AN ANALYSIS OF THE EARLY ONTOGENY OF *APLOCHEILICHTHYS JOHNSTONI* (GUNTER, 1893) FROM A LIFE HISTORY PERSPECTIVE

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by

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Corrections made
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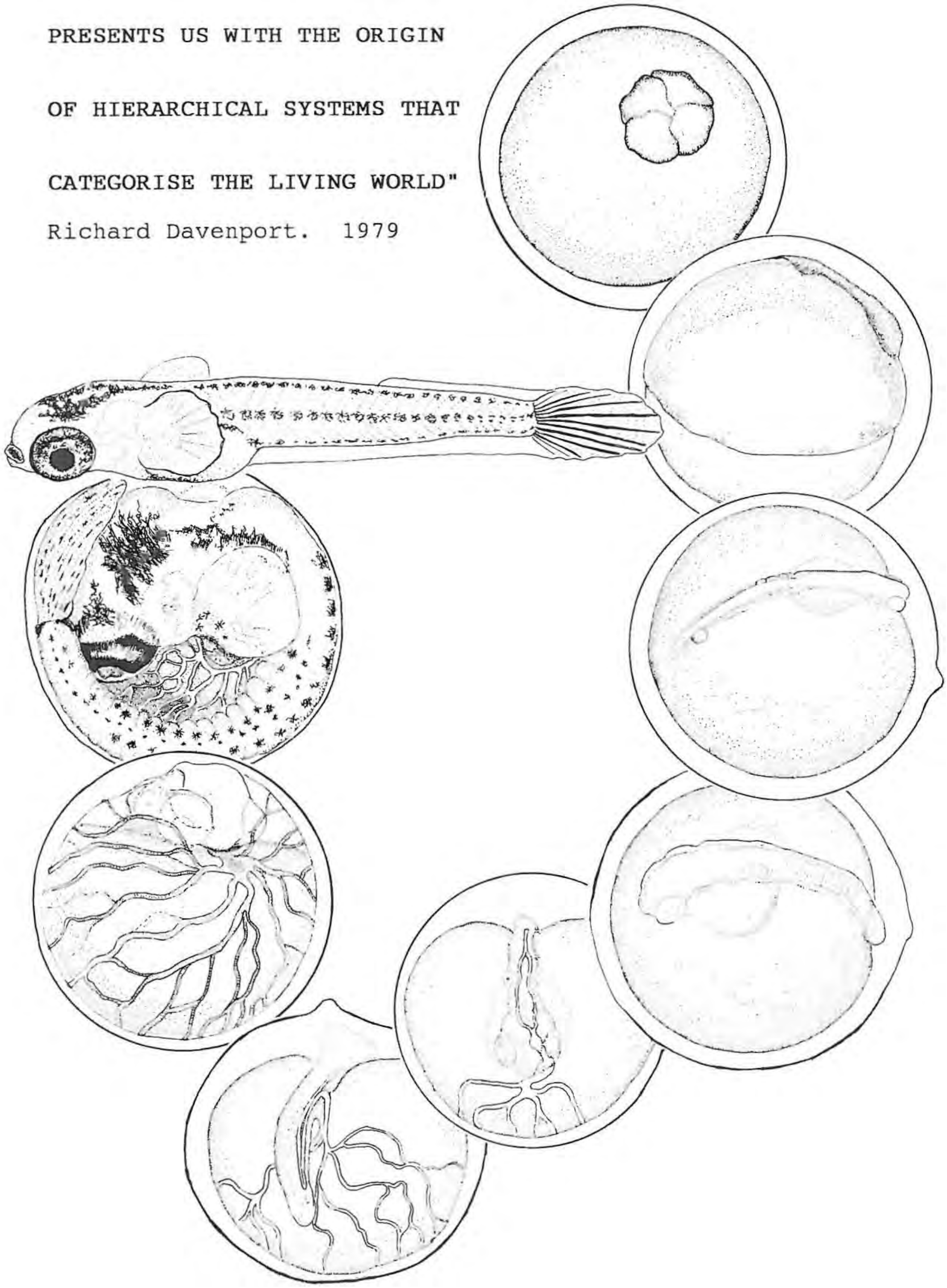
"TOGETHER WITH PHYLOGENY, ONTOGENY

PRESENTS US WITH THE ORIGIN

OF HIERARCHICAL SYSTEMS THAT

CATEGORISE THE LIVING WORLD"

Richard Davenport. 1979



CONTENTS

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This thesis is dedicated to John, Gregory and David, my family, as a token for their support and patience, especially through the writing part of the work.

ABSTRACT

The reproductive and developmental styles of *Aplocheilichthys johnstoni* closely resemble those of other oviparous cyprinodont fishes reported in the literature. Reproductively it sorts to the guild of nonguarding phytophyllic broodhiders, is a daily, fractional spawner of relatively large, adhesive eggs. The length of the embryonic period varies between 14 and 19 days and the larval period can last for up to 30 days. Sexual maturity can be attained at an age of 150 days from fertilization. The embryology is described in detail and close comparison is made with the embryology of other cyprinodonts to highlight possible phylogenetic differences. Major differences with other cyprinodonts are in the rate of development, and heterochronic shifts in the appearance of certain structures in relation to each other.

A discussion is included on the nature of development. It is suggested that development proceeds in a gradual stepwise fashion, interspersed with four major saltations, namely, fertilisation, onset of exogenous feeding, sexual maturity and death.

Chapter 1

INTRODUCTION

The union of male and female nuclei in the egg, fertilisation, initiates a sequence of events which mimics the unfolding of life itself. A single cell will, in a series of ever more complex and involved processes, give rise to an organism with complex tissues, eventually forming integrated organ systems. During this process, growth and differentiation are closely linked, with growth usually following differentiation. The differentiation of tissues, although under genetic direction, is of an epigenetic nature, with the ultimate fate of cells determined by the position in which they find themselves relative to other cell types. The epigenetic process in early development has been under scrutiny for some time by authors such as Conrad Waddington, Soren Lovtrup, Brian Hall, Eugene Balon, Louis Wolpert, and others.

Hall (1983) writes that "Development is controlled epigenetically. Evolution acts by altering development. Therefore, epigenetic control must have played an important role in the evolutionary process". He cites examples of areas where certain epigenetic processes such as cell migration, primary embryonic induction and epithelial induction of skeletal tissues are common to present day vertebrates and may suggest that epigenetic control via tissue interactions arose very early in vertebrate evolution and has been conserved with amazing fidelity. Fundamental mechanisms for evolutionary change can be found in elements of epigenetic control such as the position of epithelia and mesenchyme within the embryo, timing of cell migration, onset and termination of induction and the length of time inducers retain inductive activity, among others. Change can therefore be effected without any necessary alteration in the nature of the inductive signal or in the competence of the responding cells. In other words the longterm stability of skeletal and tissue types remains but the rate and sequence in which they appear can be altered.

The ontogenetic process not only embraces the embryonic and larval periods but the entire life history of an organism. This study, however, concentrates on the embryonic and larval periods of the ontogeny of Aplocheilichthys johnstoni (Poeciliidae). The description and analysis of the ontogenetic process is used here as a basis for testing the theory that development is of a saltatory nature as proposed by Balon (1981). It is also used as an indicator of how this particular species, as a representative of the genus, has come to terms with the environmental variables it has encountered in its evolution and of the life history style it has adopted. Study of the development sheds light on the reproductive biology which is also reported and briefly discussed. Furthermore, it is hoped that the results will be useful to field biologists in the identification of eggs and larvae.

The field of larval fish taxonomy is undergoing considerable change in its emphasis and analysis. Early workers in this field, such as Ahlstrom and Moser, concentrated on the description and naming of the pre-adult forms (Moser et al. 1983). This knowledge was basic to the understanding of fish biology and still forms an essential part in the building of our database for the aquatic systems of the planet. With the advent of ecological studies during the past thirty years workers in the field broadened their interest in larval fish to encompass their ecology and developmental style. It was, however, the closely monitored studies on live embryos and larvae initiated by Kryzanovsky and furthered by, among others, Eugene Balon and his students, which alerted many workers to the differences and similarities in the processes of development of different species. These studies have revealed the significance of various developmental events and have led to a modified nomenclature of the various stages based on developmental events instead of the arbitrary naming of stages. However the true value of this new nomenclature is by no means universally recognised (Richards 1976, Fuiman 1987).

Comparison and integration of studies on reproductive biology and developmental styles have further emended our knowledge of the way in which different fishes have responded to environmental constraints and have encouraged a holistic approach to the study of animals.

The pattern and sequence of developmental events are signposts along the phylogenetic and ecological path of a species. These events are evidence of the solutions that the particular soma has produced in response to environmental constraints. Each ontogenetic period can vary in many different ways and can occur, according to McNamara (1982), through contraction, extension or a shift in the timing of rates of development.

The timespan of each event can be indicative of a particular strategy. A short embryonic period with an extended larval period usually indicates a reproductive style involving semelparity or protracted seasonal iteroparity and the production of many small eggs with yolk of low nutrient value, in other words an 'r' selected or altricial life history style. Further analysis of the development of these animals may reveal that certain organ systems will develop in a different sequence to those of an embryo produced from a larger egg with richer nutrient content and later hatching time. The larval period itself may also become curtailed to a greater or lesser extent. The shorter larval period is usually encountered in fish in which the reproductive style involves a smaller number of richly yolked eggs spawned at frequent but variable intervals during the reproductive season. The higher nutrient value of these eggs enables the embryo to undergo extensive development without additional extraneous nutrition. This style of reproduction is usually encountered among animals which exhibit a 'K' selected or precocial life-history style. The juvenile period, when all adult functions other than reproduction have been acquired, can also be of variable length, not only inter- but also intraspecifically. The availability of adequate food can change the timing and onset of maturity and the number of eggs produced. For similar reasons, size at maturity is also variable (Pianka 1970, Stearns 1980, Balon 1981).

The efficient utilization of energy is a major influence in the life of any animal, the partitioning of available food resources between growth, maintenance and reproduction having a major influence on the style of life history exhibited. Assimilated energy not needed for maintenance will be divided between reproduction and growth (Gadgil & Bossert 1970, Stearns 1982;245). Energy expended on reproduction at any age will result in reduced growth and thus reduced reproductive potential at all subsequent ages, especially for females (Hirshfield 1980, Stearns 1980). It is, however, not only the amount of food available that is critical but the availability of the correct type of food of adequate energetic content for a particular period in development as discussed by Bruton & Allanson (1974), Bruton and Boltt (1975) and Bowen (1976, 1979) in their studies of <u>Oreochromis</u> <u>mossambicus</u> in Lake Sibaya, South Africa.

The importance of timing in developmental events has been emphasised by Wourms (1972 a) and Balon (1985). It appears from these analyses that the developmental process does not proceed in a gradual or a stepwise fashion but in a combination of the two. Some developmental periods are more susceptible to outside influences than others and consequently the time spent in each stage will vary. The synthesis by Wourms (1972 a&b) of the resting periods in cyprinodont fishes revealed very clearly that developmental diapause only takes place during stable periods in the embryonic phase such as the end of epiboly before organogenesis or before heartbeat commences or at the end of the embryonic development.

The variations seen in the amount of overlap in initiation of organ development can further reveal phylogenetic relationships within and between groups. The body of evidence necessary to reveal details of such relationships is, however, still slender.

The suggestion of Balon (1981) that the developmental process is saltatory rests on the coincidence in the completion of the development of several organ systems that bring about a new and initially vulnerable state in the competence of the animal. This vulnerable period is described as a threshold between two periods and is of shorter duration than the previous or succeeding phases. Each period consists of a number of steps during which cells are first determined and differentiated, then grow into tissues which are subsequently elaborated into organs and later organsystems as the development of each part of the system completes.

The relative timing of these events can give an indication of the ecological history of a group; an historical association with highly capricious and widely fluctuating environments will produce a developmental style in which the vulnerable stages are rapidly passed through while longer periods will be spent in the less vulnerable stages, even giving rise in extreme cases to diapause such as is seen in annual fishes, which may be threatened by drying up of the habitat (Wourms 1972). High predation pressure in a densely populated but stable environment can have a similar effect or produce concomitant behavioral modifications, as seen in many cichlids which exhibit a range of behavioral modifications which augment their particular developmental strategies in an effort to maximise reproductive success (Ribbink et al, 1983).

Association with anoxic environments typically produces temporary embryonic respiratory/circulatory systems that facilitate the uptake of oxygen. Animals traditionally associated with conditions of high dissolved oxygen have far less extensive systems.

The developmental process as discussed above exhibits a wide variety of pattern and yet for all the variety exhibited there is a certain set sequence of events which does not change much under quite widely varying circumstances.

The mechanisms of development that produce age specific pattern of survival and reproduction produce dynamic patterns recognised as plasticity, canalisation and constraint (Stearns 1982). Plasticity, canalisation and constraint allow the organism to maintain the valuable tools acquired through its evolutionary history while also giving it flexibility to adapt to the vagaries of an ever changing environment.

In order to establish these parameters the baseline of the developmental process must be established. In order to accomplish this, a study similar to the one conducted here has to be undertaken under strictly controlled laboratory conditions, with as many parameters as possible remaining constant. In this study the constants were water quality, parent population, light regime and above all water temperature. Within limitations of the availability of live food, the feeding regime of the parents and juveniles was also controlled. Once the baseline of the developmental pattern has been established, variables can then be introduced to test developmental mechanisms.

Stearns (1982) states that "developmental mechanisms could connect population genetics with life history theory to form a predictive theory of evolution more powerful than either of the first two (population genetics or life history theory) attempted."

The choice of a candidate for an embryological study such as this is influenced by several factors, not the least of which is availability of live specimens. In this particular case three major factors were involved.

Since 1982 the JLB Smith Institute of Ichthyology has been involved in a large scale investigation of fish ecology in the Okavango Swamps in Botswana. As is usual, those species of direct economic importance to man were the most intensively investigated and a report of that investigation has been submitted to the Botswana Government and will the subject of a doctoral dissertation by Mr G S Merron. However, the role of all components in an ecosystem must be determined, and as part of the ongoing studies of the area it was decided to conduct a study on an Aplocheilichthys species, starting with the reproductive biology of A. johnstoni. A. johnstoni was selected as it is the most abundant of the three Aplocheilichthys species present in the system and is probably numerically the most abundant fish species in the Okavango Delta (Merron & Bruton 1988). These small fish are of no overt economic importance and very little is known about their biology or taxonomy.

Parenti (1981) suggested that the genus may be polyphyletic, with the southern African representatives differing from others by virtue of such derived characters as the lack of an interarcual cartilage, anal fin of 14 or more rays, a robust lower jaw and the swimbladder extending beyond the first three haemal arches. A review of all the African members of the subfamily Aplocheilichthyinae is needed. Theron (1987) has studied the feasibility of using the genus in mosquito control but no in-depth biological study of any member of the genus has as yet been conducted. As Aplocheilichthys is known to feed on aquatic arthropods, their role as energy vectors, shunting food from marginal into deeper areas of the waterbody can be surmised but has never been quantified.

It is hoped that the results of this study will further contribute to the understanding of the southern African freshwater fish fauna, provide a means of identifying eggs and larvae in the field and an insight into the life history style of this member of the cyprinidontiform fish of the southern African region. It was also undertaken as part of an international collaborative investigation into the mechanisms that govern the selection of alternative life-history styles in animals. As many of the life-history theories advanced at present were formulated on the basis of information gleaned from northern hemisphere fishes it will be interesting to determine whether low latitude southern hemisphere fish fit the same patterns.

Hypothesis : As predicted by the life history model proposed by Eugene Balon (1981), A. johnstoni exhibits a saltatory developmental pattern and the reproductive strategy of a broodhider in response to predation and fluctuating environmental conditiion.

Chapter 2

MATERIALS AND METHODS

The fish

The specimens of *Aplocheilichthys johnstoni* which were used as wild parents in this study were collected from the perennial swamp in the northern regions of the Okavango Delta in Botswana. The annual floods of the Okavango River from the Angolan highlands is an integral part of the ecology of this system. The Delta consists of a series of permanent river channels, semi-permanent drainage channels, lagoons and floodplains which link up and then separate again during the course of the annual flood cycle (Merron & Bruton 1988). The collection site was near the village of Seronga in the riverine panhandle ecotone of a blind channel. There is abundant zooplankton in the marginal vegetation (Hart 1986) which consist principally of emergent macrophytes such as papyrus and reeds. In these areas of marginal vegetation, with a temperature range between 27 and 33 ^{O}C the fish lay their eggs singly, attaching them by adhesive filaments to the vegetation, thus effectively hiding them from predators since any eggs not hidden are eaten.

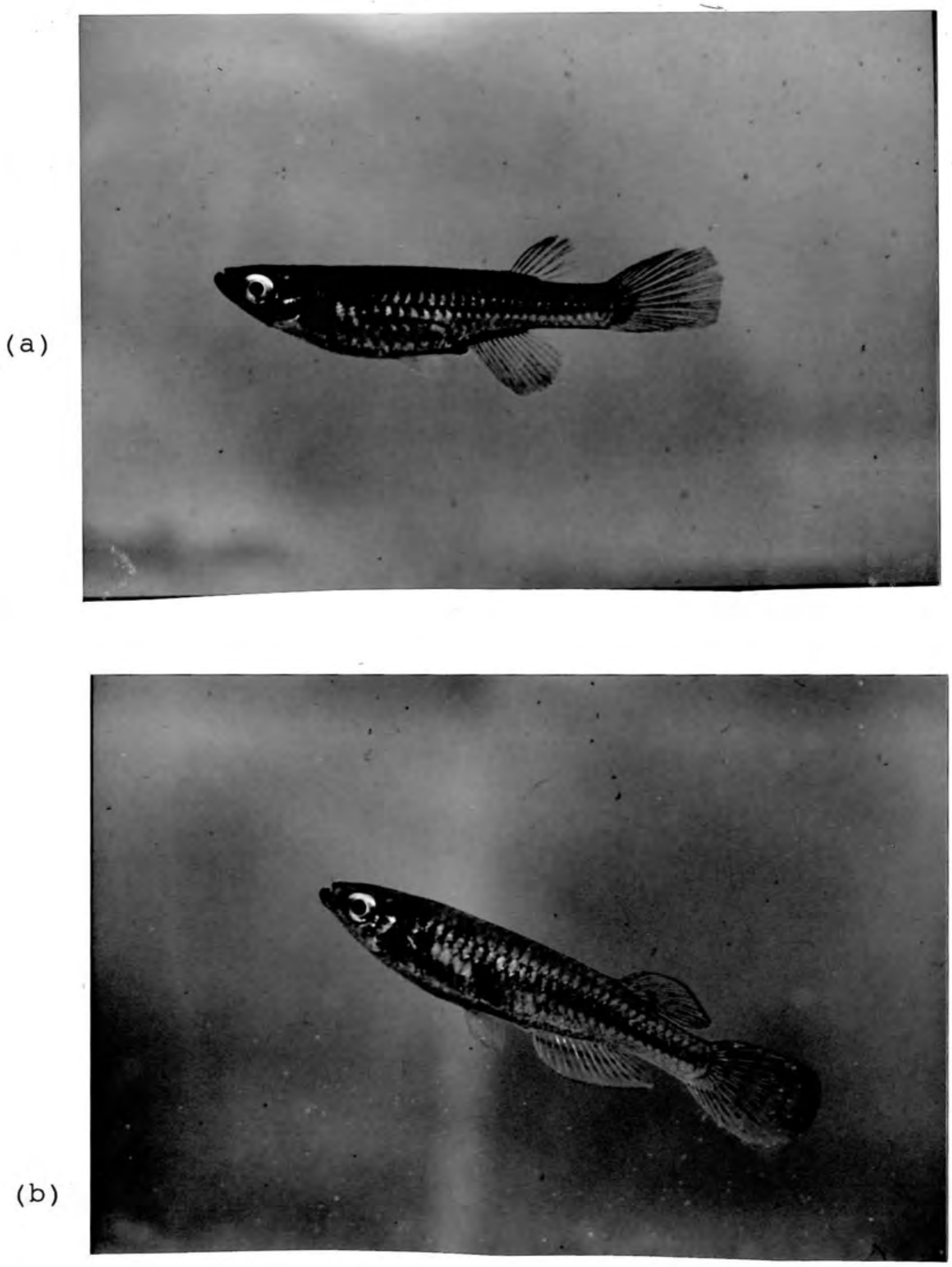


Fig. 1. Adult female (a) and male (b) of *A. johnstoni.*

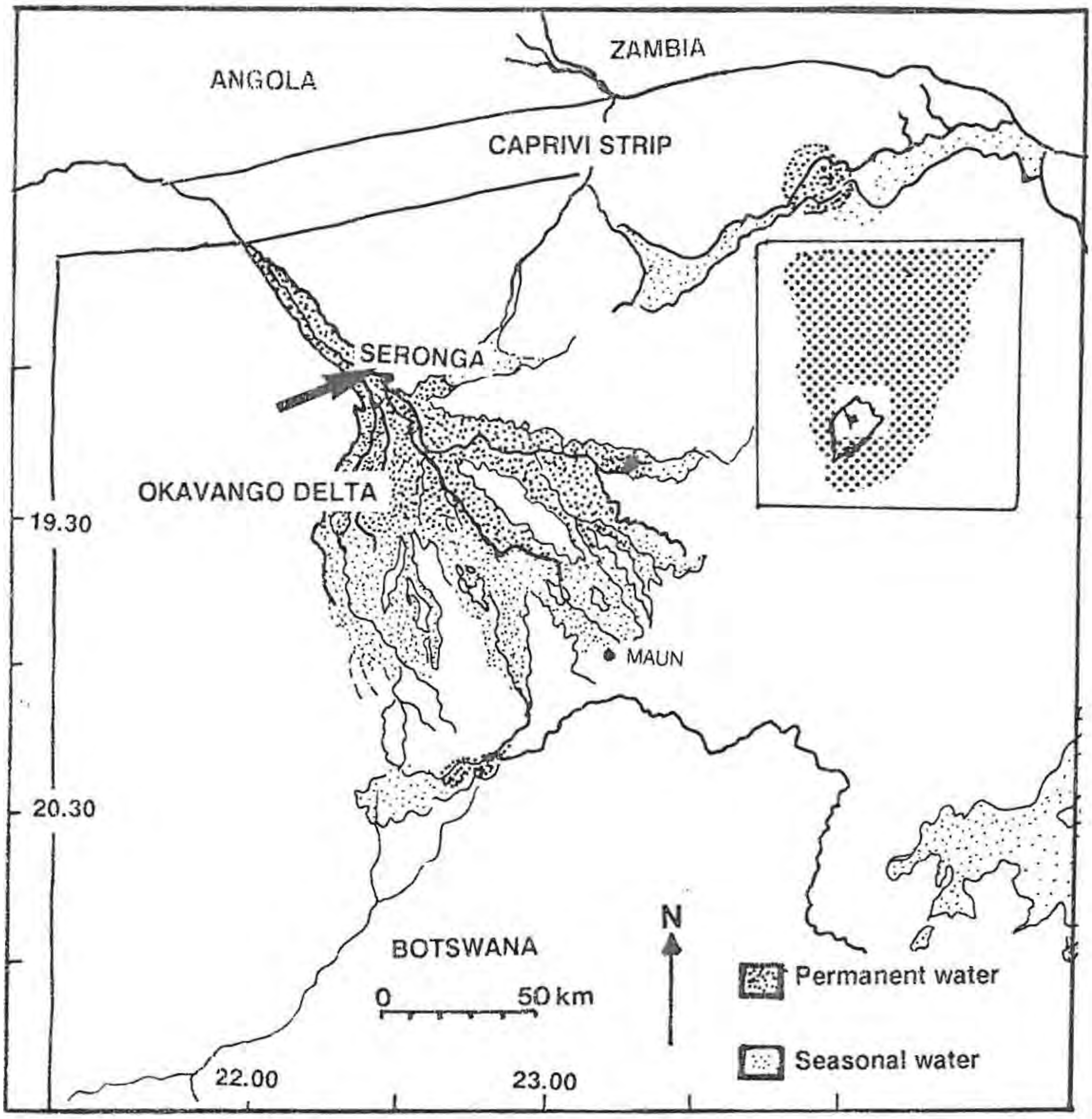


Fig. 2. Location of collection site of wild adults of A. johnstoni used in this study, at Seronga in the Okavango delta, Botswana.

Parent fish were transported to Grahamstown in insulated styrofoam-lined boxes containing plastic bags of oxygenated water by small aircraft. On arrival, these fish were acclimatised in constant temperature rooms at 25°C and a photoperiod of 16h00 light 8h00 dark. Initially the food offered was live zooplankton from the local sewage works and ponds. The major portion of this food consisted of Daphnia spp. with some aquatic insect larvae, mainly Diptera. Tetramin flakes were introduced once the mortality had been reduced and the diet then consisted of a combination of the above, fed twice a day. The parent stock was kept in aquaria of capacity 75 l at 25°C with one large undergravel filter. Green plastic grass was a preferred spawning substrate. The first eggs collected were incubated in gauze hatching boxes kept in the aquaria with the parents.

Experimental design and equipment

In designing the experimental conditions for an accurate ontogenetic series, several factors need to be considered. Factors such as light regime, temperature and water quality need to be as constant as possible, since they can influence developmental rates.

An incubation aquarium was made using a glass aquarium of 54 l capacity provided with a thermomixer pump and an aquarium box filter. The individual incubation chambers were made from soft plastic vials, volume 10 ml, by cutting out two ovals opposite one another and glueing nylon mesh over these. After drying they were soaked in water to leach out any water soluble chemicals. These chambers were suspended in a perspex sheet with a grid of labelled holes the diameter of the vials drilled in it. The vials were also numbered and in this way it was possible to document each embryo accurately. This structure was then suspended in the aquarium. The temperature was maintained at 25-26°C, the water was circulated through the vials by the thermomixer pump which maintained the oxygen content of the water at a constant level.

Breeding pairs were selected by first isolating males from females for several days. Females who were thus unable to spawn, had eggs suspended from the vent. These were selected as obviously mature and transferred to smaller aquaria with the largest of the males. Of these pairs, only two spawned successfully and were used for the first series of intensive observations. Members of each pair were separated initially by gauze partitions. In the morning, after feeding, several units of spawning grass were introduced into the tank, the partition was lifted and the pair was observed for a period of up to an hour, each spawning event being carefully timed. After two or three eggs had been laid in a particular unit of the plastic grass it, was moved to an empty aquarium. In this way the ages of the first batch of eggs were known to within a few seconds. At the end of each mating event the partition was re-inserted. In order for eggs to activate and harden they were left on the spawning grass for at least 10 minutes before being moved. Sometimes eggs were left undisturbed for some hours so that comparisons could be made between the timing of events in disturbed and undisturbed eggs.

Each grid position had a numbered datasheet (e.g. A1). Each embryo was coded according to its parental pair (e.g. Aj6), spawning time, date and vial number (e.g. 12.10.89.16h35/24) and this code was entered on the datasheet for the grid position in which the vial was kept. Each embryo thus had the following code Aj6 D5 12.10.89.16h35/24. Notes of the development were made on these sheets and drawings, made with the camera lucida, were similarly coded and filed together. After hatching, the fish were transferred to larger coded plastic cups with gauze windows and suspended in an adjacent aquarium under similar temperature and light regimes. Feeding during this developmental period proved to be problematic and was only solved satisfactorily during the second year of the experiment when water from a garden pond was found to contain sufficient Protozoa to feed the hatchlings. Larvae started feeding on Tetramin 10 days after hatching. As the juvenile period is said to terminate with the onset of sexual maturity a small number of similar aged fish was kept in a separate tank to try and establish the possible duration of this period. The group was kept in the same constant temperature room as the other experimental fish. The tank was filled with enriched pond water with a near constant supply of Daphnia spp added and supplemented by Tetramin flakes in an effort to obtain optimal growth. At 90 days, spawning grass was introduced to the tank and checked daily for eggs.

