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Functional Morphology of Cephalopods

Edited by S. v. Boletzky, P. Fioroni and A. Guerra

In memoriam
Adolf PORTMANN (1897-1982)
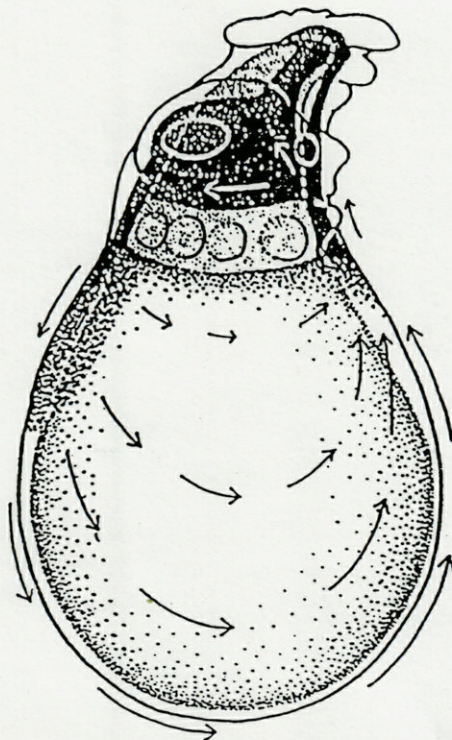
PREFACE

The second international symposium on FUNCTIONAL MORPHOLOGY OF CEPHALOPODS was held in Vigo (Spain) from 6 to 8 September 1995. It was one of five symposia taking place during the Twelfth International Malacological Congress (3-8 September), which was organized by Angel Guerra and Francisco Rocha of the Instituto de Investigaciones Marinas (IIM) on behalf of Unitas Malacologica and under the auspices of the Consejo Superior de Investigaciones Científicas (CSIC), the Sociedad Española de Malacología (SEM) and the Cephalopod International Advisory Council (CIAC). Symposia, workshops and free lectures covering a wide range of subject areas in malacology were held at the Cultural Center of Caixavigo and the adjacent Casa das Artes do Concello de Vigo. This congress was sponsored by governmental and public institutions and by private companies.

Of the thirty papers and posters presented at our symposium*, twenty one contributions are published in this volume, either as full papers or as extended summaries or abstracts (including one on octopod classification, *cf.* Abstracts * p. 367), and a Commentary by Michael Vecchione (who kindly accepted at short notice to take the place of Clyde Roper, the invited rapporteur who was prevented from coming).

Functional morphology has many facets, ranging from biophysical to ecological aspects, that are considered in an evolutionary perspective. The symposium contributions presented here deal with questions that somehow relate to adaptation and the role of environmental conditions in a given evolutionary context. Thus we are not overstraining the scope of functional morphology in publishing these papers in Vie et Milieu/*Life and Environment*, a journal devoted to general ecology and evolutionary biology.

Long-standing commitments of the journal made impossible appearance of the symposium volume before spring 1997. In return, the final publication date coincides with the hundredth anniversary of the birth of Adolf Portmann (1897-1982), zoology professor at the university of Basle from 1928 to 1970. We dedicate this volume to his memory, in grateful appreciation of the indefatigable support he gave to cephalopod research over more than one half century.

Adolf Portmann was a biologist with a keen interest in plant and animal morphology. Although much of his own work dealt with "form *beyond* function", it always built on comparative morphology viewed in functional context. Portmann was particularly fascinated by the functional aspects of developing organ systems. A fine example is his classical work on the embryonic development of the circulatory system of *Loligo vulgaris* (1926)**. In the introductory section of this study he stated (p. 406) : "If one considers the developmental stages solely as avenues leading to a goal, as inevitable transition grades before the adult stage of the animal, one tends to overlook that at any moment of development the embryo or larva is a living whole whose organs must stay in close functional relation, no matter what the final form eventually achieved." This reminder is still timely today.

There are some other, more personal reasons for our dedication : two of the three undersigned symposium convenors and volume editors were students of Adolf Portmann in Basle and in Banyuls; the Arago Laboratory was almost a second home to Portmann; and the journal Vie et Milieu/*Life and Environment* became a favourite publication platform for him and his collaborators ever since it was created in 1950.

We should like to thank the Council of Unitas Malacologica (1992-1995) and the Consejo Superior de Investigaciones Cientìficas (Madrid, Spain) for a generous grant in support of this publication. Special thanks are due to all the members of the local organising committee in Vigo, especially Ricardo Pérez Martín, Luis Ansorena, María Teresa Fernández and their colleagues from the Cephalopod Ecophysiology group at IIM, Emilio Rolán and his colleagues from SEM, and all the committee members from the universities of Vigo and Santiago de Compostela.

As invited volume editors, we gratefully acknowledge the excellent co-operation of Dr. Nicole Coineau, Managing Editor of Vie et Milieu, and of her secretarial assistants. And – last but not least – we thank all the authors who accepted to bear with our time-table of manuscript processing.

Sigurd v. BOLETZKY (Banyuls),
Pio FIORONI (Münster),
Angel GUERRA (Vigo)

* Abst. 12th Intern. Malacol. Congr., Vigo, 1995, A. Guerra, E. Rolàn & F. Rocha, eds : 59-93, 533.
** Portmann, A., 1926. – Der embryonale Blutkreislauf und die Dotterresorption bei *Loligo vulgaris*. *Z. Morph. Oekol. Tiere* **5** : 406-423.

The cover picture is adapted from a figure in Portmann (1926)

IN SITU OBSERVATIONS TEST HYPOTHESES OF FUNCTIONAL MORPHOLOGY IN *MASTIGOTEUTHIS* (CEPHALOPODA, OEGOPSIDA)

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CEPHALOPODS
SQUID
SUBMERSIBLE
DEEP SEA
BEHAVIOR
FUNCTIONAL MORPHOLOGY

ABSTRACT. – *Mastigoteuthis magna* observed *in situ* has a characteristic "tuning fork" posture that can be used as an aid for identification, even for very small squids. Observations of living *Mastigoteuthis* from submersibles in the western Atlantic Ocean enabled us to test formerly proposed hypotheses concerning functional morphology of these deep sea squids. Hypotheses supported by observations on live animals include : 1) *The large fins provide propulsion* ; simple and double sinusoidal waves move anteriorly or posteriorly, fins flap powerfully, fins roll together ventrally to squeeze water out anteriorly or posteriorly. 2) *Tentacular suckers have weak release mechanism* ; tentacular clubs on live animals feel very sticky, like fly paper, and must be firmly pulled off observer's skin and aquarium wall. 3) *Tentacular suckers have sensory function* ; tentacular tips skim along bottom to allow animal to maintain position in food-rich benthic boundary layer. Observations do not support these hypotheses : 1) *Vacuolated arms and head induce head-upwards posture* ; observations confirm a head-downwards posture maintained by constantly maneuvering fins. 2) *Ventral arms lock together to form "gutter" for feeding* ; live animals hold long ventral arms far apart with proximal part of tentacles enveloped in tentacular sheaths, allowing them to serve as non-tangling trolling lines fishing for minute prey. 3) *Well developed eyes and ink sac indicate photic zone habitat* ; all observations of *Mastigoteuthis* from submersibles are close to the bottom below 500 m, never in photic zone. We suggest *Mastigoteuthis* evolved from a *Chiroteuthis*-like ancestor ; its adaptive characters enable it to inhabit the unique trophic zone immediately above the deep sea bottom, feeding on small zooplankters with trolled, non-tangling tentacles.

CÉPHALOPODES
CALMAR
SUBMERSIBLE
EAUX PROFONDES
COMPORTEMENT
MORPHOLOGIE FONCTIONNELLE

RÉSUMÉ. – *Mastigoteuthis magna* se présente *in situ* en posture de "diapason" caractéristique permettant l'identification de l'espèce, même lorsqu'il s'agit d'individus de petite taille. L'observation de *Mastigoteuthis* à partir d'un submersible mis en action dans l'Atlantique occidental a permis de tester certaines hypothèses avancées précédemment, relatives à la morphologie fonctionnelle de ces Calmars des eaux profondes. Les hypothèses confortées par nos observations sont : 1) Les très grandes nageoires servent à la propulsion : des ondes sinusoïdales simples et doubles se propagent en direction antérieure ou postérieure, les nageoires battent vigoureusement, et s'enroulent du côté ventral en chassant l'eau vers l'avant ou vers l'arrière. 2) Les ventouses des tentacules ont un faible mécanisme de relaxation : les masses tentaculaires d'individus vivants sont très adhésives, comme du papier collant ("bandes à mouches"), et résistent fortement à toute traction exercée pour les détacher de la peau d'un observateur ou de la paroi d'un aquarium. 3) Les ventouses tentaculaires ont une fonction sensorielle : les extrémités des tentacules sont traînées au contact du fond permettant ainsi à l'animal de se maintenir dans la couche limite du benthos, riche en nourriture. Les observations ne confortent pas les hypothèses suivantes : 1) Les bras et la tête contenant des vacuoles causent l'orientation de la tête vers le haut : les observations mettent en évidence une position dans laquelle la tête est orientée vers le bas, maintenue ainsi par le mouvement continu des nageoires. 2) Les bras ventraux sont réunis pour former une "gouttière" canalisant la nourriture vers la bouche : les animaux vivants tiennent leurs bras ventraux écartés l'un de l'autre, les parties proximales des tentacules étant enveloppées dans des gaines tentaculaires, de sorte que les tentacules servent de lignes de pêche traînées pour

la capture de proies minuscules. 3) Les yeux et la poche d'encre bien développés témoignent d'un habitat en zone euphotique : toutes les observations sur *Mastigoteuthis* ont été faites près du fond, à des profondeurs dépassant 500 m, donc en aucun cas dans la zone euphotique. Nous pensons que *Mastigoteuthis* s'est développé à partir d'un ancêtre de type *Chiroteuthis* ; ses caractéristiques adaptatives lui permettent de vivre dans la zone trophique très particulière qui se trouve juste au-dessus du substrat des grands fonds, et de consommer du zooplancton de petite taille à l'aide de tentacules séparés et traînés.

INTRODUCTION

Direct observations on the functional morphology of deepsea cephalopods always have been hampered by the difficulty of access to animals that are functioning normally. As a result, most inferences about function have been based on studies of the anatomy of dead specimens. Ongoing exploration of deepsea environments using submersibles is slowly overcoming this problem by allowing accumulation of observations on cephalopods *in situ* and by enabling their gentle capture for prolonged observation in shipboard aquaria.

The research reported here continues the series of studies we are conducting on deep sea cephalopods observed *in situ* from manned and unmanned submersibles, e.g., Vecchione and Roper (1992), Vecchione *et al.* (1992), Roper and Vecchione (1996). We have compiled from a number of sources several detailed observations on *Mastigoteuthis* spp, especially *M. magna* Joubin, 1913. A submersible cruise off North Carolina in 1994 provided a particularly rich source of *M. magna* behavior captured on video tape.

Chun (1910) first described the internal anatomy of several species of immature *Mastigoteuthis* ; Dilly *et al.* (1977) provided excellent detailed observations on the anatomy of trawl-captured *Mastigoteuthis* sp. from which they inferred functional capabilities. Our observations from submersibles on living *Mastigoteuthis* in their natural habitat enable us to test several hypotheses concerning functional morphology proposed by Dilly *et al.* (1977), most notably the following :

1. Based on the vacuolated tissue of the arms, the squid probably lives with its head upwards.

2. Because of the large and complex magnocellular lobe of the brain, it is likely that propulsion is accomplished mainly by the large fins.

3. The large ventral arms may be held together to form a "gutter" for feeding.

4. The tentacular suckers probably have a poor or slowly acting release mechanism.

5. The multitude of minute tentacular suckers may have a sensory function.

6. The well-developed eyes and ink sac suggest that at least some of the adult life is spent in the photic zone where the eyes are used for the detection of prey.

MATERIALS AND METHODS

The information on *M. magna* was gathered from several sources, principally from video tapes taken during dive cruises in the western Atlantic Ocean using the *Johnson-Sea-Link I* and *II* submersibles. Also, specimens were captured and identified after having been observed *in situ*. The most extensive observations and videos were made during the cruise to "The Point" area near Cape Hatteras, North Carolina in September 1994. The bottom topography in the region of the dive sites at The Point at 700-1 000 m consists of soft sediments in a series of ridges and canyons that fan out into deeper water. Temperatures at the bottom in the canyons were generally 4.5°C. Details of the dive sites and ecosystem in relation to the cephalopod project are given in Roper and Vecchione (1996). Eight separate *in situ* video sequences that range in duration from a few seconds to about 15 minutes were recorded during the 1994 cruise. Also during this cruise, one of the specimens observed *in situ* was captured by the submersible, maintained alive and videotaped for several hours in a 1 m diameter plankton kreisel aquarium (Hamner, 1990).

OBSERVATIONS

The accumulation of video sequences of *Mastigoteuthis magna* during the past several years has revealed a strikingly habitual posture consistently seen in this species. This posture typically consists of the animal hanging or hovering head downward with the mantle vertical, posterior upward, head oblique, arms I through III held at an oblique angle toward the horizontal (approximately 45°) ; the ventral arms (IV) and the tentacles, their proximal sections tightly wrapped in the tentacular sheaths of arms IV, hang down vertically in a tuning fork (inverted Y with curved arms) configuration so that the arms and tentacles are rather widely separated (Plate I. A-F). The tentacle length of the seven specimens that could be measured extended 3-4 times the length of the mantle (Plate I. B,C,F). No active swimming or jetting is associated with the hovering posture, which appears to be held for extended periods of time. Specimens are in that posture when first encountered, continue through periods of observation and filming of up to 15 minutes, and remain essentially immobile as the submersible departs.
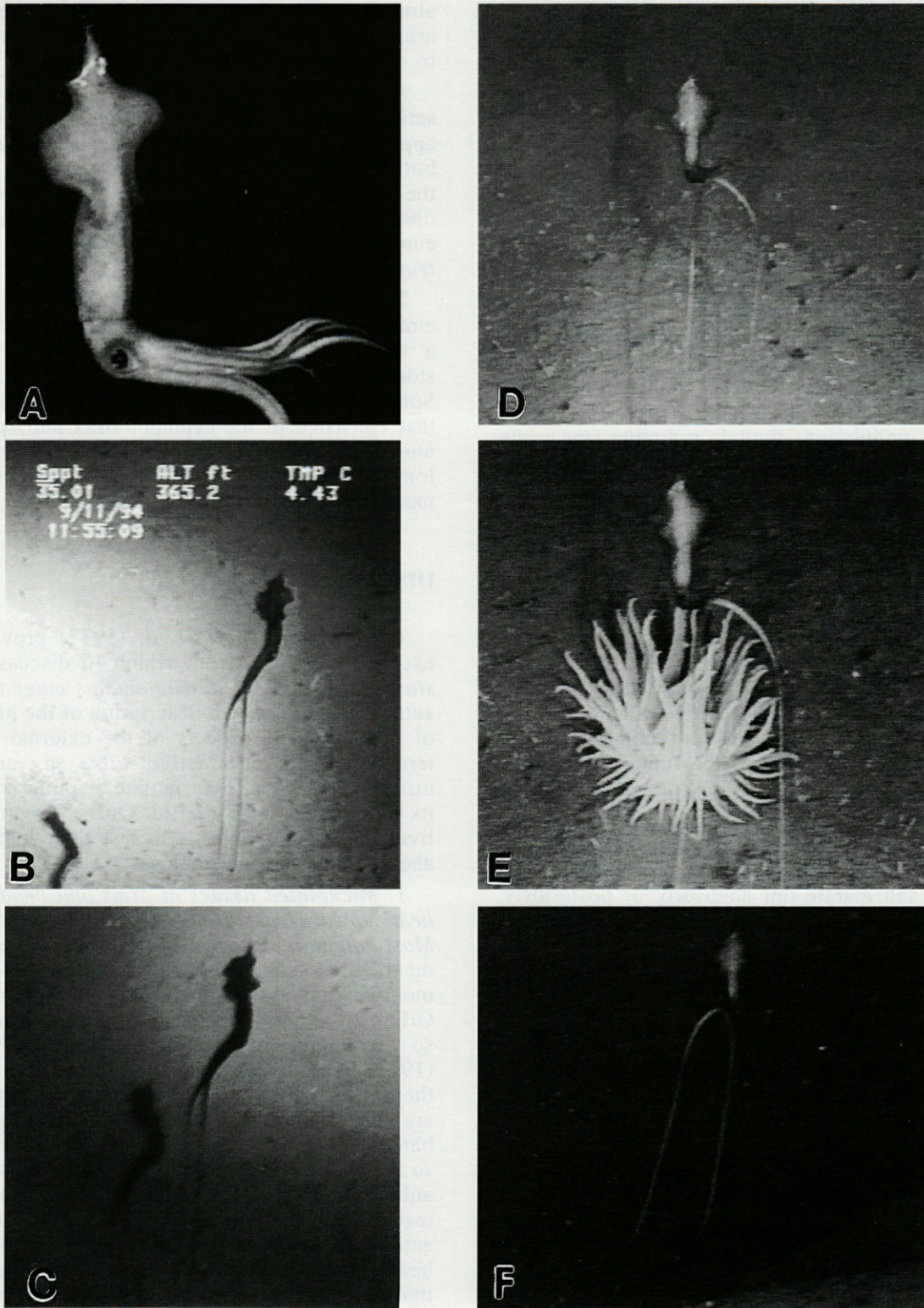
Pl. I. – *Mastigoteuthis magna* : A, In typical head-down posture. Fins show double sine wave undulation. Mucous strands (white blobs) attached to posterior end of mantle. B, In typical head-down, tuning fork posture with tips of elongate tentacles hanging close to bottom. Fins show non-synchronous double sine wave undulation. Note shadow on bottom. C, Drifting just above bottom in typical head-down, tuning fork posture. Elongate tentacles and their shadow on bottom are converging. Fins in double sine wave undulation. D, Drifting just above bottom in typical head-down, tuning fork posture with elongate tentacles and their shadows converging on the bottom. E, Drifting in typical tuning fork posture through expanded tentacles of anemone. F, In typical tuning fork posture with tentacles greatly elongate as animal suddenly drifts over steep drop-off of sea floor.

The animal's position in the water column and its vertical orientation are maintained by either simple or complex undulations of the fins. The undulations of the fins may take one of several forms, depending on the correction required to maintain vertical orientation, elevation above the bottom and, perhaps, orientation relative to the current. These include single sinusoidal undulations that originate at the anterior end of the fins and proceed posteriorly to the posterior end (Plate II. A), and those that originate at the posterior tip of the fins and proceed in a single wave to the anterior edge of the fins (Plate II. B). A new undulation begins after the completion of the preceding one. Each fin seems individually controlled, because the frequency and amplitude on one fin is different from that of the other. Complex undulations include a double sine wave progressing along the fin simultaneously (Plate I. A,B,C); these tend to be much more rapid than the single waves, and they may move either posteriorly or anteriorly. The undulations are used for relatively gentle motions that are positional corrections, not substantial locomotion. The fins also can be flapped in a strong dorsalventral beat (Plate II. C), apparently with a posterior to anterior gradient, that imparts a tailfirst locomotion that can be relatively strong when only the fins are involved; a very rapid escape reaction occurs when strongly flapped fins are combined with water ejected (jetted) from the funnel.

Another, previously unknown, function was observed for the fins. The fins sometimes are rolled together ventrally so that they overlap along the ventral midline and squeeze the water between the fins and mantle out anteriorly or posteriorly, providing a sort of low-velocity jet propulsion (Plate I. D,E).

The *M. magna* we have observed in the typical tuning fork posture have nearly always been located near the sea floor (Plate I. B-F). The animals drift along slowly above the bottom with their tentacles trailing down towards the substrate, and occasionally the tentacular tips touch the bottom. This is clear in several video sequences in which the tentacular tips and their shadows converge on the bottom sediment (Plate I. C,D). One sequence recorded over a bottom that steeply deepens shows the animal elongating its tentacles to twice their length as the sea floor drops off below it (Plate I. F). In cases where the bottom is not seen in the video, dive data show that the submersible and the *Mastigoteuthis* are just a few meters above the substrate. In the kreisel, the tentacular clubs of the squid stuck tenaciously to the glass, even when the animal vigorously tried to swim away, stretching the tentacles to many times the length of the mantle before they pulled free. When an animal is not drifting along the bottom with tentacles extended, the tentacles are contracted almost completely into the tentacular sheaths of arms IV (Plate II. A,F), possibly for protection, to reduce drag and to avoid tangling.

An observation for which we have as yet no satisfactory explanation is the occurrence of what appear to be mucus strands attached principally, but not exclusively, around the posterior end of the mantle (Plate I. A, II. B). While this is not observed on every specimen, it occurs frequently enough on *Mastigoteuthis*, as well as on *Histioteuthis*, to draw our attention.

The stomach contents of several museum specimens were examined. The stomach is lined with a very heavy, rugose, cuticular lining. Several stomachs contained a large quantity of yellow oil. Some stomachs contained a few bits and pieces that consisted of the cephalothorax and spines of unidentified copepods. The caecum is a very large, long organ in comparison to the size of the stomach and does not have a similar lining.

DISCUSSION

The work of Dilly *et al.* (1977) provides an excellent platform from which to discuss the *in situ* observations of *Mastigoteuthis magna*. These authors give a detailed description of the histology of the brain and aspects of the external and internal anatomy of *Mastigoteuthis* sp., and they utilize these data to formulate hypotheses about its habits and mode of life. Our observations on living animals enable us to test their hypotheses about functional morphology.

1. *Vacuolated tissues of arms and head induce head upwards posture.* – Most of the tissues of *Mastigoteuthis*, especially the arms, head and mantle are vacuolated and contain lightweight ammonium ions as an aid to buoyancy (Denton and Gilpin-Brown 1973). Because the ventral arms are so very large, consequently buoyant, Dilly *et al.* (1977) hypothesized that these animals live with their heads and arms vertically upwards when they are not swimming. The tentacles, on the other hand, are not vacuolated but have dense tissue, so they hang downwards. Our observations on live animals do not support the headup posture. Instead, *Mastigoteuthis* consistently use their fins actively to maintain the inverse position, with head, arms and tentacles downwards. One squid that had ceased undulating its fins briefly began rotating toward a headup position, but this was an exception, possibly caused by the squid being in water disturbed by the submersible's maneuvering.

2. *Propulsion by large fins.* – Because the magnocellular lobe of the brain is extremely welldeveloped with its motor control output going to the large fins, Dilly *et al.* (1977) deduced that the
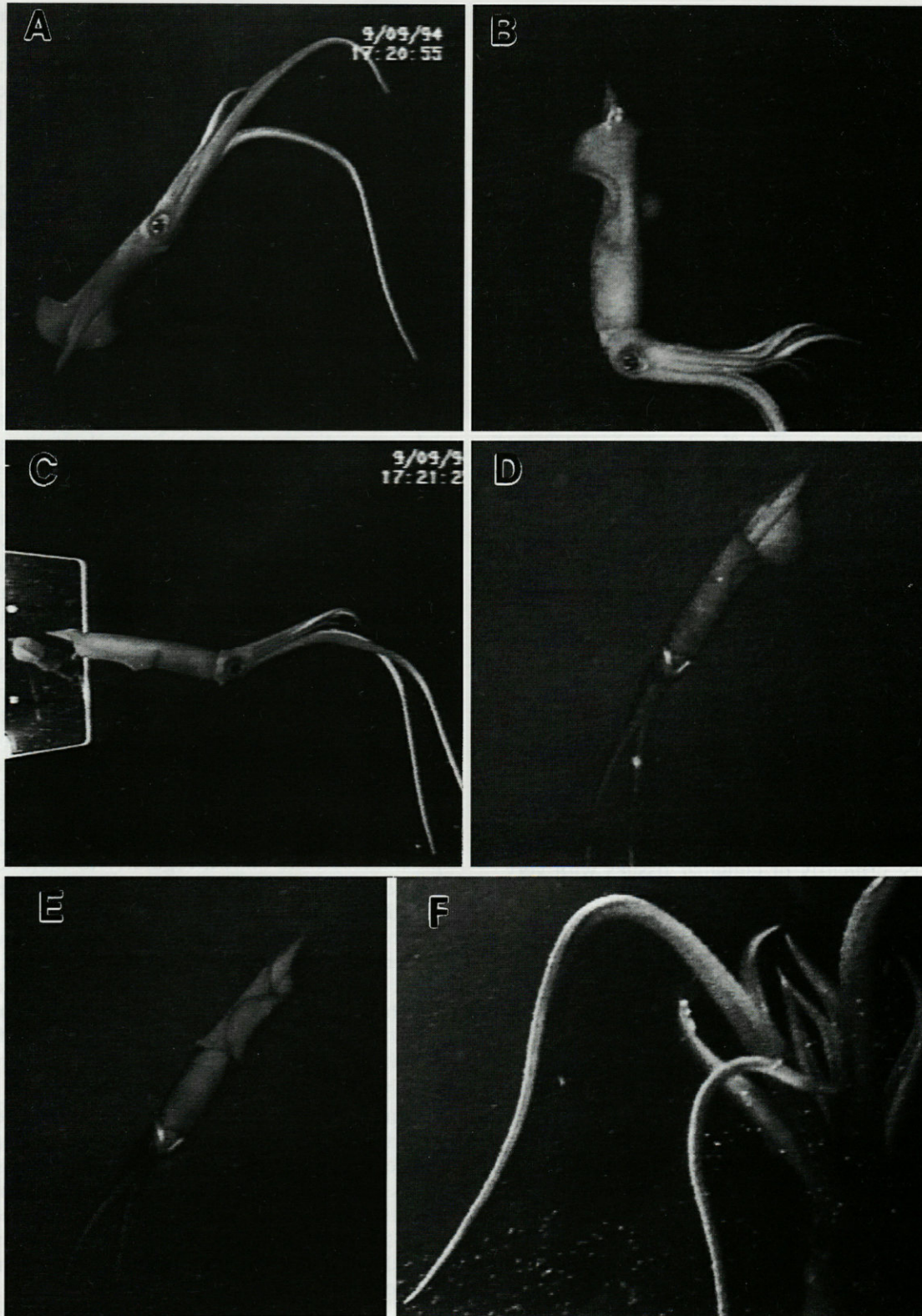
Pl. II. – *Mastigoteuthis magna* : A, With single sinusoidal undulation of fins originating at anterior end. Animal somewhat above bottom with tentacles contracted into tentacular sheaths of arms IV. B, With single sinusoidal undulation originating at posterior end. Mucous strands (white blobs) attached to posterior tip of mantle. C, With fins overlapping ventrally and motionless. Note rotation of head upward. D, With fins rolling together along ventral midline, squeezing water out posteriorly in a low-velocity jet. E, With fins overlapping along ventral midline at completion of low-velocity jetting stroke. F, In non-trolling mode off the bottom with right tentacle (left in photo) nearly completely contracted into tentacular sheath of right arm IV and left tentacle being contracted and enveloped into left arm IV tentacular sheath.

fins are the main source of propulsion for *Mastigoteuthis*. This inference is further supported by the very well-developed fin lobe that receives connections from the magnocellular lobe (Young 1977; Maddock and Young 1987). Our *in situ* observations strongly support this hypothesis and demonstrate the numerous, complex, and varied fin functions. Single and double sinusoidal wave undulations sweep anteriorly or posteriorly along the fins; fins flap powerfully in escape maneuvers; fins roll together ventrally to squeeze water out both anteriorly or posteriorly. Jet propulsion does occur through the funnel, but the fins always seem active and are dominant in both locomotion and position-holding.

3. *Ventral arms form gutter for feeding.*– Following the suggestion of Rancurel (1971), Dilly *et al.* (1977) concur that the large ventral arms may be held together by the suckers to form a gutter that captures prey. The observations and videos of live animals show that the arms virtually always are held far apart. The tentacular sheaths along the entire length of the ventral arms envelop the proximal section of the tentacles, holding them far apart, perhaps so the tentacles will not become entangled and stuck together with their multitude of minute suckers. Therefore, the large, long arms function to control the position of the tentacles, holding them as far apart as possible while they drift along near the bottom like sticky trolling lines fishing for minute prey.

4. *Tentacular suckers have weak release mechanism.* – Dilly *et al.* (1977) suggest that the extremely numerous, minute, pedunculate tentacular suckers that consist primarily of cuticular tissues with very little soft or muscular tissue, function to mechanically attach to prey, but that they are very weak and slow to release their grip. This would explain why many trawlcaptured specimens have their tentacles missing; they have been pulled off in the webbing of the net from which they could not be released. When a live *Mastigoteuthis* is handled, its tentacles feel very sticky, reminiscent of fly paper, and they have to be firmly pulled off the skin, as they do not release themselves. The hypothesis is further borne out by our observations in the shipboard kreisel aquarium, where the clubs stuck tenaciously to the glass while the animal tried to move away, stretching the tentacles to many times the length of the mantle before they release. While other squids show this sticking phenomenon, e.g., *Chiroteuthis*, they do not appear to do so to the extreme that *Mastigoteuthis* does (M. Vecchione, C.F.E. Roper, pers. observ.).

5. *Tentacular suckers have sensory function.* – A very large axial nerve along the tentacle connects directly into the large ventral magnocellular lobe, the complexity of which suggests a chemotactile function. Dilly *et al.* (1977) proposed that tentacular suckers may contain sensory cells that detect prey and direct it to the arms for transfer to the mouth. We have no observations on actual prey capture, but we observed one animal drifting just above the bottom in the feeding mode we hypothesize; it seemed to monitor its distance above the bottom with its tentacles. When the bottom dropped off suddenly, the tentacles immediately elongated to twice their length. This may result from a strong neural association between the tentacular suckers, the magnocellular lobe and the fins (Dilly *et al.* 1977; Young 1977), and it would keep the animal in the feeding zone of concentrated zooplankton in the benthic boundary layer (Dauvin *et al.*, 1995).

6. *Well-developed eyes and ink sac indicate photic zone habitat.* – Dilly *et al.* (1977) hypothesize that the large, well-developed eyes and an ink sac indicate that these animals undergo diel vertical migration into the photic zone in search of prey. None of the numerous *in situ* observations we now have on several species of *Mastigoteuthis* have occurred in the photic zone; all observations from submersibles are very close to the bottom. Roper and Young (1975) presented data on vertical distribution of trawl-captured mastigoteuthids, some by closing nets, and no captures of adults occurred in the photic zone. Shea (1995) showed the same pattern for paralarval mastigoteuthids caught in closing plankton nets. Mastigoteuthids always have been considered deep-sea inhabitants, mesopelagic to bathypelagic in vertical distribution. The first hint we had that at least some species are in fact benthopelagic came when stomach contents of macrourid rattail fishes (*Coryphenoides*) were shown regularly to include *Mastigoteuthis* and *Histioteuthis* beaks and remains (C.F.E. Roper, pers. observ.). These fishes are benthic/nearbottom feeders that do not venture far off the bottom. This revelation that mastigoteuthids are nearbottom dwellers, in spite of the longheld assumption of their midwater habitat, shows that caution is required when inferring function from anatomy in the absence of ancillary data on habitat, behavior or ecology.

An alternative explanation for the large eyes could be that vision is used to detect bioluminescence stimulated by movement of predators. It is not known yet if the ink sac secretes luminescent ink as has been observed in some other deep sea cephalopods (Herring 1977), or if the ink sac merely is a remnant of a shallow water ancestor (Vecchione 1994).

It seems counterintuitive that these squids typically are seen in a posture in which the center of gravity is higher than the center of buoyancy, because of the energy required to maintain this position. We suggest the possibility that the Mastigoteuthidae evolved from a *Chiroteuthis*-like ancestor. We have seen *Chiroteuthis*, which has smaller fins than *Mastigoteuthis* and vacuolated tis-

sues in its large head and arms, floating in an oblique head-upward orientation with the tentacles trailing downward. The mastigoteuthids have developed large, dexterous fins and complex magnocellular lobes to reverse the passive *Chiroteuthis* posture resulting from buoyancy. This allows them to use the sheathed ventral arms for better control of the tentacles with their extremely numerous, highly sensitive suckers. This complement of characters enables the mastigoteuthids to inhabit the unique trophic zone immediately above the deepsea bottom and to take advantage of the small zooplankters concentrated there (Wishner, 1980) while avoiding accidental entanglement of the tentacles.

It is acceptable, even necessary, to infer function based on morphology of dead specimens, but in order to inspire confidence, these inferences must be treated as hypotheses to be tested. Observations of live animals in their natural habitat allow tests of such hypotheses.

Because of the characteristic posture reported here, animals seen at the limits of vision from the submersible and in videotapes and photographs can now easily be identified as mastigoteuthids, even very small juveniles. In one instance, we have a one-second sequence from a transect reported on by Felley and Vecchione (1995) of a squid in the typical position that allows us to identify it as a *Mastigoteuthis*. One sequence is available on video because the observer in the submersible thought that, from a distance, the very small juvenile *Mastigoteuthis* was a cydippid ctenophore with its two tentacles hanging downward.

Portions of the video sequences upon which this paper are based are presented in the "Cephalopods in Action" web pages at the following URL on the Internet. http ://www.nmnh.si.edu/cephs.

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THE STATOCYST-OCULOMOTOR REFLEX OF CEPHALOPODS AND THE VESTIBULO-OCULOMOTOR REFLEX OF VERTEBRATES : A TABULAR COMPARISON

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HAIR CELL
STATOCYST
VESTIBULAR
OCULOMOTOR
EVOLUTION
CEPHALOPODA

ABSTRACT. – In cephalopods, the control system for compensatory eye movements, the statocyst oculomotor reflex, attains the highest level of complexity among invertebrates. In a large table, all its major components, from the receptor input to the effector output, are compared step-by-step with the equivalent components of the vertebrate vestibulo-oculomotor reflex. This direct comparison highlights the many parallels in structure and function of the two systems, and underlines the importance of the cephalopod system as an alternative invertebrate model for comparative vertebrate vestibular research.
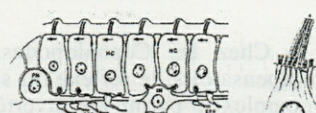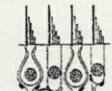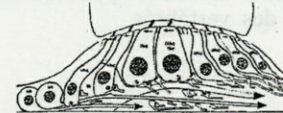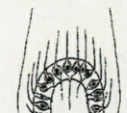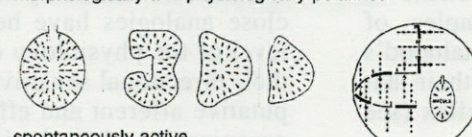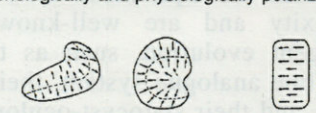
CELLULE CILIÉE
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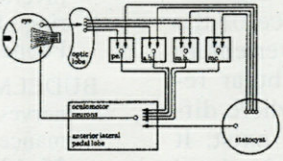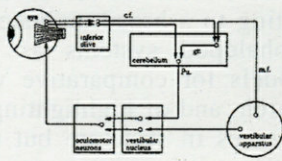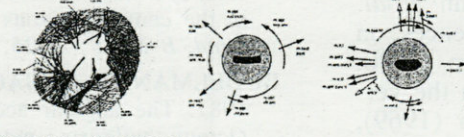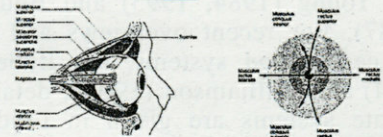
RÉSUMÉ. – Chez les Céphalopodes, le système contrôlant les mouvements oculaires compensatoirs, le réflexe dit statocyste-oculomoteur, atteint son plus haut niveau de complexité parmi les invertébrés. Tous les composants majeurs, allant du récepteur à l'effecteur, sont comparés étape par étape avec les composants correspondants du réflexe vestibulo-oculomoteur des vertébrés dans un tableau. Cette comparaison directe démontre les nombreuses similitudes de structure et de fonction dans les deux systèmes, et souligne l'intérêt du système Céphalopode en tant que modèle invertébré pour les recherches comparatives consacrées au système vestibulaire des vertébrés.

Cephalopods are one of the most highly evolved invertebrates. With only a few exceptions (e.g., *Nautilus*), they are voracious, visually-oriented and very mobile predators. Such a lifestyle necessarily requires sophisticated sense organs and an elaborate nervous system. Not surprisingly, then, during the course of evolution cephalopods – though confined to their molluscan design – developed sensory and nervous systems that are the most sophisticated of all the invertebrates; some rival the equivalent vertebrate systems in complexity and are well-known examples of convergent evolution, such as the cephalopod's lateral line analogue system, their eyes, their statocysts, and their statocyst-oculomotor reflex (see Packard 1972; Budelmann 1994).

Over the past 25 years, the structure and function of the cephalopod statocysts and statocyst-oculomotor reflex have been intensively studied at all levels and have often been compared with the equivalent vertebrate systems, the vestibular apparatus and the vestibulo-oculomotor reflex.

Areas of comparison include : the linear (gravity) and angular acceleration receptor systems; the receptor hair cells; the brain areas and afferent and efferent brain pathways involved in oculomotor control; and the eye muscle system and compensatory eye movements (compare Budelmann 1990, 1994; Williamson 1995). Although basic differences primarily in gross morphology exist between the cephalopod and vertebrate systems due to the fact that they have evolved along different evolutionary lines, a striking number of close analogies have been described, e.g., at the level of the physiology of the hair cells (including their directional sensitivity and ionic currents), the putative afferent and efferent transmitters, and the organization of the brain pathways. The main differences, despite in gross morphology, relate to : the structure of the receptor hair cells with regard to the number and arrangement of kinocilia and the presence (vertebrates) and absence (cephalopods) of stereocilia; the presence (cephalopods) and absence (vertebrates) of somata of first-order

	CEPHALOPODS	VERTEBRATES
GROSS MORPHOLOGY OF RECEPTOR ORGANS		
LINEAR ACCELERATION RECEPTOR SYSTEM	*Nautilus*: lower half of statocyst, no macula Octopods: 1 macula/statolith system, vertically oriented Decapods: 3 macula/statolith(statoconia) systems orthogonally arranged	2-3 macula/otolith(=statolith) and macula/otoconia(=statoconia) systems orthogonally arranged (utricle, saccule, lagena)
ANGULAR ACCELERATION RECEPTOR SYSTEMS	*Nautilus*: no crista/cupula system, but statocyst sensitive to angular accelerations Octopods: 9 crista/cupula segments 3-dimensionally arranged Decapods: 4 crista/cupula segments 3-dimensionally arranged	3 semicircular canals 3-dimensionally arranged ampullae containing crista/cupula systems
RECEPTOR HAIR CELLS	*Nautilus*: only primary sensory hair cells cristae: primary and secondary sensory hair cells maculae: only secondary sensory hair cells up to 200 kinocilia (9x2+2), no stereocilia/villi mechanical couplings: tip tight junctions, shaft connectors, basal connectors	only secondary sensory hair cells 1 kinocilium (9x2+2), 50-60 stereocilia/villi mechanical couplings: tip links, shaft connectors, basal connectors
LINEAR ACCELERATION RECEPTOR SYSTEM	1 hair cell type: 5,100 (*Octopus*) - 8,700 (decapods) hair cells 2 types of peripheral first-order afferent neurons: 2,000 (*Octopus*) - 5,000 (decapods)	Type-I (50%) and Type-II (50%) hair cells fishes Type-I-like (?) and Type-II hair cells amphibians only Type-II hair cells utricle: 9,200 hair cells saccule: 7,500 hair cells
ANGULAR ACCELERATION RECEPTOR SYSTEMS	3 hair cell types: primary, large, and small secondary sensory cells 2,100 (*Octopus*) - 2,600 (decapods) 2 types of peripheral first-order afferent neurons: 1,000 (*Octopus*) - 650 (decapods)	Type-I (60%) and Type-II (40%) hair cells fishes Type-I-like (?) and Type-II hair cells amphibians only Type-II hair cells 4,500 (rabbit) - 7,600 (man) hair cells
SENSITIVITY	coding of angular velocity Octopods: two 10x differently sensitive systems hair cells with different sensitivity 0.5 mV/degree cilium displacement < 0.12 µm peak/peak cilia/cupula displacement gain: 3.6 imp.s^{-1}/deg sensitive to linear acceleration	coding of angular velocity hair cells with different sensitivity 3.0 mV/degree cilium displacement 0.01 µm peak/peak cilium displacement gain: 0.11-1.6 imp.s^{-1}/deg sensitive to linear acceleration
HAIR CELL POLARIZATION AND ARRANGEMENT	morphologically and physiologically polarized spontaneously active sine-like stimulus/response correlation cosine-like directional sensitivity	morphologically and physiologically polarized spontaneously active sine-like stimulus/response correlation cosine-like directional sensitivity
HAIR CELL IONIC CURRENTS	outward delayed rectifier (I_K) potassium current outward A-type potassium current no outward calcium activated potassium channel inward sodium current inward L-type calcium current	outward delayed rectifier (I_K) potassium current outward A-type potassium current outward calcium activated potassium channel inward sodium current inward L-type calcium current

	CEPHALOPODS	VERTEBRATES
STATOLITH / STATOCONIA	Octopods: 1 statolith Decapods: 1 statolith + 2 statoconial layers calcium carbonate, statoliths with daily growth rings	otolith (= statolith) otoconial (= statoconial) layers calcium carbonate otolith (= statolith) with daily growth rings (fishes)
CUPULA	freely protruding into cyst cavity (swinging door) irregular shape and size (2 sub-systems) fibrillar (material ?)	diaphragm within ampullae; fibrillar (muco-polysaccharides, mucoproteins)
AFFERENT TRANSMITTERS and/or CO-TRANSMITTERS or MODULATORS	L-glutamate (non-NMDA) (excitatory)	L-glutamate (kainate-AMPA; few NMDA) (GABA, cAMP, adenosine ?)
EFFERENT TRANSMITTERS and/or CO-TRANSMITTERS MODULATORS	mostly inhibitory, few excitatory: **acetylcholine** (mus./nic.) (inhibitory) dopamine (excitatory and inhibitory) noradrenaline (α/β) (excitatory and inhibitory) GABA (GABA-A) (inhibitory) ATP (excitatory) NO (mostly inhibit, few excit) substance P - no effect ? CGRP (inhibitory)	mostly inhibitory, few excitatory: **acetylcholine** (inhibitory) noradrenaline GABA ATP (excitatory) NO substance P CGRP (calcitonin gene-related peptide) L-glutamate or aspartate (few reports) m-ENK (met-enkephalin), proenkephalin
AFFERENT AND EFFERENT CONNECTIONS BETWEEN HAIR CELLS AND BRAIN	non-myelinated fibers diameter between <1 µm and >12 µm 15%-25% afferent, 75%-85% efferent	myelinated fibers diameter between <1 µm and > 10 µm 90% afferent, 10% efferent
MACULAE	9,200 (*Octopus*) - 25,300 (*Sepia*) fibers	3,000 - 5,400 fibers (utricle + saccule)
CRISTAE	8,700 (*Octopus*) - 9,500 (*Sepia*) fibers	4,950 (pigeon) - 11,200 (monkey) fibers
BRAIN AREAS AND PATHWAYS INVOLVED IN INFORMATION PROCESSING	direct and indirect pathways cerebellum analogue: peduncle, ant./med. basal, and magnocellular lobes	direct and indirect pathways cerebellum
OCULOMOTOR NEURONS: LOCATION	anterior lateral pedal lobe	III.Nerve: ipsi/contralateral Oculomotor nucleus IV. Nerve: contralateral Trochlear nucleus VI. Nerve: ipsilateral Abducens nucleus
OCULOMOTOR NEURONS: TRANSMITTERS	acetylcholine (N-type; also M-type ?)	acetylcholine (N-type)
EYE MUSCLES	obliquely striated *Nautilus:* 4 muscles Octopods: 7 muscles (* recti, * oblique) Decapods: 13-14 muscles (* recti, * oblique)	cross striated 6 muscles (4 recti, 2 oblique)
EYE MUSCLE INNERVATION	Octopods: 7 nerves Decapods: 4 nerves	3 cranial nerves: Oculomotor (III) nerve: med., sup., inf. rectus, inf. oblique Trochlear (IV) nerve: sup. oblique Abducens (VI) nerve: lat. rectus
COMPENSATORY EYE MOVEMENTS	'linear' movements: postrotatory and optokinetic nystagmus rotatory movements: counterrolling, up to ±45°	'linear' movements: postrotatory and optokinetic nystagmus rotatory movements: counterrolling, up to ±10°

afferent neurons at the level of the receptor epithelia; the complexity of the efferent innervation; and the number and arrangement of the extraocular muscles. Surprisingly, in some aspects the cephalopod systems exceed the vertebrate systems in complexity, such as in the cellular organization of the receptor epithelia of the angular acceleration receptor systems, in the efferent innervation of the receptor epithelia, and in the extraocular eye muscles.

