *et al.*, 1987a) and type (sex chromosomes) of chromosomes (Eaton & Farley, 1974; Batt, 1980; Ochi, 1986; Henken *et al.*, 1987b; Schultz, 1993; Van Weerd, 1995) on growth have been observed in teleosts. Also, it is known that hybridisation between closely-related species affects growth (Rhoads, 1949; Legendre *et al.*, 1992; Van Weerd, 1995). Racial differences, as a result of genetic selection (artificial or not), may lead to differences in growth (Van Weerd, 1995; Fiorello & German, 1997). Differences in growth rate can be related to differences in ontogenetic stage (Hecht & Appelbaum, 1987), as well as adult body size itself (larger fish may have a different growth pattern than small fish) (Hogendoorn, 1983; Hogendoorn *et al.*, 1983; Buckel *et al.*, 1995), which in turn is (partially) the result of the within-species genetic differences.

However, the genetic basis of growth and development is constantly being fine-tuned because of environmental factors. These factors constitute an epigenetic influence [direct phenotypic effects according to McDowall (1994)] on development, which enable developing organisms to become adapted to the changing environment. The degree to which such influences induce variation in the 'Bauplan' morphology, reflects the degree of phenotypic plasticity. In general epigenetic control consists of factors acting from the external environment on an organism, as well as factors arising within an organism. External factors influencing growth are for example temperature (Thompson, 1952; von Bertalanffy, 1960; Batt, 1980; Hogendoorn *et al.*, 1983; Britz & Hecht, 1987; McDowall, 1994; Buckel *et al.*, 1995; Osse & van den Boogaart, 1995; Olson, 1996); oxygen levels (Avnimelech & Zohar, 1986), food level, as well as food quality (Eaton & Farley, 1974; Batt, 1980; Hogendoorn, 1983; Hogendoorn *et al.*, 1983; Hecht & Appelbaum, 1987; McDowall, 1994; Olson, 1996; Nguenga *et al.*, 1997), salinity (Thompson, 1952; Buckel *et al.*, 1995), population densities (Bok & Jongbloed, 1984; Harris, 1987), social structure within populations, frequently coupled to aggression (*e.g.,* Pomacentridae) (Fricke & Fricke, 1977; Ochi, 1986), niche occupation, resulting in so-called "growth morphs" or "ecotypes" (Bodaly *et al.*, 1991; Vuorinen *et al.*, 1993; McDowall, 1994; Nagelkerke *et al.*, 1995; Bernatchez *et al.*, 1996), seasonal fluctuations (Thompson, 1952; Jenkin, 1970; Batt, 1980; Ochi, 1986; Van Neer, 1993; Van Neer *et al.*, 1993; McDowall, 1994), required stimuli (*e.g.,* light) (Browman, 1989; Pankhurst, 1992) and toxicants (Przybylski, 1996; Nguyen, 1997). Endogenous epigenetic factors involve many kinds of interactions between the different elements constituting the *'Bauplan'*. Overall growth is known to decrease when maturation occurs (Schultz, 1993). Of more importance for this study are the changes in spatial interactions between the developing organs, which mutually affect their growth (see Discussion).

Many of these external and internal epigenetic influences come down to one basic factor: metabolism. Growth can directly be related to metabolic processes, which are influenced by temperature, oxygen level, salinity affecting osmoregulation, etc.. Seasonal fluctuations in growth rate are known to be a combination of temperature and feeding level, which can lead to differences in hormonal productions (Jenkin, 1970). Hormones in turn are known to affect the function of kidneys (which may affect nitrogen levels, important in protein synthesis) (Simone, 1990), as well as food conversion rates (Takagi *et al.*, 1992). The allocation principle[2] explains the growth changes, which are hormonally induced during metamorphosis and maturation (Simone, 1990; Bodaly *et al.*, 1991; Schultz, 1993; Vuorinen *et al.*, 1993).

[2] The allocation principle encompasses the physiological mechanism for the metabolic trade-off between energy invested in gonad growth and differentiation, and that for somatic growth and differentiation.

"Almost all anatomical, physiological, and behavioural attributes are size-related in some way" (STRAUSS, 1984), however, this size-dependency can be categorised on different levels: (1) different parts of the same structure can interact spatially with each other (BAREL, 1984; 1985), (2) different structures of an organism are in constant spatial competition with each other (WEISS & AMPRINO, 1940; CORSIN, 1961; VERRAES, 1974a, b; HANKEN, 1983b; BAREL, 1984; 1985); (3) different individuals forming a population are constantly confronted with each other, resulting in many forms of competition affecting growth (FRICKE & FRICKE, 1977; OCHI, 1986; OLSON, 1996) and (4) interspecific size-dependency can be observed in predator-prey relationships or parasite-host relationship (STRAUSS, 1984; 1990a; OLSON, 1996).

In this study, the relation between growth and development is studied in the fast growing catfish, *Clarias gariepinus* BURCHELL (1822) (HOGENDOORN *et al.*, 1983; BOK & JONGBLOED, 1984; VAN WEERD, 1995). In this species, the growth rates change substantially during ontogeny, with food conversion being very effective (HOGENDOORN *et al.*, 1983; KAMLER *et al.*, 1994; VAN WEERD, 1995). This feature, as well as the fact that maximum body length is large, make *C. gariepinus* of great importance for African aquaculture. In the context of its aquacultural importance, several studies have been done to improve growth in cultivation (BOK & JONGBLOED, 1984; AVNIMELECH & ZOHAR, 1986; REIS *et al.*, 1989; ROBINSON & BRENT, 1989; ROBINSON *et al.*, 1995). The purpose of this study, however, is focussed on the development, and its relation with growth and age. Frequently, ontogenetic stages are expressed in terms of age (VERRAES, 1974b; BOLKER & THOMSON, 1992; VANDEWALLE *et al.*, 1992; TILNEY & HECHT, 1993; HOLDEN & BRUTON, 1994; KOHNO *et al.*, 1996a, b), whereas others use size (KINDRED, 1919; BAMFORD, 1948; SRINIVASACHAR, 1958b; HOEDEMAN, 1960a; WEISEL, 1967; VERRAES & ISMAIL, 1980; SURLEMONT *et al.*, 1989; SURLEMONT & VANDEWALLE, 1991; PODOSKINA, 1993; BARTSCH, 1994; CUBBAGE & MABEE, 1996; HUNT VON HERBING *et al.*, 1996a; MABEE & TRENDLER, 1996). The question thus rises: is developmental rate more closely related to age, or more to size? Suggestions have already been made that size would be the determinant factor (OSSE & VAN DEN BOOGAART, 1995), however, no detailed ontogenetic comparison has been done yet. The effect of size or age on development can be studied by comparing the ontogenetic differentiation of specimens of similar age but different size, and of specimens of different size but similar age. This was done by raising some larval *C. gariepinus* in a spatially restricted environment, whereas other larvae, of identical age, were raised in a spatially normal environment (see I.2.3.b). This induced dwarfism (**Table I.2-11**) (**Plate III.1-1**) could be compared with observations from previous chapters (II.1.2). The main questions of this research are: (1) how does a spatially constricted environment, as an epigenetic factor, affect the overall body size of larval *C. gariepinus*, and (2) how does it affect the ontogenetic differentiation, especially the rate of ossification. Consequently, answers to these questions can provide some answers to a third question: (3) is ontogeny related to age or to size?

RESULTS

In this chapter, the results are given concerning the possible effects of size and age on the cranial ontogeny in *Clarias gariepinus*. In order to distinguish between effects related to size or those related to age, at least two references are needed. Consequently, three groups of specimens of *C. gariepinus* are described and discussed in this chapter: (1) specimens raised in a spatially constrained environment, (2) specimens raised in a spatially non-constrained environment, which are of the same age as in (1), and (3) specimens raised in a spatially non-constrained environment, which are of the same size as in (1). These groups are referred to as (1) dwarfs, (2) similar-aged specimens and (3) similar-sized specimens, respectively. Data on body lengths of the

dwarf and similar-aged specimens are given in **Table I.2-11** (see I.2). The specimen of 21.5 mm SL, discussed in Chapter II.1.2, is used for the similar-sized reference, because the body length of the dwarfs approximated its size (**Table I.2-5, p. 6**).

This chapter emphasises the comparison between the cranial osteology of these three groups, as well as between their suprabranchial organs. Additionally, to give some idea on the general cranial morphology of the dwarfs, the myology, major cranial nerves, the lateral-line system, as well as the inner ear are briefly mentioned. Such data on the normal specimens can partially be found in the previous chapters.

SIMILAR-SIZED SPECIMEN

This specimen has extensively been described in Chapter II.1.2. However, some additional features need to be discussed in the context of this chapter.

Chondrocranium - The cartilaginous skull is almost completely covered with bones (**Plates III.1-2A, 3A, 4A, 5A**). Unossified regions in the neurocranium can be observed at the posterior margin of the precerebral lamina, at the dorsal face of the otic capsules and in between all bones at the ventral side of the neurocranium (**Plates III.1-2A, 4A**). Articulation surfaces remain, evidently, cartilaginous as well (for the palatine and hyomandibular articulations). Although in earlier stages the taenia marginalis anterior was reduced, a small bar is present now between the base of the epiphysial bridge and the orbito-nasal lamina (which corresponds to the anterior taenia marginalis) (**Plate III.1-7A**). Apparently, in *Clarias gariepinus*, this structure only develops late in ontogeny.

Most rod-like parts of the splanchnocranium are cartilaginous at their ends (palatine, hyoid bars, copulae, ceratobranchials, epibranchials and infrapharyngobranchial III) (**Plates III.1-4A, 5A**). Other unossified regions involve synchondrosis (hyomandibula-quadrate, quadrate-metapterygoid, anterior ceratohyal-posterior ceratohyal, ventral hypohyal-anterior ceratohyal), articulatory facets (processus opercularis, hyomandibular articulation), as well as parts of some structures which remain largely unossified in later stages (central part of Meckel's cartilage, hypobranchials, posterior copula) (**Plates III.1-3A, 5A**). The fourth infrapharyngobranchial, however, appeared to lack any ossification in this (cleared and stained) specimen. As was mentioned in Chapter II.1, and can be derived from the dwarfs and similar-aged stages, this bone must form shortly after. The basibranchials (I, II and III) still appear to be continuous, thus forming one anterior copula (**Plate III.1-5A**). Additionally, the hypohyal cartilage appears to be connected to this copula by cartilage as well.

Some regions in the chondrocranium have become reduced (**Plate III.1-7A**), compared to the fully formed chondrocranium in the 10.0 mm SL specimen (**Plate II.1-7**). The epiphysial bridge, still connecting the left and right taenia marginalis in the 19.0 mm SL specimen (**Plate II.1-10A**), is now interrupted. The epiphysial bridge is reduced, thereby forming an isolated cartilage, encapsulated by the medial extensions of the frontal bones. The trabecular bars have become interrupted as well, as could already be observed in the 19.0 mm SL specimen (**Plate II.1-10B**).

Osteocranium - The endocranial bones are all present at this stage, with exception of the epiotic (**Plate II.1-38**). They enclose the pre- and postpineal foramen, which are separated by the ossified, medial process of the frontal bones (**Plate III.1-2A**). Interdigitations between most bones of the skull roof have formed, except for the major part of the connection between the parieto-supraoccipital and pterotic bones, and between the mesethmoid and the lateral ethmoid bones. At the ventrolateral side of the skull, the pterosphenoids have

already contacted the lateral processes of the parasphenoid bone (**Plate III.1-4A**), thereby subdividing the sphenoid fenestra in an foramen for the fasciculus opticus (optic nerve) and a foramen for the trigemino-facial complex. The prevomeral tooth plates can still clearly be distinguished from the prevomeral bone itself. The sphenotic bone has already reached the border of the trigemino-facial nerve foramen, as well as it has already surrounded the hyomandibular articulatory facet completely.

Not all canal bones already have the plate-like part, as can be observed in the adult situation. Some are still tubular (nasal, antorbital, infraorbitals I-III), whereas others are already demarcated by a small plate-like extension (infraorbital IV, preopercular and suprapreopercular) (**Plates III.1-2A, 3A**). Canal bones, covering the endocranial part of the skull, can still be distinguished from the endocranium.

The splanchnocranium is already well ossified. The small coronomeckelian bone is still separated from other mandibular bones (**Plate III.1-5A**). The dental and angular bone complexes are strongly interdigitated at the lateral face of the Meckel's cartilage. The quadrate, the hyomandibular, and the metapterygoid bones bear their dorsal, membranous outgrowths, which are still separated from each other. A total of ten branchiostegal rays is counted (**Table III.1- 1**, **Plate III.1-3A**).

Suprabranchial organ - Posterior at the articulation between the fourth cerato- and epibranchials, a small cartilaginous mass could be observed (**Plate III.1-5**). This primordium of the supportive cartilage for the arborescent trees, already bears two, small, caudally directed processes, indicating the initial, dichotomous branching of the trees.

DWARF SPECIMENS

Chondrocranium - The ethmoid region is still heavily chondrified, bearing a rather narrow ethmoid plate which is encapsulated into the mesethmoid bone complex (**Plate III.1-8A**). Laterally, the orbito-nasal lamina forms an oblique, transverse rim, lateral to the olfactory foramen, and penetrated by two foramina at its lateral margin. Ventrally, the lamina forms the articulation with the palatine. The anterior taenia marginalis connects the lamina with the disconnected epiphysial bridge. Comparable to the similar-sized specimen, the precerebral lamina and the otic capsules bear some unossified regions (**Plate III.1-2B**). The skull floor is also comparable to the similar-sized specimen (**Plate III.1-4B**).

The graphical reconstructions of the serially sectioned dwarf specimen provide a much more detailed view of cartilage resorptions. In contrast to what was observed in previous stages, the preorbital base is now penetrated by a foramen. The trabecular bars are well separated at the level of the sphenoid fenestra. The skull floor at the otic region has become highly fenestrated, compared to the 10.0 mm SL stage (**Plates II.1-7E, III.1-8B**). Lateral to the notochord and close to the pila occipitalis, a large foramen has formed. The pilae occipitales have lost contact with the otic capsules. Additional foramina have formed in the otic capsules, as well as most previously formed foramina have become enlarged substantially (*e.g.,* the foramen magnum). Surprisingly, a large foramen has formed where an anterior basicapsular fenestra is to be expected. Earlier during ontogeny, this fenestra disappears, however (**Plate II.1-9**). Whether or not these two apertures are homologous remains uncertain. For convenience, this aperture is here referred to as the fenestra basicapsularis anterior.

The splanchnocranium has undergone several resorptions as well, although all elements can still be distinguished. The palatine bears a distinct dorsal process at its rostral tip, for the connection with the antorbital bone (**Plates III.1-8A, 11A**). Meckel's cartilage is largely reduced. The rostral tips of left and right cartilages are well

separated. A small symphysial cartilage can be found in between them (**Plate III.1-8A**). Meckel's cartilage bears a distinct coronoid process, but is largely reduced at its articulatory facet. The "hyosymplectic-pterygoquadrate plate" (ARRATIA, 1992) has become reduced as well. The pterygoid process is large, but very narrow at its base. The anterior part of the hyosymplectic has become resorbed completely, as the foramen for the hyomandibular truncus can no longer be observed. Instead, the hyosymplectic bears an excavation at its anterior margin, along which the truncus passes (**Plates III.1-8A, 10A**). The opercular process has a similar morphology as the pterygoid process: a broad distal end with narrow base. Despite all these reductions, the interhyal is still continuous with both the suspensorium and the hyoid bar. These bars have become very narrow centrally (at the level of the ossification centre of the anterior ceratohyal bone). The hypohyals bear a rostral groove, through which passes the hyoid artery.

The general trend in the rod-like, cartilaginous elements of the branchial basket involves a narrowing of the central part, at the level of their ossification centres (**Plate III.1-8B**). The proximal and distal part of the cartilaginous fourth ceratobranchial even have become separated from each other. The basibranchials, previously constituting the anterior copula, have now become separated from each other, whereas the basibranchial II is still connected with the parurohyal bone (**Plate III.1-5B**). The posterior copula bears a distinct ventral and longitudinally directed rim, over which runs the transversus ventralis IV muscle[3] (**Fig. III.1-1**).

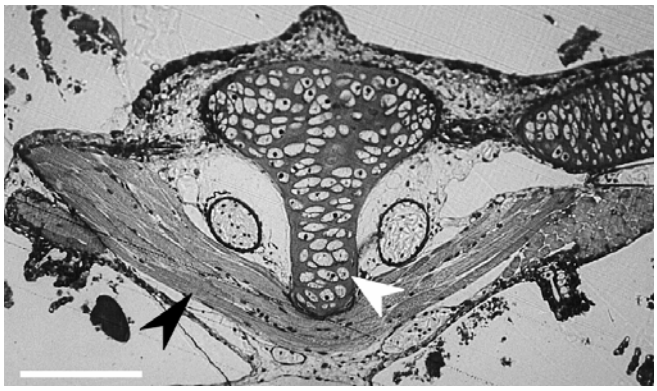

Fig. III.1- 1: Serial section at the level of the posterior copula, showing the longitudinal rim (white arrowhead), along which passes the m. transversus ventralis IV (black arrowhead) (bar = 100 µm)

Osteocranium - The overall ossification of the skull is highly comparable to the situation in the similar-sized specimen (**Plates III.1-2B, 3B, 4B, 5B, 7B**). However, some details reveal that ossification is more pronounced in the 23.3 mm SL dwarf specimen. The unossified region of the precerebral lamina and the otic capsules are smaller in the dwarf. A true interdigitation between the mesethmoid and the frontal bones has formed now. The parieto-supraoccipital bone has started to grow medially at its anteromedial border. This indicates the initiation of the subdividing of the postpineal foramen into the anterior fontanel (partly) and the posterior fontanel (**Plate III.1-2B**). In one dwarf specimen (23.3 mm SL), the pterosphenoid bone does not yet reach the parasphenoid, whereas the sphenotic does not yet enclose the hyomandibular articulation, nor does it reach the border of the trigemino-facial foramen (**Plate III.1-4B**). In the other specimens, the situation is highly comparable to the similar-sized specimen. The relation between the osteocranium and the chondrocranium is also highly comparable with the similar-sized specimen (**Plate III.1-7B**).

The membranodermal component of some canal bones has formed (infraorbital III) or has expanded. The canal bones of the endocranial part of the skull have become more incorporated in the underlying bones, as only the openings and the position of the superficial tubes can be seen externally. Whether these small differences are the result of the little difference in size (1.8 mm) or because of the dwarfism, or even a combination of both cannot be ascertained from these specimens. More specimens of the same SL would be required.

[3] This rim increases the ventrally directed force component of the transversus ventralis muscle, consequently improving the depression of the branchial arches.

No substantial differences can be noted in the splanchnocranial ossification, compared to the similar-sized specimen, except for the infrapharyngobranchials. In the small dwarf specimen (23.3 mm SL), the fourth infrapharyngobranchial bone can be distinguished now (**Plate III.1-5B**). In contrary to the similar-sized stage, the number of branchiostegal rays varies between eight and nine, the majority having nine. In two specimens, even a left-right asymmetry is present (**Table III.1- 1**, **Plate III.1-6A**). Surprisingly, those specimens having only eight rays (at both or one side) are the larger specimens. The mandibular bones have now enclosed the coronoid process partially, which is not yet the case in the similar-sized specimen (**Plate III.1-5B**). The upper pharyngeal jaw apparatus is well developed. The pharyngeal tooth plate has even formed a ridge, with which the ossified infrapharyngobranchial IV articulates (**Fig. III.1- 2**).

Table III.1- 1: Variation of the number of branchiostegal rays in normal and dwarf *Clarias gariepinus*

NORMAL	left	right	DWARF	left	right
21.0 mm SL	10	10	20.8 mm SL	8	8
			23.3 mm SL	8	8
36.2 mm SL	9	9	23.9 mm SL	8	8
37.3 mm SL	9	9	24.9 mm SL	9	9
40.8 mm SL	9	9	25.1 mm SL	9	9
41.9 mm SL	9	8	25.8 mm SL	9	9
44.1 mm SL	8	8			
51.0 mm SL	8	9			

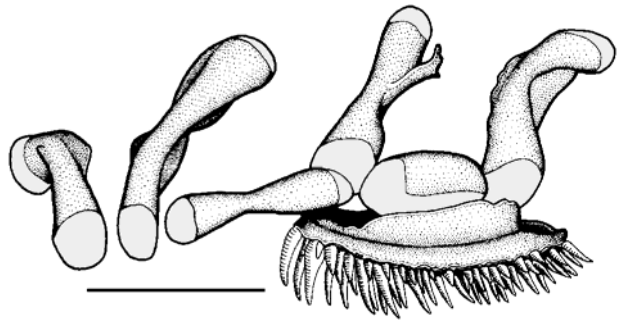

Fig. III.1- 2: Upper pharyngeal jaw apparatus in dwarf *Clarias gariepinus* (medial view) (Bar = 0.5 mm) (grey indicates cartilage, hatched areas indicate bone)

Suprabranchial organ - The arborescent trees are more primordial than in the similar-sized specimens. At the posterior margin of the fourth ceratobranchial-epibranchial articulation, two small cartilaginous elements are present. Apparently, the initial formation of the trees involves no dichotomous branching, but the separate formation of two elements.

Myology - All cranial muscles can clearly be distinguished. The adductor mandibulae complex inserts through a complex of tendons onto the lower jaw, at the level of the coronoid process (**Plate III.1-9B**). The superficial A_2A_3' is separated from the deeper A_3" by the levator arcus palatini, especially by its large tendon. The latter muscle curves dorsal to the eyeball, as is the case in the normal juvenile situation (**Plates II.2-5B, III.1-9C**). The proportion of the muscular part and the tendinous part is also comparable. Bordered dorsally by this levator muscle, lie the ocular muscles. The inferior and superior obliquus muscles run from the medioventral side of the lateral ethmoid to the ventral and dorsal side of the eyeball, respectively (**Plate III.1-9C**). The rectus muscles all attach more posteriorly to the skull, at the level of the orbitosphenoid (internal rectus) (**Plate III.1-9C**), pterosphenoid (inferior and superior rectus), as well as onto, what appears to be, the fascia of the inferior and superior rectus (external rectus) (**Plate III.1-9D**). Medial to the A_3" muscle lies the retractor tentaculi, which inserts through a stout but narrow tendon onto the posterior margin of the maxillary bone. The extensor tentaculi originates at the posterior margin of the orbito-nasal lamina (surrounded by the lateral ethmoid bone), as well as on the orbitosphenoid, and runs to the posterior part of the autopalatine, where its small tendon can be distinguished (**Plate III.1-9E**). The adductor arcus palatine muscle attaches along the complete dorsal border of the quadrate and metapterygoid bones, as well as the rostral part of the tendon sheet attaches to the entopterygoid. Posteriorly, muscle fibres are attached directly to the membranous plate of the hyomandibular bone (**Plate III.1-9F**).

The dilatator operculi bears a distinct tendon, attaching to the dorsal process of the opercular bone, and a rostral, muscular extension. However, this rostral part does not reach as far anteriorly as was observed in the normal juveniles (**Plates II.2-35C, III.1-9D**). The adductor operculi is largely covered by this dilatator, whereas its insertion is situated at the dorsal process of the opercular bone too (thus also functioning as a dilatator). The levator operculi borders the dorsal rim of the opercular bone and is well separated from the adductor muscle. In the normal, juvenile situation, these two muscles lie closer together (**Plates II.2-35C, III.1-9E**).

The musculature of the ventral side of the skull is also highly comparable to the normal, juvenile situation. The intermandibular muscle borders the protractor hyoidei anteriorly (**Plate III.1-9C**), which is well developed. The protractor is spread over the bases of the mandibular barbels, and is attached to the lower jaws and the lateroventral face of the hyoid bars (**Plate III.1-9D**). Posterior to it, lies the well developed inferior hyohyoidei muscle, which is attached to the medioventral face of the hyoid bars (**Plate III.1-9F**). The parurohyal bone is inserted on by the sternohyoideus muscle, which is already broad and short (see IV.2) (**Plate III.1-9F**). Ventral to the sternohyoideus lie the abductor hyohyoideus, where the contralateral muscles have their tendons clearly crossed over and fused (**Plate III.1-9G**). The branchiostegal rays are interconnected by a thin sheet, composed of the adductor hyohyoidei muscles. Based on the serial sections, it appeared that two separate bundles of the lateralmost adductor are attached onto the medial face of the opercular bone (**Plate III.1-9F**).

Ligaments - The maxillary bone is connected ligamentously to both the autopalatine and the premaxillary, as part of the palatine-maxillary mechanism (see II.2.2) (**Plate III.1-9B**). The opercular four-bar system requires the ligamentous connection between the interopercular bone and the lower jaw, as well as the ligament between the former and the opercular bone (**Plate III.1-9C**). The angulo-ceratohyal ligament is well developed too (**Plate III.1-9F**). The lateral wings of the prevomeral bone, bearing the tooth plates, are connected to the entopterygoid by a ligamentous strap (**Plate III.1-9E**). This entopterygoid is connected posteriorly to the metapterygoid by a comparable ligament (**Plate III.1-9F**).

Nerves - Although it is not the purpose of this chapter to describe the neurological differences between dwarfs and normal *Clarias gariepinus*, the serially sectioned dwarf specimen provides a good opportunity to situate the major cranial nerves. A short olfactory nerve (fila olfactoria, n. I) penetrates the olfactory foramen in the mesethmoid. The optic nerve (fasciculus opticus, N. II) passes through the anterior part of the sphenoid fenestra (**Plate II.1-4**), anterior to the bony bridge formed by the pterosphenoid and parasphenoid. The nerves innervating the eye muscles, *i.e.,* the oculomotor nerve (n. III), trochlear nerve (n. IV) and abducens nerve (n. VI) could hardly be observed, but follow a similar path in catfishes (MITHEL, 1964). They generally exit the braincase posterior to the pterosphenoid-parasphenoid bridge (BAMFORD, 1948; MITHEL, 1964).

