The Cycle of Activity in the Accessory Nidamental Glands from Cephalopods

A. Richard *, C. Van den Branden * * and W. Decleir * *

* Institut de biologie maritime et régionale, B.P. 41, F 62930 Wimereux, France
* * University of Antwerp (RUCA), Lab. voor biochemie, Slachthuislaan 68, B 2000 Antwerp, Belgium

INTRODUCTION

The reproductive system of female cephalopods includes a glandular system (oviduct glands and nidamental glands), which develops considerably during sexual maturation and which plays an important part in the formation of the different egg membranes. It is generally uncoloured. An exception are the nidamental glands in *Nautilus pompilius* which are composed of opaque yellowish or yellowish-green tissue (Ref. 1). Moreover all Sepioidea and Myopsida and *Ctenopteryx*, a species belonging to the Oegopsida (Ref. 2) possess accessory nidamental glands. The most striking property of these glands is their intense coloration varying from yellow to orange and red.

This paper is a comparative study of the accessory nidamental glands from *Sepia officinalis* L. (the cuttlefish), *Sepiola atlantica* d'Orbigny (the little cuttle), *Loligo vulgaris* Lamarck (the common squid) and *Alloteuthis subulata* Lamarck. These are four cephalopods which are very common on the North Sea coasts of Belgium, England, France and the Netherlands. Special attention has been given to *Sepia officinalis* L., which, since 1965, has been a main subject of intensive research at the institute of marine biology of Wimereux (France).

THE COLOUR CYCLE OF THE ACCESSORY NIDAMENTAL GLANDS

Figure 1 gives an outline of our actual knowledge of the interrelationship between the development of female gametes, vitellogenesis and the coloration of the accessory nidamental glands in *Sepia officinalis* L. These results have been obtained with cultured animals (Ref. 3), reared from the same egg cluster and kept at a constant temperature of 18°C. and in artificial light with a light-dark cycle 6:18 (Ref. 4, 5, 6, and 7). In young females, the colour of the accessory nidamental glands changes from colourless to white and then corresponds with the colour of the muscular tissue. With the ripening of the gonads the colour changes to yellow, orange and finally intense coral-red in sexually mature animals. After spawning the colour fades away to pink-red.

Figure 2 shows the endocrine mechanism controlling the development of the reproductive system in *Sepia officinalis* L. Experiments with castrated females have shown (Ref. 8) that the growth and the colour of the accessory nidamental glands do not depend on the presence of the ovary but must be correlated with the activity of the optic glands. The latter are controlled by the brain and have a secre -