The arrangement of the cephalopod and vertebrate data in a concise table form highlights all these similarities and differences. The tables have their limits, however, because uniform cephalopod and uniform vertebrate data do not exist. Also, all the known variations within closely-related taxonomic groups cannot be included and, consequently, "averaged" data often have to be used. Although the tables put special emphasis on cephalopods and, if available, describe the differences between the nautiloid, octopod and decapod systems, they neglect the differences (though much smaller) that exist between the systems of fishes, amphibians, reptiles, birds and mammals, and completely exclude the limited data that are available on the unusual systems (by vertebrate standards) of cyclostomes (hagfishes and lampreys).

Variability granted, the presentation of the data in table form has the advantage of easily demonstrating to a broad audience the importance of the cephalopod systems as alternative invertebrate models for comparative vertebrate vestibular research, and of highlighting those areas where differences in structure but not in function exist. It is the comparative research specifically in those areas that can contribute to our understanding of the basic morphological and physiological principles that underlie statocyst/vestibulo-oculomotor reflexes.

For details on the figures presented in the tables, see Lindeman (1969), Budelmann (1979, 1988, 1989, 1992), Grüsser (1983), Budelmann and Young (1984, 1993) and Budelmann *et al.* (1987). For recent overviews and references on the cephalopod systems, see Budelmann (1990, 1994) and Williamson (1995); details on the vertebrate systems are given in Lindeman (1969), Wersäll and Bagger-Sjöbäck (1974), Henn *et al.* (1980) and Lewis *et al.* (1985).

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The data shown in the tables stem from more than 100 publications. Because of space constraints, given below are only a few key references and all the sources of the illustrations used. Please contact the authors for a complete list of references.

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ASPECTS OF THE FUNCTIONAL MORPHOLOGY OF CIRRATE OCTOPODS : LOCOMOTION AND FEEDING

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CEPHALOPOD
OCTOPODA
CIRRATA
IN-SITU
BEHAVIOR
SWIMMING
MUCUS-FEEDING
ANATOMY
SUBMERSIBLE
DEEP-SEA
STAUROTEUTHIS
GRIMPOTEUTHIS

ABSTRACT. – Cirrate octopods swim by a combination of fin action and medusoid propulsion by the arm/web complex. The fins of cirrate octopods are associated with a unique cartilage-like shell in a shell sac. In cross-section, the fins have distinct proximal and distal regions, both of which are covered by a thin surface sheath of muscle. The distal region is characterized by dorsal and ventral layers of muscle somewhat similar to a typical decapod fin. In the proximal region, the fin cartilage forms a flat central core within the fin and provides skeletal support for attachment of densely packed muscle. Whereas *Stauroteuthis* maneuvers slowly by sculling with the fins, *Grimpoteuthis* swims primarily using powerful fin strokes. In *Stauroteuthis*, the mantle is extensively modified. The mantle opening closely surrounds the funnel, and the posterior mantle muscle is thickened and probably controls water flow for respiration. The "secondary web" in some cirrate species results from a modification of the way the web muscles attach to the arms. The more benthic opisthoteuthids lack this modification. The secondary web enables larger volumes of water to be trapped in the web in some postures. The entrapment of water resulting in a bell-shaped posture in *Stauroteuthis* could be related to predator defense or to feeding. Buccal secretory glands found in *Stauroteuthis* and the presence of small copepods in the digestive tract, suggest that this benthopelagic species feeds by entrapping planktonic prey in mucus.

CÉPHALOPODES
OCTOPODES
CIRRATES
IN SITU
COMPORTEMENT
NAGE
ALIMENTATION À L'AIDE DE MUCUS
ANATOMIE
SUBMERSIBLE
EAUX PROFONDES
STAUROTEUTHIS
GRIMPOTEUTHIS

RÉSUMÉ. – Les Octopodes Cirrates nagent en combinant l'action des nageoires avec une propulsion médusoïde due au complexe brachial avec sa membrane interbrachiale. Les nageoires des Cirrates sont associées à une coquille de consistence cartilagineuse logée dans un sac coquillier. En coupes transversales, on distingue une zone proximale d'une zone distale de la nageoire, les deux étant couvertes d'une mince lame de muscles. La zone distale est caractérisée par des couches musculaires dorsale et ventrale qui ressemblent à ce qu'on connaît des nageoires de Décapodes. Dans la zone proximale, le cartilage de la nageoire forme un noyau central aplati à l'intérieur de la nageoire et offre un élément de support pour l'insertion de muscles très concentrés. Alors que *Stauroteuthis* manœuvre lentement par des mouvements de godille des nageoires, *Grimpoteuthis* nage de manière efficace avec des battements vigoureux des nageoires. Chez *Stauroteuthis* le manteau est très modifié ; l'ouverture du manteau serre l'entonnoir, la musculature de la partie postérieure du manteau est épaisse et semble contrôler le flux de l'eau destiné à la respiration. La "membrane secondaire" de certains Cirrates est le résultat d'une modification dans la façon dont les muscles de la membrane interbrachiale s'insèrent aux bras. Les Opisthoteuthidés, qui sont plutôt benthiques, ne montrent pas cette modification. La membrane secondaire permet aux animaux de "saisir" des volumes d'eau plus importants lors de certaines postures. La rétention d'un tel volume d'eau qui conduit à une posture en forme de cloche chez *Stauroteuthis* pourrait être liée à la protection contre un prédateur ou à l'alimentation. Des glandes buccales chez *Stauroteuthis* ainsi que la présence de Copépodes de petite taille dans le tube digestif semblent indiquer que cette espèce benthopélagique se nourrit d'organismes planctoniques pris dans du mucus.

INTRODUCTION

Most species of cirrate octopods are fragile, and trawl-caught specimens often are badly damaged. With few exceptions, detailed descriptions of fine morphology have been lacking. Although cirrates are rare in collections, several detailed anatomical accounts were written during the late 1800s and early 1900s (e.g., Chun 1910; Grimpe 1921; Meyer 1906; Verrill, 1881), but many aspects of their anatomy remain unknown and others require confirmation. In addition, some of the early reports (e.g., Ebersbach 1915) are compromised by taxonomic confusion about the specimens in question. Some of the early work was summarized by Aldred et al. (1983) in their paper on the anatomy of Cirrothauma murrayi.

Lack of direct observations of the behavior of these animals has precluded understanding relationships between anatomical structure and function. Recent studies of deep-sea communities by deep diving submersibles have begun to rectify this situation. Many anecdotal reports (e.g., Jahn 1971; Pearcy and Beal 1973) on cirrate behavior in-situ have resulted from submersible dives. Roper and Brundage (1972) summarized many of the in-situ observations to that time, based mostly on still photographs.

Three families of the Cirrata currently are recognized (Voss, 1988a). We report here on species in two families: Stauroteuthis syrtensis in the monotypic family Stauroteuthidae and Grimpoteuthis spp. in the diverse family Opisthoteuthidae (Voss 1988a, 1988b). We include both in-situ observations from videotapes recorded from manned submersibles and dissections of specimens in good condition.

MATERIALS AND METHODS

Stauroteuthidae. A specimen of Stauroteuthis syrtensis was videotaped at a depth of 782 m off Cape Hatteras in the western North Atlantic during the submersible Johnson Sea-Link II (JSL II) dive 1991 on 21 June 1990. It was then gently collected and fixed in formalin. The specimen was kept in formalin until November 1994, when we transferred it to water for measurement and dissection as part of a study of cephalopod phylogeny (Young and Vecchione 1996). Portions of the lip and fin base of this specimen were sectioned for light microscopy after embedding in paraffin and staining with eosin and hematoxylin. Other specimens of this species were videotaped off Cape Hatteras during JSL I dives 2621 (13 October 1989) and 3725 (August 1994) and a still image that appeared to be this species was recorded at a depth of 723 m by the U.S. Navy submersible NR-1 off New England.

Opisthoteuthidae. A videotape segment lasting several minutes of a Grimpoteuthis sp. was recorded off the island of Hawaii at a depth of ca. 1500 m during PICES

V dive P5-253. A shorter sequence of the same species was recorded nearby on dive P5-236. Because we lacked an adequate specimen of this species, a G. glacialis in excellent condition was selected from the collections at the U.S. National Museum of Natural History to be dissected for comparison with S. syrtensis. It was collected in January 1968 by a 3 m Blake trawl towed by R/V ELTANIN at 470-490 m depth in the Ross Sea off Antarctica. According to S. O'Shea (personal communication), the genus Grimpoteuthis s.l. should be split into multiple genera, in which case G. glacialis may be assigned to Cirroctopus Naef, 1921. A long videotaped sequence of a small juvenile Grimpoteuthis sp. in a shipboard aquarium was also reviewed. This specimen was collected at 727 m depth off New Jersey during JSL I dive 2256, 21 September 1991.

RESULTS

Behavioral Observations on Stauroteuthis syrtensis

One of the four observations on S. syrtensis provided a prolonged video record (JSL I dive 2621). As on all occasions, this species was in a characteristic posture when first approached. The anterior-posterior axis of the body was horizontal and the web was inflated into a bell-shape with the arms separated from the primary web by the extended membranes of the secondary web (Fig. 1A-1D; also see Nesis, 1987:68). The fins are used in this posture to maintain the horizontal attitude by a gentle sculling motion, rotating at the fin base by as much as 90° (Fig. 1B). The animal was seen to contract its secondary web slowly, pulling the arms aborally into the primary web. This was followed by the animal bringing its arms and web together beginning at their proximal regions and progressing as a wavelike contraction distally, expelling the water from inside the web. Although relatively slow, this appeared to be an escape reaction. After gliding some distance with the fins wrapped ventrally around the mantle (Fig. 1E), the animal resumed the bell-like posture and then went through the escape sequence again as the submersible approached. While in the bell-like posture, the posterior end of the mantle appeared broadly rounded and inflated (Fig. 1B) whereas it was more pointed while the animal glided following contraction of the arm/web complex (Fig. 1E). When the web was inflated, the cirri, which were very long, extended laterally from the sides of the arms, against the web (Fig. 1C).

One animal (JSL II dive 1991) contacted the bottom while gliding away from the submersible but, unlike the opisthoteuthids, made no attempt to remain on the bottom. This animal was subsequently captured and examined; contact with the sea floor had damaged the posterior end of the body. "Ballooning", in which the web is ex-
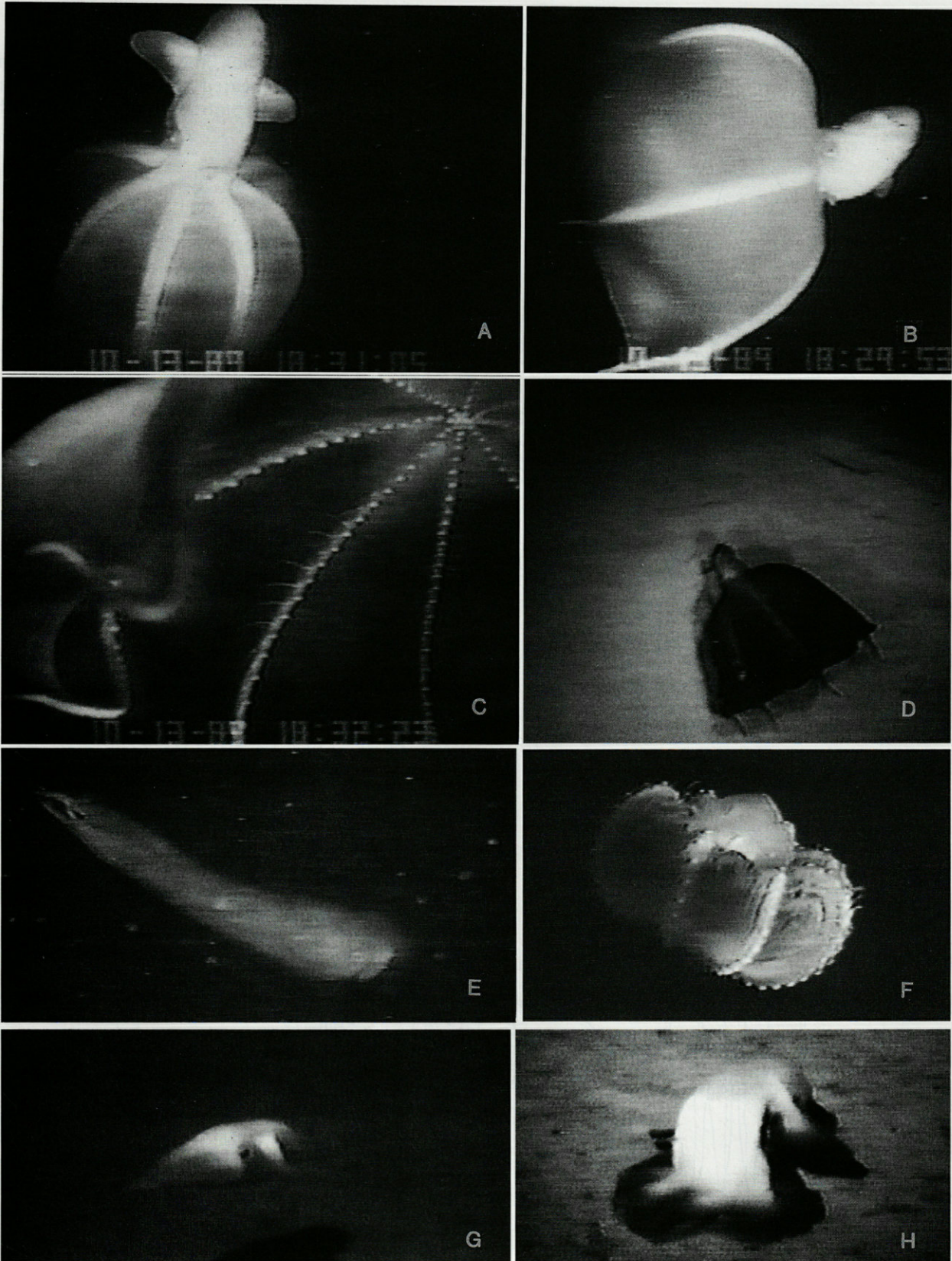
Fig. 1. – Still frames from videotapes of live animals. A-C, E : *Stauroteuthis syrtensis*, JSL I dive 2621. A. Ventral view of characteristic bell-like posture; B. Lateral view of bell-like posture. Notice fins, which are rotated, dorsolateral arm, on which the secondary web is extended along distal half and retracted along proximal half, and rounded posterior mantle; C. View inside inflated primary web, showing attachment of primary web to arm by secondary web, and long cirri extended laterally from arm; E. Animal gliding away after contraction of arm/web complex. The posterior mantle is less rounded than in Fig 1B and the fins are wrapped tightly around the ventral mantle. D. *S. syrtensis* near bottom in bell-like posture, JSL II dive 1991. This is the animal captured and dissected. E. Juvenile *Grimpoteuthis* sp. (JSL I dive 2256) floating passively in defensive posture in shipboard aquarium. F-G : *Grimpoteuthis* sp., PICES V dive P5-253. F. Swimming with strong fin strokes; G. Sitting on soft-substrate bottom.

tremely inflated and closed at the arm tips (Boletzky *et al.*, 1992), was observed very briefly once during JSL I dive 3725 following disturbance of the octopod by propeller turbulence from the submersible.

Anatomical Observations on S. syrtensis

In the fixed specimen, the general form of the animal (Fig. 2A) appeared substantially different from the video representation. The gelatinous sub-
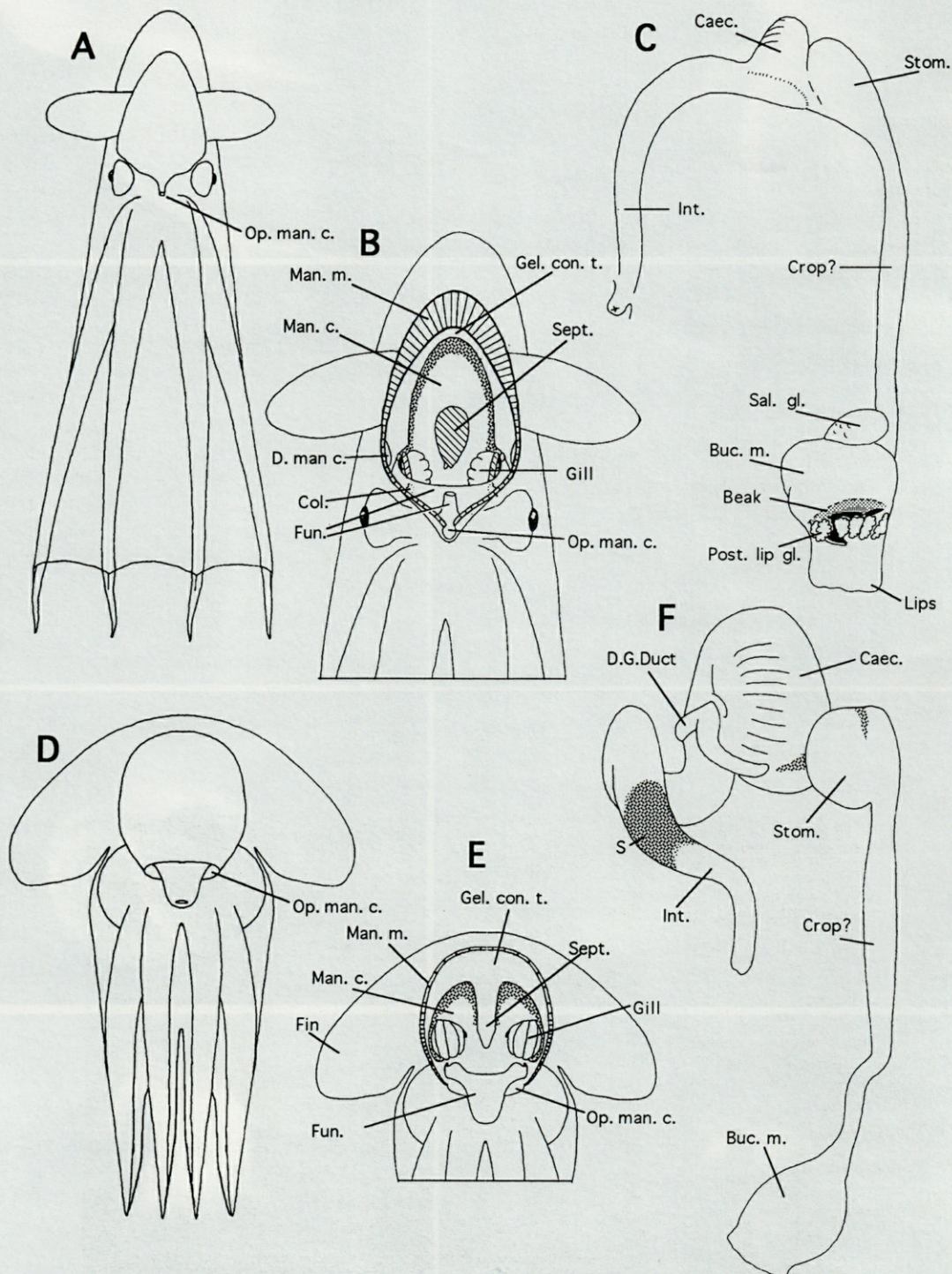
Fig. 2. – A-C : *Stauroteuthis syrtensis* (ML ca. 90 mm including expanded subcutaneous layer) ; D-F : *Grimpoteuthis glacialis* (ML = 72 mm). A, D. Ventral view of whole animal. B, E. Cut-away view showing extent and thickness of mantle wall. C, F. Digestive tract. Buc. m.-Buccal mass ; Caec.-Caecum ; Col.-Collar ; D. gl. duct-Digestive gland duct ; Fun.-Funnel ; Gel. con. t.-Gelatinous connective tissue ; Int.-Intestine ; Man. c.-Mantle cavity ; Man. m.-Mantle muscle ; Post. lip gl.-Posterior lip glands ; Op. man. c.-Opening of mantle cavity ; S.-Shading indicating turn of intestine toward viewer ; Sal. gl.-Salivary gland ; Sept.-Septum ; Stom.-Stomach.

cutaneous layer appeared to be greatly expanded in the preserved octopod and buried most of the fins. The mantle length (ML) was ca. 70 mm excluding the gelatinous layer or ca. 90 mm including it.

The mantle aperture of *S. syrtensis* is very constricted, forming a tube that, in life, closely encircles the funnel. Indeed, we briefly mistook this part of the mantle for the funnel; the funnel had been withdrawn entirely within the mantle cavity. The gills are attached ventrally in the mantle cavity. A very broad, short septum is present which, as is typical of octopods, does not extend to the posterior end of the mantle cavity. The posterior mantle wall is very thick (ca. 8 times thicker than the anterior mantle wall) and is characterized by a thick gelatinous core with long, thin, radial muscle fibers (Fig. 2B) that pass through it between the thin outer muscle layers.

The digestive tract forms a simple U-shaped loop (Fig. 2C). Salivary glands, apparently homologous with the posterior salivary glands of other cephalopods, are located on the posterior surface of the buccal mass. The straight esophagus had a slight swelling that may or may not function as a crop. Both the stomach and caecum are small and the intestine is short and simple. There are no traces of anal flaps or ink sac. The esophagus and stomach are covered externally with reddish pigment, as is the rectum. The caecum and most of the intestine are only lightly pigmented. The lateral walls of the stomach are very muscular. Folds of the caecum radiate from a central point but no clear coiling occurs. Food found in the esophagus and stomach consisted of the remains of many small crustaceans, most of which could be identified as copepods. The lips surrounding the buccal mass are honeycombed with secretory glandular tissue consisting of a system of tubules opening through pores to the exterior (Fig. 3). Although fixation is poor, the tubules seem composed largely of goblet cells filled with secretory globules. The glands are concentrated in two regions; one in the tissue surrounding the more proximal portion near the beaks (Fig. 2C) and one in the more distal part of the true lips. The glandular tissue in the latter appeared somewhat different from that of the more proximal part because it had a predominance of cells containing small granular globules while the latter had large vacuoles but virtually lacked granular globules.

A membrane, formed by a continuous shelf of muscle fibers, divides the core of each arm into longitudinal oral and aboral chambers, each of which contains loose muscle fibers. The brachial nerve is in the oral compartment. The muscle fibers in the aboral compartment form septa-like bands, or loose bundles, perpendicular to the long axis of the arm, subdividing the compartment. The primary web does not reach quite to the tips of the arms. It is joined to the arms by an "intermediate" or "secondary web" (Voss and Pearcy, 1990). This structure is a modification of the attachment of the web to the arms. Instead of attaching to the arms, the aboral muscle layer of the web is continuous. As a result, the arms can pull away from the aboral surface of the web, causing the oral surface of the web (Fig. 4A) to become the "secondary web".
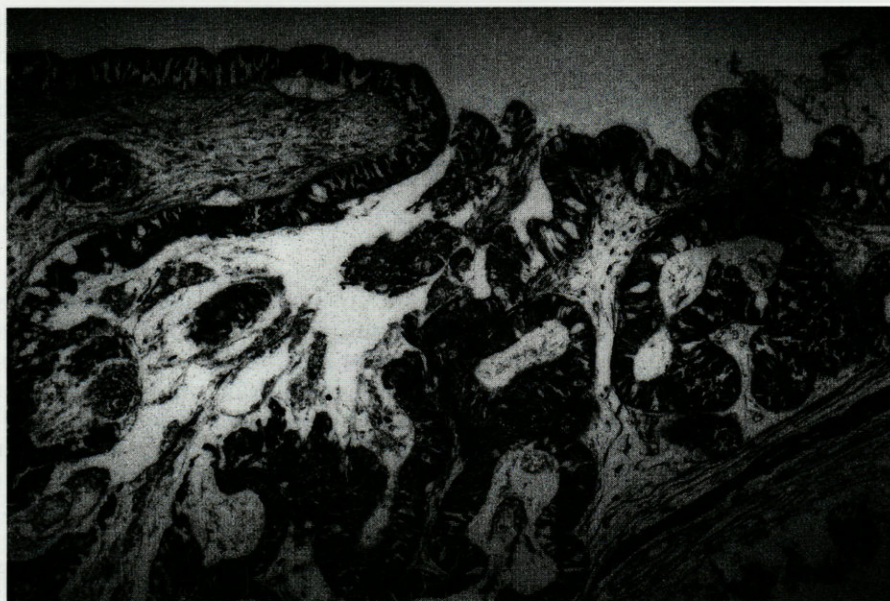
Fig. 3. – Histological section through lips of *Stauroteuthis syrtensis*, showing glandular tissue and ducts. Field width = 1.76 mm (long axis).
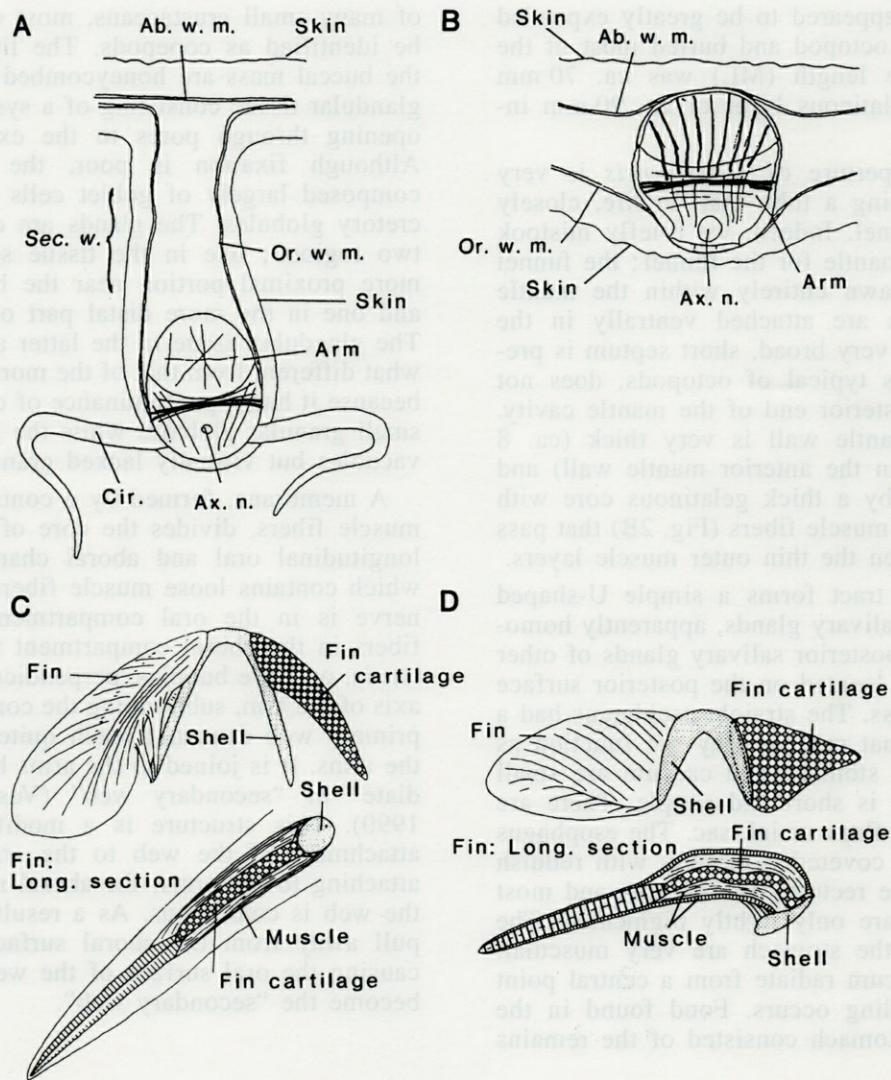
Fig. 4. – A. Cross-section of arm and web of *Stauroteuthis syrtensis*, showing independence of aboral web muscle from arm. B. Cross-section of arm and web of *Grimpoteuthis glacialis*, showing attachment of aboral web muscle to arm; this section did not pass through sucker or cirri. C. Shell and fins of *G. glacialis*. Top: Orientation of surface fin musculature on left, extent and insertion of fin cartilage on right. Bottom: Section through long axis of fin, showing proximal and distal regions. D. Shell and fins of *S. syrtensis*. Top: Orientation of surface fin musculature on left, extent and insertion of fin cartilage on right. Bottom: Section through long axis of fin, showing proximal and distal regions. Ab. w. m.-Aboral web muscle; Ax. n.-Axial nerve; Cir.-Cirrus; Or. w. m.-Oral web muscle; Sec. w.-"Secondary web."

The shell is U-shaped, with the lateral extensions (these have been called wings, horns, or arms; we refer to them below as wings) flattened dorsolaterally where the fins abut them. The posterior end of the shell, where the wings are joined, is approximately 1/3 of the mantle length from the posterior end of the mantle. The shell is tightly bound by the shell sac and the fins insert on the shell sac. However, when the shell and sac were cut to section the fin, the shell section fell easily out of the sac. The growth center of the shell is obvious, appearing like a filled tube down the center of the shell, but growth rings could not be detected with the dissecting microscope. The fins

are covered with the subcutaneous layer of gelatinous tissue continuous with that covering the rest of the animal. Beneath the subcutaneous layer are thin dorsal and ventral sheaths of surface muscle oriented obliquely to the long axis of the fin (Fig. 4D). There are smaller bundles of surface muscle along the anterior edge of the fin attached to the tips of the shell wings. Beneath this surface layer of oblique muscles, the fin is divided into distinct proximal and distal regions. The proximal region has a flexible central core which is spongy in consistency. This material is similar histologically to the fibrous connective tissue that has been referred to as reticulated cartilage in other cepha-

lopods (Nixon, in press). This cartilage flattens against the shell sac at its proximal end. Very thick bundles of heavy muscle cover the central core dorsally and ventrally, oriented parallel to the long axis of the fin and inserting on the core and on the shell sac. These appear to be the most robust muscles in the octopod. The distal region of the fin is very different from the proximal. It consists primarily of two layers of thin muscle fibers oriented transversely to the plane of the fin.

Behavioral Observations on Grimpoteuthis sp.

One *Grimpoteuthis* sp. observed off Hawaii (dive P5-236) was sitting on hard substrate. Its arm tips were tucked underneath the web except when one arm reached out. The mantle was bent ventrally (i.e. "flopped over" to lie horizontal to the ocean floor) and the fins were held close to the body. A second animal (dive P5-253) left the bottom as the submersible approached and swam ca 1-2 m above it (Fig. 1G). Swimming consisted entirely of strong flapping of the fins with a frequency of 1 complete cycle about every 3 seconds. The fins rotated to change angle of attack between upward and downward strokes similarly to the movie-frame tracings of an unidentified cirrate (probably *Grimpoteuthis*) in Figure 125 of Aldred *et al.* (1983). Neither medusoid swimming nor mantle jetting were observed. Finally it settled on soft substrate in the same benthic posture seen in the other animal (Fig. 1H). This characteristic posture is seen in other anecdotal observations, such as Figure 17D of Roux (1994), misidentified as an *Opisthoteuthis*.

Several other behavioral patterns were recorded in a long videotape sequence of a small juvenile *Grimpoteuthis* sp. in a shipboard aquarium. In addition to continuous flapping of the fins, this animal occasionally accelerated by isolated medusoid contractions of the arm/web complex. Although each pulse was executed more quickly than those of *Stauroteuthis*, the pulses did not form a rapidly recurring series as has been seen in *in-situ* videotapes of *Opisthoteuthis agassizi* (Vecchione and Roper, 1991). The juvenile *Grimpoteuthis* also sometimes stopped swimming and drifted with its arms and web curled, oral surface outward, covering the head, fins, and most of the mantle (Fig. 1F).

Anatomical Observations on Grimpoteuthis glacialis

The mantle opening of *G. glacialis* (ML = 72 mm) does not encircle the funnel tightly, and a distinct aperture is present on either side of the funnel (Fig. 2D). The skin of the specimen is tougher than that of *Stauroteuthis* and the subcutaneous gelatinous layer is not as thick. Unlike in *Stauroteuthis*, the thickness of the mantle wall does not vary greatly and is thin all around. However, the anterior region is more muscular than the posterior region. That is, the outer muscle layers make up more of the mantle thickness in the anterior region while the gelatinous core is more prominent posteriorly. The thick mantle septum, containing the mantle adductor muscle, continues to the posterior end of the mantle cavity and thereby divides the mantle cavity into right and left sides (Fig. 2E). The gills are attached dorsally.

The digestive tract of *G. glacialis* (Fig. 2F) is more complex than that of *Stauroteuthis*. Although we did not find the posterior salivary glands, Aldred *et al.* (1983) cited Ebersbach (1915) as stating that they are contained within the buccal mass of *Cirroteuthis umbellata* (= *Grimpoteuthis umbellata* fide Voss, 1988b). The middle region of the esophagus is slightly swollen as in *Stauroteuthis*. The stomach is small but the caecum very large. In this specimen the caecum contained numerous unidentified but apparently parasitic worms (F.G. Hochberg, personal communication). Unlike the condition in *Stauroteuthis*, the intestine is long and characterized by repeated bending in 3 planes. There was no external indication of lip glands. If present, they are very small compared with those of *Stauroteuthis*. The lips were not sectioned.

The arms are embedded in the thick primary web (Fig. 4B). There is no indication of a secondary web; the aboral muscle layer of the web attaches to the aboral surface of the arms. Although the web membrane extends to the arm tips, the web is reduced between the arms, especially the ventral pair. The arms are divided into oral and aboral chambers as in *Stauroteuthis*. However, in the aboral chamber, the transverse muscle fibers are not organized into septa-like bands. These muscle fibers are both more robust and oriented more consistently parallel in the oral-aboral axis than those of *Stauroteuthis*. The cirri are relatively shorter than those of *Stauroteuthis*.

The overall structure of the fins is similar to that of *Stauroteuthis,* with distinct proximal and distal regions and a thin surface sheath of muscle (Fig. 4C) beneath the skin and subcutaneous layer. The details of orientation of the surface muscles differ somewhat from those of *Stauroteuthis*, but the primary difference between the species is in the insertion of the fins on the shell/shell sac complex. The shell is basically U-shaped in both species, but whereas the central plate and core of muscle insert along the entire length of the wings of the shell in *Stauroteuthis*, this insertion is confined to the posterior 1/3 of the shell wing in

G. glacialis. The latter has a well developed set of muscles extending from the anterior edge of the fins to the anterior tips of the shell wings. These muscles apparently pull the fins close to the body when the animal is sitting on the bottom. The posterior end of the shell coincides with the posterior end of the mantle.

DISCUSSION

The mantle does not appear to function in cirrate locomotion. In *Stauroteuthis*, the mantle is extensively modified. The mantle opening seals tightly around the funnel, and the posterior mantle wall is unusually thickened. The thickened posterior mantle wall seems designed for increasing the volume of ventilating water. The gelatinous thickening is associated with long radial muscles which, when contracted, would greatly thin the mantle and thereby considerably enlarge the posterior mantle cavity for intake of water. Although the mantle is not as strongly modified in this manner in *Grimpoteuthis*, it is greatly modified in other ways by flattening in its confamilial, *Opisthoteuthis* (Meyer 1906). We saw no evidence in the videotapes that either *Stauroteuthis* or *Grimpoteuthis* uses the mantle for locomotion. Similarly, videotapes of *Opisthoteuthis* show no indications of mantle jetting (Vecchione and Roper 1991). Instead, cirrate octopods swim by fin action or by medusoid propulsion with the arm/web complex.

The relative importance of the arm/web complex and the fins for locomotion varies among taxa. In the opisthoteuthids, which apparently are the most benthic of the cirrates, the arms are strongly embedded in the primary web and there is no secondary web. *Opisthoteuthis* appears to flap its fins for stabilization and steering while it relies primarily on medusoid swimming for propulsion. This rapidly repeated expansion and contraction of the arm/web complex ejects a large volume of water at low velocity, which should be more efficient energetically than mantle jetting (R.K. O'Dor, personal communication). *Grimpoteuthis* makes much more use of strong fin motions in swimming, perhaps with an occasional isolated pulse with the arms and web. The coordinated flapping and rotation of the fins seen in videotapes of *Grimpoteuthis* should efficiently provide propulsion at low velocities (Wells and O'Dor, 1991). *Stauroteuthis*, in which the arms do not connect as strongly to the primary web because of the secondary web, seems to be a much weaker swimmer. It uses its fins for slow swimming and to maintain position when hovering with the web inflated. It escapes from threats by a single, relatively slow, contraction of the arm/web complex, or sometimes a series of isolated contractions.

Although both *Stauroteuthis* and the cirroteuthids have a secondary web, the bell-like posture that seems characteristic of the former contrasts with the umbrella-like "drogue" posture described by Roper and Brundage (1972) for cirroteuthids, which is more open and inverted. In *Stauroteuthis* the arms are arched with the aboral arc convex, trapping a volume of water within the web, along with the arms, cirri, etc. The cirroteuthids arch their arms with the oral arc convex, so that the web is stretched umbrella-like between the arms, with the suckers and cirri facing outward.

The inversion of the web by *Grimpoteuthis* (Fig. 1E) is reminiscent of a defensive posture commonly used by incirrate octopods (Packard and Sanders 1971). In addition to the videotape of the juvenile in a shipboard aquarium, this posture can be seen in a photograph of a *Grimpoteuthis* published in Nesis (1987:10). A similar posture is also known in *Vampyroteuthis* (Conniff 1996:132).

The fins of cirrate octopods are associated with a unique cartilage-like shell in a shell sac. The structure of these fins contrasts with those of decapods and adult *Vampyroteuthis* (Young and Vecchione 1996). In cross-section, the fins have distinct proximal and distal regions, both of which are covered by a thin surface sheath of muscle. The distal region is characterized by dorsal and ventral layers of muscle fibers perpendicular to the plane of the fin, both attaching to a central membrane, a condition somewhat similar to a typical decapod fin (Kier 1989). In the proximal region, the fin cartilage extends out from the shell sac along the plane of the fin, forming a flat central core. Although Robson (1932) recognized a core "of elastic consistency" in cirrate fins, he did not consider it to be cartilage. The histology of the structure, however, appears identical to the "reticulated cartilage" of other cephalopods (Nixon in press; *cf.* fibrous connective tissue in Roper and Lu 1990). Furthermore, the baselike apposition of the proximal end of the plate with the shell sac indicates homology with the fin cartilage of decapods (Young and Vecchione 1996). Thick dorsal and ventral layers of densely packed muscle, oriented parallel to the long axis of the fin, are attached to this central core and to the shell sac, not directly to the "shell vestige" as stated by Robson (1932). In contrast to the muscular hydrostat typical of cephalopod movement (Kier 1982, 1989), the spongy, flexible core of cartilage in the proximal part of the fin is a true skeletal support for the thick muscles attached to it and enables powerful upward and downward swimming strokes. The surface layer of muscles oriented obliquely to the axis of the fin is apparently responsible for the rotation of the fin

to change the angle of attack on up– and down-strokes. The proximal region of the cirrate fin is unique among cephalopods, although it shares some similarities to the paralarval fin of *Vampyroteuthis* (Young and Vecchione 1996).

Variation in shell shape among families (Voss, 1988a) is related to differences in attachment of fin muscles to the shell sac and fin cartilage. Although the U-shaped shells of *S. syrtensis* and *G. glacialis* are superficially similar, their evolutionary distinctness is highlighted by differences in fin insertion (Fig. 4C-D).

The chambered structure of the arms of cirrates appears to be convergent with that of some pelagic incirrate octopods (see Fig. 64 in Nixon and Dilly 1977), which also rely on medusoid swimming, and likely is related to the ability of the animals to contract their arms orally. Contraction of muscular septa in the aboral chamber would elongate the fluid-filled spaces between septa, causing the arms to move orally as seen in medusoid swimming. This arm structure is probably related to reduction of musculature as more muscular octopods without such a chambered arm structure also use web swimming occasionally in addition to mantle jetting.

The secondary web in some cirrate species is a modification of the attachment of the web muscles to the arms. It allows the entrapment of a large volume of water within the web in some postures. It also has the effect of separating the arms from the web so that the arms, cirri, etc. are closer to the center of the entrapped mass of water. Because the secondary web is contractile, the arms can be pulled up to the primary web, either to trap entrained organisms or in preparation for a medusoid escape pulse.

The entrapment of water could be related to predator defense or to feeding. Boletzky *et al.* (1992) and Roux (1994) proposed that the extreme inflation of the web of a cirroteuthid after contact with a submersible may have been a defense mechanism. The response of that cirroteuthid, however, was much more intense than the typical posture seen repeatedly in *Stauroteuthis*, although in one of our videotape sequences the animal briefly inflated in a similarly extreme manner.

Based on the presence of buccal secretory glands in *Stauroteuthis* and the very small crustacean prey found in the digestive tracts of this and some other cirrates (Vecchione 1987), we suggest that the benthopelagic stauroteuthids (and maybe the cirroteuthids) may feed by entrapping their prey in mucus, perhaps manipulating the mucus web with their long cirri. This may explain why the cirri of stauroteuthids and cirroteuthids are much longer than those of the bottom dwelling opisthoteuthids, which feed on benthic prey (Villanueva and Guerra 1991). The pigmented esophagus and stomach seem well suited for concealing small bioluminescent prey such as the copepods we found in them, and the muscular stomach seems adapted for processing whole copepods. Although mucus-trapping of prey has never before been reported as a feeding method for cephalopods, it is common in other pelagic molluscs (Lalli and Gilmer 1989). We can think of no way that *Stauroteuthis* could capture these small copepods without entrapping them in mucus.

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FUNCTIONAL MORPHOLOGICAL ASPECTS OF THE CEPHALIC AORTA OF *SEPIA OFFICINALIS* L.
(Mollusca : Cephalopoda)

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CEPHALOPODA
SEPIA
AORTA
CYTOLOGY
INNERVATION

ABSTRACT. – Cytological differentiations of the cephalic aorta of *Sepia officinalis* L. were analysed by LM and TEM. The origin and ultrastructure of the elastic fibres are demonstrated. The highly specialised cross striated muscle cells of the T. media with their particular Z-patch system and close interdigitations with extended areas of gap junctions represent a functional syncytium. Only the peripheral layer of muscle cells of the T.m. is innervated. The histo- und immunhistochemical findings confirm earlier pharmacological results suggesting that the vessel tonus is under a multiple, i.e. cholin-, catecholamin- and peptidergic neurocontrol. The role of 5-HT as a putative neurotransmitter in this mechanism is not yet clarified.

