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Yolk Sac Morphology in Cephalopod Embryos

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12 Text-Figures

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Dottersack-Morphologie bei Cephalopoden-Embryonen

Zusammenfassung

Das Grundmuster der Cephalopoden-Embryogenese ist gleichförmig; die gruppen- und artspezifischen Eigrößen variieren dagegen von knapp 1 mm bis etwa 30 mm. Der organbildende Teil („Embryokappe", „prospektiver Embryokörper") der epibolischen Gastrula bedeckt einen größeren (in kleinen Eiern) oder kleineren Teil (in großen Eiern) der animalen Kuppe des ungefurchten Dotters. Die Peripherie der Gastrula bildet eine Hülle für den außerhalb des prospektiven Embryokörpers liegenden Dotter; dieser aus dem Gastrularand gebildete Teil gehört zum sogenannten äußeren Dottersack, der ein transitorisches Embryonalorgan ist.

Die Ausgangsform der Dottermasse zeigt gruppentypische Varianten, von nahezu kugelig bis länglich-oval. In späteren Stadien der Organogenese wird der äußere Dottersack durch Kontraktion des Kopf-Arm-Bereiches vom Embryokörper unterscheidbar; eine Ausnahme bilden gewisse Kalmare (z.B. Ommastrephidae), bei deren Embryonen der äußere Dottersack rudimentär bleibt.

Der innerhalb des Embryokörpers liegende Dotteranteil wird als Dotterhals (Verbindung zum äußeren Dottersack) und innerer Dottersack bezeichnet. Der Dotterhals wird in fortgeschrittenen Embryonalstadien zu einem einfachen Strang, der von den umgebenden Kopforganen zusammengepresst wird. Die Form, die der innere Dottersack annimmt, ist ebenfalls durch die Lage und die zunehmende Konzentration der umgebenden Organe des Eingeweidekomplexes beeinflusst. Ein Umschlagen der relativen Druckverhältnisse zwischen innerem und äußerem Dottersack scheint in späten Embryonalstadien eine sekundäre Vergrößerung des inneren Dottersackes zu unterstützen; seine endgültige Form ist aber entscheidend bestimmt durch spezifische morphogenetische Vorgänge im spätembryonalen Eingeweidekomplex. Dadurch entstehen morphologisch klar unterscheidbare Formen des inneren Dottersackes.

In funktioneller Hinsicht erinnern die Formvarianten des inneren Dottersackes an verschiedene „Strategien" der Dottereinlagerung während einer Entwicklungsphase, die zum Schlüpfen des Jungtieres hinführt. Bei allen untersuchten Cephalopoden können die frisch geschlüpften Jungtiere für eine begrenzte Zeit ohne aktive Futteraufnahme überleben. Unter normalen Bedingungen wird aber die embryonale Nährstoffreserve des inneren Dottersackes bei Jungtieren parallel zu (jedoch unabhängig von) aktiver Futteraufnahme und damit einsetzenden Verdauungsprozessen innerhalb kurzer Zeit (Tage oder Wochen) vollständig resorbiert. Diese Gleichzeitigkeit von lecithotropher und carnivorer Ernährung ist durch die morphologische und physiologische Trennung von embryonalem (Dotterorgan) und post-embryonalem Ernährungssystem ermöglicht. Die effektive Dauer dieser Koexistenz hängt von dem zu verarbeitenden Dottervolumen und der Temperatur ab.

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Abstract

The basic pattern of cephalopod embryogenesis is uniform, whereas the specific ovum sizes vary from less than 1 mm to about 30 mm. The organ-forming part (called the "embryo cap", or the "embryo proper") of the epibolic gastrula covers a larger (in small eggs) or smaller part (in large eggs) of the animal hemisphere of the uncleaved yolk mass. The rest of the gastrula forms an envelope for the portion of yolk that remains outside the embryo cap; it constitutes the so-called outer yolk sac, a transient auxiliary organ of the embryo.

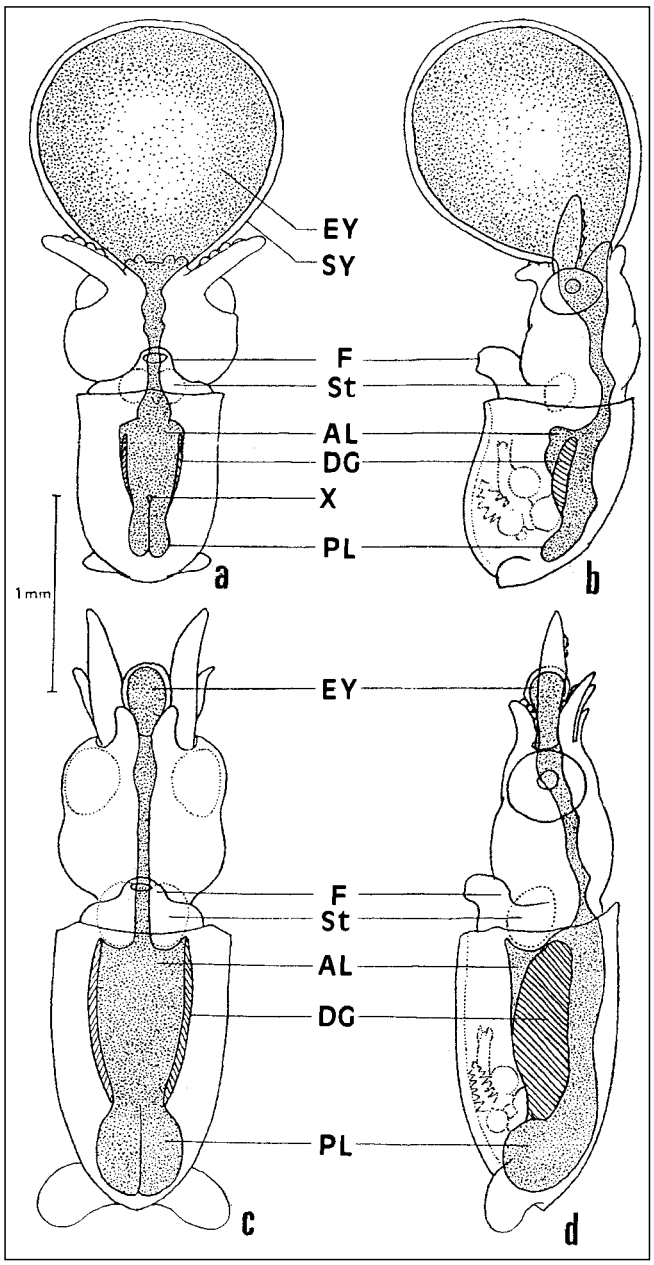
The overall shape of the yolk mass at early embryonic stages varies among systematic groups of cephalopods, from nearly globular to elongate/oval. At advanced stages of organogenesis, the outer yolk sac becomes increasingly distinct due to a constriction of the brachial and cephalic zone of the embryo proper; an exception are some teuthoid squids (e.g. the Ommastrephidae), in which the outer yolk sac remains rudimentary.

The portion of the yolk mass lying inside the embryo proper is called the inner yolk sac and yolk neck (the latter connects the inner with the outer yolk sac). At advanced embryonic stages, the yolk neck is a simple strand, which is more or less strongly compressed by the organs of the head. The inner yolk sac takes on a shape that is generated by the surrounding organs; they undergo a progressive concentration leading to a size reduction of the inner yolk sac. A shift of partial pressure between the outer and the inner yolk sac occurring at late embryonic stages appears to support a secondary enlargement of the inner yolk sac, the final shape of which is defined by special morphogenetic processes shaping the whole visceral complex of the embryo. Thus more or less distinctive morphologies of the inner yolk sac are achieved.