Also posterior to that bridge, the trigemino-facial complex branches out. Generally, a supraorbital, infraorbital and hyomandibular trunk can be distinguished, consisting of combined trigeminal (n. V) and facial (n. VII) nerves (FREIHOFER, 1978). (1) The supraorbital trunk runs anteriorly, above the eye, where it passes through a foramen in the orbito-nasal lamina (and thus the lateral ethmoid bone) (**Plate III.1-10A**). It does not split into the usual trigeminal ramus ophthalmicus superficialis and facial ramus ophthalmicus superficialis, which could also be observed in other siluriforms (MITHEL, 1964). Consequently, no certainty exists whether this branch, running into the nasal barbel has a trigeminal or a facial origin. At the base of the supraorbital trunk, a small ramus lateralis accessorius anterior is split off, which runs along the dorsolateral side of the taenia marginalis. In *Bagarius bagarius* (Sisoridae), this branch runs intracranially (MITHEL, 1964). Branches of this ramus presumably innervate the supraorbital lateral-line canal (MITHEL, 1964). (2) The infraorbital trunk has a dorsal and ventral branch. The dorsal

branch subdivides into an external and an internal ramus buccalis facialis, which run into the maxillary barbel. The external branch has a medial branch, the anterior ramus palatinum, which runs along the dorsal face of the palatine up to the premaxillary teeth (MITHEL, 1964). The ventral infraorbital branch, *i.e.,* the maxillo-mandibular trunk, immediately splits into a ramus mandibularis trigeminus (or V_2-branch) and a ramus maxillaris trigeminus (or V_3-branch). The latter runs into the maxillary barbel, lateral to its supporting rod. The mandibular ramus runs ventrally, up to the coronoid process, where it splits off an external, small branch that runs lateral to the coronoid process. The internal mandibular ramus runs along the medial side of the coronoid process, but bends along the lateral face of the main part of Meckel's cartilage. It runs anteriorly along the lower jaw, where it splits into an anterior and posterior branch, innervating the internal and external mandibular barbels, respectively (**Plate III.1-10B**). (3) The hyomandibular trunk of the facial nerve runs laterally, splitting off the ramus opercularis profundus, which innervates the opercular adductor and levator muscles (see II.2.4) (**Plates II.2-34, III.1-10B**). The trunk then passes through a foramen, which is formed by the hyomandibular bone and the preopercular bone, and no longer by the cartilaginous hyosymplectic. The trunk passes into the excavated anterior margin of the hyosymplectic (**Plates II.1-34A, III.1-10A**). Consequently, the trunk branches into a mandibular ramus and a hyoid ramus. The former runs up to the lower jaw, whereas the latter runs into the branchiostegal membrane. At the base of the trigemino-facial complex, the posteriorly directed ramus recurrens runs up to the tectum synoticum, into the epaxial muscles (**Plate III.1-10A**) (SURLEMONT, 1983).

The acoustic nerve (n. VIII) lies intracranial, contacting the inner ear (**see text box**). The glossopharyngeal nerve (n. IX) passes through a separate foramen in the chondrocranium, enclosed by the exoccipital bone (**Plates II.1-32G; III.1-8, 10B**). Several branches innervate the branchial basket, and so does the vagus nerve (n. X) does. The latter also penetrates the skull through a separate foramen in the chondrocranium, also enclosed in the exoccipital bone (**Plates II.1-32G; III.1-8, 10B**). The vagus nerve also gives off a ramus lateralis, innervating the body lateral line, and a ramus visceralis, innervating the heart and viscera (MITHEL, 1964; FREIHOFER, 1978). At the lateral face of the medulla oblongata, posterior to the root of the vagus nerve, a small hypobranchial nerve (n. XII) passes through a separate foramen in the exoccipital bone (**Plates II.1-32G, III.1-10B**).

Lateral-line system - The cranial lateral-line system is fully developed, comparable to the normal, juvenile situation (see II.1.3). All branches and superficial tubes can be recognised (**Plate III.1-11A**). The transition of the mandibular canal to the preopercular canal, however, is not yet enclosed by the splenial bones (**Plates III.1-3B, 10A**). Due to the uncompleted ossification of many canal bones, some parts of canals are still exposed.

Labyrinth organ - The inner ear is situated in the otic capsule, lateral and ventral to the metencephalon and myelencephalon. A substantial crus communis interconnects the two vertical semicircular canals with the horizontal one (**Plate III.1-11B**). The broad utriculus contains the largest of the three otoliths, *i.e.,* the lapillus. The utriculus is encapsulated by the pterotic and prootic bones (**Plates II.1-25A, 30D**). Medially, at the base of the crus, the semicircular canals are connected to the lagena, enclosing the smallest of the otoliths, *i.e.,* the asteriscus (**Plate III.1-11B**). The cartilaginous walls enclosing the lagena are ossified as the exoccipitals and the basioccipital (**Plate II.1-32C-G**). The latter bones also enclose the sacculus, which surrounds a narrow and long otolith, the sagitta (**Plates II.1-32G, III.1-11B**). Left and right part of the inner ear are interconnected by the communicans transversus canal, which posteriorly contacts the sinus perilymphaticus impar (SURLEMONT, 1983) (**Fig. I.3-1, p. 25**). It is the posterior part of this sinus which makes contact with the Weberian apparatus, at the level of the claustrum (CHARDON, 1967a; SURLEMONT, 1983; RADERMAKER *et al.*, 1989).

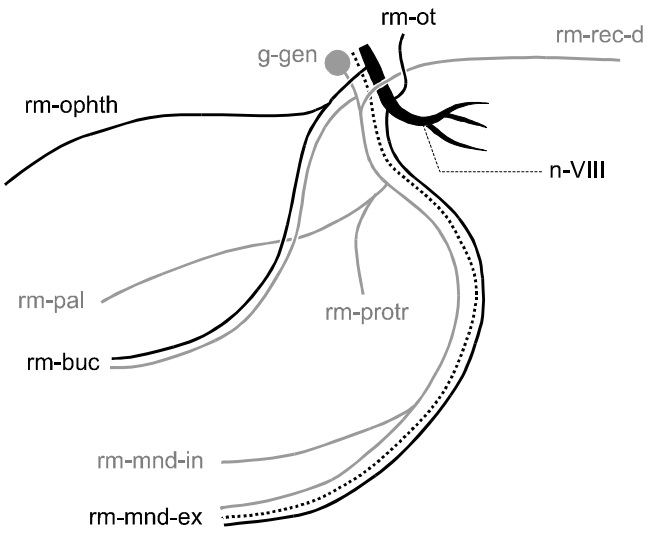

Fig. III.1- 3: Scheme of the nervus acoustico-facialis VII-VIII in fishes: solid black lines indicate branches innervating the lateral-line system, broken black lines indicate viscero-motoric branches, gray lines indicate viscero-sensorial branches (*Abbreviations:* n-VIII = nervus acousticus; g-gen = ganglion geniculi; rm-buc = ramus buccalis; rm-mnd-ex = ramus mandibularis externus; rm-mnd-in = ramus mandibularis internus; rm-ot = ramus oticus; rm-pal = ramus palatinus; rm-prot = ramus protrematicus; rm-rec-d = ramus recurrens dorsalis) [Modified from BERTIN (1958b)]

Nervus acousticus VIII in fishes:

During embryonic development of aquatic vertebrates, a single nerve complex is formed in the hyoid segment (BERTIN, 1958b). This complex consists of both sensorial and viscero-motoric nerves, bearing branches spread in the skull, as well as in the body. The nerve, innervating the labyrinth organ (n. VIII), however, does not bear a separate origin, but forms a caudal branch of the facialis root (n. VII) (**Fig. III.1- 3**). Hence, in fishes, it is suggested that for this complex, the term '*nervus acoustico-facialis (VII-VIII)*' should be used (BERTIN, 1958b; LAGLER *et al.*, 1962). The separation of the facialis and acoustic nerves in terrestrial tetrapods is coupled to the loss of those branches associated with the lateral line system (**Fig. III.1- 3**) (GOODRICH, 1958).

SIMILAR-AGED SPECIMENS

Chondrocranium - The previously exposed cartilaginous areas in the skull roof have become reduced almost completely (**Plate III.1-2C**). Ventrally, the unossified regions have become reduced as well, but can still clearly be distinguished (**Plate III.1-4C**). The ethmoid cartilage has become more narrow and elongated than in the other two stages (**Plate III.1-7C**). The isolated rod of the epiphysial bridge is reduced, whereas the remains of this bridge, contacting the taenia marginalis, are still present. The postorbital process has disappeared (see II.1.1), which was not yet the case in the two other groups. The orbito-nasal lamina is well extended laterally.

The splanchnocranium has been subjected to some additional reductions, compared to the previous specimens. The cartilage, interconnecting the epibranchials I and II with the third infrapharyngobranchials [which is suggested to correspond to the reduced infrapharyngobranchials I and II (see II.1.1)] is no longer observed (**Plate III.1-5C**). The basibranchial II has now become isolated from the parurohyal. The latter is still connected to a small cartilaginous element, presumably corresponding to the basibranchial I (see II.1.2). The hypobranchial IV, which is still fused to the ceratobranchial IV, has formed a distinct process at its posterior margin (**Plate III.1-5C**).

Osteocranium - Ossification has substantially increased, compared with the dwarf specimens. All bones of the skull roof and many of the skull floor are strongly interdigitating now. The general trend in the cranial roof bones involves the lateral and medial expansion, absolutely as well as relatively to the chondrocranium (**Plate III.1-7C**). The anterior part of the parieto-supraoccipital bone has now grown medially, contacting the contralateral one (**Plate III.1-2C**). Consequently, the posterior fontanel is completely demarcated. However, the parieto-supraoccipital bone, anterior to this fontanel has not fused, although this is the case in larger juveniles (**Plates II.1-28A, 40**). The initiation of this fusion could be observed in the 51.0 mm SL specimen. It appears that the parieto-

supraoccipital fusion occurs from the anterior border of the posterior fontanel, up to the anterior fontanel. In the similar-aged specimens, the frontal bones have continued to grow medially, thus narrowing the anterior fontanel (**Plate III.1-2C**). The cranial floor bones have expanded as well, although many of them still do not contact the surrounding bones (**Plate III.1-4C**). Exceptions are the pterosphenoids, which have contacted the frontals, the orbitosphenoids which interdigitate with the frontals, the lateral ethmoids and the parasphenoid.

Compared to the dwarf specimens, all canal bones have formed their plate-like component: the nasals, the antorbitals, the lacrimal and the second infraorbital. In all other canal bones, the plate has become expanded substantially (**Plates III.1-2C, 3C**). One exception involves the separate splenial bones, where the first of the series have formed now (**Plates III.1-3C, 5C, 6B**).

The suspensorium is noticeably more heavily ossified. The membranous outgrowths of both the hyomandibular and quadrate bones have become expanded and have started to interdigitate with each other (**Plates III.1-3C, 5C**). The metapterygoid bone has become more plate-like as well, as a dorsal and ventral apolamella have formed as an outgrowth of the perichondral bone. The entopterygoid is now substantially larger, its form is similar to that of the larger juvenile, although the gap with the metapterygoid bone is still larger (**Plates II.1-34A, D**). The preopercular bone now strongly interdigitates with the quadrate and the hyomandibular bones. The ventral hypohyal bones have contacted the anterior ceratohyal bones, at their dorsal face (**Plate III.1-5C**). Caudal to the ventral hypohyals, the dorsal hypohyals have formed but are still small. The period of the dorsal hypohyals formation can thus be reduced to somewhere between 21.5 mm SL and 40.8 mm SL (instead of 46.8 mm SL) (**Plate II.1-38**) (see II.1.2).

The first hypobranchials have enlarged substantially, which is not the case for the second ones (**Plate III.1-5C, 6B**). The crest on the fourth epibranchial, for the support of the uncinate process of the third one, has noticeably enlarged (**Plates II.1-36D, III.1-5C**). The fourth infrapharyngobranchials have become elongated, especially the anterior, unossified part, presumably for improving the articulation between the third infrapharyngobranchial and epibranchial (**Plate III.1-5C**). A total of eight (in smaller specimens) or nine (in larger specimens) branchiostegal rays could be counted (**Table III.1- 1**).

Suprabranchial organ - The additional branching of the cartilaginous support for the arborescent organs has continued. It appears that the lateral process, observed in the similar-sized specimen, now has formed additional branches (**Plate III.1-5C**). The base of the tree has broadened substantially as well.

DISCUSSION

Although some differences in skull morphology, in terms of ossification, between the dwarfs and the similar-sized specimen exist, a highly comparable ontogenetic status can be suggested. Similar-aged specimens, on the other hand, show a substantially more heavily ossified skull morphology. Small unossified regions can be observed in both the neurocranial roof and floor, which are almost completely covered with bone in the similar-aged specimens. The postpineal foramen is not yet subdivided in dwarfs and the similar-sized specimen, whereas in the similar-aged specimens, the anterior part of the parieto-supraoccipital bone complex has already formed the anterior border of the posterior fontanella. The lateral wings of the parasphenoid barely touch the pterosphenoid bones in dwarfs and the similar-sized specimen, but have already started to interdigitate in similar-aged specimens. The plate-like extensions of some infraorbital bones (*e.g.,* the fourth one) are substantially larger in similar-aged specimens, compared to the dwarfs and the similar-sized specimen. The

cartilaginous splanchnocranium has clearly become more ossified in the similar-aged specimens, compared to both the dwarfs and the similar-sized specimen. The suprabranchial organ bears several dichotomous branches in similar-aged specimens, whereas dwarfs and the similar-sized specimen only have one.

ONTOGENY: RELATED TO SIZE OR TO AGE?

Growth, as well as development is affected by many epigenetic influences. The effect of many of these factors has already been studied extensively in fishes (see introduction). However, little has been done concerning the effect of spatial constraints on a developing organism. The general notion does occur that fish, which generally experience an asymptotic growth (instead of a true indeterminate growth) (ANDREW, 1960; VON BERTALANFFY, 1960; BATT, 1980; MCDOWALL, 1994), remain small if kept in small tanks, whereas they attain larger sizes in spatially less constrained tanks. In that case, the surrounding space is the factor, directly or indirectly, lowering the asymptote of the growth curve. In order to quantify this effect, larval *Clarias gariepinus* of the same egg batch were raised in both conditions. The difference in growth is substantial: dwarf specimens reached a size, ranging between 20.8 and 32.5 mm SL, whereas normal, similar-aged specimens were between 36.2 and 58.1 mm SL. Whether dwarf specimens had already reached the phase of a decreased growth, associated with senescence in normal fish, can not be derived from this experiment.

According to MCDOWALL (1994), "size is an outcome of growth". But, as mentioned before, growth and development do not always coincide. The question then rises: can size really be used to be related to development? Based on the osteology of the cranial skeleton in artificially induced dwarfs and normal specimens of the catfish, *Clarias gariepinus*, it can be suggested that size is a better indication of developmental differentiation than age is. Theoretically, five possible scenarios could be expected: (1) development is only related to age; (2) development is only related to size; (3) development is a trade-off between size and age, (4) development is related to both size and age, as well as the input of the environment, or (5) development has nothing to do with size nor age, and is solely depending on the input of the environment. In the first scenario, the skull of the dwarf specimens could be expected to be equally ossified as the similar-aged specimens. For the second hypothesis, the skull of the dwarf specimens could be almost equal to the similar-sized specimen and less developed than the similar-aged specimen. In the third case, the dwarf specimen could show an intermediate configuration between the similar-sized and similar-aged specimens. The fourth and fifth hypotheses would be hard to distinguish from the third one, if such an intermediate configuration was to be found. Additionally, a differential response of the different elements or tissues of the 'Bauplan' cannot be overruled either, in which case no real correlation between ontogeny and size or age may be apparent. This would then require further experimental work, with a closer follow-up of the growth and the development during ontogeny.

When looking at the ossification status of the skulls, it is clear that the dwarf specimens (age 38 days posthatching, average size 24.5 mm SL) show a morphology highly comparable to that of the similar-sized specimen (age 31 days posthatching, size 21.5 mm SL), whereas the skull of similar-aged specimens is much more ossified (age 38 days, average size 43.8 mm SL). Even though the similar-sized specimen and dwarf specimens do not differ that much in age or in size, the fact that similar-aged specimens are more heavily

ossified can give a good indication that ossification, as an indicator of developmental rate, is related to size and not to age.

DWARFISM: REDUCTION IN SIZE

Reduction in size can be considered as the result of the action of a whole cascade of factors acting on the growing organism. The question here is, to what degree can dwarfism be contributed to genetic derived mechanisms and to what degree is a direct action of epigenetic factors involved, and are both mechanisms disconnectable? In earlier papers, it was believed that the mechanism responsible for a stop in growth involved the presence of certain substances in a concentration corresponding to the adult concentration (*e.g.,* the administration of an extract of adult brain tissue induced growth stop in juvenile brain) (CLARK, 1960). Nowadays, it has been demonstrated that growth influencing substances involve hormones, vitamins, prostaglandines, growth factors and cytokines (JENKIN, 1970; BATT, 1980; MARKS & POPOFF, 1988; THORARINSSON *et al.*, 1994). Many hormones, both growth hormones and non-growth hormones, have been found to invoke differences in cell proliferation (see below). In general, growth inhibition is not a direct action of hormones, but rather the result of a reduced or inhibited production of growth stimulating hormones (JENKIN, 1970). In this study, however, no research was done on the difference in hormone levels in the dwarf *Clarias gariepinus* and the two normal groups, so no suggestions on hormonal influences can be made.

The effect of temperature on growth can also be indirect, as it changes the hydrodynamic features of water. Lower temperatures increase water viscosity, which implicates different mechanical loadings and functional expectancies for larvae to survive, requiring special adaptations in the musculo-skeletal system (OSSE & VAN DEN BOOGAART, 1995). The suggestion can be made that changes in hydrodynamics of water in constrained spatial environments may have had some influence on mechanical loadings onto the developing *Clarias gariepinus* larvae as well.

Effects of population densities on growth may be an indirect consequence of spatial constraints acting on the organism. As could be observed in *Notophthalmus* (Amphibia, Salamandridae), differences in growth and the occurrence of neotenic and metamorphosed forms could be related to differences in population densities (HARRIS, 1987). Low densities evoked a higher growth rate and lesser number of metamorphosing specimens. In situations of high predation pressure and low densities of their host anemones, *Amphiprion* (Pomacentridae) pairs experience a reduced growth because of the narrow space surrounding the anemone (OCHI, 1986). Aggressive behaviour, coupled to the social structure in many pomacentrids, resulting in reduced growth of specimens of lower hierarchic levels, may be an evolutionary important feedback mechanism for controlling competition (FRICKE & FRICKE, 1977; OCHI, 1986). Although cause and result are known in these cases, little or nothing is known about the mechanism regulating it.

A factor that still cannot be neglected, as the driving force for size differences, is the genetic variability. In the specimens of *Clarias gariepinus* used in this study, changes as a result of genetic differences can to a certain degree be reduced, as both dwarfs and similar-aged specimens came from the same egg batch. The genetic differences that exist in both dwarfs and similar-aged specimens, may be (partially) reflected by the differences in size of the different dwarfs and the different similar-aged specimens (**Table I.2-11, p. 23**). One has to bear in mind that variation, which appears to be genetically dependent at first sight, may simply be a consequence of "environmental heterogeneity" (STRAUSS, 1990a).

All these examples, however, still don't give a plausible answer to the question which mechanism enabled *Clarias gariepinus* larvae to retard their growth as a response to spatial constraints. In order to unravel such a mechanism, it is essential to look at regulation of growth and development at another level of discrimination: how is cell differentiation and cell proliferation (especially during osteogenesis) controlled?

DWARFISM: RETARDATION IN OSSIFICATION

For the developmental aspect of dwarfism, and more specifically ossification, it is of importance to look at those factors influencing osteogenesis, in order to get an idea of how detection of a spatial constrained environment can evoke reduced ossifications. In general, osteogenesis involves cartilage and bone cells differentiation, proliferation, resorption, as well as matrix secretion and mineralisation (MARKS & POPOFF, 1988). The control of these processes can be divided in two important mechanisms which determine the combination and the sequence of these processes: (1) hormonal activity, controlling the biological process and (2) the mechanical loadings, controlling the epigenetic, mechanobiological processes (CARTER *et al.*, 1996).

(1) In the biological process, many hormones, that are involved in osteogenesis, are derived from the adenohypophysis, the thyroid gland and the parathyroid gland (JENKIN, 1970; MARKS & POPOFF, 1988; BONGA, 1993). Several hormones are known to have a direct or indirect effect on osteogenesis (**see text box**). The influence of pituitary and thyroid hormones can be focussed on two major processes: osteoblast proliferation and differentiation, and bone resorption by osteoclasts. However, based on experimental work, not all results are conclusive whether a certain hormone evokes bone formation or it induces bone resorption. As mentioned above, no data on hormonal involvement is obtained in this study.

HORMONAL REGULATION OF OSTEOGENESIS

Growth hormone (GH) [= somatotropine (WALLIS, 1996)] is secreted in the adenohypophysis of all fishes but not in agnathans. Within teleosts, two forms of this GH have been discovered. The activity of GH is generally mediated by insulin-like factors (IGF), playing an important role in the metabolism of thyroid hormones (BONGA, 1993). Thyroid hormones may require a transformational metabolism for their proper working. In the thyroid, tetra-iodothyronine or thyroxine(=T_4) is produced and secreted into the circulatory system. In target organs (liver and gills) T_4 is metabolised into tri-iodothyrosine (=T_3), which is the active component in osteogenesis, under the control of GH (BONGA, 1993). Calcitonin is produced in the ultimobranchial gland. In fishes this gland is situated in the connective tissue sheets surrounding the heart and is formed by neural crest derived endocrine cells (BONGA, 1993). The parathyroid hormone (PTH) observed in many vertebrates is absent in fishes [although evidence of a comparable, novel compound may be present (BONGA, 1993)]. Hormonal activity during osteogenesis targets cartilage and bone proliferation and differentiation. GH induces an increased production of IGF mRNA, where IGF is believed to be the mediating factor in birds and mammals (TAKAGI *et al.*, 1994; TAKAGI & BJÖRNSSON, 1996). In teleosts, this mediation occurs by somatomedin, an IGF-like compound (TAKAGI & BJÖRNSSON, 1996). T_4 and T_3 stimulate cartilage proliferation and maturation (MARKS & POPOFF, 1988; TAKAGI & BJÖRNSSON, 1996). T_3 induces matrix synthesis, as well as the differentiation of chondrocytes from the growing zone to the hypertrophic zone (TAKAGI *et al.*, 1994). The action of the hormones appears to be dose-dependent and bone-type related (MARKS & POPOFF, 1988; TAKAGI *et al.*, 1994; TAKAGI & BJÖRNSSON, 1996). Calcitonin inhibits osteoclast activity but has no effect on bone formation, although it plays a role in the mineralisation (MARKS & POPOFF, 1988). In teleosts, it is believed to regulate hypercalcemia levels in the blood, by stimulating calcium deposition in bone (BONGA, 1993). However, the latter author does mention that "it promotes bone formation by osteoblasts".

(2) The mechanobiological processes, related to epigenetic influences, are believed to be as equally important to normal skeletal ontogeny as the genetically derived biological processes (CARTER & WONG, 1988; CARTER *et al.*, 1991; 1996; CARTER & ORR, 1992). Skeletal structures possess the potentials to adapt to changes in mechanical loads, the process known as 'functional adaptation' (CARTER & ORR, 1992). In human ontogeny, the

mechanical strains, put onto the growing bones due to muscular attachments, are essential for normal development, as the absence of such strains results in skeletal deformations (CARTER *et al.*, 1996). The absence or presence of mechanical loads onto embryonic precursor cells, have been observed *in vitro* to be a determining factor for whether these cells will undergo chondrogenesis or osteogenesis. Osteoprogenitor cells are capable of forming both osteoblasts and chondroblasts (MARKS & POPOFF, 1988). Changes in mechanical loads during ontogeny may affect cartilage formation in different ways: (1) the orientation of chondrocytes changes, where they become arranged along stress trajectories; and/or (2) mesenchyme becomes differentiated into cartilage (WEIS & AMPRINO, 1940). The kind of mechanical load also affects cartilage ossification differently: (1) intermittent shear forces, which change the shape but not the volume of cartilage, have an accelerating effect on chondroblast proliferation, maturation, degeneration and ossification; whereas (2) intermittent compressive (or dilatational) stresses, which change the volume but not the shape of cartilage, have an inhibiting effect on them (CARTER & WONG, 1988; CARTER & ORR, 1992). Consequently, this results in a "system of tissues which are, in many ways, self-designed for their mechanical function" (CARTER & ORR, 1992). The response of bone cells is characterised by the so-called trigger response, where proliferation and differentiation of osteoblasts is increased some time after initial loading (STANFORD *et al.*, 1995). In the dwarf *Clarias gariepinus*, both cartilage and bone resorption can be observed. Cartilage resorption can be observed in many structures, which were already ossified (**Plate I.4-3C**). Reversal lines in several bones indicate that bone resorption and remodelling has already occurred (**Plate I.4-5B**). The same can be observed in similar-aged specimens. However, the comparison with similar-sized specimens could not be made, as serial sections of such specimens was not available for this study. Whether the reduced ossification in the dwarf *C. gariepinus*, compared to the similar-aged specimens, is the result of an equilibrium between osteogenesis and osteoresorption, instead of being a pure stop in osteogenesis, cannot be derived from the material used.