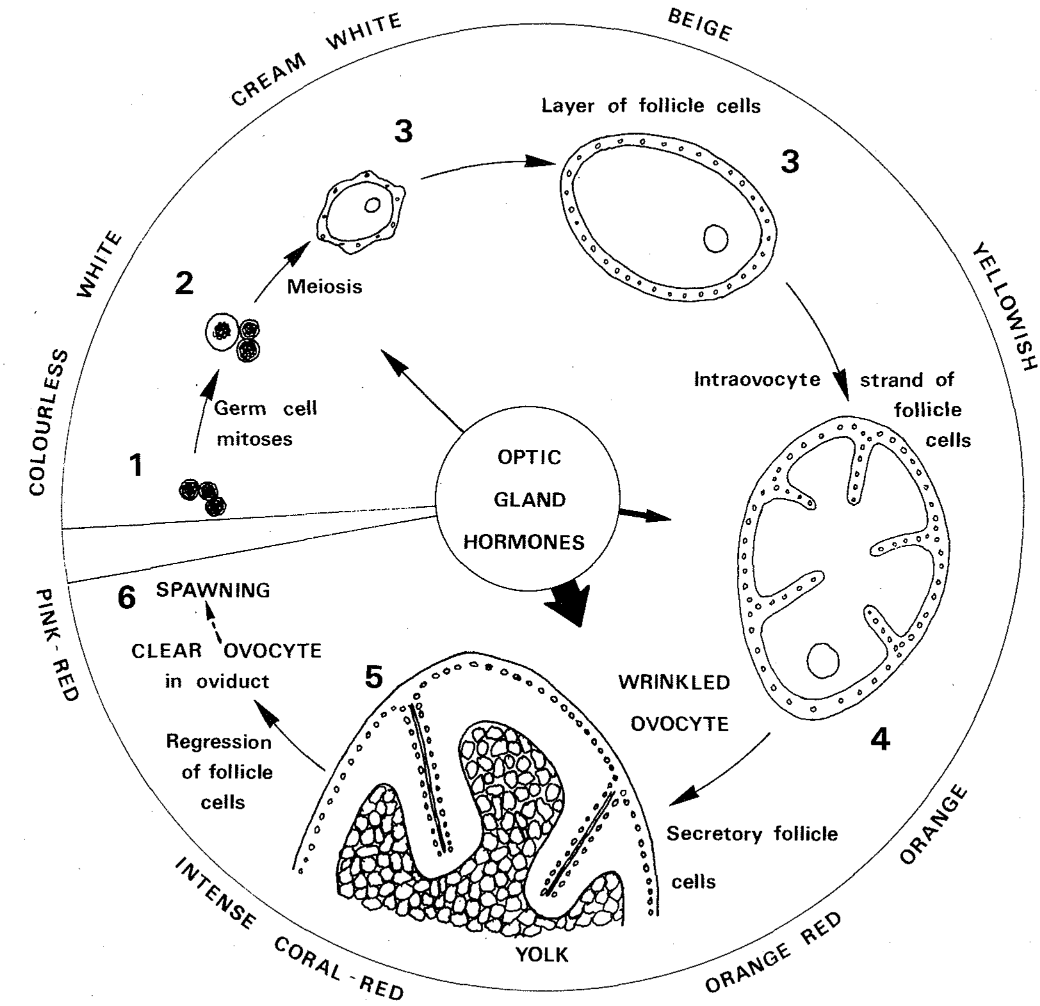

Fig. 1 : Interrelationship between development of female gametes, vitellogenesis and the coloration of the accessory nidamental glands in *Sepia*.

tory activity linked with light-dark cycles with a long dark period.

The accessory nidamental glands in *Sepiola atlantica* and *Loligo vulgaris* are relatively smaller than in *Sepia officinalis* (Fig. 3, a, b, and c) but show almost comparable colour changes.

THE SECRETORY ACTIVITY OF THE ACCESSORY NIDAMENTAL GLANDS

Our observations on *Sepia officinalis* (Ref. 9 and 10) show that during morphological differentiation the secretory cells organize themselves into tubes with increasing diameter (Fig. 4 a). Their secretory activity starts in very young animals before the first coloration of the gland appears. During this process the endoplasmic reticulum and the Golgi apparatus form granules (Fig. 4 b) which are secreted into the lumen of the tubes (Fig. 4 a). We were unable to find cellular secretory activity correlated with the colour cycle of the gland except for the