The male of one, and a female of the other spawning pair died towards the completion of the collection of the first dataset.

After a simulated winter period (10h00 light 14h00 dark) in the constant temperature rooms the extant F1 population was used to obtain the next set of information. The eggs were collected from a small population of males and females producing eggs at a set time during the day. Eggs were consequently collected at 15h30 each day and were aged from previous experience of cell division times established during the first series. Embryos of 32 or 68 cells could be aged to within 30 minutes. Due to this inaccuracy none of the embryos less than 82h00 old were used. Altogether 189 drawings were made but notes were taken from many more specimens. Of the drawings 59% were obtained from embryos of the isolated pairs and encompassed the entire embryonic as well as the larval period. These larval period drawings were made at wide intervals. The embryos from the F1 population comprised 32% of the drawings made, starting during the late embryonic period but concentrating on the larval period. Of the drawings refered to, 9% were from a pilot study.

Observations were planned around a four hour schedule. At times when new events were occurring, monitoring at more frequent intervals was necessary and more specimens were monitored. Earlier stages in the ontogeny necessitated more frequent observations than later ones. Events were monitored under a Nikon stereoscopic microscope with a double camera mounting, using both colour and black & white film, with a motordrive and remote shutter release. A C&D projection microscope was also available for confirmation of developmental events. In order to confirm earlier observations, the same embryo was used sequentially, returning it to the incubator between observations. No special apparatus was used to maintain temperature under these circumstances but the observation times were shortened.

These methods and techniques were based on those advocated by Balon and Flegler-Balon (1985).

Preservation and staining technique

Embryos were preserved in 10% buffered formalin solution. Preserved embryos and larvae were stained for cartilage and bone using the methods developed by Taylor (1967) Dingerkus & Uhler (1977) and Noakes & Balon (1980). Larvae of the same age were stained for both tissues and others for bone only. These procedures served to confirm the destructive nature of the alcian blue stain on calcified structures and it was necessary to monitor the alcian blue staining time carefully.

Graphic presentation

When the assembled drawings and notes were analysed it was soon realized that age could not be used as a definitive marker for establishing the events which could mark the steps and phases of early ontogeny as development did not proceed at the same pace in all embryos. The largest number of embryos of similar age with similar development was used to determine event boundaries. The illustrations produced are often a composite of the events at any given age using colour slides taken at the same or sometimes similar age as corroboration. Colour photographs are presented to corroborate the drawings and illustrate pigmentation. In charting the skeletal development, the youngest fish showing the most advanced skeletal development stained with only Alizarin Red S was used as a benchmark. Only specimens preserved in 10% buffered formalin were measured. All specimens resulting from this study are lodged with the JLB Smith Institute of Ichthyology, Grahamstown.

Terms used

Age is expressed in hours and minutes (00h00) until the periods elapsing between observations were longer than 12 hours, then it was changed to days (00d).

Blood vessels and other structures were named largely but not entirely using the nomenclature of Cunningham and Balon (1986) and bones were identified with the assistance of Dr P.H. Greenwood and following the conventions of Langile & Hall (1987) and Parenti (1981).

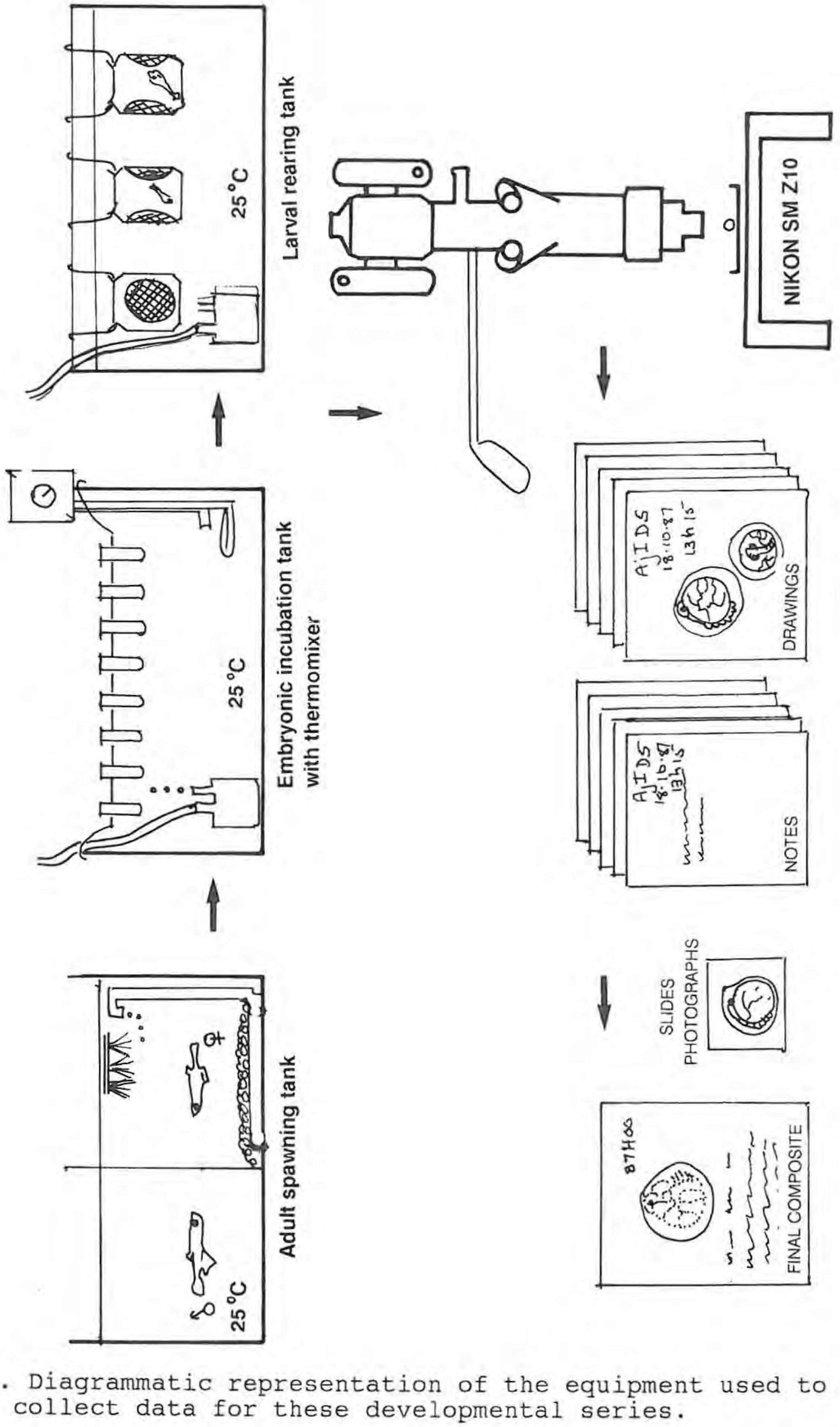


Fig. 3. Diagrammatic representation of the equipment used to collect data for these developmental series.

RESULTS

3.1 REPRODUCTIVE BEHAVIOUR

The spawning behaviour and some aspects of the reproductive biology were noted carefully while the eggs were collected.

Aplocheilichthys johnstoni is a serial spawner, depositing up to ten but usually three to five eggs singly in vegetation after a short mating dance. When observed in the laboratory, the mating was usually initiated and orchestrated by the female. When there was only a single pair in a tank the female would lead the male to the chosen spawning area with tail whisks and fin flutters. At the spawning grass she would usually swim through the patch of grass on her own, select a spot, then return to the male and lead him through the grass, stop at or near the selected place, position herself and wait for him to position himself slightly below and behind her. The egg would be rapidly expelled into the grass with a characteristic tremble from both fish. The adhesive threads on the egg entangle with the grass. The male has been observed trying to entice the female to return to the spawning area unsuccessfully after she had terminated her mating dances.

The fish used in these developmental series responded to food availability and quality by changes in their fecundity. The number of fertilised eggs produced by an isolated spawning pair varied between three and five when the fishes were fed twice daily on Tetramin flakes only but increased markedly to between six and twelve at one spawning when flakes were supplemented or replaced by Daphnia and mosquito larvae. When the food supply was reduced to one feeding per day the egg production ceased.

3.2 DEVELOPMENT

Introduction

Early ontogeny is divided into three periods, namely embryonic, larval and juvenile. The two main phases in the embryonic period are those of cleavage and organogenesis. Fertilisation consists of two separate processes, activation (the formation of embryonic membranes) and amphimixes (the fusion of the two haploid nuclei). After fertilisation the cytoplasm separates from the yolk. The cleavage phase is divided into two distinct periods of differentiation, namely blastulation and gastrulation. Blastulation involves the translation of the amino acids and proteins of the egg into those needed for the initial differentiation of the cell into blastomeres which by the completion of the blastula stage will have become differentiated into extra embryonic cells that take part in epiboly and the formation of embryonic precursers (Davenport 1979, Balinsky 1981). Prior to gastrulation there is further protein synthesis after which the differentiation of the cells into primary germ layers takes place as well as epiboly during which the yolk is enveloped by a tissue layer. At the completion of gastrulation, the process of embryogenesis starts during which the embryo with its complex organ systems is formed. The embryonic phase, which is the most complex, may take place entirely within the egg membranes or partly after hatching as a free embryo. The term hatchling used here, refers to a newly hatched individual which may be still embryonic ie dependant on endogenous food or able to feed exogenously. The transition from the embryonic to the larval period takes place when the organism begins to feed on external food sources as the yolk is being resorbed. This is the most vulnerable threshold in development.

The larval period is one of growth and the differentiation of adult structures. The larval/juvenile transition takes place when all adult structures are differentiated and have replaced larval structures such as the median finfolds. During the juvenile period further growth takes place, the skeletal system completes its ossification and the gonads mature.

Embryonic period : Cleavage phase

Fertilisation was never observed under the microscope. The demersal egg is spherical, ranging in diameter between 1.60 and 1.90 mm. When spawned it is soft, the oil droplets are scattered throughout the yolk and there is no perivitelline space. There are adhesive threads, longer than the diameter of the egg, attached to the conical micropyle area and these secure the egg to the plant onto which it is ejected. The eggs are scintillating, transparent and pale yellow and when the cytoplasmic streaming is completed the cell is a deeper yellow.

Between 00h10 and 01h30 the perivitelline space enlarges to 0.05 mm. As activation proceeds the oil droplets coalesce into larger globules and move together in the upper area, of the yolk. The chorion hardens but remains flexible and is finely textured. At 0h23 the animal pole is distinguishable as a deeper yellow cytoplasmic pool at the yolk periphery, usually on the dorsal surface ventral to the micropyle area.
Between 00h31 and 01h15 the deep yellow cytoplasmic accumulation becomes a discrete cell, rising above the level of the yolk and the oil globules are congregated in the upper surface of the yolk. Activation is now complete.

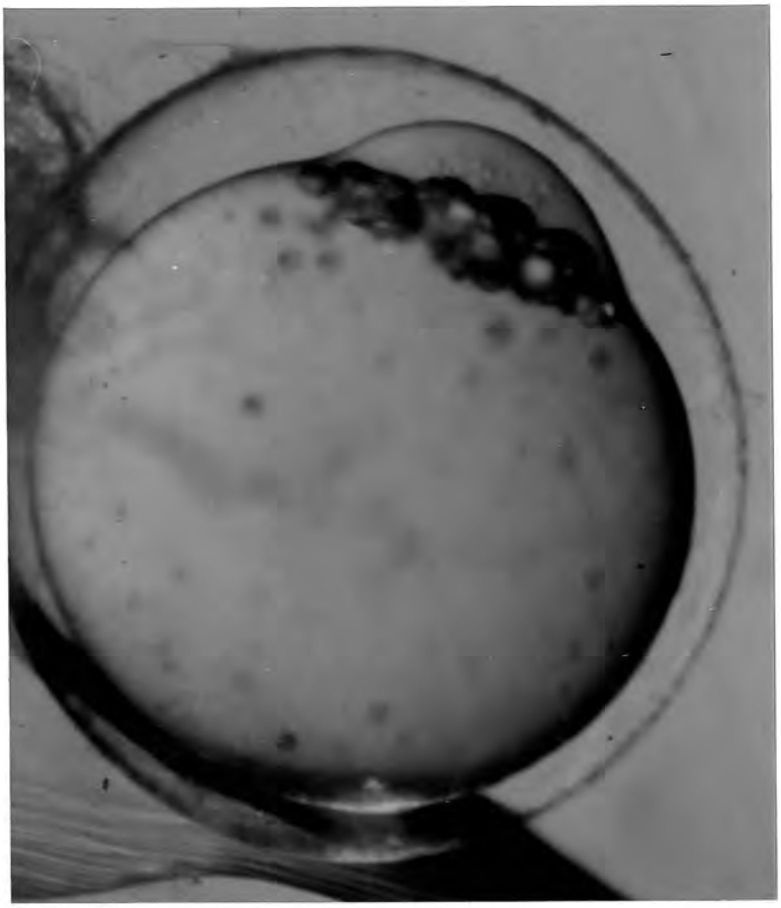


Fig. 4. Photomicrograph of fertilised egg of A. johnstoni age 01h13 prior to first cleavage.

ABBREVIATIONS FOR FIGURES ARE ON PAGE 80 AT THE END OF THIS CHAPTER.

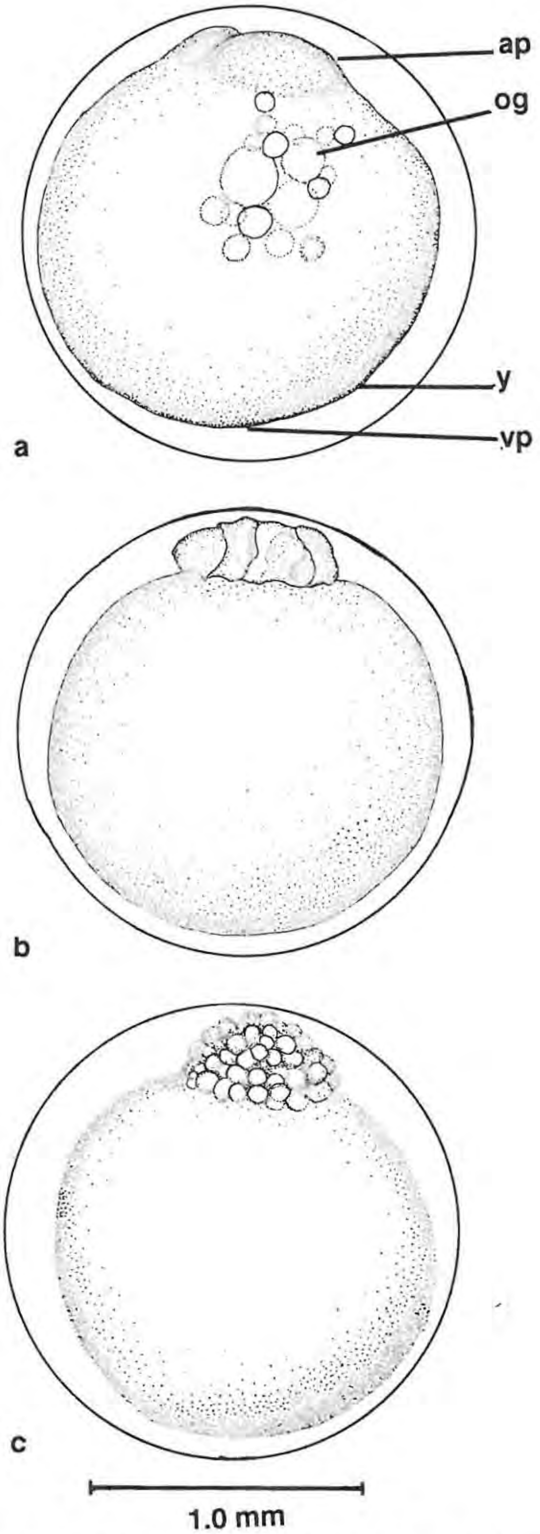


Fig. 5. Earliest cell divisions of A. johnstoni.
Oil globules omitted in all but (a)
(a) 2 cells, 02h00 old (b) 8 cells 04h00 side view
(c) 5h00 morula prior to blastular differentiation

01h45 (Fig. 5a).

The first division takes place in a meridional plane, with subsequent divisions taking place at 15 min intervals, in the same plane but at right angles to the first. The third division also takes place in the meridional plane, but there appear to be two simultaneous divisions, as each pair of blastomeres is divided.

During these early cell divisions the surface of the cell about to divide becomes dimpled and the division lines appear. When the division is complete, the new cells draw away from one another, except at their bases, rising high above the level of the yolk. They then flatten and move closer to one another until they are once more touching, when the next division commences.

At 02h15 there may be either two or four cells or the third division may commence.

At 03h15 there are four or eight cells, or the third division may be in progress (Fig. 5b).

Observations on 11 eggs showed that cell division is not a regular procedure; the rate can vary and cells of unequal size which do not always assume the same pattern are formed.

04h00-08h00 (Fig. 5c).

The fourth cell division begins on the equatorial plane six, then eight cells result. Eggs between 05h00 and 06h00 old may have as few as 32 or as many as 128 cells (a morula) the diameter of which is between 36 and 40% of the yolk diameter. Most 6h00 eggs, however, have morulae. Cell sizes range between 0.05 - 0.10 mm and the cell mass is 0.125 mm deep. By 07h00 the faster developers have a blastula and some already show signs of periblast formation at the edge. Blastodisc size is between 39 and 50% of yolk diameter (Fig. 6). The cells are smaller than before. In preserved specimens small oil globules cling to the undersurface of the blastula.

Between 06h00 and 08h00 the cells divide asynchronously in the raised cell mass. At times the cells appear to sink into the yolk surface in the center of the blastula. The larger cells on the outer perimeter of the blastula appear to be confluent with the cytoplasmic yolk membrane, observed in live specimens and confirmed on dissection of preserved material. This is a typical feature of discoidal cleavage. According to Lovtrup (1974;152) "the blastodisc remains divided by vertical walls while remaining in contact with the remainder of the egg at the lower end. Only when blastomeres become so narrow that a mitotic apparatus perpendicular to the surface of the blastodisc obeys the rules of cell division will separation occur, with the simultaneous formation of yolk nuclei".

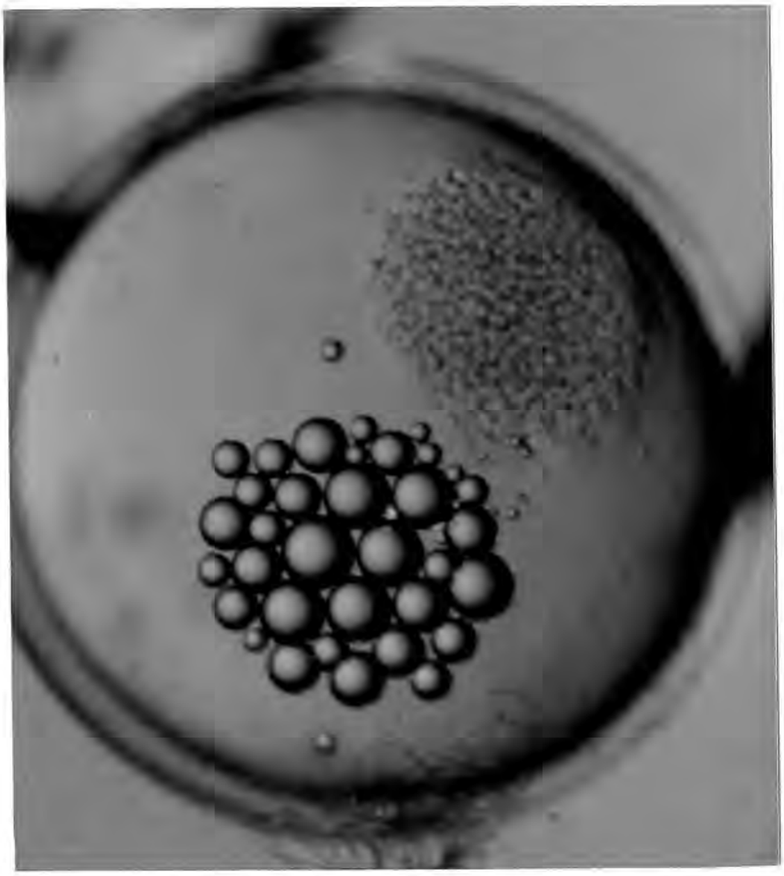


Fig. 6. 7h00 blastodisc of A. johnstoni prior to formation of syncytium.

The earliest diversification of cells into superficial epibolic yolksac cells and inner embryonic blastomeres takes place (Fig. 6). Cunningham & Balon (1985) describe a similar pattern of events in Adinia xenica (Cyprinodontoidei, Fundulidae).

08h00-10h00 (Fig. 8 a,b).
On the perimeter of the blastodisc a collar of large cells with visible nuclei can now be distinguished clearly. This is the syncytial periblast. The periblast can be seen moving away from the edge of the blastula, forming a clear collar, 0.125 mm wide, dotted with nuclei (Fig. 7,8b,). The entire blastodisc now has a diameter of 0.75 mm (73% of the yolk diameter); the cells are 0.025 mm in diameter

Between 10h00 and 13h00 the periblast widens while the blastodisc remains the same size and is still raised above the surface of the yolk.

Between 14h00 and 15h00 the blastodisc begins to enlarge and flatten. The embryonic shield appears on the edge of the disc as a deeper area of cells with a denser yellow colour (Fig. 9a). As epiboly begins, the edge of the blastodisc moves closer to the edge of the periblast which has an irregular perimeter narrowing opposite the embryonic shield area (Fig. 9b).

A period of 08h00 elapses between the formation of the syncytial periblast and the commencement of epiboly.

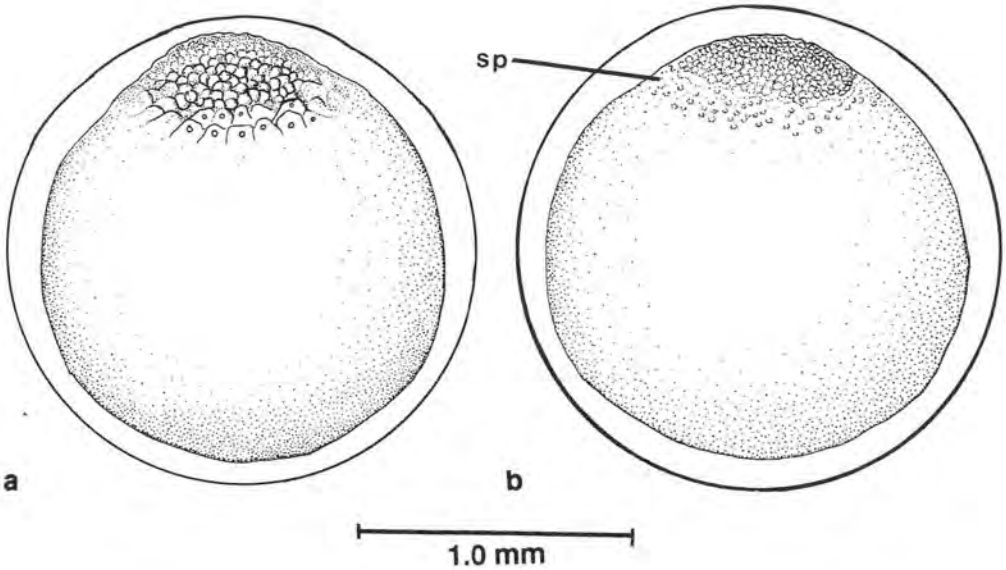


Fig. 7. Differentiation of the blastula of A. johnstoni prior to the onset of epiboly.
(a) At 08h00 with the syncytial periblast cells.
(b) At 10h00 showing the syncytial periblast with large nuclei in a collar around the blastodisc.

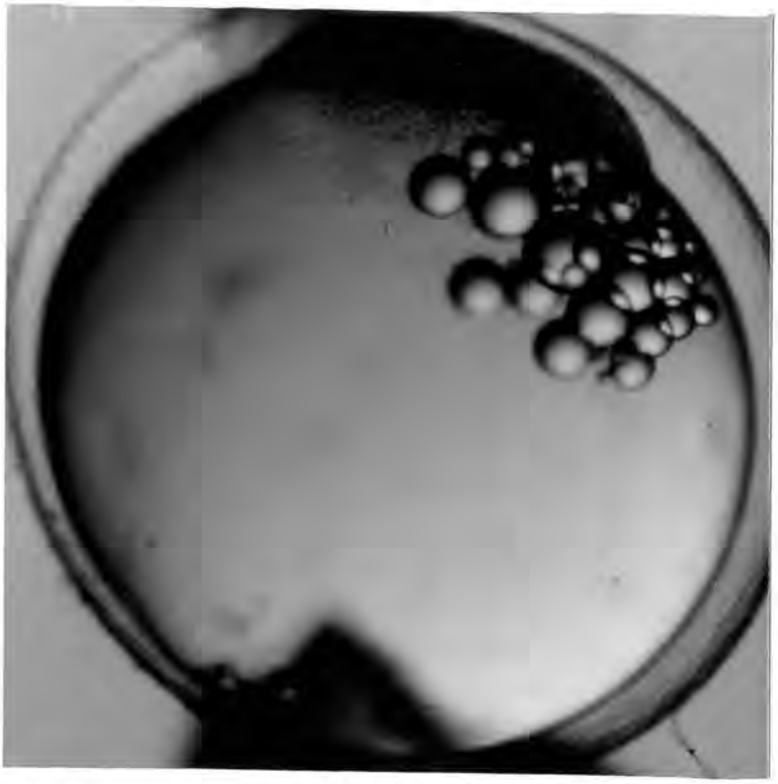


Fig. 8. Embryo of A. johnstoni at age 14h40 showing the blastodisc at the start of epiboly.