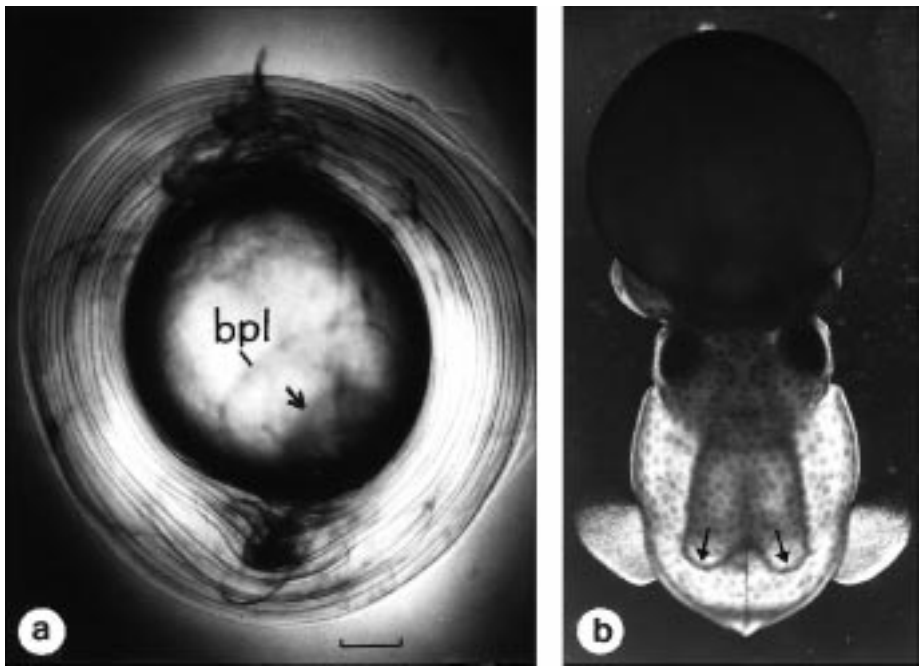
In a strictly functional perspective, the various yolk sac morphologies can be viewed as different modes of yolk storage during the developmental phase that leads to hatching of the young animal. In all the cephalopods so far studied, the newly hatched young can survive some time without foraging. Under normal conditions, however, the embryonic nutriment remaining in the inner yolk sac of the hatchling is rapidly (within days or weeks) absorbed in parallel to, but independently of digestive processes that are induced by the capture and ingestion of prey. This coexistence of lecithotrophy and active feeding is due to the morphological and physiological separation of the embryonic (yolk organ) and post-embryonic alimentary systems. The actual duration of this concomitancy is partly conditioned by the water temperature acting on the metabolism, and by the volume of the yolk reserve which in turn depends largely on the yolk storage capacity of the visceral mass.

1. Introduction

The first comparative account of yolk sac morphologies in embryos of living cephalopods was given by KÖLLIKER (1844). This author emphasized the independence of the embryonic components resulting from cellulation in relation to the uncleaved yolk mass; he described the division of the yolk mass into an outer and an inner yolk sac, and he noticed the early size reduction and the subsequent, secondary size increase and morphological differentiation of the inner yolk sac (namely the formation of posterior diverticula) at late embryonic stages. NAEF (1928) provided additional information on the embryos of several other cephalopod species, but his descriptions were mostly concerned with the surface aspect of embryos; their inner anatomy was intended to be described in a complementary volume, which unfortunately remained unfinished. However, two instructive figures prepared from histological sections were included in the published work (NAEF, 1928 Text-Figs. 42, 50). Thus, the early midgut rudiment is shown to be ring-shaped (in contrast to the erroneous description given by KORSCHELT [1892]) in Text-Fig. 42, and the subsequent closure of this rudiment around the apex of the inner yolk sac is also figured (only the position of the junction between the midgut and the ectodermic foregut is misplaced in Text-Fig. 50, as can be seen from the work of MEISTER & FIORONI [1976]). PORTMANN (1926) studied the yolk absorption in squid embryos and figured histological sections and corresponding reconstructions of the blood



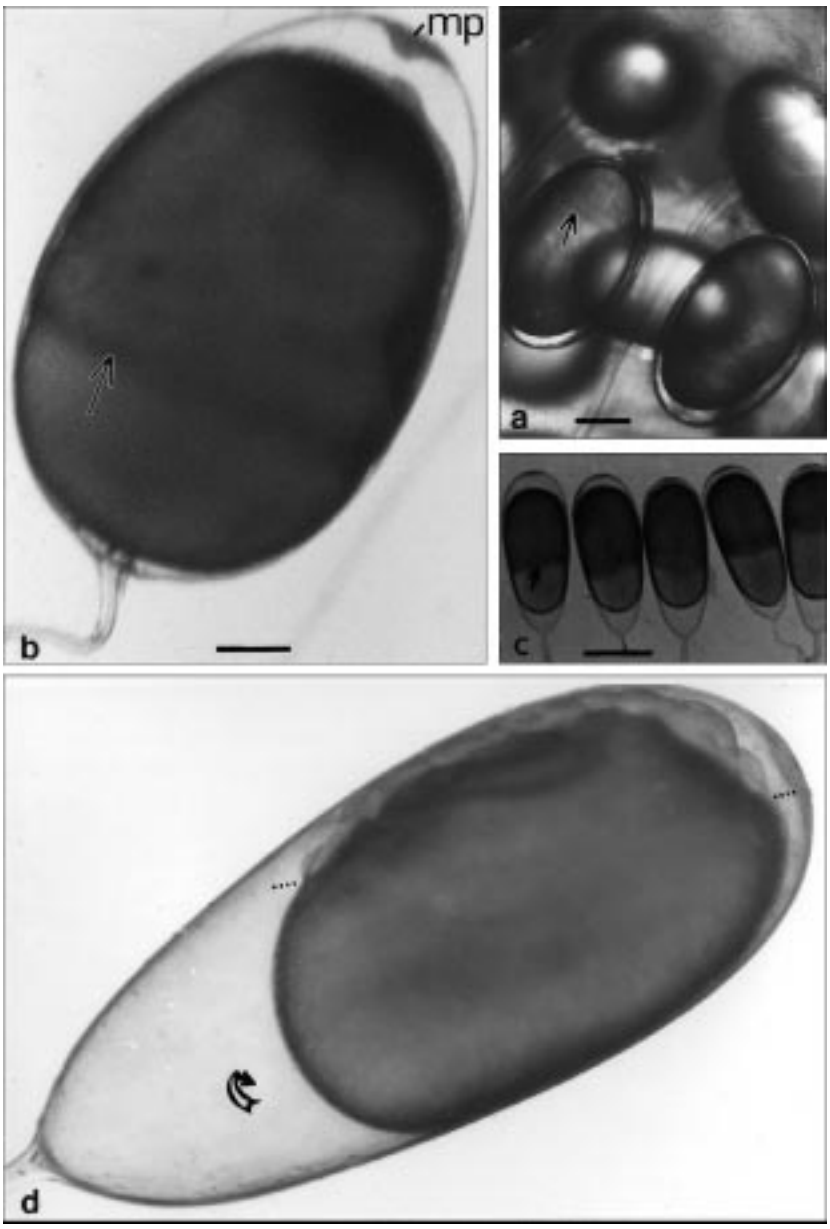
Text-Fig. 1.
Two developmental stages of the common squid *Loligo vulgaris*.
a) Dorsal view.
b) Lateral view of an embryo at stage XVI of NAEF (1928).
c) Dorsal view.
d) Lateral view of a hatchling.
The yolk mass (EY = external yolk sac, AL = anterior lobe of internal yolk sac, PL = posterior lobes of internal yolk sac) is identified by fine stippling, the diverticula of the digestive gland (DG) are marked by hatching. The main visceral organs (gills and branchial hearts, ventricle, intestine, ink sac) are indicated by dotted lines in the lateral views b and d; the fore-gut is not shown, but X marks the point where it passes, together with the cephalic aorta, between the posterior lobes of the internal yolk sac.
F = funnel; St = statocyst; SY = blood sinus of external yolk sac (modified from PORTMANN & BIDDER, [1928]).



Text-Fig. 2.
Two developmental stages of the sepiolid squid *Rossia macrosoma*.
a) Embryo at gastrular stage IV–V of NAEF (1928), tightly enclosed in the chorion which is wrapped by a spirally coiled, gelatinous envelope (the outer egg case is removed).
bpl = blastopore lip (prospective outer yolk sac envelope); small arrow points at the organ forming part of the gastrula.
b) Embryo at stage XIX–XX of NAEF, with a still very large outer yolk sac (cf. size of yolk mass in a!). The two arrows indicate the inconspicuous posterior lobes of the inner yolk sac.
Scale bar: 1 mm for a and b (modified from BOLETZKY & BOLETZKY [1973]).

spaces surrounding the yolk mass. PORTMANN & BIDDER (1928) elaborated on this study and came to the conclusion that the midgut gland ("liver") was the exclusive site of yolk absorption at late embryonic stages. Although this conclusion and some other details of functional morphology presented in the two studies (e.g. interruption of blood circulation between outer and inner yolk sac) were later found to be erroneous (BOLETZKY, 1975), the general picture they provide of yolk sac development in a myopsid squid embryo is correct. The introductory figure from the work of PORTMANN & BIDDER (1928) is therefore reproduced here (Text-Fig. 1) to illustrate the typical yolk sac development in a loliginid squid embryo approaching the stage where it normally would hatch from the egg.