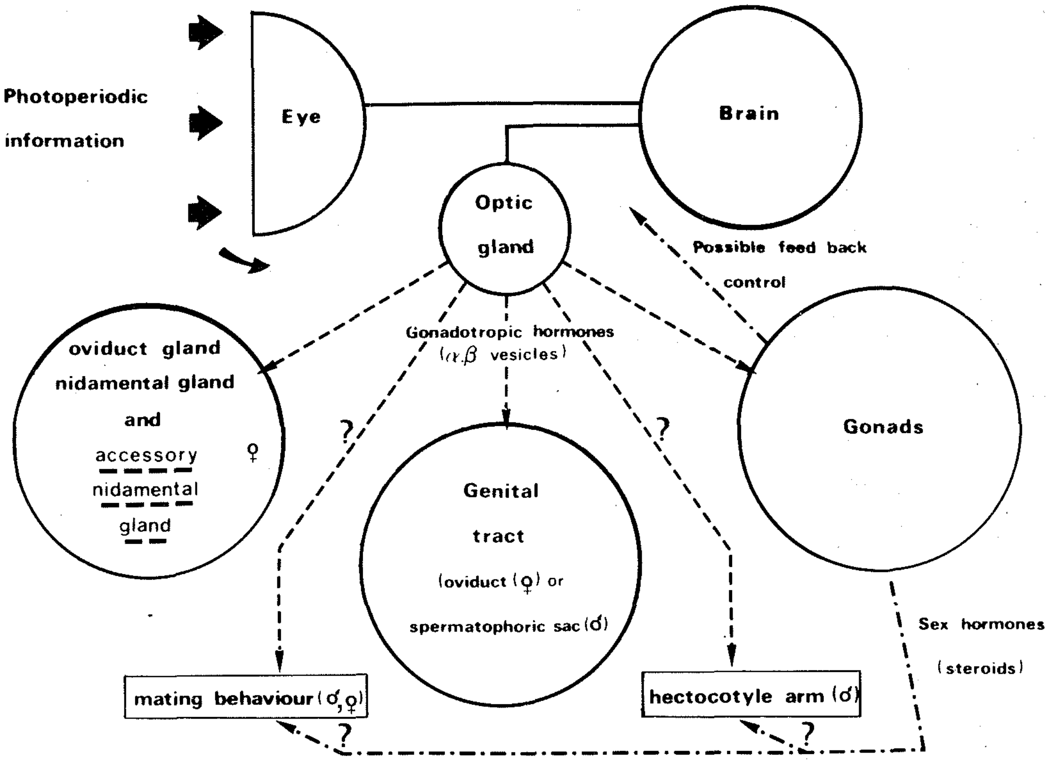

Fig. 2 : The endocrine mechanism controlling the development of the reproductive system in *Sepia officinalis* .

aspect of the secretory granules, which look completely filled with dense material in young animals, but in sexually mature animals only the central part of the granules remains osmiophilic (Fig. 4 c).

The coloration of the gland is due to the accumulation in its tubes of coloured symbiotic bacteria (Ref. 11 and 12). The glandular tissues always remain slightly beige while the content of the tubes may become intense coral-red and determine the general outlook of the glands.

STUDY OF THE SYMBIOTIC BACTERIA

By very weak homogenation and subsequent centrifugation we were able to isolate the symbiotic bacteria of the accessory nidamental glands. The general aspect of the bacteria is shown in fig. 5 and is quite different in the four cephalopod species studied. Moreover different species of symbiotic bacteria seem to occur in one host. With glands of *Sepia officinalis* and by using different culture media we succeeded in isolating eleven different types of bacteria (C. Van den Branden et al. - to be published). Five strains were not coloured but the others were respectively yellow (three strains) , yellow-orange (two strains) , and orange-red (one strain).