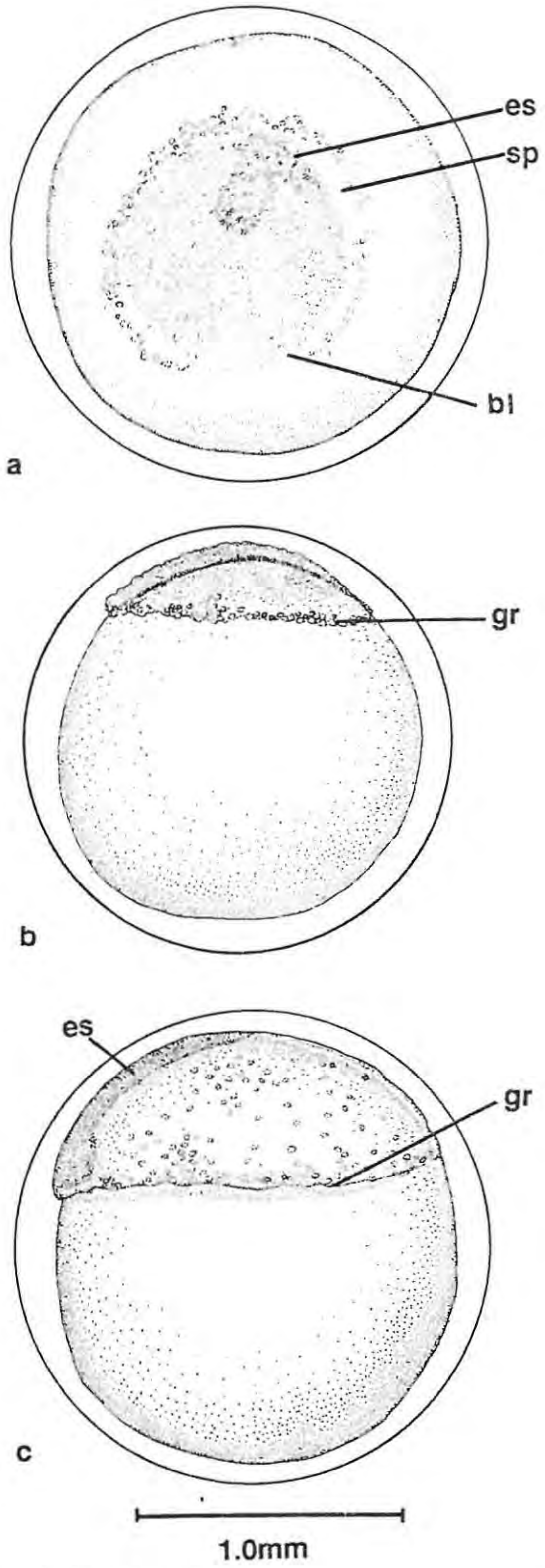


Fig. 9. Epiboly in A. johnstoni.
(a) 15h00 upper polar view of the blastodisc with the embryonic shield and the syncytial periblast.
(b) 16h00 side view of blastodisc, the germ ring has encroached the periblast.
(c) 19h00 embryonic shield lengthens.

16h00-19h00 (Fig. 9c)

The edge of the blastodisc moves up to the edge of the periblast. The blastula becomes a disc, the cell layers thins and form a cap over the top third of the yolk, 1.25 x 1.125mm diameter (72% of yolk diameter) and 0.20-0.25 mm deep. When the cell cap is peeled off the yolk the embryonic shield is clearly visible. The embryonic shield lengthens as epiboly proceeds.

20h00-24h00

Epiboly continues, 50% of the yolk surface is now covered. The cells on the leading edge are larger than those on the rest of the yolk surface already covered.

The embryonic shield does not move with the migrating cell sheet but remains in one position during epiboly. As epiboly continues the embryonic shield lengthens. Toward the end of the epibolic phase the caudal end of the shield is left a little behind the edge of the periblast.

According to Balinsky (1981) writing on generalised fish embryology, the cell layer covering the yolk surface consists of the periblast edge followed by a sheet of endo- and meso-dermal cells, with no ectodermal involvement.

The end of the cleavage phase is marked by invagination of the epidermal cells; the embryonic shield lengthens and a dorsal furrow is formed indicating invagination prior to neurulation. The caudal end of the embryonic shield lies towards the point of germ ring closure. Gross microscopic examination of preserved eggs showed that when invagination and organogenesis take place the cells create a depression in the yolk below the surface of the epidermis.

Embryonic period : Embryonic phase

The transition between primary differentiation of primordial layers of ecto and endomesoderm and organ rudiments to organ formation is difficult to pinpoint. The onset of neurulation is difficult to observe and the first clear indication of organogenesis is the formation of the anterior swelling in the brain area followed shortly by the eyebuds. Although a visible sign, this is a late marker as the notochord will already have been formed. The oil globules, which till now have been aggregated, separate and disperse throughout the yolk.

Epiboly is nearly complete. The embryo is readily distinguishable from the vitelline membrane. Viewed from the side the primordial tissue layers appear to be denser, smoother and a deeper yellow colour than the vitelline membrane which has clearly visible star-shaped cells. Incipient optic vesicles can be seen on either side of the prosencephalon, which is further away from the gastropore (Fig. 10a).

30h00-34h00, 1-5 somites (Fig. 10).
The optic vesicles are differentiated from the brain area. The spinal chord can be seen in the middle of the body. Gradually the first somite pairs of the body appear in the mesodermal tissues on either side of the notochord/neural plate. There is a thickening of the tissues midway between the head and caudal region. Cunningham & Balon (1986) identified this thickening as Henson's node and it was seen only in preserved material. By 36h00 there are as many as five somites. Kupfer's vesicle, a transient embryonic structure, forms under the caudal region. The pericardial cavity forms under the head, behind the optic vesicles. The surface of the yolksac becomes less smooth, showing faint ridges and bumps as mesodermal tissue differentiates here, as well as in the

body, to form the bloodvessels and corpuscles of the circulatory system.

36h00-48h00 (Fig. 10d).
There is a large sinus filled with blood corpuscles in the caudal area of the yolksac on which minute punctate chromatophores form. Between 44h00-48h00 the number of somites ranges from five to nine depending on the developmental rate of the particular embryo. In some specimens with seven to nine somites the optic vesicles indent. The mesencephalon enlarges. Otocysts form on either side of the rhomencephalon. The pericardium is as wide as the head. Some black and yellow chromatophores appear on the yolksac in the area between the notochord and the somites (Fig. 11).

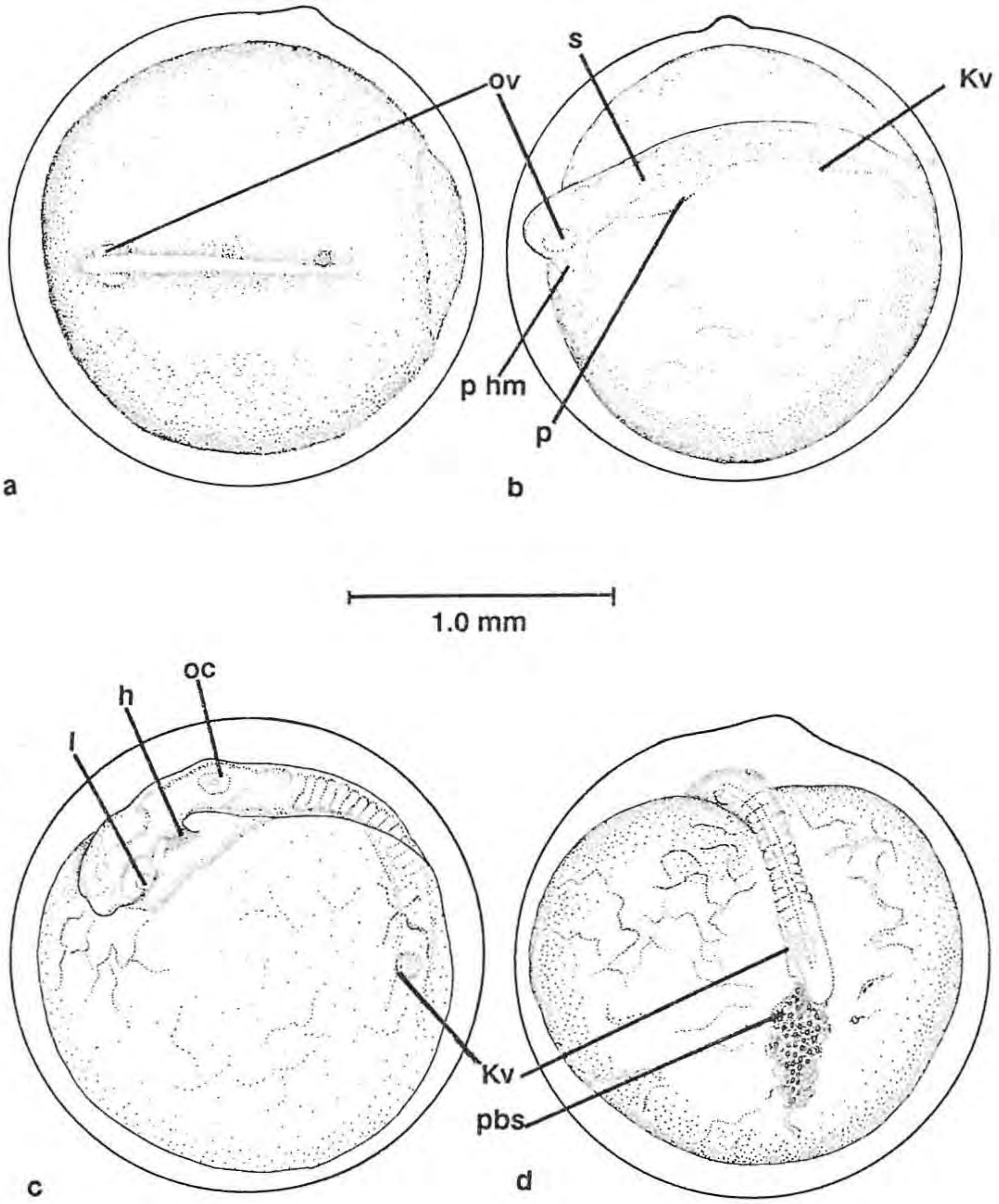


Fig. 10. Organogenesis in A. johnstoni.
(a) 31h00 Neurulation and early brain differentiation .
(b) 39h00
(c) & (d) 53h00 showing lateral and posterior views with lenses in eyes and heart in pericardial cavity, somites, tailbud and posterior blood sinus.

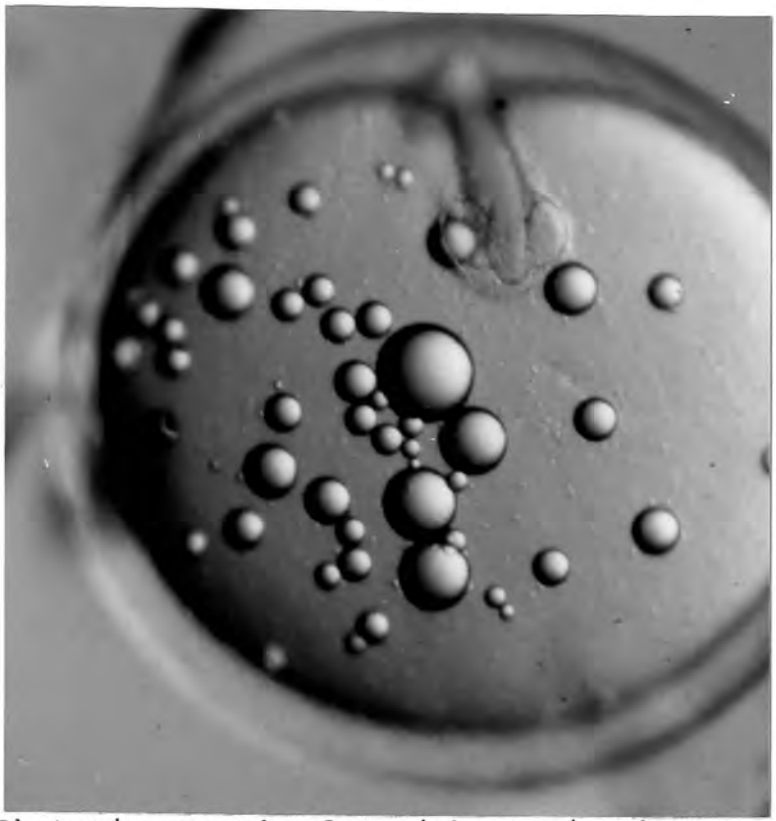


Fig. 11. Photomicrograph of A. johnstoni embryo, age 40h00 to show brain development, the position of oilglobules and pigmentation.

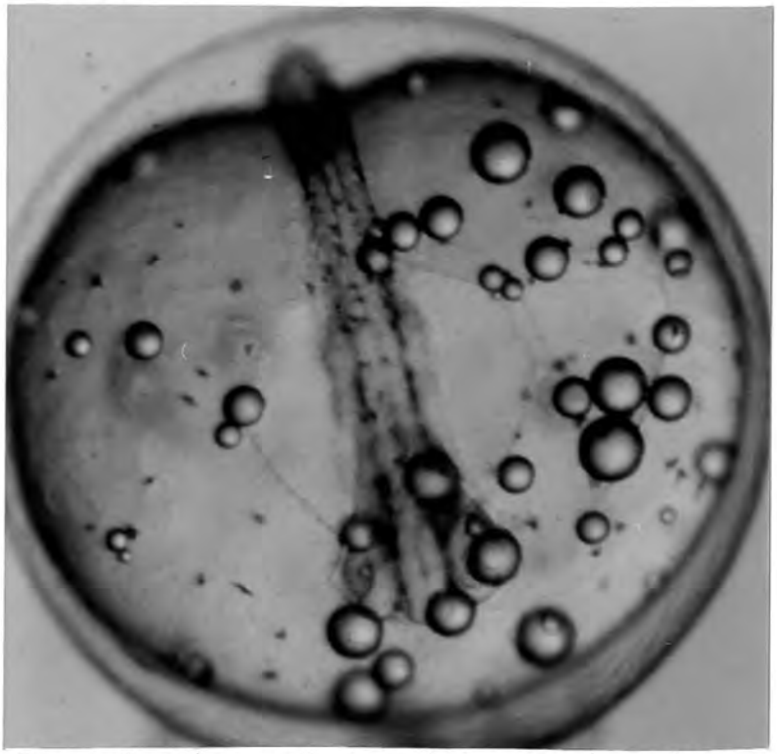


Fig. 12. Dorsal view of A. johnstoni embryo, age 52h00 showing brain and eye development as well as the start of pigmentation.

48h00-52h00, 9-13 somites. (Fig. 10c,d).

The optic cups are formed as a result of the eye vesicles coming in contact with the epidermis, thus inducing the formation of lenses (Balinsky 1981). The otocysts are growing and take on the appearance of a ring around a central cavity. The brain area shows constrictions between the fore- and mid-brain and becomes relatively larger. The pericardium has deepened and widened, extending to the level of the eyes and is now wider than the head. The simple heart tube is visible under the head just behind and medial to the eyes. Small vessels and sinuses with blood corpuscles become visible on the yolk surface, especially on the ventro-posterior aspect of the yolk where the circumvitelline vessel is clearly visible.

The tailbud becomes rounded and stands above the yolk surface, this being an indication that the posterior differentiation has been completed. The oil globules move ventral to the body.

Fig. 13. Photomicrograph of A. johnstoni embryo, age 53h00, left view showing the heart tube ventral to the midbrain, 13 somites and rounded off tailbud. Kupfer's vesicle is the dark structure under the caudal area.

The start of the heartbeat indicates the newly developed ability of muscular movement. A new phase in the development ensues with the innitiation of the circulation.

53h00-59h00, 14 somites.
An irregular heartbeat starts in some embryos. The oil globules begin to move to a position anterior to and below the head. Growth and differentiation of the brain increases as a division appears medially and the mesencephlon assumes a rounded shape. The lenses of the eyes form (Fig. 12, 13). Visible blood circulation commences toward the end of this time period, usually about 2 hours after the heartbeat has started.

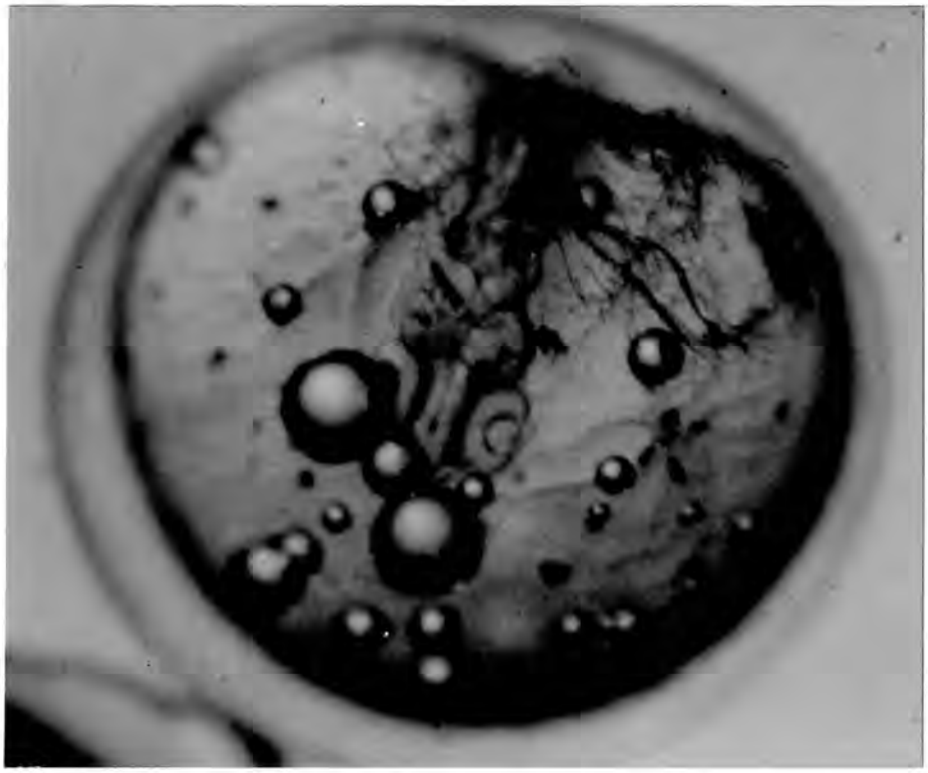


Fig. 14. Dorsal view of A. johnstoni embryo, age 59h50 to show the increase in brain complexity, and position of the eye lenses and oilglobules.

60h00-72h00

The total number of somites ranges from 14 at the start of this 12 hour period to 22 at its close. Of these, six somites formed in the caudal area which is free from the yolksac. The heart rate recorded in various specimens ranged from 34 to 127/minute with the average around 45/minute. In the latter six hours the heart rate stabilises to around 80/minute. The major feature of this period is the establishment of the basic circulatory pattern (Fig. 15a).

The blood vessels described below are faintly visible before blood corpuscle movement makes them clear. The circulatory system consists of two aortal branches from the heart which is situated under the left half of the body. These run along the ventrum of the head and unite ventral to the end of the medulla, forming the dorsal aorta which runs posteriorly below the notochord and exits onto the yolk surface above Kupfer's vesicle. The circumvitelline bloodvessel becomes branched within an hour of the first flow commencing, incorporating many of the previously formed lacunae of corpuscles. The carotid/lateral vitelline circulation is initiated between 63h00 and 66h00, when most specimens have 20 somites. From the heart, the left carotid artery loops up between the eye and brain while the right branch loops behind the eye, both run posteriorly along the lateral body walls below the otic capsules and exit onto the yolk surface on either side of the body in the region of the aortic confluence, behind the pericardial membrane (Fig. 15a & 16a). Here they become the lateral vitelline veins. All the vitelline vessels join the sinus venosus which leads the blood into the heart which has now angled anteriorly forward under the head (Fig. 15a). In a few specimens, the lateral and circumvitelline bloodflow starts almost simultaneously but in most embryos there is a variable delay of some hours. There may also be a delay between the initiation of flow in the left and right carotid vessels.

Where the dorsal aorta exits onto the yolk, the vessel forms a loop, which becomes the caudal artery and vein as it gradually lengthens with the caudal area (Fig. 16a). A blood sinus forms behind this loop under the newly forming somites. This sinus pulses in rhythm with the blood in the caudal loop. The surface of the yolk becomes more reticulated with ridges and lacunae as several new blood vessels are formed. As the circulation becomes established the pale orange corpuscles deepen to red which may indicate either the initiation or the acceleration of haemoglobin formation.

At the end of this time period the lenses are clearly formed. The fore- and hindbrain are enlarged and the midbrain is raised and rounded. The anlagen for two gillpouches are faintly visible behind and below the otic capsule in which the first otolith can now be seen. Pectoral fin anlage are seen as two small bumps on either side of the body anterior to the lateral vitelline vessels. The caudal body area lengthens rapidly during the final six hours of this interval, increasing from two to three postanal segments at 66h00 to six to eight segments at 72h00. Kupfer's vesicle diminishes in size and the body shows movement. Melanophores become larger and stellate (Figs. 15, 16).

Once the basic circulatory pattern is established, a new step in development starts with endodermal proliferation under the somites.

Circulatory and somatic development will now be described separately.

Circulatory development

72h00-80h00, 22-25 somites. Heart rate 98/min.
The carotid circulation in the head forms a reticulum, first one loop develops behind each of the eyes (Fig 15b,c) these then branch into three loops from the left and two from the right carotid artery. At 80h00 the bloodvessels around the eyes branch further around and through the eyes. A new vessel originates from the heart area at the ventrum of the

brain and flows dorsally to the median area of the midbrain, where it branches, each branch runs along the dorsal surface of the brain laterally to above the otic capsule before joining the lateral carotid artery (Fig. 15c, 16b).
The pericardium, which has been enlarging since 60h00 to form a thin membrane overlying the eyes, develops capillary circulation during this time period. The caudal loop extends further into the postanal trunk, which now has 10-14 somites and dorsal artery loops under the fourth from last segment (Fig. 16b). Posterior to the exit of the caudal vein onto the yolksac it branches sequentially, beyond which the vessels form a plexus.

80h00-88h00, 22-27 somites. Heart rate 137-166/min.
The lateral vitelline vessel moves posteriorly by developing parallel branches which course ventro-laterally and form more branches (Fig. 16b,c).

88h00-96h00 (Fig. 15c). Heart rate 116/min.
The tubular shape of the heart changes as it develops small constrictions. The vitelline circulation reticulates more as the lateral and posterior vessels anastomose. In some embryos the hepatic artery originates from the dorsal aorta behind the liver anlage. It may take one of several courses through the liver and can join the lateral vitelline vessel at a variety of places. Slightly later, in the same area, a mesenteric artery originates, it runs posteriorly in the area of intestinal anlage (Fig. 16b) and joins the caudal vein before the latter exits onto the yolksac.
96h00-104h00, 30 somites. Heart rate 124/min.
The first heart chamber, the ventricle, forms closest to the body, then the area further away from the body becomes differentiated. In the majority of the embryos observed at this stage the hepatico-mesenteric circulation is well established with the hepatic vessel branched. In some specimens the ventral vein has a double loop at the urinary bladder. This variation in then caudal vein is also reported by Cunningham & Balon (1985) for <u>Adinia</u>. The caudal sinus is absorbed and the caudal loop of the dorsal aorta reaches the end of the postanal trunk, which achieves its definative somite number during this interval.

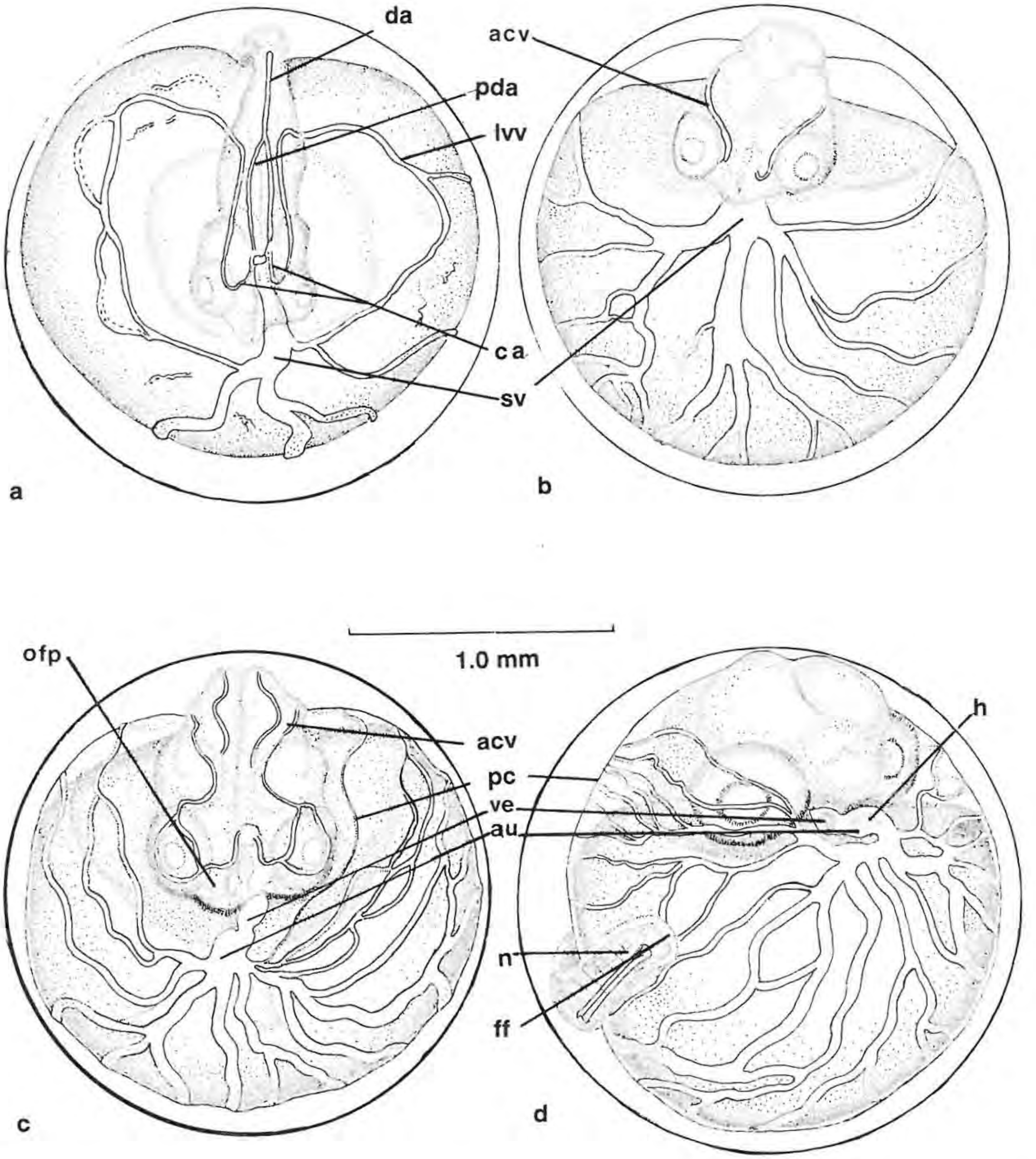


Fig. 15. Dorsal and anterior views of development taking place in A.johnstoni embryo from age 60h00 to 126h00 (5d06h00). Oil globules omitted for clarity.
(a) Dorsal view of head showing circulatory system at 60h00
(b) Anterior view of head at 80h00 showing the increase in vessel reticulation and the first cephalic branches.
(c) Similar view at 96h00 showing enlarged brain, olfactory placodes, branched cephalic and pericardial membrane circulation as well as the start of heart differentiation.
(d) Embryo at age 126h00 (5d06h00). Cephalic tissues too dense to show blood vessels. Yolk sac circulation at its apogee, heart has ventricle and auricle. Somatic segmentation completed and caudal with a small finfold.

