



HAL
open science

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

M C Martins

► **To cite this version:**

M C Martins. THE STATOLITHS OF LOLIGO VULGARIS AND L. FORBESI HATCHLINGS: PRELIMINARY MORPHOLOGICAL STUDY. Vie et Milieu / Life & Environment, 1997, pp.171-176. hal-03103568

HAL Id: hal-03103568

<https://hal.sorbonne-universite.fr/hal-03103568v1>

Submitted on 8 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

M.C. MARTINS

Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB9 2TN Scotland, U.K.

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 ou 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 (Hanon *et al.* 1989). Behavioural defects in hatchlings are strongly related to malformation of some or all statocyst elements (Colmers *et al.* 1984; Hanon *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).

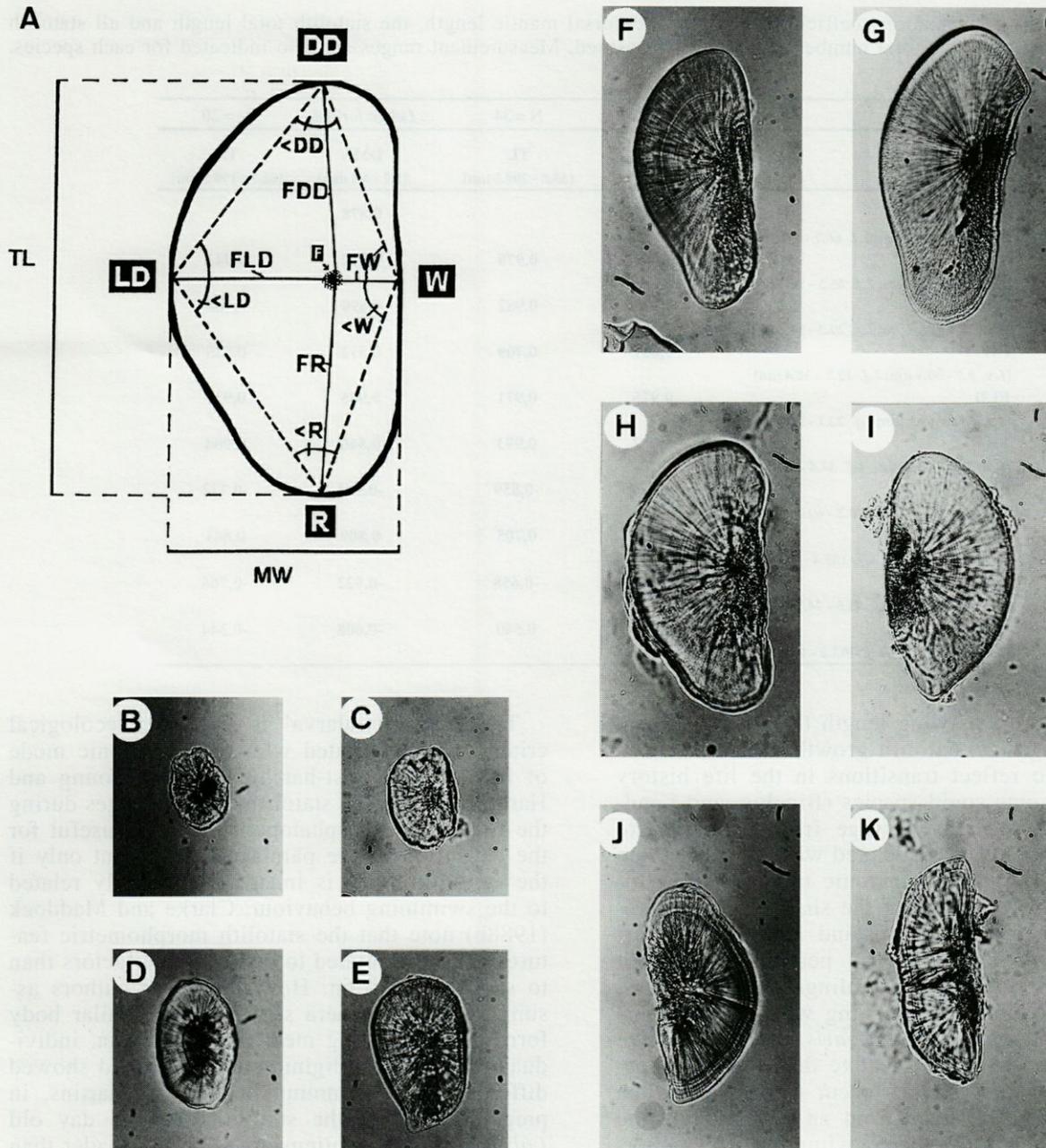


Fig. 1. - A, Hatchling statolith morphology and measurements taken, DD-dorsal dome, LD-lateral dome, R-rostrum, W-wing, TL-total length, MW-maximum width, FDD, FDL, FR and FW are the distances from the focus (F) to the dorsal dome, lateral dome, rostrum and wing, respectively; \angle DD, \angle LD, \angle R and \angle W are the angles from triangulation of adjacent sides. B to K, transmitted light micrographs of embryos and hatchlings' statoliths (scale bar = 38 μ m). 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.