The above mentioned changes in osteogenesis may lie at the basis of changes in cranial ossification patterns, observed in some miniaturised lineages of vertebrates. Trends, which are frequently observed in miniature vertebrates are: (1) reduced ossification, (2) hyperossification, especially calcification of cartilage, (3) increased variability in skeletal morphology, and (4) morphological novelties (HANKEN, 1993). Ossification in the miniature urodele, *Thorius* (Amphibia, Plethodontidae) showed that different, individual bones are reduced, especially in the anterior part of the skull. Additionally, several structural novelties are present, which can be related to 'functional adaptations' to the jaw suspension and brain size (HANKEN, 1983a, b). In neotenic *Triturus* (Amphibia, Salamandridae), cranial ossification is retarded, whereas chondrification is higher than in metamorphosed specimens, even between genetically similar specimens within the same population (ROČEK, 1996). However, neotenic specimens are larger than the metamorphosed specimens. It has to be noted, however, that the complex process of metamorphosis in amphibians can hardly be compared to the situation in fishes.

In gigantism, as a result of hormonal level increases, skulls in rats were enlarged, bearing "ruggedness especially at the attachment of the suboccipital and temporal muscles" (ASLING *et al.*, 1955). This suggests that certain skeletal modifications in enlarged skulls reflect "functional adaptations" to altered mechanical loads, coupled to the enlargements. In the experiments with the dwarf *Clarias gariepinus*, substantial changes in the normal activity patterns can, of course, be expected. As the larvae were reared in small areas, they were partially immobilised. As food was administered close to their mouth, they hardly had to search actively for food. It may thus be expected that muscular activity was lower than in similar-sized specimens. This would implicate that a reduced mechanical loading is exerted onto the skeletal elements, which could explain for the reduced

ossification. However, before any reliable comparison in functional adaptations to different mechanical loadings in the cranial morphology can be done, the true nature of these loadings has to be known into detail. Based on the experiments performed for this study, this can only be suggested on an overall basis of activity reduction.

DWARFISM: ISOMETRY OR ALLOMETRY?

Normal ontogeny in fishes is characterised by allometric growth of the different parts of the organism. In larval fishes, the anterior and posterior parts of the body show an initially larger growth rate, compared to the middle part (OSSE, 1990; OSSE & VAN DEN BOOGAART, 1995). Frequently, those allometries change into isometric changes, as ontogeny proceeds. These allometric growth rates reflect the changing functional importance of the corresponding parts or regions of the organism: a larger head enables a functional feeding and respiratory apparatus, whereas a larger tail enables increased locomotory potentials (feeding and predation avoidance) (OSSE & VAN DEN BOOGAART, 1995). Consequently, allometry itself is not the only important variable, also timing is crucial (OSSE, 1990). Differences in timing of allometric changes, coupled to a kind of metamorphosis in fishes, is frequently attributed to differences in egg size, and thus yolk sac size (OSSE & VAN DEN BOOGAART, 1995).

On the other hand, the occurrence of so-called allometries have to be carefully considered. They are related to body size, more frequently than is sometimes assumed. In some haplochromine cichlids, the relative size and shape of cranial elements, involved in trophic adaptations, appear to be related allometrically to each other. Although characters may be considered as being species-specific, trophic specialisations (BAREL, 1984; 1985), many of them can be scaled to body size (STRAUSS, 1984). However, this cannot be generalised, as was demonstrated by WILHELM (1984). Although exceptions exist, body size dependency of the size and ontogenetic differentiation of structural elements has to be taken into account, before coming to conclusions concerning evolutionary adaptive traits (STRAUSS, 1984; 1985).

Allometries in skull morphology that occur during the evolutionary process of miniaturisation, are frequently related to certain critical values of organ size. In miniature urodeles, trends in cranial morphology involve negative allometry of the brains, otic capsules and eyes (in relation to skull length), whereas the nasal capsules show an isometric relation. Miniaturisation could be rather indefinite, if it where not that in order to remain functional, certain elements require a minimal size, as for example the brain and the eyes (HANKEN, 1983b). Consequently, allometries can be seen as a reflection of competitive interactions between different elements of the skull. It can thus be expected that in the artificially induced dwarf *Clarias gariepinus*, such a spatial competition between organs will play an important role as well. A detailed morphometric study of the different organs and cranial regions could give some conclusive results on this topic.

Further research could provide some additional information on the mechanism responsible for dwarfism in *Clarias gariepinus*. Investigation towards the ontogenetic timing of the growth curve deviation in dwarfs, compared to the normal situation, could reveal information on whether the reduced growth in the dwarfs involves a lowering of the asymptote, or whether growth just stops along the normal curve. Transferring induced dwarfs after some time to a spatially non-constrained area, could provide data on whether a so-called "rebound acceleration" occurs afterwards (NEEDHAM, 1960). Although the skulls of the dwarfs appeared to be isometric to the equally sized, normal specimens, a detailed study of the growth rate of the separate elements of the '*Bauplan*' could reveal possible allometries. On the other hand, it could be checked then to what level ontogeny (and growth) does occur isometrically with the normal situation.

SIZE DEPENDENCY OF ONTOGENY: SOME CONSEQUENCES

If ontogeny is really determined solely by size, a whole evaluation of certain methodological approaches may need to be done. If age is measured in fish, by means of counting mineral deposition cycles in otoliths, scales or fin spines (SIRE *et al.*, 1993; VAN NEER, 1993; VAN NEER *et al.*, 1993; CONAND *et al.*, 1995), can it be ascertained that age, rather than developmental rate (and/or growth) is measured? Can possible, extensive fluctuations in fish growth rate, under the impact of many epigenetic factors, obscure true chronological age?

Whether or not it can be stated that the cranial morphology of dwarf *Clarias gariepinus* is the result of paedomorphoses, and more specifically a neoteny[4] or progenesis[5] (**Fig. III.1- 4**), depends on whether ontogeny[6] is considered to be related to size or age. If age is to be used, the dwarf *C. gariepinus* would indeed represent an overall paedomorphotic cranial morphology, whereas when size is to be used, no aberrant cranial morphology is present. In general, however, paedomorphoses (and thus also peramorphoses) are coupled to time, and thus age, instead of size. This poses a methodological problem: should ontogenetic stages be expressed in terms of corresponding age or size, when comparing? In the case of the ontogeny of *C. gariepinus*, it has already been noted that specimens of equal age do show differences in size, and consequently in the degree of development (see II.1.2, **Table I.2-5, p. 6**). Before an answer to this question can be provided, another central question has to be answered first: is the way researchers measure time comparable to the way cells, tissues and organisms measure time? Embryo's are believed to measure time based on cell size (HALL & MIYAKE, 1996). Thus, time, or developmental stage for these cells, is measured as size, instead of chronological time. Also, as HALL & MIYAKE (1995) stated: "physiological rates rather than chronological time can be used as a time standard in comparative studies", with physiological rates being determined by biological time (or developmental time). On the other hand, physiological time can also be expressed as the amount of energy consumption in relation to body weight. Consequently, a third measuring unit of development can be used: weight (or mass). This unit can be best discarded for two reasons: first, weight changes can be

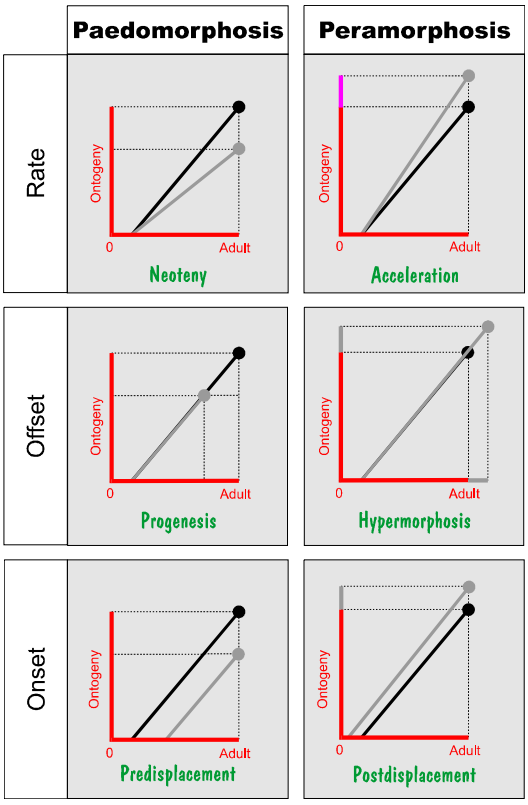

Fig. III.1- 4: Scheme of different types of paedomorphoses and peramorphoses (black curve indicates ancestral situation, grey curve indicates derived situation) [Modified from OSSE (1992)]

[4] *Neoteny:* adult phenotype characterised by a reduced growth rate, compared to the ancestral situation (onset and offset of growth remain the same) [definition according to GODFREY & SUTHERLAND (1995)]
[5] *Progenesis:* adult phenotype characterised by an early offset of growth, compared to the ancestral situation (onset of growth and growth rate remain the same) [definition according to GODFREY & SUTHERLAND (1995)]

correlated to growth but not to developmental differentiation, and second, body weight can even disguise true growth by processes like water absorption, fat deposition, … (THOMPSON, 1952; VON BERTALANFFY, 1960; BATT, 1980). This implies that the answer to the question suggests that 'time' for ontogenetic process should be related to size, instead of chronological time (whether it be the size of cells, structures or organisms depends on the discrimination level). Size-dependency of several structural traits in organisms has been demonstrated by STRAUSS (1984; 1985) as well.

However, this consequently shifts the question to: how recognising paedomorphoses (or peramorphoses)? For this study, it would imply that the 'dwarf specimens' are no dwarfs and present no paedomorphoses. In amphibians, some interspecific differences could be noted in the skull morphology, even when the skulls were reduced to equal size (HANKEN, 1983b). In that case, true heterochronies may be present. The use of size as a valid biological time would also improve the study of heterochronies in fossil material, as the lack of chronological age of the specimens generally presents the restricting factor. However, one aspect has not been taken into account: the fact that the relation between size and ontogenetic stages differs interspecifically. The relation of ontogenetic differentiation with the size of different species could be compared only, if phylogenetic affinity between them is high, for example in the ancestors of recent amphibians (SCHOCH, 1995).

CONCLUSIONS

The comparison of the developmental status between artificially induced dwarf and normal *Clarias gariepinus* (both of equal age and of equal size) suggests that ontogeny, and cranial ossification to be more specific, is to be related to size and not to age. Dwarf *C. gariepinus* show a cranial morphology which closely resembles that of similar-sized, but younger normal specimens, whereas the skull is significantly less developed, compared to the similar-aged, but substantially larger normal specimens. This invokes that when ontogenetic stages are to be compared, the factor of possible size variability for a certain age has to be taken into account. No conclusive answer can be given to the question which mechanism enables the fish to detect a restricted spatial environment and respond to it by reducing its growth and development. The reduced mechanical loadings, as a result of the experimental conditions, may partially explain the reduced ossifications. Hormonal control during stress, on the other hand, can not be demonstrated, but cannot be ruled out.

Size dependency of ontogenetic rate also invokes some methodological problems. First, special attention has to be paid to methods measuring age of teleosts, but which are based on developmental processes. Second, defining paedomorphoses (or peramorphoses) is largely dependent on how the biological time scale is defined. In the case of this study, the 'dwarf' *Clarias gariepinus* are examples of paedomorphes, if age is used as a reference, whereas no paedomorphotic 'Bauplan' can be suggested when body size is used.

[6] GODFREY & SUTHERLAND (1995) use "growth" in their definition in paedomorphoses and peramorphoses. In the context of this thesis, paedomorphoses and peramorphoses are seen as processes of different ontogenetic differentiation, instead of mere growth.

Chapter IV.1 – A shift in mouth opening mechanisms: a response to increasing functional demands

During ontogeny, larval fish have to deal with increasing nutritional and respiratory demands. As ontogeny is characterised by an increase in complexity of structural elements composing the skull, some constraints will have to be met when developing mechanisms, which enable feeding and respiration, arise at a certain ontogenetic stage. In this chapter, special attention is paid to the possible ontogenetic shift in mouth opening mechanisms in *Clarias gariepinus*, based on morphological data. Starting shortly after hatching, a total of five different mouth opening mechanism may become functional. Of these, three may remain functional in the adult situation. As could be expected, the apparatuses which enable these mechanisms show an increase in complexity, as well as an improvement in mouth opening capacity. Initially, two consecutive mechanisms allow a passive depression of the lower jaw. One of these becomes more improved, allowing an active depression. Synchronously a fourth one is formed. Both the latter mechanisms involve the coupling of the hyoid depression to the mouth opening. At about 11 mm SL the fifth mechanism is formed, better known as the opercular mouth opening mechanism. Some aspects of these mechanisms are discussed, with special attention is paid to the shift in the efficiency of the opercular mechanism during ontogeny. Some arguments are given which may suggest an ontogenetic shift in feeding type, as is frequently observed in many teleosts. Finally, the chronology of the shift in mouth opening mechanisms is coupled to several morphological, behavioural and physiological changes during ontogeny, related to feeding and respiration. Based on these features, five different phases can be defined during the ontogeny of *C. gariepinus*.

INTRODUCTION

When looking at other structures related to feeding, several adaptations can be observed in adult *Clarias* species. As THOMAS (1966) categorised, different types of adaptations can be recognised, for example (1) benthophagic adaptations, (2) planktophagic adaptations, (3) phytophagic adaptations and (4) piscivorous adaptations.

(1) Benthophagic adaptations comprise the sluggish body form, small eyes, a flat head and the specialised oral barbels. In general, most catfishes exhibit a nocturnal and benthic behaviour, in which food and prey localisation is done primarily by probing with the well developed, mobile oral barbels (ALEXANDER, 1965; 1966; THOMAS, 1966; GOSLINE, 1973; NELSON, 1994; TEUGELS, 1996) (see II.2.2). In many siluriforms the epidermal layers of these barbels contain several taste buds, as well as superficial neuromasts (ARRATIA & HUAQUIN, 1995). These sensory buds can also be found in large

numbers on the lips, and in lesser numbers spread all over the body (ARRATIA & HUAQUIN, 1995). In *Clarias gariepinus*, such buds can be observed as well (**Fig. IV.1- 1**). Sensory buds on the maxillary barbels in *Clarias* are able to distinguish between chemical and mechanical stimuli, as well as between stimuli of different chemicals (LONG & HUANG, 1995). Prey localisation in aphotic waters is also facilitated by the presence of so called ampullary organs, which enable electroreception. Such ampullary organs have been observed in *Clarias* as well (LAHIRI & KAPOOR, 1975).

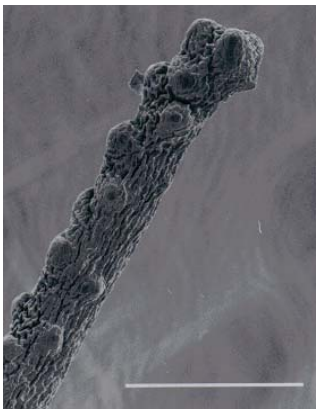

Fig. IV.1- 1: SEM image of the distal tip of the nasal barbel in a larval *Clarias gariepinus*, showing the presence of many sensory buds (scale bar = 100 µm)

(2) Planktophagic adaptations involve the non-protractile mouth, patches of small, villiform and conical teeth and long gill rakers. As a result of the adaptation to the palatine-maxillary mechanism, those structures related to mouth protrusion have become decoupled from the protrusion apparatus (see II.2.2; IV.2) (ADRIAENS & VERRAES, 1997e). Tooth batteries in *Clarias gariepinus* consist of densely packed teeth on the dental, premaxillary, prevomeral, lower and upper pharyngeal bones (see II.1.2) (**Plates II.1-31A, 32A, 33B, 36D-E**). Gill rakers can be found on the ceratobranchial and epibranchial bones of the anterior four branchial arches, with the longest rakers on the first arch (**Plate II.1-36**). The total number of gill rakers on the first arch ranges between 24 and 110 (TEUGELS, 1986). Additionally, the low suspensoria and wide mouth gapes are an indication of ram feeding behaviour (HUNT VON HERBING *et al.*, 1996b). Stomach analysis of *C. gariepinus* showed that plankton is a substantial part of their diet (GROENEWALD, 1964).

(3) Phytophagic adaptations, according to THOMAS (1966) , involve the subterminal mouth, in the form of a transverse slit, as well as the long mandibular and premaxillary dental plates. The author, in his paper, is referring to *Clarias senegalensis*, which is considered to be a synonym of *C. anguillaris* (TEUGELS, 1982). As pointed out by THOMAS (1966), BENECH *et al.* (1993) and TEUGELS (1986), *C. anguillaris* resembles *C. gariepinus* closely, and can be compared.

(4) Piscivorous adaptations are rather obvious in *C. gariepinus*: large body size, powerful adductor mandibulae complex, large tooth batteries and heavily dentigerous pharyngeal jaws. Body size is measured, reaching up to 700 mm (TEUGELS, 1986). The large adductor mandibulae muscle covers almost the complete lateral face of the orbito-temporal region (**Plate II.2-5A**).

All these characters indicate that in the adult situation, *Clarias gariepinus* is well equipped to obtain different kinds of food. Because of its large size, it encounters little problems in taking up food in all its forms. However, this is not the case for *C. gariepinus* during its early life stages. When comparing the larval skull with the adult one, it is obvious that not only size dependent feeding, but also a feeding, which is dependent of the complexity of feeding apparatuses, will have to be performed (**Plate IV.1-1**). At hatching, catfishes in general have a well developed yolk sac, which provides them with an initial food supply (BURGESS, 1989; 1992). Important is, however, that this period of endogenous feeding allows them a transition phase during which they do not have to feed actively. This period lasts for maximum four days in *C. gariepinus* (HECHT & APPELBAUM, 1987). This

gives them time to develop a functional feeding apparatus by the time they will have to feed actively, as well as it provides time for a learning process for optimising food capturing (COUGHLIN, 1994; HUNT VON HERBING *et al.*, 1996a). In *C. gariepinus*, the yolk sac is converted into larval tissue very quickly and efficiently (KAMLER *et al.*, 1994). Apart from feeding, the respiratory mechanism will have to become functional as well, as the gas exchange will no longer be sustained by cutaneous respiration in the growing larva (OSSE, 1990; HOLDEN & BRUTON, 1994; OSSE & VAN DEN BOOGAART, 1995; HUNT VON HERBING *et al.*, 1996a). The initial mechanism that will be required, both for respiratory and feeding requirements, is that for mouth opening and mouth closing, followed by mechanisms involved in volume changes of the orobranchial cavity, as well as volume changes of the opercular cavity. Later on, as the larva becomes larger, the dietary demands will change as well. Such ontogenetic shifts of diets have been observed in different groups of fishes (THOMAS, 1966; SEGNINI & BASTARDO, 1995; COOK, 1996; ROWE & CHISNALL, 1996; LOWE *et al.*, 1996; OLSON, 1996). In terms of "symmorphosis"[1], *i.e.,* the quantitative match between the capacity of an organism and the functional demand they have to cope with (GALIS *et al.*, 1994), it can be expected that changes in diet and food particle size during ontogeny will be coupled to changes in feeding mechanisms. Not only will the larva have to adapt to taking up larger food items, it may also have to increase food capturing and manipulation efficiency, as the food type changes during growth (OSSE, 1990). As GALIS *et al.* (1994) mentioned, larvae may do so by: (1) making complete use of those structures that are present, and (2) enlarging the capacity of those structures present, in response to changes in demand (phenotypic plasticity). It is, however, crucial for a developing larva to do so at low cost of energy (GALIS & DE JONG, 1988; GALIS *et al.*, 1994) or to minimise energy loss during prey capture (OSSE & DROST, 1989; OSSE, 1990). Equally important is the timing between morphological transformations, in relation to the timing of changes in functional demands and nutritional requirements (HOLDEN & BRUTON, 1994) (see II.1.2).

In this chapter, the ontogeny of the feeding apparatus in *Clarias gariepinus* is discussed. Special interest is paid to the mechanisms responsible for mouth opening, as there appears to be shift of different mechanisms, especially early during ontogeny. It has to be emphasised that the assumptions made are in a way theoretically. The assumptions are based on morphological evidence, without any kinematical or electromyographical research. Some data, however, are obtained from literature (SURLEMONT *et al.*, 1989; SURLEMONT & VANDEWALLE, 1991).

RESULTS

The ontogenetic data of those elements which constitute the feeding apparatus in *Clarias gariepinus* are already given in the chapters II.1 and II.2. It is, however, necessary to give an overview of the morphology of the different feeding apparatuses, in relation to the shift in feeding mechanisms, especially those responsible for mouth opening. In that way, it is easier to follow the process of increasing complexity of these different mechanisms. The different feeding apparatuses will be described, categorised according to the corresponding mouth opening mechanism (the corresponding standard length is mentioned also). A total of up to five different mouth opening mechanisms can be distinguished.

[1] Hypothesis defined by WEIBEL and TAYLOR (1981)

MECHANISM 1 (5.0 – 5.6 MM SL)

FORM – At hatching, the suspensorium, lower jaw and hyoid bar arise as one, single cartilaginous structure (**Plate II.1-1B**). Shortly after hatching (at about 5.0 mm SL), the musculus adductor mandibulae becomes attached to both the suspensorium and the lower jaw, the latter still being constituted of Meckel's cartilage only (**Plate IV.1-2**) (SURLEMONT *et al.*, 1989). The position of the mandibula, in relation to the neurocranium, shows that in a relaxed situation the mouth is open.

FUNCTION – As the only muscle inserting onto the lower jaw is the adductor mandibulae, no muscular contraction can result in the depression of the lower jaw. Additionally, as the lower jaw is not connected ligamentously to any other mobile element, indirect muscular depression is overruled as well. However, the fact that the lower jaw is continuous with the suspensorium, instead of articulating with it, can induce a passive depression (SURLEMONT & VANDEWALLE, 1991). Movements of the lower jaw were observed in the 5.0 mm SL larvae [5.2 mm larvae of SURLEMONT *et al.* (1989)]. Presumably, the contraction of the adductor mandibulae results in the closure of the mouth. As the natural position of the lower jaw is in a depressed manner, the closure of the mouth will strain the connection of the mandibula with the suspensorium. Due to the elastic features of this cartilaginous connection, the relaxation of the adductor mandibulae may result in a passive mouth opening (**Plate IV.1-3**). This mouth opening mechanism, however, allows a lower jaw depression not further than its natural position. This mechanism also implicates that once the connection between Meckel's cartilage and the suspensorium is lost, it can no longer be functional (**Plate IV.1-12**).

MECHANISM 2 (5.6 – 19.2 MM SL)

FORM – At around 5.6 mm SL, Meckel's cartilage starts to become detached from the suspensorium, as an articulation is formed. At this stage, some muscles have formed, connecting the lower jaw to the hyoid bar: (1) the intermandibularis posterior and (2) the interhyoideus anterior (see II.2.3) (**Plate IV.1-4**). The hyoid bar is still continuous with the suspensorium through a cartilaginous interhyal.

FUNCTION – From the moment the previous mechanism is inactivated, it can be expected that another solution has to enable a continuity in mouth opening. In this transition phase from the passive mouth opening to the mouth opening mechanisms that persist till the adult situation, the cartilaginous connection between the hyoid bar and the suspensorium might provide a weak but functional mouth opening. The contraction of the muscle complex, formed by the intermandibularis posterior and the interhyoideus anterior, will pull both onto the lower jaw and the hyoid bar (**Plate IV.1-5**). As the sternohyoideus, which could retract the hyoid bar, cannot be functional until at the 6.2 mm SL stage, it cannot prevent the hyoid bar to become elevated during the contraction of the intermandibularis and interhyoideus complex. This would partially annul the mandibular depression. However, the difference in resistance between the mandibular articulation and the hyoid connection with the suspensorium might be sufficient to abduct the lower jaw: the protraction of the hyoid bar will be resisted more than the retraction of the lower jaw will be. Theoretically, this mechanism can remain functional until the interhyal becomes detached from the hyoid bar (or the suspensorium) (**Plate IV.1-12**).

MECHANISM 3 (6.2 MM SL – …)

FORM – A clear articulatory facet has formed now between the lower jaw and the suspensorium (**Plate IV.1-6**). At the retroarticular process of Meckel's cartilage, a ligament is attached, running to the lateral face of the cartilaginous hyoid bar.

FUNCTION – As the larvae become larger, the mouth opening for feeding and respiration must be more important. It is consequently necessary that mouth opening should become improved. From 6.2 mm SL, it appears that mouth opening can occur through a more direct muscular action onto the lower jaw (SURLEMONT & VANDEWALLE, 1991). One mechanism involves the coupling of the hyoid depression, through contraction of the sternohyoideus muscle and the hypaxials, to the abduction of the lower jaw. The angulo-ceratohyal ligament [*i.e.,* the mandibulo-hyoid ligament of HUNT VON HERBING *et al.* (1996b), "ligament mandibulo-hyoïdien" of SURLEMONT and VANDEWALLE (1991); ligamentum mandibulo-hyoideum of VERRAES (1977); ligamento hioideo-mandibular of DE LA HOZ and ALDUNATE (1994)] enables this coupling (**Plates IV.1-7; IV.1-12**). The depression of the hyoid bars can already be observed in the 6.2 mm SL larvae [6.8 mm larvae of SURLEMONT & VANDEWALLE (1991)]. The maximal gape is then reached, once the working line of this ligament comes to run through the mandibular articulation.

MECHANISM 4 (6.2 MM SL – …)

FORM – The intermandibularis posterior and interhyoideus anterior muscles have fused now, thus forming the protractor hyoidei muscle (**Plate IV.1-8**). The sternohyoideus muscle interconnects the rostral tip of the hyoid bar with the cleithral bone of the pectoral girdle. At the posterior margin of this cleithral bone, the hypaxial musculature is attached.