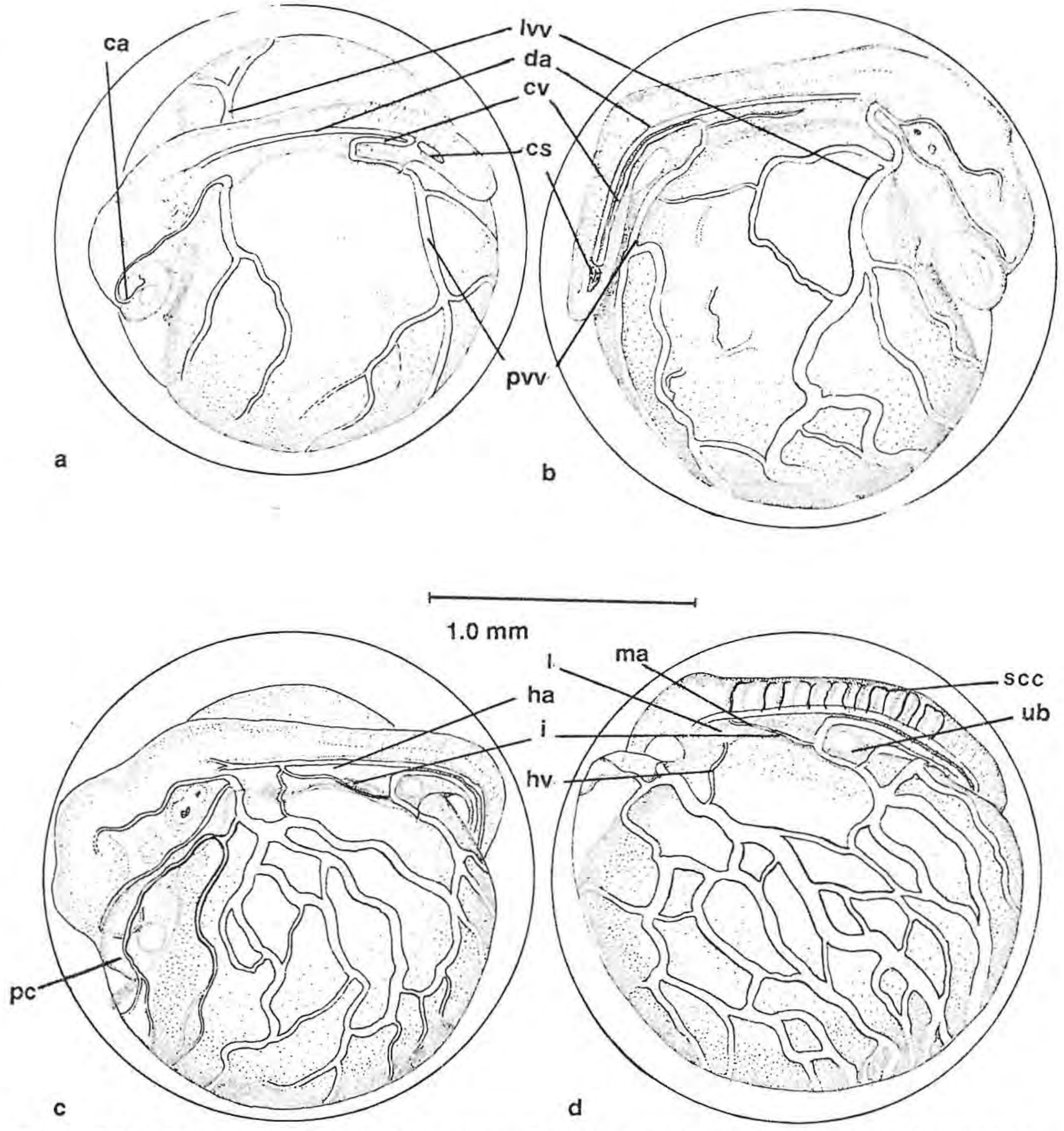


Fig. 16. Lateral views of circulatory and somatic development of A. johnstoni embryos at ages 72h00 to 114h00 covering the same development as in Fig. 15, but at other intervals
(a) Left lateral view at 72h00 showing basic circulatory pattern and the endodermal growth ventrad of the somites.
(b) Right lateral view at 80h00 showing the otic development, mesenteric vessel and branching of the vitelline circulation.
(c) Left lateral view at 108h00 showing proliferation in the vitelline network, the hepatic artery and vein joining the left lateral vitelline vein. Mesenteric vessel omitted to show the intestinal lumen. Otic capsule omitted.
(d) Left lateroposterior view, age 114h00 showing segmental circulation devlopment, structural differentiation of the as the pectoral bud.

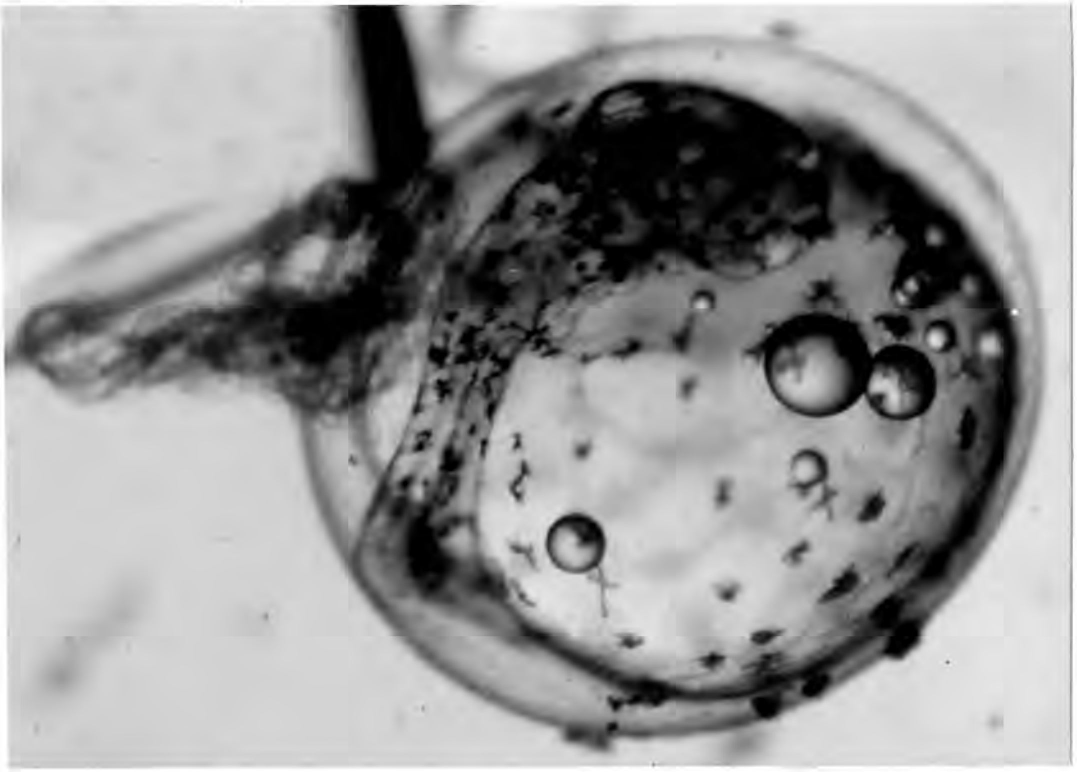


Fig. 17. Photomicrograph of A. johnstoni embryo, age 84h00 showing angled caudal region of body, chromatophores and oilglobules.

Somatic development

72h00-88h00, 22-28 somites (Fig. 16a,b).
Two otoliths are present and the auditory capsules are lengthening posteriorly. At 72h00 there is a proliferation of cells below the dorsal aorta which elevates the body in an angular position over the egg (Fig. 16b & 17). This is endodermal growth which precedes the differentiation of the digestive system. Thirteen to 16 tail somites form, an indication of accelerated growth.

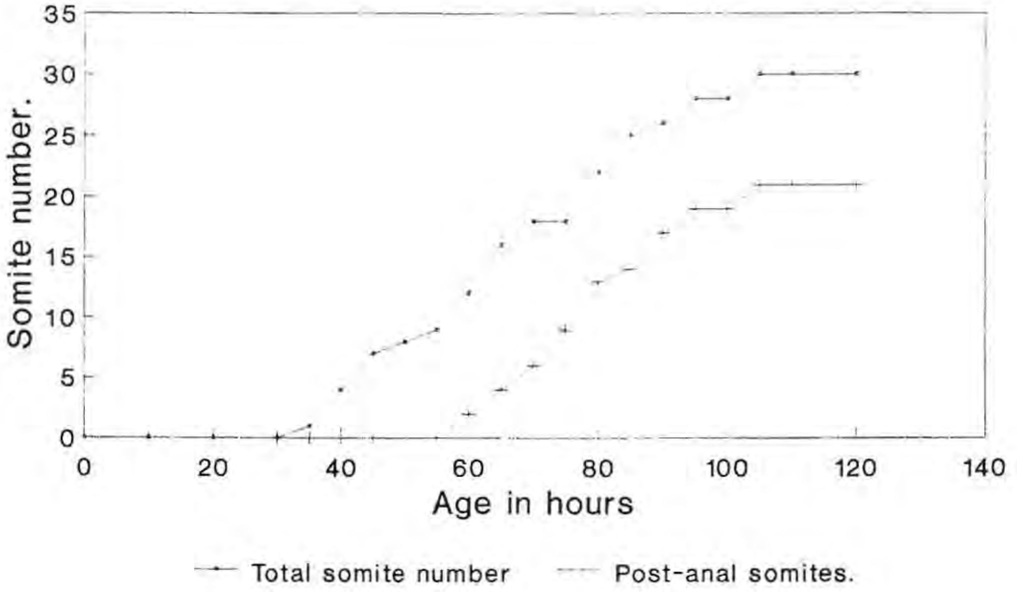


Fig. 18. Rate of somite differentiation during the embryonic period of A. johnstoni.

Between 80h00 and 88h00, eye pigmentation starts to form. The midbrain undergoes rapid growth, bulging above the eyes. A small spherical lumen appears in the tissues at the posterior juxtaposition of the body and the yolksac. This is the anlage for the urinary bladder. The liver anlage appears immediately behind the exit of the lateral vitelline vessel on the left side of the embryo. At the end of this interval a few more advanced embryos have a small hepatic artery flowing through the liver and joining the left lateral vitelline vessel. The tissues ventral to the aorta begin to show some structural differentiation.

88h00-96h00.

The otic capsules grow posteroventrally and assume a bean-shape. According to Sokolowski & Popper (1987) their study of the development of the inner ear of Opsanus tao (Batrachoidea) revealed that the general patterns of gross development in bony fishes is similar not only within the group but among jawed vertebrates. For this reason the development observed in A. johnstoni was compared on a gross level to that described for O.tao and the underlying processes deduced. Thus it can be assumed that when the otic capsule becomes bean shaped that this is a stage in the development when the anterior, future utricular area is differentiating from the posterior, pars inferior or future saccular area. Behind the eyes and under the otic capsules there are three gill pouches. In the interior of the snout region some buccal differentiation takes place. The intestinal anlage becomes differentiated and structured. Consequently a small coelomic space is visible. The liver is larger and some embryos show a faint yellow spot on the medial aspect which is the gallbladder.

The pectoral finbud, although larger, is still in the embryonic position, parallel to the long axis of the body. The caudal trunk has 20 to 21 somites and a narrow median finfold around the caudal peduncle.

96h00-104h00.
The definitive number of somites (30) is formed. The body now encompasses 50% of the yolk circumference. The intestine is no longer smooth but appears structured and a lumen can be seen with some difficulty. The melanophores have increased in size and on the body area, especially on the dorsal surface of the head, are more numerous.

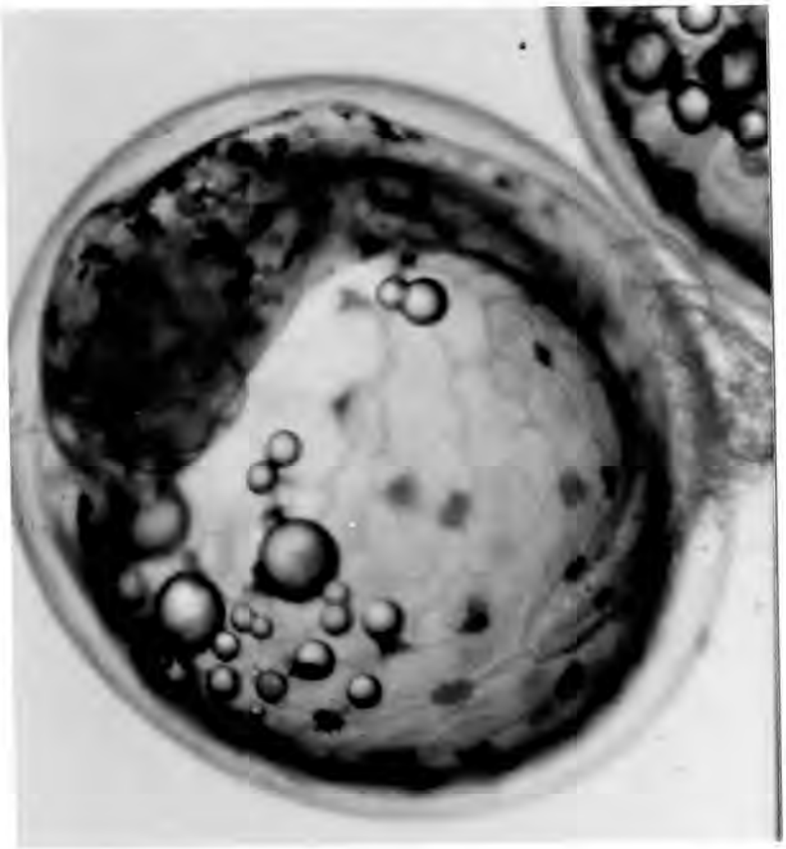


Fig. 19. Photomicrograph of A. johnstoni embryo at age 102h00

During the previous 30 hour period the basic pattern in the circulatory system has been formed, the internal organs have differentiated but not elaborated and the final somite number has been laid down. The brain and sensory organs have undergone an extensive increase in size and complexity. The next step is the development of the secondary circulatory system and the final structural differentiation of the internal organs.

Circulatory development

104h00-120h00. Heart rate 115 to 126/min.
Once the major bloodvessels and the basic embryonic circulation are established the secondary circulation in the segments of the body is initiated. As the size of tissues requiring perfusion increases, the finer bloodvessels are

established in them, many as permanent structures. By 108h00, in some fish, the segmental myotomal circulation has formed in the segments above the liver anlage and by 114h00 (Fig 16d) there are capillaries in at least eight to 14 central somites. The hepatic circulation has two branches. The superficial circulation in the eyes is another indication that capillary circulation is developing. The pericardial membrane overlying the eyes now has an extensive capillary network. The vitelline circulation forms a network of interconnected vessels.

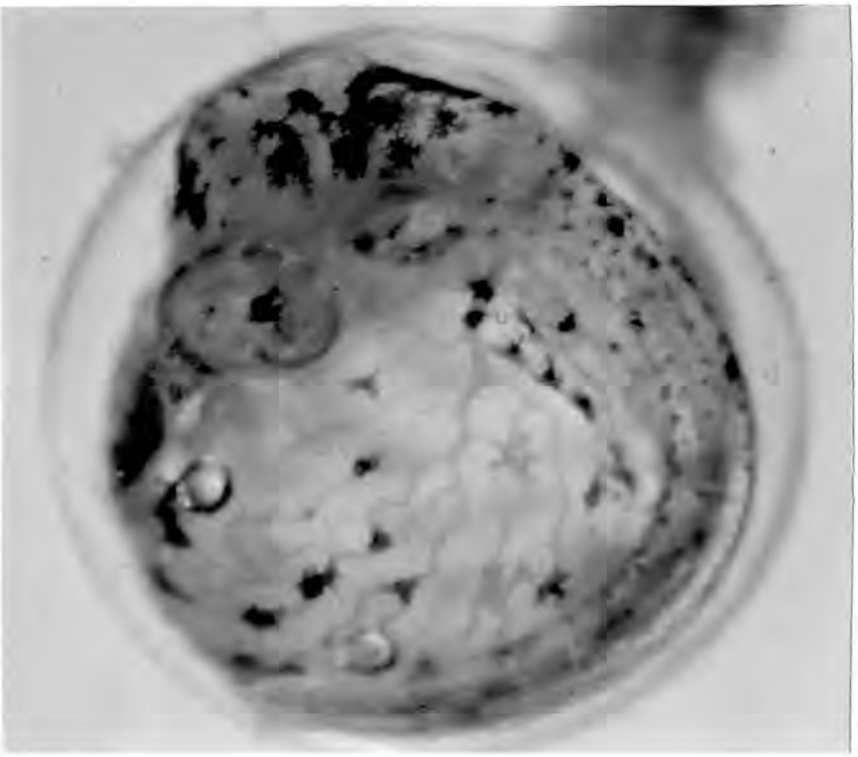


Fig. 20. Photomicrograph of A. johnstoni embryo, age 5d05h00.

120h00-132h00. 5d00.
Variation in developmental rates can be highlighted by a summary of information gleaned from a batch of sibling eggs of similar age which were closely monitored in order to gain information on the sequence of blood vessel appearance.

At 122h00 there were mesenteric and hepatic vessels.
At 123h00 there were mesenteric, hepatic and segmental vessels.
At 126h00 there were mesenteric, hepatic, segmental and pectoral vessels.

Thus in some embryos the segmental circulation may only start some 15 hours after it was first seen in the more advanced fish in which the first segmental vessels were seen at 108h00. At 140h00 the heart rate is 121 beats/minute, and the heart has moved in under the head and become S-shaped with a constriction between the two chambers. The hepatico-mesenteric circulation is branched. Since 126h00 the pectoral fin has one vessel flowing ventrodorsally between the peduncle and the finfold; in the caudal lobe there is one circulatory loop.

Somatic development

104h00-120h00. 28-30 somites.
Olfactory placodes are present on either side of the snout. Eye pigmentation increases and the buccal cavity forms as a groove inside the snout as it lengthens. The otic capsule differentiates further as a ring forms around the perimeter and the bean shape becomes more distinct. Between 120h00 and 132h00 the capsule undergoes rotation and the anterior utricular area is divided from the posterio-ventral saccular area. According to Sokolowski & Popper (1987) " an invagination of the medial and lateral walls of the dorsal roof .. forms the vertical semicircular canal". There are four gill pouches. The head depth is 35% of the diameter of

the egg. The pectoral fins are still in embryonic positions but enlarge during the next 8 hour period.

5d00.

As the organs become larger, the coelomic cavity and the bladder expand and the yolk is reduced. The intestine is a well-developed straight tube and in preserved material a lumen can be seem. The differentiation into stomach and intestine starts at about 122h00 with a bend developing between the first and second third of the intestine. The notochord turns up at the caudal end and the caudal lobe expands. The narrow finfold surrounding the caudal grows and expands into the ventral finfold first, followed shortly by a small dorsal finfold and by 140h00 the caudal lobe is discoidal. Body length occupies 75% of the egg diameter or perivitelline space.

By 140h00 the mouth aperture forms and the gill pouches elongate on the ventral surface of the head. The expansion of the body lumena serves to keep the diminishing yolk sac close to the surface of the egg which no doubt facilitates gaseous exchange. The pectoral fins are differentiated into a lobe and finfold with three pigment spots and start to rotate to the perpendicular position toward the end of this interval.

Pigmentation consists of large stellate melanophores on the yolksac concentrated mostly on the blood vessels with smaller spots on the body with one expansive cluster of large spots on the nape area (Fig. 20).

By 144h00 a valve has formed between the ventricle and auricle of the heart. A small swimbladder, only visible in dissected specimens, lies against the dorsal body wall above the stomach.

During the next step the skeleton differentiates. A description of skeletal development is now included at this point since changes occur in this system.

Skeletal differentiation

Skeletal differentiation takes two forms: (a) certain elements are formed first in cartilage which is later invaded by osteoblasts and (b) superficial bones are formed by direct ossification of epidermal or membranous tissues. (de Beer 1971). The uptake of stain by structures is not neccessarily a definitive indication of skeletal development. Alcian blue is absorbed by mucupolysaccharides and in the transition stage between the chondrified and ossified states there can be a time when neither alcian blue nor alizarin red S will be absorbed satisfactorily and structures will remain unstained. These transparent structures can be regarded as bone in formation. In this analysis it is not the nature (chondral or dermal) but rather the presence of the skeletal structure which is of greatest importance. For this reason it was decided that the differentiation within the caudal lobe should be used as an indication of skeletal formation rather than the enlargement of the lobe which starts at 5d00.

7d00

At this age the earliest evidence of the skeletal differentiation is seen with the appearance in the caudal fin of rays which are distinguishable from the surrounding finfold tissue.

8d00

The mouth opening which appears on day 7 in some specimens begins to take on an inverted U shape and the tissues in the jaw area stain slightly darker than the surrounding tissues with alcian blue. The uptake of this stain indicate the the presence of cartilage. The caudal fin has three or four rays and by 9d00 five or six rays.

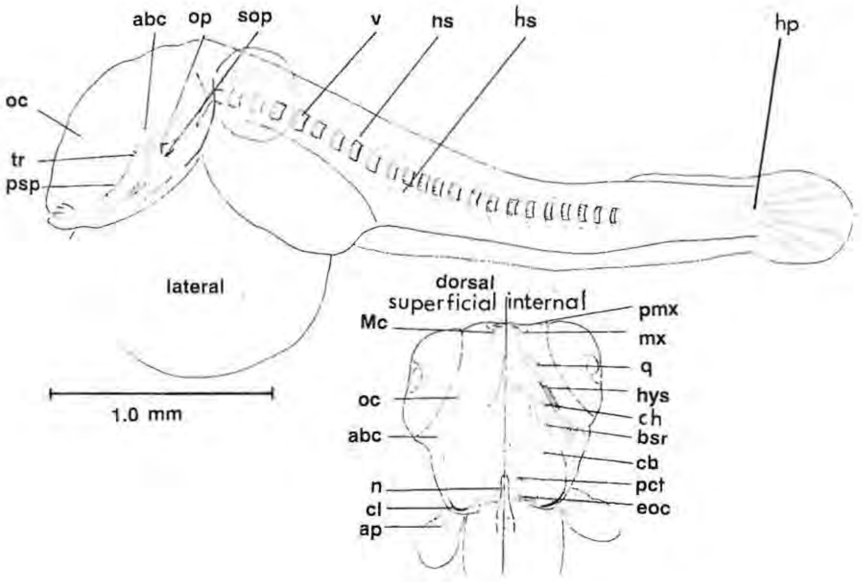


Fig. 21. Skeletal development of ecdysed embryo at 10d00. Cartilaginous structures depicted by stipples only, ossified structures by solid lines. (a) Lateral view of entire embryo, (b) Dorsal superficial and interior views of the of head.

Skeletal differentiation accelerates from 10d00 in the majority of embryos observed. By 10d00 old most fish show six to eight definite caudal rays and the hypural plate in the caudal fin skeleton, which stain blue.

The head region is only slightly chondrified but nevertheless the structures are well differentiated and discernible. Clearly visible are:

In the suspensorium and jaws: Meckels cartilage, the angulo-articular, quadrate and hyosymplectic.

In the neurocranium: the exoccipital struts and parachordal cartilages around the anterior end of the trabeculae and a thin layer of bone around the parasphenoid are also visible.

The visceral arches have cartilaginous ceratohyals and faintly discernible ceratobranchials.

In specimens which have not been stained for cartilage, Alizarin red S stain is absorbed very slightly by the following structures in a few embryos: the premaxilla and maxilla and a thin ridge on the ventral area of the dentary, the middle area of the hyosymplectic. In the branchial area two branchiostegal rays and the cleithrum are visible.

The notochord takes up stain very slightly in those areas where the centra are differentiating.

The first caudal finrays to have differentiated also absorb some red stain.

11d00.

The lower jaw and snout grow longer as the underlying structures develop further. The orbital cartilages extend onto the edge of the auditory capsule laterally and to the ethmoids anteriorly. The auditory capsules take up blue stain in an uneven manner, the utricular area being much paler than the saccular area. In the pharyngobranchial area, the ceratohyals, the basihyal and the first faint indications of the ceratobranchials chondrify.

In the pectoral fin the actinosal plate takes up stain as an undifferentiated sheet.

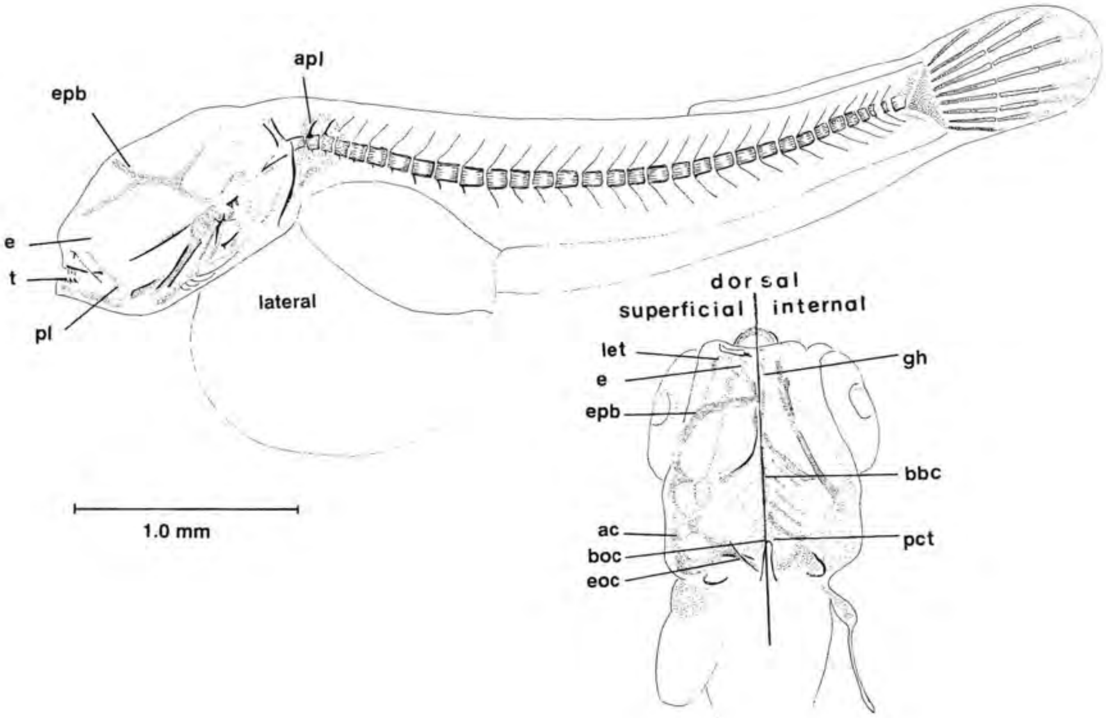


Fig. 22. Skeletal development in an ecdysed embryo at 12d00. As in Fig. 21 cartilage stippled and bone solid lines.

Fig. 23. Ventral view of the skeletal development in the head of an embryo 13d00 old of <u>A. johnstoni</u>. Stained only for cartilage with Alcian blue.

12 to 17d00.

The delicate ossification of some dermal bones continues but the major feature of this period is the extensive chondrification of the internal skeleton.

Upper jaw: Premaxillae with two to three teeth; maxillae ossified. Chondrification starts in the palatines which appear to form a unit with the quadrate. The ethmoid plate and processes which are the anterior extentions of the cranial trabeculae differentiate. As time passes the ethmoid plate becomes more defined and cups around the dorsal head of the palatoquadrate. (Fig. 24)

Suspensorium and lower jaw: The dentary ossifies around Meckel's cartilage and develops dorsal flanges. By 14 days there are teeth on the dentaries. Thin sheets of bone form around the articular and quadrate cartilages and thicker rods form the symplectics. There is a small red protrusion ventral to the anguloarticular, the retroarticular. The hyomandibula has a patch of ossification around the fossa. Other ossified structures are: the preopercula, opercula, sub-opercula, anterior prootic bars and ossified processes in the sphenoid areas.