CEPHALOPODA
SEPIA
AORTE
CYTOLOGIE
INNERVATION

RÉSUMÉ. – Les différenciations cytologiques de l'aorte céphalique de *Sepia officinalis* L. sont analysées par microscopie photonique et électronique. L'origine et l'ultrastructure des fibres élastiques sont mises en évidence. Les cellules musculaires striées très spécialisées de la Tunica media avec un système particulier de z-patches et des jonctions gap étendues dans les domaines d'interdigitations cellulaires étroites représentent un syncytium fonctionnel. Seule, la couche musculaire périphérique de la T.m. est innervée. Les observations histo- et immunohistochimiques confirment des résultats pharmacologiques anciens indiquant que le tonus du vaisseau est sous contrôle multiple, c'est-à-dire cholin-, catécholamin- et peptidergique. Le rôle de 5-HT comme neurotransmetteur éventuel dans ce mécanisme n'est pas encore clairement établi.

INTRODUCTION

The cephalic aorta of coleoid cephalopods represents a good example of a high pressure "Windkessel" vessel in invertebrates that shows structural and functional properties corresponding to those of larger vertebrate arteries (Bourne 1982; Shadwick & Gosline 1983, 1985 a,b; Schipp 1987).

In comparison to the cephalic aorta of nautiloids the coleoid aorta has a thick wall, but a relatively low volume and a low extension coefficient. *In situ* it shows tonic pulse waves running with a higher frequency (F:30-50/') and being under a higher pressure (Sys./Dias. : 294-390/98-196 Pa) than the peristaltic pulse waves of *Nautilus* (Gosline and Shadwick 1982; Schipp and Kleemann 1994).

This study aims at presenting some further cytological and histochemical aspects of the cephalic aorta of *Sepia officinalis*. They could contribute to a more comprehensive understanding of the tonic functions and the neuronal control of this vessel type (Schipp *et al.* 1991; Schipp and Fiedler 1994).

MATERIALS AND METHODS

Juvenile *Sepia officinalis* L. (mantle length : 3-6 cm) from the Bassin d'Arcachon (Atlantic Ocean) and adult animals (mantle length : 9-10 cm) from the Mediterranean near Banyuls-sur-Mer were used in this study. All animals were anaesthetised by 1.5 % ethanol/seawater before dissections were carried out.

LM-Methods : For the histological analyses vessel preparations fixed in Bouin's solution or 3.5 % glutar-

aldehyde PBS were embedded in paraffin or araldite. Araldite sections (1 μm) were studied in the phase-contrast microscope. Paraffin sections (6 μm) were stained with the Masson differential coloration or the nerve silver impregnation after Bodian.

TEM-Methods: Tissue specimens fixed with 4% glutaraldehyde and 1.5% OsO4 (PBS; pH 7.3; 1100 mOsm) were embedded in araldite and the ultrathin sections viewed in a Philips EM 300.

The freeze-etching preparations were made with a modified method after Bachmann et al. (1969). The fixed specimens, frozen in liquid nitrogen (– 196 °C) were cleaved at – 100 °C under 10^{-6}-10^{-7} Torr and rotatory-shadowed with platinum and carbon in a Balzers Mikro-BA3 at an angle of 45°. Replicas were floated in a detergent including NaClO and after rinsing in A. dest. mounted on uncoated copper grids.

Histochemical methods: specific staining of elastic fibres was obtained by aldehyde fuchsin (Gomori 1950) and the PAS-reaction demonstration of catecholaminergic nerves by glyoxyl acid induced fluorescences (de la Torre and Surgeon, 1976, modified by Barber, 1982); localization of the acetylcholinesterase (AChE, E.C. No.: 3.1.1.7) and the non-specific cholinesterase (EC. No: 3.1.1.6) was made after Karnowsky and Roots (1964) using ISO-OMPA as inhibitor of the non-specific Ch-E. Immunhistochemical and cytochemical localization of FMRFamide and serotonine (5-HT) were made using the peroxidase-anti-peroxidase (PAP) reaction and colloidal gold (∅ 15 nm) as tracer (Sternberger et al. 1970).

RESULTS

Morphological findings

The wall of the cephalic aorta of Sepia officinalis L. consists of 3 layers (Plate I A): 1. Tunica intima (T.i.). It is composed of an incomplete endothelium and a PAS-positive lamina basalis which in semi-adult and adult animals is lined with an elastic layer that shows a positive reaction to aldehyde fuchsin. 2. Tunica media (T.m.). Depending on the age of the animals it is built by a varying number of circular muscle layers which are surrounded by a network of collagenous fibres. In adult and semi-adult animals elastic fibres take also part in the intercellular matrix. Most of them are circularly arranged, but there are also singular radially running fibres connecting the T. intima with the T. media and the peripheral wall area. 3. Tunica adventitia (T.a.). Its inner part is built by longitudinal and transversal muscle fibres that are peripherally lined by a different number of layers of fibrocytes and circular and transversal collagenous fibres. In addition to numerous afferent and efferent (= exchange) vessels (vasa vasorum), there are, locally, close formations of polyaxonal nerve fibres. Their terminal endings enter into the peripheral T.m., but do not occur within its middle and inner part.

Cytological findings

According to TEM analyses the elastic fibres which are typical for the T.i. and T.m. of older animals, are in close contact with the sarcolemma of bordering muscle cells. They are built up by bundles of fibrils that are connected by an amorphous electron dense material. The fibrils have nearly the same diameter (11-16 nm) as those of the adjacent collagenous fibres but do not show their typical periodic cross striation (Plate I C, D).

The muscle cells have only a small number of sarcosomes. They are closely interdigitated; their extended areas of gap junctions which show the typical pattern of hexagonal connexions in freeze-etching TEM-preparations are one of their main characteristics (Plate I E). The extended velum-like Z-patches anchored by 10 nm filaments within the cytoskeleton and in hemidesmosomes of the sarcolemma can be seen as a further special feature of these muscle cells (Plate I B, II A). The T-system is represented by funnel-shaped tubules deeply entering the muscle cells at the level of the Z-patches. Numerous close membrane contacts between SR-tubules and the sarcolemma occur especially in the area of gap junctions (Plate I E).

The neuro-muscular junctions

The terminal nerves reach only the peripheral muscle cells of the T.m. In contrast to the polyaxonal nerve fibres of the T.a. they contain few axons only which are surrounded by a sheath of Schwann cells on one side only. In the synaptical area the non-sheathed sides of the axons are in close and extensive contact with the post-synaptic membrane (= sarcolemma) and often seem to be deepened into crypt-like sarcolemmal invaginations comparable to the neuro-muscular junctions of the motor endplate. The intersynaptic cleft has a width of 15-20 nm. The axons contain different types of vesicles: large transparent vesicles (∅ 138 ± 25 nm), dense cored vesicles (∅ 102 ± 23 nm), small dense bodies (∅ 170 ± 45 nm) and accumulations of small transparent synaptical vesicles at the presynaptic membrane (∅ 62 ± 11 nm). Large dense bodies (± 540 ± 365 nm) are typical for the Schwann cells (Plate II A, B).

Histochemical results

The ACh-E- as well as the Ch-E-reactions revealed strong Hatchet-Brown colorations within the whole area of the T.i. (= lamina basalis) and distinct spot- or fibre-like pigmentation at the border of the T.a. and the T.m., i.e. the area of the neuro-muscular junctions; but the non-specific
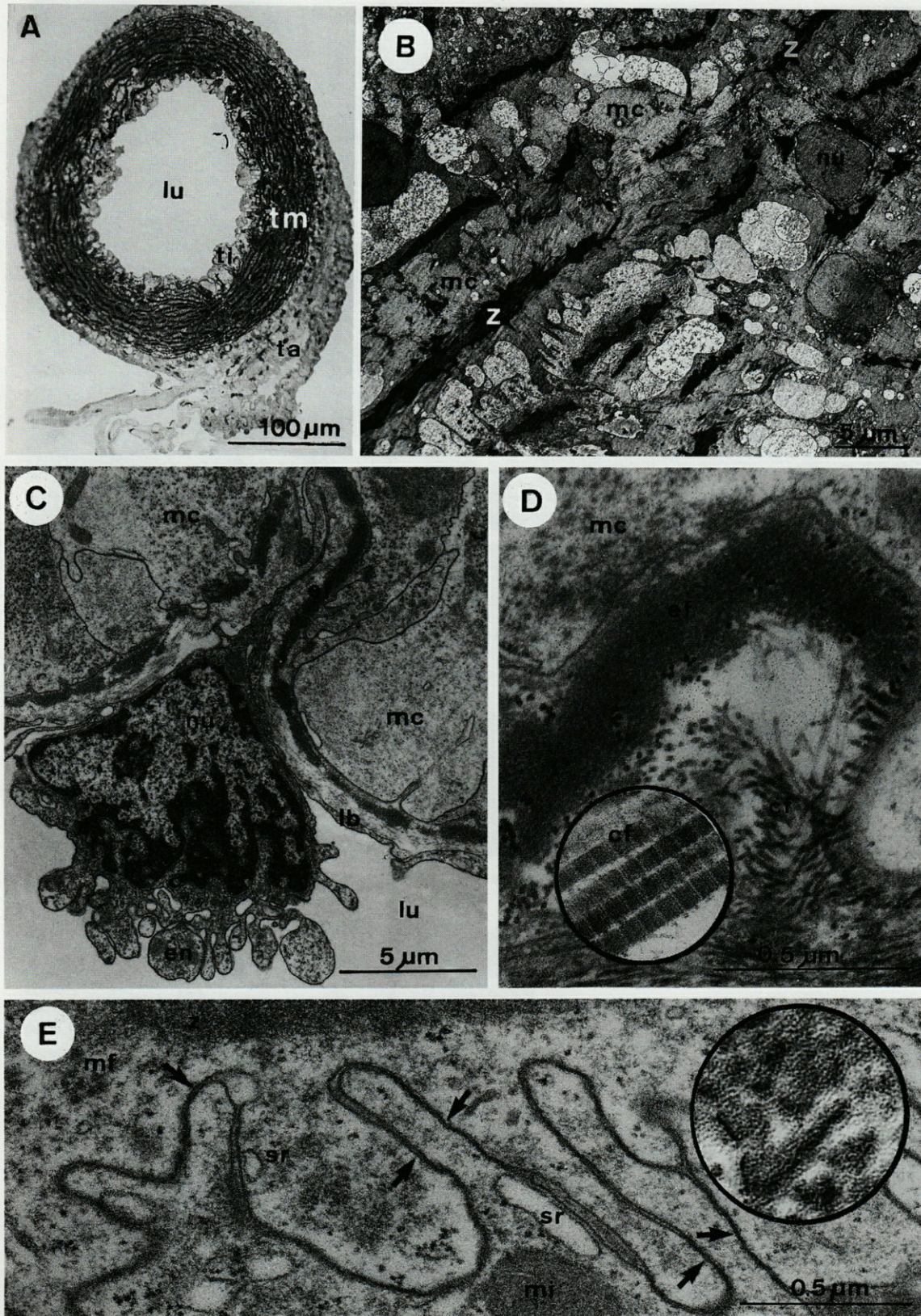
Pl. I. – Cephalic aorta of a juvenile *Sepia officinalis*. A, Total cross section alcian blue stained (in LM). B, Cross section from the T. media in TEM with circular muscle cells that are closely interdigitating. C, The area of the T. intima shows elastic fibres in the stage of forming in close contact with muscle cells. D, partial TEM section from C at higher magnification (inset magnification : 69900X). E, Close interdigitations of muscle cells within the T.m. with numerous gap junctions (arrows) in close contact with sr-tubuli in TEM and a freeze-etching preparation (inset magnification : 110000X). ax, axon; cf, collagenous fibres; db, dense bodies; ef, elastic fibres; en, endothelium; lb, lamina basalis; lu, lumen; mc, muscle cell; mi, mitochondrium; nu, nucleus; sc, Schwann cell; sr, sarcoplasmic reticulum; ta, Tunica adventitia; ti, Tunica intima; tm, Tunica media; z, z-patches.
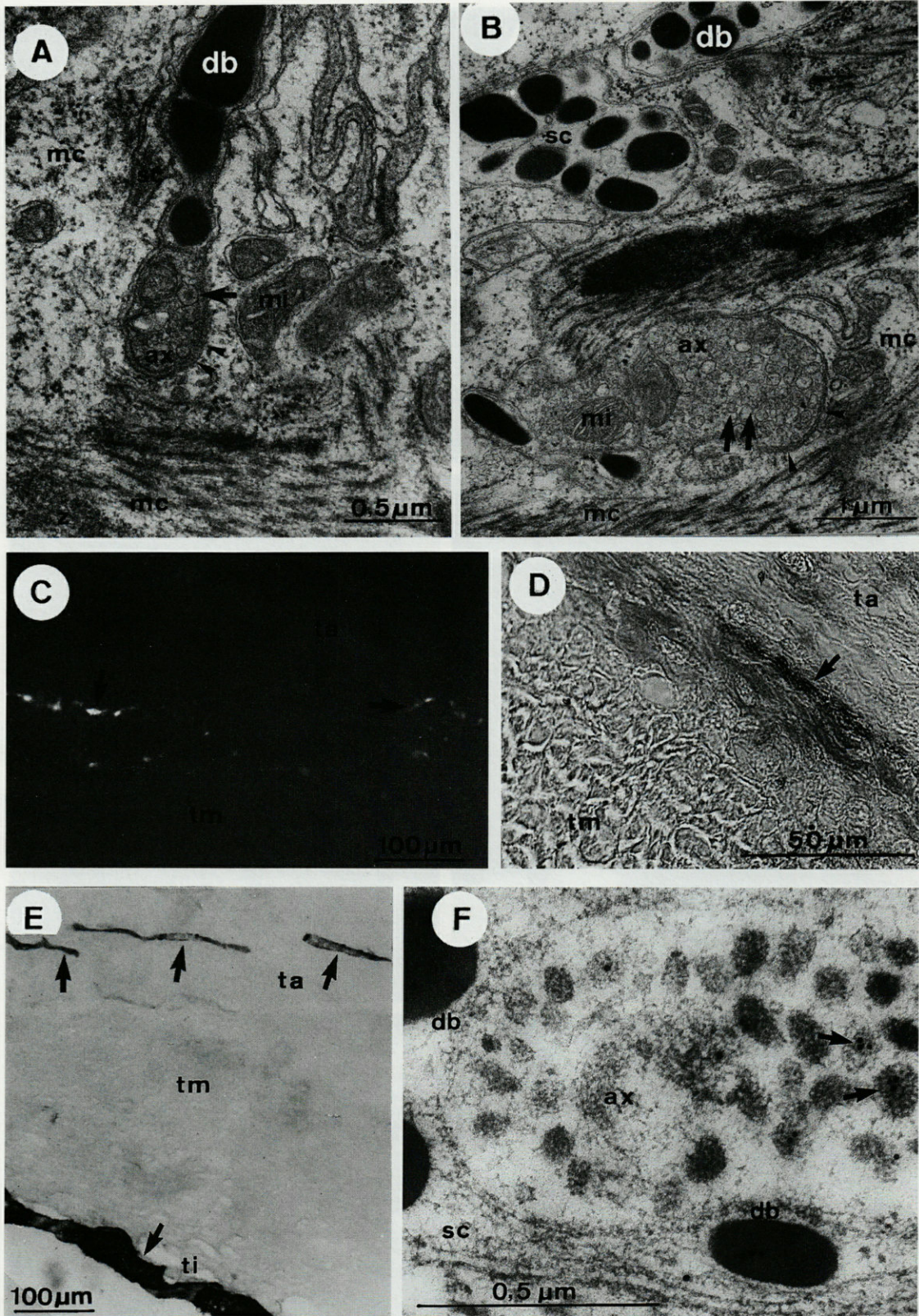
Pl. II. – Innervation of the cephalic aorta of *Sepia officinalis*. A, B, Neuromuscular synapses with dense cored vesicle (arrow) and transparent vesicles of different sizes (double arrow), synaptic area (arrow heads). C, Glyoxyl acid induced fluorescences in the border area of t.a./t.m. (arrows). D, Immunhistochemical reaction against FMRFamide (arrow) within terminal nerves of border area t.a./t.m. E, Acetylcholinesterase-reaction of the nerve endings of the t.a. and the t.m. F, Immunogold reaction against FMRFamide within electron dense granules (arrows) and dense bodies of a Schwann cell. Abbreviations as in Plate I.

Ch-E-reaction was stronger at the T.i. than at the area of the neuro-muscular junctions where a stronger reaction of the ACh-E was obvious (Plate II E).

Glyoxyl acid-induced fluorescences (E max : 480 nm) were localized only within the area of the neuro-muscular junctions at the border T.a./T.m. These were distinct but not frequent and showed a fibre- or bead string-like pattern (Plate II C).

The immunhistochemical methods applied produced positive reactions of the polyclonal-A.B. against FMRFamide but not of that against 5-HT. FMRFamide positive PAP reactions with spot- or fibre-like colorations occurred within the area of the neuro-muscular junctions at the border of T.a./T.m., and in larger nerve fibres within the peripheral T.a., singular spot-like PAP-colorations could also be detected within the T.m. (Plate II D). Small dense bodies within terminal axons and larger ones within the Schwann cells were traced by the immunogold-reaction against this peptide (Plate II F).

DISCUSSION

The structure of the T.i. of the cephalic aorta of adult *Sepia officinalis* resembles that of the adult *Octopus* aorta (Barber and Graziadei 1967; Shadwick and Gosline 1983). In both instances there is an incomplete endothelial layer but a continuous lamina basalis which is built by a collagenous network and a layer of elastic fibres.

As to our findings showing that in juvenile animals elastic fibres are not yet established, comparable results from octopods do not exist. The close contact of the elastic fibres, especially of their initial cores of formation in juvenile animals, to the sarcolemma as well as their substructure and diameter of fibrils suggest that the elastomere protein and/or its precursor substances are synthetized and secreted by the muscle cells. Furthermore it is probable that these precursor substances penetrate into an already preformed collagenous network. By the increasing cross linking of its fibrils the latter seem to lose their typical cross striation and to get a secondary amorphous substructure of high electron density.

The second structural peculiarity that is responsible for the special elastic/tonic properties of this vessel type, in sepioids as in octopods, is the highly specialised muscle system of the T.m. with its irregular cross striation. The extended velum-like Z-patches look similar in sepioids and octopods. They can be interpreted as a special structural adaptation of this high pressure vessel since they are not established in the nautiloid aorta that is under a distinct, lower pressure (Bourne *et al.*

1978; Shadwick and Gosline 1983, 1985a, b; Schipp and Kleemann 1994).

Our TEM analysis revealed, in the area of the close interdigitations between the muscle cells, extended gap junctions with the typical connexon-pattern which are in a close topical relationship to SR-tubules and their marginal cisterns. These structures suggest an electrical coupling of the T. media muscle system, thus representing a functional syncytium.

Our LM- and TEM findings indicate that only the peripheral muscle cells of the T.m. are reached by nerve endings forming close neuro-muscular junctions comparable to the axon-muscle couplings of the motor endplate; this means that these peripheral muscle cells probably have a pace-maker function influencing the cells of the inner T.m. via the gap junctions.

The histochemial results about the ACh-E activity, and the catecholamines indicating fluorescence within terminal nerves of this area correspond to the cytological finding that transparent as well as small dense cored neuro-vesicles were detected within the terminal axons. These structural evidences for a dual cholinergic-catecholaminergic innervation of the cephalic aorta of *Sepia* are in accordance with findings on other cephalopod vessels (Andrews and Tansey 1983; Schipp 1977; Kleemann and Schipp 1996) as well as earlier physio-pharmacological results on the *Sepia* aorta (Schipp *et al.* 1991; Schipp and Fiedler 1994).

The positive immunhisto- and cytochemical reactions against FMRFamide can probably be related to medium-sized dense bodies within the terminal axons localized by TEM. They are also in accordance with functional findings about vasodilatatory actions of this peptide on isolated aorta preparations precontracted by dopamine (Schipp *et al.* 1991).

The role of 5-HT in the neuroregulation of this vessel remains to be clarified. In pharmacological tests it showed also a vessel tonus decreasing effect, but the histo– and immunhistochemical method applied in this work did not reveal this neurotransmitter; a possible humoral action of this and other neurotransmitters via the bloodstream has to be taken into consideration.

The function of the high ACh-/BUT-Ch-E-activity (EC No : 3.1.1.7/3.1.1.8) within the lamina basalis of the T.i. is not yet clarified either; the findings confirm similar strong reactions of these enzymes in other coleoid and nautiloid vessels and heart organs and suggest that the lamina basalis represents an extended ACh-/Ch-barrier of the circulatory system of cephalopods, possibly protecting the inner wall area against cholinesters circulating in the blood (Schipp 1977, 1987; Kling 1986; Kleemann and Schipp 1996).

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SOME HISTOLOGICAL AND CYTOLOGICAL ASPECTS OF SMALL ARTERIES IN *NAUTILUS POMPILIUS* AND *N. MACROMPHALUS*

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NAUTILUS
ARTERIES
CYTOBIOLOGY
INNERVATION

ABSTRACT. – Small arteries of nautiloids are composed of a three-layered wall. Terminal nerve fibres are well established within the tunica adventitia. Only there, a high acetylcholinesterase activitiy and catecholamines could be detected. Immunohistochemical attempts to localize the neuropeptide FMRFamide yielded positive reactions within nerve fibres of the tunica adventitia. The three-layered wall of the afferent branchial vessel (ABV) shows some structural pecularities : the multilayered tunica media is also well innervated and a marginal sinus is established. Three different vesicle types are distinguished in the axons of the nerve fibres. Longitudinally arranged fibres of obliquely striated muscle cells reach from the tunica media into the collagenous network of the tunica adventitia.

NAUTILUS
ARTÈRES
CYTOBIOLOGIE
INNERVATION

RÉSUMÉ. – Les petites artères de Nautiloidés sont composées d'une paroi structurée en trois couches. Des fibres nerveuses terminales sont bien représentées dans la tunique "adventitia". C'est dans cette dernière que l'on trouve une forte activité de l'acétylcholinestérase et des catécholamines. Des essais immunohistochimiques réalisés pour localiser le neuropeptide FMRFamide démontrent des réactions positives dans les fibres nerveuses de la tunique adventitia. Le vaisseau branchial afférent (ABV) à trois couches indique des particularités structurales : la tunique "media" à multiples couches est également bien innervée et il y a un sinus marginal. On peut distinguer trois types de vésicules dans les axones des fibres nerveuses. Les fibres longitudinales des cellules musculaires obliquement striées font saillie dans le réseau des fibres de collagène de la tunique "adventitia".

INTRODUCTION

The structure of the artery wall of coleoid cephalopods with its three layers : tunica intima, tunica media and tunica adventitia (Jullien *et al.* 1957 ; Smith 1963 ; Barber and Graziadei 1967a, 1967b ; Alvarado *et al.* 1969 ; Kawaguti 1970 ; Kurtscheidt 1980, unpubl. ; Schipp 1987b ; Mangold and Bidder 1989) resembles that of vertebrate vessels and fullfills a "Windkessel-function" (Gosline and Shadwick 1982 ; Shadwick and Gosline 1983 ; Schipp and Kleemann 1994). The cephalic aorta of *Nautilus* shows a similar functional-morphological aspect ; but apart from the three afore-mentioned layers there is a further tunica, the tunica periadventitia surrounding a marginal sinus (Kleemann 1994). No details are known about the structure and function of smaller nautiloid arteries.

MATERIAL AND METHODS

Histological methods (Masson's trichrome after Goldner, Aldehydfuchsin after Gomori, PAS-reaction and toluidin blue coloration) and electron microscopical analyses were used to describe the wall structure of small arteries of Nautiloids (hepatico-columellar artery, proventricular artery, hepatic artery, afferent branchial vessel). The following technics were also used : immunohistochemical reaction against FMRFamide using the peroxidase-anti-peroxidase method as a tracer (Sternberger *et al.*, 1970 ; van Leeuwen, 1986), the acetylcholinesterase-reaction (AchE E.C. No. 3.1.1.7 ; Karnowsky and Roots, 1964), and the glyoxylicacid induced fluorescence (GIF) after de la Torre and Surgeon (1976) and Barber (1982).

Specimens of *Nautilus macromphalus*. Sowerby, 1849 (shell diameter 14-16 cm ; net body weight 250-400 g) from the Coral reefs of New Caledonia and of *N. pompilius* Linné, 1758 (shell diameter 9-11 cm ; net body weight 200-270 g) from Philippine costal waters were used.

RESULTS

The tunica intima is composed of a large conti-
nuous PAS-positive lamina basalis and an in-
complete endothelium (Fig. 1, 2, 4). The tunica
media is usually composed of 1-4 layers (depen-
ding on the vessel caliber) of circularly arranged
fibres of obliquely striated muscle cells (Fig. 1,
2, 3, 4) and an elastic network (Fig. 1). The tunica
adventitia is generally composed of a collagenous
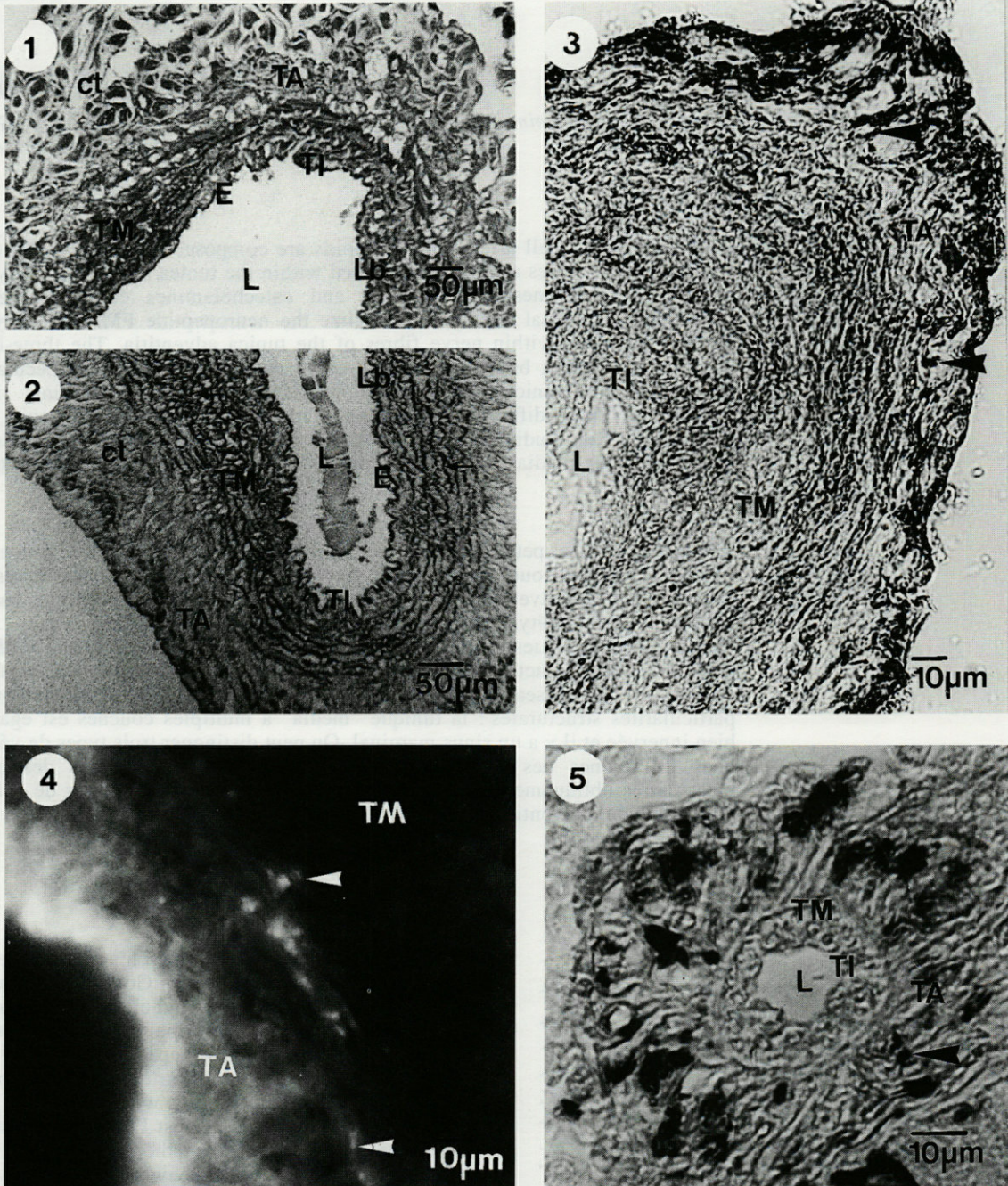
Fig. 1-5. – Cross-sections of small arteries of *Nautilus pompilius* : 1, Left proventricular artery. PAS- positive reaction
of the lamina basalis and the loose connective tissue in the tunica media and tunica adventitia. 2, Elastic fibres (◀)
in the tunica intima and tunica media of the hepatico-columellar artery (Aldehydfuchsin after Gomori). 3, Acetyl-
cholinesterase activity (◀) in the tunica adventitia of an arteriole of the foregut. 4, Catecholamine fluorescence (◀)
in the tunica adventitia of the hepatico-columellar artery. 5, FMRFamide reaction (◀) in nerve bundles of the tunica
adventitia of the hepatic artery.
Abbreviations : Ax, axon ; Ce, coelom epithelium ; cf, collagen fibrils ; cm, circularly arranged muscle fibres ; ct,
connective tissue ; E, endothelial cell ; G, glia cell ; L, lumen ; Lb, lamina basalis ; lm, longitudinally arranged muscle
fibres ; Mi, mitochondrion ; Ms, marginalsinus ; N, nucleus ; Nb, polyaxonal nerve fibre ; SR, sarcoplasmic reticulum ;
TA, tunica adventitia ; TI, tunica intima ; TM, tunica media ; tT, transverse tubule ; zp, z-patch.

network with few scattered muscle cells; it contains vasa vasorum as well as a large number of polyaxonal nerve fibres. The acetylcholinesterase was demonstrated within nerve fibres of the tunica adventitia of an arteriole of the foregut (Fig. 3). In the tunica adventitia of the hepaticocolumellar artery we observed fluorescent fibres, which revealed a bluish-green colour typical for catecholamines (Fig. 4). Immunohistochemical attempts to localize the neuropeptide FMRFamide yielded positive reactions within nerve fibres of the tunica adventitia in all small arteries investigated (Fig. 5).

Whereas in the other small arteries terminal nerves occurred only in the tunica adventitia, the tunica media of the afferent branchial vessel of *N. macromphalus* is densely innervated (Fig. 6). The collagenous network (periodicity of the collagen fibrils 54-64 nm) of the tunica adventitia is penetrated by longitudinally arranged fibers of obliquely striated muscle cells with few sarcosomes. Deep invaginations of the sarcolemma on the level of the z-patches are seen as a specialized t-system. The sarcoplasmatic reticulum has direct membrane contacts to the sarcolemma (Fig. 8). The axons of the peripheral nerves within the
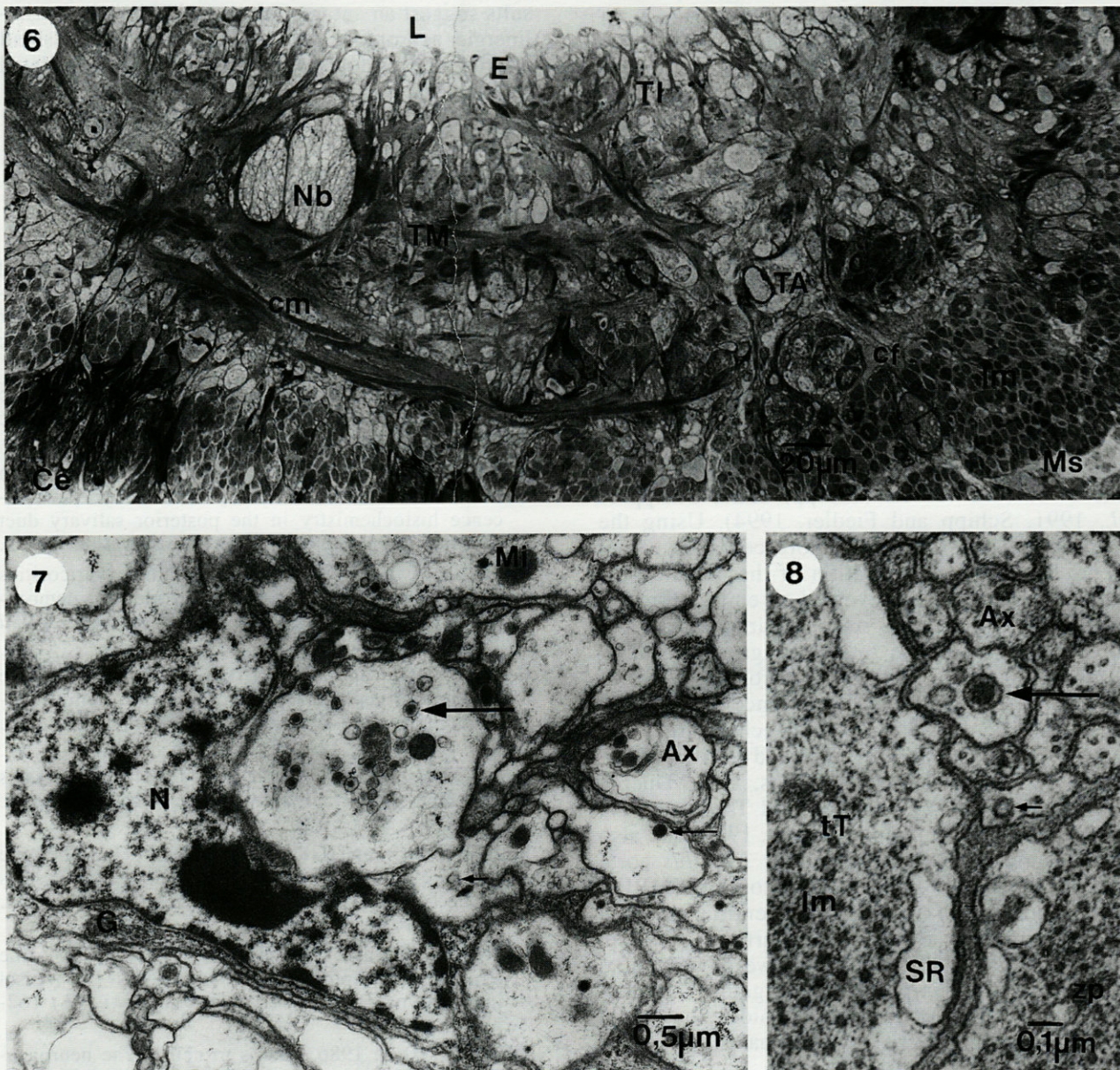
Fig. 6-8. – 6, Cross-section of the three layered vessel wall of the afferent branchial vessel (ABV) of *Nautilus macromphalus*. The obliquely striated muscle cells of the tunica media are well innervated. The longitudinally arranged muscle fibres belong to the tunica adventitia. (Semithin section, toluidin blue). Fig. 7-8 : TEM-sections of the afferent branchial vessel (ABV). 7, Polyaxonal nerve fibre in the tunica adventitia with transparent vesicles (→), dense cored vesicles (⟶) and osmiophilic vesicles (→) and dense bodies within the glia cell. 8 : Neuromuscular synapsis. The terminal axons contain dense cored (⟶) and transparent (→) vesicles (abbreviations : see Fig. 1-5).

vessel wall contain dense cored (⌀ 83-98 nm), transparent (⌀ 46-56 nm) and osmiophilic (⌀ 72-97 nm) vesicles, neurofilaments, neurotubuli and single mitochondria (Fig. 7). The axons are surrounded by glia cells with dense bodies (⌀ 180-239 nm). For the neuromuscular synapses in the vessel wall an intersynaptic cleft of 13-21 nm is typical.

DISCUSSION

Like the coleoid arteries (Smith, 1963; Barber and Graziadei, 1965, 1966, 1967a, 1967b; Kawaguti, 1970; Schipp, 1987) the small arteries of nautiloids possess a three layered wall. Considering the network of elastic fibres and the numerous circularly arranged muscle cells within the tunica media it seems probable that these vessels function as "Windkessel" too (Gosline and Shadwick, 1982; Schipp and Kleemann, 1994). These findings correspond to those on the coleoid cephalic aorta as well as on small vessels of the midgut, mantle and gills of *Sepia officinalis* (Jullien *et al.*, 1957). The occurrence of numerous elastic fibres within the tunica media is in accordance with the cephalic aorta of *Nautilus*, which is also seen as a "Windkessel"-vessel (Gosline and Shadwick, 1982; Kleemann, 1994).

The acetylcholinesterase localization in nerves of the tunica adventitia indicates that acetylcholine acts as neurotransmitter in the vessel control like in coleoid vessels (Schipp, 1987, Schipp *et al.*, 1991, Schipp and Fiedler, 1994). Using the GIP-method we observed fluorescent nerve elements in the tunica adventitia of the hepatico-columellar artery, which revealed an emission maximum ($Em_{max} = 480$ nm) characteristic of catecholamines. These results suggest a catecholaminergic component in the neuronal control of small arteries. Previous fluorescence histochemical studies have shown that catecholamines are widely distributed in the tunica adventitia of coleoid vessels (Arluison and Ducros, 1976; Ducros and Arluison, 1977; Andrews and Tansey, 1983; Kurtscheidt, 1980 unpubl.; Schipp, 1987) and also in the tunica periadventitia of the *Nautilus* aorta (Kleemann, 1994). The localization of FMRFamide in nerves of the tunica adventitia provides another similarity to the coleoid arteries (Schipp, 1987; Schipp *et al.*, 1991); but we have to note that this reaction is not specific against this peptide, but acts also against all amides that carry the sequence FM at their c-terminal side.

TEM-analysis of the afferent branchial vessel revealed a specialized muscle system in the tunica adventitia. The sarcoplasmatic reticulum has direct membrane contacts at invaginations of the sarcolemma; these diad-like junctions are probably involved in the intracellular Ca^{2+}-mediated electromechanical coupling of the muscle contraction.

According to Dorsett (1986) the ultrastructural differences of the molluscan neurovesicles are correlated with their respective, different transmitter content. The three different vesicle types detected in nerves of the afferent branchial vessels give a hint for a coexistence of acetylcholine, catecholamines and peptides. In terminal axons of the aorta of *Nautilus* (Kleemann and Schipp 1996) and *Sepia* (Schipp, 1987, Schipp *et al.*, 1991, Schipp and Fiedler, 1994; Schipp, 1995) there are also three different vesicle types. Together with our histochemical and immunohistochemical findings these results suggest an antagonistic catecholaminergic-cholinergic neuroregulation of the tonus of the small arteries of nautiloids, which is probably modulated by peptides.

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AURICULAR-VENTRICULAR INTERACTING MECHANISMS IN THE SYSTEMIC HEART OF THE CUTTLEFISH *SEPIA OFFICINALIS* L. (CEPHALOPODA)

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CEPHALOPODA
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CUTTLEFISH
CIRCULATORY SYSTEM
HEART
PACEMAKER

ABSTRACT. – Our physiological examinations on the systemic heart of the cuttlefish *Sepia officinalis* L. revealed that the auricle as well as the ventricle each has a myogenic automatism of its own. The results presented in this paper support the hypothesis that the ventricular pacemaker is located within the atrio-ventricular junction, while the auricular myogenicity seems to be diffuse. Because examinations of perfused systemic hearts indicated that the ventricular automatism is influenced by the pulsating auricle, it is supposed that the ventricular pacemaker is subordinated to the myogenicity of the auricles. According to histological and physiological examinations, the concept of a hydro-mechanical interaction between the auricle and the ventricle has been developed. It suggests that the auricle transforms the peristaltic contractions of the efferent branchial vessel into a kind of pulse wave, which causes the opening and stretching of the AV-valves during the ventricular diastole, inducing a reflex-like contraction of the ventricle. Proceeding from this hypothesis, it is assumed that the auricles are the primary pacemakers of the coleoid cephalopod systemic heart.

CEPHALOPODA
SEPIA OFFICINALIS
SEICHE
SYSTÈME CIRCULATOIRE
CŒUR
PACEMAKER

RÉSUMÉ. – Mécanismes interactifs auriculo-ventriculaires du cœur de la Seiche *Sepia officinalis* L. (Cephalopoda). Nos recherches physiologiques sur le cœur systémique de *Sepia officinalis* L. ont démontré que l'auricule, comme le ventricule, ont chacun un automatisme myogénique qui fonctionne séparément. Les résultats que nous présentons soutiennent l'hypothèse que le pacemaker ventriculaire est localisé à la jonction atrio-ventriculaire tandis que la myogénicité auriculaire semble être diffuse. Les études portant sur des cœurs systémiques perfusés indiquant que l'automatisme ventriculaire est influencé par l'auricule contractant-décontractant, on suppose que le pacemaker ventriculaire est subordonné à la myogénicité des auricules. Les études histologiques et physiologiques permettent de développer la conception d'une interaction hydro-mécanique entre l'auricule et le ventricule. Celle-ci suggère que l'auricule transforme les contractions péristaltiques du vaisseau branchial efferent en une sorte d'onde de pression qui fait que les valves-AV s'ouvrent et s'étendent pendant la diastole ventriculaire produisant ainsi une contraction réflexe du ventricule. En se basant sur cette hypothèse on pourrait conclure que les auricules sont les pacemakers primaires du cœur systémique des Céphalopodes Coleoidés.

INTRODUCTION

Numerous physiological investigations on isolated ventricles of bivalves, gastropods and cephalopods (for review see Krijgsman & Divaris 1955) revealed a myogenic, stretch-dependent automatism of the molluscan heart. E.g. an isolated, 'Straub-cannulated' ventricle of *Sepia officinalis* L. shows only rhythmical contractions if it is stretched by a filling pressure of at least 20 mm water-column. A further increase of the pressure leads to a linear acceleration of the frequency up to a maximum value of 58 ± 12 beats/min (Kling 1985). This correlation between filling pressure and excitation is characteristic of molluscan ventricles and has been described by several authors who examined the ventricles of gastropods (*Aplysia* : Straub 1904; *Helix* : Willems 1932; Almquist 1973; *Dolabella* : Matsui 1945), bivalves (*Mercenaria* : Smith 1985) and cephalopods (*Eledone* : Smith 1981a; *Octopus* : Foti *et al.* 1985). However, though a lot of work has been done to investigate the physiology of the molluscan ventricle, all attempts to localize a distinct pacemaker area within the systemic heart have so far failed. For that reason it is still being discussed where the automatic activity of the molluscan ventricle originates. In general it is proposed that the rhythmicity of the molluscan heart is governed by a diffuse myogenicity, i.e. the automatic activity originates in each myocardial cell (Krijgsman & Divaris 1955 ; Hill & Welsh 1966). Some authors, however, observed *in vivo* (Wells 1979), *in vitro* (Smith 1981b; Wells 1983) and even in tissue culture (Versen, unpublished) that the origin of the cephalopods' ventricular contractions is located in the area of the atrio-ventricular junction. Proceeding from these observations, it is a matter of interest that in whole systemic heart preparations of *Sepia officinalis*, perfused synchronously via both auricles, the auricles and the ventricle contracted as *in vivo*, i.e. alternately with the same rate of beats (Jakobs 1991a, b). However, although Jakobs used the same filling pressure as Kling (1985) in the 'Straub-cannulated' ventricle preparations, the perfused systemic hearts contracted in an evident lower frequency (17 ± 7/min.) than the 'Straub-cannulated' organs.

These results suggest that the myogenic automatism of the ventricle is being influenced by the auricles. Proceeding from the assumption that there is an interaction between both compartments of the coleoid cephalopod systemic heart, it seems probable that the supposed ventricular pacemaker is located next to the auricle, i.e. within the AV-junction.

The following observations and experiments were carried out to elucidate the presence of an auriculo-ventricular interaction within the systemic heart of *Sepia offcinalis* L. and to give further evidence that the myogenic automatism of the cephalopod ventricle is organized into nodal pacemaker areas.