FUNCTION – The depression of the lower jaw can also be coupled muscularly to the depression of the hyoid bar. The pectoral retraction or fixation (through the contraction of the hypaxials) and the depression (or retraction) of the hyoid bar (through the contraction of the sternohyoideus) can be coupled to the contraction of the protractor hyoidei (OSSE, 1969) (**Plate IV.1-9**). In that way the protractor hyoidei depresses the lower jaw.

MECHANISM 5 (11.1 MM SL – …)

FORM – The majority of cranial bones have formed at the 11.1 mm SL stage (**Plate II.1-38**). The structures of importance for this mechanism are: (1) the opercular bone, (2) the interopercular bone, (3) the suspensorium, (4) the ligaments attached to the interopercular bone, (4) the lower jaw and (5) the levator operculi muscle. The opercular bone is already well developed, being triangular in shape. The interopercular bone could not yet be discerned at this stage. However, a ligamentous connection appears to run from the retroarticular process of the lower jaw, up to the anteroventral border of the opercular bone[2]. Consequently, this ligament can functionally replace the interopercular bone in this apparatus. The interopercular bone is present shortly after, at 11.6 mm SL. The lower jaw is now composed of two bone complexes, partially surrounding the Meckel's cartilage (**Plate II.1-23**). At the retroarticular process, the attachment site of the angulo-ceratohyal ligament and the angulo-interopercular bone are marked by small processes (**Plate II.1-33A**). Inserting on the dorsal margin of the

[2] VANDEWALLE *et al.* (1985) mentioned the absence of this ligament up to the 21.4 mm stage of *Clarias gariepinus*.

opercular bone, the levator operculi muscle can already be distinguished from the 6.2 mm SL stage [6.8 mm stage of SURLEMONT & VANDEWALLE (1991)] (**Table II.2-1, p. 137**). It runs to the lateral face of the neurocranium (see II.2.4). **Plate IV.1-10** shows the elements involved in a 125.5 mm SL juvenile *Clarias gariepinus*.

FUNCTION – This opercular four-bar system is well known to play an important role in mouth opening in many teleostean fishes (AERTS & VERRAES, 1984). In this case, the contraction of the levator operculi makes the opercular bone to rotate around the opercular process of the suspensorium (**Plate IV.1-11**), consequently retracting the interopercular bone. As a result, the interopercular bone pulls onto the retroarticular process of the lower jaw, thereby opening the mouth. In the case of the 11.1 mm SL specimen, where the interopercular bone can not yet be observed, it is possible that the ligament connecting the retroarticular process with the opercular bone functionally replaces the interopercular bone. In this model, the suspensorium acts as a *frame*, onto which a *crank* (represented by the opercular bone) and a *follower* (represented by the retroarticular process of the lower jaw) are hinged. The follower and the crank are connected to each other by the *coupler* (represented by the interopercular bone and the angulo-interopercular ligament) [terminology according to AERTS & VERRAES (1984)].

DISCUSSION

A SHIFT IN MOUTH OPENING MECHANISMS DURING ONTOGENY

Mouth opening in fishes occurs by a wide range of mechanisms, going from very simple to a whole complex of integrated couplings. Simple mechanisms involve the coupling of hyoid depression to the mandibular depression (OTTEN, 1982; DE LA HOZ & ALDUNATE, 1994; HUNT VON HERBING *et al.*, 1996b). Mouth opening becomes more complex, once four-bar systems are responsible (ANKER, 1974; AERTS & VERRAES, 1984; WESTNEAT, 1990; 1994). More complex mouth opening mechanisms involve the coupling of mandibular depression to a whole set of other mechanisms: neurocranial elevation (MULLER, 1987), intracranial angulation (LAUDER, 1980b), maxillary rotation (WESTNEAT & WAINWRIGHT, 1989; WESTNEAT, 1990), premaxillary protrusion (WESTNEAT & WAINWRIGHT, 1989; WESTNEAT, 1990) and even lower jaw protrusion (WESTNEAT & WAINWRIGHT, 1989).

As the complexity of such a mechanism is related to the number of elements involved, it can be expected that the complexity of these mechanisms will increase during ontogeny. Consequently, a shift in mouth opening mechanisms during ontogeny is not unfavourable. Such a shift appears to be present in *Clarias*, but has been observed in many other teleosts as well (VERRAES, 1977; OTTEN, 1982; LIEM, 1991; HUNT VON HERBING *et al.*, 1996b; 1997). In general, two types of mouth opening mechanisms are mentioned: (1) the hyoid mechanism and (2) the opercular mechanism. The presence of a passive mouth opening mechanism has generally been overlooked. It was SURLEMONT *et al*. (1989) who mentioned the role of the cartilaginous connection between Meckel's cartilage and the suspensorium in mouth opening. This mechanism can remain functional until a true mandibular articulation is formed, thus at 5.6 mm SL. This, however, would implicate that between this moment and the onset of the hyoid mouth opening mechanisms (at 6.2 mm SL) (see below), the larvae would not be able to open their mouth (**Plate IV.1-12**). Although the larvae then still perform endogenous feeding, mouth opening will become more crucial for respiratory purposes. It is consequently to be expected that a mechanism should enable mouth opening during that phase. In *Clarias gariepinus*, this mechanism presumably involves the cartilaginous interhyal, which is continuous with both suspensorium and hyoid bar, as well as some

muscles connecting the hyoid bar to the lower jaw (*i.e.,* the intermandibularis posterior and the interhyoideus anterior). Again, this mechanism would rely on the elastic properties of cartilaginous connections, with mouth opening enabled by their passive recoil, after being tightened.

The hyoid mechanism involves the coupling of the hyoid depression to the mandibular depression, through the ligamentous connection between both structures (*i.e.,* the ligamentum angulo-ceratohyale of the present study). The presence of this ligament is believed to be related to the length of the lower jaw and the interopercular bone (VERRAES, 1977). Teleosts with long lower jaws and short interopercular bones tend to possess this ligament. The ligament can be observed in larval stages of catfish (*Clarias gariepinus*), salmons (*Oncorhynchus mykiss*) and cod (*Gadus morhua*) (VERRAES, 1977; HUNT VON HERBING *et al.*, 1996b), as well as in many adult teleosts (DE LA HOZ & ALDUNATE, 1994). In cichlids (*Astatotilapia elegans*) and embiotocids (*Micrometrus minimus*), such a ligament is absent (OTTEN, 1982; HUNT VON HERBING *et al.*, 1996b). As mouth opening is crucial, even in larval fish, it can be expected that in the latter teleosts another mechanism will have to allow mouth opening. It was observed that another hyoid system is responsible for mouth opening: the hyoid depression is coupled to a mandibular depression through the contraction of the protractor hyoidei (DATTA MUNSHI & SINGH, 1967; OTTEN, 1982). In *C. gariepinus*, the protractor hyoidei is connected to both the lower jaw and the hyoid from 5.0 mm SL on (**Table II.2-1, p. 137**). However, the role of the protractor hyoidei, as a mouth opener, does not depend on its presence and its insertions only, but also on the position of its working line in relation to the mandibular joint (OTTEN, 1982). As long as this working line runs ventral to the mandibular joint, a contraction of the protractor hyoidei will enable mandibular depression. However, once the working line comes to lie above the articulation, the muscle shifts from being a mouth opener to being a mouth closer. This could be observed in a cichlid, where at six days posthatching, the mouth can no longer be opened by the protractor hyoidei muscle (OTTEN, 1982) (**Plate IV.1-13**). As in cod the protractor hyoidei remains a mouth closer, the presence of the angulo-ceratohyal ligament is crucial for allowing mouth opening early during development (HUNT VON HERBING *et al.*, 1996b). In *C. gariepinus*, the protractor hyoidei remains a mouth opener during ontogeny. This means that, theoretically, both hyoid mechanisms, *i.e.,* with the ligamentous and the muscular connection, can generate a mandibular depression.

One parameter of the cranial morphology of a developing larva that plays a crucial role in the function of the protractor hyoidei is the quadrate angle (**Plate IV.1-14**). This angle is an indication for the ventral and rostral displacement of the mandibular joint during ontogeny (OTTEN, 1982; HUNT VON HERBING *et al.*, 1996b). A large quadrate angle generally implicates that the mandibular joint comes to lie ventral to the protractor hyoidei working line. In *Astatotilapia elegans*, this angle increases rapidly (up to about 90°), followed by a much slower increase. The bending point is situated just before six days posthatching, the moment the protractor hyoidei shifts its function (**Plate IV.1-13**) (OTTEN, 1982). In *Gadus morhua*, the quadrate angle is larger during the entire ontogeny, raising up to its maximum (about 160°) at the moment the opercular mechanism becomes functional. The maximal quadrate angle at the moment the opercular mechanism becomes functional appears to be the general trend in three of the five species mentioned (not in *A. elegans* and *M. minimus*) (**Plate IV.1-14**). The low quadrate angle in *Clarias gariepinus* supports the idea of the mouth opening function of the protractor hyoidei during the entire ontogeny, whereas the same can be suggested for *Oncorhynchus mykiss*. The low values in *C. gariepinus* can be explained by the dorsoventrally flattening of the skull, as well as the elongation of the lower jaw. Low quadrate angles are observed in most species at the moment the yolk sac is resorbed (except for *A. elegans*).

Kinematically, both hyoid mechanisms can hardly be distinguished from each other. However, the ligamentous hyoid mechanism involves a simultaneous depression of the hyoid bar and the lower jaw (HUNT VON HERBING *et al.*, 1996b), whereas in the muscular hyoid mechanism, the hyoid depression is observed to follow the mandibular one shortly after (OTTEN, 1982). In 6.2 mm SL larvae, the hyoid depression was observed to initiate shortly after the mandibular depression, which might indicate that the muscular hyoid mechanism is responsible for mouth opening (SURLEMONT & VANDEWALLE, 1991). If so, the question can be raised concerning the functionality of the ligamentous hyoid mechanism.

Apart from the direct ligamentous connection, an indirect connection between the hyoid bar and the lower jaw is possible also. In several teleosts, a ligamentous connection is found between the hyoid bar and the interopercular bone, the latter being ligamentous connected to the lower jaw [*e.g.,* Cottidae (YABE, 1991); Bagridae (BHIMACHAR, 1933)]. In *C. gariepinus*, no true ligament could be discerned, but the fact that the interopercular bone lies close against the lateral face of the hyoid bar, to which it is connected by undifferentiated connective tissue, may be sufficient for some force transfer.

The opercular mechanism is observed in most adult teleosts, where it functionally replaces or assists to the hyoid mouth opening mechanisms (ANKER, 1974; OTTEN, 1982; DE LA HOZ & ALDUNATE, 1994). The contraction of the levator operculi during mouth opening has been demonstrated by electromyographs (OSSE, 1969; ELSHOUD, 1978; LAUDER & LIEM, 1980; LAUDER, 1981; LIEM, 1984; WAINWRIGHT & TURINGAN, 1993). As mentioned above, this mechanism functionally replaces the hyoid mechanism during ontogeny in a cichlid fish, at the same moment that the protractor hyoidei shifts from being a mouth opener to being a mouth closer (**Plate IV.1-13**) (OTTEN, 1982). In this species, the ontogenetic shift is thus crucial for the survival. The shift from hyoid mechanism to opercular mechanism occurs very early in ontogeny in those species which lack an angulo-ceratohyal ligament (after 6% of the larval period) (HUNT VON HERBING *et al.*, 1996b). Apparently, in cichlids the opercular mechanism may play an initial, major role in respiration, as it becomes functional prior to the complete yolk sac resorption. In most other teleosts, however, the opercular mechanism becomes functional after the yolk has been resorbed (**Plate IV.1-14**). In these cases, this shift may be related to a possible shift in feeding types (see below).

The opercular four-bar system can be categorised as being kinematically efficient or force efficient (AERTS & VERRAES, 1984). A kinematically efficient system implicates that little opercular rotation will induce a substantial mandibular rotation (or depression). This, however, implicates that this system is force inefficient and that the input force will have to be large (or the output force will be low). In their paper, AERTS & VERRAES (1984) made a model to measure the efficiency (kinematical or force) of four-bar systems in four different groups of teleosts (Scorpaenidae, Serranidae, Balistidae and Labridae). In this study, it is attempted to apply the data of large juvenile *Clarias gariepinus* (127.0 mm SL) onto that model, in order to compare it with other teleost groups. Additionally, the efficiency of the system is calculated for three other ontogenetic stages of *C. gariepinus* (11.6 mm SL, 12.7 mm SL, 21.5 mm SL), in order to see whether any shift occurs during ontogeny. The values of the mandibular depression, the kinematic efficiency[3], the force efficiency[4], the output velocity[5] and the output force[6] of different ontogenetic stages of *C. gariepinus*, compared with other teleosts are given in **Plates IV.1-15-19**. The opercular system of juvenile *C. gariepinus* appears to be very low in kinematic efficiency, compared to the other teleosts compared (**Plate IV.1-16A**). For a certain opercular rotation (*e.g.,* 10°), the lower jaw is only rotated ventrally for 1°, whereas the values in the other groups are much higher (Scorpaenidae: 34°; Balistidae:

[3] The *kinematic efficiency* is defined as the ratio of the mandibular rotation (in degrees) to the corresponding opercular displacement (in degrees).
[4] The *force efficiency* is the inverse of the kinematic efficiency, and is defined as the ratio of the mandibular depression force and the opercular retraction force.
[5] The *output velocity* gives the relation between the input velocity at the interopercular-opercular connection and the output velocity at the rostral tip of the lower jaw (the ratio of the lever arm of the opercular bone and the length of the lower jaw is incorporated).

48°; Labridae: 41°) (**Plate IV.1-15A**). When looking at the output force diagram, the curve of *C. gariepinus* lies much higher than that of the other groups, especially during the initial opercular rotation of 10° (**Plate IV.1-19A**). During ontogeny, a striking shift occurs in the efficiency of the opercular system. The kinematic efficiency is very low early during ontogeny (11.6 mm SL), but gradually rises as the larva grows (**Plate IV.1-16B**). A peak is obtained in the 21.5 mm larva, where the kinematic efficiency is much higher than in the previous two stages. However, the values appear to decrease heavily at some point during further ontogeny, as in the 127.0 mm specimen, the kinematic efficiency is even lower than in the 11.6 mm larva (**Plate IV.1-16B**). When looking at the output force factor, this trend is the same (but inverse then), although the values of the 11.6 mm and 12.7 mm larvae come to lie close to each other (*e.g.,* for an opercular rotation of 10°: 0.22 in 11.6 mm and 12.7 mm, 0.13 in 21.5 mm and 0.39 in 127.0 mm). When putting all the data together, all ontogenetic stages of *C. gariepinus* can well be distinguished from the other teleosts. However, the output force factor of the 21.5 mm larva equalises that of balistids at an opercular rotation of 12° (**Plate IV.1-19C**). For even further rotations, the output force is lower than that of balistids (**Plate IV.1-19C**).

Although in cichlids there is an acute shift from the hyoid mechanism to the opercular mechanism, there is no indication that an overlap of functionality of both mechanisms is not possible in other teleosts. As can be derived from **Plate IV.1-12**, an overlap of the presence of mouth opening apparatuses (those that enable the hyoid mechanisms and the one that enables the opercular mechanism) occurs in *Clarias gariepinus*. WESTNEAT (1990) observed in labrids that the moment of maximal opercular rotation does not always coincide with the maximal gape, but frequently precedes it. This indicates that mouth opening is not solely depending on the opercular mechanism. AERTS *et al.* (1987) proposed that in cichlids, the role of the opercular mechanism involves the triggering of mouth opening, whereas the mandibular depression is exerted by the contraction of the hyoid muscles (protractor hyoidei and/or sternohyoideus). It is generally observed that during the preparatory phase of a suction action, the mouth is closed (COOK, 1996). In this phase, the protractor hyoidei is responsible for the mouth closure, if of course its working line lies above the mandibular articulation (LAUDER, 1981). The position of the working line above this articulation is believed to be a typical feature of suction feeding fish. In that way, the protractor hyoidei compresses the buccal cavity as much as possible during this preparatory phase, which will increase the suction force during the expansion or suction phase. Additionally, by doing so, the lower jaw is kept under tension in an elevated position. If now the working line of the protractor hyoidei is lowered by the opercular four bar system (through an initial mouth opening), the mouth opening can be triggered in a very rapid manner (AERTS *et al.*, 1987). In non-suction feeding fishes, the working line of the protractor hyoidei remains below the mandibular articulation and contraction of this protractor, together with the sternohyoideus and hypaxials, then contribute to a fast depression of the lower jaw (LAUDER, 1981).

In some teleosts, the epaxials are known to play an important role as well in this system (MULLER, 1987). However, the presence of the highly modified Weberian apparatus, involving the broadened parapophyses and the fused vertebral centra, may pose some spatial constraints on the elevation possibilities of the neurocranium in *C. gariepinus*.

In some siluriforms, the opercular mouth opening mechanism is lost, as the interopercular bone and the angulo-interopercular ligament are lost (SCHAEFER, 1987). This is a synapomorphy of Loricariidae and Astroblepidae (SCHAEFER & LAUDER, 1996). In these groups, a new connection has formed between the lower jaw and the hyoid bars. A cartilaginous plug is present between the hypohyals and the mandibular symphysis (SCHAEFER & LAUDER, 1986). Additionally, the protractor hyoidei is subdivided into two parts: one dorsal part inserting on the lower jaw (and the hyoid bar) in a direct manner, the other part inserting onto this cartilaginous plug. The

[6] The *output force* factor gives an idea of the progression of the force transmission during the opercular rotation (the ratio of the lever arm of the levator operculi and the

contraction of the dorsal part would enable mandibular depression. Such a dorsal subdivision of the protractor hyoidei can also be observed in *Clarias gariepinus* (**Plate II.2-25B**) (see II.2.3).

A SHIFT OF FEEDING TYPE DURING ONTOGENY

It is generally observed that with age the prey capture success increases. Consequently, mouth opening will have to be improved in order to occur faster (COUGHLIN, 1994; COOK, 1996). As it has been estimated that the prey size of 0.6 times a fish mouth size gives the greatest energetic benefit in relation to time cost, the gape size will be important also (GILL, 1997). Changes in feeding mode generally involve changes in ram- or suction-actions during feeding (COOK, 1996). These changes are the result of ontogenetic alterations in morphology and behaviour. In *Lates calcarifer*, the capture efficiency in early larvae (*i.e.,* 10-20 hours after hatching) largely depends on the size of the larvae, whereas in older larvae (*i.e.,* 60-70 hours posthatching) the feeding ability was greatly improved by the ontogenetic differentiations of the skull. At that stage, structures have formed which allow suction/grasping and manipulation of prey, whereas earlier prey capturing was depending on suction abilities only (KOHNO *et al.*, 1996a). Because of the fact that early larvae live in "an environment with a density 800 times and viscosity 30 times that of air", they require a highly effective mechanism for prey capturing (OSSE, 1990). This could be done by producing a high forward thrust, in order to engulf prey, or by producing a high suction force. However, as OSSE (1990) stated that when "larvae start external feeding, their suction forces must be high because little contribution of swimming and none of protrusion is available", many fish larvae start as suction feeders (OSSE, 1990; KOHNO *et al.*, 1996a). In carp larvae of 6.5 mm, a negative pressure inside the orobranchial cavity has been estimated to reach –300 Pa (DROST *et al.*, 1988). Suction feeding has been observed in other teleostean larvae as well, *e.g.,* in *Amphiprion perideraion* from three days posthatching (COUGHLIN, 1994). On the other hand, other feeding types have been suggested for larval *Chanos chanos* (Gonorhynchiformes), which appears to be adapted to straining (or strict ram feeding) (KOHNO *et al.*, 1996b). It can also be observed that the swimming ability of these larvae is already well developed.

One crucial aspect for enabling suction feeding is the presence of the opercular cover (OSSE, 1990). This implicates that for cod, suction feeding is presumably only possible after three to four weeks posthatching (about 6 mm larvae), as the opercular bone fails to develop until then (HUNT VON HERBING *et al.*, 1996a). In *Clarias gariepinus*, the opercular bone is the first bone to develop (already present at 4.1 mm SL, one day posthatching). Initial orobranchial expansions are theoretically possible from 5.0 mm SL on (SURLEMONT *et al.*, 1989) (**Table II.2-2, p. 148**). These involve the abduction of the suspensoria, the elevation of the neurocranium and the depression of the lower jaw (see II.2.4). Functional teeth are observed shortly after (at about 6.0 mm SL) (**Plate II.1-15**). At that stage, however, the larva still possesses a yolk sac, implicating that active feeding is not crucial yet. It may be possible that any suction action at that time is for respiratory purposes mainly.

Mouth opening mechanisms are of great importance for suction feeding fish, as a fast mouth opening is advantageous. For those fish, the speed of mouth opening will be improved during ontogeny. For the opercular mouth opening mechanism, the kinematic efficiency is a good indicator. It can consequently be suggested that during early ontogeny, *C. gariepinus* increases its mouth opening speed (maximal values calculated for the 21.5 mm SL stage), which later on drastically drops again (**Plate IV.1-16B**). It can thus be suggested that larval *C. gariepinus* performs suction/grasping feeding, where the importance of suction becomes less as the larvae become larger (at least when larger than 21.5 mm SL). Apart from changes in mouth opening, changes in the overall skull form are essential also. Suction feeding is improved if the skull

length of the lower jaw are incorporated).

changes from tube-like to cone-like, implicating pointed snouts and high heads (ALEXANDER, 1965; 1967a; MULLER & OSSE, 1984; HUNT VON HERBING *et al.*, 1996b). On the other hand, species with low suspensoria and broad mouths are better adapted to perform ram feeding (HUNT VON HERBING *et al.*, 1996b). In *C. gariepinus*, head and suspensorial depths tend to decrease (in relation to head length) during ontogeny. In the juvenile and adult specimens, the mouth is far from pointed, but rather broad and flat (**Plate II.2-25A**). This does not imply that juvenile and adult *C. gariepinus* do not perform any suction. As demonstrated by the experiments of ALEXANDER (1970), catfishes with dorsoventrally flattened heads (*e.g., Ictalurus*, Ictaluridae) do generate a suction force during prey capturing. This force, however, is lower then any other teleost tested in the experiments. The fact that *C. gariepinus* becomes cannibalistic at 8 mm length, is an indication that feeding must occur by suction/grasping, in which grasping is most important. Feeding on prey of about the same size as the predator would require an enormous suction force, if feeding occurred only by suction.

A SHIFT OF FUNCTIONAL DEMANDS DURING ONTOGENY

The shift of mouth opening mechanisms and the shift in feeding types can be expected to be a response to a shift in functional demands, which the developing larva experiences. As mentioned above, an ontogenetic shift in diet is present in most teleosts, as a response to increasing nutritional demands. The timing of the mouth opening mechanism formation and the functional demands will be crucial for the survival of the larva. As observed in some cichlids, the timing of the shift between two mechanisms can be that acute that within one day the larva is doomed to die of starvation or to be able to feed (OTTEN, 1982). It would, though, be advantageous that a safety factor should be incorporated, in order to reduce such vulnerable moments during ontogeny (GALIS *et al.*, 1994). This can be done in different ways: (1) the elements of a certain apparatus can be formed prior to the moment that the involved mechanism is needed, or (2) the functioning of different mechanisms can chronologically overlap: when one mechanism becomes inoperative, the functional demand can still be dealt with by the other. Although functional demands for feeding and respiration are absent in viviparous embiotocids (feeding is intraovarian, whereas respiration occurs through enlarged and highly vascularised median fins), a synchronous shift does occur of the protractor hyoidei becoming a mouth closer, and the opercular mouth opening mechanism becoming functional (LIEM, 1991). In this case, a new reproductive strategy reduces the changes of mortality because of possible asynchronies between ontogenetic functional shifts of cranial elements.

Mouth opening will be of importance for feeding and respiratory movements during ontogeny. In this study, it is attempted to investigate to what extent a relation can be found between the timing of the different mouth opening mechanisms during ontogeny, and some morphological, behavioural or physiological changes, related to nutritional or respiratory demands (**Plate IV.1-20**). Based on these data, five main phases can be recognised in the life history of *Clarias gariepinus*.

PHASE 1: Initially, at hatching, no mouth opening is possible. Respiration must occur through cutaneous diffusion, as no sign of any gills are observed. The hyoidean vascular net may function as a respiratory organ (GREENWOOD, 1955). The ventilation of the boundary layer of water, surrounding the larva, occurs through undulatory movements of the notochord, which already results in forward locomotion. Feeding is completely dependent on the yolk sac (HECHT & APPELBAUM, 1987; KAMLER *et al.*, 1994).

PHASE 2: The first movements of the lower jaw are possible: mouth closing through contraction of the adductor mandibulae and a passive mouth opening through mechanism 1, followed by mechanism 2. These restricted mandibular movements may allow some volume changes of the buccal cavity. At this moment, the gill filaments have started to form, indicating a possible respiratory shift. The formation of the opercular cover, as well as its initial movements, may take part in the initial respiratory pumping mechanism. As the yolk sac is still substantial at this stage, the movements of the mouth are presumably for respiratory purposes only. However, this phase spreads close to the end of the yolk sac period, indicating that active feeding will become necessary at the end of this phase.