Neurocranium: On the dorsal aspect of the head two orbital commissures extend from the ethmoid plate posteriorly to behind the eyes. The epiphyseal bars join these commisures between the eyes. The sphenotic bar or basicapsular commissure is a chondrified spur which leads from the post-orbital end of the commissure ventromedially behind the eyes to the parasphenoid which is further ossified. The parachordal cartilages on either side of the notochord have expanded anteriorly to form the basal plate to the braincase, which is lightly ossified. Lateral to this plate, forming the walls of the brain and the medial areas of the otic capsules, are the prootics. Behind the prootics, on either side of the notochord, the exoccipitals also ossify.

Pharyngo-branchial area: Ventrally the narrow glosso- and basihyal are cartilaginous but the ceratohyals have ossified central bars, each with three ossified branchiostegals.

central bars, each with three ossified branchiostegals. There are two to three pharyngeal teeth on the interior surface of each of the last ceratobranchials which are more robust than the preceding four elements and ossify before them. The dorsal pharyngo-branchials also carry teeth. By 14 days all five ceratobranchials have an outer bone sheath. There are three anterior pairs of basibranchials ossified in the copula. The glossohyal expands anteriorly, is spade-shaped and still cartilaginous. The hyoid complex is attached to the hyosymplectic by the small interhyal. Dorsal pharyngeal teeth proliferate and a toothplate forms.

The pectoral fins: The cleithra are two narrow ossified structures and the cartilaginous actinosal plates gradually differentiate during this interval so that by 17 days there are scapulocoracoids and five radials which are all still cartilaginous. There are no pectoral rays at 12d00 and chondrification starts in the dorsal aspect of the fin at about 15d00 and at 17d00 seven to nine rays are formed (Fig.24).

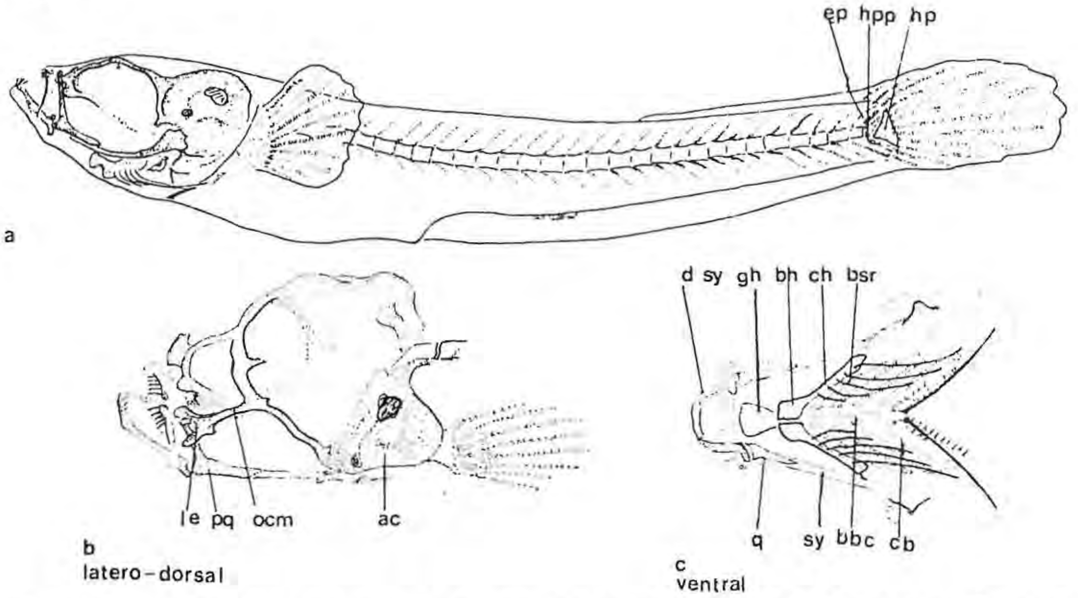


Fig. 24. Lateral view the cartilagenous skeleton of a larva of *A. johnstoni* age 17d00. The cartilage is stippled and some bone indicated in solid lines.
(a) lateral view of entire skeleton.
(b) enlarged laterodorasl view of head showing the ethmoid.
(c) Venral view.

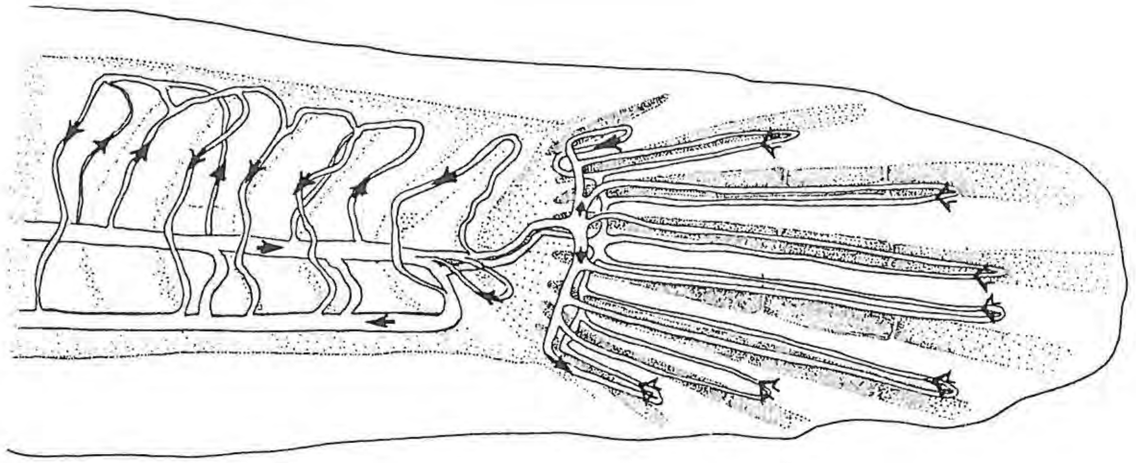


Fig. 25. Enlarged view of the capillary circulation in the caudal area of 12d00 embryo of A. johnstoni.

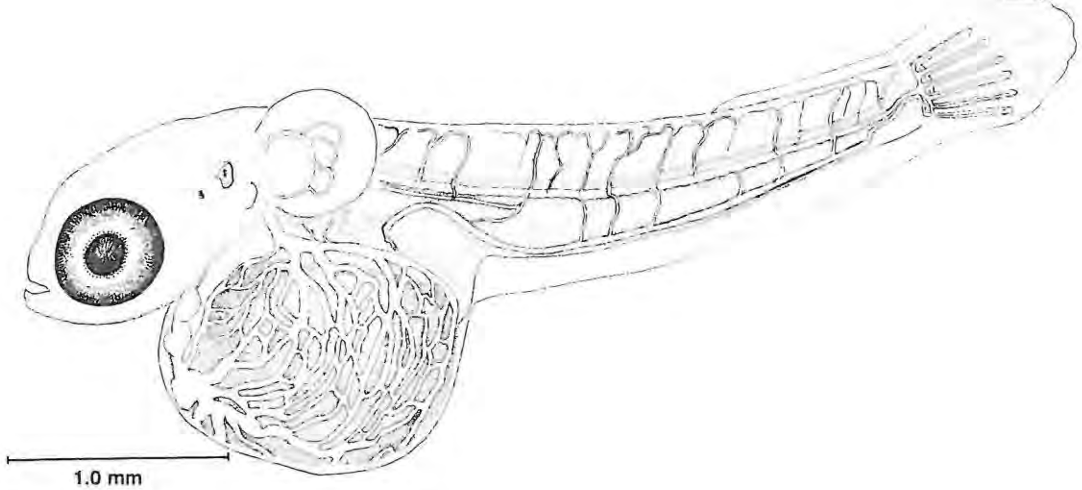


Fig. 26. Left lateral view of entire embryo at 12d00 old showing circulatory development of A. johnstoni

can cease with consequent arrest of the bloodflow when the embryo is disturbed. The heart is now in a completely ventro-cephalic position. There is a small blood vessel in the body wall above the liver. By the end of this period there are six to eight circulatory loops in the caudal fin.

12d00. Heart rate 141/min.
The segmental and cutaneous circulation increases in complexity with deep and superficial capillaries connecting the dorsal artery and the caudal vein. At intervals the segmental vessels in which the flow is dorsad fuse, then loop ventrally around the vertebrae to join the ventral vein. These connections at vertebrae 6/7, 13/14, 19/20, 24/25 and 27/28 show a stronger flow than others (Fig. 26 & 27).
The bloodvessels on the lateral aspect of the yolksac (Fig.26) are still in the embryonic form but the lateral vessels have diminished in size and number and move closer together as the yolk sac itself diminishes in size. The flow lessens in the posterior vessels and shifts towards the anterior vessels which will eventually form the Ducts of Cuvier. By 14 days (Fig. 27 a,b) the shift and reduction is already clearly visible. Once the hypaxial muscles grow down the yolksac, blood vessels are concentrated on the ventral aspect of the abdomen and the reticulation is further simplified. The largest volume of caudal blood still returns to the heart via the posterior vitelline vein. The development of the anterior postcardinal vein is obscured from view by the development of the hypaxial muscles.

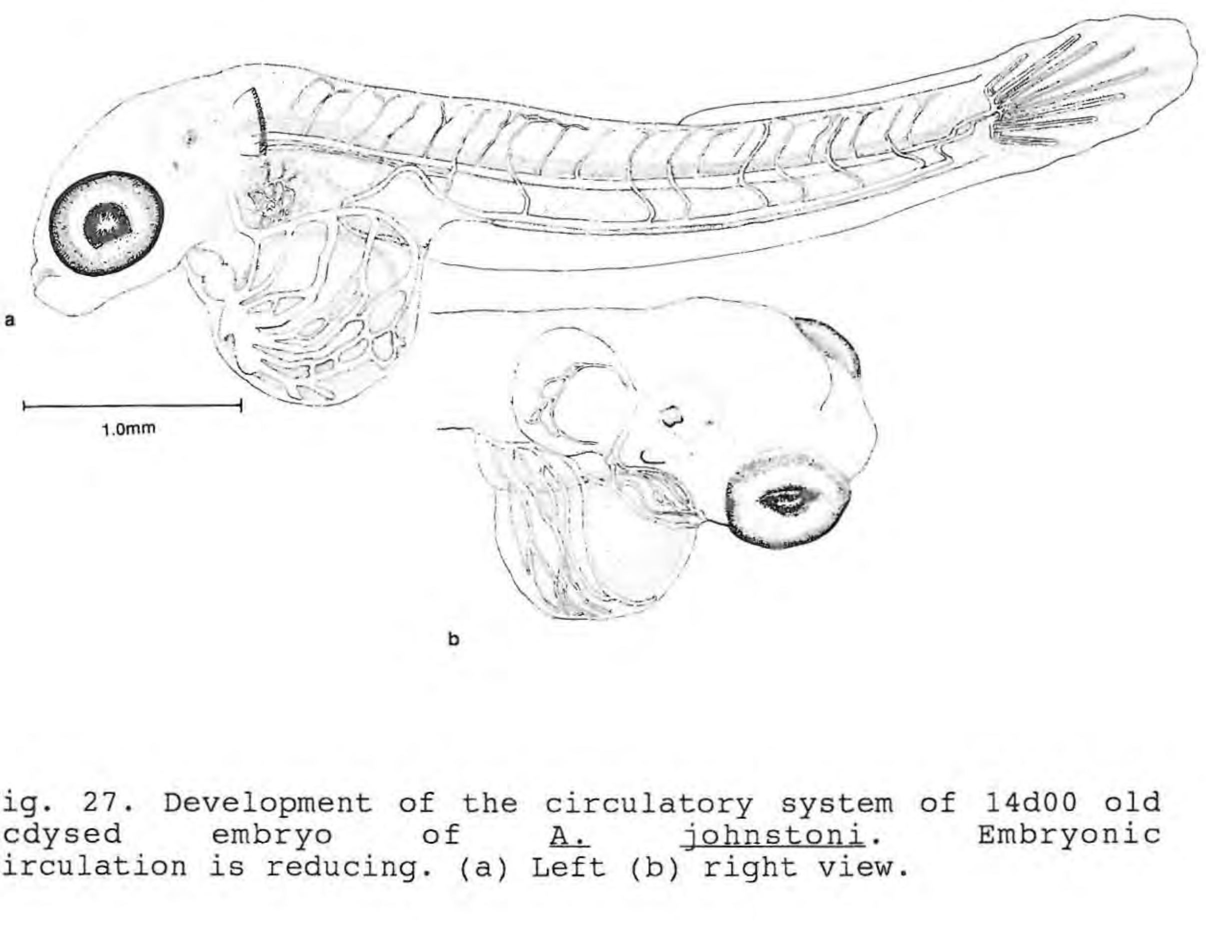


Fig. 27. Development of the circulatory system of 14d00 old ecdysed embryo of A. johnstoni. Embryonic circulation is reducing. (a) Left (b) right view.

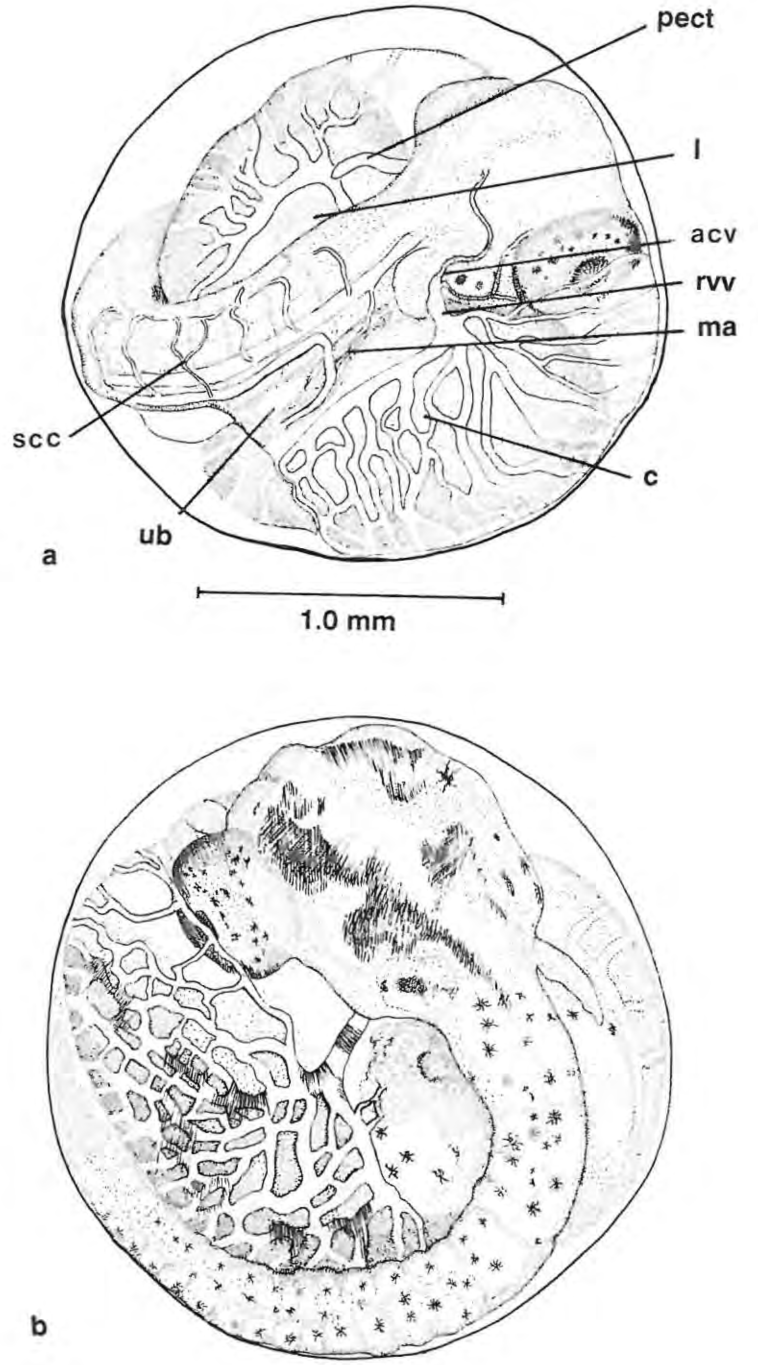


Fig. 28. Embryos at (a) 7d06h00 and (b) 8d00 showing circulatory and somatic development.

be ecdysed, a delicate procedure, not always accomplished with success. From day seven the eyes darken, a few iridescent spots develop and the pupils grow around the translucent lenses. The lenses then appear to darken but this most probably reflects the development of the retina between days eight and 11.

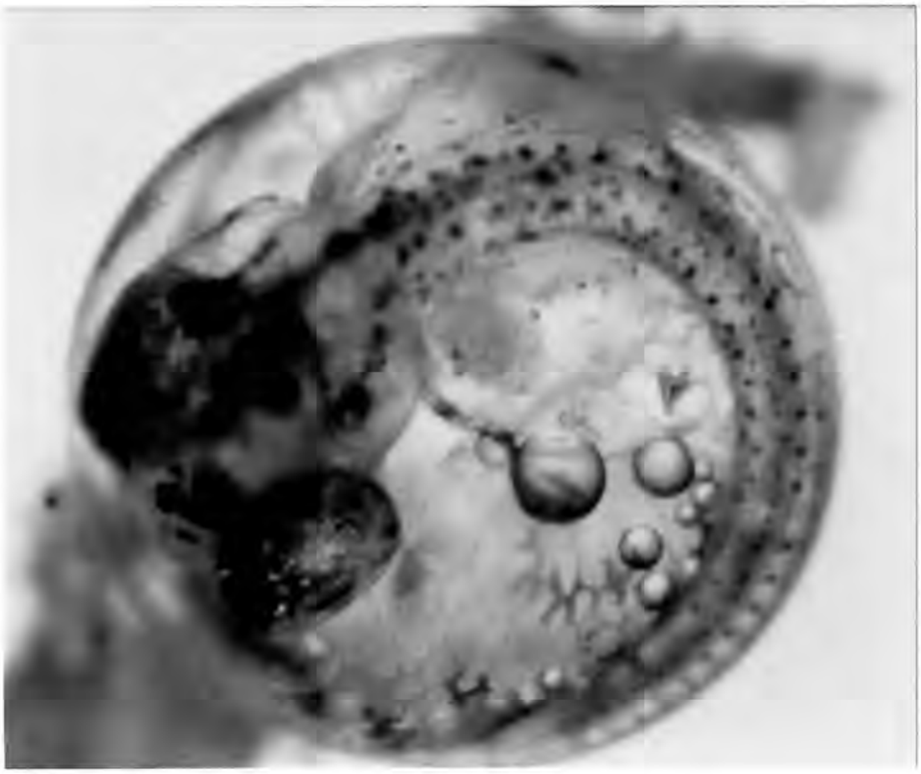


Fig. 29. Latero-dorsal view of 8d00 old embryo of A. johnstoni showing development in general but in particular the eyes, ears, pigmentation, liver and circulation.

On day seven the mouth aperture breaks through in some specimens and assumes an inverted U-shape from day eight. The branchial chambers are clearly defined, the gills are tucked underneath the large otic capsules. By day nine, three otoliths are present which is an indication of the final stage of otic differentiation which according to Sokolowski and Popper (1987), involves the addition of the lagena which forms as a dorsocaudal budding of the saccule wall and its

stage of otic differentiation which according to Sokolowski and Popper (1987), involves the addition of the lagena which forms as a dorsocaudal budding of the saccule wall and its otolith. Between 12 and 15 days the semicircular canals of the labyrinth reach adult levels of development.
The jaws and opercula become mobile between 10 and 11 days.

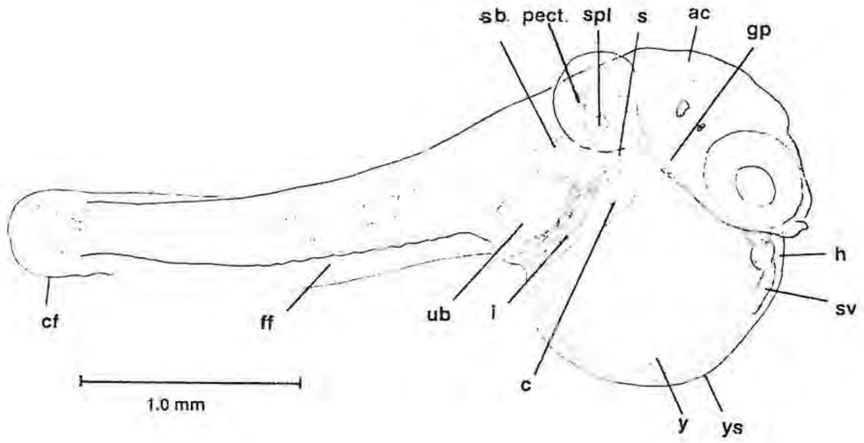


Fig. 30. Right lateral view of an ecdysed 10d00 embryo of A. johnstoni to show the somatic development.

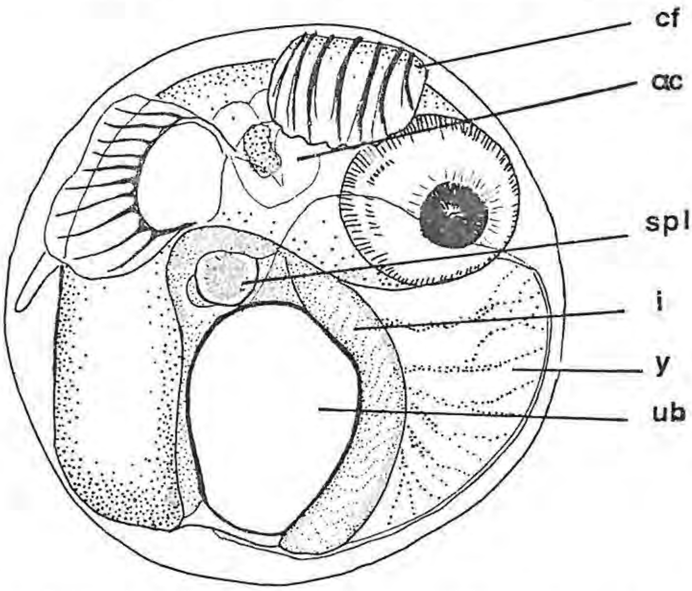


Fig. 31. Diagrammatic representation of the arrangement of internal organs in 16d00 old embryos of A. johnstoni, showing the enlarged urinary bladder.

The body cavity expands and the urinary bladder has become considerably enlarged pushing the gut away from the trunk muscles towards the floor of the coelom (Fig. 31). By day eight the intestinal lumen is clearly visible, the stomach area is better defined and the surface of the gut becomes more structured. From nine days onwards the intestine increases in length and consequently becomes more convoluted in the coelomic space. In live specimens the gallbladder become defined and the yellow bile pigment darker, the liver grows from 0.25 x 0.13 mm to 0.30 x 0.20 mm in size by 10 days and medial to the gallbladder there is a faint pink area indicating the position of the spleen. This becomes a discrete round, bright red structure by 16 days (Fig. 32b). Behind that, closer to the dorsal artery and dorsal to stomach, is the swimbladder, a round clear structure. There is a proliferation of cells in the area immediately adjacent and ventral to the myotomes of the trunk; these are the hypaxial muscles forming. There are melanophores on the caudal finfold on 7d00 and from 10d00 pigment develops on the dorsal body cavity wall.

The postanal trunk circumference expands and the body length grows from its previous 80 to 90% of egg circumference to more than 100% when it overlies the head (Fig. 32). The pectoral fin is motile.

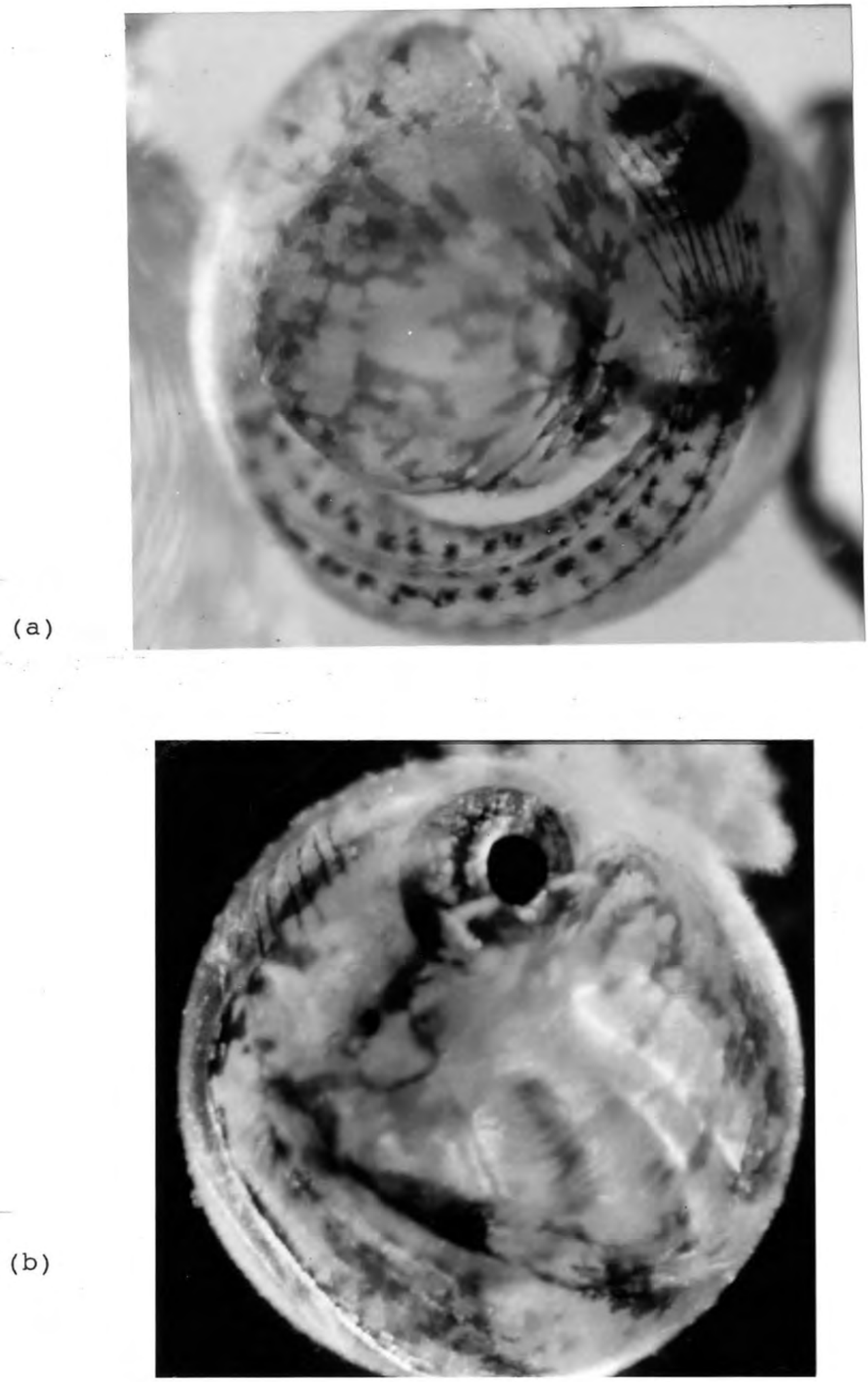


Fig. 32. Embryo of A. johnstoni at (a) 13d00 and (b) 16d00.

The eyes, mouth and opercula move. The capillaries in the pericardial membrane atrophy and the extent to which the pericardial membrane overlies the head lessens from as early as 11d00 but usuallly from 14d00 and usually disappears by 16d00 when the flexure of the head over the yolk straightens. Orange/yellow pigment has been deposited on the ventrum of the head and the dorsum of the coelomic cavity. As the hatching period approaches the ecdysed embryos appeared more larval and the size of the yolk in the sac diminished. This enables a clear view of the structure of the sac.