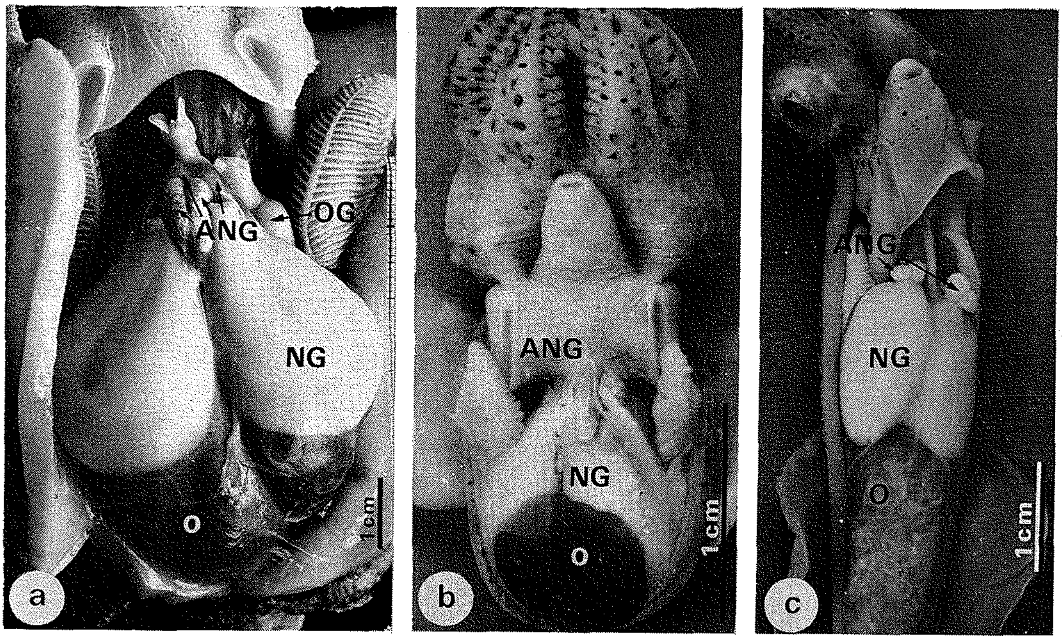

Fig. 3 : The localisation of the accessory nidamental glands (ANG) in a) *Sepia officinalis*, b) *Sepiola atlantica* and c) *Alloteuthis subulata*.
N.G. : Nidamental glands.
O. : Ovary.
O.G. : Oviduct glands.

THE STUDY OF THE PIGMENTS

We have demonstrated earlier (Ref. 9 and 12) that the pigments in the accessory nidamental glands from *Sepia officinalis* were carotenoids and that the main pigment (with a concentration of about 50 % of the total carotenoid content) is an orange-red xanthophyll which we have called SEPIAXANTHINE. We are actually studying the chemical structure of this pigment (G. Britton et al. - to be published). It is a carotenoid glycoside with probably one aromatic ring and probably one or two supplementary hydroxyl-groups. The accompanying pigments are β-carotene, γ-carotene, phytoene, phytofluene, lycopene etc. Most of these are typical microbial pigments.

Figure 6 shows our first results towards the identification of the pigments in isolated bacterial strains. After saponification the extracts from the yellow and yellow-orange strains were brightly yellow and their spectra in ether all correspond with the spectrum of β-carotene (Fig. 6 a). The pigment from the orange - red strain is clearly different. It can only be dissolved by polar solvents and its spectrum in ethanol looks like the spectrum of a crude extract from the accessory nidamental glands (Fig. 6 b). The eventual identification of the pigment in the orange-red bacterial strain as sepiaxanthine (Fig. 6 c) is a matter of further research.