PHASE 3: This phase starts at the transition from endogenous to exogenous feeding. As the yolk sac period is terminating, adaptations to active feeding become essential. The onset of this phase is characterised by the formation of the hyoid mouth opening mechanisms (mechanisms 3 and 4) (6.2 mm SL). At this stage, teeth have already formed, indicating the potential of active feeding. At the age of three days posthatching (6.5-7.0 mm SL), oxygen consumption reaches a maximal peak for yolk sac larvae of *C. gariepinus*, raised at 25°C (KAMLER *et al.*, 1994). Respiratory requirements will consequently become more important. At 8 mm length, *C. gariepinus* larvae show a cannibalistic behaviour, in which they catch other larvae by the tail, swallow it up to the head, partly digest it, followed by the biting off of the head [cannibalism type I of HECHT & APPELBAUM (1987)]. This indicates that extensive mouth opening is essential and that a powerful adductor mandibulae is needed. At the end of this phase, the opercular mouth opening mechanism becomes functional, presumably as a preparation to the fully carnivorous feeding.

PHASE 4: This phase is characterised by the digestive system, which becomes completely functional now. At 11.5 mm SL, the stomach has formed a clear pylorus, functional glandular cells, whereas the pH has dropped below 5 (VERRETH & VAN TONGEREN, 1989). As mentioned, all mouth opening mechanisms, which are present in the juveniles, are present now. This means that the cannibalistic behaviour can fully be exploited now.

PHASE 5: The last phase is characterised by the shift in respiratory mechanisms. At 20 mm length, the suprabranchial organ is formed (HAYLOR & OYEGUNWA, 1993). This structural innovation, which is typical of clariids, enables them to perform aerial respiration (GREENWOOD, 1961). However, the apparatuses present at that stage appear to be sufficient to allow the required respiratory movements, as now major changes in related structures can be observed. Mandibular and hyoid depression enable the uptake of an air bubble, which is then transported into the suprabranchial cavity by the opposite actions, as well as the opening of the epibranchial part of the third branchial cleft. After gas exchange, the air bubble is transported into the opercular cavity through the opercular abduction, as well as the contraction of muscles of the suprabranchial cavity. Finally, the adduction of the opercular cover presses the air bubble out through the opercular slit (VANDEWALLE & CHARDON, 1991).

A SHIFT IN MOUTH OPENING MECHANISMS DURING EVOLUTION

Finally, a brief comment can be given concerning the evolutionary background of mouth opening mechanisms. The hyoid mechanism, in which hyoid depression is ligamentously coupled to mouth opening, is suggested to be a synapomorphic feature of Teleostomi (LAUDER, 1980a; LAUDER & LIEM, 1980) (**Plate IV.1-21**). It is only from the halecostomian level on that the opercular mechanism takes part in mouth opening (LAUDER, 1980a; LIEM, 1991). The increasing importance of the opercular bone is also suggested by modifications, which increased its mobility, from the halecostomian level on (SCHAEFFER & ROSEN, 1961). Apparently, it seems that the ontogenetic shift in mouth opening mechanisms is reflected in the evolutionary trend for improving mouth opening.

CONCLUSIONS

OSSE (1969) listed six different mouth opening mechanisms in fishes: (1) the contraction of the sternohyoideus and the extension of the protractor hyoidei, (2) the opercular mechanism, (3) the mechanism in which a special abductor mandibulae muscle is involved, (4) the hyoid mechanism with the coupled contraction of the sternohyoideus (for hyoid fixation) and protractor hyoidei (for mandibular depression), (5) the mechanism which involves the ligamentous connection between the interopercular bone and the hyoid bar, (6) the passive abduction of the lower jaw due to absence of tension in the adductor mandibulae. Based on this study, as well as the study of SURLEMONT & VANDEWALLE (1991), two more mechanisms can be added, as well as a modification of mechanism (6). This modification involves the passive depression of the lower jaw due to the relaxation of the adductor mandibulae, but more important due the elastic features of the cartilaginous connection between the mandibula and the suspensorium. Two new mechanisms involve the elastic features of the interhyal with some hyoid muscle action, and the ligamentous hyoid mechanism.

During ontogeny, the shift of a total of five different mouth opening mechanisms in *Clarias gariepinus* guarantees a functional mouth opening mechanism from shortly after hatching. The increase in functional demands on this mechanism is reflected in the increasing complexity and capacity of the successive mechanisms, ranging from a restricted, passive mouth opening to a coupling of different, active mouth opening mechanisms. The shift in mechanisms during ontogeny can also be related to a shift in different feeding types, as a response to an ontogenetic shift in diet. Certain parameters of these mechanisms indicate that suction capacity (relative to body size) may be higher in larval fish, then in the juvenile and the adult fish. Several features indicate that grasping becomes functional early during ontogeny as well.

The increase in complexity of the mouth opening mechanisms during ontogeny is reflected in the evolutionary shift of mouth opening mechanisms: hyoid mechanisms precede the opercular one.

Chapter IV.2 – The loss of the interhyal: an adaptation to a benthic behaviour[1]

In larval *Clarias gariepinus* the cartilaginous interhyal is continuous with the dorsal and ventral part of the hyoid arch. During later ontogeny, the interhyal becomes reduced and is even completely lost in the juvenile and adult specimens. The connection between the suspensorium and the hyoid bar is replaced by a stout ligament, *i.e.,* the ligamentum hyomandibulo-ceratohyale. It is hypothesised that this reduction and functional replacement of the interhyal will reduce the mobility of the hyoid bar. Some additional morphological evidence can be given to support this hypothesis: (1) a short skin fold between the lower jaw and the hyoid bar, (2) short suspensoria, (3) a short sternohyoideus muscle, (4) the position of the sternohyoideus in relation to the hyoid bars, and (5) an unfolded branchiostegal membrane. Some arguments are given which suggest that an extensive hyoid depression is not needed in *C. gariepinus*: (1) an extensive hyoid depression would come into conflict with benthic stability, (2) the aquatic respiration does not require an extensive hyoid depression as a suprabranchial organ enables aerial respiration, (3) a broad skull implicates that less hyoid depression is needed for increasing the volume of the orobranchial cavity and (4) a sudden and substantial depression of the skull floor is not required for suction feeding because adaptations to benthic behaviour and the development of the palatine-maxillary mechanism make a powerful suction feeding impossible.

INTRODUCTION

The interhyal, also referred to as the stylohyal (DAGET, 1964), forms in most teleosts the connection between the dorsal and ventral part of the hyoid arch, respectively the hyosymplectic and the ceratohyal. In a generalised teleost, this interhyal initially consists of a cartilaginous, bar-like structure, which is already, or becomes isolated from the cartilaginous hyoid arch early during ontogeny. In *Clarias gariepinus*, and in most Siluriformes, the interhyal remains continuous with both the hyosymplectic and the ceratohyal (HOEDEMAN, 1960b; HOWES & TEUGELS, 1989; ARRATIA, 1990; ADRIAENS & VERRAES, 1997c) (see II.1.1). In a generalised teleost, the cartilaginous interhyal becomes ossified during ontogeny, except at its two articulatory facets. These articulations come into contact with the hyomandibular and the posterior ceratohyal bones. In catfishes the interhyal, if present, becomes ossified or remains cartilaginous. An ossified interhyal is present in *Callichthys* (Callichthyidae). A rudimentary interhyal can be observed in some primitive species, such as *Diplomystes* (Diplomystidae), as well as highly specialised species like *Nematogenys* (Trichomycteridae) and *Loricaria* (Loricariidae), where the dorsal articulation between the interhyal and the hyomandibular bone is lost (ARRATIA, 1990). In *C. gariepinus* a reduction of the interhyal occurs during ontogeny until it is completely lost (ADRIAENS & VERRAES, 1997c). The loss of the interhyal appears to be a feature observed in several groups of Ostariophysi. In the gonorhynchiform

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Phractolaemus ansorgii (Phractolaemidae) (DAGET, 1964) and the cypriniform *Gobio gobio* (Cyprinidae) (VANDEWALLE, 1975) the interhyal is lacking. In the Siluriformes, the absence of the interhyal appears to be common, although some exceptions do occur, as mentioned above (ARRATIA, 1990).

Commonly, in the adult situation, the interhyal forms two articulatory facets: a dorsal one with the hyomandibular bone and a ventral one with the posterior ceratohyal bone. In general, these articulations are of the ball and socket type, although variation is present in some teleosts. In several cases the interhyal-ceratohyal connection becomes ligamentous (KARRER, 1967; OSSE, 1969; ANKER, 1974; 1989; BIRDSONG, 1975; VANDEWALLE *et al.*, 1982). In *Lipophrys pholis* (formerly *Blennius pholis*) (Blenniidae) the dorsal articulation also becomes ligamentous (VANDEWALLE *et al.*, 1982). In some teleosts, additional ligaments are present, connecting the interhyal to surrounding structures, other than the posterior ceratohyal bone. In *Gasterosteus aculeatus* (Gasterosteidae) a ligamentum interhyalo-suspensoriale is present, connecting the interhyal to the preopercular bone (ANKER, 1974), as is also the case in *Microgobius* (Gobiidae) (BIRDSONG, 1975) and *Haplochromis* (Cichlidae) (ANKER, 1989). In *Ammodytes* en *Embolichthys* (Ammodytidae) a ligament connects the bony interhyal with the interopercular bone (PIETSCH & ZABETIAN, 1990). In those teleosts where the interhyal is absent, a ligament is present between the suspensorium and the hyoid (VANDEWALLE, 1975; ARRATIA, 1990). This is also the case for *C. gariepinus*, where a stout ligament runs from the hyomandibular bone to the posterior ceratohyal bone. The absence of the interhyal in adult *C. gariepinus* and the presence of a ligament was also noted by NAWAR (1954), however, no functional interpretation of this transformation was given.

The loss of the interhyal and its replacement by a stout ligament in *C. gariepinus* will probably influence the mobility of the hyoid. As stated by ANKER (1989), the interhyal plays an important role in the four bar system of the hyoid, the interopercular bone, the lower jaw and the suspensorium. In this system the length of the interhyal is related to the movement range of the hyoid, and thus of the lower jaw. Apart from their role in the opening of the mouth, the hyoid bars play an important role in the depression of the mouth floor and the lateral expansion of the orobranchial cavity (AERTS, 1991). Both these actions are crucial for generating a substantial suction force into the mouth cavity.

ARRATIA (1990) stated that the loss of the interhyal and its replacement by a ligament is a result of the strongly dorsoventral flattening of the skulls. This chapter deals with the functional significance of the loss of the interhyal in *C. gariepinus* and its possible effect on the movements of the hyoid. The interhyal and surrounding structures are studied in three ontogenetic stages of *C. gariepinus*, as well as additional data on the ontogeny is given in chapter II.1.1 and II.1.2.

RESULTS

Some morphological features will be given, concerning three ontogenetic stages of the suspensorium, interhyal and hyoid bar in *Clarias gariepinus*. The overall ontogeny of these structures is discussed in chapter II.1.1 and II.1.2, and will consequently not be repeated here. The results in this chapter will be restricted to some, previously not mentioned details that are relevant to understand the ontogenetic transformation and the impact on hyoid depression.

7.2 MM SPECIMEN

The interhyal forms a continuous bar between the hyosymplectic and the ceratohyal (**Plate IV.2-1**). Muscles, related to hyoid depression and elevation can be observed also. The sternohyoideus inserts onto a ventral process of the anterior copula (see II.2.3). The protractor hyoidei inserts onto the ceratohyal, connecting it with the lower jaw (see II.2.3). A ligamentous connection is present between the ceratohyal and the lower jaw. This ligamentum angulo-ceratohyale runs from the posterolateral face of the ceratohyal up to the ventrocaudal face of the retroarticular process of Meckel's cartilage (see IV.1).

46.8 MM SL SPECIMEN

The suspensorium is completely ossified in this stage (see II.1.2). The interhyal still lacks any ossification. At its connection with the ceratohyal, the cartilaginous tissue consists of strongly packed, large chondrocytes with little intercellular matrix, thus appearing to be articular cartilage (*e.g.*, fibro-cell rich cartilage). A true connection, as could be observed in smaller specimens, however, cannot be observed anymore. The connection of the interhyal with the hyosymplectic, on the other hand, is still more firmly. This connection is situated in between the hyomandibular bone and the quadrate bone, at the level of the cartilaginous area, which presumably corresponds to the unossified symplectic. The interhyal is covered laterally by the preopercular bone (**Plate IV.2-2**). VANDEWALLE *et al.* (1985), however, mentioned that in a 21.4 mm SL specimen of *Clarias gariepinus* "les barres hyoïdiennes deviennent articulées … par rapport au suspensorium". The reduction of the interhyal appears to occur extremely gradually, as this situation at 21.4 mm SL is not different from that at 46.8 mm SL.

Medially to the interhyal a stout ligament is present, connecting the hyomandibular bone to the posterior ceratohyal bone. This ligamentum hyomandibulo-ceratohyale is attached to the medial face of the hyomandibular bone, ventrally to the posterior insertion of the adductor arcus palatini. It runs up to the dorsal face of the posterior ceratohyal bone, right in front of the articulation with the interhyal.

The muscles involved in hyoid depression and elevation are completely formed in this stage (ADRIAENS & VERRAES, 1997d) (**Table II.2-2, p. 148**) (see II.2.4). The sternohyoideus connects the pectoral girdle with the hyoid bar, by means of the parurohyal bone and the paired ligamenta parurohyalo-hypohyalia. The protractor hyoidei is well developed and has become differentiated into a pars dorsalis, a pars lateralis and a pars ventralis (**Plate II.2-24B**) (see II.2.3). The pars dorsalis is attached to the lower jaw through two tendons, whereas the other parts insert musculously onto the lower jaw and a median aponeurosis.

125.5 MM SL SPECIMEN

The interhyal is completely absent now. The only firm connection remaining between the suspensorium and the hyoid bar is the stout ligamentum hyomandibulo-ceratohyale (**Plate IV.2-3**). This ligament is attached to a ridge on the medial face of the hyomandibular bone and runs up to the dorsal face of the posterior ceratohyal bone. The morphology of the sternohyoideus and protractor hyoidei muscles corresponds to the previous stage (**Plates II.2-25, II.2-27C**).

DISCUSSION

According to VANDEWALLE *et al.* (1985) and SURLEMONT & VANDEWALLE (1991), the functional significance of a cartilaginous interhyal, continuous with the dorsal and ventral part of the hyoid arch, is related to the mechanical properties of cartilage. Such a connection assists in a passive recovery displacement of the hyoid bars after their depression. For the role of the continuous interhyal in mouth opening I refer to Chapter IV.1.

ANKER (1989) stated that the range of the movement of the hyoid increases with a longer interhyal. The loss of the interhyal and its functional replacement can thus be expected to influence the movements of the hyoid bar. Moreover, the rotation of an interhyal can be related to the abduction of the lower jaws as well as the abduction and the rotation of the hyoid bars (ANKER, 1974). The replacement of a skeletal element through a ligament can be functionally interesting when only tensile forces have to be applied. When compressed, the ligament would become folded, thus reducing the ventral excursion of the posterior part of the hyoid, during its forward and backward swinging. Consequently, the main movement of the hyoid bar would involve an anteroposterior translation instead of a rotation (**Fig. IV.2- 1**).

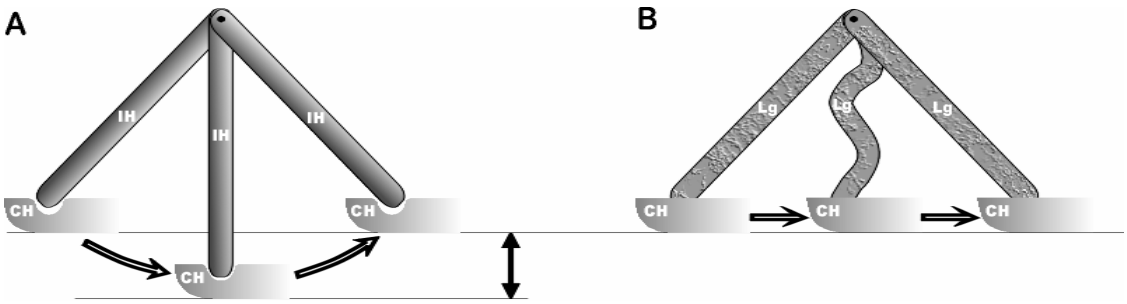

Fig. IV.2- 1: Scheme: A. Rotation of an interhyal (IH), with a ventral excursion of the hyoid bar (CH); B. replacement of a rigid interhyal with a ligamentum hyomandibulo-ceratohyale (Lg), with an anteroposterior translation of the hyoid bar

As the interhyal is lacking in adult *Clarias gariepinus*, it can be expected that this will affect the rotation possibilities of the hyoid bar, more specifically, its depression. Some morphological evidence can be given which supports this hypothesis:

1. The rostral tip of the hyoid bar is connected to the lower jaw through skin and connective tissue. To what extent the hyoid can be displaced ventrally, partially depends on the length of that connection, as well as the depression possibilities of the lower jaw itself. Cross sections show that the skin fold between lower jaw and hyoid bar is rather short, only allowing a restricted ventral displacement of the hyoid tip (**Plate IV.2-4A**). The lower jaw can be depressed very little also, as a short skin fold between the mandibula and the maxillary barbel restricts it (**Plate II.2-9**).

2. During hyoid depression, the posterior tips of the hyoid bars are abducted. This action assists in the expansion of the orobranchial cavity through abduction of the suspensoria (AERTS, 1991). A strong abduction of the suspensoria would require a strongly developed articulation with the skull. In most teleosts, the suspensorium articulates with the skull both anteriorly (palatine) and posteriorly (hyomandibular). In most non-ostariophysan teleosts, the posterior articulation consists of two consecutive ball and socket joints (BAREL *et al.*, 1976b; BARLOW *et al.*, 1968; OSSE, 1969; GARDINER,

1973; ANKER, 1974; BENMOUNA *et al.*, 1984; MESTERMANN & ZANDER, 1984; VANDEWALLE *et al.*, 1982; BRILL, 1988; BELLWOOD & CHOAT, 1990; ARRATIA & SCHUILTZE, 1991). These allow a firm attachment and substantial mobility in a mediolateral direction (see II.2.4). In Siluriformes, not only is the palatine articulation lost (see II.1.1 and II.2.2) but the posterior articulation is established by a ridge, instead of ball and socket articulations (ARRATIA, 1992). This may be considered as an adaptation to the loss of the palatine connection, in order to withstand torque forces on the hyomandibular articulation more adequately. However, this adaptation may suggest a reduction in rotation possibilities of the suspensorium in the mediolateral direction. A supportive argument is the fact that because of the dorsoventrally flattening of the skull, the suspensorium has become less high. Such a shortening will affect the lateral displacement of the ventral margin of the suspensorium during rotation, and consequently the abduction of the posterior tips of the hyoid bars (**Fig. IV.2- 2**). As ALEXANDER (1970) stated, in fish with dorsoventrally flattened heads, the depression of the hyoids plays a relatively more important role for increasing the volume of the orobranchial cavity than in fish with laterally depressed heads. In the latter, the abduction of the suspensorium is far more important.

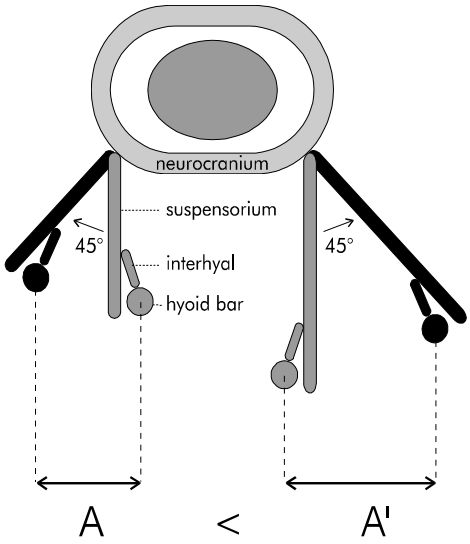

Fig. IV.2- 2: Scheme of the abduction of the suspensoria: A and A' indicate the difference in the lateral displacement of the posterior tip of the hyoid bars in short (A) and high (A') suspensoria

3. Muscular evidence for a reduced depression of the hyoid bars in *Clarias gariepinus* can be given as well. The architecture of the sternohyoideus, which is responsible for the depression of the hyoid bar, indicates that in *C. gariepinus*, a contraction of the muscle will not generate a substantial displacement of the rostral tip of the hyoid bars. In those teleosts, where a large depression is needed, the sternohyoideus muscle is rather long and slender (ANKER, 1974; 1989) (**Plate IV.2-5**). In *C. gariepinus* the muscle consists of three myomeres, separated by myocommata, as is the case in most teleosts (WINTERBOTTOM, 1974; ADRIAENS & VERRAES, 1997d) (see II.2.3). The muscle is rather broad but short. This architecture implicates that during contraction, a larger contraction force can be generated, but a reduced displacement of the rostral tip of the hyoid bar will take place. What has to be taken in account is the protagonist function of the inferior obliquus muscle, which is the hypaxial muscle inserting on the posterior border of the cleithral bone. This muscle plays an important role in the retraction of the pectoral girdle, and thus in the retraction of the hyoid bar if contraction is synchronous with that of the sternohyoideus (OSSE, 1969; MULLER, 1987). However, cross sections show that in *C. gariepinus*, the ventral part of the inferior obliquus muscle is rather small.

4. The contraction force of the sternohyoideus onto the hyoid bars is spread over three expansion movements: (1) depression of the mouth floor, (2) abduction of the suspensorium and the opercular bone, and (3) opening of the mouth. However, in case the action line of this contraction

force comes to lie in or above the horizontal plane through the hyoid bars, the depression of the mouth floor is annulled. In case the hyoid bars come to lie in or lateral to the vertical plane of the suspensoria, the abduction of suspensoria and opercular bone is absent (DE VISSER & BAREL, 1996). If these two conditions are fulfilled, the contraction of the sternohyoideus will only cause the depression of the lower jaw. Now, in *Clarias gariepinus* the skull has become dorsoventrally flattened to a large extent. This has resulted that the hyoid bars have come to lie in a horizontal plane, whereas the sternohyoideus has taken position in between these bars, also in an approximately horizontal plane (**Plates II.2-27C; II.2-36A**). The contraction of the sternohyoideus will consequently have a reduced hyoid depression activity. Secondly, the long hyoid bars in *C. gariepinus* would reduce the hyoid angle with the medial plane, when adducted [= α_{add} of DE VISSER & BAREL (1996)]. This is, however, partially compensated by the broadening of the skull, resulting in an α_{add} of approximately 38° (in the 100 day old specimens) (**Plate II.2-27C**). But, as mentioned in argument 2, the short suspensoria restrict the abduction possibilities of the hyoid bar anyway [= α_{max} of DE VISSER & BAREL (1996)]. It thus appears that in *C. gariepinus*, the contraction force of the sternohyoideus is mainly converted into a retraction of the hyoid bar, resulting in the opening of the mouth. The fact that in *C. gariepinus* the skeletal interhyal is replaced by a pliable ligament, might also implicate that mouth opening can, hypothetically, be performed without any depression of the hyoid bars (**Fig. IV.2- 1**) (see IV.1). In that case, a contraction of the sternohyoideus might retract the hyoid posteriorly, without any ventral rotation around the interhyal.

5. A large expansion of the orobranchial cavity involves an expansion of the branchiostegal membrane. This membrane can be extended from a folded situation, due to the support by a series of branchiostegal rays and some muscles acting onto them (WINTERBOTTOM, 1974; ADRIAENS & VERRAES, 1997d). In *Clarias gariepinus*, this membrane is well developed but rather firm (**Plate II.2-9**). However, cross sections show that the thick branchiostegal membrane is not folded in an adducted position (**Plate IV.2-4B**), which implicates a restricted expansion possibility (**Fig. IV.2- 3**). This may be an additional indication of a restricted expansion of the orobranchial cavity.

6. Aquarium observations show that *Clarias gariepinus* exerts a restricted depression of the hyoid during respiration. Also, manipulation of fixed material suggests that little depression of the hyoid bars is possible.

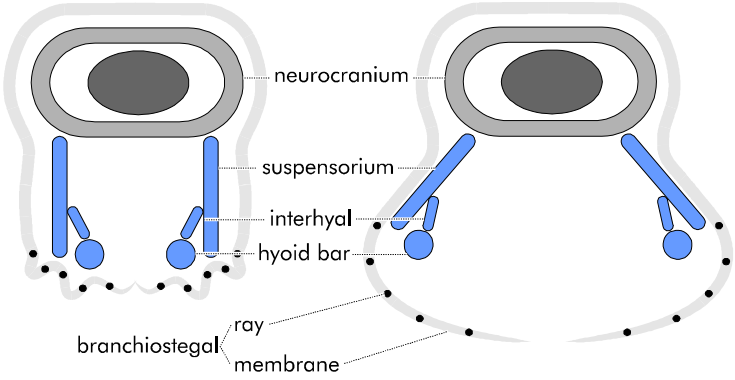

Fig. IV.2- 3: Scheme of the unfolding of the branchiostegal membrane during suspensorial abduction and hyoid depression

Apart from arguments indicating the restricted possibility of extensive hyoid depression, some arguments can be given which indicate that an extensive hyoid depression is not needed in catfishes as *Clarias gariepinus*:

1. Like many benthic fishes, *Clarias gariepinus* has a strongly, dorsoventrally flattened head. This enables them to stay close to the bottom, thus reducing pressure as well as the friction drag of moving water, and to stabilise their benthic postures (ALEXANDER, 1965). However, if respiration would require an extensive hyoid depression, this stabilisation would completely perish.