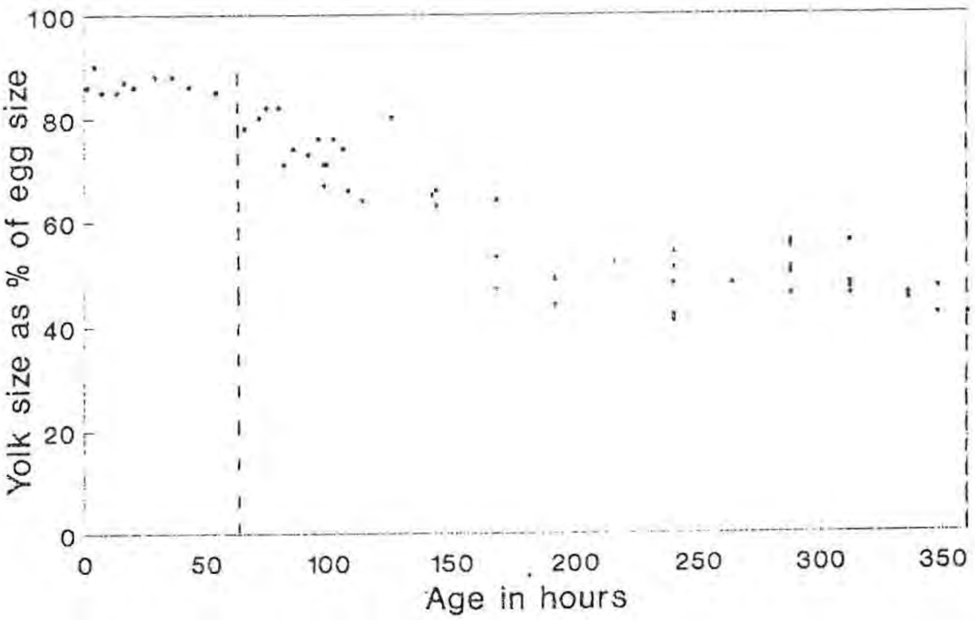


Fig. 33. Graphic presentation of the rate at which the yolk reduces during embryonic development. Broken line indicates start of heartbeat.

The pattern exhibited by the depletion of the yolk in the graph above merits some comment. The reduction of the yolk starts after the bloodflow has commenced at 60h00. There is then a gradual reduction in the size of the yolksac to about 50% of egg size at about 180h00 (7d12h00).
After this time the size of the yolksac is apparently maintained at between 40 and 60% until 15d00. Thereafter measurement becomes increasingly difficult due to the distortion of the yolk by the enlarging tail of the embryo. It was evident that the distention of the urinary bladder increased as the yolk depleted. It is surmised that this is due to the retention of excretory products as the bladder decreases markedly in size if the embryo is ecdysed. Why the yolksac should apparently remain at such a stable size is not immediately clear especially as the size of the embryo is still increasing during this time period as the tail grows in length and circumference.

Hatching pattern and variation in the population

A group of eggs, spawned in a two hour period by the F1 population of fish (second dataset) were monitored to observe hatching. The relative levels of development of these 14d00 embryos are described now to highlight the different rates of development. The relative size of the fish was used as an indicator of development. There is a continuum of sizes present in this batch which could be divided into three categories, viz.

1) The end of the caudal peduncle does not reach the edge of the eye, the caudal fin is over the eye but does not reach the top of the brain. Heart rate 100 beats per minute.

2) The caudal peduncle reaches the outer rim of the eye and the end of the caudal fin lies over the brain but does not reach the opposite eye or otic capsule. Heart rate ranges

from 100 to 150 beats per minute.

3) The end of the caudal peduncle reaches the inner rim of the eye or the top of the head and the caudal fin lies over the top of the head, reaching to the opposite otic capsule. Pigment dark especially on the yolk. Heart rate ranges from 100 to 139 beats per minute.

The age at hatching of *A. johnstoni* embryos ranges from 13 to 21 days (Fig.34) and no doubt the range in the sizes described above has some bearing on the hatching time.

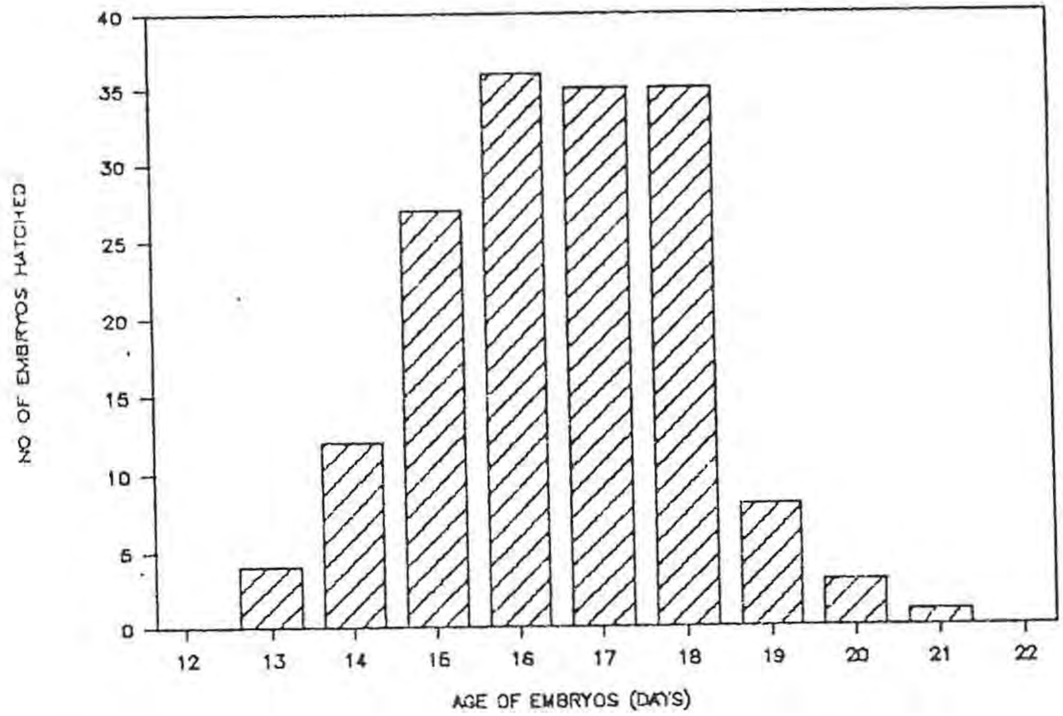


Fig. 34. Hatching intervals of *A. johnstoni*. Information collected from progeny of both wild and F1 parents.

Embryos of newly fertile small adult fish took 20-24 days to hatch and were not included in this graph.

The newly hatched free embryo or "hatchling" is in the transitional stage between endogenous and exogenous feeding. Hatchlings of A. johnstoni have all systems for external feeding fully functional; the eyes can move, the jaws are functional and the respiratory organs also appear to be functional as the opercles are mobile. The gut is convoluted and differentiated into stomach and intestine and shows peristalsis. If there is any yolk material remaining it is usually scant or simply the residue of the largest oil globule. The vitelline circulation has reduced in extent. This signifies the end of the embryonic period and the start of the larval period.

Larval period

The fish are now free swimming and are dependent on external nourishment. Consequently the rate of development could be influenced by the available food. Due to their small size, these fish are difficult to feed under laboratory conditions. The problems encountered with feeding were only solved in the second half of the experimental period when the hatchlings were reared in enriched natural pond water. As the developmental rates recorded for the various fish varied widely due to these circumstances, the specimens with the most advanced development were used as a benchmark of the rate at which development could proceed.

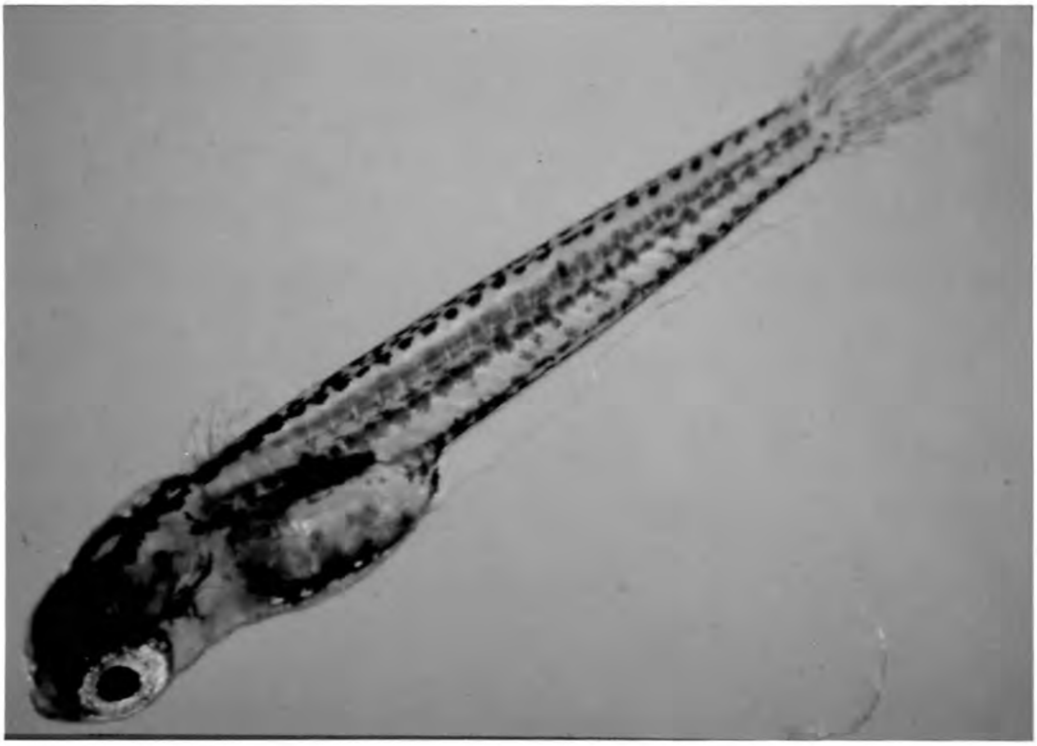
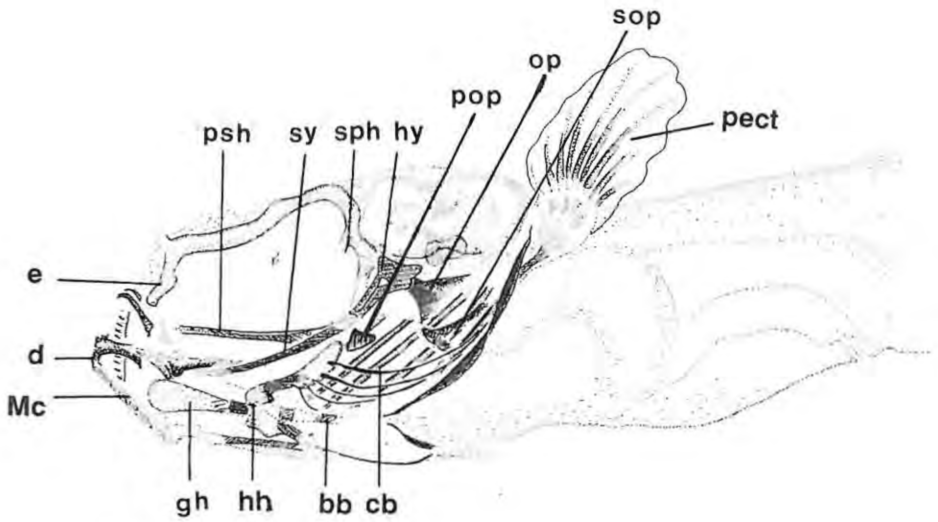
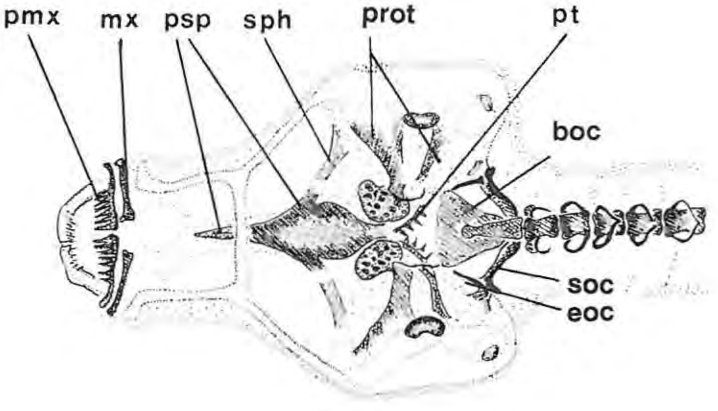


Fig. 35. Lateral view of hatchling of A. johnstoni at age 18d00 showing pigmentation and remnants of yolk. Note the short snout.



a

ventro-lateral



b

dorso-internal

Fig. 36. Skeletal development of larva of A. johnstoni at 27d00.
Cartilage stippled and ossification in solid lines. Structures in transition from cartilage to bone rendered in a combination of stipples and lines.

Due to the decalcifying effect of alcian blue as mentioned in Chapter 2, unstained areas were taken to be newly ossified. After hatching most fish exhibited the following state of ossification:

There are six teeth in each of the premaxillaries.

The maxillae have expanded lateral flanges. The toothed dentaries have ossified to near adult shape around Meckel's cartilage and the anguloarticular is a T shaped bone, the head of which articulates with the quadrate. At this stage the quadrate is a bar except for the condylar region where some expansion of the articular surfaces has occurred. The quadrate, hyosymplectic, basi/glossohyal, ceratohyal and (auto) palatine, epiphyseal bar, as well as most of the branchial skeleton still absorb some blue stain especially on the articular surfaces as could be expected. The parasphenoid is more heavily ossified and expanded laterally with the anterior basicapsular processes formed. The epiorbital area on either side of the orbital bar ossifies as thin, cancellous plates. The prootic bars are ossified as are the exoccipitals and the sphenotic bar which ultimately will articulate with the parasphenoid processes.

All the vertebral centra (usually 30) are ossified as are both the haemal and neural arches and spines. The anterior neural spines are developing zygapophyses.

The pectoral fins have ten rays, seven of which have ossified bases. The caudal fin has eleven ossified rays.

At 27 days the bones stain a deeper red and are larger and sturdier. The chondrified structures are paler blue. Ossification occurs in those bones forming the base of the brain cavity.

The opercular complex shows more ossification. Five ceratobranchials are now stained red but the anterior flanges of the ceratohyals are still cartilaginous.

At 30 days the median fins start to develop with a thickening

of the anal fin and later in the dorsal fin position.
By 33 days the most advanced larvae have five cartilagenous rays and pterygiophores in the dorsal fin and ten fully or partially formed rays and five pterygiophores in the anal fin. The caudal fin has eight ossified rays on the hypural plate, plus three dorsal and five rays ventral to these. The nasal and the lateral ethmoid bones ossify, while posterior to the hyomandibular a small patch of ossification can be seen in the pterotic region (Fig. 37).

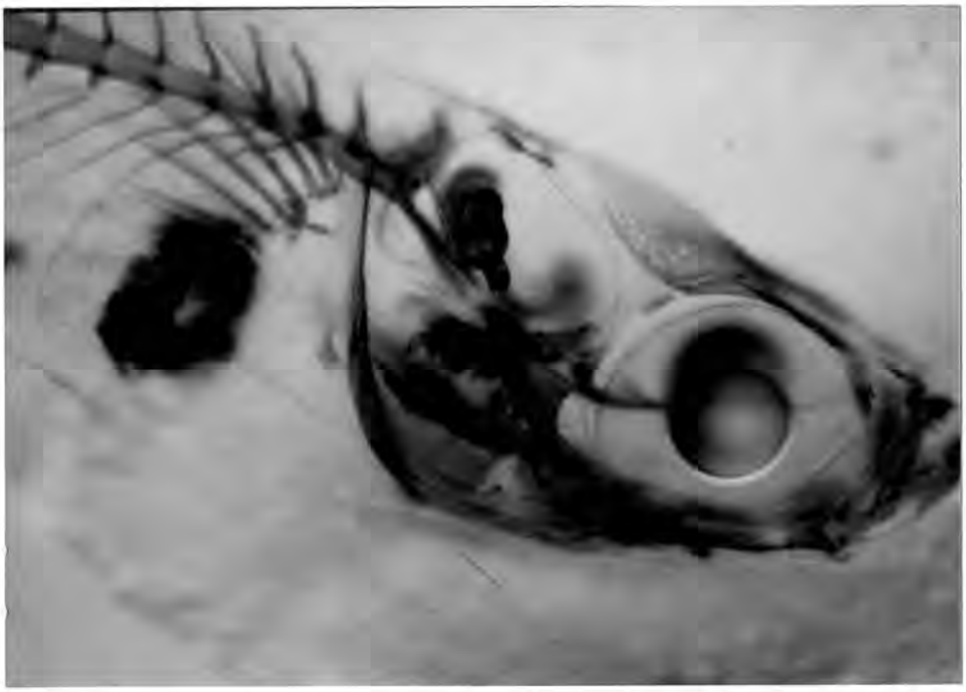


Fig. 37. Lateral view of the head of A. johnstoni at age 35d00. Stained with alizarin red S.

At 37 days old the larvae had four dorsal and 14 anal rays

At 37 days old the larvae had four dorsal and 14 anal rays ossified. The cranial skeleton had the following new bones (Fig. 37): Premaxillary ascending processes, lachrymal and dermo-palatine as well as the mesopterygoid in a pre-otic position. The quadrate was expanded and developed a dorsal flange. The frontal plates extend behind the eyes as far as the parietals. The supracleithrum rests on the epiotic adjacent to and behind the pterotic. The entire branchial skeleton is ossified: three or four basibranchials, five ceratobranchials, epibranchials and dorsal and ventral pharyngeal teeth. The position and origin of the pharyngeal teeth have been described previously. The upper teeth are found on a toothplate which is situated below the prootic and slightly in front of the ventral teeth. This toothplate fuses with the pharyngobranchial bones. The ceratohyals are not totally ossified and only the central areas of the interhyals are.

There are seven ossified and three chondrified ribs. The paired fins are nearing complete ossification. In the pectorals the coracoids and the scapulae are formed in bone in the adult shape but only the cores of the radials are ossified. Pelvic finrays are differentiated but do not stain red.

At 40 days there are three or four scales on the lateral aspect of the body, which signify the onset of the juvenile transition. There are now the adult number of eight dorsal and 15 anal rays and the anterior pterygiophores are ossified. The pelvic fins have five ossified rays and an ossified girdle. Within the next six days squamation will proceed rapidly to completion and sufficiently nourished larvae will undergo juvenile transition at about 50 days although some will still have a vestigial median finfold.

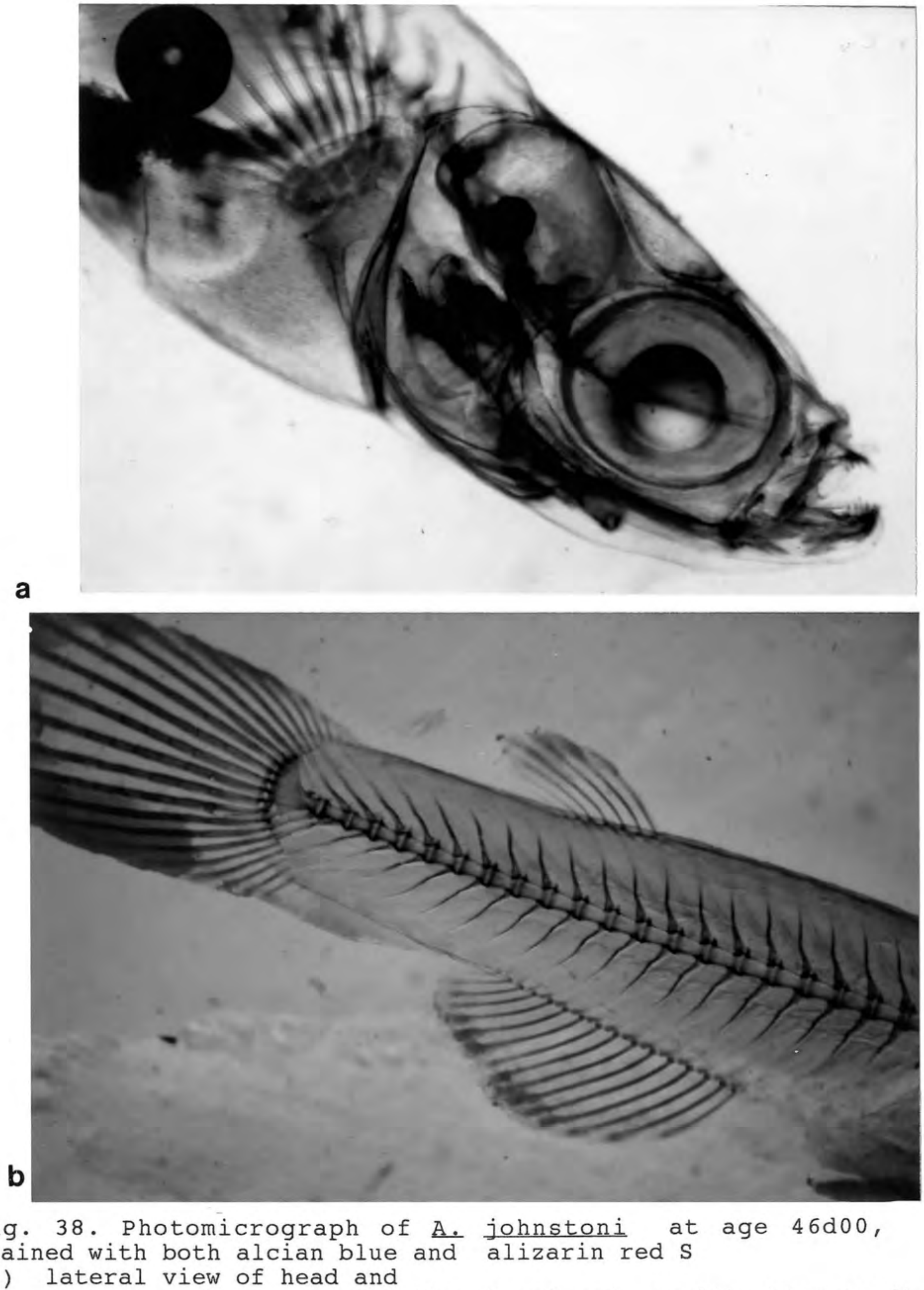


Fig. 38. Photomicrograph of *A. johnstoni* at age 46d00, stained with both alcian blue and alizarin red S
(a) lateral view of head and
(b) caudal area to display the level of ossification (red).

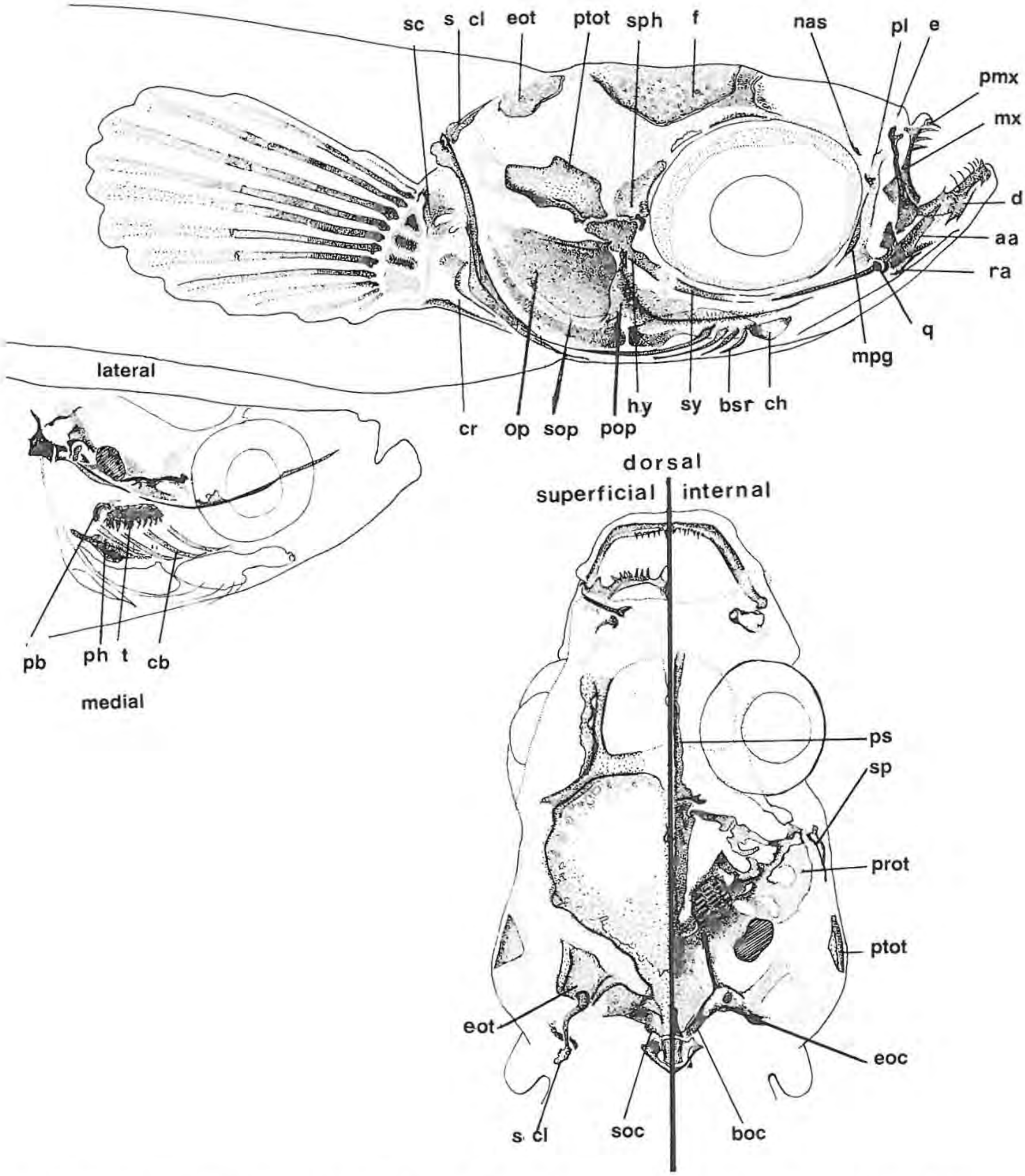


Fig. 39. Prejuvenile skull of <u>A. johnstoni</u> 46d00 old; cartilage in fine stippling, ossification heavy stippling with solid outline.

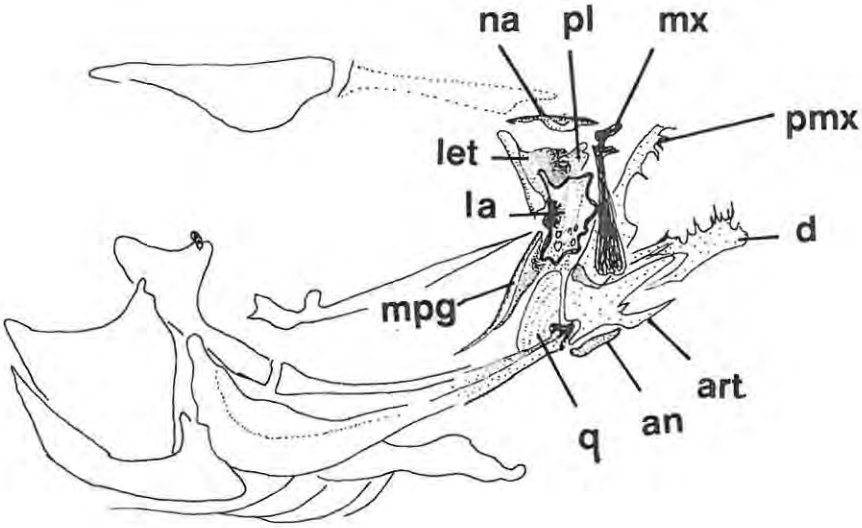


Fig. 40. Enlarged view of the snout area of juvenile A. johnstoni to display positions of bones.