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

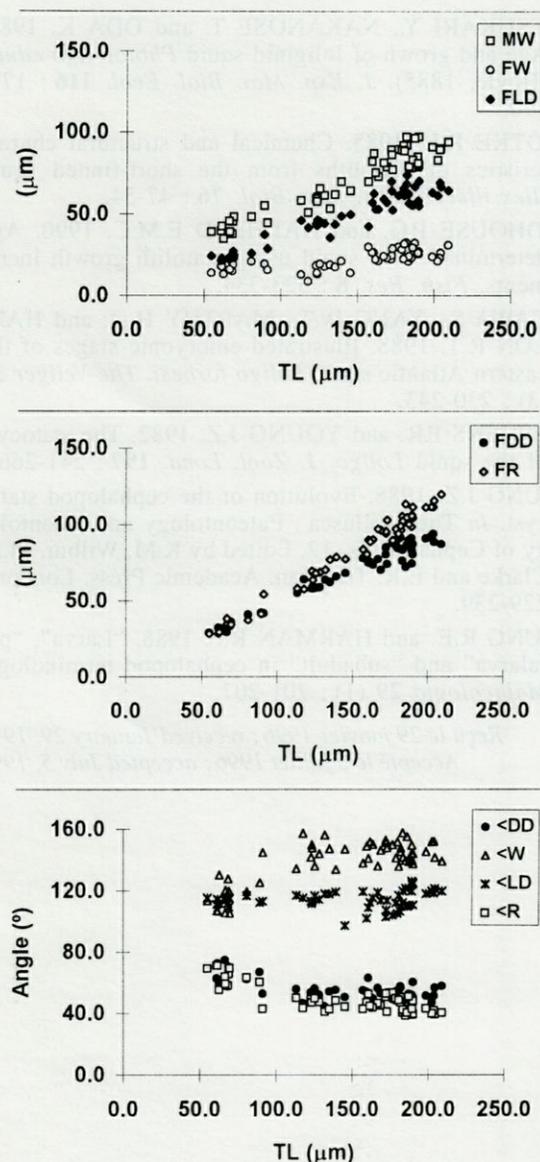


Fig. 2. – *Loligo vulgaris* and *Loligo forbesi* hatchling statolith measurements plotted together against the total length of the statolith. All statolith lengths are in micrometres (μm) and the angles in degrees ($^{\circ}$).

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.

ACKNOWLEDGMENTS. – The author is indebted to Professors P. Boyle (Dept of Zoology, University of Aberdeen), L. Coelho and P. Andrade (UCTRA, University of Algarve) who granted the possibility to work at both universities. Thanks go to M. Gaspar, M. Neves dos Santos, S. Marreiros and J.P. Jorge for providing the *Loligo vulgaris* egg clusters. J. Reis, J. Quintela and V. Bettencourt are thanked for their help during the rearing of *L. vulgaris* hatchlings at the PRODEP (University of Algarve). The author is also indebted to R. Parry and D. Hardy for supplying the *Loligo forbesi* egg clusters and to S. Hoskins (Dept of Zoology, University of Aberdeen) for help during collection of these samples. This work is a part of a PhD thesis of M.C. Martins, funded by J.N.I.C.T. – Portugal (BD-1429/91-IG, 3606/94). Two anonymous referees are thanked for useful comments on the manuscript.

REFERENCES

- BIGELOW K.A. and LANDGRAF K.C. 1993. Hatch dates and growth of *Ommastrephes bartramii* paralarvae from Hawaiian waters as determined from statolith analysis. *In* Recent Advances in Fisheries Biology, Edited by T. Okutani, R.K. O'Dor and T. Kubodera, Tokai University Press, Tokyo : 15-24.
- BOLETZKY S. V. 1974. The "larvae" of Cephalopoda : A review. *Thalassia Jugoslavica* **10** (1/2) : 43-76.
- BOLETZKY S. V. 1987. On eggs and capsule dimensions in *Loligo forbesi* (Mollusca : Cephalopoda). *Vie Milieu* **37** (3/4) : 187-192.
- BUDELMANN B.U. 1980. Equilibrium and orientation. *Oceanus* **23** (3) : 34-43.
- CLARKE M.R. 1966. A review of the systematics and ecology of oceanic squids. *Adv. Marine Biol.* **4** : 91-300.
- CLARKE M.R. 1978. The cephalopod statolith – an introduction to its form. *J. mar. biol. Ass., U.K.* **58** : 701-712.
- CLARKE M.R. and MADDOCK L. 1988a. Statoliths of fossil coleoid cephalopods. *In* The Mollusca : Paleontology and Neontology of Cephalopods. Vol. 12, Edited by K.M. Wilbur, M.R. Clarke and E.R. Trueman, Academic Press, London : 153-168.
- CLARKE M.R. and MADDOCK L. 1988b. Statoliths from living species of cephalopods and evolution. *In* The Mollusca : Paleontology and Neontology of Cephalopods. Vol. 12, Edited by K.M. Wilbur, M.R. Clarke and E.R. Trueman, Academic Press, London : 169-184.
- COLMERS W.F., HIXON R.F., HANLON R.T., FORSYTHE J.W., ACKERSON M.V., WIEDERHOLD M.L. and HULET W.H. 1984. "Spinner" cephalopods : defects of statocyst suprastructures in an invertebrate analogue of the vestibular apparatus. *Cell Tissue Res.* **236** : 505-515.
- DILLY P.N. 1976. The structure of some cephalopod statoliths. *Cell Tiss. Res.* **175** : 147-163.
- HANLON R.T., BIDWELL J.P. and TAIT R. 1989. Strontium is required for statolith development and