2. An extensive hyoid depression is probably not required in Clariidae for respiratory purposes. Clariidae have developed a specialised accessory breathing organ, called the suprabranchial organ or arborescent organ (WILLEM, 1951; GREENWOOD, 1961; DATTA MUNSHI, 1961; POLL, 1977). This organ enables these species to capture atmospheric oxygen and allows them to survive in tropical swamps and ponds with low oxygen levels. *Clarias* is also known to make terrestrial excursions between ponds, which is facilitated by aerial respiration (THOMAS, 1966; SPINAGE, 1970; BABIKER, 1984). The process of swallowing and transporting an air-bubble is partially caused by movements of the hyoid bars (HELLIN & CHARDON, 1981; VANDEWALLE & CHARDON, 1991). With the mouth at the water surface, hyoid depression enables the suction of a bubble. The consequently elevation of these bars then presses the bubble from the orobranchial cavity into the suprabranchial cavity. The expel of the bubble from the latter cavity, through the opercular cavity is caused by a second elevation of the hyoid bars. In abnormal *C. gariepinus*, in which the hyoid bars were immobilised, VANDEWALLE & CHARDON (1991) noted that aerial respiration did not occur, although opercular movements were possible. Concerning aquatic respiration, two possible pumping mechanism may be involved: a pressure pump system and a suction pump system (see II.2.3). In the pressure pump system, water is pushed through the gills through elevation of the hyoid bars, whereas in the suction pump system the abduction of the opercular bones suck the water through the gills. It is argued that in bottom-living fishes, the suction pump system is more important for creating a water flow through the gills, than the pressure pump system is (HUGHES, 1970; BALLENTIJN, 1972). Thus, hyoid depression (and consequently elevation) is needed for respiratory purposes (both aquatic and aerial), however, not extensively.

3. The dorsoventrally flattening of the skull can also be seen in terms of broadening. The skull of *Clarias gariepinus* can be considered as a broad one. When compared to most other small eyed catfishes, clariids have a lower eye diameter/interorbital distance ratio, indicating the broader skulls (**Plate IV.2-6**) (BOULENGER, 1909-1916; 1915; STEINDACHNER, 1914; PELLEGRIN, 1923a; 1923b; 1928; GILTAY, 1929; HOLLY, 1930; TREWAVAS, 1936; SCHULTZ, 1942; POLL, 1945; 1954; 1967a; 1967b; DAGET, 1954; MYERS & WEITZMAN, 1954; 1966; CRASS, 1960; BECKMAN, 1962; POLL & GOSSE, 1963; BLACHE, 1964; THYS VAN DEN AUDENAERDE, 1964; ALFRED, 1966; ROMAN, 1966; POLL & DAGET, 1968; LOISELLE & WELCOME, 1971; POLL *et al.*, 1972; GLODEK, 1976; THYS VAN DEN AUDENAERDE & DE VOS, 1982; DE VOS & LEVEQUE, 1983; NIJSSEN & ISBRÜCKER, 1983; 1987; 1990; STEWART, 1985; 1986; STEWART & PAVLIK, 1985; TEUGELS, 1986; VARI & ORTEGA, 1986; RISCH, 1987; MAHNERT & GÉRY, 1987; ROBERTS, 1990; TEUGELS, & THYS VAN DEN AUDENAERDE, 1990; REIS & SCHAEFER, 1992; LUCENA *et al.*, 1992). The broad skull, in for example *C. gariepinus*, implicates that for a certain degree of hyoid depression, the volume increase of the orobranchial cavity will be larger than what would be the case in a narrow skull. This means that less hyoid depression has to be performed for obtaining the same amount of volume increase (**Plate IV.2-7**).

4. A sudden, substantial volume increase of the orobranchial cavity would be essential for performing suction feeding behaviour. Movements of the hyoid bars is one of the mechanisms responsible for producing this large negative pressure in the orobranchial cavity (MULLER, 1987; AERTS, 1991). In generalised suction feeding teleosts, powerful suction is frequently associated with a protrusion of the anterior parts of the mouth, *i.e.,* the premaxillary and maxillary bones (MOTTA, 1984). The premaxillary bone of such teleosts bears a notable ascending process. This process is shorter in biting species, compared to suction feeding ones (WITTE, 1984). In *Clarias gariepinus*, such an ascending process is completely lacking (**Plate IV.2-8D**). The maxillary bone has been completely decoupled from the feeding apparatus as it has become incorporated into the palatine-maxillary mechanism (ADRIAENS & VERRAES, 1997a) (**Plate IV.2-8C**) (see II.2.2). Additionally, the presence of large patches of narrow, conical and villiform teeth on the prevomeral bone and lower jaw, as well as a large adductor mandibulae complex, may indicate an adaptation to powerful grasping and holding prey with the oral jaws, which is not the case in suction feeding fishes (ALEXANDER, 1970; GOSLINE, 1973). All these features suggest that *C. gariepinus* is unable to perform substantial suction feeding in which extensive hyoid depression would be needed. Aquarium observations also show that *C. gariepinus* swims up to potential food, which is then engulfed from right in front of its mouth.

CONCLUSIONS

The ontogenetic shift of the interhyal morphology may be a reflection of its functional shift during ontogeny. Early during ontogeny, the interhyal is continuous with the hyosymplectic and the hyoid bar, which appears to assist in mouth opening at that stage. As the skull becomes enlarged, the elastic properties of a continuous interhyal becomes negligible and muscles take over completely. The reduction of the interhyal starts with the formation of an articulation between the interhyal and the hyoid bar. The connection between suspensorium and hyoid bar, however, is already strengthened by the formation of a ligament. Eventually, the interhyal is lost, supposedly as an adaptation to the benthic behaviour. The arguments presented indicate that the interhyal can functionally be replaced by a ligament, as the mechanical properties of a skeletal interhyal are not required. The impact of the loss of the interhyal and some other adaptations on the restricted hyoid depression possibilities are a reflection of the absence of a need to an extensive hyoid depression.

Chapter V.1 - General discussion

Studies on cranial morphology of vertebrates, and more specifically fishes, have abundantly been done. These studies have served different purposes and have been approached from different disciplines. As the adult, or completely formed skull is the end result of an ontogenetic process, the 'Bauplan' involves much more than it reveals. Along the process of development, at any time, the slightest deviation of the normal developmental curve may result in a substantially different adult skull. Much more can be learned about adult skull morphology, if the ontogenetic background is known. In this chapter, I have opted to present a general discussion on the results of this thesis, for some topics referring to citations from literature as a starting point. For a discussion on the ontogeny itself, *i.e.,* the formation of the elements of the skull 'Bauplan', I refer to the separate discussions in the previous chapters.

V.1.1 - METHODOLOGY

- "The relation between function and form in an element is of an acausal character. It is a relation or correlation instead of a causality" (DULLEMEIJER, 1974)

A causal relation between form and function[1] was the general idea of many early morphologists. Form has been expressed as the causal result of its function, *i.e.,* in teleology[2], whereas others, like GEOFREY ST. HILAIRE saw it the other way around: function was considered to be the effect of the organisation (*i.e.,* the arrangement and structure) of an organ (ASMA, 1996). True causality of form, however, has to be found in morphogenetic processes. The question can be raised whether or not a true causality of function exists. I think that the "cause" of function can only be cut back in an evolutionary context and not in a teleological *'vis vitalis'* context. This aspect becomes especially important when looked at from an ontogenetic point of view, as a developing larva continuously has to struggle with changing environmental factors, as well as the constraints of the 'Bauplan' within. As the larva is growing, certain needs, like respiration or feeding, will arise and will have to be fulfilled in order to survive. In order to satisfy these needs, certain mechanisms will have to be formed, using these 'Bauplan'-parts that are present at that moment. For such a part, the initiation of movement, or the shift from one function to another, will have an impact on the mechanical loading onto that element, which will consequently induce certain changes in one or more of the parameters of form (*i.e.,* presence, shape, size, position or structure). In general, function cannot be seen as a causality of form, but function may be seen as an indirect cause to changes in form *s.l.*, especially during ontogeny.

[1] According to VERRAES (1989) a distinction should be made between "*function*" and simple "*action*". Actions involve all movements, whether they assist in the maintenance of the organism or not, whereas functions imply contributions to self-maintenance. According to DULLEMEIJER (1974), "even *being* a form can be regarded as a function".
[2] In the *teleology*, as applied by CUVIER, the functions of an organism are regarded as the "cause" of that organism's 'Bauplan' (ASMA, 1996).

- "It is a developmental study in functional morphology (*sensu* DULLEMEIJER, 1974) rather than a morphogenetical study dealing with the causation of growth" (OTTEN, 1982)

The study presented here can be described as such, where causal factors at the morphogenetic level are beyond its scope. However, I would say that, based on ontogenetic evidence, as presented in this thesis, some argumentation on indirect causal or non-causal relations between phenomena (be it form or function) may be suggested, as described in the previous paragraph.

V.1.2 - CHANGES IN FORM DURING CRANIAL ONTOGENY: INSEPARABLE FROM FUNCTION

When reading earlier papers and books on comparative morphology, it is astonishing how people managed to discuss endlessly and write monographs on morphology without mentioning anything on function. Once you start to get familiar with some functional interpretations, it becomes obvious that it is practically impossible to discuss form without ending with some functional involvement. This is certainly the case for comparative morphology, but maybe less obvious but as important for ontogeny. In order to demonstrate the unity of form and function, I will discuss the entanglement based on each of those parameters defining form.

V.1.2.a - Presence

- "In morphology, the 'events' are (1) the presence (existence) itself of the Bauplan of the organism ..." (VERRAES, 1989)

Ontogeny can be expressed as a sequence in the formation of 'Bauplan' elements, where certain elements are formed prior to others. In *Clarias gariepinus*, at first the basis of both the neurocranium and the splanchnocranium is formed. This basis consists of cartilage only. Ossification initially involves the splanchnocranium (at about 4 mm SL), whereas the bulk of the neurocranial ossifications only starts much later (at 10 mm SL). The onset of muscle formation occurs almost simultaneously with that of the chondrocranium, whereas some of them become functional shortly after. All the muscles that finally are formed and become functional, are related to movements of splanchnocranial elements. The major events during early ontogeny in *C. gariepinus* thus seem to be concentrated on the splanchnocranium. It is a fact that the kinetic possibilities of the skull parts vary substantially in teleost fishes. The elements of the neurocranium constitute an akinetic system, whereas the splanchnocranium is highly kinetic. Consequently, function becomes involved. From a kinematical point of view, the neurocranium can be considered as an internally immovable[3] suspension unit, from which the splanchnocranium is anchored in a highly movable manner. Those structures that arise early during ontogeny involve splanchnocranial elements which are part of the mechanisms enabling buccal expansions, and thus play a crucial role in functions such as respiration and feeding.

[3] The neurocranium is not immovable when considered in relation to the vertebral column, for example.

- "It is a well-known fact that many organs or elements always occur simultaneously that is, functionally must occur simultaneously"

(DULLEMEIJER, 1974)

The presence or absence of a certain organ or element is frequently related to the presence or absence of a certain function. However, it can be argued that a kind of critical level has to be reached before a newly formed organ can become functional. For example in *Clarias gariepinus*, the palatine is involved in the palatine-maxillary mechanism where it provides the articulation for the maxillary bone. The presence of the palatine bone can already be observed from 5.0 mm SL on, although the maxillary bone is still absent, as well as the retractor and extensor tentaculi muscles are still lacking. Consequently, in this stage the function of the palatine is not similar to that of later stages. It can be argued if simply being connected to the maxillary barbel rod is a function of the initial palatine or not. If not so, it would be better to regard the presence of the palatine as a preformation for the later palatine-maxillary mechanism. On the other hand, as DULLEMEIJER (1974) mentioned, the presence itself can be regarded as a function of a 'Bauplan'-element. In that case, the palatine would only experience an ontogenetic shift in function. As can be derived from the study dealing with the shift in mouth opening mechanisms, such preformations may be crucial in order to avoid critical periods during ontogeny.

V.1.2.b - Shape

Changes in shape and size are the most obvious expressions of the ontogeny of 'Bauplan' elements. The overall shape of the skull of larval *Clarias gariepinus* changes substantially. Larval skulls are short compared with the juvenile situation. Short structures have the advantage that lever action on them is low, and thus less mechanical loads have to be exerted onto them. This is especially important for the elements involved in powerful biting. As larval *C. gariepinus* become carnivorous at about 8 mm length, and the elements of the feeding apparatus have not yet become fortified through ossification (10.0 mm SL stage), a short lower and upper jaw will improve biting force for a certain adductor mandibulae muscle. Additionally, shorter skulls reduce friction drag, compared to long skulls. The shortness of the skull as a hydrodynamic advantage in *C. gariepinus*, can only be effective once the yolk sac has been resorbed.

Ontogenetic changes in shape can be observed in practically all elements composing the 'Bauplan', as growth is not isometrical. The shape of bones, for example, appears to be related to the shape of the underlying cartilage. This is evidently the case for perichondral bones, but is also the case for dermal bones. For example, the frontal bone of *Clarias gariepinus* arises as a gutter-like bone which closely follows the dorsal side of the cartilaginous skull roof (commissura spheno-septalis, pons epiphysialis and taenia marginalis posterior). From that ossification centre, the plate-like part of the bone becomes expanded, especially in the medial and posterior direction. Such a close connection between the shape of the chondrocranium and certain dermal bones can be found in the parieto-supraoccipital, the dermosphenotic, the dermopterotic, the preopercular bones, etc. It is, however, not surprising that such a close relation exists. As ossification is an adaptation to increase the resistance against mechanical loading, dermal bones would be able to resist highest loads if they are anchored, instead of lying completely separated. The only, non-perichondral bones that arise during

ontogeny, without any close contact with cartilage are the sesamoid bones (*i.e.,* the parurohyal and the entopterygoid). But here again, their formation is linked to mechanical loading, in this case at the level of the ligament or tendon in which they are formed. For these bones, it can thus be expected that the shape of these bones will be related to the shape of this ligament or tendon. This can clearly be observed. The parurohyal bone arises as a paired structure, with each element being forked distally. The paired origin is related to the paired nature of the ligaments in which they arise, whereas the forked distal tip enables a more powerful attachment of the head of the sternohyoideus, which fits into the fork. The entopterygoid bone is formed in the ligamentous sheet between the metapterygoid and the prevomeral bones, where its triangular form can be derived from the form of the ligament: the broad ligament tapers as it runs forward and attaches to the prevomeral bone.

V.1.2.c - Size

As the ontogenetic transformations of the 'Bauplan' elements occur allometrically in *Clarias gariepinus*, their changes in size cannot be discussed without referring to changes in shape. It may, however, be opportune to refer to the discrepancy between the ontogenetic changes in size (and shape) of the chondrocranium and the osteocranium. Initially, the chondrocranium constitutes both the material and functional skull, whereas the bony skull is only fragmented. As growth continues, the osteocranium becomes more and more functional, although completely depending on its anchoring to the chondrocranium. Eventually, the chondrocranium becomes completely enclosed by the osteocranium, whereas the anchoring function of the latter is lost. At this moment, lateral expansions of the bony skull, away from the chondrocranium, can occur unrestrainedly (so to say). However, at the level of the chondrocranium, the increase in size of the bones is depending on the cartilaginous growth zones, underlying the sutures. The bony skull is thus partially dependent of the underlying chondrocranium, *i.e.,* for its growth. Concerning its functionality, the osteocranium remains partially dependent of the chondrocranium as well: many articulatory facets correspond to the articulations of the initial chondrocranium. Consequently, the function of the chondrocranium in juvenile *C. gariepinus* can be defined as: (1) providing growth centres for the osteocranium, and (2) providing articulatory facets, without having to form "secondary cartilages" (which can not be observed in *C. gariepinus*).

V.1.2.d - Position

During the ontogeny of the skull of *Clarias gariepinus*, changes in position were not abundant. The position of the skeletal elements, in relation to surrounding structures, remains fairly constant. Changes in the position of muscles, however, can be observed. The normal position of the adductor operculi in adult fish is between the neurocranium and the opercular bone. This can be observed in the late larval and juvenile *C. gariepinus*. Early during ontogeny, however, this adductor runs from the neurocranium to the lateral face of the cartilaginous suspensorium. The difference in form *s.l.* can now hardly be explained by morphological-ontogenetic evidence only. This would only be the case if the muscle would lie in the direction of the opercular bone, but would not reach it yet because of its early developmental state. This is, however, not the case in *C. gariepinus*: the direction of the adductor attaching to the suspensorium is different than if it would be directed to the opercular bone. Consequently, the ontogeny of this muscle not only involves an increase in size, but also a displacement

of its ventral head, which eventually becomes attached to the opercular bone. This shift in position or insertion of this muscle can be explained functionally. Early during development, the opercular bone is still rudimentary, being less important in respiration and feeding than the buccal cavity is. Expansions (and constrictions) of this cavity create a more substantial volume increase than the expansion of the opercular cavity would do. Consequently, the movements of the suspensorium can be expected to be more important for a larva than those of the gill cover. A functional shift, through a shift in position of a muscle during ontogeny, can thus enable a more optimal usage of skeletal elements, in order to improve the capacity of certain mechanisms.

The increasing importance of the opercular pumping system, for respiratory functions, becomes apparent during further ontogeny of *C. gariepinus*. At the early larval stage, all movements of the opercular bone appear to be possible: (1) elevation (levator operculi), (2) dilatation (dilatator operculi) and (3) adduction (levator operculi and adductores hyohyoidei). Later on, the insertion of the adductor operculi shifts from the suspensorium to the opercular bone, at its processus dorsalis (thus lateral to the opercular articulation with the suspensorium). This implies a functional shift from being a suspensorial elevator to an opercular dilatator, and thus not an adductor, as the name would suggest. The protagonist action of the dilatator and adductor operculi improve opercular dilatation, for creating a suction force in the opercular cavity, whereas the levator operculi presumably has a double function: the adduction and the elevation of the opercular bone.

V.1.2.e - Structure

- "Cranial morphogenesis comprises a sequence of interactions between tissues and structure that differ in their response capabilities" (HANKEN, 1983b)

Ontogenetic changes in the structure of the 'Bauplan' element are evident as well: the chondrocranium becomes substantially replaced by the osteocranium. This sequence, first cartilage than bone, is not only phylogenetically determined, but is also developmentally crucial. The typical features of cartilage and bone allow the ontogenetic transformations to occur at the degree they do. Cartilage enables a faster growth than bone does, as well as cartilage can be more quickly remodelled than bone. On the other hand, bone can resist stronger forces, especially those that are unevenly exerted onto an element, and can function as a storage of calcium. A newly hatched larva is immediately confronted with certain requirements. It is consequently crucial that (1) certain structures are present at the moment the larva is about to hatch, or (2) a basic 'Bauplan' has to be formed very quickly shortly after hatching. In *Clarias gariepinus*, the latter is observed. When comparing the chondrocranium of a 5.6 mm SL specimen with that of a 6.0 mm SL specimen, the changes in size and shape of the chondrocranium are far more substantial, than is the case for the osteocranium (even when comparing between two later stages with the same time interval). The changes in the osteocranium may be more related to gradually increase of mechanical loading, although this is not applicable for all elements (*e.g.* toothed bones).

Although in *C. gariepinus*, no clearly demarcated period of metamorphosis can be distinguished, the remodelling capacity of cartilage remains important. Some examples of a chronologically different remodelling of similar structures in *C. gariepinus* can be distinguished. Initially, a single cartilaginous plate, consisting of the pterygoquadrate, the hyosymplectic, Meckel's cartilage, the hyoid bar and the

anterior branchial elements, are formed. Later on, the connection with the lower jaw is lost, followed by that with the branchial arches. It is, however, much later that the hyoid bar becomes separated from the remaining hyosymplectic-pterygoquadrate plate. As can be derived, the time difference appears to be related to the functionality of this connection: (1) the connection with the lower jaw is lost to allow more active mouth opening, (2) the connection with the branchial arches is lost, presumably allowing greater mobility for respiratory purposes, and (3) the interhyal disappears, but becomes replaced (functionally) by a ligament as the skull becomes more and more dorsoventrally flattened.

V.1.3 - Changes in Functional Demands during Cranial Ontogeny: The Skull as a System of Functional Units

The skull 'Bauplan' can be viewed from different angles. From a pure morphological viewpoint, the skull can be subdivided in all the composing bones (canal bones, anamestic bones, cartilage bones, …), ligaments, muscles, nerves, etc. A histological survey of the skull will describe different types of cartilage, different types of bone, sensory organ composition, etc. However, as this thesis involves a functional morphological approach, it is evident that the skull is looked upon from a functional point of view. Consequently, the skull can be divided in units, which give an indication of functionality: *i.e.,* functional units [or functional components according to Verraes (1981)] (**Fig. I.1-1, p. 2**). The functional units that will be discussed here only involve those skeletal elements that are part of the skull and which undergo actions (or movements) in order to maintain the biological roles[4] of the organism. The branchial skeleton will be regarded as one functional unit (as the different branchial elements were not studied, as well as any argumentation concerning intrabranchial actions in ontogenetic stages is extremely difficult).

When looking at the ontogeny of the skull in terms of functional units and coupling between these units, a clear trend in increasing complexity can be observed. Four different types of coupling are distinguished: (1) connection, (2) articulation, (3) muscle, and (4) ligament. Of course, many more elements are interconnected through undifferentiated connective tissue, but these are not considered. In the case of *Clarias gariepinus*, the initial skull 'Bauplan' (4.1 mm SL) is fairly simple: only six functional units can be distinguished, of which four of them constitute a single, continuous element (I will here not go into detail again about which connections exist, as this is extensively discussed in previous chapters) (**Plate V.1-1A**). Any muscular insertion is lacking yet. The only links between these units involve articulations or a connections through cartilage. At about only one millimetre larger (5.2 mm SL), some new articulations have formed, as well as muscles start to interconnect units (**Plate V.1-1B**). These muscular connections may consequently imply the onset of actions. At 6.7 mm SL, more muscles have formed, whereas previous connections have become articulations. Ligamentous connections are present as well at this stage (**Plate V.1-1C**). In the juvenile situation, a complex network has formed of interconnected functional units. Every element is directly or indirectly suspended at the neurocranium (**Plate V.1-1D**).

[4] "An element of an organism fulfils a '*biological role*' when it contributes directly or indirectly to the maintenance of the whole organism in space and time" (Verraes, 1981).

- "Both the embryo and the juvenile have to survive while growing. This means that particular structures not only have a biological role in their 'adult' size, but also a sequence of other roles for each intermediate stage" (GANS, 1976)

Early ontogeny actually represents a dangerous period in fish life history: they have to maintain biological roles, although they get little equipment along the way. Consequently, in a manner of speaking, they continuously have to improvise how to use the available structures optimally. This means that certain structures may serve different purposes along the process of ontogeny. This also implies that the timing of shifts in function may be crucial. As one structure may become unusable for a certain function (*e.g.* mouth opening), its function has to be taken over by another structure simultaneously, in order to be able to maintain that function. Such a critical period can be avoided by overlapping functionalities, as can be observed in the shifts in mouth opening mechanisms. A critical period in mouth opening, as can be observed in cichlids, cannot be observed in *Clarias gariepinus* as most of the mouth opening mechanisms overlap chronologically.

- "... the whole organisation is so tied together during growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified" (DARWIN, 1859)

The sequence of functional roles of elements of the 'Bauplan' can also be seen on an evolutionary time scale, next to a chronological, ontogenetic time scale. Catfishes, in general, demonstrate such a coupling of 'Bauplan' elements. In the ancestral, presiluriform otophysans, the palatine is continuous with the pterygoquadrate, whereas the latter is connected to the hyosymplectic. The palatine thus functions as a support of the suspensorium for its articulation with the neurocranium. This implies that the palatine can hardly be distinguished as a functional unit, but takes part in the suspensorium as functional unit. Decoupling of structures has frequently been suggested to be a "key evolutionary innovation" in vertebrates, as it frequently involves a decoupling of function. Consequently, new functions arise. The decoupling of the palatine of the pterygoquadrate in siluriforms is a perfect example, as it has allowed the development of a novel sensory mechanism, the palatine-maxillary mechanism. However, this also implies that its previous function as suspensorial attachment is lost. If the remaining part of the ancestral suspensorium would have remained in its original configuration, the following would be present: a hyosymplectic articulating with the neurocranium, with a pterygoquadrate attached to its anterior margin, bearing no articulation anymore with the neurocranium. Consequently, the suspension of the lower jaw, through the pterygoquadrate would be fairly unstable. This has become compensated in catfishes, as a separate, cartilaginous pterygoquadrate never arises in catfishes (it may secondarily become separated again in Ariidae), as it is completely fused to the hyosymplectic. This provides a stronger, indirect connection of the lower jaw, through the suspensorium, to the neurocranium. The synchondrosis between the pterygoquadrate and the hyosymplectic also allows strong interdigitations between the perichondral bones.

This fusion now also implicates that the mechanical loading onto this hyosymplectic will be larger, as it sustains all the forces acted upon from both the lower jaw and the hyoid. Additionally, in the ancestral

situation, torque forces can be resisted adequately through both connections (palatine and hyosymplectic), wide apart from each other. As the anterior connection is lost in Siluriformes, torque forces onto the hyosymplectic will increase substantially. The way this hyosymplectic articulates with the neurocranium will consequently be of great importance as well. A single articulation, along the whole dorsal margin of the hyosymplectic can be more advantageous than a double-headed articulation, which can be observed in most teleosts. A double-headed articulation is especially interesting if anteroposterior forces have to be endured. The presence of a single-headed articulation of many Characiformes (ARRATIA, 1992), from which the Siluriformes are believed to be derived (FINK & FINK, 1996), may be considered as a kind of preadaptation, which allowed the decoupling of the palatine in Siluriformes.