In figures 39 and 40 the level of ossification at the larval/juvenile transition is displayed.

Circulation

During the larval period the remaining embryonic aspects of the circulatory system are replaced by the adult form. After hatching, the vitelline circulation moves ventrally becoming concentrated on the midventral line (Fig. 41). The posterior vitelline vessel carries most of the blood on the remains of the yolksac while the flow in the lateral vitelline vessels becomes concentrated in the anterior ducts which ultimately form the ducts of Cuvier under the cleithra. This change is usually completed in two to three days after hatching, the period of the transition depending on the developmental state of the individual at hatching.

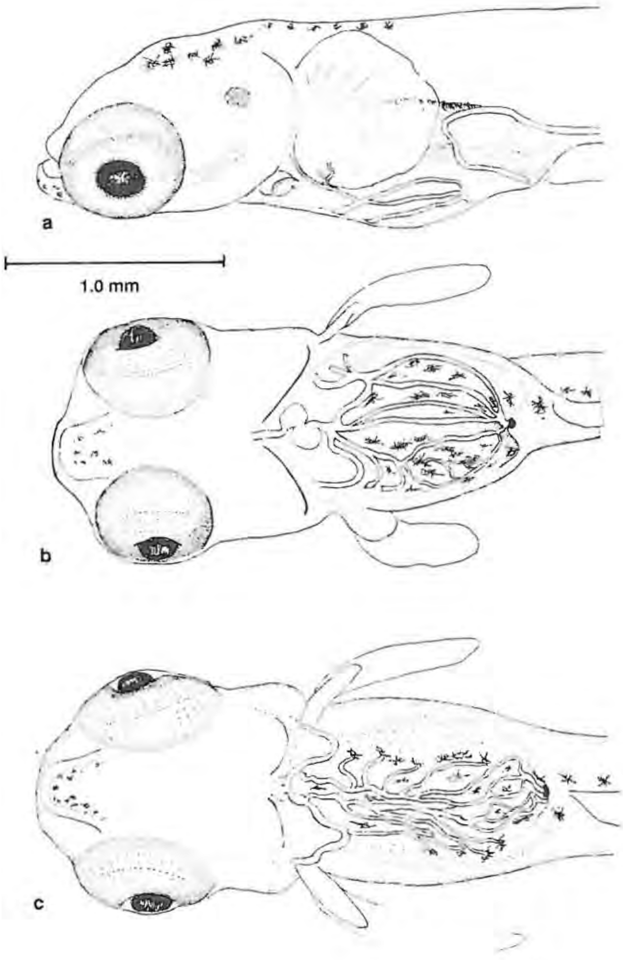


Fig. 41. Anterior aspect of larvae of *A. johnstoni* to indicate the reduction in the embryonic circulation.
(a) Age 17d00 lateral, (b) ventral view of same larva
(c) Ventral view of larva age 19d00 showing vessel reduction.

The other visible change in the circulatory system is the replacement of the superficial (embryonic) caudal vein which runs along the ventral body wall close to the surface, by a profundal (adult) caudal vein which runs immediately ventral to the dorsal artery through the haemal arches. The caudal vein thus developed will link with the postcardinal vein.

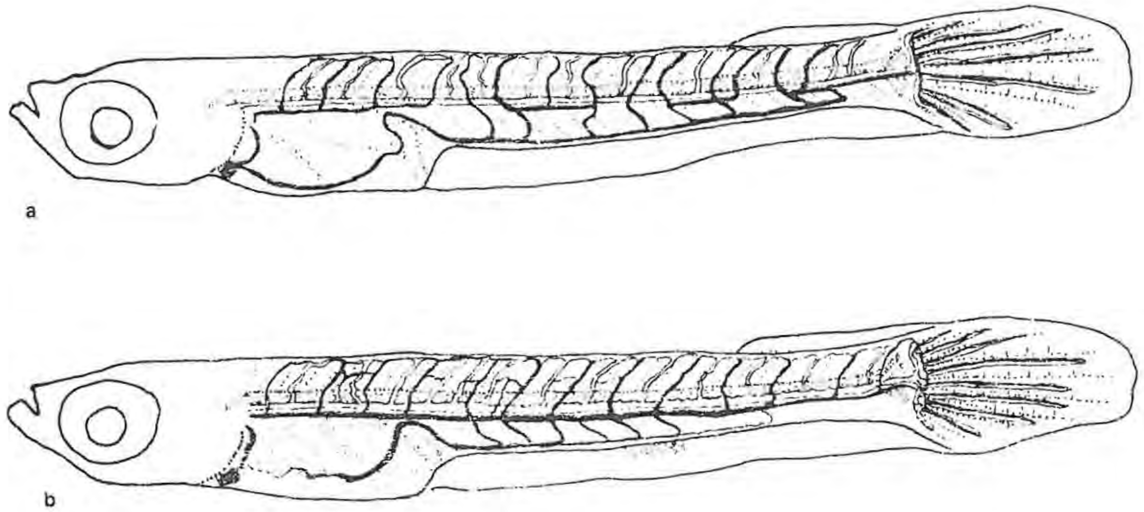


Fig. 42. Diagrammatic representation of the formation of the profundal caudal vein in larval *A. johnstoni*.
(a) At the start about 21d00 (b) when the profundal is comlpete about 25d00

After hatching, the blood vessels connecting the aorta with the superficial caudal vein along the final haemal spines, will atrophy starting with the most posterior. A vessel running forward under the the dorsal artery will remain, thus initiating and the profundal vein (Fig. 42). The rest of the profundal vein develops from a series of connecting capillaries from the dorsal aorta either via the dorsal myotomes or directly to the superficial caudal vein along the haemal spines. The vessel will form sections under the aorta in which the blood will flow forward before running down to the superficial vein (Fig 42). A continuous profundal vein is thus established in some fish by age 25 days and a double caudal venous circulation is evident for several days. During this period reverse flow from the superficial to the profundal has been observed. The superficial caudal vein atrophies and by 40 days there are only vestiges left in the anal region. In this area above the body cavity the postcardinal vein is formed in a similar fashion as the profundal vessel with which it ultimately unites.

Somatic development and growth

After hatching the first significant event in somatic development is the activation of the swimbladder which fills within an hour or two of hatching. During the larval period the swimbladder expands to occupy a third of the pre-anal region.

The prehatching larvae of 12 to 14 days old range in standard length from 1.90 - 3.65 mm with an average of about 2.04 mm. At hatching when some larvae are 14 but most 16 to 18 days old sizes range from 4.2 - 5.5 mm with an average of 4.66 mm. The standard length remains fairly constant, increasing only one to three mm during the following 10 days after which length increases more rapidly (Fig. 43)

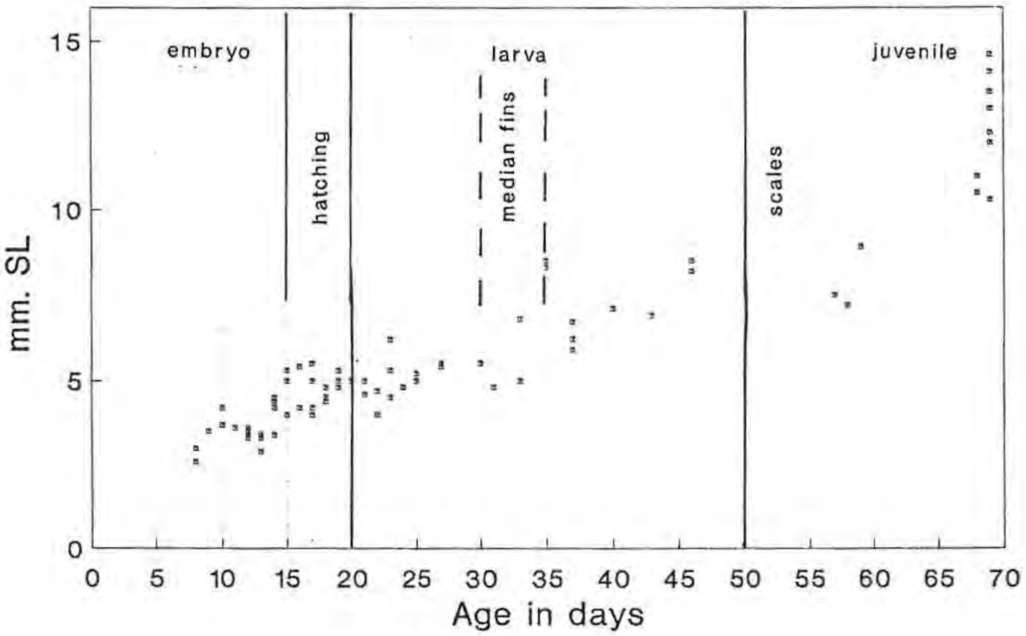


Fig. 43. Graphic presentation of the growth rate of embryos, hatchlings and larvae of A. johnstoni

The growth rate of the specimens in this sample seems to progress in a stepwise fashion. The measurements were taken from all the larvae and late embryos obtained during this study, so some received sufficient food while other were undernourished. After hatching and from 20d00, when the yolk reserves have been totally used, growth and developmental rate can be influenced by nutrition. Despite this extraneous variable, it appears as though length increase is minimal while differentiation of the skeleton progresses. At about 35 days there is an increase in length. At this time the skeletal ossification reaches an advanced stage and the median fins complete ossification. By this age in most specimens studied, the adult (profundal) caudal vein is fully formed. The rapid increase in size between 60 and 65 days takes place at the start of the juvenile period. At this time squamation has been completed and the skeleton is fully formed. An experiment with a larger number of larvae under a carefully controlled feeding regime would corroborate or refute these observations.

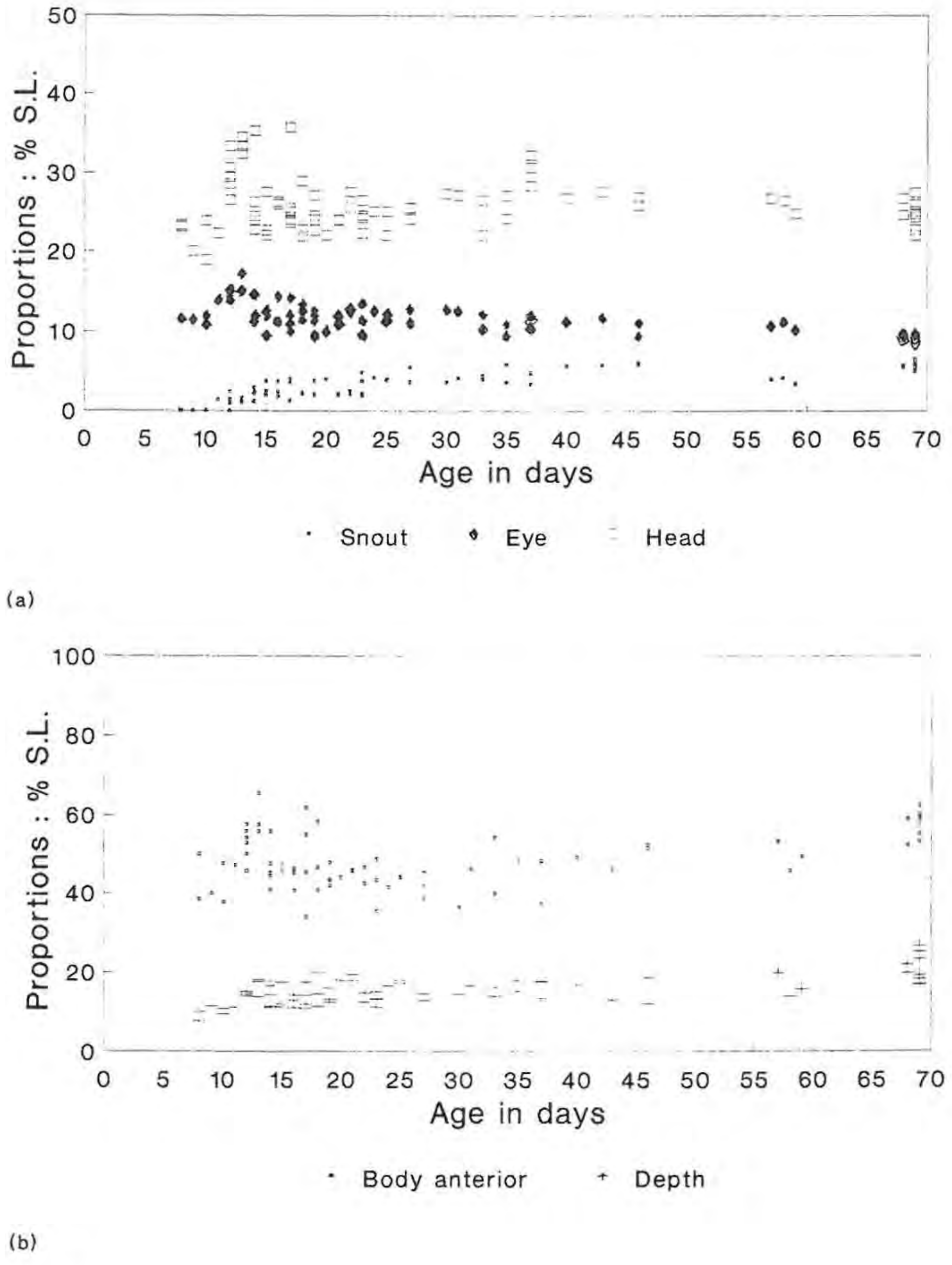


Fig. 44. Body proportions (expressed as % of standard length) during late embryonic, larval and early juvenile periods of <u>A. johnstoni</u>. (a) Changes in the eyes, snout and head. (b) Changes in pre-anal body length and depth.

The snout length increases rapidly prior to hatching, which reflects the growth and development of the jaw elements from 8d00 onwards. Once the snout has reached 5% of standard length (SL) it remains constant at about that relative size.

Between days 12 and 15 the anterior part of the body, especially the head, shows a marked increase in size relative to the standard length. However this proportion diminishes as the caudal trunk grows to its maximum prehatching length prior to hatching between days 15 and 18.

The depth of the embryonic and juvenile fish remains fairly constant at about 18% of SL until the juvenile transition is complete. The increase in depth at 70 days can be regarded as an indication of the change in the body shape of the fish as they lose the slender elongate larval body and assume the adult body form. (Compare figs. 1 & 35)

Juvenile period

The juvenile period has not been extensively investigated. As stated before, growth and ossification are dependent on food availability and any statement on the duration and timing of events during this period can only be made keeping this variable in mind.

As the juvenile period is said to terminate with the onset of sexual maturity, a small group of similar aged fish was kept in a separate tank to try and establish the duration of the juvenile period (See chapter 2). The first eggs were collected from this group when the fish were 150 days old and long before average adult size had been achieved. This is an indication of the length of the juvenile period. As the fish fed to satiation, but were kept under laboratory conditions this time period can be used as a reasonably accurate but not definitive indication.

List of abbreviations for figures

aa = angulo-articular
abc = anterior basicapsular commisure
acv = anterior cardinal vein
apl = actinostial plate
au = auricle
bb = basibranchial
bh = basihyal
boc = basioccipital

c = coelom
cb = ceratobranchial
ch = ceratohyal
cr = coracoid
cv = caudal vein
d = dentary

e = ethmoid
ep = epural
eot = epiotic
f = frontal

gh = glossohyal
gr = germring
h = heart
hh = hypohyal
hpp = parhypural
hv = hepatic vein
hys = hyosymplectic

i = intestine
Kv = Kupfer's vesicle
l = lens
le = lateral ethmoid
lvv = lateral vitelline vein
ma = mesenteric artery
mpg = metapterygoid

n = notochord
ns = neural spine
oc = otocyst
ofp = olfatory placodes
op = operculum

p = pericardium
pbs = posterior blood sinus
pcp = pericardial capillaries
pct = parachordal cartilages
pect = pectoral fin
p hm = pericardial headmembrane
pl = palatine
pop = pre-operculum
prot = prootic
pt = pharyngeal teeth
pvv = posterior vitelline vein
q = quadrate

ra = retroarticular
s = somatic segmentation
scc = segmental circulation
s cl = supracleithrum
sop = suboperculum
sp = syncytial periblast
spl = spleen
sv = sinus venosus
t = teeth
ub = urinary bladder
v = vertebra
vp = vegetal pole
y = yolk

ac = auditory capsule
ap = animal pole
art = articular
bbc = basibranchial copula
bl = blastodisc
bsr = branchiostegal rays

ca = carotid artery
cf = caudal finrays
cl = cleithrum;
cs = caudal blood sinus
da = dorsal aorta

eoc = exoccipital
epb = epiphyseal bar
es = embryonic shield
ff = fin fold

gp = gill pouches
ha = hepatic artery
hp = hypural
hs = haemal spine
hy = hyomandibular

la = lachrymal
li = liver
Mc = Meckels cartilage
mx = maxilla

nas = nasal
ocm = orbital commissure
og = oilglobules
ov = optic vesicles

pb = pharyngobranchial
pda = paired dorsal aortae
pg = pterygoid
pmx = premaxilla
pq = palatoquadrate
psh /ps= parasphenoid
ptot = pterotic

rvv = right vitelline vein
sb = swim/ airbladder
sc = scapula
soc = supra occipital
sp = sphenotic
st = stomach
sy = symplectic
tr = trabecula
ve = ventricle
ys = yolksac

Chapter 4

DISCUSSION

Introduction

The early ontogeny of an organism is largely a consequence of the interaction between the internal and external environment of its progenitors.

Ontogeny can also be described as an interaction of the historical and the immediate. The historical aspect is represented in the phylogeny, morphology and genotype of the animal while the immediate is represented by the growth and reproductive ability of the animal as a consequence of the quality and quantity of the available food and space and the efficiency with which its particular metabolism utilizes these resources. Immediate influences may result in long-term changes of the genotype, through mutations, with resultant change in the phylogeny of the descendants of a reproductive event. A more subtle influence is the epigenetic one whereby the environmental influence affects the expression of the genotype in such a way as to change the phenotype or the life-history style of the animal (Lovtrup 1974, 1984, Gordon & Jacobson 1978, Balon 1985, Swain & Lindsay 1986).

Of the three metabolic categories of growth, reproduction and maintenance into which available energy is partitioned, reproduction is that area where the environmental interaction with the genotype can be the most direct. The maternal contribution, through the extranuclear internal environment of the egg can influence the expression of the genotype directly through, among other things, the quality of the cytoplasmic nutrients, during the developmental process. (Boulekbache 1981)

External environmental factors such as temperature and water quality can also have a direct influence on development. Temperature may influence the rate of development (Boulekbache 1981, as well as the fixation of certain meristic characters, as reported by Fahy (1983) and Swain & Lindsey (1986) among others. Water quality, in terms of the solutes which change the pH, salinity and amount of dissolved oxygen, can also affect development by slowing down the metabolic rate as well as influencing the health of the larvae. However, despite wide environmental variations, siblings will develop along remarkably similar lines, thus supporting the concept of developmental canalization (vide Waddington 1942, Waddington & Robertson 1966 & 1975, and Stearns 1982).

Patterns exhibited in the ontogeny in terms of saltatory development, plasticity and heterochronies

Development is a hierarchical process, " ... a succession of qualitatively different kinds of processes, ... with the product of one step forming the starting point of the next" (Maynard Smith, 1983 p. 38).

The boundaries of developmental intervals are found at the transition from one state to another. Activation and fertilization transform a single haploid cell with a limited lifespan into a diploid organism of infinitely greater potentialities and longer lifespan. Activation can therefore be taken as a boundary or threshold. Generally, the translation by mitochondrial RNA of amino acids and proteins present in the cytoplasm of the unfertilised egg to those required for blastulation takes place at activation (Davenport 1979). The division of this cytoplasm into many cells forming a blastula is the next homeorhetic interval with the interface between blastulation and epiboly-gastrulation as the transition. During the cleavage phase there are two distinct intervals reported in the literature. Firstly at the start of cell division oxygen consumption of the embryo increases by 50% from that of the fertilized egg (Boulekbache 1981). On a molecular level, during the first 30 minutes after fertilisation, protein synthesis occurs rapidly but then remains at a constant level during cleavage (Davenport 1979). In A. johnstoni particularly, the first cell is separated from the yolk by 01h15, which is an external, visible indication of the above processes described in general.

Secondly the cells formed during blastulation become determined at a early stage of cleavage (Devillers 1961) into future embryonic and enveloping cells. From the scant experimental evidence reported by him it appears as though the exact stage at which this occurs may be species or group specific. The stage at which this determination occurs can only be fixed in an experimental fashion. The result can, however, be seen in A. johnstoni at 7h00 when the larger enveloping cells are clearly distinguishable from the smaller deep blastomeres. The fact that early embryogenesis does not proceed in a gradual way is thus evident at the molecular level.

Prior to gastrulation there is another escalation in the rate and range of protein synthesised, as the differentiating blastomeres require proteins not available from translational control but which have to be formed from newly manufactured mRNA.

Terner (1979) suggests that at early cleavage the nuclei do not transmit information actively but by midblastula stage the rates of gene transcription and morphogenetic activity increase. This suggestion corresponds with Davenport (1979) and Balinsky (1981) who report changes in cell structure of the blastomeres, which are fairly simple initially and then become increasingly complex as the cells are differentiated prior to the formation of primordial tissue layers. It is possible to detect the results of this process in A. johnstoni between 10h00 and 14h00, prior to the start of the epibolic process.

At the conclusion of this process, the ectoderm and the endomesoderm so formed are then ready for the process of organogenesis. This transition is the next threshold.

The formation of tissue layers is of necessity preliminary to the following sequences as they form the substrate for subsequent organ formation. For example, the earliest interaction with organogenetic results is that which takes place between presumptive notochord and presumptive ectoderm which results in neural differentiation (Hall 1983).

The formation of organs and organ-systems does not follow the same rigid sequential pattern as the previous sequences and appears to be more flexible according to the dictates of the historical environment of the particular organism. However there are certain broadly definable sequences. If these sequences, cited below, are analysed, it will be noted that the structures formed are derived from firstly ectodermal cells followed closely by structures of mesodermal origin and lastly those of endodermal origin. The nervous system must precede any other as it is the controller. Segmentation of the embryonic body and basic muscular elements (mesodermal) take place very slightly later than initial nervous differentiation, no doubt in order to provide a framework for the nerves.

The circulatory system (mesodermal) follows. This is the transport system, moving nutrients and oxygen to, and waste products from the tissues, thus facilitating differentiation. The formation of the digestive/excretory system (endodermal) follows later (Balinsky 1981) and with the skeletal (ecto & mesodermal) system, the sequence of appearance of their various elements is plastic and can overlap variously, dictated no doubt by the environmental constraints imposed on the organism. (See Table 1 for the timing of these events in _A. johnstoni_).

The above discussion illustrates the hierarchical nature of development, and from it the epigenetic events during the developmental process can be deduced, during which succeeding juxtapositions of different cell types will elicit different and ever more complex processes (Hall 1983).

The fact that some events appear in a rigid sequence whilst others show flexibility of occurrence raises the question of the relative importance of canalisation and plasticity. Canalisation is a feature of the system which is built up by natural selection,... it ensures the development of the normal, that is, optimal type..' (Waddington 1942). Waddington's proposal of an epigenetic landscape, whereby cells are either canalised in paths essential to the successful development of the organism or allowed alternative routes which open up greater developmental possibilities, provides a plausible explanation of the mechanisms responsible for the phenomena of similarity and diversity within species from very different environments. Stearns (1982) correctly states that plasticity and canalisation can be seen as antonyms. Stearns (1986), discussing adaptation and constraint, deals with similar effects but ascribes more rigid genetic control mechanisms to the processes. For more recent and extensive discussions on this topic see Balon (1989) and Bruton (1989) as well as several other authors in the same publication.

Given that the hierarchical nature of development and its underlying genetic and epigenetic controls have been demonstrated above, the nature of the developmental process will now be discussed. Yeremeyeva (1967) translated by and cited in Balon (1981) suggests that the anlagen of new organs and their new functions appears at the boundaries of developmental steps during the transition from one step to another ... a transition of quantity into quality. During each step accumulated quantitative and qualitative changes of structure and function of the organism gradually result in small changes of the relationship with the environment - until a certain limit is reached - then a transition into a new step of development occurs. Balon (1981) explains that these steps "will only achieve functional significance in a combination of meaningful associations"; the recognition of such combinations constitutes a major difficulty in the application of the theory of saltation. Thresholds occur at the end of steps, defined as a "combination of abrupt functional changes" during ontogeny and is a median time from the first to the last appearance of such change in individuals. The distinction between change in structure and change of function is not always clear; other aspects of biochemical, physiological, morphological and ethological nature will need to be considered. Whether development can be described as truly saltatory is still an issue of debate as evidenced by Greenwood (1989) who favours one of "gradual processes expressed in a stepped pattern."

The discrepancies between the interpretation of these two authors cited above seem to be centered on the exact meaning and implication of the word 'saltation'. Balon (1989) equated saltation with Bohr's quantum jumps. When he discusses the effect that the 'noise' of individual variation has on the ability of the observer to pinpoint thresholds (see below), he moves further in the direction of seeing the developmental process as one of leaps of rapid change between homeorhetic states. Greenwood (1989), on the other hand, would prefer to categorise ontogeny as 'a stepped continuum'. I would suggest that apart from the problem caused for Greenwood by Balon's use of saltation, there is a great deal of agreement between the authors on the nature of the ontogenetic process. They agree upon the hierarchical nature of development and the fact that the various steps in ontogeny are the necessary precursors to subsequent steps interspersed by times during which, through congruence of development of structures a new or altered potential comes about, i.e. they agree about thresholds. What they disagree about is what constitutes a saltation. Greenwood regards the presence of continuities at either end of a threshold as preclusive to the existence of saltation. I would venture to suggest a compromise: Let the term 'saltation' be reserved for those occasions when radical qualitative change takes place which alters all aspects of that organism rapidly, i.e. fertilisation, the change from endogenous to exogenous feeding, the attainment of sexual maturity, and death. It should therefore be agreed that development is a series of homeorhetic states linked by thresholds during which new developmental processes come into play.

From my observations on *A. johnstoni* I would venture to suggest the following events as thresholds;
Completion of cytoplasmic separation & start of cleavage (01h00-01h10);
First differentiation of blastomeres (07h00-08h00);
The initiation of epiboly (10h00);
Definition of the primordial layers (invisible);
Initiation of organogenesis (difficult to pinpoint, circa 26h00);
Start of heartbeat (53h00- a functional threshold);
Endodermal proliferation (70h00- 72h00);
Skeletal differentiation (06d00-07d00);
Brachycardia (11d00 - a functional threshold, and perhaps a phylogenetic remnant);
Completion of skeletal differentiation with formation of pelvic fins and adult circulation (36d00);
Scalation starts (43d00);
Resorption of all finfolds (juvenilisation) (variable 55d00);
Cessation of sexual activity (unknown);

Saltations, on the other hand, would then be ascribed to the following events and times for *A. johnstoni*:

Fertilisation and activation (0h00-0h10)
Exogenous feeding (18d00-21d00 depending on hatching time)
Sexual maturity (about 150d00)
Death (unknown).
It then becomes clear that saltations occur at the thresholds of periods and further that the larval /juvenile transition could be seen as an artificial separation. The developmental style displayed by holometabolic insects is an extreme illustration

of saltatory development.