MATERIAL AND METHODS

Advanced juvenile (mantle-length 8-11 cm; body weight 60-110 g; Bassin d'Arcachon) and adult animals (mantle-length 11-17 cm; body weight 200-400 g; Mediterranean near Banyuls-sur-Mer) of both sexes of *Sepia officinalis* L. were used in this study. All animals were anaesthetized by 1.5 % ethanol/seawater (SW) before surgical procedures and dissections were carried out.

Scanning Electron Microscopy : The preparations were fixed in 4 % formalin/SW and postfixed with 2 % osmium tetroxide in sodium cacodylate buffer (0,1M; pH 7,4 ; 1 000 mOsm). The specimens were dehydrated through a graded series of acetone, critical point dried (Technics, Alexandria), sputter coated (Polaron equipment) and viewed with a scanning electron microscope (Jeol Ltd., Tokyo).

Light microscopy : Material fixed in buffered formalin or Bouins solution was embedded in paraffin. Paraffin sections (7 µm) were stained with Massons trichrome modified by Goldner and Bodian's nerve coloration.

Physiological Preparations : Filtered aerated seawater-glucose solution (1.7 g/l; pH 8.3) was used as physiological solution, bathing- and perfusion medium. All experiments were performed at temperatures of 18-20 °C.

a. Ring-shaped preparations : Ring-shaped segments (length 4-5 mm) of the left or right auricle, the atrio-ventricular junction (AV-junction), the central part of the ventricle and the ventricular area next to the cephalic aorta were mounted on stainless steel clasps. These preparations were isometrically suspended (auricle 1 ± 0.5 cN; AV-junction 2.5 ± 0.5 cN; ventricle 0.5-4 cN; ventricle/cephalic aorta 2.5 ± 0.5 cN) in a 50 ml water jacketed organ bath with one clasp anchored and the other fixed to a strain gauge (Statham UC2). The pressure transducer was connected to a DC bridge amplifier (HSE type 300) and its signals were registrated on a thermographic recorder (Watanabe Mark V).

b. Auricle-ventricle preparations : The isolated systemic heart, including both auricles, was mounted in a flat organ bath and the genital and renal artery as well as the cephalic aorta were closed by ligatures. In each experiment one auricle was used to place an input cannula inside the ventricle. All potential electrical connections between this auricle and the ventricle were prevented through the fixation of the cannula by a ligature at the AV-junction. The second input cannula was placed into the other auricle and fixed by a ligature at the end of the efferent branchial vessel. For the 'open-valve' preparations, the AV-valve of this auricle was kept intact and functioning. For the experiments in which the hydro-mechanical coupling between auricle and ventricle should be interrupted, the AV-valve was sealed with a tissue adhesive (Histoacryl, Braun Melsungen).

The tightness of the seal was tested after the experiments by filling of the auricle with a 0.1 % Evans Blue/Seawater solution. Both input cannulas were connected with separate perfusion reservoirs which enabled controlled preload pressures for the auricle (4 cm H_2O) and for the ventricle (9 cm H_2O). To record the pressure pulse and the contraction rate of the isolated preparations, pressure transducers (auricle : HSE W101 ; ventricle : surgical 'single-use' transducer, Braun Melsungen) were installed between the cannulas and the perfusion reservoirs. The signals of the transducers were amplified by two HSE bridge amplifiers and displayed simultaneously on a two-channel xt-recorder (Watanabe Mark V).

RESULTS

Ring-shaped preparations

Whereas all examined auricles (n = 25) showed, with (0.5-1 cN) or without stretching, spontaneous rhythmical contractions (3.5 ± 1/min.) for several hours, remarkable differences between the tested ventricle-preparations could be recorded (Fig. 1, Aa).
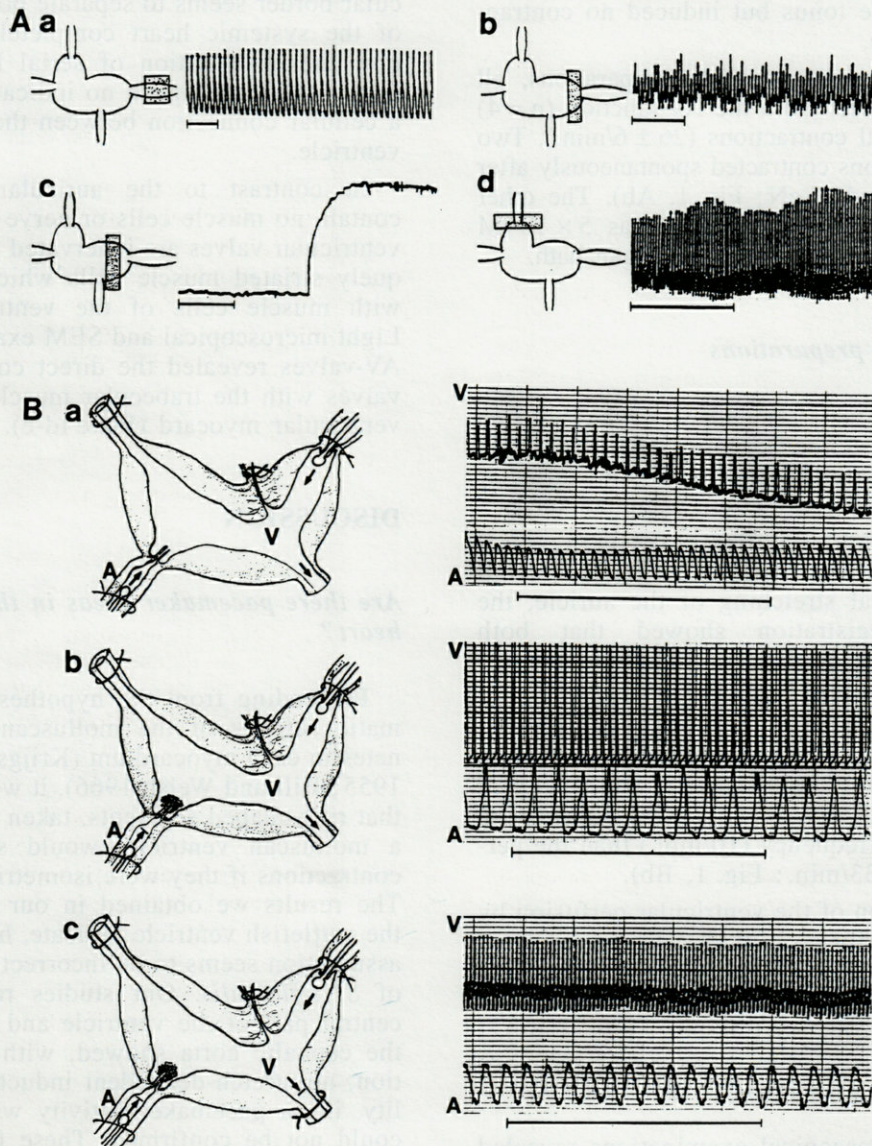
Fig. 1. – A, Actograms of isometrically suspended ring-shaped preparations taken from different parts of the systemic heart of *Sepia officinalis* (Scale bar 1 min.). a, Spontaneous contractions of an auricle preparation. b, Stretch-induced contractions of an AV-preparation. c, Increasing tonus of a ventricle-preparation after catecholamine stimulation. d, Catecholamine-induced contractions of a preparation from the ventricular area next to the cephalic aorta. B, Simultaneously recorded actograms of the auricle-ventricle preparations (Scale bar 1min.). a, 'Open valve' preparation ; auricle (A) and ventricle (V) contract with the same frequency. b, Occlusion of the AV-valves ; auricle and ventricle contract with different frequencies. c, Closure of the posterior aorta ; the increasing ventricular frequency does not affect the auricular contraction rate.

Only one of the ring-shaped segments (n = 6), taken from the ventricular area next to the cephalic aorta, showed spontaneous contractions (35/min.) when they were isometrically suspended (3 cN). Two of these specimens started to contract for a short period as we applied 5×10^{-6} M noradrenaline into the organ-bath (Fig. 1, Ad). The other preparations didn't show any contractions, neither by an increase of the stretching force nor by a higher noradrenaline stimulation (10^{-5}M).

The ring-shaped preparations from the middle area of the ventricle (n = 2) showed, even if they were forcefully stretched (4 cN), no contractions. The application of noradrenaline (10^{-5}M) led to an increase of the tonus but induced no contractions (Fig. 1, Ac).

In contrast to these ventricle preparations, all ring-shaped segments from the AV-junction (n = 4) showed rhythmical contractions (26 ± 6/min.). Two of these preparations contracted spontaneously after they were stretched (3 cN; Fig. 1, Ab). The other two preparations started to contract as 5×10^{-6}M noradrenaline was applied into the organ bath.

Auricle-ventricle preparations

The experimental arrangement we had chosen to perfuse the systemic heart of *Sepia officinalis* provided the possibility to record the auricular and the ventricular contractions simultaneously.

Though we had to use different filling pressures (auricle : 4 cm H_2O; ventricle 9 cm H_2O) to avoid an unphysiological stretching of the auricle, the simultaneous registration showed that both compartments were contracting with the same frequency (20 ± 10/min.; Fig. 1Ba).

As we interrupted the luminal connection between the auricle and the ventricle by sealing the AV-valves, the unperfused auricle, still stretched with a filling pressure of 4 cm H_2O, showed an obviously lower frequency (10/min.) than the perfused ventricle (33/min.; Fig. 1, Bb).

The interruption of the ventricular perfusion by closing the posterior aorta with a ligature did not affect the contraction frequency of the auricle (10/min.), but led to a remarkable acceleration of the ventricular frequency (60/min.; Fig. 1, Bc).

Morphological examinations

The light-microscopical examinations revealed that the auricular wall consists of three layers : the epi-, myo– and the endocard. The epicard is built by cubic cells of the coelom epithelium with a continuous lamina basalis, connected to the myocardium by a thin layer of collagenous fibers. The muscle cells of the myocard branch several times and show a clear oblique striation. At the base of the gills, where the auricular valves form a sharp border between the efferent branchial vessel and the auricle, the muscle cells are circularily arranged similar to those whithin the Tunica media of the efferent branchial vessel (Plate Ia). Towards the middle area of the auricle, however, the muscle fibers near the auricular lumen are longitudinally orientated and the outward running fibers show a transversal-circular orientation (Plate Ib). Whithin the auricular myocard next to the ventricle, all muscle fibers show only a longitudinal orientation (Plate Ic). There they insert on a collagenous layer which separates the auricle from the ventricle (Plate Id). This auriculo-ventricular border seems to separate both compartments of the systemic heart completely, because until now the examination of serial longitudinal sections of this area gave no indication that there is a cellular connection between the auricle and the ventricle.

In contrast to the auricular valves, which contain no muscle cells or nerve fibers, the atrioventricular valves are innervated and contain obliquely striated muscle cells which are connected with muscle cells of the ventricular myocard. Light microscopical and SEM examinations of the AV-valves revealed the direct conjunction of the valves with the trabecular muscle bundles of the ventricular myocard (Plate Id-e).

DISCUSSION

Are there pacemaker areas in the coleoid heart?

Proceeding from the hypothesis that the automatic activity of the molluscan ventricle originates in each myocardium (Krijgsman and Divaris 1955; Hill and Welsh 1966), it would be expected that ring-shaped segments, taken from any part of a molluscan ventricle, would show rhythmical contractions if they were isometrically suspended. The results we obtained in our examinations of the cuttlefish ventricle indicate, however, that this assumption seems to be incorrect for the ventricle of *S. officinalis*. Our studies revealed that the central part of the ventricle and the area next to the cephalic aorta showed, with a single exception, no stretch-dependent induction of contractility, i.e. a pacemaker activity within these areas could not be confirmed. These findings seem to contradict the results of Kling (1985), who described a stretch-dependent automatism of the cuttlefish ventricle. But it must be considered that the 'Straub-cannulation' of the ventricle will stretch each part of the organ, also the AV-junction. And here we found a stretch-inductable contractility in our preparations.
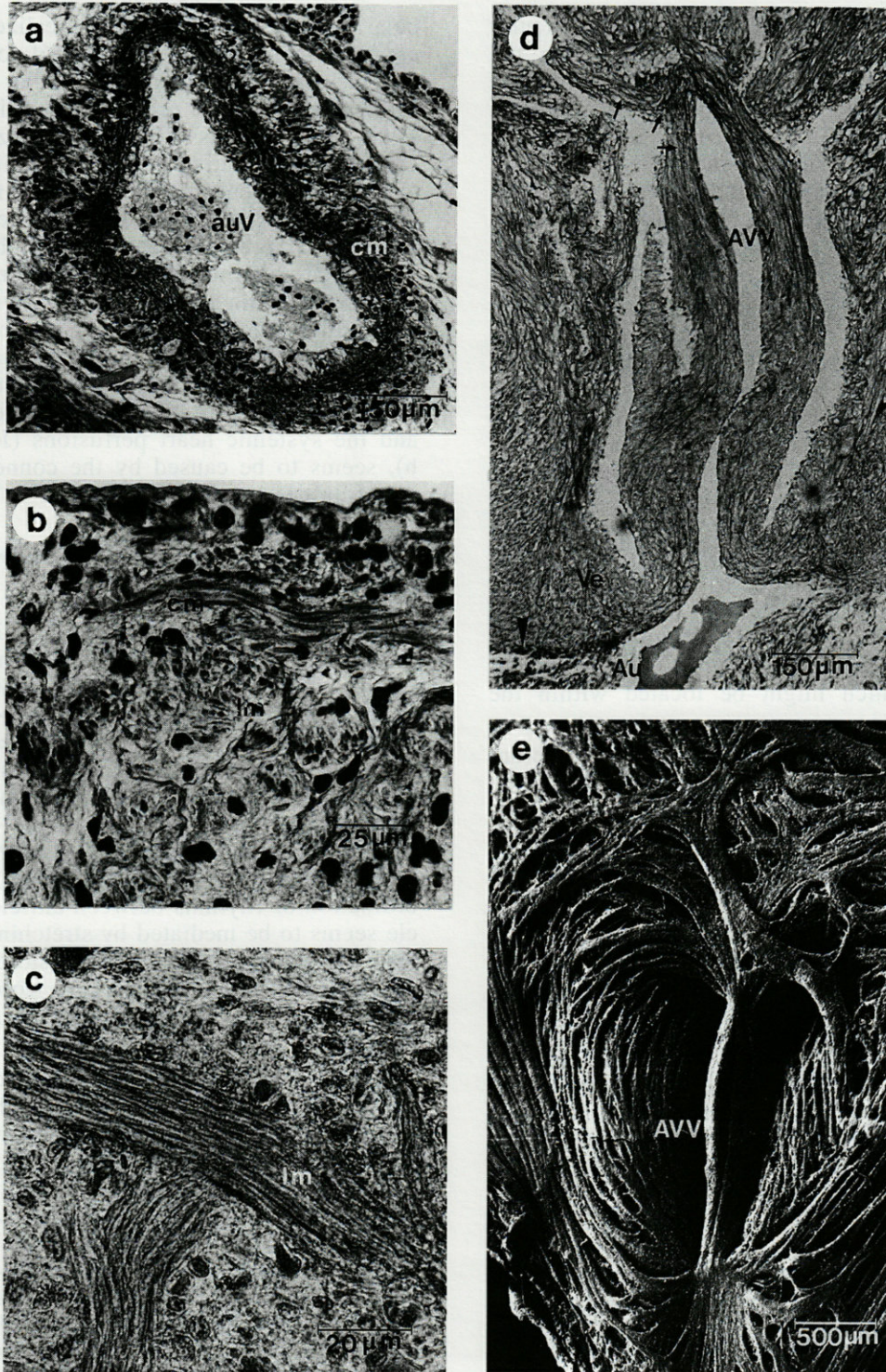
Pl. I. – a, Cross section of the auricular area next to the gills with circularily orientated muscle fibers (cm). The auricular valves (auV) contain no muscle fibers. b, Cross section of the middle area of the auricle with circularily (cm) and longitudinally (lm) orientated muscle fibers. c, Longitudinal section of the auricular area next to the ventricle with longitudinally orientated muscle fibers (lm). d, Longitudinal section of the AV-valves (AVV). A connective tissue layer (➤) separates the auricle (Au) from the ventricle (Ve). The muscle fibers of the valves are connected with the ventricular myocard (→). e, SEM-view of the AV-valves (AVV) and the inner surface of the ventricle. Note the ramifications of the trabecular network and its conjunction with the AV-valves.

Abbreviations: Au, auricle; auV, auricular valves; AVV, Atrio-Ventricular valves; cm, circular muscle fibers; lm, longitudinal muscle fibers; Ve, ventricle.

Since Skramlik (1941) observed a particular sensitivity of the *Octopus* ventricle within the AV-junction, other authors also supposed that the myogenicity of the cephalopod ventricle is not diffuse (Wells 1979; Wells 1983; Smith 1981b; Wells and Smith 1987). Though we are still not able to localize the assumed pacemaker precisely, our results support the hypothesis of Smith (1981b) that the diffuse automatism of the cephalopod ventricle is in fact organized into nodal areas, located in the region of the auriculo-ventricular junctions.

The results of Jakobs (1991a, b) suggest, however, that the ventricular pacemaker only works autonomously if the ventricle is isolated and stretched alone. In complete systemic heart perfusions the ventricles contract with the same frequency as the auricles, but though the same filling pressure was used, obviously slower than in 'Straub-cannulated' preparations. Based on these findings and the observation that also *in vivo* the auricles and the ventricle contract with the same rate of beats, it must be considered that the ventricular automaticity seems to be subordinated to a further pacemaker, which might be located within the auricles.

These connections between the efferent branchial vessels and the ventricle have not been studied as much as the ventricle. Some authors, who observed that isolated oyster auricles are able to beat by themselves with a regular rhythm, concluded that they seem to function as a whole with a similar mechanism as the ventricle (Jullien and Morin 1931; Oka 1932). Our studies revealed, however, that the mechanism of the auricular contractility seems to be different from those of the ventricle. Corresponding to the findings with the oyster auricle, we observed that isolated auricles of the cuttlefish systemic heart show slow regular contractions (3.5 ± 1/min.). But in contrast to the ventricular preparations, each part of the auricular ring-shaped segments contracted without any stretching and the frequency was not accelerated if the preparations were isometrically expanded. The assumption that the missing nerve control might be the reason for the low contraction rate of the isolated auricle was disproved, as we perfused some auricles (n = 5), using the same filling pressure as in the auricle-ventricle examinations. The contraction rate of the perfused auricles was accelerated to the same values (20 ± 10/min.; Versen, 1996 unpubl.) we obtained in the 'open-valve' preparations. In these experiments, the ventricle contracted simultaneously with the same frequency as the auricle, though it was directly stretched with a considerably higher filling pressure than the auricle.

Based on these results it is stated that both compartments of the systemic heart have their own myogenic automatism. But while the ventricular automatism seems to be localized in distinct areas, there are no indications until now, to contradict the assumption that the auricular myogenicity originates from each myocardium (Hill and Welsh 1966). Despite this different organization of the myogenic automatism, the examinations of isolated auricles (Versen, 1996 unpub.) and ventricles (Kling 1985) revealed that in both compartments, the luminal pressure seems to be the mediating factor that coordinates the contraction of the muscle fibers within the auricular as well as in the ventricular myocard. However, the results of the 'open-valve' experiments indicate that the considerable discrepancy between the ventricular frequencies of the 'Straub-cannulated' examinations and the systemic heart perfusions (Jakobs 1991a b), seems to be caused by the connection of the ventricle to a pulsating auricle.

How do the auricles influence the ventricular contractility?

The means by which the auricles influence the ventricular automatism are still being discussed. Based upon morphological examinations, it had been supposed that there might be a cellular conduction pathway between both compartments of the systemic heart (*Sepia*: Jakobs and Schipp, 1992). Examinations of the systemic hearts of gastropods (*Helix*: Willems 1932; *Dolabella*: Matsui 1945) and bivalves (*Crassostrea*: Uesaka *et al*. 1987a, b) indicated, however, that the coordination of rhythms between auricle and ventricle seems to be mediated by stretching. Nevertheless, the morphological and physiological results we obtained in our studies gave good reasons to suppose that there might be a different form of interaction.

The histological examinations indicated that the specific orientation of the muscle fibers within the auricular myocard enables the auricle to transform the peristaltic contractions of the efferent branchial vessel into a contraction mode which creates a kind of a pulse wave. I.e. each auricular contraction pushes the haemolymph with a fast increase of pressure towards the AV-valves, causing an opening of the valves in the ventricular diastole. The SEM-examinations of the AV-valves suggest that an opening of the valves will stretch them as well as the neighbouring ventricular areas. Stretch sensitive cells within this area could create an excitation if the valves were stretched. The multiple ramifications of the ventricular muscle cells and their close coupling would permit the transmission of this excitation from cell to cell, inducing a ventricular contraction wave which originates at the AV-junction (Kling and Schipp 1987).

Though this theory is only based upon histological examinations, nevertheless it corresponds in

many respects with the results of several authors, e.g. the alternate contractions of the auricle and the ventricle (Uesaka *et al.* 1987a, b), the sensitivity of the AV-junction (Skramlik 1941) or the spreading of the ventricular contraction wave (Smith 1981b; Wells and Smith 1987).

Is there a hydro-mechanical interaction between auricle and ventricle?

Because also an electrical- or a stretch-mediated interaction between the two compartments would explain the observations described above, we repeated the auricular-ventricular experiments, with the difference that the AV-valves were sealed with a tissue adhesive. This occlusion of the valves only interrupted the luminal connection between the auricle and the ventricle, while a potential electrical or mechanical interaction should not be affected. The results of these experiments show that though we used the same filling pressures as in the 'open-valve' experiments, the auricle and the ventricle contracted with different frequencies. While the auricular contraction-rate still corresponds with the frequencies we obtained in the isolated auricle perfusions, the perfused ventricle obviously contracted faster than in the 'open-valve' preparations respectively complete systemic heart perfusions (Jakobs 1991a, b). Assuming that the electrical and the mechanical connection between the auricle and the ventricle was not interrupted, it must be supposed that the independent contractility of the auricle and the ventricle in this experiment could only be caused by the occlusion of the luminal connection between the both compartments.

The assumption that only stretching is the mediating factor for the coordinate contractility of the systemic heart (Uesaka *et al.* 1987a, b) does not explain why the auricular frequency was not accelerated, induced retrogradely by the increasing contraction rate of the ventricle, after we closed the posterior aorta with a ligature. The frequencies we recorded here correspond with the ventricular contraction rates of the 'Straub-cannulated' preparations (Kling 1985), indicating that the ventricular frequency is considerably higher if the AV-valves are disabled and the ventricle, or the AV-junction which contains the ventricular input-cannula, is stretched by a constant luminal pressure.

These results suggest that the coordination between the independent automatisms of the auricle and the ventricle is caused by a hydro-mechanical interaction between the compartments. According to our results and the findings of other authors, it is supposed that there is a ventricular pacemaker within the AV-junction, being subordinated to the auricular automatism, which acts as the primary pacemaker of the systemic heart.

Though there are still many questions left which have to be answered for the complete understanding of the cuttlefish systemic heart physiology, the results presented here, substantiate the hypothesis that the auricles are not only connections between the efferent branchial vessels and the ventricle, but have a definite regulatory function in the circulatory system of cephalopods.

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HISTOCHEMICAL DETECTION OF DIFFERENT NEUROTRANSMITTERS IN THE DIGESTIVE TRACT OF *NAUTILUS POMPILIUS* L. (CEPHALOPODA)

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NAUTILUS POMPILIUS
DIGESTIVE TRACT
CATECHOLAMINES
ACETYLCHOLINESTERASE
FMRF-AMIDE

ABSTRACT. – In the organs of the digestive tract of *Nautilus pompilius* catecholamines and serotonin have been shown in the nerve endings by fluorescence histochemical methods. HPLC analysis confirm that noradrenaline and dopamine occur in all organs, while in the midgut gland only adrenaline is found. Using immune-histochemical methods against the tetrapeptide FMRF-amide and serotonin, positive reactions have been demonstrated within terminal nerves of the tunica muscularis of the oesophagus, crop, stomach, caecum, midgut and rectum. Additionally, histo- and cytochemical proof of acetylcholinesterase was found in the tunica muscularis of the oesophagus, crop, stomach, caecum, midgut, and rectum. The findings give evidence that – similar to the coleoids – an antagonistic cholinergic-aminergic neuroregulation, which seems to be modulated by FMRF-amide, also exists in the digestive tract of *N. pompilius*.

NAUTILUS POMPILIUS L.
ACÉTYLCHOLINESTÉRASE
FMRF-AMIDE
CATÉCHOLAMINES
FMRF-AMIDE

RÉSUMÉ. – La présence de catécholamines et de sérotonine dans les formations terminales nerveuses des organes digestifs de *Nautilus pompilius* est démontrée par des méthodes de fluorescence histochimique. La chromatographie liquide à haute pression (HPLC) confirme que la noradrénaline et la dopamine prédominent dans tous les organes, tandis que dans la glande digestive, seule l'adrénaline a été détectée. Des méthodes immuno-histochimiques, permettent de mettre en évidence des réactions positives contre le tétrapeptide FMRFamide et la sérotonine dans les nerfs terminaux de la tunique musculaire de l'oesophage, du jabot, de l'estomac, du caecum, de l'intestin et du rectum. Les réactions histochimiques de l'acétylcholinsetérase sont également positives dans la tunique musculaire de l'oesophage, du jabot, de l'estomac, du caecum, de l'intestin et du rectum. Il existe aussi – comme chez les Coléoidés – une neuro-régulation antagoniste-cholinerque-aminergique du système digestif de *N. pompilius* qui est probablement modulée par le FMRF-amide.

INTRODUCTION

Not many investigations were carried out on the innervation of the digestive tract of *Nautilus pompilius*. Only Young (1987) reports that the oesophagus of *Nautilus* is innervated by sympathic nerves which originate in the buccal ganglion, and there are no findings about the putative neurotransmitters within the terminal nerves of the digestive system of nautiloids. But the digestive tract of coleoid cephalopods was investigated by

topographical (Fage and Racovitza 1913, Alexandrowicz 1928), histochemical, and pharmacological (Andrews and Tansey 1983) methods. These authors found catecholamine fluorescences in the gastric ganglion and in the nerves which innervate the oesophagus, crop and stomach. Furthermore investigations on the innervation of the posterior salivary glands of *Octopus* showed that ^{3}H-labelled serotonin is taken up into nerve endings of the posterior salivary glands, whereas ^{3}H-marked noradrenaline is not taken up (Martin and Barlow 1972). Chiba and Yaku (1979) also found histo-

chemically in the nerve endings serotonin as well as dopamine fluorescences.

Our histological studies on *Nautilus pompilius* show that the digestive tract especially the foregut is densely innervated. For this reason the different organs of the digestive tract are investigated by histochemical methods and the high pressure liquid chromatography (HPLC), and the results are compared with those of the coleoids.

MATERIAL AND METHODS

Three adult *Nautilus pompilius* from the Celebes Sea (Southern Philippines) were used for this study. For the fluorescence and enzyme histochemical investigations tissue samples of the oesophagus, crop, stomach, caecum, and rectum were embedded in Tissue tec® and frozen in liquid nitrogen. The specific acetylcholinesterase (E.C. Nr. 3.1.1.7) has been localized according to the direct thiocholine method of Karnovsky and Roots (1964). For the fluorescence histochemical investigations the unfixed cryostat sections were stained according to Falck and Hillarp (1962) and Barber (1982).

For the immune histochemistry the digestive organs (except the midgut gland) were fixed in Bouin solution without acetic acid (immune reaction against FMRF-amide) and in 4% saline formalin (immune reaction against serotonin), embedded in paraffin (melting point 51°-53°C), and stained according to Van Leeuwen (1986).

For the HPLC-analysis the tissues were portioned and frozen in liquid nitrogen. They were broken down by adding 0,1 M perchloric acid, in which 2,7 mmol EDTA was dissolved (1 000 ml perchloric acid/1 000 mg tissue) followed by ultrasounie treatment. The homogenate was centrifuged (12 000 g, 10 minutes at 4°C), and the supernatant was frozen (Kime and Messenger, 1990). Before preparation the samples were diluted 1 : 10 and 1 : 100 with 1,0 M Tris/HCl buffer, pH 8,6. The preparation of the samples and the eluation of the catecholamines was carried out according to a modified method of Adams *et al.* (1987).

For electron microscopy, small pieces of oesophagus, crop, stomach, caecum, midgut and rectum were fixed in 4% glutaraldehyde in 0,1 M cacodylate buffer at pH 7,4 adjusted to seawater osmolarity (1 000 mOsm) with sodium chloride, postfixed in 1,5% osmium tetroxide in the same buffer, dehydrated in ethanol, and embedded in Epon. Thin sections were stained in uranyl acetate and counterstained with lead citrate (Reynolds, 1963). For the immunocytological studies, small pieces (diameter 1 mm) of the above mentioned organs were fixed in 0,5% glutaraldehyde, postfixed in 1,5% osmium tetroxide, dehydrated, and embedded in LR-White (Griffond *et al.* 1986). The immuncytological analyses were carried out according to a modified method of Varndell *et al.* (1982) using immunogold as tracer. The cytochemical proof of the acetylcholinesterase was carried out according to Karnovsky (1964).

RESULTS

The digestive tract of *Nautilus* consits of the buccal mass, the foregut which is widened to a crop, the stomach, the vestibulum, the caecum, the midgut and the rectum. The caecum is connected with the midgut gland by the ductus hepatopancreas (Griffin 1900 ; Bidder 1966, 1976 ; Boucaud-Camou and Boucher-Rodoni 1983 ; Westermann and Schipp 1992). Light microscopical examinations show that the tunica mucosa is situated luminally and is subdivided into a lamina epithelialis mucosae, a lamina propria mucosae and a lamina muscularis mucosae. The lamina epithelialis mucosae of all organs of the digestive tract is a columnar epithelium containing goblet cells which secrete acid and neutral mucopolysaccharides and glycolipids. The tunica muscularis borders this layer peripherically and is followed by a tunica adventitia consisting of connective tissue. Stomach, midgut, and rectum are surrounded peripherically by an isoprismatic tunica serosa. A tela submucosa, as found in vertebrates, is lacking in nautiloida (Fig. 1). The electron microscopical investigations show a dense innervation of the tunica muscularis of the digestive organs. The terminal axons contain transparent (diameter 55-70 nm) as well as dense cored (diameter 80-100 nm) and osmiophilic vesicles (diameter approx. 130 nm, Fig. 2).

Glyoxylic-induced fluorescences with an emission maximum of 480 nm has been detected within the nerve endings of the lamina propria mucosae and the tunica muscularis in the digestive system as well as in the midgut gland, which indicates catecholamines as neurotransmitters (Fig. 3). In the midgut gland the catecholamine fluorescences could be observed only in the tunica muscularis of the ductus hepatopancreas. Additionally, fluorescences with an emission maximum of 520 nm could be demonstrated in the tunica muscularis, which suggests that serotonin there occurs as neurotransmitter, too. No fluorescences could be detected in the lamina epithelialis mucosae.

The HPLC-analysis showed that mainly dopamine and noradrenaline are found in the oesophagus, crop, and in the stomach, whereas adrenaline predominates in the midgut gland. In the caecum dopamine, adrenaline and noradrenaline were found in almost equal concentrations. In the midgut and rectum dopamine, and adrenaline predominate, while noradrenaline was only found in low concentration (Fig. 5a+b).

Using immune histochemical methods against serotonin (Fig. 4) and the family of the FMRF-amides (Fig. 6a), immune precipitations against both neurotransmitters could be found within the nerves of the tunica muscularis of the oesophagus, crop, stomach, caecum, midgut, and rectum. With the immunogold method, positive immune reac-
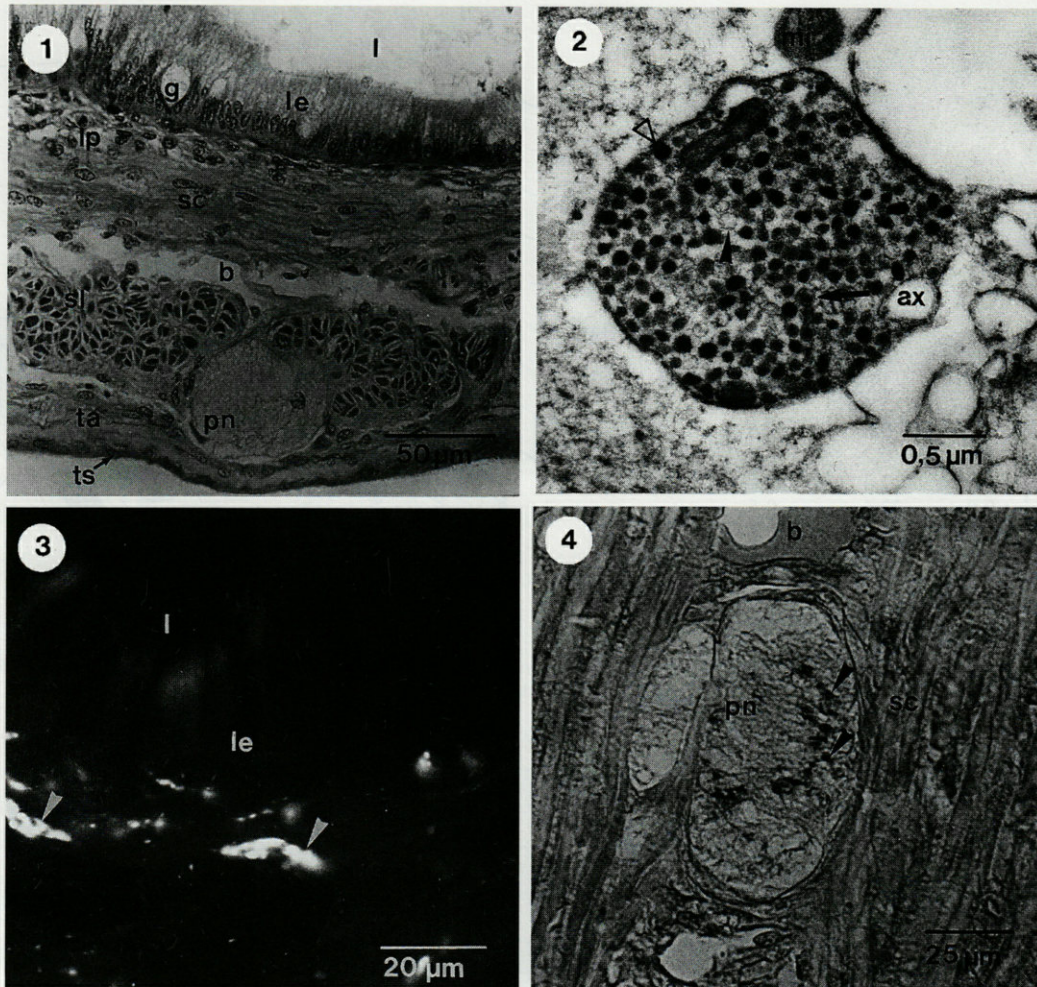
Pl. I. – Fig. 1 : Cross section of the midgut wall of *Nautilus pompilius*.
Fig. 2. – Terminal nerve fibre within the tunica muscularis of the stomach of Nautilus pompilius with (→) dense cored vesicles, (➤) transparent vesicles, (▷) osmiophilic vesicle.
Fig. 3. – Cross section of the crop wall of Nautilus pompilius; (➤) catecholamines fluorescences. tunica serosa.
Fig. 4. – Cross section of the tunica muscularis of the crop wall of Nautilus pompilius; immune reaction against serotonin within nerve fibres (➤).
Abbreviations : ax : axon; bl : blood sinus; g : goblet cell; l : lumen; le : lamina epithelialis mucosae; lp : lamina propria mucosae; mi : mitochondria; my : myofilaments; pn : polyaxonal nerve fibre; sc : stratum circulare; sl : stratum longitudinale; ta : tunica adventitia; tm : tunica muscularis; ts : tunica serosa.

tions against the tetrapeptide FMRF-amide have also been detected cytologically within the axons of the lamina propria mucosae and the tunica muscularis (Fig. 6b). Enzyme histo– and cytochemical reactions of the specific acetylcholinesterase yielded positive results in the tunica muscularis of all organs of the digestive tract of *N. pompilius* (Fig. 7a+b).

DISCUSSION

According to our findings in the neuromuscular systems of the digestive tract of *N. pompilius* probably an antagonistic cholinergic aminergic

transmitter mechanism exists, in which FMRF-amide may play a modulating role.

Our examinations show that the catecholamines and serotonin found in all organs of the digestive tract, can probably be related to the dense cored vesicles with a diameter of 80 to 100 nm, detected in the lamina propria mucosae and in the tunica muscularis, and according to Dorsett (1986) dense cored vesicles of this size store monoamines, especially catecholamines. In the lamina epithelialis mucosae of the examined organs of the digestive tract of *N. pompilius*, however, fluorescences could not be observed. It can be assumed that there are no enterochromaffine cells which can be demonstrated in the posterior salivary glands of

5b

5a

Organ	Noradre-naline	Adrena-line	Dopa-mine
Oesophagus	119,0	1,6	141,1
Crop	8,6	0,0	69,5
Stomach	4,2	0,2	8,0
Caecum	2,8	2,4	7,6
Midgutgland	0,0	22,4	0,5
Midgut	1,4	3,8	7,6
Rectum	1,6	8,8	36,3

ng/(g tissue)
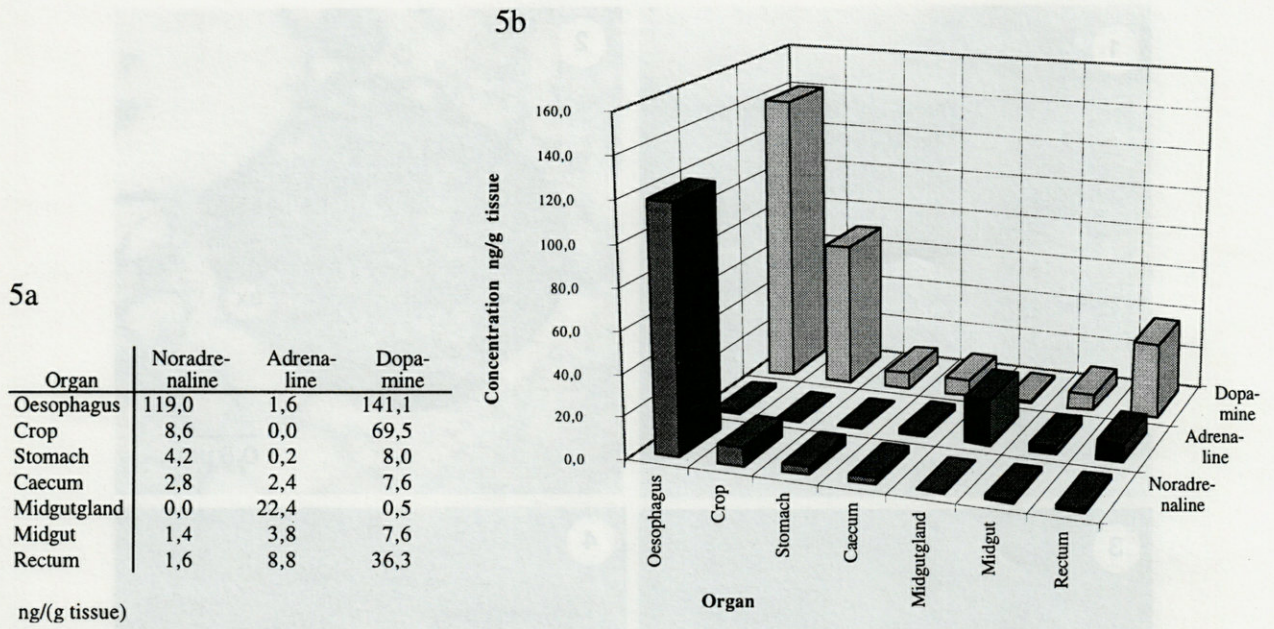
Fig. 5. a+b. – Table of catecholamine content in the digestive tract of *Nautilus pompilius*; b, diagram of the distribution of the catecholamines in the digestive tract of *N. pompilius*.
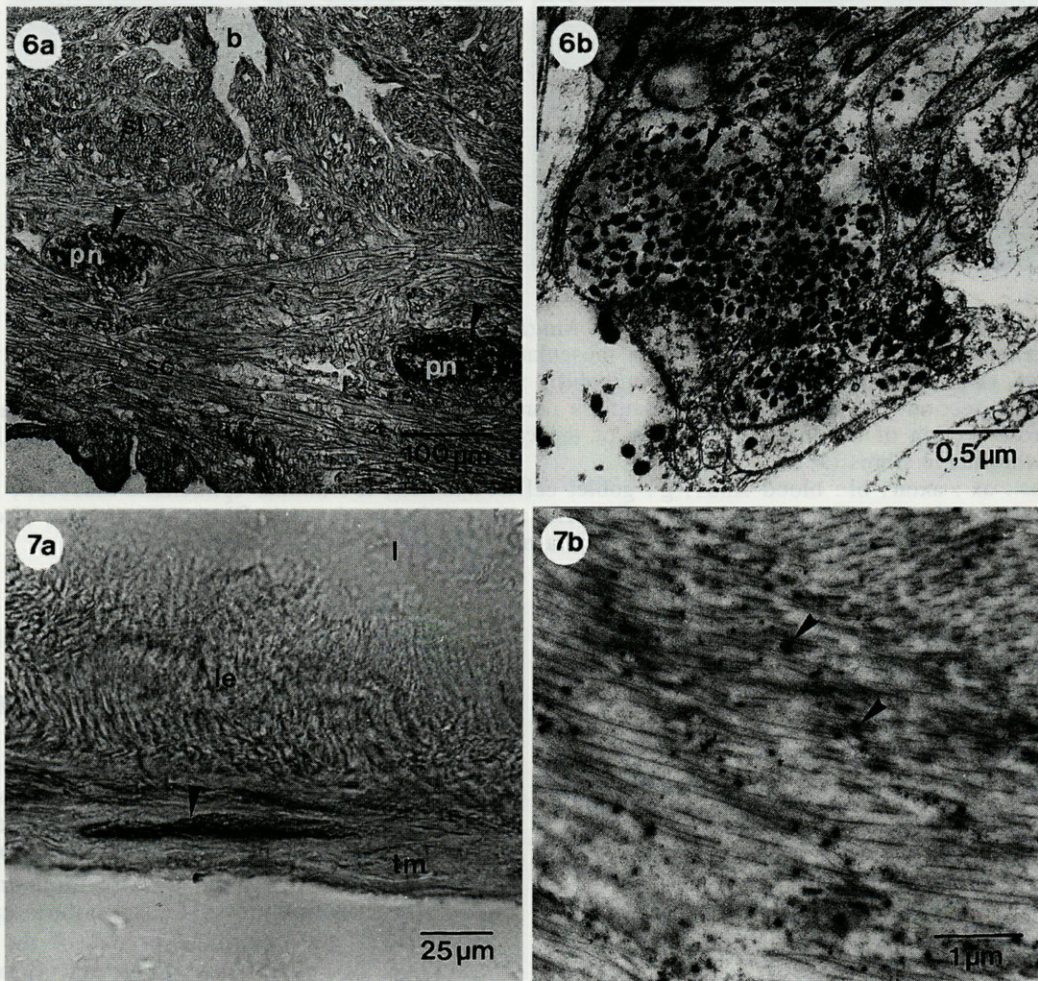
Pl. II. – Fig. 6. Immune reaction against FMRF-amide in the digestive tract of *Nautilus pompilius*. a, PAP method, (➤) immune precipitates within polyaxonal nerves in the tunica muscularis of the oesophagus. b, immunogold method, single axon in the tunica muscularis of the rectum with (→) dense cored, vesicles, (➤) transparent vesicles and (▷) immune precipitates.