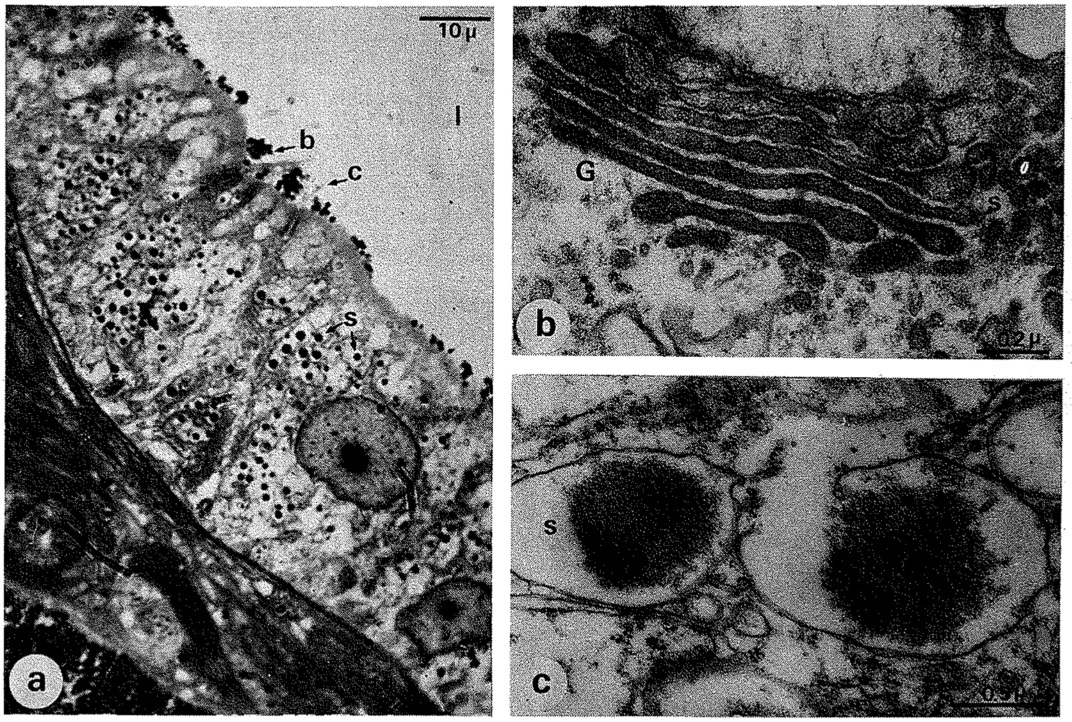

Fig. 4 : The secretory process in the accessory nidamental glands from *Sepia officinalis*.
a) Secretory cells and tubes with bacteria.
b) Golgi apparatus with small secreted vesicles (s).
c) Secreted vesicles in a gland of a mature cuttlefish.
b : Symbiotic bacteria - c : Cilia of secretory cell
G : Golgi apparatus - l : Lumen of the tube - S : Secreted vesicles.

DISCUSSION

Many female cephalopods possess accessory nidamental glands composed of a system of tubes. In the glandular epithelium granules are formed by the Golgi apparatus and subsequently secreted into the lumen of the tubes. Besides this and in correlation with sexual maturation the lumen becomes progressively filled with several types of symbiotic bacteria. These findings suggest a possible correlation between the cellular secretion and the subsequent development of symbiotic bacteria. This, however, needs further research. The bulk of the bacteria contains an orange-red pigment, which we have called sepiaxanthine in the case of *Sepia officinalis* and which gives the gland a typical coral-red appearance in sexually mature animals. After spawning the colour of the gland has become pink-red and the number of symbiotic bacteria has decreased significantly. The gland has a muscular layer for contraction and several less defined openings which lead into the mantle cavity.

All these results point to the existence, in cephalopods, of an organ which appears to be unique in the animal kingdom and for the moment we can only guess at its physiological significance.

A first possibility is that the accessory nidamental glands contribute to the formation of the egg envelopes and in this way complete the action of the oviduct - and nidamental glands. An argument against this idea is the fact that we could not