In contrast to the considerable variation in yolk sac morphology observed in the late embryonic stages of (especially decabrachian) cephalopods, there is very little variation during the early embryonic stages. This may seem surprising given the enormous differences in egg sizes among



Text-Fig. 3.
Gastrular stages of
a) the veined squid *Loligo forbesii* (stage IV),
b) the pelagic octopod *Argonauta argo* (stage VI–VII),
c) the common, bottom-dwelling *Octopus vulgaris* (stage VI–VII),
d) an early post-gastrular stage of *Octopus vulgaris* (stage VIII).
Straight arrows in a–c point at the edge of the blastopore lip (prospective outer yolk sac envelope); mp = micropyle area of the chorion.
In d, the embryo is about 5 times enlarged compared to c, to show an instantaneous situation during the first reversal. The broad, curved arrow indicates the sense of rotation of the embryo inside the chorion (clockwise when seen from the micropyle at the upper right). The dotted line indicates the approximate limit between the embryo proper (above) and the outer yolk sac (below). At the end of the first reversal, the embryo proper comes to lie at the side of the chorion stalk, at the lower left (cf. Text-Fig. 6).
Scale bars: 1 mm in a and c, 0.1 mm in b.

cephalopods: indeed an ovum may measure from less than 1 mm to about 30 mm in length, depending on the species or group (NAEF, 1928; ARNOLD & CARLSON, 1986; HOCHBERG et al., 1992). Yet cleavage (blastulation) and germ layer formation (gastrulation) are very similar, in fact basically identical in terms of the overall pattern. The only major difference is that the initial cap of embryonic cells that forms the prospective "embryo proper"(during epibolic gastrulation) may cover a larger or smaller part of the animal hemisphere of the uncleaved yolk mass. Generally speaking, the relative size of this embryo cap is inversely proportional to the absolute size of the ovum: it is relatively small in the large eggs, which measure from several millimeters to a few centimeters in length (Text-Fig. 2a), whereas it is relatively large in the small eggs measuring about 1 or 2 mm in diameter (Text-Fig. 3b,c).

The periphery of the cap-like early gastrula can be viewed as a blastopore lip (Text-Fig. 2a), which rapidly broadens and grows out from the embryo proper to cover the entire surface of the yolk mass (NAEF, 1928). Its edge finally closes like an iris diaphragm over the former vegetal pole of the zygote, thus providing a cellular envelope for the uncleaved yolk mass; the latter acquires a syncytial state that persists throughout development (ARNOLD, 1971; BOLETZKY, 1988a, b). The cellular yolk envelope has a densely ciliated surface (cells of ectodermic origin) and an underlying network of muscular elements (cells of mesodermic origin). The latter generates peristaltic waves of surface contractions that drive the early blood circulation, first through the laminar space between the envelope and the syncytial yolk surface, and soon also through the developing venous system of the embryo proper (BOLETZKY, 1968). The whole complex lying outside the developing body of the animal is called the external or outer yolk sac. The connection between the outer and the inner yolk sac is maintained throughout embryonic development; in fact both the yolk syncytium and the blood spaces surrounding it remain continuous. This continuity is meaningful for the functioning of the entire complex, which is termed the "yolk organ" (NAEF, 1928).

From its initially simple (hemispherical or nearly globular) shape, the outer yolk sac may change in aspect more or less drastically during embryonic development; in certain decapods it becomes nearly triangular in outline (Text-Fig. 11b), whereas in others it remains globular (Text-Figs. 1, 2b). The outer yolk sac always remains undivided. In contrast, the yolk mass lying inside the embryo proper, which forms the so-called yolk neck and the inner yolk sac, may undergo a true subdivision. Here the variations in inner yolk sac shape are viewed from the standpoint of developmental and evolutionary morphology.

2. The Yolk Mass in Cephalopod Development

2.1. Shapes of Cephalopod Oocytes and Zygotes

The overall shape of the yolk mass at late oogenetic and early embryonic stages varies, among systematic groups, from almost globular to elongate/oval (Text-Figs. 2, 3a). The smallest cephalopod ova, which measure somewhat less than 1 mm, are nearly globular. Larger ova are more elliptical in outline, generally showing a slightly more pointed end at the animal pole. Especially in the finless octopods, the ovum is elongate, sometimes almost cylindrical (with hemispherical ends).

Whatever the specific shape of the ovum, it is defined by the envelope (the so-called chorion), which has been formed at late oogenetic stages from special secretions of the follicular cells. Inside the chorion, the yolk mass is held together by the egg cortex and its deep cytoplasmic extensions, which establish a three-dimensional network of strands traversing the whole oocyte. The convex surface thus obtained is essential for the onset of superficial cleavage at the beginning of embryonic development (ARNOLD, 1971).

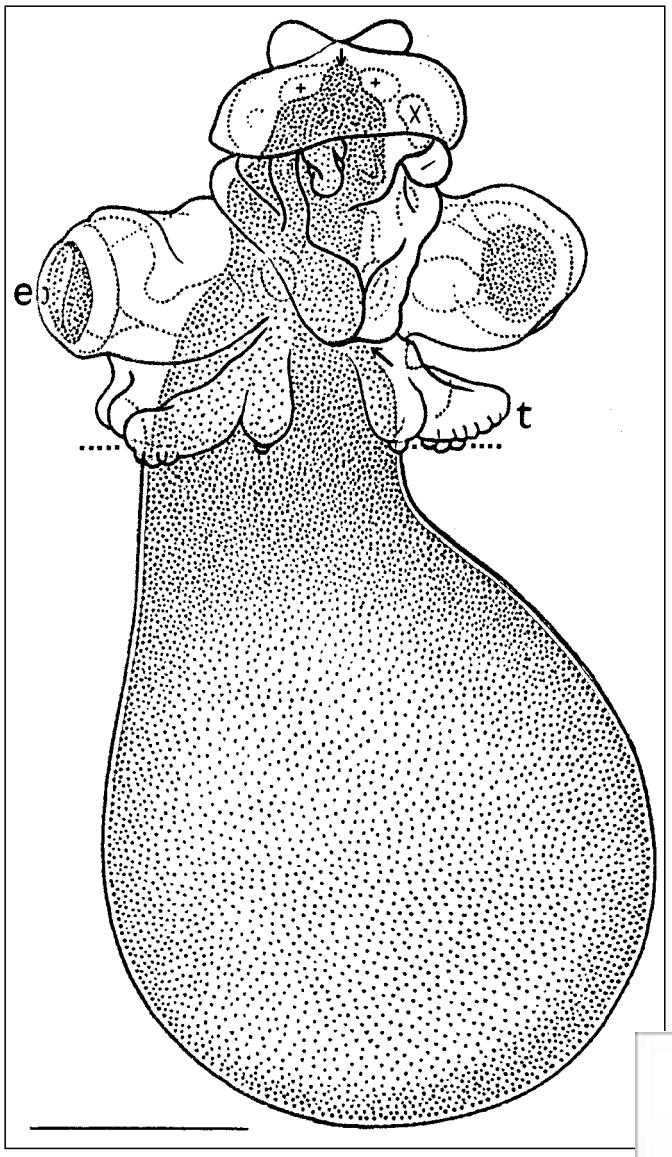
The fibrillar network of cytoplasmic strands traversing the oocyte, along with the egg cortex, may modify the shape of the yolk mass at early embryonic stages. In octopod eggs, this complex of cytoplasmic elements generates alternating torsions around the longitudinal axis of the egg (ORELLI, 1960). These slow twisting movements, which become clearly visible only in time-lapse cinematography, are recognizable from the oblique, opaque lines appearing in the yolk mass of gastrular stages (Text-Fig. 3c). Torsion ceases at the end of gastrulation when the yolk becomes covered by the cellular envelope (PAINLEVE et al., 1958). Apart from a change to a more globular shape, as observed during these early octopodan stages (especially in embryos taken from the chorion; see BOLETZKY, 1971a), no major modifications of the shape of the yolk mass occur between the end of gastrulation and the onset of organogenesis. The plasticity of the yolk mass at the end of gastrulation is recognizable during a very peculiar process that occurs in the embryos of all "incirrate" octopods except *Argonauta*. Shortly before the outer yolk sac envelope is completed, its ciliature generates a rotation of the whole embryo around the longitudinal axis of the chorion. By the time the yolk sac is completed (closure of the former blastopore lip!), the direction of the ciliary beat begins to swing around in a co-ordinated fashion, resulting in a gradual reversal, so that the effective beat of the cilia points into the opposite direction (BOLETZKY, 1971a). The whole embryo follows this change of ciliary beat direction by undergoing a progressive overall deformation, which is imposed by the narrow chorion, until the embryo cap arrives at the side of the chorion stalk (Text-Fig. 3d).