Fig. 7 a+b. – Acetylcholinesterase activity (➤) in the tunica muscularis of a, the caecum (lightmicroscopical) and b, the rectum (electronmicroscopical).

the octopods (Chiba and Yaku 1979). These findings are in agreement with those of Andrews and Tansey (1983). They also found catecholamines in the gastric ganglion and in the nerves, which innervate the oesophagus, crop, and stomach of *Octopus vulgaris*, whereas none was found within the wall of the caecum.

Our HPLC-analysis showed that in the midgut gland adrenaline predominates (22,4 ng/g); scarce amounts of dopamine (0,5 ng/g) and noradrenaline have been found. The fluorescence histochemical examinations showed that catecholamine fluorescences only exist in the tunica muscularis of the ductus hepatopancreas, but not in the glandular tissue. So an adrenoendocrine function of the midgut gland can be ruled out.

In addition, the high content of noradrenaline (119 ng/g) and dopamine (141,1 ng/g) is conspicuous in the oesophagus. This confirms the high innervation density of this organ already shown by lightmicroscope. In all other organs dopamine is located in a higher concentration than noradrenaline and adrenaline.

The comparison of the digestive tracts of *Octopus vulgaris* (Juorio and Killick 1973) and *N. pompilius* shows that in both species dopamine and noradrenaline have the highest concentration in the oesophagus. In the oesophagus and crop of *O. vulgaris* the content of noradrenaline is three times higher than that of dopamine, while in the foregut of *N. pompilius* dopamine predominates. In all other organs of the digestive tract (stomach, caecum, and intestine) of both species dopamine and noradrenaline exist in approximately the same concentration.

So the HPLC-analyis confirms the fluorescence histochemical results that in the organs of the digestive tract of *N. pompilius* noradrenaline or dopamine act as a putative neurotransmitter.

Using immune-histochemical methods positive reactions against FMRF-amide and serotonin in the polyaxonal nerves of the tunica muscularis of the digestive tract of *N. pompilius* could be shown. In regard to the immune reaction against FMRF-amide, however, it has to be pointed out that this reaction is not only directed against this peptide, but also against all amides with the sequence FM at their c-terminal end. Also immune reactions against FMRF-amide in the axons of the polyaxonal nerves in the tunica muscularis of the digestive tract could be observed. Furthermore, the electron microscopic findings showed that osmiophilic vesicles (diameter approx. 130 nm) exist in the axons of the polyaxonal nerves of the lamina propria mucosae and the tunica muscularis. According to Dorsett (1986), these are peptide storing vesicles. Based on our findings it can be assumed that FMRF-amide as well as serotonin represent putative neurotransmitters in the nerve endings. Also in the nerve endings of the midgut

gland (Ruth, 1993) and the tunica adventitia (Kleemann, 1994) of *Nautilus* immune reactions against FMRF-amide could be shown as well, whereas they could not locate serotonin in these organs.

Peptides also take part in the neuroregulation of the circulation of the coleoids. In the neurosecretorial nerve endings of the vena cava of *Octopus vulgaris* peptides of the FMRF-amide family were shown (Martin *et al.* 1981). By immune histochemical methods FMRF-amide could be located in the nerve fibres of the aorta cephalica (Schipp *et al.* 1991), the nervus cardiacus, the lobus visceralis, and the vena cava of *Sepia officinalis* (Jakobs 1991). Pharmacological investigations on the branchial hearts of *S. officinalis* show that FMRF-amide has any effect on the branchial heart beat when applied alone, but it counteracted the positive inotropic actions of noradrenaline (Fiedler 1992). On the smooth muscle of *Mytilus edulis* FMRF-amide (10-6 M) enhanced the contractions produced by doses of acetylcholine below the ED_{50}, but had no effect on contractions produced by acetylcholine at doses greater than the ED_{50} (Raffa and Bianchi 1986). Low concentrations of FMRF-amide (0,5 nM and above) induce rhythmic contractions of the tentacle retractor muscle of *Helix* (Cottrell *et al.* 1983). Cottrell *et al.* (1990) could also show that the neuropeptide FMRF-amide activates a ligand-gated ion channel in the neurons of *Helix* and they suppose that FMRF-amide is a depolarizing neurotransmitter.

Tansey (1980) describes acetylcholinesterase activity in the nerves of the digestive tract of *Octopus*. This enzyme could also be located in the tunica muscularis of the digestive tract of *Nautilus pompilius*, and transparent vesicles with a diameter of 50 to 70 nm were found by electron-microscopical examinations in the lamina propria mucosae and in the tunica muscularis of the digestive organs. According to Dorsett (1986) the transparent vesicles contain the transmitter acetylcholine. The proof of the acetylcholinesterase in the digestive tract of *N. pompilius* indicates that acetycholine acts as a neurotransmitter.

As these results base only on histochemical methods, pharmacological studies on the digestive tract of *N. pompilius* have to follow to get more detailed evidences about the effects of the different transmitters.

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STRUCTURE OF THE SO-CALLED OLFACTORY ORGAN OF OCTOPODS AFTER HATCHING : EVIDENCE FOR ITS CHEMORECEPTIVE FUNCTION

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ELEDONE MOSCHATA
OCTOPUS VULGARIS
OLFACTORY ORGAN
HATCHING
ULTRASTRUCTURE

ABSTRACT. – The structure and histology of the so-called olfactory organ are characterised in octopods, for both the benthic (*Eledone moschata* Schneider) and the planktonic (*Octopus vulgaris* Cuvier) developmental type. At the adult-like hatching stage of *E. moschata* the pseudostratified epithelium of the already pit-shaped olfactory organ consists of two epithelial and three sensory cell types. In contrast to its shape at the hatching stage the organ of *O. vulgaris,* with a brushborder of microvilli and cilia, is only at its juvenile stage (planktonic larva) becoming pit-shaped and there composed of one epithelial and four sensory cell types. At hatching the organ is more developed in species of the benthic than of the planktonic developmental type. Strong evidence for the chemoreceptive function of this organ is provided.

ELEDONE MOSCHATA
OCTOPUS VULGARIS
ORGANE OLFACTIF
ÉCLOSION
ULTRASTRUCTURE

RÉSUMÉ. – Les caractéristiques de la structure et de l'histologie du "dit" organe olfactif sont mises en évidence pour les deux types de développement chez les Octopodes, le type benthique (*Eledone moschata* Schneider) et le type planctonique (*Octopus vulgaris* Cuvier). À l'éclosion les jeunes d'*E. moschata* sont déjà semblables aux adultes. L'organe olfactif présente une dépression ; l'épithélium pseudo-stratifié est composé de deux types de cellules épithéliales et de trois types de cellules sensorielles. Contrairement à son aspect à l'éclosion, l'organe olfactif d'*O. vulgaris,* recouvert par des cils et des microvillosités, présente une dépression seulement au stade juvénile (larve planctonique) ; l'épithélium comprend un type de cellules épithéliales et quatre types de cellules sensorielles. À l'éclosion l'organe olfactif est plus développé chez les espèces au développement benthique que chez les espèces au développement planctonique. La fonction chémoréceptive de cet organe est démontrée.

INTRODUCTION

The so-called olfactory organ of dibranchiate cephalopods, situated behind the eyes, has been well described for the decapods *Lolliguncula brevis* (Emery, 1975), *Loligo vulgaris* (Wildenburg and Fioroni, 1989) and *Sepia officinalis* (Wildenburg 1990). Ultrastructural studies of the organ in adult octopods were performed by Woodhams and Messenger (1974, *Octopus vulgaris*) and Emery (1976, *Octopus joubini*) and briefly by Wildenburg (1995, *Octopus vulgaris, Eledone moschata*). The present study characterises, for the first time in detail, its structure and histology in octopods after hatching, both for the benthic (*Eledone moschata* Schneider) and the planktonic (*Octopus vulgaris* Cuvier) developmental type.

MATERIALS AND METHODS

Specimens, identified according to Naef (1921/28), originated from the Laboratoire Arago at Banyuls-sur-mer (France), where their spawn was collected. Part of it was reared in Münster (Germany) using artificial seawater.

For LM and TEM the embryos were fixed for 1.5 h at 4°C in 2 % OsO_4 dissolved in $K_2Cr_2O_7$ and 70 % filtered seawater (1:9 ; pH 7.4), dehydrated via graded ethanol series and embedded using Spurr's medium. Ultrathin sections, stained with 1 % lead citrate, were examined in a TEM Phillips 201. For SEM specimens were fixed in Bouin's solution, dehydrated via graded ethanol series, critical-point dried with CO_2, sputtered with gold and viewed in a Hitachi SEM H-530.

Numbering of cell types is after Wildenburg and Fioroni (1989) and after Wildenburg (1990).

RESULTS

At the adult-like hatching stage of *E. moschata* the big, already pit-shaped olfactory organ (Fig. 1A, B) is situated at the mantle-derivation at the 'neck' of the hatchling. The organs' pseudostratified epithelium consists of two epithelial and three sensory cell types (Fig. 4A). In contrast to its oval shape at the hatching stage (Fig. 1C) the organ of *O. vulgaris* becomes pit-shaped (Fig. 4C) at its juvenile stage (planktonic larva). It is covered by a brushborder of microvilli and by cilia (Fig. 1D) and composed of one epithelial and four sensory cell types (Fig. 4B).

Epithelial cells are interspersed between the sensory cells. Cells of type 1 in *E. moschata* (Figs. 2A, 4A) as well as in *O. vulgaris* (Fig. 4B) have a continuous apical seam of microvilli, but lack cilia, in contrast to cells of type 2 (Fig. 2B, 4A), which do not occur in *O. vulgaris*.

In both species most receptors have some kind of ciliated cavity, varying from a small distal pocket to a spacious ciliated cave. Sensory cell type 1 (Fig. 3B, 4A(c), B(b)), very similar in both species, is apically equipped with a small pocket of cilia, which opens to the surface of the organ via a porus. Sensory cell type 2 appears in two variations in the organ of *E. moschata* at hatching. Type 2a (Fig. 4A(d)) shows a large ciliated cavity, which is filled with a dense matrix. Cilia project from all sides towards the centre of the cavity, and microvilli are found between their bases. Type 2b is very similar, but the cavity is sealed by a dense granule, which is sometimes penetrated by a few cilia. This receptor lies in part below the basal lamina (Figs. 3A, 4A(e)). In the planktonic *O. vulgaris* this cell type occurs only above the basal lamina and is never provided with a granule (Fig. 3B, 4B(c)). Sensory cell type 4 of the hatched *E. moschata* (Fig. 2C, 4A(f)) is located completely in a subepithelial position between assemblages of axons and blood vessels, and type 4' of the juvenile *O. vulgaris* (Fig. 4B(d)) lies mainly subepithelially and is probably connected to the surface of the organ via a narrow isthmus and a small cilia pocket. Both possess an oval, spacious ciliated cavity with an osmiophilic matrix. In *O. vulgaris* the cavity is partly divided by cytoplasmic protrusions like in sensory cell type 2b (Fig. 3A) of *E. moschata*. In the juvenile *O. vulgaris* a unique sensory cell type with one kinocilium and several stereocilia (type 5; Fig. 3C, 4B(e)) occurs, mainly in the marginal areas of the olfactory pit (Fig. 4C).

DISCUSSION

The epithelial cell types and sensory cell type 1 described here resemble very much the respective cell types in the decapods (Wildenburg and Fioroni 1989; Wildenburg 1990). Also sensory cell type 2 and its variations seem to be a common receptor type for deca- and octopods.

In contrast to the decapods there are also subepithelial cell types in the octopod olfactory organ. Similar receptors like the subepithelial sensory cell types 4 (*E. moschata*, at hatching) and 4' (*O. vulgaris*, planktonic) have been detected in other molluscs (e.g. Crisp 1971; Zylstra 1972). The function of their large ciliated cavities, which are situated far from the surface of the organ, is still unknown. These cell types could represent ontogenetic stages of other sensory cell types.

Sensory cell type 5, only occurring in the planktonic *O. vulgaris*, probably represents a mechanosensitive cell type (Barber 1974), suggesting a double function of the olfactory organ in the planktonic *O. vulgaris*.

At hatching the olfactory organ of both decapods and octopods, is further developed in species of the benthic than of the planktonic developmental type as shown by the already pit-shaped organ of the bottom living adult-like hatchlings of the benthic developmental type e.g. *Eledone moschata*, *Sepia officinalis* (Wildenburg 1990), in contrast to the bulging organ of the pelagic larvae of the planktonic developmental type e.g. *Octopus vulgaris*, *Loligo vulgaris* (Wildenburg 1989). This is in agreement with the different hatching states of the different cephalopod species and can be interpreted as an adaptation of the hatched animal to the specific environmental conditions during the post-embryonic time (Fioroni 1977). In principle, the yolk content of the eggs determines the developmental type. Yolk richness leads to the benthic and a low amount of yolk to the planktonic developmental type (Fioroni 1977).

Given the structural aspects, a detailed comparison with chemosensitive organs of other molluscs (e.g. Crisp 1971; Bonar 1978; Chia and Koss 1984; Haszprunar 1985a, b) as well as results of behavioural (Otis and Gilly 1990; Gilly and Lucero 1992) and physiological experiments by other authors (Brismar and Gilly 1987; Gilly and Brismar 1989; Lucero *et al.* 1992), this organ appears to have mainly a chemoreceptive function.
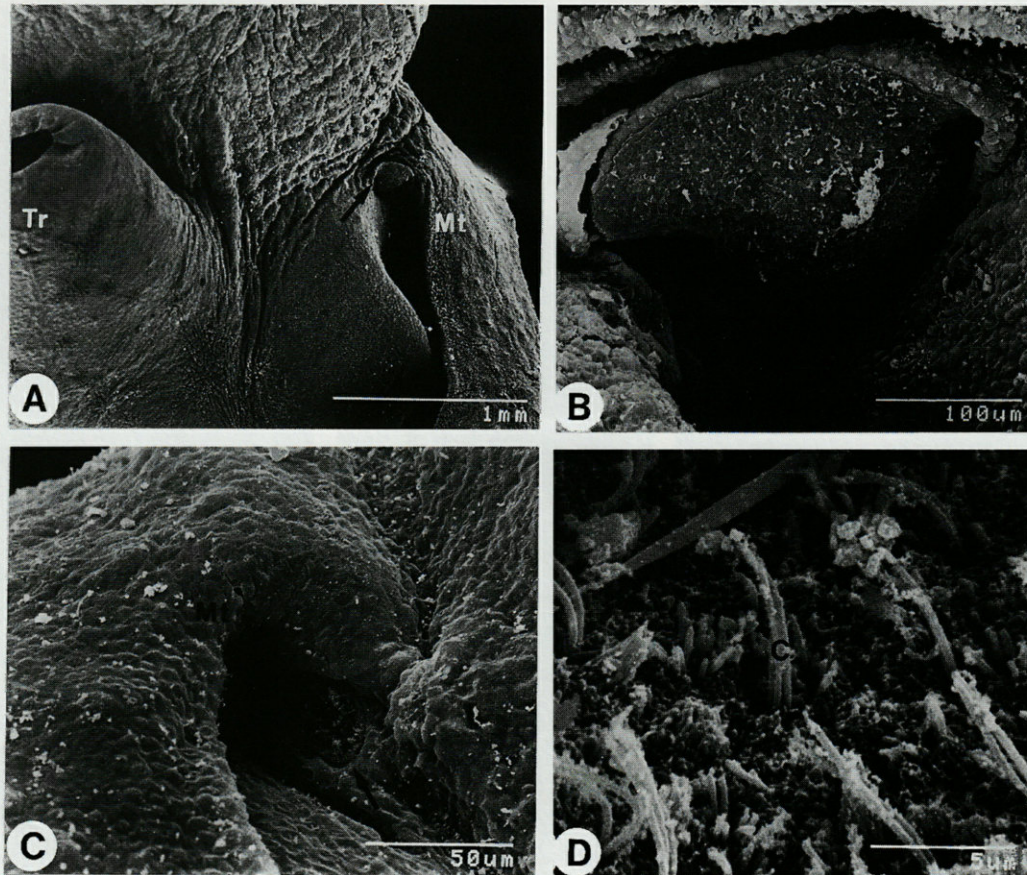
Fig. 1. – SEM pictures of the olfactory organ in octopods at the hatching stage. A, Ventral view of *E. moschata*, the olfactory organ (arrow) is situated at the mantle-rim (Mt) in the 'neck' area; Tr funnel. B, *E. moschata*, surface of the olfactory organ. C, Position of the oval olfactory organ (arrow) of *O. vulgaris* in the area of the mantle-rim (Mt) at the 'neck'. D, *O. vulgaris*, detail of the surface of the organ, cilia (C) originating in the depth of the cells.
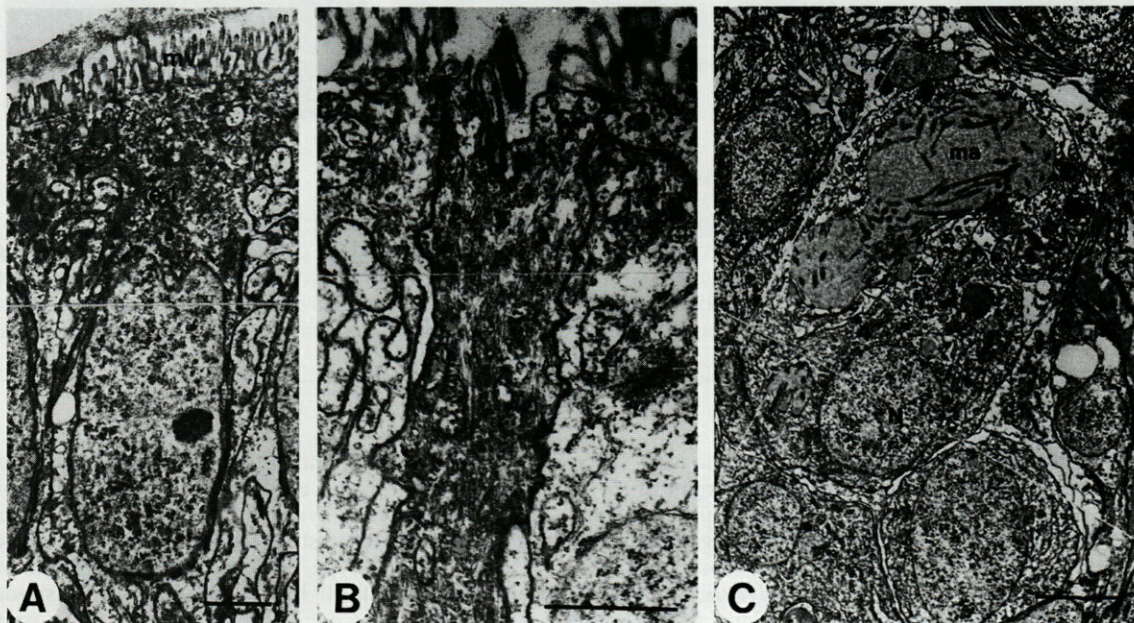
Fig. 2. – TEM pictures of cell types of the olfactory organ of *E. moschata* at hatching. A, Epithelial cell type 1 (e1), only with microvilli (mv); scale bar 2 μm. B, Apical part of epithelial cell type 2, with cilia; scale bar 2 μm. C, Completely subepithelial sensory cell type 4, ma matrix, N nucleus; scale bar 5 μm.
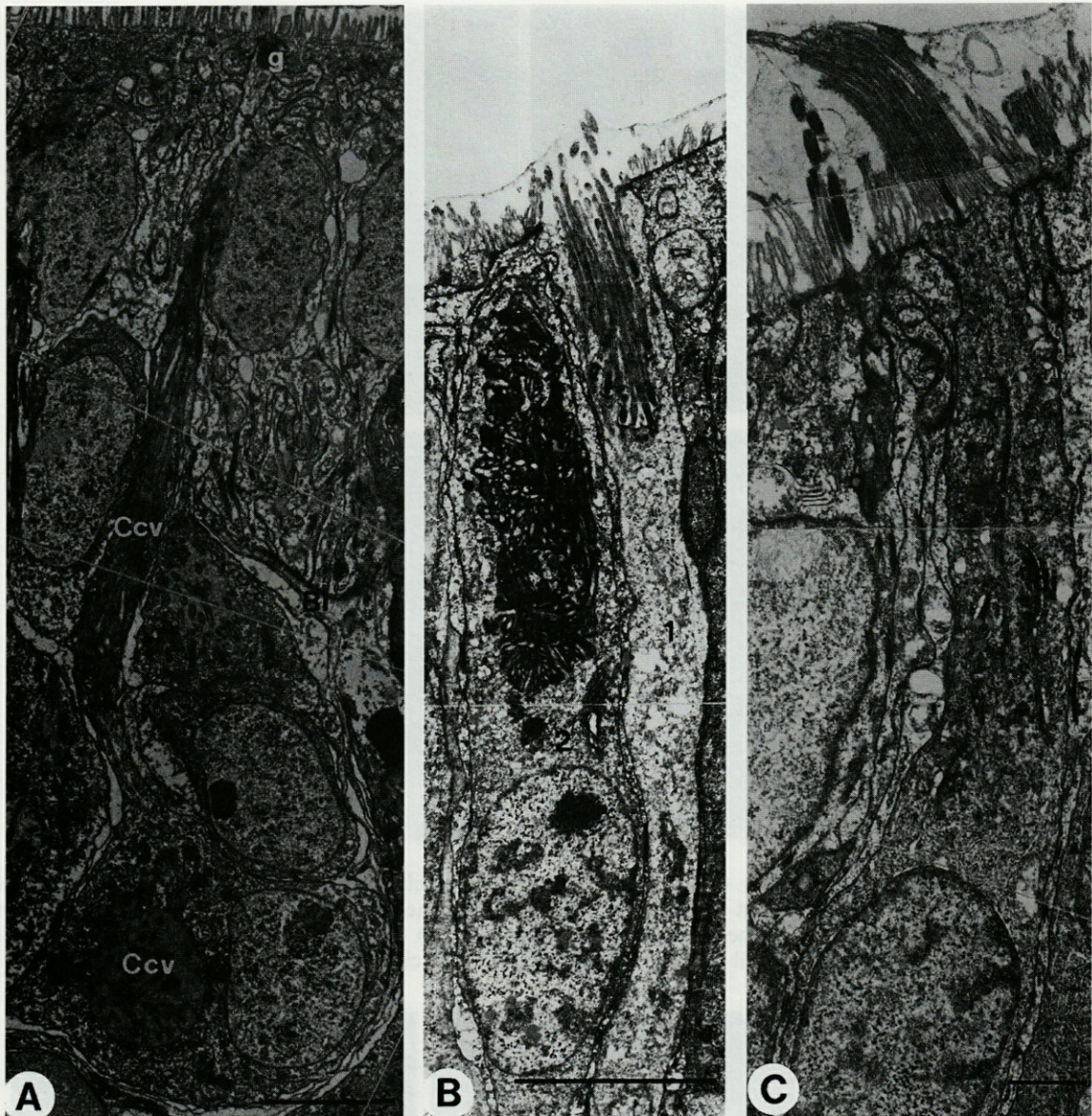
Fig. 3. – TEM pictures of the sensory cell types in octopods. A, *E. moschata* at hatching, sensory cell type 2b, in part below the basal lamina (Bl). The ciliated cavity (Ccv) seems to be divided by cytoplasmic parts, note the granule (g) at the apical part; scale bar 5 μm. B, *O. vulgaris* at the planktonic stage, sensory cell type 1 with an apical cilia pocket and type 2 with a spacious ciliated cavity; scale bar 10 μm. C, *O. vulgaris* at the planktonic stage, sensory cell type 5, cell apex with one kinocilium and several 'stereocilia' (microvilli), di dictyosome; scale bar 2 μm.

Fig. 4. – Diagrammatic representations of the cell types and composition of the olfactory organ in octopods. A, Epithelial and sensory cells in the olfactory organ of *E. moschata* at hatching; a) epithelial cell type 1; b) epithelial cell type 2; c) sensory cell type 1; d) sensory cell type 2a; e) sensory cell type 2b; f) sensory cell type 4. B, Epithelial and sensory cells in the olfactory organ of *O. vulgaris* at the planktonic stage; a) epithelial cell; b) sensory cell type 1; c) sensory cell type 2; d) sensory cell type 4; e) sensory cell type 5. C, Schematic representation of the olfactory organ of planktonic *O. vulgaris*, scale bar 20 μm. Ax axon(s), Bl basal lamina, bmv brushborder of microvilli, bv blood vessel, C cilium, Ccv ciliated cavity, cf ciliary foot, Cp ciliated pocket, cr cilia rootlet, di dictyosome, el epithelial cell, Ep epidermis, F filaments, g granule, gly glycogen, I isthmus, M mitochondrium, ma matrix, Mb microtubuli, mv microvilli, N nucleus, nu nucleolus, po porus, r ribosomes, rER rough endoplasmatic reticulum, St 'stereocilia', V vesicle, 1 sensory cell type 1, 2 sensory cell type 2, 4' sensory cell type 4', 5 sensory cell type 5.
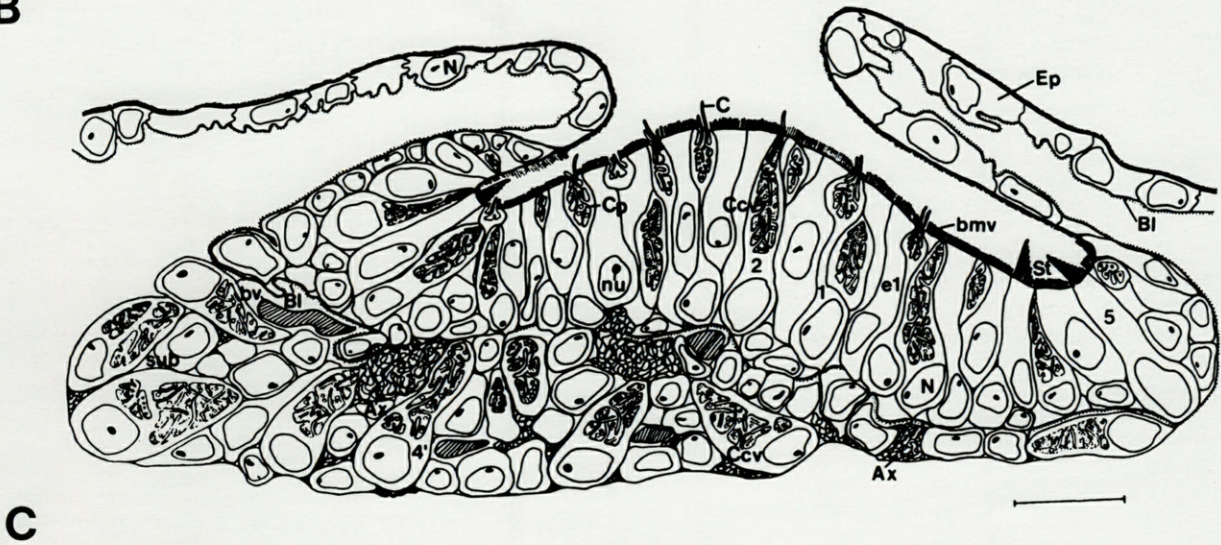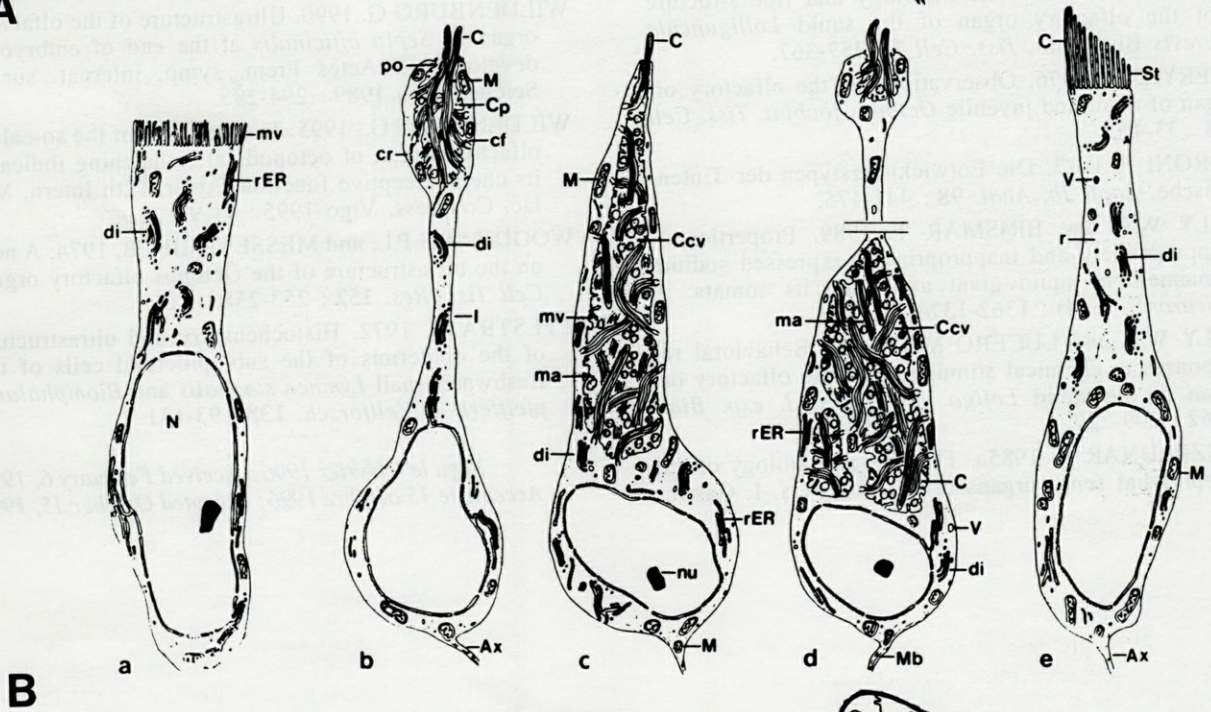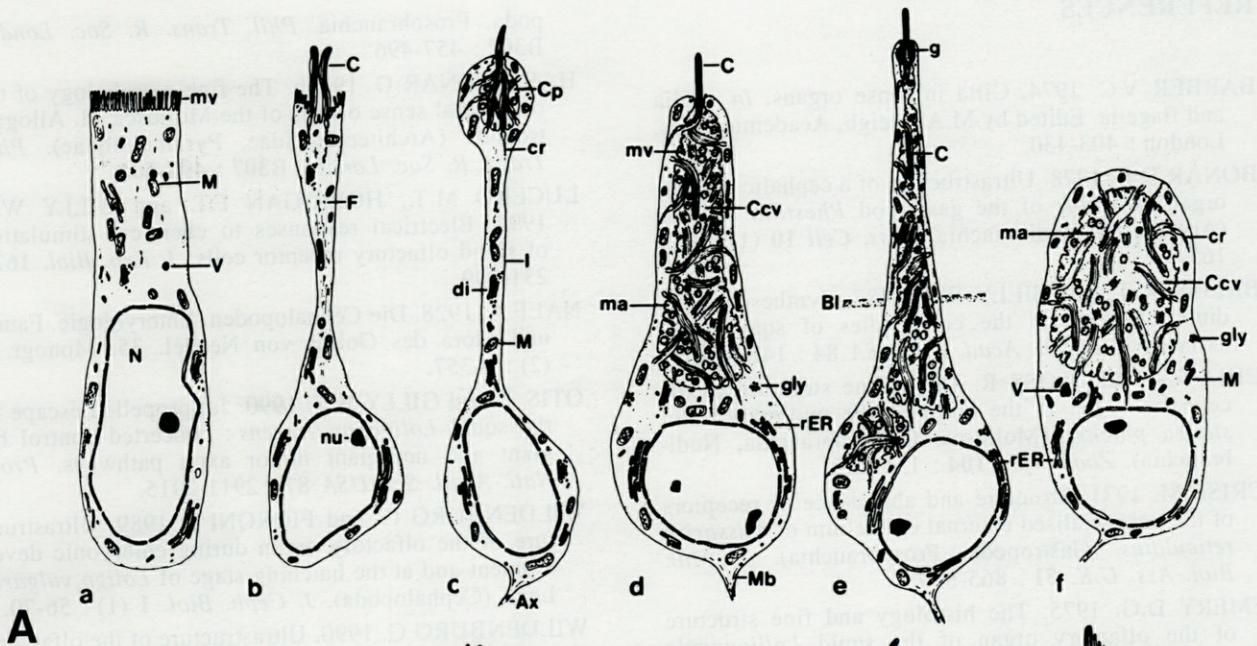
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CILIA IN THE EPIDERMIS OF LATE EMBRYONIC STAGES AND PARALARVAE OF *OCTOPUS VULGARIS* (MOLLUSCA : CEPHALOPODA)

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OCTOPUS VULGARIS
PARALARVAE
EPIDERMIS
CILIATED CELLS

ABSTRACT. – Scanning electron microscope analyses have shown ciliated cells in the epidermis of paralarval *Octopus vulgaris* Cuvier, 1797 in contrast to earlier studies that claimed the existence of these cell types only in decapods. Ciliated cells occur either in special arrangements (in the epidermal lines, the olfactory organ, the funnel pockets, and the external yolk sac) or randomly scattered over the body surface (on the arms, the suckers, the head, the funnel, and the mantle). Devoid of cilia are the growing tips of the arms, the cornea, the margin of the eye, the funnel aperture, and the inner side of the mantle. The functions of the previously described cell types are listed and possible functions of the newly described cells are discussed.

OCTOPUS VULGARIS
PARALARVES
ÉPIDERME
CELLULES CILIÉES

RÉSUMÉ. – Les analyses faites au microscope électronique à balayage ont montré que l'épiderme paralarvaire d'*Octopus vulgaris* Cuvier, 1797 contient des cellules ciliées, contrairement à ce qui a été dit dans les publications antérieures qui prétendaient que ce type de cellule ne se trouve que chez les Décapodes. Les cellules ciliées sont soit disposées de manière particulière (lignes épidermiques, organe olfacteur, poches de l'entonnoir, sac vitellin externe) soit dispersées à la surface du corps (bras, ventouses, tête, entonnoir, manteau). Les extrémités des bras, la cornée, le pourtour des yeux, l'ouverture de l'entonnoir, et la face intérieure du manteau sont dépourvus de cils. Les fonctions des types de cils déjà décrits sont rappelées, et les fonctions éventuelles des cellules ciliées décrites pour la première fois sont discutées.

INTRODUCTION

Studies on integument cells of embryonic cephalopods have mostly concentrated on specialized cell types (Fioroni 1962, 1963; Boletzky 1982). As to the presence of ciliated cells, the epidermis of octopod embryos – in contrast to the one of decapod embryos – was regarded to be devoid of ciliated cells the only exception being the external yolk sac, the suckers, and the olfactory organ (Boletzky 1982; Boletzky and Fioroni 1990; Fioroni 1962, 1963, 1978; Graziadei 1964; Woodhams and Messenger 1974; Packard 1988). Scanning and transmission electron micrographs, however, clearly show that ciliated cells do occur

in the epidermis of late embryonic stages and paralarvae of *Octopus vulgaris* (see also Lenz *et al.* 1995).

MATERIAL AND METHODS

Late embryonic stages and paralarvae of *Octopus vulgaris* from the western Mediterranean Sea (Banyuls-sur-Mer, Villefranche-sur-Mer) were fixed with Bouin solution for 24 hours, with 2 % osmium-tetroxide (OsO$_4$) dissolved in 0.5 % K$_2$Cr$_2$O$_7$ in 70 % sea water at 4°C or with 2.5 % glutaraldehyde dissolved in 90 % sea water at room temperature for 1.5 hours at 4°C. For scanning electron microscopy (SEM) specimens

were dehydrated in graded ethanol solutions, critical point dried (with CO_2) and gold-coated. Measurements of ciliary length were made on SEM pictures; therefore, values indicate minimal length.

RESULTS

The ciliated cells in the epidermis of *Octopus vulgaris* occur either in the epidermal lines or in special arrangements on the external yolk sac, in the olfactory organ, and on the funnel pockets, or as individual cells on the arms, on the suckers, on the head, and on the mantle (Fig. 1-3).

Epidermal lines

In the epidermal lines the ciliated cells (primary sensory cells) are located on the arms, head, mantle and funnel (Fig. 1, 2B, C). The dorsal, dorsolateral, ventrolateral and ventral lines are paired, i.e. occur on either side of the head and on the left and right arms, whereas there is only one in the middle of the funnel. The ciliated cells of the epidermal lines have an elongated apical surface bearing up to 6 long (10 µm) cilia and short microvilli. These ciliated cells are the most conspicuous on the embryo due to their ciliary length and their linear arrangement.

The number and density of the ciliated cells differ on the arms, head, and mantle. In the arm lines they occur in greater distances to each other, whereas on the head, especially around the eyes, the cells of the dorsolateral and ventrolateral lines occur in broad bands or multiple rows (between 3 and 6). In the dorsal lines the ciliated cells are not separated but arranged in a continuous line from the bases of the arms towards the end of the lines (Fig. 2B, C). The funnel line has the most complex structure of all epidermal lines. Cross-sections show two or three accessory cells in the centre of the line; their long microvilli can be seen in scanning electron micrographs (Fig. 1B). The accessory cells are lined by one or two ciliated cells on each side and corresponding ciliated cells occur laterally at a short distance. The parallel arrangement of ciliated cells in a double row in the funnel line is probably related to the embryonic development of the funnel tube, which arises from two initially separated funnel folds. Thus, the funnel line can be regarded as an originally paired structure.

Single ciliated cells and group-arranged ciliated cells

Additionally to the ciliated cells of the epidermal lines, there are single ciliated cells scattered all over the body surface and other group-arranged ciliated cells (Fig. 2, 3). They occur on the exter-
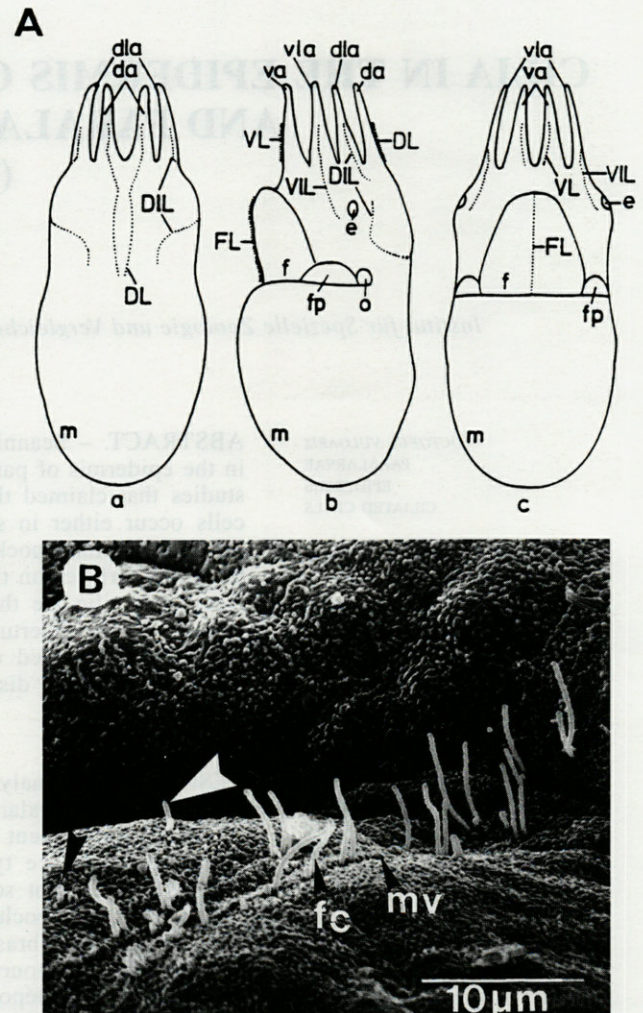
Fig. 1. – Epidermal lines of *O. vulgaris* paralarva. A, Schematic drawing; dotted lines indicate course of epidermal lines (from left to right: dorsal, lateral, ventral). B, Funnel line (SEM). Note the microvilli (mv) of the accessory cells and the ciliated cells in the immediate neighbourhood to the line (Black and white arrow heads on the left).
Abbreviations: a arm, c cilia, da dorsal arm, DL/dl dorsal line, dla dorsolateral arm, DlL/dll dorsolateral line, e eye, f funnel, fc ciliated cells of the funnel line, FL funnel line, fp funnel pocket, h head, kb Kölliker's bristle, m mantle, me mantle edge, mv microvilli, o olfactory organ, sc single ciliated cell, va ventral arm, VL ventral line, vla ventrolateral arm, VlL/vll ventrolateral line, y external yolk sac.

nal yolk sac, the arms, the suckers, the head, the olfactory organ, the funnel, the funnel pockets, and the mantle. The ciliated cells on the suckers and the olfactory organ are described elsewhere (Graziadei, 1964; Woodhams and Messenger, 1974).

The epithelium of the external yolk sac consists of cells that bear very numerous cilia during the rotation of the embryo (Boletzky and Fioroni,
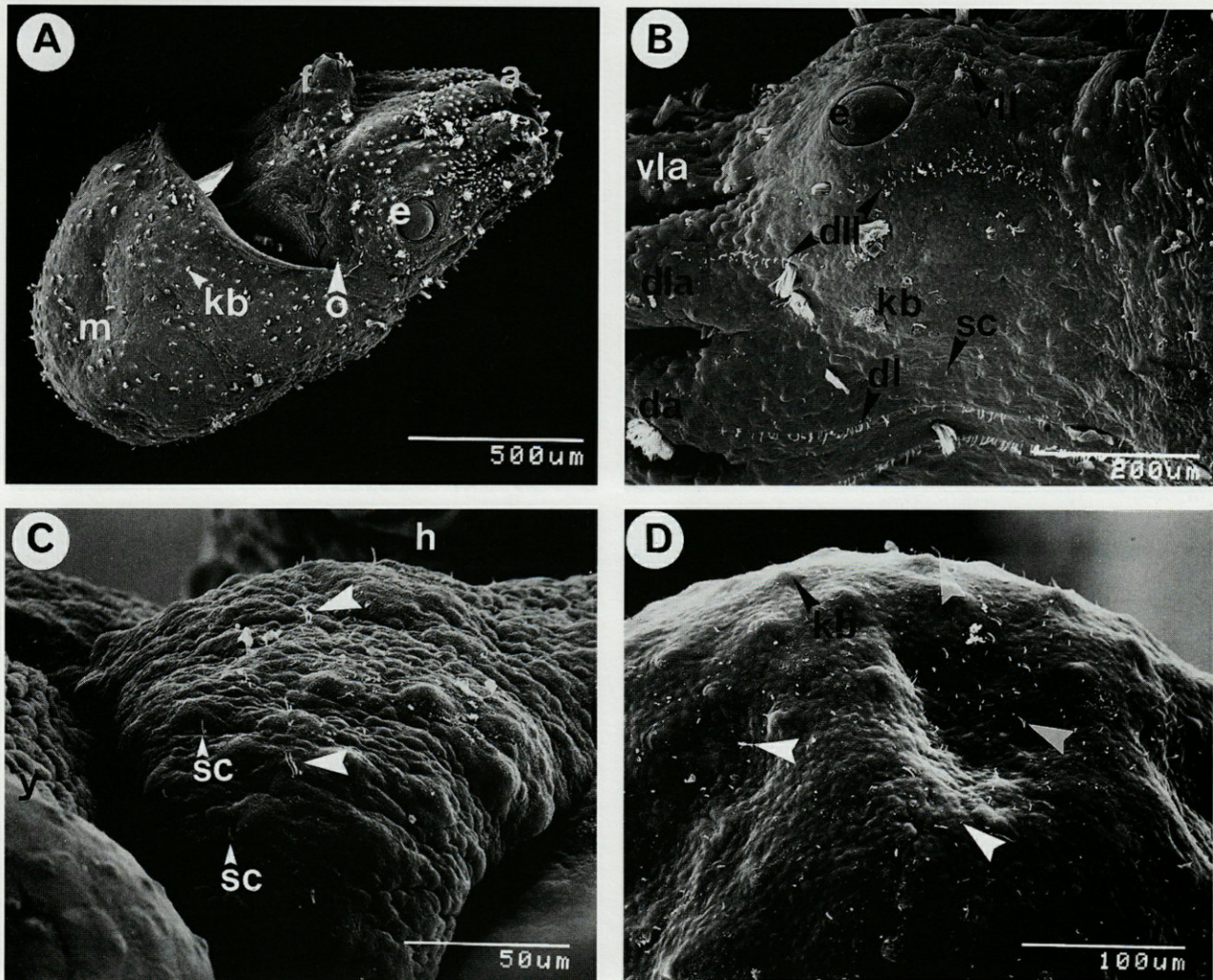
Fig. 2. – A, Paralarva of *O. vulgaris*, lateral view. B, Head (lateral view) with dorsal, dorsolateral, ventrolateral line, and single ciliated cells. C, Dorsolateral arm with single ciliated cells and cilia of dorsolateral line (large white arrow heads). D, Mantle top with single cilia (arrow heads). *Abbreviations* : see Fig. 1.