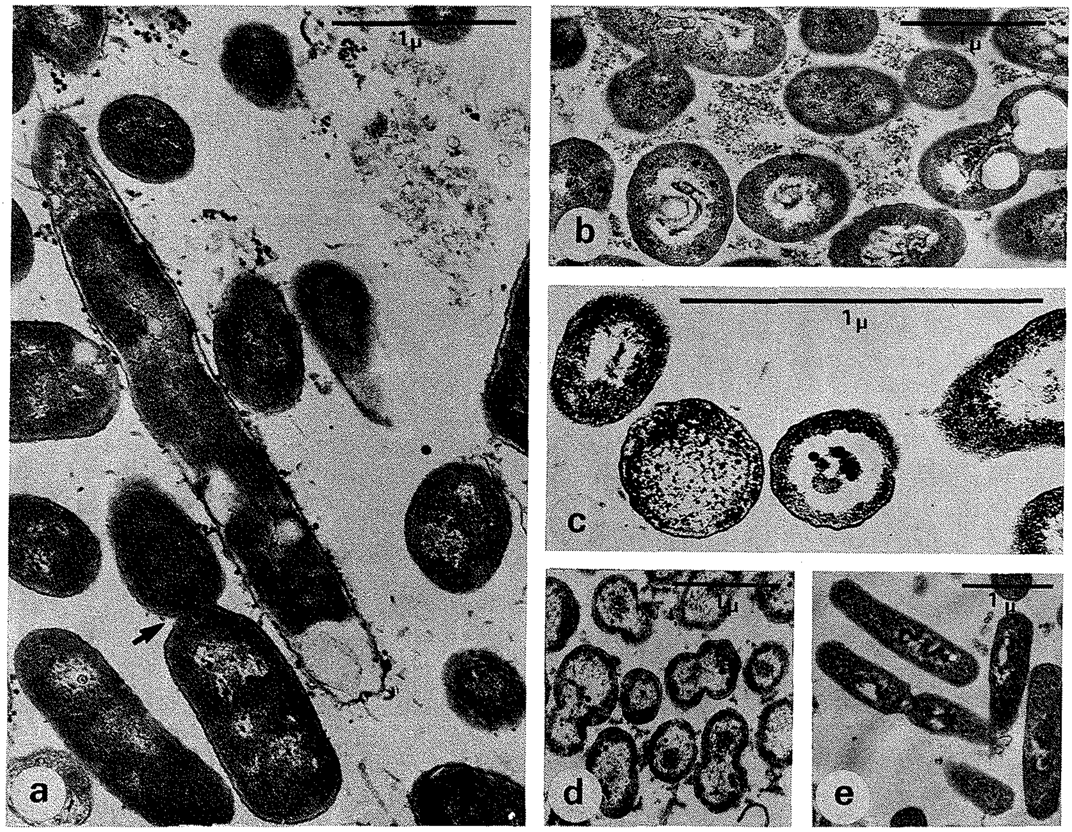

Fig. 5 : Aspect of the symbiotic bacteria in accessory nidamental glands from a) *Sepia officinalis* , b) *Loligo vulgaris* , c) *Alloteuthis subulata*, d) and e) *Sepiola atlantica* (with very different bacteria in the same animal).

detect any sepiaxanthine in eggs or egg membranes, but the possible occurrence of symbiotic bacteria in these structures remains to be studied.

A second possibility is a role in reproductive behaviour. Fishermen in the region of Boulogne (France) frequently use female cuttlefish in a cage to attract the males. The secretion of the accessory nidamental glands might act as a pheromone, but until now we never found any such effect with isolated glands or gland extracts. The destruction of the glands in very young animals or the injection of bactericide agents and the subsequent study of reproductive behaviour might solve this problem.

Another successful technique to capture cephalopods is the "pêche au lamparo" by Mediterranean fishermen. Many cephalopod species have indeed luminescent organs with symbiotic bacteria which play a part in sexual attraction. Although male cuttlefish are attracted by light, a light producing organ is unknown in this species and we have never seen any luminescence in isolated accessory nidamental glands or extracts of them. A light organ is known to occur in *Sepiola atlantica* and several other Sepiolidae but these animals have also accessory nidamental glands which lie very close to the luminescent organs.

A last possibility is to consider the accessory nidamental glands as a primitive organ with lost function (Ref. 13). We consider this hypothesis as highly im-