2.2. Modifications of the Original Shape of the Ovum

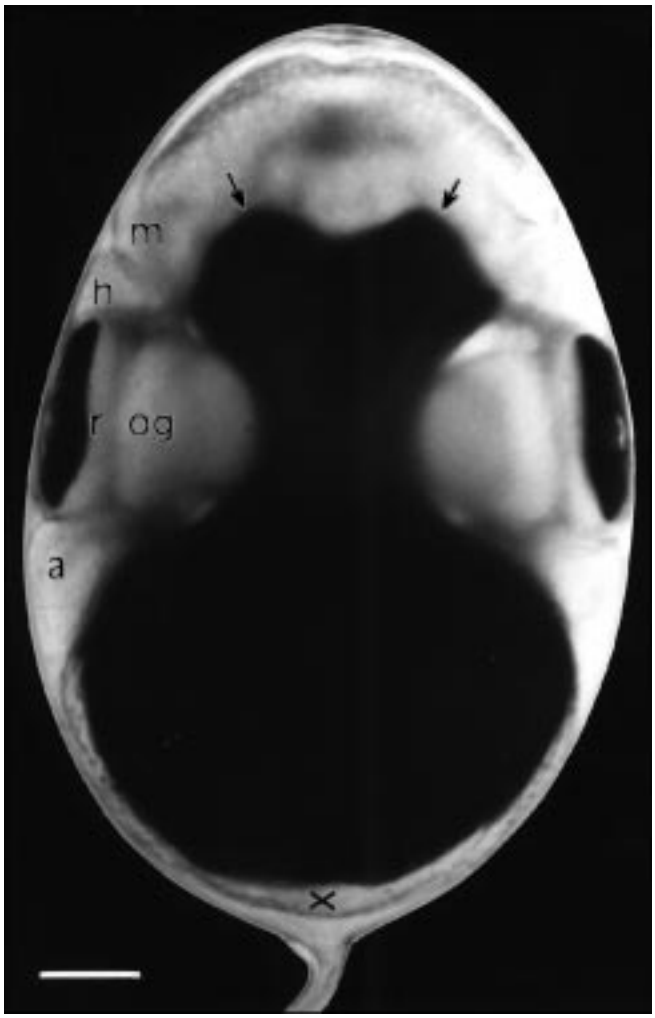
2.2.1. Formation of an Outer Yolk Sac

Following the closure of the yolk envelope, incipient differentiation of organ rudiments in the embryo cap marks the beginning of actual organogenesis. This early phase of patterning in the organogenetic zone requires (much like superficial cleavage) the presence of a convex substrate surface such as the one offered by the uncleaved yolk mass. A semi-solid substrate is indeed necessary for the establishment of embryonic cell complexes that form the early organ rudiments. Experiments altering the physical properties of that substrate show that organ rudiments can develop normally only if the embryo cap is stretched out on the convex yolk substrate (MARTHY, 1985).

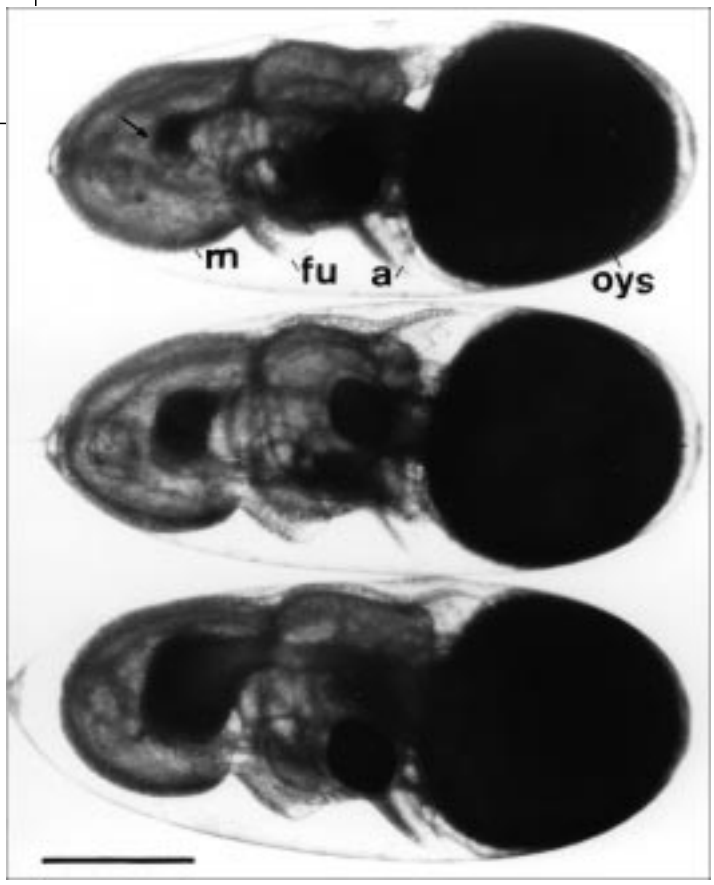
At more advanced stages of organogenesis, the outer yolk sac becomes increasingly distinct, due to the progressive contraction of the brachial and cephalic zone (Text-Figs. 4, 5, 6a). The latter constricts the yolk mass into an increasingly narrow strand (the so-called yolk neck), which connects the outer yolk sac with the rest of the yolk that lies inside the embryo proper. An exceptional



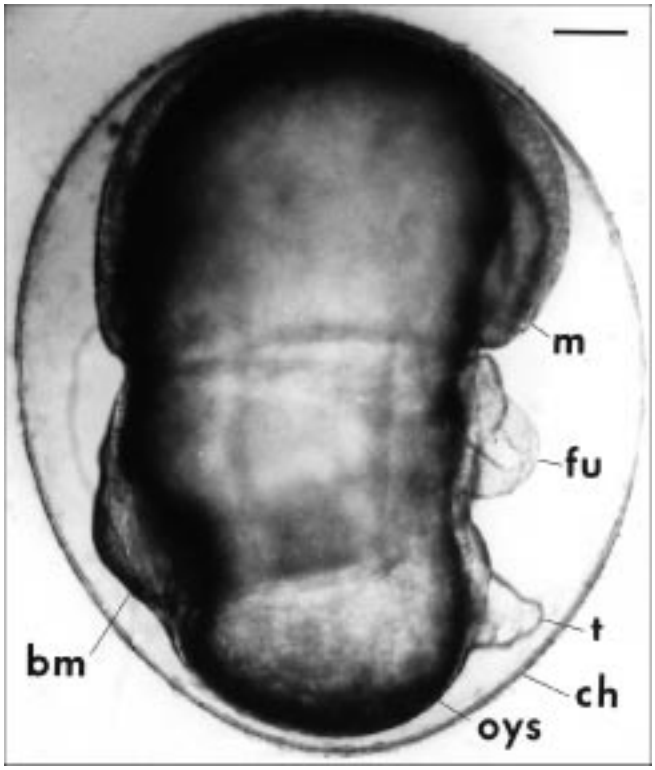
Text-Fig. 4 (upper left).
An embryo of *Loligo vulgaris* at stage XI–XII of NAEF, showing the apical "papilla" (small arrow) of the inner yolk sac, shaped by the midgut rudiment.
+ marks the posterior blood sinus; X the branchial heart lumen; the larger arrow points at the anterior end of the funnel tube, which begins to form by fusion of the medial ridges of the rudiment; the dotted line indicates the separation between the embryo proper (above) and the outer yolk sac (below); e = eye; t = tentacle.
Scale bar: 0.5 mm.



Text-Fig. 5 (upper right).
An embryo of *Argonauta argo* at stage XIII–XIV.
The arrows point at the slight elevations of the inner yolk sac on either side of the midgut complex. Note the well developed outer yolk sac (below, x indicating the blood sinus).
a = arm; h = head (posterior part, behind retina [r] and optic ganglion [og]); m = mantle (anterior part).
Scale bar: 0.1 mm.



Text-Fig. 6. ►▷►
Embryos of *Octopus vulgaris* at stages XV (above), XV–XVI (middle), and XVI (below), in lateral view.
Note the strong size increase of the inner yolk sac (arrow) from top to bottom.
a = arms; fu = funnel; m = mantle.
Scale bar: 0.5 mm (modified from BOLETZKY [1988a]).



Text-Fig. 7.
An embryo (inside its chorion membrane, ch) of the ommastrephid squid *Illex coindetii* at stage XIII of NAEF, in lateral view.
Note the rudimentary outer yolk sac (oys) which forms a dome-shaped protuberance below the buccal mass (bm) and the tentacles (t).
fu = funnel; m = mantle.
Scale bar: 0.1 mm (modified from BOLETZKY [1988b]).

able from the prospective yolk neck; its size reduction and form change is due to the pressure exerted by the surrounding organs of the visceral mass. In addition, special processes of organ formation may modify the shape of the inner yolk sac. The effect of such an organogenetic process is visible at the apex of the inner yolk mass. The contraction of the clasp-shaped midgut rudiment forms a roughly circular depression in the yolk surface (Text-Fig. 4), then squeezes out the yolk apex in the form of a thin papilla, and finally cuts the remaining apical strand when the stomach rudiment becomes a tubular structure (BOLETZKY, 1967). This process, which proceeds concomitantly with the cephalic contraction forming the yolk neck, is basically identical in all cephalopod embryos.