1990). When rotation ceases a continuous loss of cilia occurs. Consequently the number of cilia ranges from one or very few to numerous (Fig. 3C). The cells have a polygonal apical surface and do not show microvilli.

Ciliated cells are absent from the distal, growing tips of the arms, but some cells with few cilia occur at their proximal parts (Fig. 2C). On the head, funnel, and mantle ciliated cells are surrounded by non-ciliated epithelial cells. The apical surface of the single ciliated cells is round or ellipsoid and has microvilli. The length of its cilia decreases from anterior to posterior, i.e. they are shorter on the head than on the mantle.

The ciliated cells on the funnel pockets are arranged in groups of 2 or 3; they are separated by epithelial cells. The cilia are short and form 1 or 2 tufts of 2-4 surrounded by microvilli (Fig. 3A, B).

Devoid of cilia are – in addition to the tips of the arms – the cornea and the margin of the eyes, the lips, the funnel aperture, and the inner side of the mantle.

Table I. – Location of ciliated cells, and length and number of cilia per cell in the epidermis of paralarval *Octopus vulgaris*.

part of body	length of cilia (µm)	cilia per cell
epidermal lines	10	up to 6
external yolk sac	3	1 to numerous
arms	2-5	1-3
head	2-8	1-4
funnel	3-6	1-3
funnel pocket	1-2	2-4
mantle edge	2-4	1-4
mantle	2-12	1-4
mantle top	1-8	1-5
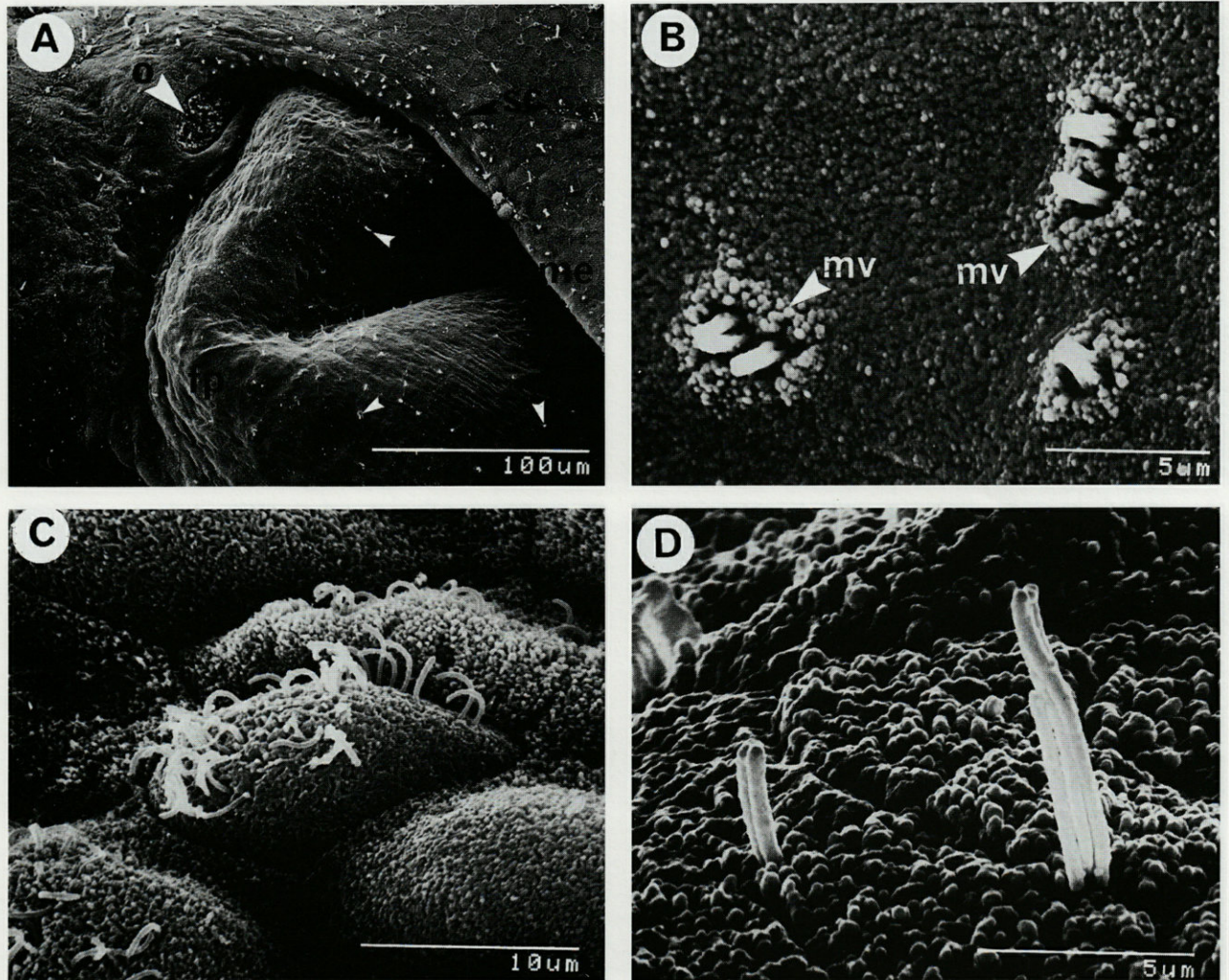
Fig. 3. – A, Funnel pocket (small arrow heads show groups of cilia on the funnel pocket; large arrow head shows cilia of the olfactory organ). B, Ciliated cells of the funnel pocket. C, Cilia on the external yolk sac. D, Single ciliated cells on the mantle. *Abbreviations* : see Fig. 1.

DISCUSSION

Ciliated cells in the epidermis of coleoid cephalopods have been described at ultrastructural, physiological, and behavioural levels. Several serve as mechanoreceptors. In morphology and ultrastructure the epidermal lines of *Octopus vulgaris* largely correspond to those of decapods (Lenz *et al.* 1995) ; therefore it is reasonable to assume that they are homologous in both groups and function as a lateral line analogue organ as shown for sepoid and teuthoid decapods (Budelmann and Bleckmann 1988; Bleckmann *et al.* 1991). The ciliated cells on the suckers and the olfactory organ have been suggested to be mechano- and chemoreceptors (Graziadei 1964; Woodhams and Messenger 1974; Wildenburg and Fioroni 1989; Gilly and Lucero 1992; Lucero *et al.*

1992). On the external yolk sac the cilia are non-sensory, but beating and cause the rotation of the embryo around its longitudinal axis; they are responsible for the first inversion of the embryo (Boletzky and Fioroni 1990). They presumably keep the chorionic fluid in motion, too. After the end of the rotation and the partial loss of cilia, they probably loose their role in the circulation of the chorionic fluid by then. The groups of ciliated cells on the funnel pockets of *O. vulgaris* might be mechanoreceptors as considered for the equivalent cells of *Loligo vulgaris* Lamarck 1798 (Sundermann-Meister 1978). They may function as sensors for the contact between the funnel pockets and the mantle. If motile the single ciliary cells on the body surface may functionally replace the ciliated cells on the external yolk sac. This needs to be verified by *in vivo* studies. The chorionic fluid would still be kept in motion and thus

oxygen exchange and respiration would be facilitated. Also, the motion of the chorionic fluid and thus of excretory products could contribute to detoxification. Another possible function of the newly described ciliated cells on the mantle may be to prevent the embryo from sticking to the chorion after rotation has stopped.

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oxygen exchange and respiration would be facilitated. Also, the motion of the chorionic fluid and thus of excretory products could contribute to detoxification. Another described ciliated cells on the mantle may be to prevent the embryo from sticking to the chorion after rotation has stopped.

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DEVELOPMENTAL ASPECTS OF EMBRYONIC INTEGUMENT IN *ALLOTEUTHIS MEDIA* (CEPHALOPODA LOLIGINIDAE) : A SCANNING ELECTRON MICROSCOPICAL STUDY

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CEPHALOPODS
EMBRYONIC DEVELOPMENT
CILIATURE PATTERN
GLANDULAR CELLS

ABSTRACT. – The embryonic integument of teuthoid cephalopods shows two obvious features. First the development of an elaborate pattern of ciliated cells and second the differentiation of a dense cover of glandular cells. These epithelial structures are illustrated by scanning electron microscopy in hatchlings of the midsize squid *Alloteuthis media*. Squid embryos have three different sets of locomotory multiciliated cells. The cells of the outer yolk sac carry a uniform ciliation. On the embryo proper cilia are arranged in separated tufts, and finally a system of ciliary bands forms on the dorsal and ventral mantle surface. A further type of cilia in the epidermal lines of the head and the arms serves as a mechanoreceptor. The development of epithelial glandular cells in the form of goblet cells reaches its highest amount in the hatching stage. The ciliature pattern as well as the glandular cells occur in a functional context of development inside the chorion and of the mode of hatching through the envelopes of the egg mass.

CÉPHALOPODES
DÉVELOPPEMENT EMBRYONNAIRE
STRUCTURE CILIAIRE
GLANDES

RÉSUMÉ. – Le tégument embryonnaire des Calmars présente deux caractéristiques évidentes : d'une part une structure ciliaire complexe et d'autre part la différenciation d'une couche dense constituée de cellules glandulaires. Ces structures épithéliales sont mises en évidence à l'aide d'un microscope électronique à balayage sur des Calmars de taille moyenne *Alloteuthis media* (L.) au moment de l'éclosion. Les embryons de Calmars présentent trois séries différentes de cellules multiciliaires locomotrices. Les cellules du sac vitellin externe présentent une structure ciliaire uniforme. Sur l'embryon, les cils sont disposés en touffes séparées et les surfaces dorsale et ventrale présentent un système ciliaire disposé en bandes. Un autre type de cils implantés sur les lignes épidermiques de la tête et des bras a une fonction mécanoréceptrice. Le développement des glandes muqueuses atteint son degré maximal lors de l'éclosion. La formation de la structure ciliaire et des glandes est imposée par la structure des enveloppes de la masse des œufs dans un contexte fonctionnel déterminé par le développement à l'intérieur du chorion et la manière d'éclore.

INTRODUCTION

The integument of embryos and larvae can be considered a multifunctional organ system, which serves as a turn-table for the interaction between the organism and the external medium. Therefore ciliary systems are common and responsible for movement, feeding, protection or sensory functions (Nielson 1987). A dense pattern of motile cilia is an obvious feature in the embryonic epithelia of teuthoid squids during post-cleavage development. These multiciliated cells are shed after

hatching. Arnold and Williams-Arnold (1980) described in detail the embryonic development of this ciliature pattern in *Loligo pealei*. As Boletzky (1982) reported, this ciliature is not as well elaborated in sepiid embryos. In sepiolid embryos the entire ciliary band system is missing. In the incirrate octopods a dense embryonic ciliature occurs only on the outer yolk sac.

Beside the numerous locomotory cilia a further type of cilia has been described by Sundermann (1983) in the epidermal lines, the so-called "Drüsenlinien" of Naef (1928) on the head and arms.

Budelmann and Bleckmann (1988) demonstrated that these cells serve as a sensory receptor system for detecting water movements.

A further integumental differentiation is the development of glandular cells, which cover the whole embryo. A comparative description was given by Fioroni (1978).

This short contribution will represent some of the morphological features of the squid integument from an *A. media* hatchling by scanning electron microscopy and will discuss the functional morphology of this organ system.

MATERIALS AND METHODS

Egg strands of *Alloteuthis* were obtained from samples collected off the shore of Laboratoire Arago, Banyuls-sur-Mer (France). They were maintained under running sea-water at a mean temperature of $15°C$. Embryonic development was observed from early organogenetic stages to hatching. The embryonic stages referred to are those of Naef (1928) in combination with those of Arnold (1965) given in brackets. Embryos were taken from the chorion, washed in seawater and fixed in 2,5 % glutaraldehyd/seawater at $4°C$. They were dehydrated through a graded series of ethanol and critical-point-dried with CO_2. The examination was carried out with a CamScan 44 scanning electron microscope at 15 KV at the University of Hamburg, Geological and Paleontological Institute.

RESULTS

The onset of organogenesis at the end of gastrulation is also the onset of differentiation of integumental structures. When the outer yolk sac envelope is nearly closed, a shallow depression forms the boundary between the embryonic area of blastoderm and the extra-embryonic area (future external yolk sac). Organ rudiments morphologically arise first as thickened placodes, foldings or contractions of the embryo proper at stage VII (16) (Fig. 1). The formation of the various organ primordia like shell gland, mantle, optic vesicles, gills, arms and statocysts defines the following developmental stages.

Two different types of cilia can be recognized from stage VII (16) onward. First, a uniform pattern of cilia appears on all cells of the future outer yolk sac (Fig. 2). By hatching stage XX (30) (Fig. 4) they develop into an elaborate system of partly branched bands with a mean width of 9.5 µmm (Fig. 6).

The glandular cells, which cover the whole embryo in the form of goblet cells are most obvious by their partly empty vacuoles (Fig. 6) in the hatching stage XX (30). A further glandular system, which develops in a Y-shape arrangement

at the mantle apex is the so called Hoyles organ. It is notable that the system of ciliary bands radiates from this organ (Fig. 5).

In the system of epidermal lines, which forms on the head and partly on the arms sensory cilia are best visible in the dorsal L1 and L2 line on each side of the head (Fig. 7). Most of these hair cells are elongated in the antherior-posterior axis of the embryo and the cilia are standing in one row. Further details of these sensory cells, as well as the lateral and ventral lines were not examined here.

DISCUSSION

The general integumental differentiation, the development and placement of the ciliature pattern in *Alloteuthis* correspond to those reported for *Loligo pealei* by Arnold and Williams-Arnold (1980). The ciliature of the outer yolk sac envelope generates a first constant circulation of the intrachorionic fluid and a slow movement of the embryo inside the egg case. In the incirrate octopods these cilia are responsible for the first embryonic inversion (Boletzky and Fioroni 1990). The ventilation guarantees the necessary gradient for an effective O_2/Co_2 respiration and the metabolic exchange through the chorion. The cover of multiciliated ectodermic cells of the outer yolk sac is the only cilia system, which appears in all main lines of the Coleoidea and also in *Nautilus* (Arnold and Carlson 1986). It resembles the unspecific uniform ciliation in early free swimming planula-like gastrula stages of bivalve larvae (Bandel 1988, pers. obs.). Most probably this is based on the same genetic larval program for ectodermic ciliation.

The ciliature on the integument of the embryo proper supports the movement of the intrachorionic fluid. Arnold and Williams-Arnold (1980) observed the general direction of particle flow caused by the beating cilia on the embryonic integument. The ciliary tufts were described by them as so called "paddle-type cilia", because of their tendency to form plasma membrane swellings at the distal end of the cilia. The investigation of *Alloteuthis media* could not confirm these observations in any type of cilia.

The phenomenon of paddle cilia or discocilia has been reported in ciliary systems of different invertebrate larvae (*cf.* Campos and Mann 1988). Sundermann (1991) observed similar membrane swellings in a hitherto not further described type of cilia at *Sepia* hatchlings. Haszprunar (1985) found these kinds of cilia also in the chemoreceptor epithelia of the molluscan osphradium. Since the discovery of this type of cilia there has been a continuing discussion whether this phenomenon
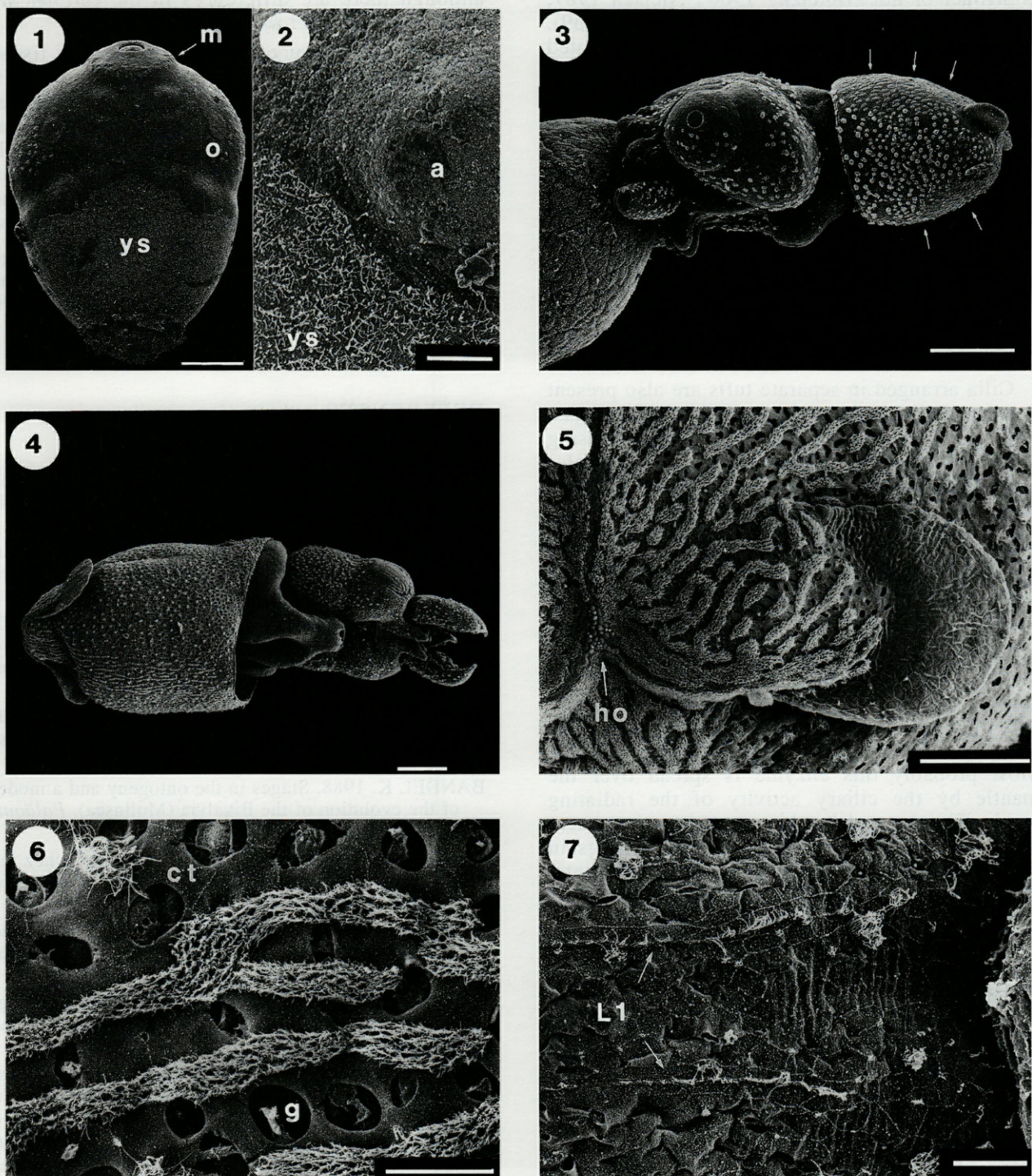
Pl. I. – Scanning electron micrographs of *Alloteuthis media* illustrating the development of the integument at different embryonic stages. Fig. 1, ventral view of stage VII (16) with organ primordia. Note ciliary tufts, visible as small light patches on mantle margin and optic vesicles. Fig. 2, detail of stage VII (16) at the boundary of the outer yolk sac and the embryo proper with arm primordia. Note the uniform pattern of ciliated cells of the outer yolk sac. Fig. 3, lateral view of stage XVI (28). Arrows indicate ciliary bands on dorsal and ventral mantle. Fig. 4, ventrolateral overall view of hatching stage XX (30). Fig. 5, stage XX (30) detail of dorsal mantle apex with ciliary band system radiating from Hoyles organ. Fig. 6, stage XX (30) detail of ciliary band system, ciliary tufts and goblet cells. Fig. 7, stage XX (30) detail of head showing the two dorsal epidermal lines L1. a = arm primordia, ct = ciliary tuft, ho = Hoyles organ, g = goblet cell, m = mantle margin, o = optic vesicles, ys = yolk sac. Scale bars 0.25 mm (Fig. 1, 3, 4), 0.03 mm (Fig. 2), 0.1 mm (Fig. 5) and 0.3 mm (Fig. 6, 7).

is artifact or not (Boletzky 1980 ; Nielsen 1987, Campos and Mann 1988 ; Short and Tamm 1989). In several publications it has been demonstrated that paddle cilia are a preparation artifact, caused by osmotic stress during fixation. In our investigation the embryos were fixed without any additional buffer system in glutaraldehyd/seawater. It should be mentioned, that the phenomenon of paddle cilia can be observed in different functional types of cilia, such as ciliary tufts of cephalopods, compound cilia in bivalve veligers and the osphradial sense organs of adult molluscs. Therefore the terms paddle shaped cilia or discocilia should not be used as a character in phylogenetic discussions (Eernisse *et al.* 1992 ; Haszprunar 1996).

Cilia arranged in separate tufts are also present on the mantle surface of some intracapsular gastropod veligers (pers. obs.). This is not surprising as the formation of the mantle tissue is homologous in all conchiferan taxa (Bandel 1982 ; Hopkins and Boletzky 1994). The elaborate system of ciliary bands on the dorsal and ventral mantle as demonstrated here in the example of *Alloteuthis* can be regarded as a characteristic adaptation of coleoid cephalopod embryos to the mechanism of hatching. This cilia system beats in metachronic waves and supports gliding of the embryo across the gelatinous envelopes of the egg mass. The chorion and the surrounding egg cases are dissolved by a specific enzyme produced by the hatching gland or Hoyles organ (Boletzky 1989). Most probably this enzyme is spread over the mantle by the ciliary activity of the radiating bands. This type of hatching apparatus is present in cuttlefishes and in squids embedding eggs in gelatinous envelopes produced by the nidamental glands. So far as known there is only little data about eggs and egg masses of the so-called oceanic or oegopsid squids. For example species of the family Enoploteuthidae are known to spawn single pelagic eggs with no more than one gelatinous envelope (Young and Harman 1985). Presumably the ciliary apparatus is reduced in these taxa similar to sepiolids and incirrate octopods.

The development of the numerous mucous or goblet cells in the hatching stage represents not only an integumental preparation for the post hatching phase. According to Marthy (1974), there is evidence for an immunological significance of these glandular cells.

The mechanosensory system of epidermal lines is, beside the eyes and the statocyst organs, one of the most sophisticated receptor systems evolved by modern Coleoidea. Immediately after hatching it is jointly responsible for a coordinated swimming behaviour and capture of prey (Budelmann and Bleckmann 1988). The system of epidermal lines is present in all coleoid hatchlings,

although there are differences in the cellular arrangement (Lenz *et al.* 1995).

The development of cilia on ectodermic tissue and their arrangement in specific patterns is a common feature of embryos and larvae. The cephalopod embryos represent a special transformation of the multiciliated cell pattern of the molluscan embryogenesis as a functional adaptation to a direct development inside the chorion and the mode of hatching through various egg envelopes.

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THE STRUCTURE OF SUCKERS OF NEWLY HATCHED *SEPIA OFFICINALIS, LOLIGO VULGARIS,* AND *OCTOPUS VULGARIS*

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SEPIA OFFICINALIS
LOLIGO VULGARIS
OCTOPUS VULGARIS
NEWLY-HATCHED
SUCKERS

ABSTRACT. – Scanning and transmission electron microscope studies allow a comparison of the state of differentiation of the suckers of the newly-hatched benthic *Sepia officinalis* Linné, 1758, and planktonic *Loligo vulgaris* Lamarck, 1798, and *Octopus vulgaris* Cuvier, 1797. These analyses may help to correlate the differences in the suckers with the divergent developmental types of the three species and give further information about the functional morphology of the suckers at hatching.

SEPIA OFFICINALIS
LOLIGO VULGARIS
OCTOPUS VULGARIS
FRAÎCHEMENT ÉCLOS
VENTOUSES

RÉSUMÉ. – Nos études en microscopie électronique à balayage et à transmission permettent de comparer l'état de différenciation des ventouses chez les animaux fraîchement éclos, soit benthiques (*Sepia officinalis* Linné, 1758) soit planctoniques (*Loligo vulgaris* Lamarck, 1798 ; *Octopus vulgaris* Cuvier, 1797). Ces analyses pourraient aider à définir d'éventuelles corrélations entre différences de structures au niveau des ventouses et les différences de type de développement parmi les trois espèces, tout en apportant de nouvelles informations sur la morphologie fonctionnelle des ventouses au moment de l'éclosion.

INTRODUCTION

Behavioural observations on the post-embryonic life of cephalopods show that the suckers even of hatchlings perform a remarkable variety of functions (Boletzky 1974, 1977, 1979, 1987). A detailed morphological and histological study of the suckers of postembryonic cephalopods, however, is lacking. In this paper the suckers of three cephalopods of different developmental types (Fioroni 1977) are analysed and an attempt is made to compare the differentiation and the functional morphology of the suckers of *Sepia officinalis, Loligo vulgaris,* and *Octopus vulgaris* at hatching.

MATERIAL AND METHODS

Newly-hatched specimens of *S. officinalis* (dorsal mantle length (dml) approximately 8 mm), *L.vulgaris* (dml : 3 mm), and *O. vulgaris* (dml : 1,5 mm) were obtained from the western Mediterranean Sea (Banyuls-sur-Mer, France). The dorsal mantle length was measured on SEM-pictures. The tissue was fixed with Bouin's solution or in 2 % OsO_4 dissolved in 0.5 % $K_2Cr_2O_7$ in 70 % seawater. The material for transmission electron microscopy was dehydrated in ethanol and embedded in Spurr's medium (Spurr, 1969). Ultrathin sections were stained with lead citrate (Reynolds, 1963). Material for scanning electron microscopy was dehydrated in ethanol, critical point dried with CO_2 and coated with gold. In *S. officinalis* the most highly differentiated suckers on the outer rows of all four arms and the suckers of the middle region of the tentacle club were examined. In *L. vulgaris* the developmental state of the arms – compared with *S. officinalis* – is quite different at hatching; thus, only the largest suckers on the base of the ventral arms and on the tentacles were analysed. In *O. vulgaris* the central one of the three suckers on each arm was investigated, because it shows the highest differentiation on the outer surface of the infundibulum.

RESULTS

Beside the differences in the arrangement of the suckers on the various arms and within one arm there exist species-specific differences in number and size : *S. officinalis* (Fig. 1 A) exhibits
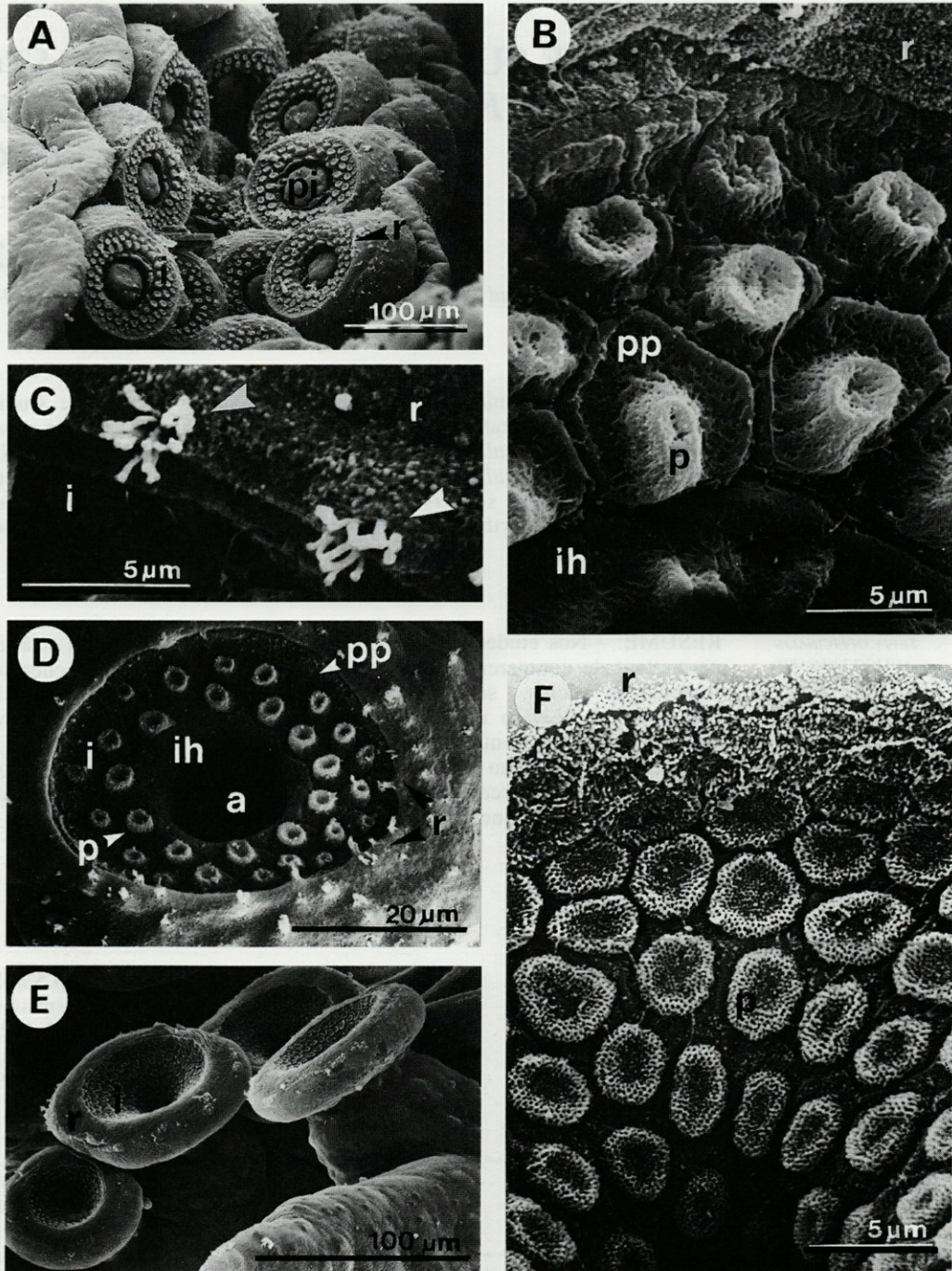
Fig. 1. – A, Four longitudinal rows of arm suckers of *S. officinalis*. B, Polygonal processes with pegs cover the infundibulum of a sucker of *S. officinalis*. C, On the outside of the suckers tufts of cilia emerge through pores (arrow head), here on the rim of a sucker of the cuttlefish. D, In the squid the infundibulum is endowed with two rings of polygonal processes with small pegs. E, In *O. vulgaris* the infundibulum is encircled by a rather plain rim. F, The infundibulum of a sucker of *O. vulgaris* at higher magnification shows almost rounded flat pegs with small pores. a acetabulum, i infundibulum, ih inner horny ring, p peg, pi piston epithelium, pp polygonal process, r rim.

adult-like morphology at hatching with a high number of suckers which develop during an intensive phase of growth in late embryogenesis (Fioroni 1977). In the less developed paralarvae of *L. vulgaris* (Fig. 1 D) and *O. vulgaris* (without

this phase) only a small number of suckers is present. For example, in *O. vulgaris* only three suckers are present on each arm (Fig. 1 E), but the suckers are relatively large compared with the short arms (Nolte & Fioroni 1983).

In *S. officinalis* the outer surface of the infundibulum of the arm suckers shows rings of polygonal processes which are provided with a projecting peg, each with a concave surface (Fig. 1 B). The processes and the pegs are covered with small pores. They decrease in size from the center of the infundibulum to its periphery. Here the processes lack pegs or only little pegs are formed. The distal half of the inner horny ring, where the processes are fused, has blunt projections, while the remaining half is smooth. The diameter of the infundibulum of the most highly differentiated suckers on the tentacle club is larger than that of the arms. Their whole inner horny ring is still smooth.

In the arm suckers of *L. vulgaris* the infundibulum is endowed with fewer and smaller pegs whose concave upper surface is well marked (Fig. 1 D). At the edge of the infundibulum small processes without pegs are prominent. Like in *S. officinalis* the cuticle forming a peg contains a system of canals penetrated by microvilli (Fig. 2 B, see Haas 1989). The inner horny ring is smooth and has no teeth. In general, the infundibulum of the tentacles resembles that of the arm suckers.

In *O. vulgaris* (Fig. 1 F) the infundibulum possesses very flat but numerous pegs which are already endowed with minute pores. Like in the two decapod species the concavity on the upper surface of the pegs is obvious. The infundibulum is encircled by a plain rim. The circumferential marginal folds which surround the infundibulum in suckers of adult animals are not yet present.

In the suckers of the cuttlefish large cells exist below the epithelium of the piston (Fig. 2 C). They seem to have no contact with nervous tissue. Similiar cells are found in the squid but they are not as conspicuous as in *S. officinalis*. Nevertheless, in both decapods numerous nerve fibres exist beneath the infundibulum and the rim of the muscular wall and a thick nerve bundle in the peduncle can be traced into the brachial nerve. In *O. vulgaris* nerve fibres appear in the walls of the acetabulum and infundibulum and run along the extrinsic muscular system of the sucker. In the subacetabular region a concentration of nerve fibres can be observed. In all species investigated, ciliated cells are common on the rim but rare at the lateral regions of the sucker (Fig. 1 C). Ciliated cells were not observed in the piston or in the
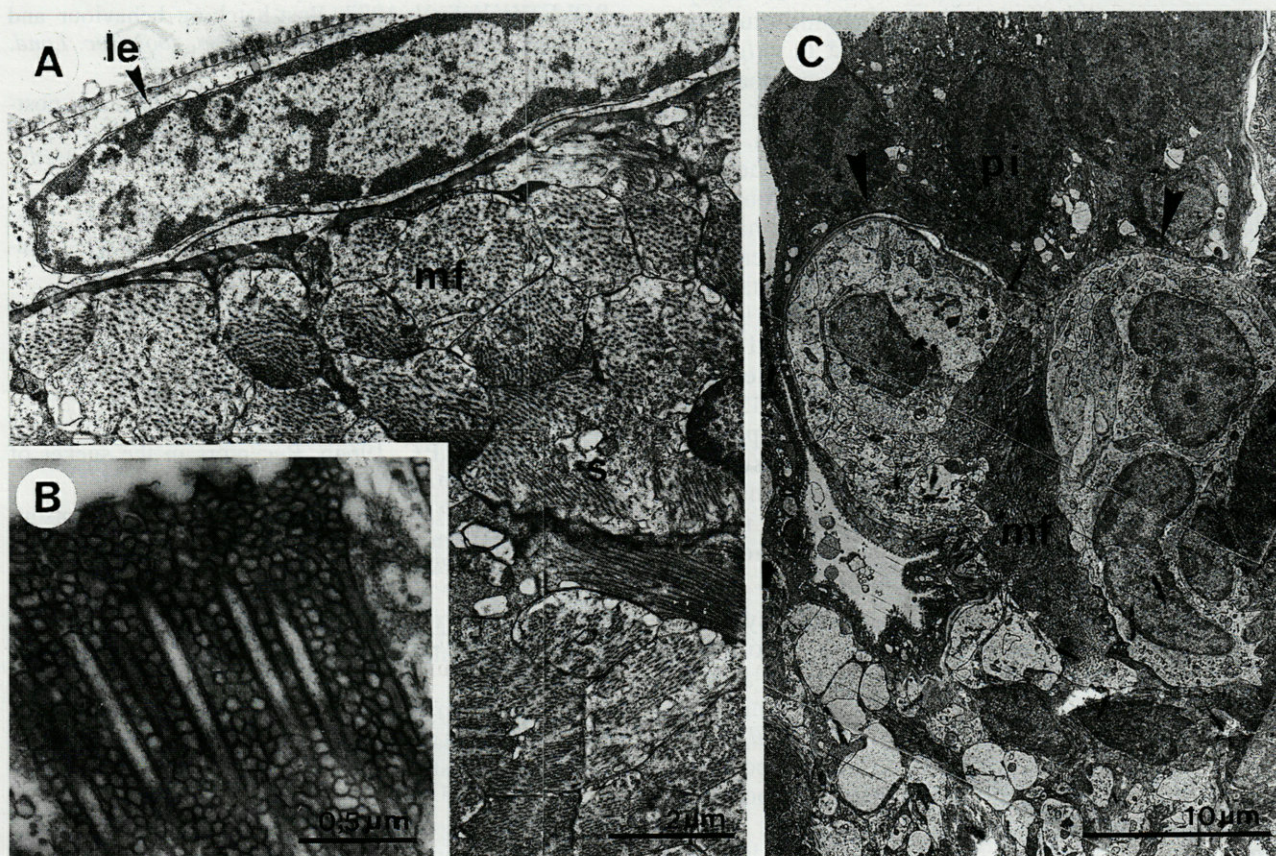

Fig. 2. – A, The main sphincter muscle of a sucker of *S. officinalis* with densely packed muscular fibres. B, The cuticle of the pegs of all investigated specimens – here in *L. vulgaris* – is formed by a system of canals which are penetrated by microvilli. C, In *S. officinalis* large cells (arrow head) below the piston epithelium seem to lack nervous tissue. le lateral epithelium, mf muscular fibres, pi piston epithelium, s main sphincter muscle.

epithelium of the infundibulum (Graziadei, 1964a, 1964b).

In *S. officinalis* the intrinsic muscular tissue of the sucker walls and the peduncle is well differentiated (Fig. 2 A). The suckers of *L. vulgaris* possess highly developed muscular tissue (circular and longitudinal muscular fibres) in the peduncle but lack well-differentiated muscle in the sucker wall. In *O. vulgaris* the acetabulum roof includes radial muscle fibres which extend between an inner and outer fibrous connective tissue (Kier & Smith 1990). But they are not as densely packed as the corresponding fibres in the suckers of the cuttlefish. Nevertheless, radial, circular, and meridional fibres are differentiated in the infundibulum and acetabulum wall. No well marked sphincter muscle can be found at hatching stage but thick extrinsic muscular bundles attach the sucker to the arm.

DISCUSSION

In general, the main features of the outer surface of the sucker show an obvious resemblance to those of the adult suckers (Nixon & Dilly 1977). Even in the hatchlings the infundibulum is provided with pegs : *S. officinalis* and *L. vulgaris* are endowed with a relatively small number of tall pegs, whereas in *O. vulgaris* many flat pegs appear on the surface of the infundibulum. The pegs may provide friction to aid suction adhesion of the suckers of the hatchlings. In addition, the epidermis of the rim and the lateral regions are dotted with ciliated cells which probably enable the hatchlings to respond to chemical and tactile stimuli from the environment (Graziadei 1964a, 1964b; Graziadei & Gagne 1976). A typical subacetabular ganglion was not observed in the two decapods (Guérin 1908; Martoja & May 1956). The large cells below the epithelium of the piston may secrete the collagenous connective tissue of the subacetabular region (Haas 1989). In the subacetabular region of *O. vulgaris*, however, nerve fibres exist and may establish the connection to the prospective subacetabular ganglion.

In *S. officinalis* the intrinsic muscular system is very well developed and corresponds more or less to the situation in the suckers of the adult. The retardation in the development of the muscular tissue in the wall of the suckers of *L. vulgaris* may be compensated by a well differentiated one in the peduncle which probably plays an important role in the mechanism of attachment : The contraction of the musculature of the peduncle leads to an extension of the acetabulum and thus enlarges the suction effect. In *O. vulgaris* the muscular system of the suckers is more highly differentiated than expected from the outward appearance.

The various developmental stages of the suckers of the hatchlings reproduce almost exactly general morphological differences between the two developmental types (benthic, planktonic) of cephalopods : *S. officinalis* is equipped with highly differentiated suckers which seem to be able to act like the adult ones, whereas the suckers of *L. vulgaris* are less developed. The suckers of the planktonic *O. vulgaris*, however, are more advanced than those of the planktonic squid. This advanced development may be required for the benthic way of life which follows soon after. But the suction process is probably not as effective as in adults because of the still missing marginal fold which is important for forming a tight seal (see Nixon & Dilly 1977). Nevertheless, the suckers of both planktonic cephalopods investigated seem to have a sufficiently effective adhesive function according to the demands in the early postembryonic life.

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STRUCTURE AND FUNCTION OF THE DUCT OF KÖLLIKER IN PARALARVAE OF *LOLIGO VULGARIS* LAM. (CEPHALOPODA)

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CEPHALOPODA
DUCT OF KÖLLIKER
LOLIGO VULGARIS,
PARALARVA

ABSTRACT. – Newly hatched paralarvae of *Loligo vulgaris* have a well developed duct of Kölliker, composed of two cell-types. It is divided into seven different regions. The duct is not a rudimentary organ but seems to have hydrostatic functions. The duct has an external opening into the connective tissue.

CEPHALOPODES
CANAL DE KÖLLIKER
LOLIGO VULGARIS
PARALARVE

RÉSUMÉ. – Les nouveau-nés (paralarves) de *Loligo vulgaris* possèdent un canal de Kölliker qui se compose de deux types cellulaires ; il se divise en sept régions distinctes. Il ne s'agit donc pas d'un organe rudimentaire ; le canal semble avoir des fonctions hydrostatiques. Il s'ouvre vers le tissu conjonctif.

INTRODUCTION

During formation of the statocysts of *Loligo vulgaris* a duct develops on either side of the embryo by invagination of the epithelium. This duct persists after the separation of the statocysts from the epidermis, as an appendix of the lateral walls of each of the statocysts. It was discovered by Kölliker (1844) in *Loligo vulgaris, Sepia officinalis* Linné, 1758 and *Argonauta argo* Linné, 1758 and consequently named "duct of Kölliker". Since its discovery it received only little attention and it was considered to be an ontogenetic relict of the ectodermal invagination. In *L. vulgaris* it has been described to have a closed end in the cartilage surrounding the statocyst (Hamlyn-Harris, 1903). Stephens and Young (1982), however, showed that the duct crosses the cartilage and opens ventrally into the connective tissue. The following morphological and ultrastructural data describe the course of the duct, answer the question wether the duct opens to the connective tissue, and give a survey of its ultrastructure.

MATERIALS AND METHODS

18 hatchlings of *Loligo vulgaris* [stage XX of Naef 1921/28)] from Banyuls-sur-Mer, western Mediterranean sea, France, were examined. The specimens were fixed in 2 % OsO_4 dissolved in 0,5 % $K_2Cr_2O_7$ in 80 % seawater, pH 7,2-7,4, embedded in Durcupan (Fluka, Switzerland), and cut on a LKB-Ultrotom III. The ultrathin sections were stained with 1 % lead citrate and examined with a Zeiss EM 900 transmission electron microscope (TEM). The spatial orientation follows the swimming position of the adult squid : arms = anterior, mantle tip = posterior, funnel = ventral.