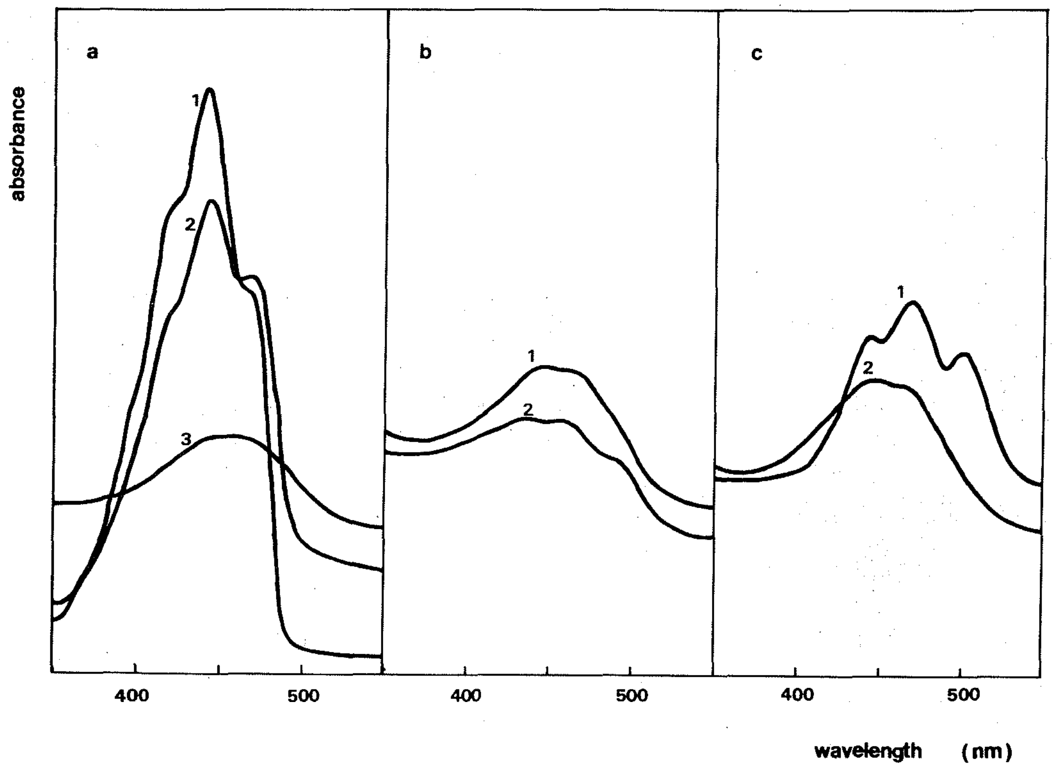

Fig. 6 : Spectral characteristics of the pigment extracts from the accessory nidamental gland of *Sepia officinalis* and from the coloured bacterial strains isolated from it.

a) Spectra in ether of the pigment extracts from the yellow and orange - yellow strains (1), the orange-red strain (3) compared with β-carotene (2).

b) Spectra in methanol of the pigment extracts from the accessory nidamental gland (2) and from the orange-red strain (1).

c) Spectra in methanol of sepiaxanthine (1) and the pigment extract from the orange-red strain, after purification by thin layer chromatography on silicagel in 40 % ethanol / ether (2).

probable and the right answer to the problem of the physiological role of the accessory nidamental glands from cephalopods remains a challenge for further scientific research.

REFERENCES

1. Haven N. (1977) Reproduction of marine invertebrates, Academic Press N.Y.

2. C.F.E. Roper, Systematics and Zoogeography of the Worldwide Bathypelagic Squid *Bathyteuthis* (Cephalopoda Oegopsida), U.S.Nat.Mus.Bull. 291, 92 (1969).

3. A.Richard, L'élevage de la seiche (*Sepia officinalis* L. - Mollusque Cephalopode), 10th Eur. Symp. Mar. Biol. 1, 359 (1975).

4. A.Richard, Action de la température sur l'évolution génitale de *Sepia officinalis* L., C.R.Acad.Sc.Paris 263, 1998 (1966).

5. A.Richard, Rôle de la photopériode dans le déterminisme de la maturation génitale femelle du Céphalopode *Sepia officinalis* L., C.R.Acad.Sc.Paris 264 , 1316 (1967).

6. A.Richard, Analyse du cycle sexuel chez les Céphalopodes : Mise en évidence expérimentale d'un rhytme conditionné par les variations des facteurs externes et internes, Bull.Soc.Zool.Fr., 95, 461 (1970).

7. A.Richard (1971) Contribution à l'étude expérimentale de la croissance et de la maturation sexuelle de *Sepia officinalis* L. Thèse de l'Université de Lille I.

8. A.Richard et J.Lemaire, Détermination et différentiation sexuelles chez la seiche *Sepia officinalis* L. (Mollusque Céphalopode), 8 th Eur.Mar.Biol. Symp. 574 (1973).

9. C.Van den Branden, A.Richard, J.Lemaire et W.Decleir, La glande nidamentaire accessoire de *Sepia officinalis* L. : Analyses biochimiques des pigments des bactéries symbiotiques, Ann.Soc.r.Zool.Belg. - In the press.

10. A.Richard et J.Lemaire, Etude cytologique des glandes nidamentaires accessoires des Céphalopodes décapodes. Arch.Biol. In the press.

11. U.Pierantoni, Organi luminosi, organi simbiotici e glandiola nidamentale accessoria nei Cephalopodi, Bull.d.Soc.Napoli, 30, 34 (1918).

12. W.Decleir and A.Richard, A study of the orange-red pigment from the accessory nidamental glands of the cephalopod *Sepia officinalis* L. Biol. Jb. Dodonaea, 40, 188 (1972).

13. A. Naef (1923). Die Cephalopoden Systematik. Monograph 35,2 - Fauna und Flora Neapel.