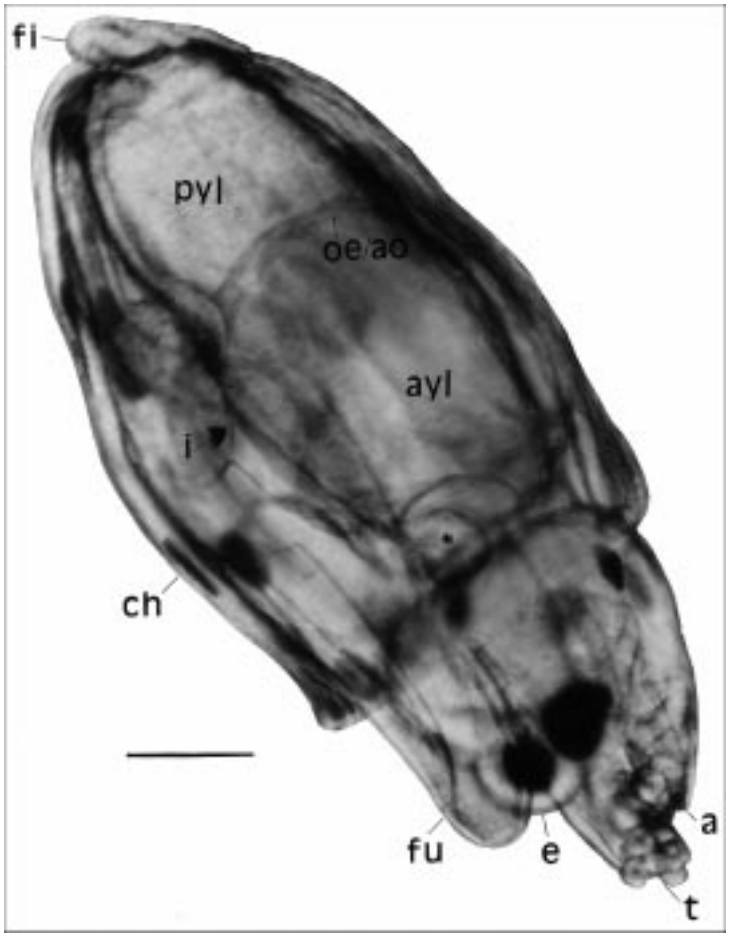
A difference between decapods and octopods exists in the position, relative to the inner yolk mass, of the paired midgut diverticula that form the digestive gland. When (around stage XII of NAEF [1928]) the stomach rudiment takes shape, the digestive gland diverticula in decapod embryos begin to extend in cephalic direction to approach a situation similar to what is shown in Text-Fig. 1. In octopods, on the other hand, the diverticula soon combine to form a solid complex, accomodating the unpaired ink sac rudiment in its middle part (BOLETZKY, 1968). Henceforward the paired origin of the digestive gland is recognizable in octopods only from the paired ducts connecting it to the caecum (like in decapod juveniles after disappearance of the inner yolk sac).

The overall contraction of the embryo cap continues to constrict the inner yolk mass, which tends to become ever smaller up to stages XIII, XIV or XV of NAEF (1928). In decapod embryos, the posterior end of the inner yolk sac begins to extend caudally from stage XIII onward, forming

situation exists in some teuthoid squids such as the Ommastrephidae, which produce very small eggs. In the embryos of these squids the outer yolk sac is rudimentary and forms an inconspicuous dome in the center of the arm crown (Text-Fig. 7). At later embryonic stages, the outer yolk sac rudiment disappears completely (Text-Fig. 8). Apart from this minor modification in the general pattern of cephalopod embryogenesis, a distinct outer yolk sac is formed in all groups of cephalopods, even in the very small embryos of *Argonauta* (Text-Fig. 5) and of *Idiosepius pygmaeus* (NATSUKARI, 1970). Starting out from a subglobular form, which may be conserved throughout development, the outer yolk sac can become pear-shaped (Text-Figs. 4,10) or roughly triangular (Text-Fig. 11a,b).

2.2.2. Differentiation of an Inner Yolk Sac

The portion of the yolk mass that lies inside the embryo proper is called the inner yolk sac and yolk neck (see above). Since the yolk neck is increasingly compressed by the organs of the head, it undergoes some changes in outline (Text-Figs. 4, 9, 11). At first the inner yolk sac is not easily distinguish-



Text-Fig. 8.
A hatchling of *Illex coindetii* in lateral view.
Note the large inner yolk sac with an anterior yolk lobe (ayl) and the two posterior yolk lobes (pyl) behind the passage of the oesophagus and the anterior aorta (oe/ao).
a = arm; ch = chromatophore; e = eye; f = fin; i = ink sac; t = tentacle; * = statocyst.
Scale bar: 0.3 mm (modified from BOLETZKY [1974]).

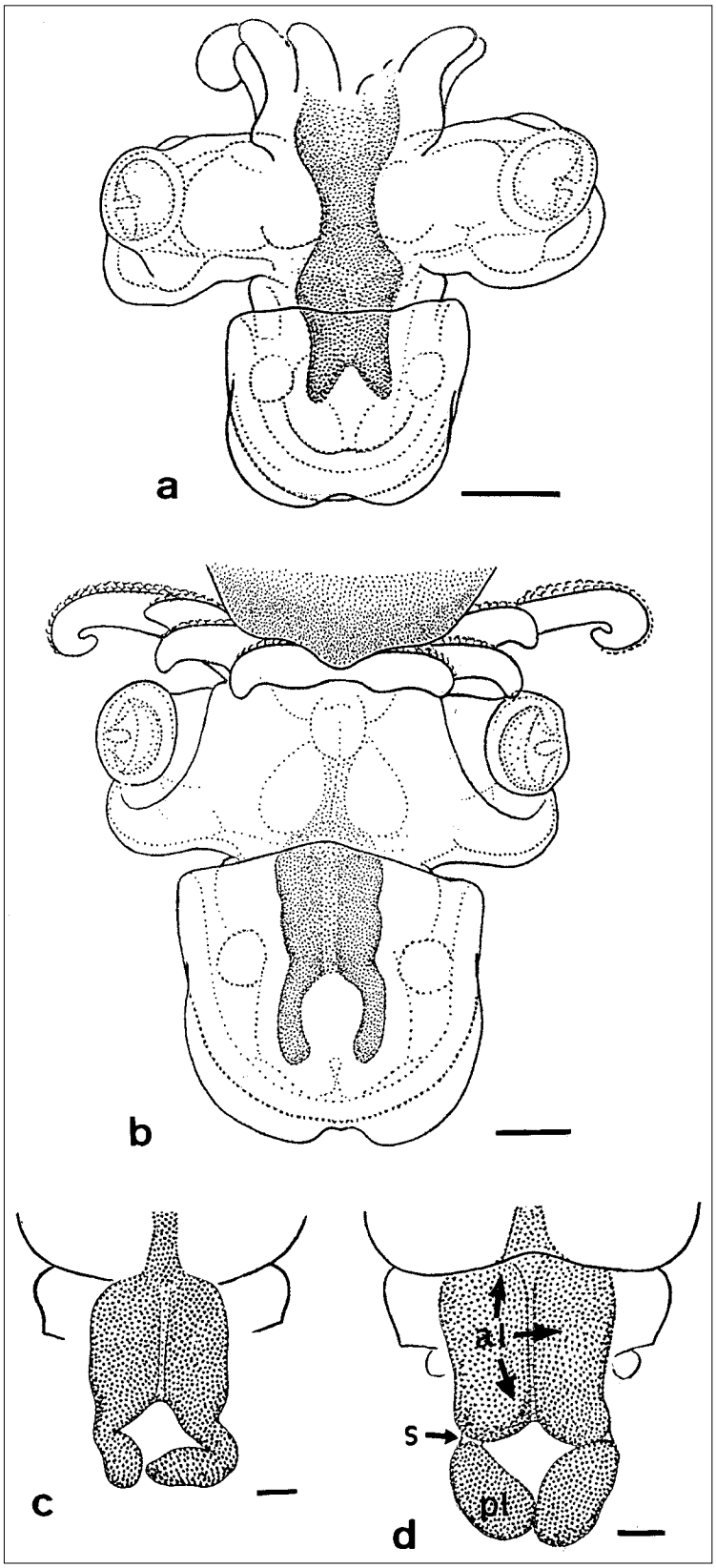
two finger-like processes on either side of the stomach (i.e. parallel to the line where the central strand of yolk has disappeared when the stomach was completed!). These caudal yolk diverticula are called the "posterior lobes" of the inner yolk sac, while the unpaired mass lying before the midgut complex is called the "anterior lobe" of the inner yolk sac. Posterior diverticula are never formed in octopod embryos (Text-Figs. 5, 6).