RESULTS

Structure of the duct

According to their intensity of staining in semithin sections and variable electron density in TEM the cells of the single-layered epithelium of the duct of Kölliker can be described as two different types : the type-1 cells which are electron light and the type-2 cells which are electron dense (Fig. 1). The type-1 cells are highly prismatic and have more numerous interdigitations apically than basally.

The type-2 cells have an irregular shape (Fig 1). Because of their high electron density the total content of cell organelles is difficult to determine. In both cell types, the large number of mitochondria and Golgi vesicles indicates a high energy turnover. Lateral interdigitations are more numerous between type-1 and type-2 cells than between type-2 cells only. Mitochondria are found regularly in all interdigitations. Intermediary filament strings, which are clearly distinguishable in the plasma of type-1 cells, are not seen in type-2 cells. Both cell types bear long cilia, except the
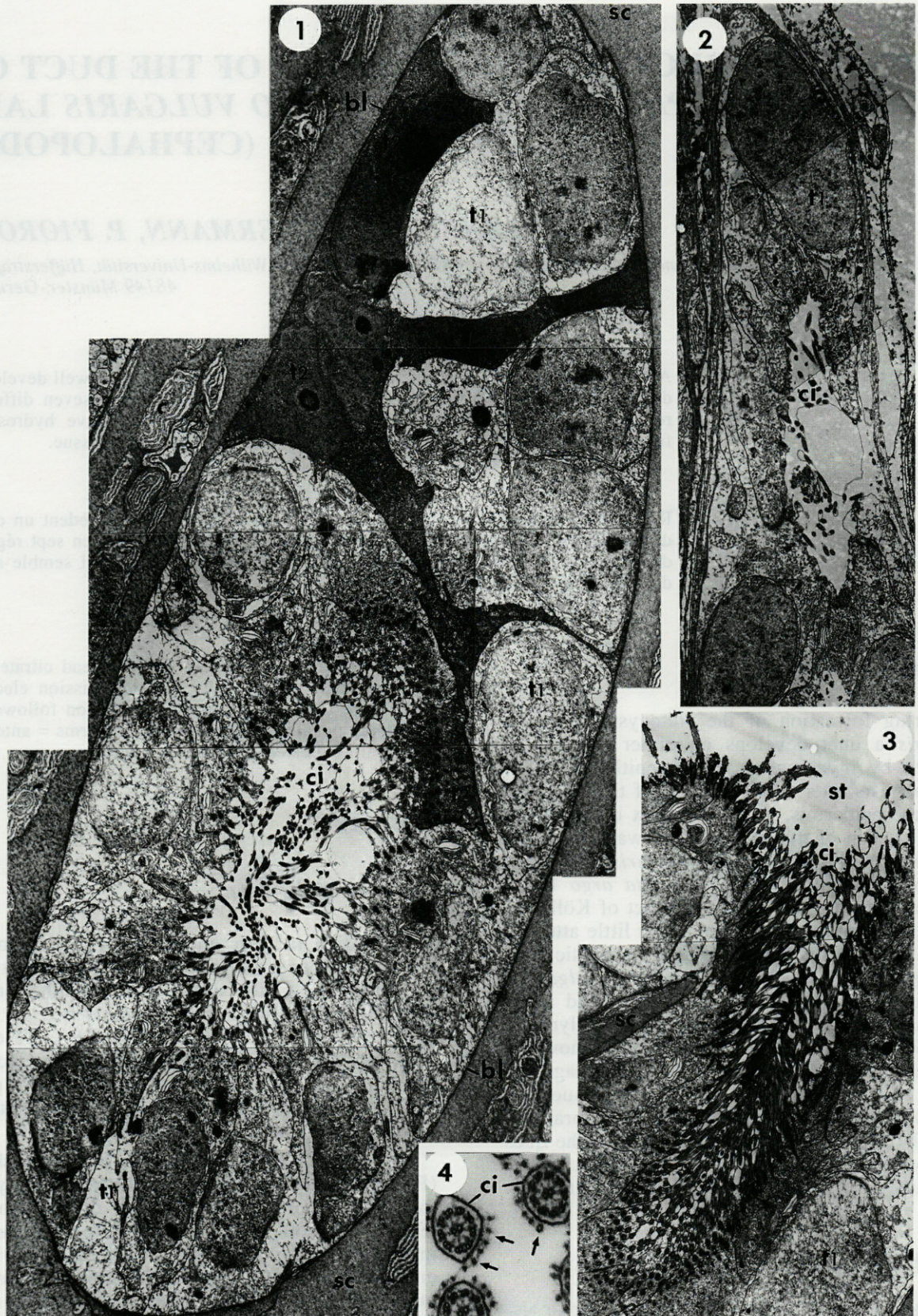
Fig. 1-4. – TEM-sections through the duct of Kölliker. Fig. 1, 5175x. Part C, surrounded by cartilage, with type-1 and type-2 cells. Fig. 2, 5175x. Part G, aperture into the surrounding connective tissue. Fig. 3, 5175x. Part A, aperture to statocyst lumen. Fig. 4, 49.500x. Part B, cilia surrounded by microtubular structures (arrows), a = aperture, bl = basal lamina, c = cartilage cell, ci = cilia, sc = statocyst cartilage, st = statocyst, t1 = type-1 cell, t2 = type-2 cell.

peripheral cells of the bulge around the opening of the duct into the statocyst lumen (Fig. 3). The ciliature in part B of the duct (see below) is different from that of other parts. In regular intervals around the cilia are thin, microtubular-like structures (Fig. 4). They are connected to each other and to the cilia by a substance of medium electron density (Fig. 4). These connections combine approximately 150-200 cilia to bundles. The number of cilia per cell decreases from part C to part G (Fig. 1 and 2). No obvious signs of degeneration in the epithelium of the duct were seen.

Course of the duct

From the aperture in the statocyst lumen the duct runs into the statocyst cartilage (Fig. 3). Right after entering into the cartilage, it turns in ventro-caudal direction. In the region underneath the primordium of hamulus 3 it turns in anterior direction and then leaves the cartilage and its lumen narrows. Surrounded by a thin layer of connective tissue it continues in anterior direction between the outer surface of the cartilage and a haemolymph sinus. At its end, the duct opens into the connective tissue dorsal to the funnel muscle and ventral to the edge of the statocyst cartilage; the opening points to the nearby epidermis (Fig. 2). In this area the lumen of the duct has an ampulla-like dilatation (Fig. 2).

The duct can be divided into seven parts (A – G), according to the different width of its lumen, the variation in ciliature of the epithelial cells, and the cell types (which are highly prismatic in parts A-D) (Fig. 5):
Part A : Aperture to statocyst lumen; only type-1 cells.
Part B : Region with widest lumen of entire duct (diameter ca. 17.4 μm), type-1 and type-2 cells.

Part C : Region between B and cephalic turn; lower stags of cilia, type-1 and type-2 cells.
Part D : Region of cephalic turn; lumen narrows continually; number of cilia as in C; type-1 and type-2 cells.
Part E : Transition from cartilage to connective tissue; thinnest part of duct with smallest lumen (diameter ca. 1.4 μm); flattened epithelial cells, only type-1 cells.
Part F : Ampulla-like dilatation of lumen; number of cilia, form and type of epithelial cells as in E.
Part G : Aperture into connective tissue; only type-1 cells.

DISCUSSION

The examination of the duct of Kölliker at the hatching stage proves that the duct shows no signs of degeneration but rather represents a well differentiated structure. Therefore, it is rather unlikely that it is only an ontogenetic relict without any function.

The duct of Kölliker reaches its greatest diameter in B and C. If there were a metabolic exchange between the duct epithelium and the fluid inside the canal, endolypmph would most probably occur in this part of the duct. However, no secretion vacuoles or anything similar can be found in the apical surfaces of the cells. Also the fact that some coated vesicles and secretion grains of undefinable content can be recognized in the area of the Golgi apparatus of type-1 cells, is not sufficient support for the assumption that the epithelial cells have a secretory function. Therefore, we suppose that the duct of Kölliker is not involved in the production of the statocyst endolymph.

The experiments of Stephens and Young (1982) showed that after injection of coloured substances into the statocyst the fluid in the lumen of the duct also becomes coloured and that, therefore, a transport of endolymph into the duct must exist. Our investigations show that the duct runs a short distance in the surrounding tissue outside the statocyst cartilage and then opens into it very close to the epidermis.

All these facts indicate that the duct of Kölliker receives endolymph from the statocyst. This hypothesis is supported by the fact that the ciliary beat is directed from the statocyst lumen into the duct (Budelmann, 1990) and that the lumen of the duct is larger at its beginning (Parts A-C) than at its end. Consequently, it is reasonable to assume that one essential function of the duct of Kölliker is to receive endolymph from the statocyst; and thus to regulate the volume of the endolymph inside the statocyst. Where the endolymph secretion occurs remains to be seen.
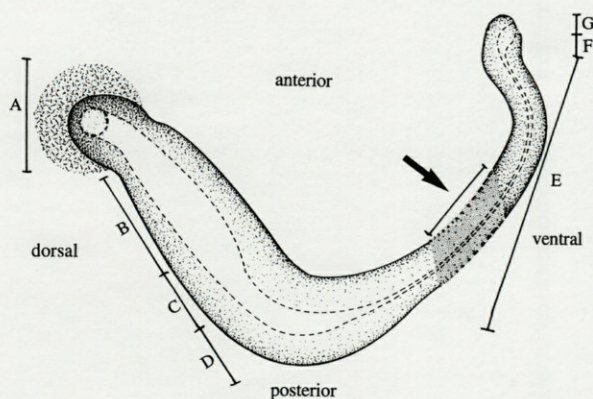

Fig. 5. – Diagram of the course of the duct of Kölliker (parts A – G). (sc = statocyst cartilage, arrow = transition from statocyst cartilage to connective tissue).

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INTRASPECIFIC SHAPE VARIABILITY IN STATOLITHS OF THREE CEPHALOPOD SPECIES

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CEPHALOPODA
STATOLITH
ELEDONE CIRRHOSA
TODARODES SAGITTATUS
SEPIA OFFICINALIS

ABSTRACT. – The intraspecific variations in the morphology of the statoliths from three species of cephalopod from the NW Mediterranean have been studied using image analysis systems. The analysis of the shape revealed intraspecific differences throughout the growth of *Eledone cirrhosa*. On the contrary *Sepia officinalis* and *Todadores sagittatus* demonstrated a large intraspecific homogenity.

CEPHALOPODA
STATOLITHE
ELEDONE CIRRHOSA
TODARODES SAGITTATUS
SEPIA OFFICINALIS

RÉSUMÉ. – Les variations intraspécifiques dans la morphologie des statolithes de trois espèces de Céphalopodes du NW de la Méditerranée ont été étudiées au moyen d'un système d'analyse d'images. L'analyse de la forme révèle des différences intraspécifiques durant la croissance chez *Eledone cirrhosa*. Au contraire *Sepia officinalis* et *Todarodes sagittatus* montrent une grande homogénéité intraspécifique.

INTRODUCTION

The techniques of image analysis have been used in studies of cephalopod morphology (Ragonese and Jereb 1995), and the studies of statoliths from juvenile and adult specimens. These have thrown light on certain phylogenetic relationships among the cephalopods (Clarke and Maddock 1988a, 1988b). However, the existence of changes in the morphology of the statolith in the course of growth could alter the result of the analysis of shape (Clarke 1978; Morris and Aldrich 1984; Guerra and Sánchez 1985; Clarke and Maddock 1988b). The objective of this study is to determine the changes of statolith shape of three species of cephalopods in the course of postlarval growth. Also the study tries to determine if there is an influence of the sex and sexual stage on the intraspecific variability of the statoliths.

Three species were selected because they are very different in their taxonomy and behaviour.

Sepia officinalis (Sepiidae) occurs in coastal waters and the continental shelf mainly on sand and muddy bottoms. Immediately after hatching animals live in close contact with the bottom. The species is known to be relatively tolerant to salinity variations (Guerra 1992).

Todarodes sagittatus (Ommastrephidae). This neritic and oceanic species is a powerful swimmer. Vertical migrations by day and night in relation to feeding activity have been observed.

Eledone cirrhosa (Octopodidae). This is a truly benthic species. In the Mediterranean its typical depth range is between 50-300 m. The hatchlings are certainly planktonic but this phase probably lasts only a matter of days during which most of them remain relatively close to the bottom (Boyle 1983).

MATERIAL AND METHODS

Statoliths from these three species of cephalopods of the Northwestern Mediterranean (Catalan Sea), were collected by trawls. The sex, the sexual stage and the mantle length (ML) of each individual were determined, in order to find if these influence statolith shape.

Differences in statolith shape were analyzed using Fourier series, calculated from a numerical description of the anterior and lateral outlines of the statolith. These techniques have been used with success in the studies of the shape variability in otoliths and stock discrimation of fishes (Castonguay *et al.* 1990; Lombarte and Castellón 1991; Campana and Casselman 1993).

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In order to detect the existence of allometric differences between the various parts of the statolith throughout growth the study has been carried out using various measurements of the statoliths.

In order to obtain the digitized statolith outline we used the program KRONOMORPHOS. It consists of the following processes :

1. Capture and calibration of the frontal and lateral image of the statolith, previously drawn.

2. Obtaining measurements from the statolith. Area, perimeter, width, length, thickness of the frontal and lateral view of the statolith for the three species. Also the same measurements have been taken for the frontal and lateral view of the lateral dome, and the wing in *Sepia officinalis* and *Todarodes sagittatus* (Fig. 1).

3. Obtaining a numerical spectrum related to the shape. This process consists of the following phases.

a – Obtaining an image from the external outline of the statolith.

b – Selection of 100 equidistant points on the outline. The initial point of the numerical spectrum has to be the same. In this case the tip of the rostrum.

c – Calculation of the distance to the centre of gravity of the N points from the perimeter following a clockwise direction (Fig. 2A).

d – Dividing the distances calculated by the maximum distances (Fig. 2B).

4. Calculation of the first 20 Fourier shape descriptors from the numerical spectrum (Fig. 2C).

Multivariate analysis was used to classify the statoliths. Euclidean distance and the UPGMA aggregation algorithm were employed because of the results high cophenetic correlation coefficient (Lombarte and Castellón 1991).

RESULTS

A negative allometric growth was observed in relation to the length of the statolith for some measurements related to the thickness in *E. cirrhosa* and *S. officinalis*, such as the thickness and area in the two species. In the case of *S.officinalis* a negative allometric growth was also observed for the thickness of the lateral dome. In *T. sagittatus* all the parameters showed an isometric growth (Table I).

The cluster analyses from the Fourier parameters showed clear shape changes throughout the growth of the statolith of *E. cirrhosa* (Fig. 3A).
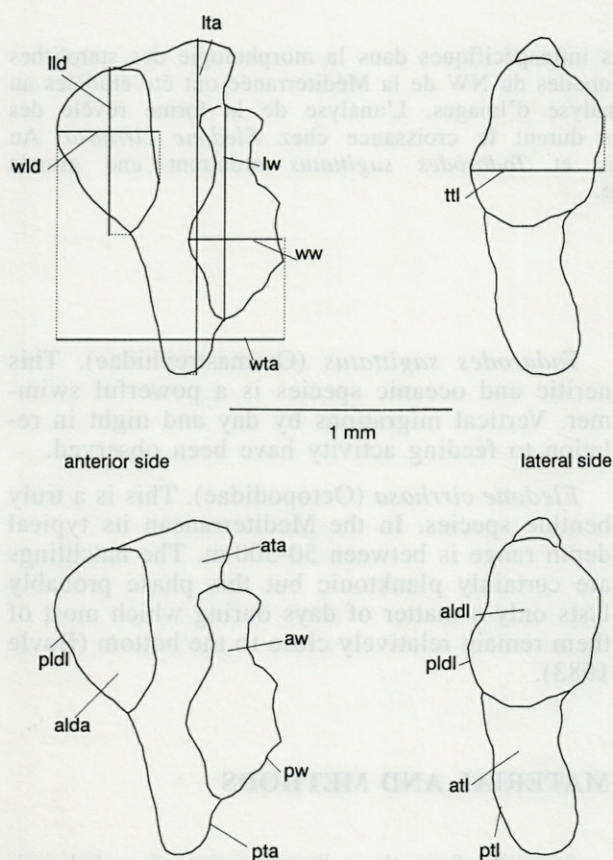

Fig. 1. – Measurements taken on each statolith by a digital image processing system. alda, area lateral dome in anterior view. aldl, area lateral dome in lateral view. ata, area total statolith anterior. atl, area total statolith lateral. aw, area wing. lld, length lateral dome. lta, length total statolith. lw, length wing. plda, perimeter lateral dome anterior. pldl, perimeter lateral dome lateral. pta, perimeter total anterior. ptl, perimeter total lateral. pw, perimeter wing. ttl, thickness total statolith lateral. wld, width lateral dome. wta, width total statolith anterior. ww, width wing.

Tabl. I. – Growth of the biometric parameters of the statoliths in relation to the frontal length of the statolith in *Sepia officinalis*, *Todarodes sagittatus* and *Eledone cirrhosa*. b, slope. se, standard error. a, area. p, perimeter. w, width. l, length. t, thickness. ta total statolith anterior side. lda lateral dome anterior side. w wing. tl total statolith lateral side. ldl lateral dome lateral side.

		S.officinalis		T.sagittatus		E.cirrhosa	
		Allometry		Allometry		Allometry	
		b	se	b	se	b	se
a	ta	1.77	0.11	2.01	0.10	2.15	0.07
p	ta	0.91	0.04	1.03	0.03	1.03	0.03
w	ta	0.73	0.09	1.16	0.09	1.14	0.06
a	lda	1.27	0.48	1.96	0.16		
p	lda	0.65	0.38	0.93	0.07		
w	lda	0.62	0.14	1.24	0.10		
l	lda	0.70	0.50	0.75	0.09		
a	w	2.04	0.20	1.94	0.24		
p	w	1.00	0.09	0.92	0.07		
w	w	0.94	0.16	1.14	0.15		
l	w	1.02	0.07	0.88	0.07		
a	tl	1.61	0.11 ** -	2.02	0.14	1.76	0.09 ** -
p	tl	0.85	0.07	0.94	0.05	0.92	0.04
t	tl	0.58	0.15 ** -	0.77	0.13	0.75	0.05 *** -
a	ldl	1.27	0.13 ** -	1.91	0.14		
p	ldl	0.62	0.11 ** -	0.93	0.06		

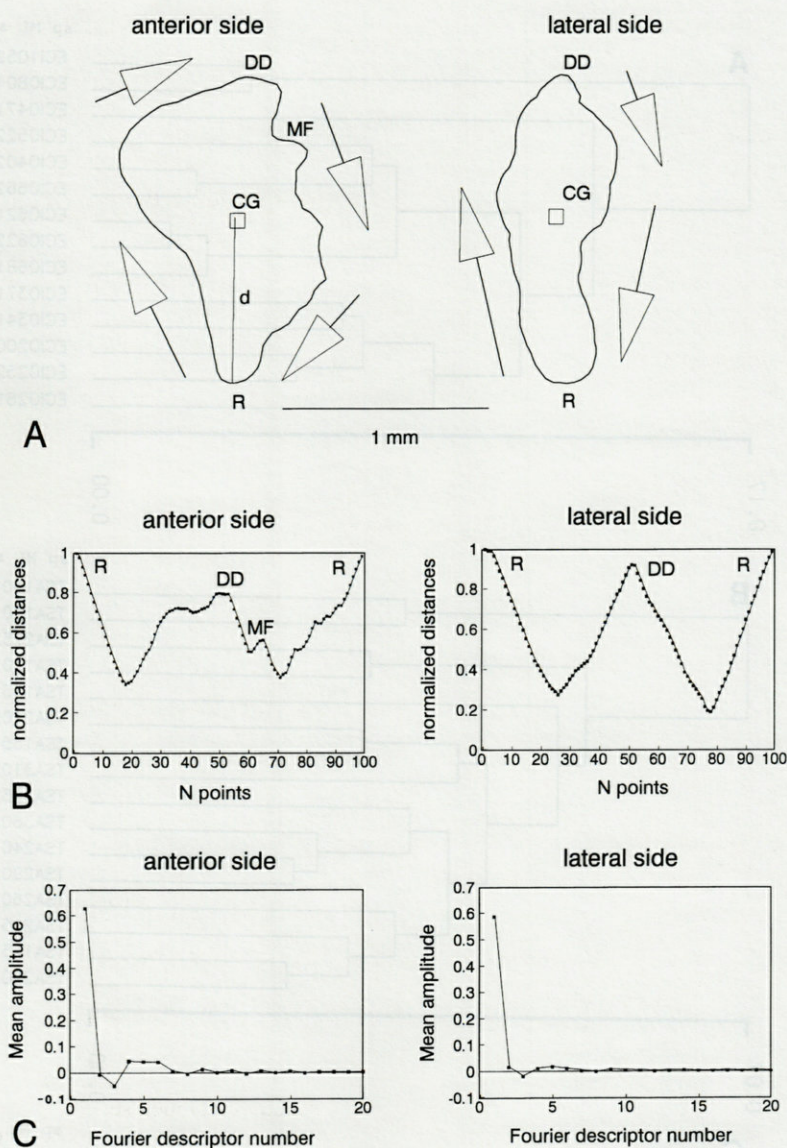** p>0.01, *** p>0.001. Significantly different from isometric growth.

Fig. 2. – Illustration of the process of numerical transformation of an outline of the statoliths of *Todarodes sagittatus*. A, Calculation of the distance (d) to the centre of gravity (CG) of the N points from the perimeter following a clockwise direction (see arrows). B, Spectra for the numerical transformations of statoliths in terms of normalized distances. C, 20 first Fourier descriptors. DD, dorsal dome. R, rostrum. MF, medial fissure.

The youngest individuals were characterized by a greater relative size of the dorsal dome.

In the case of *T. sagittatus* (Fig. 3B) the differences in shape were much smaller showing greater chaining (C), and a smaller maximum separation distance (D) and were related to the lesser development of the rostrum length of the statolith in small-sized individuals (less than 14 cm).

S. officinalis showed a high degree of shape homogeneity among all the statoliths analyzed (Fig. 3C).

In the three cases no differences in shape were observed between the sexes. Only in *E. cirrhosa* is there a relationship between shape and the sexual stage.

DISCUSSION

The observed shape variability of *E. cirrhosa* is related to the growth of the animal, so that a morphological study of their statoliths will have to take this factor into account. This heterogeneity throughout growth in *E. cirrhosa* may be related to the special structure of the statoliths of the octopods (Clarke and Maddock 1988b).

In marked contrast, *Todarodes* and *Sepia* statoliths were characterized by homogeneity of their outline shape, a fact which can facilitate the use of outlines in setting up a comparative morphological study of postlarval statoliths. Such homogeneity is similar to that observed in the statoliths
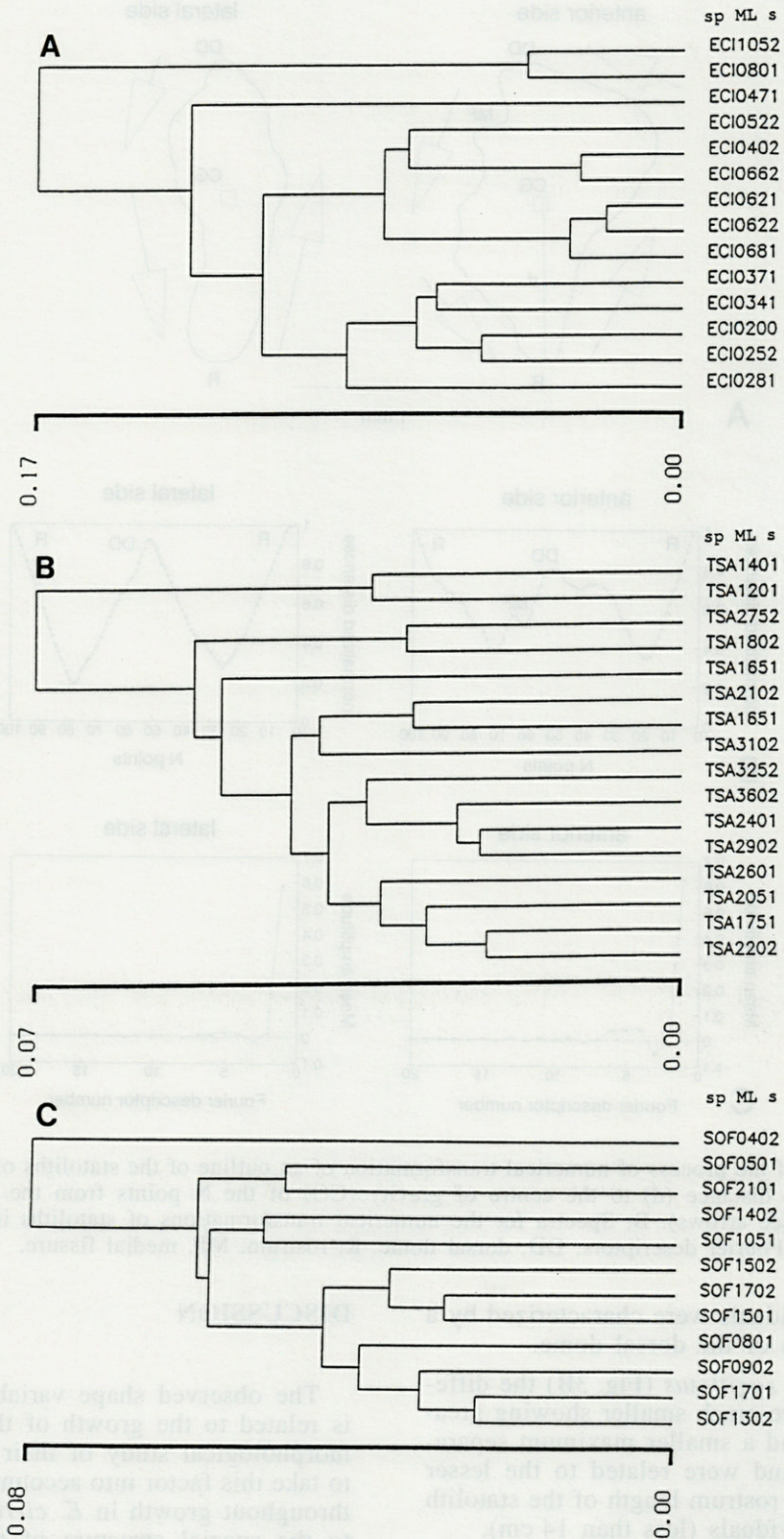
Fig. 3. – Intraspecificic hierarchical classifications of 20 first Fourier parameters from statolith shape similarity calculated on the basis of Euclidean distance. A, *Eledone cirrhosa* (ECI), cophenetic correlation coefficient (CCC = 0.948); chaining (C = 0.333); and maximum distance (D = 0.17). B, *Todarodes sagittatus* (TSA), CCC = 0.778; C = 0.419; D = 0.07. C, *Sepia officinalis* (SOF), CCC = 827; C = 0.419; D = 0.08. sp, species. ML, mantle length in mm. s, sex (1 = male, 2 = female).

of the juvenile and adult stages of *Illex illecebrosus* (Ommastrephidae) in which the larger size changes occur in the paralarval period. These changes are related to changes in the biology and ecology during this period of life (Morris and Aldrich 1984). Morphological changes in statoliths are related to changes in the sense of equilibrium (Budelman *et al.* 1987; Hanlon and Budelman 1987). The limited variability observed in juveniles and adults could be related to the rapid growth and the short life of these organisms.

This homogeneity throughout the growth of the statolith contrasts with that observed in the growth of fish otoliths. In this group, morphological changes have been observed related to changes of behaviour and habitat that are produced throughout the postlarval cycle of the fish, these being more extensive than in cephalopods (Lombarte and Castellón 1992; Lombarte and Lleonart 1993).

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THE STATOLITHS OF *LOLIGO VULGARIS* AND *L. FORBESI* HATCHLINGS : PRELIMINARY MORPHOLOGICAL STUDY

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CEPHALOPODA
LOLIGO
STATOLITHS
MORPHOMETRY
ERRATIC BEHAVIOUR

ABSTRACT. – This paper presents a comparative morphometric study of the statoliths of *Loligo vulgaris* and *L. forbesi* late embryos and hatchlings. Correlation between statolith deformities and abnormal swimming behaviour was also assessed. *L. vulgaris* and *L. forbesi* embryos and hatchlings were obtained by maintaining eggs, collected from the wild, in closed natural seawater systems. A total of 70 individuals (*L.v.* n = 44, *L.f.* n = 26) showing normal and abnormal swimming behaviour were sampled, their statoliths dissected and examined. Abnormal behaviour was not significantly related to deformation or absence of the statoliths, in spite of 25 % of all erratically swimming individuals having at least one deformed statolith. The statolith length was positively correlated with the dorsal mantle length as well as with all other statolith linear measurements. The relative dimensions of all statoliths did not differ significantly between the two species, except for the slightly larger focus-lateral dome length (FDL) in *L. forbesi* hatchlings.

CEPHALOPODE
LOLIGO
STATOLITHES
MORPHOMETRIE
COMPORTEMENT ANORMAL

RÉSUMÉ. – Une étude comparative de la morphométrie des statolithes d'embryons et de nouveau-nés de *Loligo vulgaris* et *L. forbesi* est présentée. La corrélation entre les déformations des statolithes et le comportement anormal a été examinée. Les œufs de *L. vulgaris* et *L. forbesi* ont été récoltés en mer et amenés à l'éclosion dans des systèmes fermés d'eau marine naturelle. Les individus de ces deux espèces nageant normalement et anormalement ont été séparés, leurs statolithes disséqués et examinés. Le comportement anormal des nouveau-nés n'est pas fortement associé à la déformation où à l'absence de statolithes, tandis que 25 % des individus nageant anormalement avaient au moins un statolithe déformé. La taille des statolithes est corrélée à la longueur dorsale du manteau des individus et aux autres dimensions des statolithes. Les dimensions relatives des statolithes des deux espèces ne sont pas différentes, à l'exception de la dimension focus-dome latéral (FDL) de ceux des nouveau-nés de *L. forbesi*.

INTRODUCTION

The importance of cephalopod statoliths as functional, evolutionary, chronological and ecological indicators is reported in numerous papers (Clarke 1966; Stephens and Young 1982; Radtke 1983; Young 1988; Clarke and Maddock 1988a, 1988b). The statoliths of cephalopods are contained within two fluid-filled cavities, the statocysts, located posteriorly to the cranial cartilage. Research work has shown that the statocyst is involved in the equilibrium and orientation of cephalopods, therefore functionally analogous to the vestibular apparatus of vertebrates (Budelmann 1980).

Cephalopod statoliths are composed of calcium carbonate in the aragonite crystal forms, arranged

in an organic matrix (Dilly 1976; Radtke 1983). Regular deposition of crystals form increments that have been used to estimate age and growth in squid (Rodhouse and Hatfield 1990). Crystal deposition depends upon the ionic concentration of the statocyst fluid and that of the development medium (Dilly 1976; Morris 1991). Absence or low Sr^+ concentration in the experimental culture medium deters the mineralization of the embryonic statoliths in different cephalopod groups (Hanlon *et al.* 1989). Behavioural defects in hatchlings are strongly related to malformation of some or all statocyst elements (Colmers *et al.* 1984; Hanlon *et al.* 1989).

In the Loliginidae, the statolith primordium appears in the course of embryonic development (Segawa *et al.* 1988). Possibly due to the difficulty in obtaining cephalopod eggs, embryonic

statolith growth has received little attention. Conversely, hatchling statoliths have been used in studies estimating age and growth of older individuals (Natsukari *et al.* 1988; Natsukari and Komine 1992). All statocyst elements grow and change their shape during the life of the squid (Maddock and Young 1984), and the adult statolith shape is a very useful tool for the study of evolutionary relationships (Clarke and Maddock 1988a, b).

In a separate study on the swimming behaviour of hatchlings (Martins, *in preparation*), it was observed that *L. vulgaris* swims near the surface for longer periods than *L. forbesi*. Some individuals of both species show erratic or abnormal swimming behaviour during the early post-hatching phase. The main purpose of this study was to assess if this abnormal behaviour was related to the absence or deformation of the statoliths, and to determine if the differences in swimming behaviour were in any way associated to different statolith dimensions.

MATERIALS AND METHODS

Loligo vulgaris and *L. forbesi* egg clusters were collected off the southern coast of Portugal and off the northwestern coast of Scotland, respectively. *L. vulgaris* early embryos and hatchlings were reared at 15°C, and *L. forbesi* late embryos and hatchlings were reared at 11°C. Natural seawater was used in both experiments and the water quality monitored throughout. After hatching, *L. vulgaris* hatchlings were fed live zooplankton, and *L. forbesi* hatchlings were fed a mixture of *Artemia* sp. nauplii in diluted Liquifry®. Late embryos, from Naef's (1928) stages XVI-XVIII, and hatchlings of both species were sampled individually for the dissection of statoliths. The swimming behaviour of each hatchling was recorded as abnormal if it was erratical, i.e. spinning. Hatchlings showing normal swimming behaviour moved actively in the water column in both horizontal and vertical directions.

The dorsal mantle length (DML), in millimetres, was taken from each individual prior to the dissection of the statoliths. The statoliths were dissected with fine needles, washed in 70% ethanol and mounted in DPX on glass slides. Whenever possible both left and right statoliths were mounted. Each statolith was viewed by transmitted light microscopy and the digitized image was measured (Fig. 1A) using the image analysis system PCImage™. Statoliths were recognised and defined as deformed when their form differed significantly from the form of most statoliths at the same stage (Fig. 1I,J,K). Statolith total length (TL) and maximum width (MW) measurements were adapted from those referred to by Clarke (1978) for adult statoliths. The lengths taken from the focus to the ends of the dorsal dome (FDD), lateral dome (FLD), wing (FW) and rostrum (FR) were adapted from Natsukari *et al.* (1993). The angles at the dorsal dome (< DD), lateral dome (< LD), wing (< W) and rostrum (< R) were calculated by triangulation of adjacent sides. Deformed statoliths

were not measured. All measures were taken in triplicate by the author on separate digitized images, and their coefficients of variation were less than 10%. Linear correlation coefficients were computed with the statistical package MINITAB without data transformation. Descriptive statistics was performed after size correction of linear measurements, dividing them by the statolith total length. These ratios were arctangent transformed before analysis.

RESULTS

All *L. vulgaris* and *L. forbesi* embryos or hatchlings sampled had both statoliths (Fig. 1B to 1K). Embryonic statoliths were round at stages XVI-XVII (Fig. 1B, C), becoming oval in shape by stage XVIII (Fig. 1D, E), and attaining the hatchling 'bean-shaped' statolith form (Fig. 1F, H) at pre-hatching stages. In *L. vulgaris* this shape does not change significantly during the first week post-hatching (Fig. 1G). Growth rings were visible in most statoliths from stages XVIII (Fig. 1D, E), but their counts will be presented in a separate report (Martins, *in preparation*). Statoliths from seventy individuals (*L.v.* n = 44; *L.f.* n = 26) were dissected and analysed, of which sixteen hatchlings showed abnormal behaviour (*L.v.* n = 10; *L.f.* n = 6). Abnormal behaviour was not significantly correlated to the presence of deformed statoliths ($r^2 = 0.341$, n = 16). Ca 25% of the hatchlings showing abnormal behaviour had one or both statoliths deformed (Fig. 1J, 1K). However, 5 to 6% of hatchlings with one apparently deformed statolith (Fig. 1I) swam normally during the first two days after hatching.

No significant differences were found between the dimensions of the right and left statoliths in both species (t = – 0.19, d.f. = 35, p < 0.05). At hatching, *L. forbesi* individuals are larger than those of *L. vulgaris* (Boletzky 1987). After one week, *L. vulgaris* individuals fed adequately are similar in size, DML and TL, to *L. forbesi* 1 day old hatchlings. The other statolith dimensions did not differ between the two species, except for the slightly larger focus-lateral dome length (FDL) and the smaller lateral dome angle (< LD) in *L. forbesi* hatchlings (t = – 2.64, d.f. = 47, p < 0.05).

All measurements taken were linearly correlated to the dorsal mantle length or the statolith total length (Fig. 2). Of all linear measurements, the focus-rostrum length exhibits the highest growth rate and the focus-wing length the lowest growth rate (Fig. 2). The negative correlation between the angles < R and < DD with both DML and TL also indicates that the statolith at these stages grows mainly in length (Table I). In *L. forbesi* statoliths the negative correlation between the angle < LD with both DML and TL confirms the more laterally extended lateral dome (Table I).
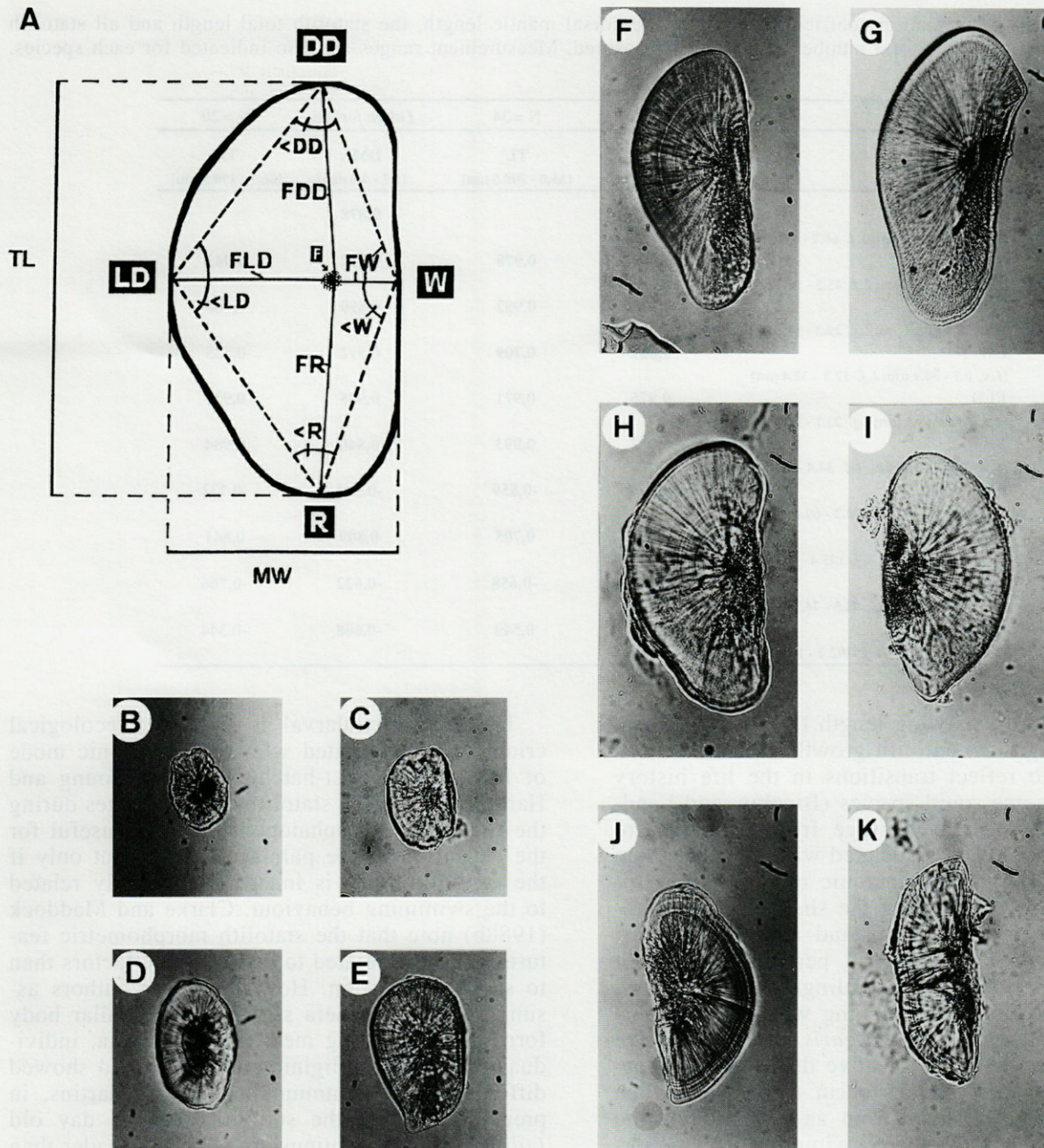
Fig. 1. – A, Hatchling statolith morphology and measurements taken, DD-dorsal dome, LD-lateral dome, R-rostrum, W-wing, TL-total length, MW-maximum width, FDD, FDL, FR and FW are the distances from the focus (F) to the dorsal dome, lateral dome, rostrum and wing, respectively; <DD, <LD, <R and <W are the angles from triangulation of adjacent sides. B to K, transmitted light micrographs of embryos and hatchlings' statoliths (scale bar = 38 µm). B and D, statoliths of *Loligo vulgaris* embryos from stage XVI (Naef 1928). C and E, statoliths of *Loligo forbesi* embryos from stage XVII (Naef 1928). F, *L. vulgaris* statolith of 1 day old hatchling (2.8 mm DML). G, *L. vulgaris* statolith of 6 days old hatchling (3.3 mm DML). H, *L. forbesi* statolith of 1 day old hatchling (3.8 mm DML). I, statolith from a 1 day old *L. forbesi* hatchling with normal swimming behaviour (3.6 mm DML). J, deformed statolith of 1 day old *L. vulgaris* hatchling that swam erratically (2.5 mm DML). K, deformed statolith of 1 day old *L. forbesi* hatchling (4.0 mm DML) showing abnormal swimming behaviour.

DISCUSSION

Information on the development and growth of the loliginid statoliths at embryonic and pre-hatching stages is scarce, usually restricted to the indication of their presence or absence. This study shows that the statolith shape and size of both species changes from mid-embryonic to hatching stages. Statolith dimensions increase linearly, with the rostrum length exhibiting the highest growth

Tabl. I. – Linear correlation coefficients between the dorsal mantle length, the statolith total length and all statolith measurements. N is the total number of statoliths measured. Measurement ranges are also indicated for each species.