- "Most zoologists will probably agree that the bodies of animals are intricately integrated in their design and that morphological change must proceed without disturbing their integration" (ALEXANDER, 1975b)

The intricately integration of 'Bauplan' elements cannot only be expressed in terms of spatial constraints, but also in the coupling of different mechanisms, through the coupling of functional units. The integrated design can be demonstrated in *Clarias gariepinus* in a evolutionary context. The trend has been observed that within the Clariidae, the increasing size of the adductor mandibulae is coupled to the increasing reduction of the canal bones covering this muscle in *C. gariepinus*. The integration of mechanisms, however, is something that can be derived from this study. As a developing larva is forced to use its available structures in an optimal way, that is by using as few elements as possible, the same is the case for the juvenile situation. When summarising the interconnections between the different functional units, some interesting observations can be made concerning to what degree these functional units constitute an integrated system. When looking at the impact of a certain action of a single functional unit, it becomes apparent that this evokes a whole cascade of movements of other functional units. Some examples are given, where the maximal number of actions are suggested. This means that muscular connections between two functional elements are considered to remain fixed, meaning that if the origo or insertio starts to move, the muscle will maintain its length (**Plate V.1-2**).

(1) If the mouth is closed in a juvenile *Clarias gariepinus*, through the contraction of the adductor mandibulae, some functional units are pulled along as well: the interopercular bone is pulled anteriorly, which in its turn makes the ventral tip of the opercular bone to rotate anteroventrally (**Plate V.1-2A**). The ligamentous connection with the hyoid bar will induce the elevation of the latter, which will consequently pull onto the pectoral girdle as well as the branchial basket, through the parurohyal bone. The adduction of the mouth also brings forward the mandibular barbels, which lie embedded in the protractor hyoidei at the ventral face of the mandibula.

(2) The impact of a pectoral girdle retraction on surrounding functional units may be even greater (**Plate V.1-2B**). The sternohyoideus may induce the depression of the hyoid bar, which will depress the lower jaw (either ligamentously or muscularly). The retraction of the parurohyal bone, coupled to the depression of the hyoid bar, may also induce the depression of the branchial basket. The lower jaw may be depressed additionally, through the attachment of the hyoid bar to the interopercular bone. The impact of the protractor hyoidei contraction during jaw depression may influence the orientation of the mandibular barbels. The pharyngoclavicular muscles provide the connection between the pectoral girdle and the posterior branchial elements. The depression of the hyoid bars evokes an abduction of

the suspensoria, which will pull along the lower jaws and the opercular bone. The ligamentous connection between the suspensorium and the palatine may alter the orientation of the palatine, which may consequently change the orientation of the maxillary and nasal barbels. Additionally, the movements of the palatine will alter the volume of the nasal sacs. This means that actions for respiratory or feeding purposes become coupled to actions for sensory purposes (both olfaction and taste).

(3) The single contraction of the levator arcus palatini, for the suspensorial abduction, may evoke a whole set of other actions. Due to its articulation with the opercular bone, the latter becomes abducted, in reference with the skull, whereas due to the levator operculi, the opercular bone may become adducted in reference to the suspensorium. Suspensorial abduction results in the abduction of the distal parts of the lower jaw, which is enabled by the non-fused mandibular symphysis. The connections of the interopercular bone with both the mandibula and the opercular bone will consequently result in the lateral displacement of this bone as well. Abduction of the suspensoria also implies that the caudal tips of the hyoid bars will become abducted, which are also connected to the abducted interopercular bone. Anteriorly, the abducted suspensorium will pull onto the distal tip of the palatine, thereby presumably inducing its lateral displacement. This may consequently result in the retraction of the maxillary barbel, as well as a displacement of the base of the nasal barbel. Additionally, the consequently medial displacement of the rostral tip of the palatine will induce a decrease in the volume of the nasal sacs. This means that, again, respiration and feeding mechanisms are coupled to olfaction and taste mechanisms, whereas only the contraction of a single muscle is required.

(4) The opercular four-bar system requires the contraction of the levator operculi. This four-bar system then involves the coupling of opercular elevation to mouth opening, through the interopercular bone. The connection between the interopercular bone and the hyoid bar, however, couples this four-bar system to hyoid depression which may consequently evoke the depression of the branchial basket. Depression of the mandibula and the hyoid bar will also alter the position of the mandibular barbels. In summary, the contraction of a single muscle, the levator operculi, may result in both the depression of the lower jaw, the hyoid bar and the branchial basket.

V.1.4 - CRANIAL ONTOGENY: GENETICS OR EPIGENETICS?

Although no true genetic research has been involved in this study, some considerations concerning this topic can be derived from the material studied. Some control on genetic differences could be obtained by using gynogenetic material for some parts of the study, although even here, the genome cannot be considered as being identical between the specimens of the gynogenetically obtained egg batch (due to crossing over during first polar body formation).

- "Results of studies of the mechanics of bone growth and morphogenesis emphasize the degree to which adult bone is not under direct genetic control; instead, they emphasize the degree to which modification in bone form is usually a biomechanical consequence of genetic changes, primary targets of which are adjacent structure"

(HANKEN, 1983b)

Different hypotheses have been proposed and discussed concerning to what degree the ontogeny of the skull is subjected to genetic control, or epigenetic control. Epigenetic control can be considered as all those stimuli that act onto the genetic expression. Factors may arise from outside the organism, or from within. External factors comprise several possibilities, as summarised in Chapter III.1. Epigenetic factors from within the organism range from hormones to simple contacts between two abutting structures. Epigenetic control of cranial ontogeny can only be distinguished from genetic control, if certain parameters surrounding or within the organism are altered in one of two genetically identical specimens. Consequently, the study of aberrant cranial morphology contributes to the understanding of normal ontogeny, and may even be crucial. One of these hypotheses states that the initial formation of the skull is self-differentiating, whereas once functions begin, the epigenetic control takes over. Another hypothesis suggests that the growth and differentiation of cartilage is genetically determined, whereas that of bone is epigenetically regulated by cartilage (HERRING, 1993). Craniofacial abnormalities in the chondrocranium have already demonstrated the close relationship between the form of bone and that of the underlying cartilage. However, cartilage can hardly be designated as being solely genome dependent, as influences of mechanical loadings onto cartilage are known to be inductive factors for cartilage differentiation. In normal *Clarias gariepinus*, the dermal bones clearly follow the border of the chondrocranium as well. The study of the aberrant 'Bauplan' morphology, as a result to the exposure of certain teratogens, can reveal additional information on this subject.

- "Four distinct features of skeletal morphology are associated with miniaturization of adult body size in vertebrates: reduced ossification, hyperossification, increased variability, and morphological novelty. ... Nevertheless, the high frequency with which dwarfed taxa display them is evidence of the significant effect that miniaturization may have on skeletal development, and especially bone growth"

(HANKEN, 1993)

It may be obvious that in species, or specimens of reduced size, the skeletal development has become reduced as well. The same can be observed in the artificially induced dwarfs of *Clarias gariepinus*, where the ontogenetic state of differentiation is much reduced compared to the normal sized specimens of equal age. As suggested, not all skeletal reductions in dwarfs may be considered as true paedomorphoses, at least not when size (instead of chronological time or age) is used as the biological time frame. This appears to be the case in *C. gariepinus*. Although more experiments are necessary, it may be suggested that the spatial constraint (or freedom) on a developing and growing larva has an overall effect on the skeletal ontogeny of the skull. No substantial, differential effect on the different parts of the skull 'Bauplan' can be observed, as the dwarfs closely resemble the normal situation of a similar size. However, as said before, this is only a preliminary study which needs confirmation from other experiments.

- "The adult skeleton of dwarfed forms is the product of both phylogenetic and ontogenetic constraints and functional modification mediated by natural selection"

(HANKEN, 1993)

One of the ontogenetic constraints, causing dwarfism, is the spatial environment, surrounding the developing organism. HANKEN (1983b) also mentioned that "one of the most evolutionary plastic

vertebrate features is adult body size". It can be argued to what degree the phenotypic plasticity, expressed here in terms of size variability within one specimen, lies at the basis of the evolutionary plasticity. A high phenotypic plasticity in body size may contribute to survival in changing environments. If the effect of epigenetic influences is able to modulate the size to a large extent, a wider range of niches can be occupied: smaller size implicates different food, different behaviour, different habitat exploration, etc. The initial change in body size may consequently induce more differential changes in 'Bauplan' elements, as for example adaptations to a certain feeding type become advantageous. This means that what initially may have been a direct consequence of the spatial constraint on the 'Bauplan' morphology, may subsequently lead to changes in this 'Bauplan' as a result of changing functional demands. Consequently, the degree to which the phenotypic plasticity of size enables a response to a changing environment may be crucial to the survival of a species. A high phenotypic plasticity may also be of great importance for the dispersal of species, which may eventually enable speciation, mediated by natural selection.

Chapter VI.1 - Conclusions

The ontogenetic study of the cranial 'Bauplan' of the African catfish, *Clarias gariepinus*, as presented here has given conclusive results on some of the questions, as mentioned in Chapter I.1, whereas some suggestions can be made, based on this results, in order to answer other questions. The following conclusions can be given, in correspondence with the questions posed in Chapter I.1:

1. The form *s.l.* of both the chondrocranium and the osteocranium has been described in detail. The comparison of the ontogeny of *Clarias gariepinus* with that of other catfishes, based on data from the literature, has provided evidence on the formation of structures, which had not been described yet, as well as it has revealed the possible presence of structures which have frequently been overlooked (*e.g.,* the infrapharyngobranchials I and II). The study of the ontogeny of the osteocranium has allowed to distinguish all bones present, even though certain distinctions were not made in previous studies of the adult skull. The ontogenetic study revealed fusions between bones (*e.g.,* the ethmoid complex, sphenotic and pterotic bones), as well as paired origins of bones that become unpaired in the juvenile situation (*e.g.,* the parurohyal bone). However, a conclusive result can not be given concerning possible fusions of certain bones, which has been suggested in the literature (*i.e.,* between the supraoccipital and the parietal).

2. The cranial musculature has been studied into detail, in four different ontogenetic stages. Together with data from the literature, a clear view has been obtained how the muscles in the cranium arise during ontogeny, and which structures they interconnect. Ontogenetic evidence of the phylogenetic changes in muscle configuration of catfishes, in relation to the palatine-maxillary mechanism, can not be revealed. Evidence suggests that the separation of the retractor and extensor tentaculi from the adductor mandibulae complex and the adductor arcus palatini, respectively, occurs at the blastema level, or even not. Some suggestions can be made concerning the composition of the adductor mandibulae complex, as well as the origin of the retractor tentaculi. Specialisations of the protractor hyoidei, in relation to the mandibular barbels, as well as to the mandibula can be revealed.

3. The fully developed skull of *Clarias gariepinus* consists of a rigid endocranium, which is bordered laterally by plate-like bones. Several bones, especially plate-like bones, have frequently been named wrongly. The true nature of these bones can be derived, partially from the relation with the cranial lateral-line system, and partially through the ontogenetic evidence. The detailed study of the juvenile skull also revealed the presence of certain bones, which previously had been overlooked (*e.g.,* the epiotic). Some bones of the juvenile skull in *C. gariepinus* demonstrate specialisations, compared with other catfishes. The plate-like extensions of the infraorbital bones, as well as the suprapreopercular and the nasal bones are considered to be secondarily derived. All muscles that have been observed in generalised catfishes, are present in *C. gariepinus*. Specialisations can be observed in some of the opercular muscles, as well as the protractor hyoidei muscle.

4. A close relationship can be observed between the chondrocranium and the osteocranium. Dermal bones follow the margins of cartilaginous structures, whereas perichondral ossification frequently initiates around

articulatory facets (leaving the latter cartilaginous). Ossification showed some correspondence with the insertion site of muscles, although insertion is observed prior to ossification. Form changes can be observed in several bones, which appear to be an adaptation to increased muscle size. The formation of certain processes and plates provides larger attachment surfaces (*e.g.,* membranous plates of the suspensorial bones), as well as it may be part of a safety mechanism for preventing dislocation because of increased muscle power (*e.g.,* the sphenotic spine of the suspensorium).

5. The examination of ontogenetic events, coupled to data from the literature on feeding, respiration, behaviour and so on, can provide a possible ontogenetic scenario of a continuum of compromises between what is present and what has to be performed. A possible relation between function and ontogeny of form can be revealed at several levels. First, chondrocranial features, indicating special adaptations for respiration, for maxillary barbel sensation, and for feeding can be observed. Secondly, the sequence of bone formation can be coupled to the sequence of certain functional events during ontogeny. Arguments can be given which suggest that this sequence is largely determined by the life history of larval fish (or the other way around!). Thirdly, an ontogenetic shift in the use of elements of the 'Bauplan', in order to construct functional mechanisms for biological roles like respiration and feeding, can be observed. Two major conclusions can be derived: (1) the complexity of the apparatuses, enabling these mechanisms, increases during ontogeny, which may seem evident, and (2) a close relationship exists between the timing of the onset of functionality of the different mechanisms and the onset of corresponding functional demands.

6. Some novelties can be observed in the skull of *Clarias gariepinus*. The functional consequence of these novelties fitted nicely in the hypotheses concerning the function of the several mechanisms of the 'Bauplan'. For example, the adaptations for a benthic behaviour of many catfishes, results in different consequences on different parts of the cranial 'Bauplan'. Dorsoventrally flattening of the skull has its consequences on the effectiveness of the buccal cavity, for the increase of its volume. Adaptations can be found at several levels: the depression possibilities of the hyoid bar are restricted, which is no substantial disadvantage as the skull is broad, and (2) a shift in the adductor operculi muscle indicates the increased importance of the opercular pumping system (in contrast to the buccal system).

7. The testing of teratogens on the cranial ontogeny in *Clarias gariepinus* proved to be difficult, in order to obtain conclusive results. Several teratogens are tested, which can yield a deformed 'Bauplan' that can reveal important information on the spatial interactions between the elements of that 'Bauplan'. Teratogens were found that can get data on the relation between bone, cartilage and muscles, as well as the adaptive force to perform vital functions. Useful cranial deformities that are obtained involved: (1) agnathia, (2) microphthalmia, and (3) oral barbel deformities. Information on spinal column deformities are obtained as well. Because of time restrictions, however, only the effect of dwarfism is used for further studies.

8. The teratogens are especially picked because of their directed action on a certain type of tissue. In that case, based on background information of the teratogen, a discrimination between a direct teratogenic effect on the 'Bauplan' elements, and the indirect effects would be allowed. Such indirect effects would presumably arise as a result of the change in spatial and mechanical interactions due to the former effects. As no abnormal skulls, as a result of the teratogens, are studied, no further information on this topic can be

given. In case of the dwarfs, however, some possible factors inducing reduced growth can be eliminated (temperature, water quality, ...). However, whether or not the reduction in size is a direct effect of the reduced spatial environment, or is indirectly caused by hormonal activity as a result of stress, or caused by reduced functional demands, can not be revealed.

9. Growth of larval *Clarias gariepinus* appears to be partially determined by the amount of surrounding space. Although a vague notion of this existed, the present experiments enabled to quantify this phenomenon. Dwarf specimens are substantially smaller than normal specimens.

10. The overall ontogenetic differentiation of the cranial 'Bauplan' in dwarf *Clarias gariepinus* is influenced substantially. Compared to its equally aged, normal situation, the skull demonstrated a reduced state of ossification. All muscles, as observed in normal ontogeny, can be discerned. Compared to younger, but equally sized specimens, a highly comparable ossification status can be observed. This provides additional proof of the close relation between size and ontogeny, instead of age and ontogeny, as is already suggested from evidence of the normal ontogeny.

11. The timing of ontogenetic events and functional demands, as well as the formation of novelties in the skull of *Clarias gariepinus* provided some evidence on the causal and functional relations between 'Bauplan' elements. As the effects of the teratogenic activity on the constructional morphology of the skull can not be studied, no evidence can be given on the direct causal and functional relation between 'Bauplan' elements. Additionally, as the induced dwarfism results in an overall reduced ontogeny of the skull, and no differential effect on the separate elements can be observed, this study can not provide evidence either. However, some considerations can be given concerning evolutionary relations between different elements, or different mechanisms (*e.g.,* the consequences of the formation of the palatine-maxillary mechanism).

Chapter VI.2 - Summary

A general summary of is given, following the same arrangement as the thesis. A summary of each chapter can as well be found at the beginning of each respective chapter.

VI.2.1 - General Introduction

I.1 - This study comprises the ontogeny of the cranial 'Bauplan' of the African catfish, *Clarias gariepinus* Burchell (1822) (Siluriformes; Clariidae). In broad outlines, the study encompasses a descriptive part, which served as the basis for the functional-morphological part. The cranial ontogeny is studied during both normal and abnormal development, with the general aim to distinguish adaptations and epigenetic control of cranial ontogeny.

I.2 - Eggs of *Clarias gariepinus* are obtained from the Laboratory of Ecology and Aquaculture (KULeuven), and reared in our laboratory, thus providing material for collecting an ontogenetic series. A total of 62 different specimens of different age and size are used.

Depending on the size and aim of the specimens, different fixatives are used. Specimens are used for dissections, for *in toto* clearing and staining, as well as for serial sectioning. A total of 15 of these are serially sectioned and stained. Serial sections are used for detailed observations, as well as computer-based three-dimensional reconstructions.

The study of the abnormal ontogeny required standardised anomalies, for allowing functional and epigenetic interpretations. Consequently, the effect of different teratogens on the ontogeny of larval *Clarias gariepinus* is tested. Larvae are exposed to a total of six different teratogens, of which some are known to have a very targeted action: (1) 2,4-dinitrofenol, (2) colchicine, (3) diazo-oxo-nor-leucine, (4) β-amino-propio-nitril, (5) retinoic acid, and (6) malathion. Additionally, the effect of spatial constraints is also tested on larval *C. gariepinus*, resulting in dwarfism. Due to time restrictions, only this dwarf, abnormal ontogeny is used for a detailed morphological study.

I.3 - The study object, *Clarias gariepinus*, is a catfish species, belonging to the family of the Clariidae. Catfishes are characterised by a set of specialisations involving the oral barbels, the palatine-maxillary mechanism, the Weberian apparatus, etc. Clariidae are considered to a specialised catfish family, showing a heavily ossified skull in generalised species, like *C. gariepinus*. This species is generally well known because of its economic value in aquaculture.

I.4 - In this study, data on both body sizes [total length (TL), standard length (SL) and preanal length (PAL)] and age (days posthatching) are collected. The specimens used in this study can be categorised into larval and juvenile specimens. Some considerations on this terminology are given. Also, a preliminary survey is given of the different types of skeletal tissues that can be observed in *Clarias gariepinus*. At least six different types of cartilage and four different types of bone can be discerned.

VI.2.2 - Ontogeny of the Skull

II.1 - The study of the ontogeny of the cranial skeleton is subdivided into three parts: (1) the ontogeny of the chondrocranium, (2) the ontogeny of the osteocranium, and (3) the cranial lateral-line system, with the ontogeny of related bones.

The chondrocranium largely corresponds to the generalised catfish configuration, where adaptations in relation to reduced eye size and the dorsoventrally flattening of the skull can be observed. The skull corresponds to the platybasic type, where modifications related to the trabecular bars, the hypophyseal fenestra, the ethmoid region and the position of the olfactory nerves can be noted. Reductions in 'Bauplan'-elements can be observed as well. Most of them are non-specific and occur in most catfishes (*e.g.,* the pila lateralis, the commissura lateralis, the myodomes), whereas others have only been observed in some catfish species (*e.g.,* the reduction in the taenia marginalis anterior and the tectum synoticum). The neurocranium, as well as the splanchnocranium are well developed in *Clarias gariepinus*. Typical catfish splanchnocranial configurations observed, involve the isolated palatine and the single hyosymplectic-pterygoquadrate plate. Other transformations, observed in some other siluriforms, are present as well (*e.g.,* in relation to the interhyal, Meckel's cartilage and the suspensorium). All branchial elements of a generalised siluriform, can be observed. Evidence of this study suggests that even the infrapharyngobranchials I and II are present, although they have frequently been misinterpreted or overlooked in other studies.

The ontogeny of the osteocranium appears to be related to the changes in functional demands in the developing *Clarias gariepinus* larva. The first bones to ossify are the opercular bones, which arise at the moment the opercular skin fold begins to move. Other early ossifications involve dentigerous bones, which arise shortly before the transition of endogenous feeding to exogenous feeding. The expansions of the enlarging branchiostegal membrane becomes supported by the increasing number of branchiostegal rays. The parasphenoid covers up the large hypophyseal fenestra, thereby protecting the above lying brains against damage during food intake. Hyoid bones arise, which attribute to the reinforcement of the hyoid bar, as well as the attachment of muscles to it. The skull becomes fortified in a relative short period, as most perichondral bones arise rather simultaneously, especially at the level of the notochord and articulations. At this moment, larval *C. gariepinus* are known to initiate cannibalistic behaviour, which implies increased mechanical loads onto these articulations. The suspensorium becomes attached to the skull anteriorly through a ligament, which later on even becomes ossified, forming the sesamoid entopterygoid bone. The adding of dermal bones (both canal and anamestic) eventually covers the chondrocranium completely. Additional dentition approximately coincides with the moment the opercular four-bar system and the digestive system may become functional.

The cranial-lateral line system, together with its related bones, are well developed in *Clarias gariepinus*. All major lateral-line canals can be distinguished: (1) the supraorbital canal, (2) the infraorbital canal, (3) the preoperculo-mandibular canal, (4) the otic canal, (5) the postotic canal, and (6) the temporal canal. One canal, the supratemporal canal, is missing, although a pit-line can be observed at its presumed position. In most specimens, a supraorbital commissure is present. Other pit-lines involved, are: (1) the vertical pit-line, (2) the horizontal pit-line, (3) the oral pit-line, (4) the anterior pit-line, (5) the middle pit-line, and (6) the posterior pit-line. All canal bones, as observed in generalised catfishes, can be recognised, with exception of the extrascapulars. Fusions can be suggested between the posttemporal bone and the supracleithral bone, as well as between the parietals and the supraoccipital bone. At the mandibular articulation, the preoperculo-mandibular canal is enclosed by a set of small canal bones, here referred to as splenial bones. Several canal bones in *C. gariepinus* have become secondarily plate-like, a supporting evidence of the specialised character of *Clarias*.

II.2 - The ontogeny of the cranial muscles is studied into detail in three different ontogenetic stages, combined with data from literature (SURLEMONT and co-workers, see references). The muscles are discussed according to their morphological and functional coherence: (1) the adductor mandibulae complex, (2) the tentacular muscles, (3) the hyoid and intermandibular muscles, and (4) the opercular and suspensorial muscles. A general asynchrony in muscle development can be observed.

The morphology and the composition of the adductor mandibulae complex in catfishes have been a subject of many discussions so far. Part of the reason for this involves the differentiation of a part of this complex into a tentacular retractor muscle. The remaining part enables the closure of the mouth, and is well developed in *Clarias gariepinus*. The ontogeny, as well as data from the literature, may suggest that in *C. gariepinus* the following two parts can be distinguished: (1) a lateral A_2A_3'-part, which is only little differentiated, and (2) a medial A_3"-part, in which a superficial and a deeper part can be distinguished. Caudally, both muscles are separated from each other by the levator arcus palatini muscle, whereas rostrally at the insertion on the lower jaw, they are fused to each other. The A_1 and A_ω cannot be observed in *C. gariepinus*.

A typical feature of catfishes is the presence of a palatine-maxillary mechanism, which involves the muscular retraction and extension of the maxillary barbel. In *C. gariepinus*, such a mechanism can be observed as well. Already early during ontogeny (7.2 mm SL) both skeletal and muscular elements are present and appear to be functional: (1) the isolated palatine, articulating with the neurocranium, (2) the maxillary bone, enclosing the maxillary barbel and articulating with the rostral tip of the palatine, (3) the retractor tentaculi, running between the maxillary bone and the suspensorium, and (4) the extensor tentaculi, connecting the palatine with the neurocranium. Later on, the articulation between the palatine and the neurocranium becomes reinforced by ossifications surrounding the articulatory facets (*i.e.,* the os autopalatinum and the os lateroethmoideum, resp.), whereas the attachment of the retractor tentaculi becomes improved. The morphology of the elements of the palatine-maxillary mechanism in *C. gariepinus* suggests that barbel retraction and extension occurs through palatine rotation, although some sliding is possible as well.

The hyoid muscles, including the intermandibular muscle, arise in a clear asynchrony: the formation and insertion of the intermandibularis and protractor hyoidei muscle occurs first of all. The hyohyoideus inferior and the sternohyoideus follow, whereas the hyohyoideus abductor and adductor muscles are the last. The ontogenetic origin of the protractor hyoidei can clearly be distinguished, as it arises through the fusion of the intermandibularis posterior and interhyoideus anterior. This protractor hyoidei is well developed in *C. gariepinus*, where it is expected to play an important role in the mechanisms for respiration and feeding. This can be derived of its size, as well as its secondary subdivision in a superficial part, which presumably controls the position and direction of the mandibular barbels, and a deeper, tendinous part, presumably for mouth opening. The tendons of the contralateral hyohyoideus abductor muscles become crossed over. The adductores muscles interconnect the branchiostegal rays, as well as the last one is connected to the opercular bone.

In *Clarias gariepinus*, already early during ontogeny three opercular and two suspensorial muscles are present and insert onto the involved skeletal elements. The muscles present are: (1) the levator operculi, (2) the adductor operculi, (3) the dilatator operculi, (4) the adductor arcus palatini, and (5) the levator arcus palatini. Again, an asynchrony can be concluded, which has an impact on respiratory actions during ontogeny. The muscles that arise first are part of the apparatus, which allows the pressure pump system to create a negative pressure in the buccal cavity, and subsequently pushes the water through the gills. The suction pump system, creating a negative pressure in the opercular cavity, is suggested to become more important later during ontogeny. This can for example be derived from the fact that the adductor operculi in juvenile *C. gariepinus*

actually assists the dilatator operculi, in abducting the opercular bone. The muscles involved in aquatic respiration, also appear to be functional during atmospherical respiration, as can be derived from the literature.