At later embryonic stages, the further differentiation of the visceral mass apparently tips the pressure balance between the outer and the inner yolk sac, so that yolk from the outer sac "flows" into the inner sac (MARTHY, unpubl. time-lapse film [1976]). In octopod embryos, the resulting size increase of the inner yolk sac is very distinct (Text-Fig. 6), but in comparison to that observed in decapod embryos, the storage capacity of the inner yolk sac of octopod embryos seems more limited. The posterior yolk sac lobes formed by the decapod embryos provide some additional storage volume (Figs. 8–12).

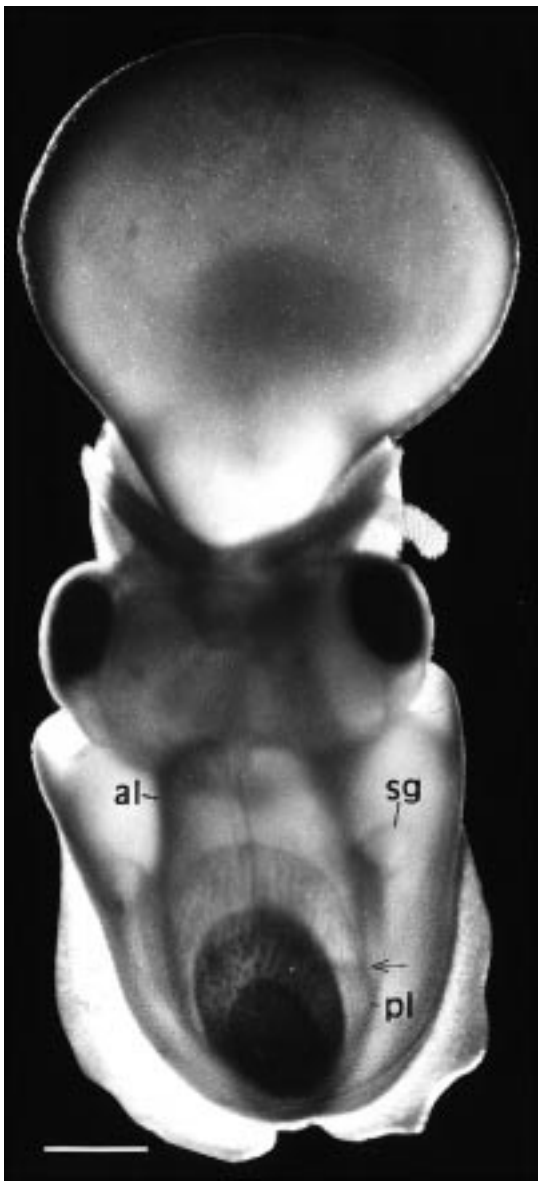
In some taxa, more or less distinctive morphologies of the inner yolk sac can be recognized. In *Sepia* embryos the posterior diverticula corresponding to those of squid embryos become subdivided, each one forming a distinct terminal lobe by a true segmentation of the yolk mass in each posterior lobe (Text-Figs. 9, 10). A completely different, peculiar "four finger" pattern is observed in certain sepiolid embryos; it is characterized by the formation of two lateral lobes in addition to the (unsegmented) posterior lobes. But this pattern exists only in the embryos of species belonging to the sub-family Sepiolinae (Text-Figs. 11, 12c), whereas in embryos of Rossiinae and Heteroteuthinae only the two admedian diverticula corresponding to the well-developed posterior lobes of teuthoid squid embryos are recognizable; in *Rossia* they are very small (Text-Figs. 2b, 12a,b), in contrast to the large diverticula developed in *Heteroteuthis* (BOLETZKY, 1978).

3. Functional Aspects

In a strictly functional perspective, the various yolk sac morphologies can be viewed as different modes of yolk storage during the developmental phase that leads to hatching. During this phase, of course yolk continues to be used to fuel both the embryonic metabolism and the growth and differentiation of tissues. Since the blood lacuna surrounding the yolk syncytium in the outer yolk sac remains connected to the circulatory system of the growing embryo, there is no immediate functional need for yolk transfer to the inner yolk sac prior to hatching (see further below on *Eledone moschata*). The generalized yolk transfer from the outer to the inner yolk sac is likely to serve a function related to post-hatching life. As far as is known to date, newly hatched cephalopods can survive some time without food (from a few days to several weeks, depending on the juvenile physiology and ecology). Under normal conditions, the embryonic nutriment remaining in the inner yolk sac of the hatchling is absorbed independ-



Text-Fig. 9.
Embryonic stages XIII (a), XV (b), XVII–XVIII (c), XVIII (d) of the common cuttlefish *Sepia officinalis*, in dorsal view.
The outer yolk sac is not shown (only its base is visible in b). Note the continuous size increase and change in shape of the inner yolk sac, with development of slender posterior lobes from a to b. The situation of the inner yolk sac after removal of the mantle and visceral organs is shown in c and d, stage XVII–XVIII with the onset of "segmentation" in the posterior lobes, stage XVIII with distinct posterior lobes (pl) that may now be called terminal lobes; they remain connected to the anterior lobe (al) solely by the blood sinus (s).
Scale bar 0.5 mm.



Text-Fig. 10.
An embryo of *Sepia officinalis* at stage XVIII of NAEF, in dorsal view.
The outer yolk sac (above) is still very large. The inner yolk sac has a fair-sized anterior lobe (al) and the two distinct posterior lobes (pl) shown in Text-Fig. 9d. These posterior lobes are partly hidden by the two first chambers of the cuttlebone, but the separation between the anterior lobe and the right posterior lobe is visible (arrow).
sg = stellate ganglion.
Scale bar: 1 mm.

ently of the onset of the digestive processes, which are induced by capture and ingestion of prey. This coexistence of lecithotrophy and active feeding is made possible by the physiological independence of the embryonic and post-embryonic alimentary organs. As long as some yolk is left, the syncytium continues to release the products of "yolk digestion" into the blood stream, no matter whether or not active foraging and digestion of food has started.

The duration of the overlap between embryonic and post-embryonic energy supply is conditioned by the environmental temperature which acts on the whole metabolism, yolk absorption included, and by the volume of the yolk reserve that remains to be absorbed, which in turn depends on the yolk storage capacity of the visceral mass prior to hatching. Given the greater storage capacity of the inner yolk sac in decapod embryos, one might expect a longer co-existence of the two modes of energy supply in comparison to octopod embryos. Does this mean that decapod hatchlings may survive in the absence of prey much longer than octopod hatchlings? In approaching this question, one has to consider – in addition to the physiological factors related to water temperature and its influence on developmental rates – the extent to which the juvenile animal can use body tissues as an energy reserve (e.g. lipids stored in the digestive gland, or muscle proteins from which amino acids can be remobilized).