	Loligo vulgaris	N = 34	*Loligo forbesi*	N = 20
	DML [1.5 - 3.3 mm]	TL [55.0 - 209.5 µm]	DML [1.7 - 4.4 mm]	TL [66.7 - 190.5 µm]
TL [*L.v.* 55.0 -209.5 µm; *L.f.* 66.7 - 190.5 µm]	0,987		0,878	
MW [*L.v.* 34.8 - 93.7 µm; *L.f.* 45.5 - 96.7 µm]	0,965	0,978	0,912	0,941
FDD [*L.v.* 28.9 - 93.8 µm; *L.f.* 29.3 - 91.6 µm]	0,975	0,982	0,899	0,968
FW [*L.v.* 8.7 - 30.9 µm; *L.f.* 12.8 - 32.4 µm]	0,667	0,709	0,512	0,525
FLD [*L.v.* 21.5 - 69.1 µm; *L.f.* 22.1 - 73.7 µm]	0,975	0,971	0,905	0,933
FR [*L.v.* 28.3 - 120.0 µm; *L.f.* 34.4 - 111.6 µm]	0,982	0,993	0,846	0,984
<R [*L.v.* 38.4 - 70.9°; *L.f.* 40.2 - 69.2°]	-0,857	-0,859	-0,543	-0,772
<W [*L.v.* 106.7 - 157.2°; *L.f.* 105.4 - 157.5°]	0,719	0,705	0,809	0,841
<DD [*L.v.* 42.4 - 72.6°; *L.f.* 48.5 - 74.7°]	-0,662	-0,658	-0,622	-0,766
<LD [*L.v.* 97.5 - 126.2°; *L.f.* 102.2 - 124.0°]	0,511	0,540	-0,608	-0,344

rate and the focus-wing length the lowest growth rate. Changes in statolith growth axes have been assumed to reflect transitions in the life history stages of some squid species (Bigelow and Landgraf 1993), such as passage from paralarval to juvenile forms also associated with a change from the planktonic to the nektonic mode of life (Boletzky 1974). Comparing the shapes of the statoliths of *Loligo* hatchlings and adults (Natsukari and Komine 1992) we can perceive significant changes. In this study, hatchlings were not reared beyond the first post-hatching week. During this week the statolith of *L. vulgaris* increased in size but did not change in relative dimensions. Apparently, the main changes occur at hatching when the late embryo shifts from an activity limiting chorionic space to a free swimming life. Observing closely the late embryos, one can detect an increased embryonic activity towards the late developmental stages, i.e. the embryo swirls or bounces back and forward inside the chorion when disturbed. Generally the late embryos remain quiescent due the tranquillizing action of the perivitelline fluid (Marthy *et al.* 1976). The factors triggering hatching are still poorly understood, and the hatching time undefined (Boletzky 1974). Embryos at stage XIX that hatch prematurely are also able to swim actively after losing their outer yolk sac and their statolith is already 'bean-shaped' (*pers. obs.*). Hence, there must be a stage in later embryonic development when the embryos reach the minimum morphological and physiological conditions that allow them to survive as free-swimming individuals.

The term 'paralarva' is based on ecological criteria and associated with the planktonic mode of life at early post-hatching stages (Young and Harman 1988). The statolith shape changes during the life cycle of cephalopods could be useful for the definition of the paralarval stage, but only if the statolith shape is indeed functionally related to the swimming behaviour. Clarke and Maddock (1988b) note that the statolith morphometric features are more related to evolutionary factors than to statolith function. However, these authors assumed that the genera studied have similar body form and swimming methods. In aquaria, individuals of the two loliginid species studied showed differences in swimming behaviour (Martins, in preparation), and the statoliths of one day old *Loligo forbesi* hatchlings are slightly broader than those of *Loligo vulgaris*. In newly hatched squids the most developed statocyst element is the statolith (Stephens and Young, 1982; Maddock and Young, 1984). The relative size of the statocyst decreases with growth, and the volume and flow of endolymph is restricted by the growth of some of its elements, including the statolith (Young, 1988). Fastest movers have the greatest restriction of flow (Maddock and Young 1984). Boletzky (1987) indicated speeds between 5 and 10 cm sec^{-1} for *L. forbesi* swimming by sustained backward jetting. Although sufficient evidence is not presently available, the broader statolith of *L. forbesi* hatchlings could be related to a more efficient swimming.

The incidence of deformed statoliths in both species was lower than the 16% mentioned by
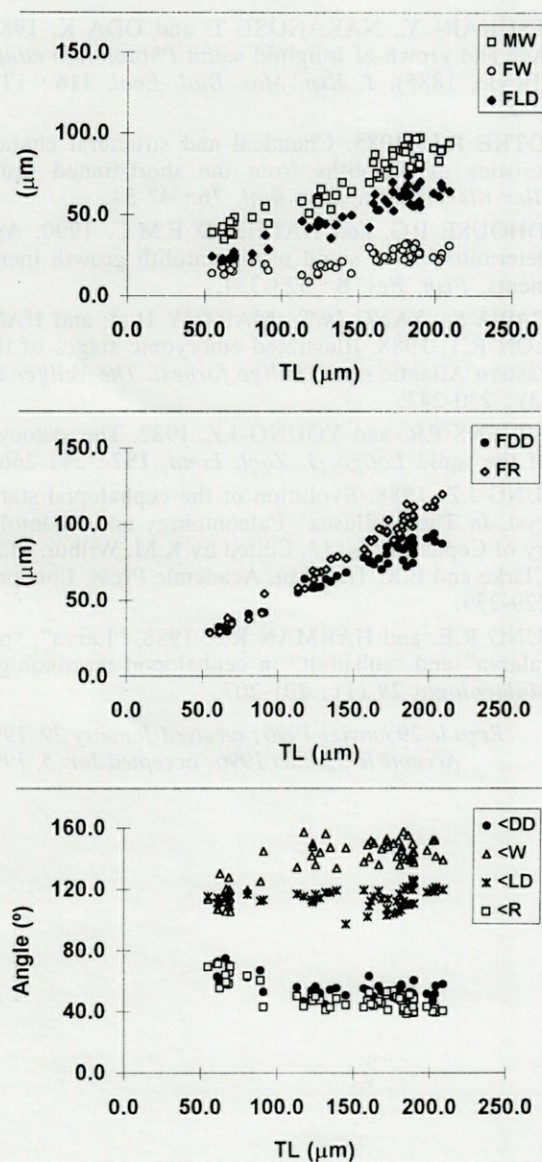
Fig. 2. – *Loligo vulgaris* and *Loligo forbesi* hatchling statolith measurements plotted together against the total length of the statolith. All statolith lengths are in micrometres (μm) and the angles in degrees (°).

Hanlon *et al.* (1989). Statolith deformities were not the sole cause of abnormal behaviour of the hatchlings in both loliginid species studied. Other deformities within the statocyst may account for erratic swimming, but verification of their occurrence in such small animals is difficult and this was not approached in this study. Debilitation due to starvation may also have contributed to the erratic behaviour of hatchlings. Further research work is necessary to investigate the significance of the statolith differentiation in the evolution, chronology and behaviour of cephalopods throughout their life cycle.

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THE RADULA SUPPORT MUSCLES OF CEPHALOPODS

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The radula supports, or "bolsters", are elongated structures whose function is obscure (Young, 1993). They are attached at the back and sides but free in front. Their muscles are all transverse, attached laterally, but free centrally and therefore not pulling on the radula. However there is a firm membrane around the muscles and contraction of the transverse muscles causes the bolster to elongate and push out the front end of the radula ribbon. They therefore work on the same hydromuscular principle as the tentacles (Kier 1982). In Nautilus and Decapods there are watery rods in the bolsters which presumably become rigid on compression. In Octopods the bolsters are purely hydromuscular. Experiments with *Octopus* have shown that the bolsters are seen to elongate when stimulated.

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THE CLASSIFICATION OF OCTOPODS

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The cirrates are so different from other 8-armed cephalopods that they should be classified in a distinct Order Cirroctopoda (Young 1989). This leaves us free to use the Order Octopoda as defined by Leach 1818 for the rest of them. His definition was given long before any cirrates were discovered. It is then possible to avoid the term Incirrata and to divide the Octopoda into three suborders, using the characteristics of their adaptation to different depths. Benthoctopoda are the common bottom-living forms. The Bathypelagoctopoda include *Japetella, Vitreledonella, Eledonella* and *Amphitretus*, which are alike in many ways in spite of the difference in their radular teeth. Epipelagoctopoda includes *Argonauta* and its allies with autotomised hectocotylus. This is a natural classification and avoids the necessity of calling common octopuses "incirrate and heterodont".

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THE LIFE OF YOUNG *SEPIA OFFICINALIS* ON THE BOTTOM

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Sepia officinalis is relatively large when it hatches. This takes place as light turns to darkness, and the hatchling crawls away to hide amongst the sea grass and algal beds, amongst which its egg had been attached. The habits of the hatchling are similar to those of the largely bottom-living adult. In a paper to be published (Nixon and Mangold, in prep.) we review the information available on the growth and development of the animal, of its central nervous system, chromatophores and colour patterns, behaviour and prey capture in its first few weeks of life after hatching. These aspects will be correlated as far as possible with changes in morphology and in the lobes of the brain. The development, habits, habitat and behaviour of *Sepia officinalis* will be compared with *Octopus vulgaris* during the same period, the former being bottom-living and the latter planktonic, although the adults of both species are benthic and coastal living.

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NEUROTRANSMITTERS IN THE PALLIOVISCERAL LOBE OF *SEPIA OFFICINALIS* L.

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The pallioviseral lobe of *Sepia officinalis* L. is an intermediate motor centre and innervates the mantle, the funnel and the viscera (Boycott, 1961). Catecholamines and 5-hydroxytryptamine (5HT) are found in the brain of *Sepia officinalis* by formaldehyde-induced fluorescence (Tansey 1980). Parr (1989) demonstrated 5HT-immunoreactivity in the visceral ganglion. Using the HPLC-method, Kime & Messenger (1990) measured dopamine, noradrenaline and 5HT in different parts of the CNS of coleoids, and the existence of noradrenaline was confirmed immunohistochemically (Marschinke, 1992). For these examinations we used specimens of *Sepia officinalis* L. (mantle-length 75-115 mm) caught in Arcachon (France). The following methods were used: the peroxidase-anti-peroxidase-method, the glyoxylic-acid-induced fluorescence (Barber, 1982) and the High Pressure Liquid Chromatography-analysis (Adams *et al.*, 1987; Kime & Messenger, 1990). The 5HT-immunoreaction in the pallioviseral lobe of *Sepia* was weak but distinctly localized in some neurons (Ø 20-30 µm) situated medio-laterally (Fig. 1). The neuropil hardly reacted to the 5HT-antibodies. Strong FMRFamide-immunoreactions were shown mostly in small perikarya as well as in the neuropil (Fig. 2) and also between fibres of the visceral nerves. Some perikarya showed a positive immunoreaction with both antibodies (Fig. 3a-c), suggesting a coexistence of 5HT and FMRFamide in the same neuron. In the lobe blue-green catecholamine-fluorescence with an emission maximum of 480-490 nm was mostly situated in the neuropil. Very strong and diffusely spread fluorescence as well as extended fluorescent streaks were typical for the neuropil (Fig. 4a-b). Distinct yellow-fluorescence (emission maximum : 520-530 nm) indicating 5HT, were only found in a few perikarya (Ø 20-40 µm) (Fig. 4c). Diffuse yellow background fluorescence occurred in the whole pallioviseral lobe. The HPLC-analysis confirms the fluorescence– and immunohistochemical examinations. Dopamine (648,72 ng/g), noradrenaline (331,74 ng/g) and adrenaline (40,07 ng/g) as well as 5HT are found. The dominant hormone is dopamine, whereas adrenaline has the lowest concentration. 5HT is found only in low quantities in the pallioviseral lobe (0,116 ng/g) and in the visceral nerves (0,054 ng/g). Parr (1989) located 5HT-immunoreactive cell bodies in the visceral lobe of some cephalopods, but details of the size are missing, so that a comparison is not possible. Jakobs (1991) found FMRFamide positive immunoreactions in singular perikarya of the visceral lobe of *Sepia officinalis*. The fluorescence and the histochemical results indicating catecholamines and 5HT as neurotransmitters confirm the findings of Tansey (1980). Earlier HPLC-analyses showed noradrenaline as well as dopamine in the visceral lobe of some cephalopods (Parr, 1989; Kime & Messenger, 1990), but adrenaline has never been measured in this part of the brain before. The higher dopamine concentration agrees with other statements in literature (Juorio & Barlow 1974). The coexistence of 5HT and FMRFamide has so far not been found in cephalopod neurons, but the results of these examinations confirm this co-localization.

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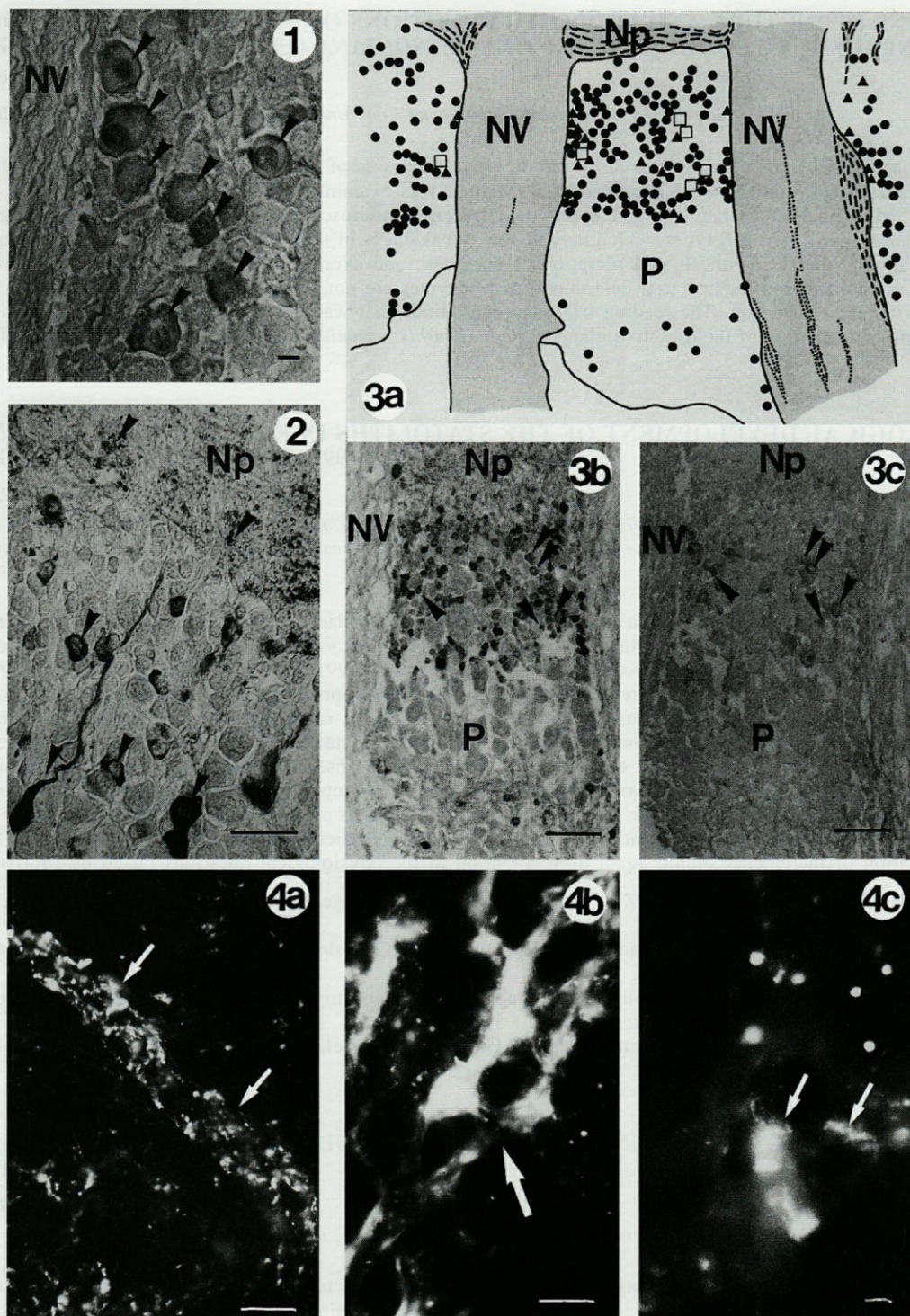
Fig. 1. – 5HT-immunoreaction (→) in a horizontal section of the palliovisceral lobe of *Sepia officinalis*. PAP-method. NV = Nervus visceralis. Scale bar = 10 μm. Fig. 2, FMRFamide-immunoreaction (→) in a horizontal section of the palliovisceral lobe of *Sepia officinalis*. PAP-method. Np=neuropil. Scale bar = 30 μm. Fig. 3, Localization of 5HT– and FMRFamide-immunoreaction in horizontal sections of the palliovisceral lobe of *Sepia officinalis*. a) Two parallel sections (b + c) have been projected in one diagram : FMRFamide (●), 5HT (▲) and the co-localization of both (□). The neuropil appears light-grey. b) FMRFamide-immunoreaction, c) 5HT-immunoreaction. → = Immunoreactions in identical perikarya. NV = visceral nerve, Np = neuropil, P = perikarya. Scale bar = 100 μm. Fig. 4 – Glyoxylic acid-induced fluorescence (⇒) in the palliovisceral lobe of *Sepia officinalis*. a)-b) blue-green catecholamine-fluorescence, c) yellow 5HT-fluorescence. Scale bar = 20 μm.

FINE STRUCTURE AND PROPOSED FUNCTIONS OF THE DIGESTIVE GLAND OF THE TROPICAL NEARSHORE SQUID *SEPIOTEUTHIS LESSONIANA* (CEPHALOPODA : LOLIGINIDAE)

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The digestive gland plays a multifunctional role in the digestive processes of cephalopods. The fine structure of this gland in the tropical nearshore loliginid squid *Sepioteuthis lessoniana* was examined in wild caught and laboratory maintained animals using histological, histochemical and cytological (electron microscopy) techniques. The digestive gland of *S. lessoniana* is characterised by 4 cell types, which may be fonctional stages of the one digestive cell. The following functions were proposed for the gland : synthesis and secretion of enzymes ; and excretion of wastes. Examination of wild caught animals and a laboratory-based feeding trial determined that enzymes are not stored in the digestive gland, but are rapidly removed soon after their secretion. The feeding trial also determined that wastes are continually removed from the gland. The fine structure and function of the digestive gland of *Sepiotheuthis lessoniana* is similar to that of other loliginid squids.

MORPHOLOGICAL DEVELOPMENT OF THE STATOLITHS IN EMBRYONIC *LOLIGO VULGARIS* (LAMARCK, 1799) (CEPHALOPODA : LOLIGINIDAE)

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Interest in recent years in age determination of cephalopods has resulted largely from their increased commercial value. Attention has been focused on the use of statoliths to determine age, and various authors have reported the evidence of rings connected with age in squid statoliths (see review in Jereb *et al.*, 1991). Morris (1988) proved that increments are distinct before hatching in *Alloteuthis*. The present study of structural development in the *Loligo vulgaris* embryo reconfirms increment formation before hatching. Egg masses of *Loligo vulgaris* were raised from early developmental stages (VIII, Naef, 1923). From stage XI, when it becomes possible to observe the statoliths under a dissecting microscope (50x), statoliths were removed for examination. A total of 108 statoliths were observed. The method for cleaning and clearing utilized was adapted from that used by Morris and Aldrich (1984). The total length of the statoliths was measured and ranged from 18.9 µm to 147.2 µm (embryonic stages XI to XX). Photographs of the morphological features of the embryonic statoliths in *Loligo vulgaris* visualize the increment marks, but the periodicity of these marks is uncertain. This lack of temporal correlation of increments should again draw attention to the physiological questions raised by Morris (1988).

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PUFFING SMOKE-RINGS UNDER WATER : THE FUNCTIONAL MORPHOLOGY OF CEPHALOPOD INK EJECTORS

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Coleoid cephalopods are known under various vernacular names that allude to the habit of inking. The Danish designation "Blaeksprutte(r)" (Steenstrup, 1849) is particularly suggestive of ink squirting above water, as celebrated in song ("with spots of the squid juice that's flyin' around" ; see Scammell 1991). The undoubtedly *defensive* function of inking is more evident under water. Here "ink squirting" has a special meaning which differs from the amusing variant in air ; in water cephalopods *release* a certain volume of ink "embedded" in a much larger volume of water expelled through the funnel tube. Bottom-living animals, especially when hidden from sight, may remain immobile when they expel ink-carrying water through the funnel ; the resulting ink "clouds" or "rings" can have a startling effect on a potential attacker. If a large volume of ink is expelled, it may form a sort of "smoke-screen" ; this method is probably more typical of behaviour under confinement than in the field. Cephalopods roaming about on the bottom or in the open water *jet away* when frightened, leaving an ink "dummy" to the attention of the attacker. Popular literature abounds with photographs of inking octopuses, cuttlefish or squids, but the technical aspects of ink ejection are rarely considered. The book "Kingdom of the Octopus" (Lane 1960) does approach this problem, describing the observations of Schäfer (1956) ; Plate 21 shows a laboratory photograph of squid ink "being discharged from a pipette" ; the ink appears to form a "phantom squid". However, a pipette is a rather incomplete representation of an ink ejector as it functions in a living cephalopod, because in the latter the ink

is retained by sphincters (Girod 1882). Thus momentary removal of sphincter muscle contraction is necessary to release a (generally small) portion of the stored ink. The ink duct empties into the rectum, close to the anal opening. An unsolved question is whether the peculiar rectal cells with their long, filamentous processes prevent ink from penetrating into the intestine (Bidder 1950). When leaving the anal outlet, the strand of ink appears to be guided for a short distance by the anal valves. It is interesting that species lacking a functional ink sac have no anal valves (Boletzky 1971). However, personal observations made on young and adult cuttlefish (*Sepia officinalis*) up to a size of 100 mm ML show that, after removal of the anal valves, normal ink clouds can be produced (and the mantle cavity is not soiled with ink!). These observations contradict the hypothesis that anal valves are necessary to minimize turbulence "so that the strand of pigment mixed with mucus from the ink gland remains intact until it has passed the funnel tube" (Boletzky 1987). The undeniable morphogenetic relationship between ink sac and anal valve reduction thus needs to be viewed from a new angle. The shape of an ink dummy depends largely on the quantity of mucus produced along with the pigment, but it is not yet clear how this proportionality is controlled. This question arises also with regard to the ejection of luminous secretions (with or without pigments) by certain sepiolid squids like *Heteroteuthis* (Harvey 1952). Especially the role of the funnel organ as a potential source of additional mucus to "envelop" the ink mass remains to be clarified. Although organ reductions do not necessarily provide a definite answer (as demonstrated by the anal valves!), it is nevertheless interesting that in *Ameloctopus litoralis*, not only the ink sac and anal valves are lacking, but in addition the funnel organ is greatly reduced (Norman 1992). Along with the anatomy and physiology of (1) the different glands secreting the components of an ink cloud and (2) the neural control of muscular movements (sphincters and locomotors), a comprehensive description of the functional morphology of cephalopod ink ejectors should integrate the hydrodynamics of both the mantle cavity and the outer body surfaces during and immediately after the generation of a jet. In the water column, it is the *moving* cephalopod as a whole that acts as an ink ejector – in contrast to the immobile bottom dweller puffing "smoke-rings" from a shelter.

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COMMENTARY ON THE INTERNATIONAL SYMPOSIUM ON FUNCTIONAL MORPHOLOGY OF CEPHALOPODS

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"These arms (the tentacles, with hooked clubs) *cannot bring what they caught* (prey) *to the mouth, they hand it over to the 8 shorter liparms* (the arms). *I think they* (the tentacles) *are rather organs to fix the body to a not very rocky sea bottom, like an anchor, and would like to call them fixation arms rather than prey catching arms."* Translated from the original generic diagnosis of *Onychoteuthis* Lichtenstein, 1818.

I chose to title this paper the same as that of the rapporteur for the first international symposium on this topic (Hulet 1982). That symposium was conducted in 1980, 15 years previous to the present one. The organizers of the current symposium felt rightly that the time had come to take another look at the functional morphology of cephalopods. Cephalopod research in general has expanded greatly since 1980, partly because of the formation and continuing efforts of the Cephalopod International Advisory Council (CIAC), under whose auspices the current symposium was organized. Furthermore, interest in funtional morphology has flourished during this period, as evidenced by several symposia and review papers (e.g., *American Zoologist*, volumes 28 and 31; *Netherlands Journal of Zoology*, volume 40).

Wake (1992) defined functional morphology as "the study of the way that form, or structure, causes, permits, and even constrains organisms to function or perform." Understanding the relationship between form and function requires that questions of "what", "how", and "why" be answered about the structure of an organism (Aerts 1990). Because each structure is a complex system, these questions of form and function may be asked at various levels of integration (Lauder 1990). Thus, for instance, functional questions may be asked about biochemical systems, the properties of cells or tissues, or the geometry and physical mechanics of organs or appendages. Questions at the organismal level may address how the general form of an organism affects the efficiency of its interactions with its physical and biotic environment, or the ways these levels are integrated. Because the form of an organism is not static in time, ontogenetic changes in function at all of these levels may be considered.

One reason for renewed interest in functional morphology over the last couple of decades is its importance to other disciplines (Gans and Gasc 1992). In particular, the optimization (or lack thereof) of function in ecological interactions is a flourishing field of study (Alexander 1988). Such studies may involve detailed investigation of biomechanics in individual species or comparisons of multiple species, focussing either on analogous structures or on homologous structures.

Distinguishing analogs from homologs is an essential aspect of systematics. Many papers have been written about the relationship of functional morphology to systematics and the study of evolution (e.g., Lang 1990; Lauder *et al.* 1995) because of the long-established importance of morphological studies in these disciplines. Understanding the details of structure and function for morphological features may aid in inferring evolutionary history by distinguishing homology from analogy. Conversely, evolutionary systematics may point out historical constraints on morphology and aid the ecomorphologist in understanding patterns of adaptation. One reason that it is important to understand the ontogeny of the form/function relationship is because heterochrony, or changes in timing of the development of various structures, has been shown to be an important factor driving evolution (Gould 1977). Functional aspects of heterochrony can be important for understanding these changes in ontogenetic timing.

Knowledge of functional morphology has practical applications as well. For example, knowing how sensory systems operate may allow improved design of harvesting methods (e.g., Flores *et al.* 1978). Understanding the functional morphology of prey capture (e.g., Kier 1982) could be important for selecting food to be offered to animals being reared for either experimental or aquaculture purposes.

Direct observation of functions is difficult for many cephalopod species, but studies of this

group can be quite rewarding. Similarities between cephalopods and fishes (Mangold and Fioroni 1970; Packard 1972) comprise one of the classic examples of convergence. Work on cephalopods also has led the way toward understanding phenomena, such as counterillumination (Young, 1978) and nerve and brain function (Young, 1938, 1961), with general applicability to other groups.

It is informative occasionally to examine trends in a scientific discipline, to highlight strong areas of recent research as well as areas perhaps in need of additional attention. By comparing the contributions to the two cephalopod symposia, one can get an idea of how this field of inquiry has progressed over the intervening decade and a half. I have reviewed the submissions to both symposia and found that they could be separated roughly into categories dealing with (1) tissues, organs and organ systems, (2) ontogeny and development, and (3) behavior.

The 1980 symposium was conducted during the annual meeting of the American Malacological Union and published in volume 23 of the journal *Malacologia*. Only 7 papers were presented, all orally, of which 6 were published. A contribution by S. Brocco on *Octopus* salivary glands was not published. Four papers (57%) dealt with organs/systems, 2 (29%) with development, and one (14%) with behavior. Thirty papers were submitted for the 1995 symposium, evenly divided between oral and poster presentations. Of these, 15 (50%) addressed questions of organs, etc., 11 (37%) concerned development, and 4 (13%) behavior. My general impression in comparing the symposia is that there is a similar distribution of interest among these arbitrary categories, but that there has been a remarkable increase in interest in the field of cephalopod functional morphology over the 15 years.

When teaching students about the many elegant features of cephalopods, the most common question I encounter is some variation of : "What does it do and how does it do it?". In large part, these are questions of functional morphology. In many cases I have to explain that we really are not sure. Many morphological structures have been described for which discussions of the structures have included speculation about their possible functions, even though direct observations or experiments to determine functions often are limited or non-existant. This is particularly true of cephalopod species other than the common squids, cuttlefishes, and octopods found in coastal waters near major marine laboratories (Vecchione 1995). Based on the healthy increase in interest revealed by attendance and contributions to this symposium, great progress can be expected in the near future.

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ANALYSE D'OUVRAGE
BOOK REVIEW

Ammonoid Paleobiology, 1996. Edited by Neil H. Landman, Kazushige Tanabe, and Richard A. Davis. Plenum Press Corporation (233 Spring Street, New York, NY 10013-1578, USA; or: 88/90 Middlesex Street, London E1 7 EZ, United Kingdom). 857 pages. Price: $ 79.50 in US and Canada; $95.40 elsewhere (only prepaid orders accepted).

"Ammonoid Paleobiology" is the 13th volume of the Plenum Press series "Topics in Geobiology". In the preface, the editors put their work in perspective by reminding the reader of an earlier volume of the series: "This book is viewed as a synopsis of our current knowledge on ammonoid paleobiology. It is by no means complete. It contains both reviews of previous work and new material. Each of the editors of this book was involved in some way with the book previously published by Plenum Press on the biology of *Nautilus*. We regard this volume as a companion piece on ammonoids."

There are seven parts of differing lengths. Part I, titled "Phylogenetic Perspective", contains one chapter by T. Engeser on "The Position of the Ammonoidea within the Cephalopoda". The introduction provides an unintentional illustration of the problem arising with names (e. g. Coleoidea) when they are used to designate both a group and a lineage: "... the Ammonoidea are more closely related to the Coleoidea than to the other ectocochleate cephalopods, i. e. the Nautiloidea", a later remark saying: "... it is still worth the effort to state more precisely the position of the Ammonoidea within the Coleoidea clade (=Neocephalopoda)". But section 3 and Fig. 2 clarify this issue by defining the group of all living and fossil forms other than nautiloids as "the Coleoidea lineage (=Angusteradulata)": "The Angusteradulata comprise the Coleoidea, the Ammonoidea, the Bactricoidca, and at least some orthoceratids...". Thus the long supposed sister group relationship between the Ammonoidea and the Coleoidea appears unchallenged.

Part II deals with the "Structure of Hard and Soft Tissues" and begins with a chapter by M. Nixon on the "Morphology of the Jaws and Radula in Ammonoids". After brief descriptions of the buccal masses in Nautiloidea and Coleoidea, the author summarises what is known of ammonoid mouthparts, with special attention to the aptychus-shaped lower jaws, and discusses their functional morphology with regard to the whole buccal mass. Aspects of food and feeding are also reviewed.

Chapter 3, by L. Dogushaeva and H. Mutvei, describes the "Attachment of the Body to the Shell in Ammonoids". The authors note that "despite the effects of diagenesis, many ammonoid shells have retained visible muscle, ligament, and mantle attachments scars"; they review the terminology of these scars and compare them (and their functional background) to what is known from the living *Nautilus* and from modern squids.

Chapter 4, by C. Kulicki, deals with "Ammonoid Shell Microstructure" from embryonic to adult stages. It provides detailed decriptions of the ultrastructure of the shell, its earliest ornamentation in the so-called Ammonitella (embryonic shell), and of subsequent modifications of the shell wall and septa, showing how these structures are built from aragonite crystals laid down in variously structured layers. The problem of secondary modifications of these crystalline structures (e. g. ultimate transformation to calcite) due to diagenetic events is also addressed.

Chapter 5, by R.H. Mapes and R.A. Davis, describes "Color Patterns in Ammonoids". Starting out from the earliest description (Orbigny, 1842), the authors survey the literature since that early record, noting that instances of actual pigment preservation are relatively rare. They discuss other color effects and address the question whether ammonoid shells may have been partly transparent (an idea for which they find little evidence). Color patterns being unknown in Paleozoic ammonites (including those from strata where other cephalopods and gastropods with preserved colors exist), it is concluded that coloration appeared only in Mesozoic ammonoids; for the latter, the authors consider possible functions of coloration, e. g. in predator/prey relationships, but also as a potential mechanism of "waste disposal".

Chapter 6, by K. Tanabe and N.H. Landman, deals with the "Septal Neck-Siphuncular Complex of Ammonoids". It provides a detailed survey of the various forms of septal neck architecture and corresponding nomenclature, of their respective distribution among suborders, and of the ontogenetic changes that these architectures undergo in different taxa within the greater groups.

Part III addresses questions of "Buoyancy, Swimming, and Biomechanics", starting with a chapter on "Buoyancy and Hydrodynamics in Ammonoids" by D.K. Jacobs and J.A. Chamberlain.

The authors review earlier concepts on the osmotic mechanisms involved in the extraction of fluid from a shell chamber (allowing it to become gasfilled). The role of gas "decoupling" of chamber fluid from the extraction site on the siphuncular tissue is discussed (concluding that the decoupling argument has "little merit"), and the various energetic implications of swimming relating to shell shape and ornamentation are reviewed.

Chapter 8, by T. Okamoto, presents "Theoretical Modeling of Ammonoid Morphology". It shows how mathematical growth models are able to generate a wide variety of shell morphologies that match very closely the forms actually observed in fossil records (including the most bizarre heteromorphs), and offers ideas on how these forms can be analysed in terms of their hydrostatic properties under different conditions of surface ornamentation.

Chapter 9, by A.G. Checa and J.M. Garcia-Ruiz, is devoted to "Morphogenesis of the Septum in Ammonoids". It proposes a "viscous fingering model" as an explanation of the essentially fractal patterns of suture line lobulation in ammonoid septa. The authors discuss also the so-called pseudosutures, pseudosepta and cameral membranes in the theoretical framework proposed.

Chapter 10, by R.A. Hewitt, describes the "Architecture and Strength of the Ammonoid Shell" in relation to habitat depth. It offers an extensive tabulation of bathymetric data and a discussion of habitat depth limits. In two concluding sections, Hewitt provides a detailed "History of Bathymetric Calculations" covering the past 30 years, and a survey of "External Shell Adaptations".

Part IV covers "Growth". Chapter 11, by N.H. Landman, K. Tanabe and Y. Shigeta, describes "Ammonoid Embryonic Development" based on a structural analysis of the Ammonitella and a comparison of size (tabulation in an Appendix) and shape of this embryonic shell in different ammonoid forms. Four different models extracted from the literature of the last three decades are discussed, with considerations on the posthatching mode of life and its relation to reproductive strategies.

Chapter 12, by H. Bucher, N.H. Landman, S. M. Klofak and J. Guex, addresses the questions of "Mode and Rate of Growth in Ammonoids". Growth stages are described as changes of shell morphology prior to the adult condition, with reflections on reproductive modes. Taking growth rates in living *Nautilus* for comparison, the authors analyse septal spacing, chamber volume and linear growth of the shell venter, and find a likely exponential increase in the time of chamber formation (correlated with a nearly exponential, though fluctuating, increase in chamber volume).

The generalized growth curve constructed for ammonoids is similar to what is known from many living cephalopods. Age at maturity as estimated from shell and jaw growth structures ranges from 1 to about 5 years for most forms, up to 20 years in one instance.

Chapter 13, by R.A. Davis, N.H. Landman, J.-L. Dommergues, D. Marchand and H. Bucher, deals with "Mature Modifications and Dimorphism in Ammonoid Cephalopods". Various kinds of late-ontogenetic shell modifications are recognized; they are discussed with regard to their morphogenetic causes and their possible biological roles (especially those relating to reproduction). Different types of dimorphism are described and reviewed with a special focus on the often elusive sexual dimorphism and the related problems of their systematic significance. Further complications due to ostensible or well-documented cases of polymorphism are also reviewed.

Part V covers "Taphonomy" and contains one chapter titled "Ammonoid Taphonomy" by H. Maeda and A. Seilacher. The authors raise the question: "Why should taphonomy be part of paleobiology?"; their answer is in the observation that "... there exists no ammonoid that does not carry a taphonomic overprint that must be intellectually removed before paleobiological analysis can begin". They discuss conditions of animal death, soft-part preservation, necrolysis, and encrustation either before or after death, and the circumstances under which dead animals sink to the sea floor. Finally transport, sediment invasion ("infill"), preservation in nodules, compaction and dissolution are discussed.

Part VI is devoted to "Ecology" and begins with a chapter on "Ammonoid Pathology" by R. Hengsbach. It provides a brief section on terminology and a review of the literature dealing with paleopath(olog)y in ammonites. Problems of nomenclature (forma types) are discussed and illustrated. The known paleopathies are tentatively classified in 4 categories as being related to mechanical injuries, parasitoses, genetic defects, and conditions of the environment.

Chapter 16, by G.E.G. Westermann, describes "Ammonoid Life and Habitat". The author considers ammonoids as generally pelagic swimmers, drifters, and vertical migrants, and notes: "The majority of ammonoids are known from epeiric seas" (i. e. epicontinental seas surrounded by land). Westermann distinguishes *epeiric, neritic,* and *oceanic* biomes; he assumes an epipelagic-mesopelagic boundary at about 240 m of depth (lower limit of chamber liquid removal by osmotic pumping) and concludes that mesopelagic ammonoids had to rise regularly to the epipelagic layer to compensate for flooding. The chapter covers ammonoid shell shape and growth stages, conse-

quences for poise, stability and mobility in the various groups, their occurrence at different depths and in different biotopes. Various scenarios (showing animals with questionable soft-parts) are given in full-page figures; a large table lists the facies and habitats of selected adult ammonoids.

Part VII is devoted to "Biostratigraphy and Biogeography". Chapter 17, by R.T. Becker and J. Kullmann, covers "Paleozoic Ammonoids in Space and Time", beginning with Pre-Devonian ammonoids identified as the uncoiled bactritids. Devonian, Carboniferous and Permian ammonoids are reviewed in separate sections, each with illustrations of stratigraphic ranges, phylogenetic relationships and biogeographic reconstructions. The concluding section covers "Systematics and Stratigraphic Distribution of Paleozoic Ammonoids" in the form of a list of taxa (from order to family level) with their respective stratigraphic ranges.

Chapter 18, by K.N. Page, deals with "Mesozoic Ammonites in Space and Time". The author introduces the 13 ammonoid suborders and their respective stratigraphic distributions from the Lower Triassic to the Upper Cretaceous, and then reviews the successive faunal provinces and realms. A full-page figure summarises the distribution of ammonoid faunal provinces throughout the Mesozoic for 25 geographic areas. In his concluding section, Page places emphasis on the observation that ammonoid faunas were dominated by very widely distributed genera only in the latest Cretaceous, and that "distinct provincialism appears to have lingered on in only one restricted area, namely, the North American Interior".

Chapter 19, by J. Wiedmann and J. Kullmann, describes "Crises in Ammonoid Evolution" in separate sections devoted to Paleozoic Ammonoids (starting in the Lower Devonian, bactritids being omitted) and Mesozoic Ammonoids, respectively. Synthetic figures prepared from works of different authors show the great faunal breaks and subsequent radiations to the Upper Cretaceous. Finally the authors pose the question : "Do Mesozoic extinctions have a common cause ?" and note, for all the Mesozoic system boundaries : "... the pattern of ammonoid extinction was perfectly comparable, if not identical, at all these boundaries... the decline was continuous, and no sudden extinction occurred". They conclude : "There is no need to involve a major cosmic impact to explain the final decline of ammonoids".

Chapter 20, by P. Ward, discusses "Ammonoid Extinction", with the special goal "to differentiate the various factors that may have been involved in diversity changes within the group". The chapter concentrates on the Cretaceous ammonoid faunas and considers competition and predation as major biological factors along with environmental factors such as sea-level changes and especially anoxic events. For the final extinction, the conclusion is the opposite of that drawn in the preceding chapter, since Ward states : "The final extinction of the ammonoids was undoubtedly brought about by the effects following the impact of the Chicxulub Comet... The virtual extinction of the plankton following this impact was probably the actual killing mechanism of the ammonites...".

The book closes with a glossary (ca 280 terms explained) and an index of taxonomic and geographic names, and of various designations and technical terms.

In contrast to "Nautilus – The Biology and Paleobiology of a Living Fossil" (1987, volume 6 of the series "Topics in Geobiology"), "Ammonoid Paleobiology" deals with "dead fossils", so any statement on biological processes and mechanisms remains hypothetical. However, the chain of arguments linking the known living cephalopods with the (only partly known) fossil forms can – and should – be forged from both ends. Many chapters of this book use biological information taken from the neontological literature. In return, cephalopod biologists can learn a lot by reading "Ammonoid Paleobiology", and they will realize how closely the incomplete knowledge of fossil forms may resemble our limited knowledge of many living deepsea cephalopods. We are only beginning to understand, for example, how squids like *Mastigoteuthis* use their tentacles (Roper & Vecchione, this volume on "Functional Morphology of Cephalopods"). Our biological view of living cephalopods is sometimes much closer to paleobiological conjecture than we would like to admit.

I recommend "Ammonoid Paleobiology" to any one taking an interest in the evolutionary history and general biology of cephalopods. This very attractive, richly illustrated book is available at a reasonable price.

S. v. BOLETZKY

INSTRUCTIONS AUX AUTEURS

TEXTE

Les manuscrits, dactylographiés en double interligne sur le recto seulement des feuilles numérotées (ne pas excéder 20 pages) sont présentés en trois jeux complets, sous leur forme définitive.

Le titre du manuscrit doit être le plus court possible; il est suivi du prénom et du nom de l'auteur (ou de chacun des auteurs) ainsi que de l'adresse (ou des adresses) du Laboratoire dans lequel a été effectué le travail.

Chaque manuscrit comportera :
— un résumé en français de 15 lignes maximum figurant en début d'article, suivi de sa traduction en anglais,
— des mots clés français et anglais (6 au maximum) permettant un traitement rapide par les revues analytiques,
— un titre abrégé pour haut de page (60 signes et espaces au plus),
— la traduction anglaise du titre de l'article,
— une liste hors-texte des légendes des illustrations et leur traduction en anglais,
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Les noms scientifiques (genres, espèces, sous-espèces) figurent en italiques ou soulignés d'un seul trait.

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GRIFFITHS C.L. and J.A. KING, 1979. Some relationships between size, food availability and energy balance in the Ribbed Mussel *Aulacomya ater. Mar. Biol.* **51** (2) : 141-150.

EAGLE R.A. and P.A. HARDIMAN, 1977. Some observations on the relative Abundance of species in a benthic community. *In* Biology of Benthic Organisms. Edited by B.F. Keegan, P.O. Ceidigh and P.J.S. Boaden, Pergamon Press, Oxford-New York, 197-208.

Le titre des périodiques doit être abrégé d'après les règles internationales (World list of Scientific Periodicals).

Les notes infrapaginales et les remerciements seront aussi brefs que possible.

ILLUSTRATIONS

Les figures au trait doivent être exécutées à l'encre de chine sur papier calque assez fort, bristol, carte à gratter, papier millimétré bleu. Il est exigé des lettres et chiffres « transfert » par caractères autocollants pour le lettrage et la numérotation, dont la taille tient compte de la réduction à supporter. Les figures sont regroupées au maximum en planches dont la justification pleine page est 17 × 24,35 cm une fois réduites (penser à retrancher la légende de ces dimensions); largeur d'une colonne : 8,1 cm. Méthode des « rectangles homologues » pour la réduction : tracer les diagonales d'un rectangle de 17/24,35 cm, les prolonger; tout rectangle admettant ces prolongements comme diagonales correspondra à la justification après réduction. Indiquer le numéro d'ordre des figures en chiffres arabes et le nom de l'auteur au crayon bleu au recto ou au dos. Ne pas inscrire de légende sur les illustrations.

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La revue publie gratuitement 2 planches au trait pleine page et 1 seule planche en simili; les illustrations supplémentaires ou en couleurs sont facturées aux auteurs.

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Un jeu d'épreuves accompagné du manuscrit est envoyé à l'auteur qui doit retourner l'ensemble après correction et indication de la place des illustrations dans le texte, dans un délai maximum de 15 jours.

Les tirages à part, facturés directement par l'imprimeur, doivent être commandés dès réception de l'imprimé spécial joint aux épreuves.

INSTRUCTIONS TO AUTHORS

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The manuscript should by typed double-spaced on one side of white paper, format A4, pages numbered (max, 20 pp), and sent in definitive form, in triplicate, to the editor.

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— a french summary of 15 lines max., followed by the english translation,
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— short version of title (60 signs and spaces max.),
— english translation of the full title,
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— a separate list of numbered tables with their legends in english and scientific names (genera, species, sub-species) should be written in italics or underlined once.

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The title of a journal must be abbreviated according to the rules set by the World list of Scientific Periodicals.

Footnotes and acknowledgments should be as brief as possible.

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Functional Morphology of Cephalopods

Edited by S. v. Boletzky, P. Fioroni and A. Guerra

SOMMAIRE/*SUMMARY*