From some selected publications consulted, (Fahy (1983), Lindsey & Arnason (1981) & Swain & Lindsey (1986) on the influence of environmental changes on development, I would venture to suggest that the most sensitive times in development are those when new tissues or organs are being differentiated and not when growth takes place. It seems logical that the period of determination or differentiation rather than the following homeorhetic state of growth should be vulnerable to outside influences. I therefore disagree with Balon (1989) when he says that "during a homeorhetic step changes would interfere; they may occur without penalty at the far from stable thresholds" and "during a stabilized state, cells and tissues differentiate,.." If the intervals when diapause appears in cyprinodonts are considered, further clarity is achieved on the degree of vulnerability of certain developmental steps. Diapause occurs only at times of low vulnerability such as after the first differentiation of blastomeres and before the deep blastomeres re-aggregate to undergo tissue differentiation (Wourms 1972), at the end of the initial ectodermal and mesodermal differentiation and before the circulation starts or at the end of the embryonic development when differentiation and most growth is completed (reviewed in Cunningham & Balon 1986 b). These tactics are often found in genera which live in habitats which undergo severe and unpredictable perturbations. Cunningham & Balon (1986 a) state that.. "it appears that options for resting or diapause may be limited to step boundaries, i.e., to thresholds at which the summation of the step has been reached but the new direction of development has not yet begun. The embryo is then at the transition to the new step, ready for the switch. If conditions are not sufficient for subsequent development, a resting interval delays the switch instead". I would suggest that the least sensitive times during development are those at the conclusion of steps and before a threshold is entered (see also Wourms 1972 a & b, Alberch 1980).

A new description of the nature of development would then read as follows: ontogeny is an hierarchical stepped continuum of alternating vulnerable and stable states with rapid saltatory intervals brought about by the synergistic effect of congruent events or maturation of systems which bring about a total change in the life history style of the organism.

As Balon (1981) stated above, it does indeed become increasingly difficult to determine step borders as development progresses and as the complexity of the organism increases. Perhaps it is that the thresholds become wider as more systems are to be integrated. This may consequently give the impression of gradual change. However if the times of change or unstable periods are viewed against the entire period of the life span they are certainly of shorter duration and could therefore be viewed as thresholds between times of homeorhesis (ie, puberty is a threshold but ageing is an homeorhetic state). Against which parameters does one measure developmental speed or rate? The entire developmental time (lifespan), absolutes of hours and days or by conversion of absolutes to proportions of life-times?

It is easy to determine steps which occur early in the ontogeny of A. johnstoni such as blastulation/epiboly, organogenesis, start of circulation or skeletal differentiation. Even more dramatic are those which could be taken as saltations ie fertilization and exogenous feeding, which in this species, coincides with hatching. If it was possible to follow one embryo closely for extended periods, such boundaries may become clearer. However, because of the multi-specimen approach of these studies at present, the step boundaries are obscured by wide ranges in developmental rates. This has been observed and reported in other detailed ontogenetic studies (Cunningham & Balon 1985).

Intraspecific developmental plasticity has obvious adaptational advantages, making a wider range of phenotypic options available to the species' genome should it encounter unexpected or unusual environmental perturbations. Here can be cited the wide ranges of developmental rates encountered throughout this study culminating in the age at hatching which varied by as much as 5 days. Coupled with the serial, fractional spawning habit of this species, the wide range in size and developmental stage of young released into the environment over the extended breeding period becomes apparent and the obvious advantage of this in an even moderately capricious situation is self evident.

In Table 1 below a summation of the ontogenetic events of A. johnstoni is given following the example of Cunningham & Balon (1985). The time sequences at the left of the table present variation in initiation times as well as completion times of the various steps.

Summary of ontogenetic events in <u>A.johnstoni</u>.	
Timing of start finit	Ontogenetic events in each step
0h00 - 1h45	activation, perivitelline space cytoplasmic separation
1h45 - 10h00	cleavage, syncytial periblast, movement of blastomeres
10h00}-{26h00 15h00} {28h00	epiboly, embryonic shield
26h00}-{33h00 28h00} {35h00	organogenesis, optic vesicles, brain, neural keel, pericardial cavity, epiboly completes (ectodermal organs)
33h00}-{53h00 35h00} {60h00	segmentation(mesodermal), Kupfer's vesicle otocysts, lenses, vitelline network, heart contractions, pigment
53h00}-{70h00 60h00} {72h00	blood flow, muscle contraction, vitelline plexus forms, cephalic circulation, endodermal anlagen
70h00}-{4d16h 72h00} {5d00	formation of gut, bladder, liver, gillpouches, pectoral buds, buccal cavity final somite number, ventral finfold, escalation in yolk absorption, brain and sensory growth and development, hepatico-mesenteric artery and vein,
4d16h0} {7d00 5d00 } {8d00	segmental, fin and gill circulation, internal and heart differentiation, gallbladder, fin growth, eye pigmentation, swimbladder formed
7d00}-{10d00 8d00} {11d00	skeletal formation, caudal lepidotrichia, eye guanine, swimbladder, liver growth, bile pigment, mouth opens.
10d00}-{12d00 11d00} {14d00	brachycardia, pectoral, eye and mouth movement, respiratory movements, greater flow through Cuvier's ducts, maximum chondrification and some calcification, dorsal coelom pigment.
14d00-20d00	hatching, first exogenous feeding, filling of swimbladder, formation of profundal (adult) caudal vein,
21d00-36d00	closure of ventral bodywall, completion of profundal caudal and atrophy of superficial (embryonic) vein, median fins formed,
36d00-43d00	development of pelvic fins, superficial caudal vein closes, pigmentation changes, scalation
43d00-5?d00	final median fin counts, absorption of entire finfold, adult proportions.

Table 1. A summary of the major ontogenetic events occuring in each step of the early ontogeny of <u>A. johnstoni</u>.

Interspecific heterochronies can be important markers in phylogeny (Paine & Balon 1985). As the number of ontogenetic investigations on representatives of orders and families increases, the possibility of doing a comparative analysis of representatives of these groups is enhanced.

Cunningham & Balon (1986,b) attempted an exercise like this by comparing the developmental styles of several cyprinodont fishes. The information was abstracted from work published by other authors and molded into a comparable form by recalculating the developmental time to temperature units. The species of fish compared were Fundulus heteroclitus (Armstrong & Child 1965), Austrofundulus myersi (Wourms 1972) and Platypoecilus maculatus (Tavolga 1940). The relationships of these species are as follows according to Parenti (1981): The members of suborder Cyprinodotoidei sort to the families **Fundulidae** (Adinia and Fundulus, Div.1) and **Poecilliidae** (Platypoecilus and Aplocheilichthys, Div. 2 Sept 2.) while the suborder Aplocheiloidei is represented by the family **Rivulidae** (Austrofundulus).

Cunningham & Balon (1986 b) assigned a number to each ontogenetic event in the order of their appearance in Adinia xenica. (The list can be seen on the graph from this publication in Fig. 45.) The events in all the other species, including A. johnstoni, are here assigned the same number but because of the heterochronies in occurrence in different species the numbers do not follow an ordinal sequence.

As the experiments were conducted under a similar temperature regime in A xenica and A. johnstoni the comparison is perhaps more valid than those for the other three species considered. When the combination of the sequence and rate of developmental events in A. johnstoni is analysed and compared with the others, its uniqueness is revealed.

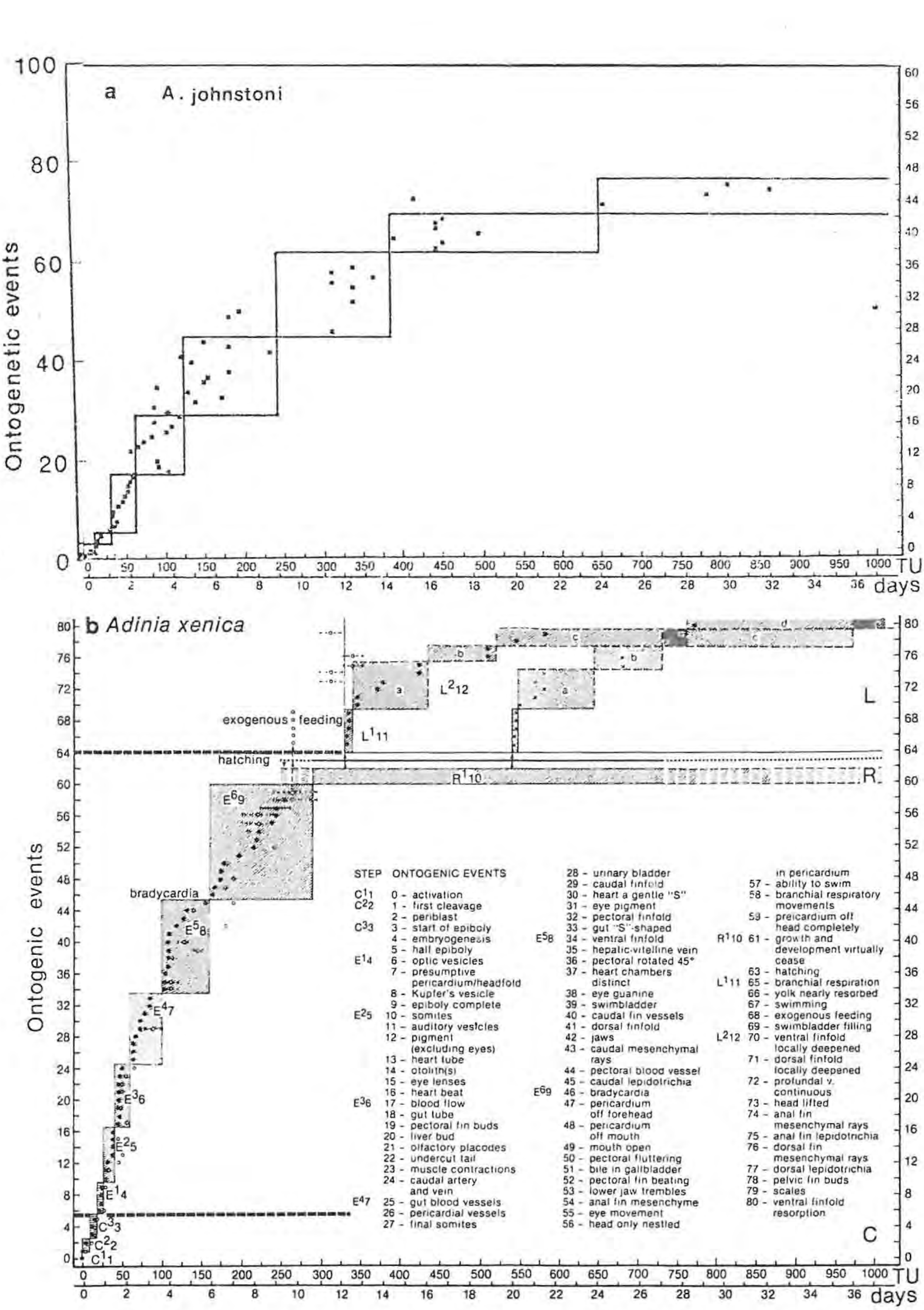

a
A. johnstoni
Ontogenetic events
0
20
40
60
80
100
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
TU
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
days
b Adinia xenica
Ontogenic events
exogenous feeding
hatching
bradycardia
$C^{1}1$
$C^{2}2$
$C^{3}3$
$E^{1}4$
$E^{2}5$
$E^{3}6$
$E^{4}7$
$E^{5}8$
$E^{6}9$
$R^{1}10$
$L^{1}11$
$L^{2}12$
a
b
c
d
L
R
C
STEP ONTOGENIC EVENTS
$C^{1}1$ 0 - activation
$C^{2}2$ 1 - first cleavage
2 - periblast
$C^{3}3$ 3 - start of epiboly
4 - embryogenesis
5 - half epiboly
$E^{1}4$ 6 - optic vesicles
7 - presumptive pericardium/headfold
8 - Kupfer's vesicle
9 - epiboly complete
$E^{2}5$ 10 - somites
11 - auditory vesicles
12 - pigment (excluding eyes)
13 - heart tube
14 - otolith(s)
15 - eye lenses
16 - heart beat
$E^{3}6$ 17 - blood flow
18 - gut tube
19 - pectoral fin buds
20 - liver bud
21 - olfactory placodes
22 - undercut tail
23 - muscle contractions
24 - caudal artery and vein
$E^{4}7$ 25 - gut blood vessels
26 - pericardial vessels
27 - final somites
28 - urinary bladder
29 - caudal finfold
30 - heart a gentle "S"
31 - eye pigment
32 - pectoral finfold
33 - gut "S"-shaped
$E^{5}8$ 34 - ventral finfold
35 - hepatic-vitelline vein
36 - pectoral rotated 45°
37 - heart chambers distinct
38 - eye guanine
39 - swimbladder
40 - caudal fin vessels
41 - dorsal finfold
42 - jaws
43 - caudal mesenchymal rays
44 - pectoral blood vessel
45 - caudal lepidotrichia
$E^{6}9$ 46 - bradycardia
47 - pericardium off forehead
48 - pericardium off mouth
49 - mouth open
50 - pectoral fluttering
51 - bile in gallbladder
52 - pectoral fin beating
53 - lower jaw trembles
54 - anal fin mesenchyme
55 - eye movement
56 - head only nestled in pericardium
57 - ability to swim
58 - branchial respiratory movements
59 - preicardium off head completely
$R^{1}10$ 61 - growth and development virtually cease
63 - hatching
$L^{1}11$ 65 - branchial respiration
66 - yolk nearly resorbed
67 - swimming
68 - exogenous feeding
69 - swimbladder filling
$L^{2}12$ 70 - ventral finfold locally deepened
71 - dorsal finfold locally deepened
72 - profundal v. continuous
73 - head lifted
74 - anal fin mesenchymal rays
75 - anal fin lepidotrichia
76 - dorsal fin mesenchymal rays
77 - dorsal lepidotrichia
78 - pelvic fin buds
79 - scales
80 - ventral finfold resorption

Fig. 45. Developmental pattern displayed by (a) *A. johnstoni* and (b) *Adinia xenica*
Ontogenetic events assigned the same number as in graph (b) from Cunningham and Balon (1986 b). These numbers (events) are plotted against the time of first appearance (X axis). Step thresholds are indicated by horizontal lines while the width of each block indicates duration of each step. The differences in both the rate of development and the variation in the appearance of structures can be clearly seen.

If the graphs resulting from these analyses are compared the difference in the developmental rates is clear, graph (a) having a more gradual slope than graph (b). There are several heterochronies revealed in the appearance of certain structures and some structures appear outside the step boundaries as is the case all the other species considered.

If a timetable of the developmental rate of the two species is drawn up, further discrepancies are revealed.

Comparison between developmental rates of

Events.	Aplocheilichthys johnstoni	Adinia xenica
activation, pervitelline space cytoplasmic separation.	1h45	1h30
cleavage, syncetial periblast movement of blastomeres.	9h00	10h00
epiboly, embryonic shield.	16h00	7h00
organogenesis starts, neural keel, optic vesicles, pericardial cavity, epiboly completes.	8h00	14h00
segmentation, Kupfer's vesicle, otic vesicles, lenses, vitelline network, heart contractions, pigment.	7h00	6-7h00
blood flow, muscle contraction, vitelline plexus forms, cephalic circulation, endodermal anlagen.	19h00 +	
formation of gut, bladder, liver, gillpouches, pectoral buds, buccal cavity, final somite number, finfold, escalation in yolk absorption.	48h00 =	
brain and sensory growth & development hepatico-mesenteric artery and vein.	67h00	17h00
segmental, fin and gill circulation, internal and heart differentiation, gallbladder, fin growth, eye pigment, swimbladder formed.	56h00	36h00
skeletal formation, caudal lepidotrichia, eye guanine, swimbladder, liver growth, bile pigment, mouth opens.	96h00	67h00
brachycardia, pectoral, eye and mouth movement, respiratory movements, greater flow through Cuvier's ducts, maximum chondrification, calcification dorsal coelom pigment.	96h00	112h00
hatching period or resting stage	14-19d	10-27d
first exogenous feeding, filling of swimbladder, formation of posterior profundal vein.	72h00	96h00
closure of ventral bodywall, completion profundal vessel, median fins formed.	18d	3d
development of pelvic fins, superficial caudal vein ceases, pigmentation changes, scalation.	7d	11d
final median fin counts, absorption of entire finfold, adult proportions.	+8	3d
total time till juvenile is formed.	++50d	50d

Table 2. Comparison between developmental rates of A johnstoni and Adinia xenica at $25^{o}C$.

As can be seen from this comparison in Table 2, the time intervals of the developmental phases and steps in these two species differ markedly. During the embryonic phase, the steps during which organogenesis occurs have the greatest differences. The differences are;
A. johnstoni, E6, 67h00; E7, 56h00; and E8, 96h00; for
Adinia. xenica, E6, 17h00; E7, 36h00; and E8, 67h00;
The embryo can be considered to be in a vulnerable state, as all organs are developing and the embryo is incapable of sustaining life outside the egg membranes. An abbreviated period in this state would be advantageous in a highly perturbed environment, such as that reported for Adinia. This marked increase in this interval in A. johnstoni could be an indication that the environment of Aplocheilichthys is of a more stable nature with less risk of exposure during the embryonic period. The tidal nature of salt marshes which Adinia inhabits has a shorter cycle of flux than the marginal vegetation of large bodies of fresh water that are subject to an annual flood such as the Okavango swamps, where A. johnstoni is found. Developmental rates are more comparable during the later stages when the embryonic differentiation reaches completion and only increase when an increase in size is taking place.

The next major difference in the developmental pattern is the fact that Adinia xenica undergoes a diapause of long but variable duration while A. johnstoni has no discernible diapause, although the age at hatching can vary by as much as 5 days. Brachycardia is seen in both species but heartblock was never found in A. johnstoni. Although the embryonic period of A. xenica is much abbreviated, the larval period is extended and the entire larval development seems to be of a similar duration to that of A. johnstoni. It can be suggested tentatively that this difference of timing is a response to different types of environmental pressures in the evolution of these species. A. johnstoni is more subject to biotic pressures such as predation while A. xenica is threatened more by abiotic factors such as an unpredictable environment.

It is difficult to detect the causes of the heterochronies in the appearance of structures and systems as it is not clear if they are under genetic control or the consequence of epigenetic interactions, i.e., an ecophenotypic response. Some measure of certainty can be achieved if the ontogenies of congenerics are compared. Shared features could then be taken to be of genetic origin.

The heterochronic differences between A. johnstoni and A. xenica are enumerated here with those of A. xenica indicated in brackets.

In A. johnstoni:

1. Epiboly is completed before optic vesicles form (earlier).
2. The tailbud becomes free from the yolk membrane (undercuts) at the time heartbeat commences (earlier).
3. The internal organs and their circulation form later and in a different sequence, i.e., liver before gut while the hepatic circulation is not delayed as in A. xenica.
4. Branchial pouches and finfolds appear before the segmental circulation starts (reversed).
5. Finfold development proceeds rapidly dorsally and ventrally without a delay in between as in A. xenica.
6. The ossification of the skeleton starts several days before hatching which is earlier than in A. xenica.

There are numerous other smaller differences. If the numerical assignments of developmental events developed by Cunningham and Balon (1986 b) are used (See Fig. 45b), the sequence in A. johnstoni would be a scatter instead of proceeding sequentially.

A * before a numeral is an indication of a shift in an event. The events are arranged in the sequence in which they appear in A. johnstoni.

1 2 3 4 5 *9 6 7 8 10 11 12 13 14 15 16 *22 17 21 23 24 25 28 *31 *20 *35 *19 *26 *18 30 *27 *29 *41 *34 *40 32 36 *44 37 *33 *39 38 43 49 *42 50.

This comparison serves to emphasise how the ontogeny could reflect the phylogenetic distance of the two genera, being as they are on two separate divisions of the same suborder (Parenti 1981).

Life-history style, phenology and reproductive strategy of *A. johnstoni*.

A. johnstoni is a small fish, the adult male seldom exceeding 5 cm in total length. It is found in shallow waters on the edges of lakes and swamps in marginal vegetation. It is a predacious topfeeder, with mouthparts modified to feed on zooplankton from the water surface. It in turn forms the food of piscivorous fish such as the African pike *Hepsetes odoe*, and the sharptooth catfish *Clarias gariepinus*, as well as birds. The role of *A. johnstoni* in the food web can therefore be categorized as a vector, shunting energy from the marginal regions of the aquatic ecosystem into deeper waters. As a prey fish the greatest life-threat is from predation. Other possible major constraints on life, such of oxygen availability and nutrition, are of lesser importance as the surface water is well oxygenated, and there is an abundance of arthropod life in this habitat.

Small size is an advantage to this fish as it slips easily between dense aquatic vegetation which could provide refuge from larger predators. In the laboratory *A. johnstoni* exhibits a typically evasive locomotory style; long periods are spent among surface vegetation, swimming is rapid and darting and a rapid escape response is elicited by any large object moved over the surface or on the water.

Miller (1979) reviews the adaptiveness and implications of small size in fish extensively and suggests that "reduction in size among fishes is... an appropriate adaptation for increasing net energy gain by individual capture of items from a food resource composed of small organisms, and also enables pursuit of the latter in a complex substrate or spatially restricted environment". This aspect of course, is especially true for cyprinodonts. Despite these advantages, small size does present some unique problems.

The influence that size has on the reproductive style of a fish is important in fish less than 10 cm standard length when adult. A combination of a changeable environment in shallow water and small adult size seems to have produced a life-history style of high reproductive effort and early sexual maturity (Schaffer 1974, Simpson 1979). However, large numbers of eggs in a small body can produce problems such as hydrodynamic instability with poor locomotory performance and predation attraction because of the small muscle mass associated with small size. Large gonadal tissue growth can also be impossible to achieve in terms of food intake ability as small fish do have a higher catabolic rate per unit mass than larger fish. For these reasons a move away from semelparity towards iteroparity is seen in many smaller fish. By frequent spawning over extended periods small fish can produce a higher number of larger eggs which could be beyond their physical and metabolic capacity if they were to partition energy into the production of a single reproductive effort once a season and grow at the same time (Miller 1979, Hirshfield 1980). Although this trend is common among some groups of small fishes not all have evolved the same life-history styles, as is reported by Miller (1984) for gobies. According to Foster (1967) and Parenti (1981) an early and regular breeding habit and long developmental period, coupled with the ability to survive desiccation, are to be considered a unique developmental pattern for cyprinodontiform fishes. As Miller (1979; 279) points out, the repeat spawning reproductive style points to an ancestry in tropical or warm conditions with no sharply defined seasonal cutoffs. This reproductive mode has proved advantageous to fishes, invading more temperate higher latitudes despite seasonal limitations.

Iteroparity also produces year classes of widely differing sizes thus giving offspring a much greater chance of survival in a fluctuating environment.

According to the ecological classification of reproductive styles into guilds by Balon (1975), the spawning pattern exhibited by A. johnstoni places it into the category of phytophylic non-guarding broodhiders. Whether it is an obligatory phytophil is not certain, as in the laboratory eggs are deposited on other substrates such as gravel if no plants are available.

The embryo of A. johnstoni spends its entire embryonic development in the protection of the egg casing hidden in plants and emerges as a fully functional free embryo, able to feed exogenously within a few hours. Balon's (1981) observation that, with the advancement of hiding strategies, the transition from endogenous to exogenous feeding is more sudden and the interval of mixed feeding brief or nonexistent is certainly borne out in this case. If a female topminnow produces only a few eggs per day the energy investment per egg will be better rewarded if the eggs produced are as large as possible because the resulting larger, better developed larvae will have better survival potential. Hempel (1979) attests that predation and starvation are the main causes of larval mortality. In the protected environment of vegetation beds where predation of conspecifics is a major threat, it would be advantageous to be swift and agile at hatching, able to feed efficiently and to grow to a size at which predators could be evaded efficiently.

The question about the life-history style of this species which is the most difficult to answer is where on the continuum between altricial and precocial extremes A. johnstoni fits. If one considers the suites of characters which have been ascribed to the altricial and precocial life-history styles and attempts to ascribe these to the life history style exhibited by A. johnstoni a situation, as depicted in table 3, overleaf emerges.

Altricial characters	A.j.	Precocial characters	A.j.
Many small eggs	N	Few large eggs	Y
One spawning/season	N	Many spawning/season	Y
Rapid embryonic dev.	~	Slow embryonic dev.	Y
Larva simple	N	Well developed larva	Y
No parental care	Y	Parental care	~
High larval mortality	?	Low larval mortality	?
Early sexual maturity	Y	Delayed sexual maturity	N

Table 3. A comparison of some altricial and precocial life-history characteristics and their presence (Y) or absence (N) in *A. johnstoni*. ~ indicates uncertainty and ? unknown.

The unique suite of characteristics evolved by *A. johnstoni* includes: relatively large eggs produced in small numbers at each spawning; a fractional spawning pattern over an extended period; an embryonic period which can be considered long when compared with the short life of the adult but relatively short when compared with that of some other species; a well-developed hatchling with a short period of mixed feeding; a larval period longer than the embryonic one but shorter than the juvenile period; early sexual maturity (early spawned individuals can spawn within the same season). To this suite of characters can be added a heavily vascularised yolksac but no temporary respiratory organs on the finfolds or other parts of it body.

Unlike cyprinodonts of the suborder Aplocheiloidei which includes annuals, the egg is not resistant to desiccation and there are no apparent diapause periods. If all the above mentioned facts of size, reproductive strategy and developmental style are taken together, a picture emerges of a coloniser species which occupies a transitional position in the continuum from the ancestral altricial life-history style towards a more precocial one, with the overriding influence of small size as a major constraint.

A detailed investigation of the sizes and energetic content of fish eggs and clutch sizes relative to maternal body mass could yield some valuable insights. An analysis performed by Miller (1979) of the relationship of egg to body size in cyprinids of the north temperate regions, revealed that a a greater percentage of primary reproductive effort is expended by small than by larger species per offspring.

In conclusion the reproductive and developmental style of _A. johnstoni_ can be summarised as follows. The reproductive strategy adopted is one of serial spawning, apparently over an extended period. The relatively large eggs are hidden and undergo an extended embryonic development when compared with some other members of the Cyprinodontiformes. There is no diapause period but the fully formed embryo remains in the egg envelope for several days before hatching. The larval period is longer than the embryonic period and it appears as though energy is utilised for differentiation first and then growth follows. All larvae observed remained much the same length for the early part of the larval period, only undergoing rapid growth during the latter part. Sexual maturity can be reached within five months of fertilisation.

These tactics produce a strategy of a hidden early ontogeny, spread over an extended period, which maximises the survival potential of the offspring of a preyfish in a moderately capricious environment.

From a skeptical stance at the outset of this investigation, I have come to understand the reasons which prompted Balon to suggest that ontogeny is of a saltatory nature. However, after the objections of Greenwood to the description of the entire developmental process as saltatory had been considered, a modified description of the developmental process is proposed. This suggests that development is a stepped continuum with a few saltatory episodes during its progress. The step boundaries have been examined and underlying causes to some have been suggested and consequently the boundaries assigned by other workers are questioned. Several further areas of research are suggested.

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