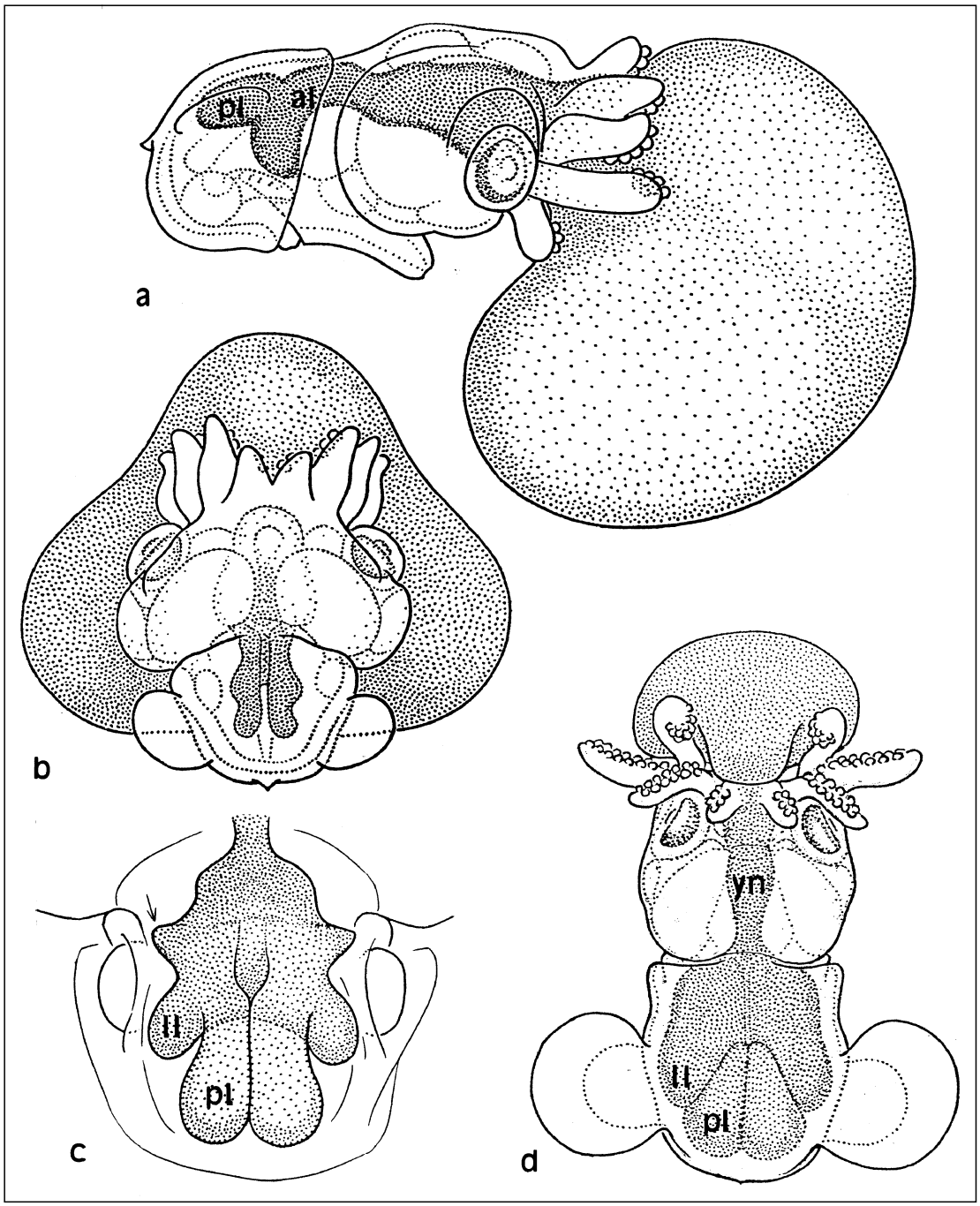
Two interesting examples may be mentioned here. One is the condition of the hatchling of the Mediterranean lesser octopus *Eledone moschata*, in which the inner yolk sac is completely emptied at late embryonic stages (much like in *Octopus maya*, cf. BOLETZKY, 1975, FIORONI & BOLETZKY, 1990). Thus the (benthic) young animal is devoid of a yolk reserve, yet it is able to survive for up to three weeks without food even at relatively high temperatures. On the other hand, *Rossia macrosoma* can survive without food for nearly three months if kept at a temperature of only 8–9°C (BOLETZKY, 1994). In contrast to *Eledone moschata*, *Rossia* hatchlings have a large yolk reserve, but in addition to this energy source, other materials were mobilized for survival under starving conditions, as indicated by the emaciated aspect of the muscular tissue, especially of the mantle (BOLETZKY, unpubl. observations). In both instances, however, survival without food intake is made possible by the very economic life style of the benthic hatchlings. Rearing experiments indeed suggest that a planktonic/micronektonic life style does not permit survival for more than a few days without foraging (BOLETZKY & HANLON, 1983).

4. Evolutionary and Phylogenetic Aspects

At the very end of yolk absorption, which normally terminates during the earliest juvenile stage, the definitive "disappearance" of the inner yolk sac reveals the fact that the yolk syncytium is a transient element of the venous part of the circulatory system (BOLETZKY, 1975). It is eliminated, much like a dissolving thrombus, from the lumen of the hepatic vein and adjacent vessels. Its positional relationship with the digestive gland once seemed to support the hypothesis of PORTMANN & BIDDER (1929), suggesting a close functional relation between the yolk syncytium and the digestive gland. The inner yolk sinus, which at later stages forms an elaborate network of yolk vessels surrounding the large surface of the inner yolk sac (Text-Fig. 12), is indeed an important part of the circulatory system of the visceral mass; however, its close anatomical association with the digestive gland does not reflect a special physiological connection. But in terms of evolutionary origin and phylogenetic relationships, this anatomical link could be a useful guide for attempts to reconstruct the relevant parts of fossil forms.

There may be also anatomical relationships between the embryonic anatomy of the visceral mass and adjacent parts of the body, especially the shell complex. Thus one may view the posterior lobe "segmentation" of the inner yolk sac in *Sepia* embryos as possibly related to the strongly modified shell (the rather flat cuttlebone). Whether this view is correct may become clear once the embryonic development of *Spirula* is known; in this peculiar decapod, the posterior lobes of the digestive gland occupy the small, hemispherical "living chamber" of the shell (CHUN, 1910). If *Spirula* embryos turn out to have subdivided posterior yolk lobes, such a "segmentation" would no longer appear as specially related to the particular situation seen in the *Sepia* shell. If, on the contrary, *Spirula* embryos do not show the posterior lobe segmentation, the idea of a correlation between such a segmentation and the typical cuttlebone shape could be pursued further.

A related question is whether the formation of two normal posterior lobes in the inner yolk sac is somehow related to an evolutionary