VI.2.3 - Abnormal Development

III.1 - The study of the cranial morphology in the artificially induced dwarfs is studied, in order to try unravel the relation between ontogeny and size or ontogeny and age. Additionally, the abnormal ontogeny may also provide information on normal ontogeny, as it may provide insight in the epigenetic control of skull formation. The larvae that were grown in the spatially constrained environments are substantially smaller than their similar-aged counterparts, which were reared in normal conditions. A detailed comparison of the skull morphology of these dwarfs with two normal skulls is done. One reference skull involves the similar-aged specimens, whereas the other reference skull involves a similar-sized specimen (in relation to the dwarf) of younger age. The ontogenetic state of the dwarf skulls, expressed as ossification status, is clearly less than that of the similar-aged, normal specimens, but is fairly comparable to the similar-sized, normal specimen. How the feedback from the constrained environment to a reduced development occurs, can, however, not be revealed in this study. A suggestion is made that hormonal regulation, as a result of stress, might be responsible, as well as a change in functional demands. The fact that ontogeny appears to be more related to size than it is to age, also implies that certain methodological questions can be posed in relation to age-determination and heterochronies.

VI.2.4 - Ontogeny: About Making Compromises between What is Present and What has to be Performed

IV.1 - A developing fish larva experiences continuous changes in nutritional and respiratory demands, which it has to cope with in order to survive. The 'Bauplan' composition of early larvae, however, is much less complex than that of juveniles, consequently restricting its functional possibilities. It is therefore of great interest for these early larvae that the available structures are optimally used. In this chapter I have paid special attention to the ontogenetic change in one mechanism involved in respiration and feeding: the mouth opening in *Clarias gariepinus*. During ontogeny, a total of up to five possible mouth opening mechanisms can be distinguished in larval *C. gariepinus*. Three of them presumably remain functional during the juvenile and adult stage, whereas the other do not. As the complexity of the cranial 'Bauplan' increases during ontogeny, so does the complexity of these mouth opening mechanisms, which may improve mouth opening capacities. Initially, two consecutive mechanisms allow a restricted, passive mouth opening. Later on, the depression of the mandibula becomes coupled to the depression of the hyoid bar, whereas eventually (at 11 mm SL) the last of these mechanisms may be functional: the opercular four-bar system. The shift in mouth opening mechanisms, as well as the shift in parameters of a certain mechanism, may be related to the shift in feeding type, as frequently observed in teleosts. The sequence of mouth opening mechanisms is compared to some changes related to behaviour and structures involved in feeding or respiration, which allows to distinguish five different phases during the life history in *C. gariepinus*.

IV.2 - A feature that has been observed in some catfishes involves the reduction, and even the loss of the interhyal. The detailed ontogeny of the interhyal in *Clarias gariepinus* is described, whereas some considerations on its functional significance are given. Initially, the interhyal is continuous with both the suspensorium and the hyoid bar. Later on, these connections are lost, and the interhyal even becomes reduced completely. The connection between the suspensorium and the hyoid bar is replaced functionally by a ligamentous strap. Based on morphological evidence of this study, as well as data from the literature, it is hypothesised that the loss of the interhyal implies a reduced mobility of the hyoid bar. Supporting evidence can be found: (1) a short skin fold between the mandibula and the rostral tip of the hyoid bar restricts the hyoid depression, (2) short suspensoria prevent substantial lateral displacements of the distal tips of the hyoid bars during depression, (3) a short sternohyoideus implies a short displacement of the hyoid bar, (4) the position of the sternohyoideus, in relation to the hyoid bars, suggest little depression action during contraction (but mainly retraction), and (5) substantial buccal cavity expansions would be little compensated with branchiostegal membrane expansions. Some arguments can be given which suggest that an extensive hyoid depression is even unnecessary in *C. gariepinus*: (1) benthic stability would be overruled, (2) aquatic respiration is partially replaced by atmospherical respiration, (3) broad skulls require less hyoid depressions for certain buccal volume increases, and (4) structural adaptations have made a powerful suction feeding mechanism impossible.

Chapter VI.3 - Samenvatting

De algemene samenvatting van dit proefschrift volgt de indeling van het geheel. Een Engelse samenvatting van elk afzonderlijk hoofdstuk kan eveneens worden teruggevonden aan het begin van elk hoofdstuk.

VI.3.1 - Algemene Inleiding

I.1 - Dit onderzoek behandelt de ontogenie van het craniale 'Bauplan' van de Afrikaanse katvis, *Clarias gariepinus* Burchell (1822) (Siluriformes: Clariidae). In grote lijnen kan gesteld worden dat dit proefschrift een beschrijvend deel omvat, dat als basis fungeert voor een functioneel-morfologisch gedeelte. De studie naar de craniale ontogenie bij *C. gariepinus* gebeurde zowel tijdens de normale als een abnormale ontwikkeling, waarbij de algemene doelstelling was het achterhalen van specifieke adaptaties die aanwezig zijn, alsmede het trachten achterhalen van het epigenetisch aspect van de controle tijdens de craniale ontogenie.

I.2 - Eitjes van *Clarias gariepinus* werden verkregen via het Laboratorium voor Ecologie en Aquacultuur (KULeuven), welke dan verder werden opgekweekt in het laboratorium in Gent. Dit maakte mogelijk dat voldoende materiaal kon worden verzameld, teneinde een ontogenetische reeks op te stellen. In totaal werden 62 verschillende specimens, van verschillende leeftijd en lengte, gebruikt voor deze studie.

Afhankelijk van de grootte en de doeleinden van de specimens werden verschillende fixatieven gebruikt. Gefixeerd materiaal werd gebruikt voor dissecties, *in toto* opheldering en kleuringen, evenals seriële reeksen van dwarse doorsneden. In totaal werden 15 verschillende stadia verwerkt tot seriële coupereeksen. Deze reeksen werden uiteindelijk gebruikt voor gedetailleerde observaties, waarbij enkele stadia werden gebruikt voor het genereren van drie-dimensionele reconstructies via de computer.

De studie van de abnormale ontogenie vereist dat abnormaliteiten op een gecontroleerde manier kunnen worden geïnduceerd, om zodoende functionele en epigenetische vraagstellingen te kunnen beantwoorden. Derhalve werd het effect van verschillende teratogene stoffen op de ontwikkeling van de schedel bij *C. gariepinus* getest. Larvale *C. gariepinus* werden blootgesteld aan zes verschillende teratogenen, waarvan sommigen een zeer specifieke, gekende werking vertoonden: (1) 2,4-dinitrofenol, (2) colchicine, (3) diazo-oxo-nor-leucine, (4) β-amino-propio-nitril, (5) retinylzuur, en (6) malathion. Eveneens werd de invloed van ruimtelijke beperking, rondom een zich ontwikkelende larve, getest. Wegens tijdsgebrek zijn enkel de gegevens van dit laatste experiment verwerkt geworden. Dit experiment leverde namelijk dwergvormen van *C. gariepinus* op.

I.3 - De soort gebruikt voor deze studie, *Clarias gariepinus*, behoort tot de katvissen of Siluriformes, en meerbepaald tot de familie van de Clariidae. Katvissen onderscheiden zich van andere teleosten door de aanwezigheid van een aantal specialisaties, waaronder de aanwezigheid van verschillende monddraden, een palatinum-maxillaire mechanisme, het gespecialiseerde apparaat van Weber, enz. De familie van de Clariidae worden algemeen beschouwd als zijnde gespecialiseerd, waarbij een typisch, sterk verbeende schedel kan worden waargenomen bij de meer gegeneraliseerde clariide soorten (waaronder *C. gariepinus*). Deze soort is eveneens bekend dankzij zijn grote economische waarde, waarbij hij dan ook op grote schaal wordt gekweekt voor consumptie.

I.4 - In deze studie werden gegevens verzameld van zowel leeftijd (in dagen na ontluiken) als lichaamslengte [in totale lengte (TL), standaard lengte (SL) en preanale lengte (PAL)]. De specimens gebruikt in dit onderzoek kunnen worden beschouwd als larvale en juveniele stadia, waarbij enkele beschouwingen betreffende deze definities worden besproken. Een summier overzicht van de verschillende types skeletale weefsels in *Clarias gariepinus* is vermeld. Hierbij wordt melding gemaakt van zes verschillende kraakbeen types, evenals vier verschillende types been, die kunnen worden teruggevonden in de verschillende seriële coupereeksen.

VI.3.2 - ONTOGENIE VAN DE SCHEDEL

II.1 - De studie naar de ontogenie van het craniale skelet kan worden onderverdeeld in drie delen: (1) de ontogenie van de kraakbenige schedel, (2) de ontogenie van de benige schedel, en (3) het kop-lateraal systeem, tezamen met de ontogenie van de gerelateerde beenderen.

De opbouw van het chondrocranium komt grotendeels overeen met dat van een gegeneraliseerde katvis. Hierbij kunnen de typische aanpassingen aan de gereduceerde ooggrootte en het dorsoventraal afplatten van de schedel worden waargenomen. De schedel is platybasisch, waarbij gerelateerde, structurele veranderingen zijn opgetreden in de trabeculaire staven, het hypofysaire venster, de ethmoid streek en de positie van de reukzenuwen. Reducties van bepaalde onderdelen van het 'Bauplan' kunnen eveneens worden waargenomen. Meestal betreft het de afwezigheid van structuren zoals bij de meeste katvissen het geval is (vb. de pila lateralis, de commissura lateralis, de myodomen), alhoewel andere afwezigheden slechts bij sommige katvissen zijn waargenomen (vb. de reductie van de taenia marginalis anterior en van het tectum synoticum). In *Clarias gariepinus* is zowel het neurocranium als het splanchnocranium goed ontwikkeld. Het splanchnocranium in *C. gariepinus* komt overeen met de algemene katvis configuratie, zoals o.a. de isolatie van het palatinum (los van de rest van het suspensorium) en de enkelvoudige hyosymplecticum-pterygoquadratum plaat. Andere structurele veranderingen, aanwezig bij sommige katvissen, kunnen eveneens worden onderscheiden (vb. het onderling verband tussen het interhyale, het kraakbeen van Meckel en het suspensorium). Alle onderdelen van het branchiale skelet, zoals kan worden waargenomen bij de meeste katvissen, kunnen worden onderscheiden in *C. gariepinus*. De gegevens van deze studie laten suggereren dat ook de infrapharyngobranchialia I en II aanwezig zijn, alhoewel deze veelal verkeerd geïnterpreteerd of genegeerd zijn geworden in verschillende studies.

De ontogenie van het osteocranium blijkt grotendeels in verband te staan met de verschuivingen in functionele vereisten die zich voordoen in een ontwikkelende larve. Het eerste been dat gevormd wordt is het operculare, dat blijkt te ontstaan op het moment dat de eerste bewegingen van de operculaire huidplooi zich voordoen. Kort daarop worden getande beenderen gevormd, die ontstaan kort voor de overgangsfase van endogene naar exogene voeding. De expansiecapaciteit van het branchiostegale membraan wordt verbeterd door de continue vorming van ondersteunende branchiostegale stralen. Het grote hypofysaire venster wordt afgedekt door het parasphenoid, wat ondermeer bijdraagt tot de bescherming van de bovenliggende hersenen tegen mechanische beschadiging tijdens de voedselopname. De vorming van hyoidbeenderen draagt bij tot de versteviging van deze staaf, alsmede de versteviging voor spieraanhechting. Een versnelling van schedel verbeningen treedt op, waarbij een groot aantal perichondrale beenderen worden gevormd in een relatief korte periode. Deze verbeningen concentreren zich rondom de chorda dorsalis en rondom articulaties. Op dit ogenblik beginnen de larvale *C. gariepinus* een kannibalistisch gedrag te vertonen, wat impliceert dat de mechanische belasting op deze structuren die instaan voor voedselopname, sterk zal

toenemen. Het suspensorium wordt vooraan aan het neurocranium vastgemaakt via een ligament, dat later zelfs zal verbenen, ter vorming van het entopterygoid. De bijkomende vorming van dermale beenderen, zowel kanaalbeenderen als anamestische beenderen, zorgt ervoor dat het chondrocranium geleidelijk aan volledig wordt afgedekt. De vorming van bijkomende, getande beenderen gebeurt nagenoeg simultaan met het functioneel worden van het operculaire vierstangen systeem, alsmede het volledig operationeel worden van het spijsverteringsstelsel.

Het kop-lateraal systeem, alsmede de hiermee gerelateerde beenderen, zijn goed ontwikkeld in *C. gariepinus*. De belangrijkste kanalen van het systeem kunnen worden onderscheiden: (1) het supraorbitaal kanaal, (2) het infraorbitaal kanaal, (3) het preoperculo-mandibulair kanaal, (4) het otisch kanaal, (5) het postotisch kanaal, en (6) het temporaal kanaal. Het supratemporaal kanaal is echter afwezig, wat ook het geval is bij de meeste katvissen. Een pitlijn kan wel worden waargenomen op de plaats waar dit laatste kanaal zou moeten verwacht worden. In de meeste specimens kan een supraorbitale commissuur worden onderscheiden. Andere pitlijnen die aanwezig zijn, omvatten: (1) een verticale pitlijn, (2) een horizontale pitlijn, (3) een orale pitlijn, (4) een voorste pitlijn, (5) een middelste pitlijn, en (6) een achterste pitlijn. Alle kanaalbeenderen, zoals ze kunnen worden waargenomen in een gegeneraliseerde katvis, kunnen worden onderscheiden, met uitzondering van de extrascapularia. De fusie tussen (1) het posttemporale en het supracleithrum, en (2) het supraoccipitale en de parietalia kunnen worden gesuggereerd, alhoewel geen eenduidig bewijs kan worden teruggevonden. Ter hoogte van de mandibulaire articulatie kunnen enkele beentjes worden onderscheiden die het preoperculo-mandibulair kanaal omgeven. Deze beentjes worden beschouwd als splenialia. Veel van de kanaalbeenderen in *C. gariepinus* zijn secundair plaatvormig geworden, wat de gespecialiseerde positie van *Clarias* (binnen de katvissen) ondersteunt.

II.2 - Een gedetailleerde studie van de craniale musculatuur gebeurde aan de hand van drie verschillende ontogenetische stadia van *Clarias gariepinus*, waarmee gegevens uit de literatuur (SURLEMONT en medewerkers) werden vergeleken en aangevuld. De spieren worden in vier delen beschreven, gebaseerd op hun morfologische en functionele coherentie: (1) het adductor mandibulae complex, (2) de maxillaire monddraadspieren, (3) de hyoid en intermandibulaire spieren, en (4) de operculaire en suspensoriale spieren. Een algemene asynchronie kan worden achterhaald in de vorming en de functie-initiatie.

De morfologie en samenstelling van het adductor mandibulae complex bij katvissen is reeds verschillende malen een punt van discussie geweest in de literatuur. Dit is gedeeltelijk te wijten aan het feit dat dit complex bijdraagt tot de vorming van een retractor tentaculi spier. Het adductor complex zelf verzorgt de sluiting van de mond en is zeer sterk ontwikkeld in juveniele *C. gariepinus*. De gegevens van de ontogenie, alsmede gegevens uit de literatuur, tonen aan dat in *C. gariepinus* de volgende twee delen kunnen worden onderscheiden: (1) een lateraal A_2A_3'-deel, dat slechts weinig gedifferentieerd is, en (2) een dieper gelegen A_3"-deel, dat verder kan opgesplitst worden in een oppervlakkig en een dieper deel. Achteraan zijn beide delen van elkaar gescheiden door de levator arcus palatini. Vooraan, ter hoogte van hun insertie op de onderkaak, zijn ze echter gefusioneerd. Een A_1, noch een A_ω-deel kunnen worden onderscheiden.

Een typische kenmerk voor katvissen is de aanwezigheid van een palatinum-maxillaire mechanisme, welke een gecontroleerde beweging van de maxillaire monddraden toelaat. In *C. gariepinus* kan reeds vroeg in de ontogenie (7.2 mm SL) de betrokken skeletale én musculaire onderdelen worden onderscheiden, en zijn deze waarschijnlijk reeds functioneel: (1) het palatinum, gescheiden van de rest van het suspensorium en articulerend met het neurocranium, (2) het maxillaire been, dat de basis van de maxillaire monddraad omgeeft en dat articuleert met de rostrale tip van het palatinum, (3) een retractor tentaculi spier die het maxillare

verbindt met het suspensorium, en (4) een extensor tentaculi spier die het achterste deel van het palatinum met het neurocranium verbindt. Tijdens de ontogenie wordt de articulatie tussen het palatinum en het neurocranium verstevigd door been, dat het articulatiefacet omgeeft (komt overeen met het os autopalatinum en het os lateroethmoideum, resp.). Daarnaast wordt de aanhechting van de retractor tentaculi, zowel aan het maxillare als aan het suspensorium verbeterd. De morfologie van de onderdelen van het monddraadmechanisme in *C. gariepinus* laat suggereren dat het palatinum voornamelijk een rotatie ondergaat, alhoewel een beperkte translatie eveneens mogelijk is.

Een ontogenetische asynchronie in de vorming en functie-initiatie kan eveneens worden waargenomen in de hyoid en intermandibulaire spieren: de vorming en insertie van de intermandibulaire spier en de protractor hyoidei gaat alle andere hyoidspieren vooraf. De sternohyoideus en de hyohyoideus inferior volgen, waarna de hyohyoideus abductor en adductor spieren als laatste aanwezig zijn. De opbouw van de protractor hyoidei kan duidelijk worden achterhaald: het ontstaat door de fusie van de intermandibularis posterior en de interhyoideus anterior. Deze protractor hyoidei spier is stevig ontwikkeld in *C. gariepinus*, waar ze waarschijnlijk een belangrijke rol speelt in mechanismen voor respiratie en voedselopname. Dit kan o.a. worden afgeleid uit zijn grootte, alsmede uit de gedifferentieerde opbouw: een oppervlakkig deel, dat de positie en de oriëntatie van de mandibulaire monddraden controleert, en een dieper deel, dat via de tendineuze insertie op de onderkaak bijdraagt tot de mandibulaire depressie. De tendons van de hyohyoideus abductor vertonen een gekruist verloop. De adductores spieren vormen een dun spierblad die de verschillende branchiostegale stralen met elkaar verbinden, en eveneens de laatste straal verbindt met het operculare.

Reeds vroeg in de ontogenie kunnen bij *C. gariepinus* drie operculaire en twee suspensoriale spieren worden onderscheiden, die zich reeds hebben vastgehecht op het skelet. Deze spieren zijn: (1) de levator operculi, (2) de adductor operculi, (3) de dilatator operculi, (4) de adductor arcus palatini, en (5) de levator arcus palatini. Ontogenetische gegevens en de literatuur duiden eveneens op een asynchronie, wat een impact op het respiratiemechanisme veronderstelt. De spieren die eerst worden gevormd maken deel uit van het apparaat dat het drukpomp systeem verzorgt tijdens de respiratie. Hierbij wordt een onderdruk gecreëerd in de buccale holte, waarna het opgezogen water door de kieuwen wordt gedrukt. Het zuigpomp systeem, daarentegen, dat een onderdruk in de operculaire ruimte induceert, wordt verondersteld belangrijker te worden later in de ontogenie. Dit kan ondermeer worden afgeleid uit het feit dat de adductor operculi, door een evolutieve verschuiving in insertieplaats, een protagonist van de dilatator operculi is geworden (in tegenstelling tot de antagonist, zoals gewoonlijk wordt waargenomen bij Teleostei). Twee operculaire spieren staan dus in bij *C. gariepinus* voor de abductie van het kieuwdeksel. Deze spieren, die dus de aquatische respiratie verzorgen, staan eveneens in voor de atmosferische respiratie.

VI.3.1 - Abnormale Ontwikkeling

III.1 - Het onderzoek naar de craniale morfologie van artificieel geïnduceerde dwergvormen van *Clarias gariepinus*, werd uitgevoerd, zodoende enig inzicht te verschaffen in de relatie tussen de ontogenie en de lichaamslengte, enerzijds, en tussen de ontogenie en de leeftijd, anderzijds. Even belangrijk is de bijdrage dat zo een studie kan leveren tot het beter begrijpen van de normale ontogenie, daar het kan toelaten enig onderscheid te maken tussen epigenetische en genetische aspecten van de controle van de schedelontwikkeling. Larvale *C. gariepinus*, die worden opgekweekt in een beperkte ruimte, zijn opvallend kleiner dat deze die in normale omstandigheden opgroeien. Een gedetailleerd onderzoek, ter vergelijking van

het kopbouwplan tussen deze beide werd dan ook uitgevoerd. Als referentie werden twee groepen specimens gebruikt: (1) normale specimens van dezelfde leeftijd (maar dus verschillende lengte), en (2) een specimen van gelijkaardige lengte (maar dus verschillende leeftijd). De ossificatie van de dwergvormen is opvallend minder dan in even oude, normale schedels, maar was nagenoeg identiek aan deze in normale, even grote schedels. Hoe de juiste feedback gebeurt tussen het waarnemen van een ruimtelijk beperkte omgeving, resulterend in een vertraagde ontogenetische differentiatie, kon echter niet worden afgeleid op basis van deze gegevens. Het kan worden gesuggereerd dat een hormonale regulatie, als gevolg van stress, een mogelijke vertraging van de ontogenie induceert, evenals dat de veranderingen in functionele vereisten hiertoe bijdragen. De algemene conclusie dat de ontogenie grotendeels bepaald wordt door de lichaamslengte, en niet door de leeftijd, heeft echter enkele methodologische gevolgen, meerbepaald in methodes van leeftijdsbepalingen bij vissen en het beschrijven van heterochronieën.

VI.3.1 - Ontogenie: Het Maken van Compromissen tussen Wat Voorhanden is en Welke Functies Moeten Worden Uitgevoerd

IV.1 - Een zich ontwikkelende larve wordt voortdurend geconfronteerd met veranderende functionele vereisten, wat o.a. voeding en respiratie betreft. Ze dient dan ook in staat te zijn te kunnen repliceren op deze veranderingen, zodoende de overlevingskansen te verhogen. De opbouw van het 'Bauplan' van zo een larve is echter veel minder complex dan in een juveniele situatie, wat grote beperkingen op de functionele exploitatie mogelijkheden van dit bouwplan legt. Het is dusdanig van cruciaal belang dat de larve in staat is om die structuren die aanwezig zijn, op een zo optimaal mogelijke manier weet te benutten. In dit hoofdstuk is de aandacht gegaan naar één bepaald onderdeel van mechanismen die instaan voor respiratie en voedselopname, nl. de mondopening in *Clarias gariepinus*. Gedurende de ontogenie worden niet minder dan vijf verschillende mondopeningsmechanismen gevormd in de larvale periode. Drie ervan blijven hoogst waarschijnlijk functioneel gedurende de juveniele en adulte periode. Naarmate de complexiteit van het craniale 'Bauplan' toeneemt, zo neemt eveneens de complexiteit van de mondopeningsmechanismen toe, wat een mogelijke verbetering van de mondopening toelaat. Heel vroeg in de ontogenie wordt de mandibulaire depressie mogelijk gemaakt door twee, elkaar opvolgende mechanismen, die volledig of gedeeltelijk gebaseerd zijn op een passieve kaakdepressie. Later gebeurt de depressie actief, waarbij deze gekoppeld wordt aan de depressie van het hyoid. Nog later (11 mm SL) treedt het vijfde mechanisme in werking, nl. het operculair vierstangen systeem. De verschuivingen tussen de verschillende mechanismen, alsmede de verandering van parameters binnen een mechanisme, kunnen mogelijks gerelateerd worden aan verschuivingen in voedselopname types, wat veelal kan worden waargenomen bij Teleostei. Wanneer de verschuiving in mondopeningsmechanismen wordt vergeleken met enkele veranderingen in verband met voedings- en respiratie gedrag en -structuren, kunnen een vijftal fasen tijdens de ontogenie worden gedefinieerd.

IV.2 - Een kenmerk van verschillende katvissoorten houdt de reductie, en zelfs het verlies, van het interhyale in. In dit hoofdstuk wordt een getailleerde beschrijving van de ontogenie van het interhyale gegeven, waarbij enkele reflecties, aangaande het functioneel belang ervan, worden besproken. Aanvankelijk is het interhyale continue met zowel het suspensorium als het hyoid. Later gaan beide connecties verloren, waarbij een ligament een

nieuwe verbinding verzorgt. Uiteindelijk verdwijnt het interhyale volledig, en wordt het functioneel vervangen door dit ligament. Deze gegevens van de morfologie, alsmede gegevens uit de literatuur, laten het toe een hypothese voorop te stellen die de reductie van het interhyale koppelt aan een gereduceerde depressiecapaciteit van het hyoid. Argumenten die deze hypothese ondersteunen zijn: (1) de huidplooi tussen de onderkaak en de rostrale tip van het hyoid is opvallend kort, (2) een dorsoventraal afgeplatte schedel impliceert lage suspensoria, wat resulteert in een beperkte laterale verplaatsing van de distale uiteinden van de hyoidstaven tijdens hun depressie, (3) een korte sternohyoideus spier laat slechts een beperkte verplaatsing van het hyoid toe, (4) de positie van deze spier, in relatie tot de hyoidstaven suggereert dat de contractie eerder een retractie dan wel een depressie zal teweegbrengen, en (5) het gering opgeplooide, branchiostegale membraan laat slechts een geringe expansie toe. De hypothese kan nog verder worden ondersteund door enkele argumenten die suggereren dat een grote hyoid depressie in *Clarias gariepinus* niet nodig is: (1) de bentische stabiliteit zou verloren gaan, (2) aquatische respiratie is grotendeels vervangen door atmosferische respiratie, (3) brede schedels induceren een groter volume-expansie dan smalle schedels, bij éénzelfde hyoid depressie, en (4) structurele adaptaties hebben een extensieve zuigvoeding nagenoeg onmogelijk gemaakt.

Chapter VII.1 - References

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