thus normal swimming behaviour of hatchling cephalopods. *J. exp. Biol.* **141** : 187-195.

MADDOCK L. and YOUNG J.Z. 1984. Some dimensions of the angular acceleration receptor systems of cephalopods. *J. mar. biol. Ass., U.K.* **64** : 55-79.

MARTHY H.-J., HAUSER R. and SCHOLL A. 1976. Natural tranquilliser in cephalopod eggs. *Nature* **261** : 496-497.

MORRIS C.C. 1991. Statocyst fluid composition and its effects on calcium carbonate precipitation in the squid *Alloteuthis subulata* (Lamarck, 1798) : towards a model for biomineralization. *Bull. Mar. Sci.* **49** (1-2) : 379-388.

NAEF A. 1928. Die Cephalopoden. *Fauna Flora Golf. Neapel* **35** (II) (Embryology monograph).

NATSUKARI Y. and KOMINE N. 1992. Age and growth estimation of the european squid, *Loligo vulgaris*, based on statolith microstructure. *J. mar. biol. Ass., U.K.* **72** : 271-280.

NATSUKARI Y., MUKAI H., NAKAHAMA S. and KUBODERA T. 1993. Age and growth estimation of a gonatid squid, *Berryteuthis magister*, based on statolith microstructure (Cephalopoda : Gonatidae). In *Recent Advances in Fisheries Biology*, Edited by T. Okutani, R.K. O'Dor and T. Kubodera, Tokai University Press, Tokyo : 351-364.

NATSUKARI Y., NAKANOSE T. and ODA K. 1988. Age and growth of loliginid squid *Photololigo edulis* (Hoyle, 1885). *J. Exp. Mar. Biol. Ecol.* **116** : 177-190.

RADTKE R.L. 1983. Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus*. *Mar. Biol.* **76** : 47-54.

RODHOUSE P.G. and HATFIELD E.M.C. 1990. Age determination in squid using statolith growth increments. *Fish. Res.* **8** : 323-334.

SEGAWA S., YANG W.T., MARTHY H.-J. and HANLON R.T. 1988. Illustrated embryonic stages of the eastern Atlantic squid *Loligo forbesi*. *The Veliger* **30** (3) : 230-243.

STEPHENS P.R. and YOUNG J.Z. 1982. The statocyst of the squid *Loligo*. *J. Zool. Lond.* **197** : 241-266.

YOUNG J.Z. 1988. Evolution of the cephalopod statocyst. In *The Mollusca : Paleontology and Neontology of Cephalopods*, 12, Edited by K.M. Wilbur, M.R. Clarke and E.R. Trueman, Academic Press, London : 229-239.

YOUNG R.E. and HARMAN R.F. 1988. "Larva", "paralarva" and "subadult" in cephalopod terminology. *Malacologia* **29** (1) : 201-207.

Reçu le 29 janvier 1996; received January 29, 1996
 Accepté le 5 juillet 1996; accepted July 5, 1996

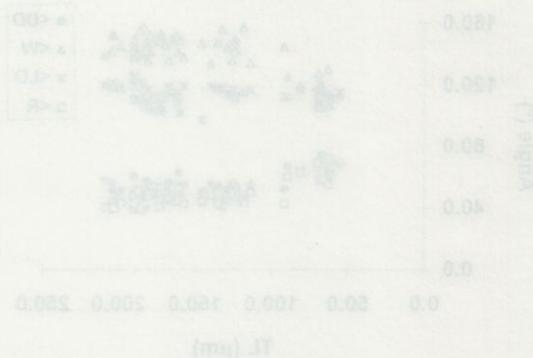


Fig. 2 - Loligo vulgaris and Loligo forbesi hatchling statolith measurements plotted against the total length of the statolith. All statolith lengths are in micrometers (µm) and the angles in degrees (°).

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