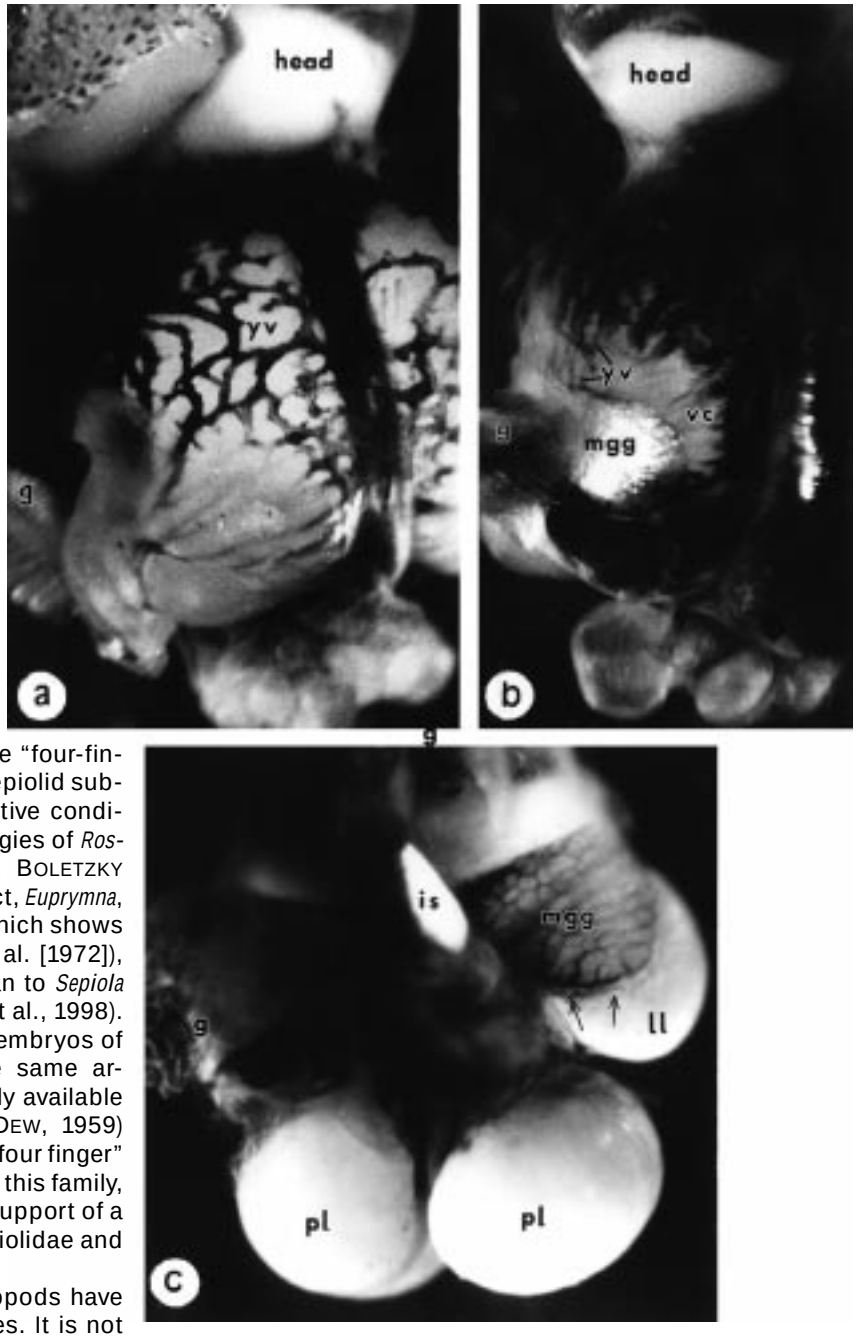


Text-Fig. 11.
Embryos of *Sepiola* sp. Stages XIV (a: lateral view, b: dorsal view), XV (c: dorsal view of partly dissected mantle complex), XIX (d: dorsal view), at different magnifications (overall lengths of embryos 3–5 mm). Note the well developed posterior lobes (pl) and the ventral extension of the anterior lobe (al) of the inner yolk sac at stage XIV (a, b). At stage XV (c), the posterior lobes (pl) are further enlarged and become adjacent to each other above the passage of the oesophagus and anterior aorta (cf. Text-Figs. 1, 8) while the ventral extension of the anterior lobe is drawn out laterally to form two lateral lobes (ll). In contrast to these "new" lobes, the lateral extensions (arrow) in the anterior part of the inner yolk sac are transient. At stage XIX (d), the outer yolk sac has grown small due to yolk absorption and to the continued yolk transfer, via the yolk neck (yn) to the inner yolk sac. The lateral lobes (ll) of the inner yolk sac are comparable in size to the posterior lobes (pl). The bulging dorsal surfaces of the latter (cf. c) simulate a separation from the anterior lobe; there is no yolk segmentation like that observed in *Sepia* (Text-Fig. 10).

trend toward mantle elongation and concomitant "stretching" of the digestive gland complex. Conversely, one may ask whether the absence of posterior lobe formation in the embryos of both finned and finless octopods (BOLETZKY, 1978–79) reflects a morphogenetic "abbreviation" related to a general paedomorphic trend (as suggested by other developmental truncations in octopod embryogenesis). For all these questions, at least partial answers may be found through careful comparison of the whole morphogenetic networks that are responsible for the shaping of a viable embryo, from which a viable juvenile and adult animal can develop.

The wide variety of yolk storage modes that exist in living cephalopods does not tell us anything about greater or lesser "evolutionary success". At most one may surmise that total absence of yolk storage before hatching (as observed in the relatively large, bottom-living young of *Eledone moschata* and *Octopus maya*) would be counterselective for a planktonic post-hatching life style. A truly quantitative assessment of the varying modes of yolk storage is particularly difficult in comparative considerations, because cephalopods have no well-defined hatching stage. There is a phase of "hatching competence" (BOLETZKY, 1994) that ranges, from late embryonic stages (before complete absorption of the outer yolk sac) which permit markedly premature hatching, to virtually juvenile conditions in strongly delayed hatching (after absorption of the greater part of the inner yolk reserve). In general the former is related to high and the latter to low temperatures (within the range of the temperature adaptation of the species considered).

From what is known about yolk sac morphologies in decapods, one may conclude that formation of one pair of posterior lobes in the inner yolk sac is a generalized pattern (expressed in decapod embryos of all sizes, including those forming no distinct outer yolk sac). Subdivision of



Text-Fig. 12.
Hatchlings of *Rossia macrosoma* (a, b) and *Sepietta obscura* (c), dissected after injection of the circulatory system with a carbon suspension.
a) Dorsolateral view of visceral mass and the posterior head surface; the mesenteric sinus (cf. WINKLER, 1915) forms a broad "vessel" between the head (above) and the visceral organs (below). Note the network of yolk vessels (yv) covering the inner yolk sac.
b) The same preparation is presented in a ventrolateral view, showing yolk vessels connected to the midgut gland (mgg) and the cephalic vein (vc) hiding the mid-ventral part of the yolk vessels.
c) The posterior lobes (pl) and the left lateral lobe (ll) with the adhering midgut gland lobe (mgg) are seen from the ventral side. In this preparation, the injected carbon suspension is visible in the larger vessels and in the midgut gland and its immediate vicinity (arrows), but not in the bulging terminal parts of the posterior and lateral lobes, where the yolk sinus is strongly compressed.
g = gill, is = ink sac.

these lobes (i.e. formation of distinct "terminal" lobes) might be a group-specific pattern of sepiid cuttlefishes, unless the embryonic development of *Spirula* will tell us a different story. Likewise, the "four-finger" pattern observed in embryos of the sepiolid subfamily Sepiolinae might represent a primitive condition, although the inner yolk sac morphologies of *Rossia* and *Heteroteuthis* (no lateral lobes; cf. BOLETZKY [1978]) seem to suggest the opposite. In fact, *Euprymna*, an ostensible member of the Sepiolinae which shows the typical four-finger pattern (ARNOLD et al. [1972]), appears more closely related to *Rossia* than to *Sepiola* according to molecular data (NISHIGUCHI et al., 1998). This also raises the question whether the embryos of (at least some) Sepiadariidae show the same arrangement as *Sepiola* and *Euprymna*. The only available description of a sepiadariid hatchling (DEW, 1959) does not mention its inner anatomy. If the "four finger" pattern of the inner yolk sac existed also in this family, it could be viewed as a synapomorphy in support of a "bobtail" monophylum containing the Sepiolidae and the Sepiadariidae (BOLETZKY, 1995).

The embryos of finned and finless octopods have an inner yolk sac devoid of posterior lobes. It is not yet known whether this condition is established in their likely sister group, the Vampyromorpha. Whatever the outcome of future studies on the embryonic development of *Vampyroteuthis*, the shape of the inner yolk sac of octopods surely reflects a secondary simplification and thus represents a homoplasy in comparison with the situation observed in *Nautilus* embryos (TANABE et al. [1991]).

5. Concluding Remarks

So far no indication is available about yolk storage modes in the embryos of fossil cephalopods. Both belemnites and ammonites must have produced rather small eggs, and their extinction at the end of the Cretaceous period may have been related to environmental conditions that were unfavourable for the small, likely planktonic hatchlings. No matter whether these hatchlings had a large yolk reserve (like extant ommastrephid hatchlings) or a small yolk reserve (like the equally small *Argonauta* hatchlings), that reserve may have been insufficient to carry them through a juvenile period marked by food limitations in terms of available living zooplankton of appropriate size.

Conversely, it seems likely that rather large hatchlings either survived as scavengers, or they were able to catch (either immediately after hatching or after some post-hatching growth based on yolk absorption) benthic or demersal prey, probably deposit feeders.

The variety of yolk sac morphologies observed in the embryos and hatchlings of living cephalopods is ontogenetically derived from a uniform pattern of early embryogenesis. There is no reason to doubt that this pattern was already typical for the earliest Cephalopoda. The subsequent diversification in the shaping of yolk organs during advanced stages of embryonic development should be viewed in relation to the differentiation of the circulatory system of the visceral mass.

A detailed morphological analysis, especially of the venous system of living cephalopods, may provide some

hints as to past developmental patterns that cannot be reconstructed from the available embryological data.

In contrast to the "delayed" results of a lengthy, rather tedious work of dissecting, histological treatment and anatomical reconstruction of specimens, necessary to carry out a morphological analysis, immediate information may be gained by watching live cephalopod embryos under a dissecting microscope. Moreover, results of reconstructions should always be checked by live observations to avoid the pitfalls of artifacts due to shrinkage during fixation etc. All the figures shown here were prepared from living embryos or supra-vital preparations (Text-Fig. 12). Ink injection of the circulatory system is a simple method requiring very little preparation; embryos are taken from their capsules, anaesthetized with 1–2 % ethanol in sea water, injected with india ink or medical carbon suspension, and partly dissected to expose deeper parts of the body if necessary (cf. BOLETZKY, 1968). After observation, the specimens are fixed in Bouin's solution or 4 % formalin, and later preserved in 70 % ethanol. In such supra-vital preparations, the outer and/or the inner yolk sac may be punctured to permit better ink filling of yolk vessels than what is shown in Text-Fig. 12, but such highly artificial conditions may also produce misleading pictures (the blood sinus and vessels surrounding the inner yolk sac normally are strongly compressed, laminar spaces!). Good photographic documents available in publications may also allow readers to pick up the relevant information from an illustration. For example, the morphology of the inner yolk sac with its two posterior lobes in hatchlings of *Idiosepius pygmaeus* is clearly recognizable in a paper by NATSUKARI (1970, Text-Figs. 31–33).

Yolk sac morphologies are variable at the level of individual development, and thus are somewhat elusive expressions of morphogenetic programs in normal cephalopod embryogenesis. Under altered developmental conditions, the minor variations normally observed among individual embryos become more conspicuous and may thus be sensitive indicators of environmental stress (BOLETZKY, 1971b). This offers new perspectives for experimental work dealing with limiting conditions in terms of reversible versus irreversible perturbations of embryonic development (MARTHY, 1978–79). Ultimately such analyses may be of interest to cephalopod paleobiologists investigating conditions related to past extinction events.

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