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Respiration of *Sepia officinalis* during embryonic and early juvenile life

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Abstract

Adult *Sepia officinalis* L. were caught in June 1984, in the coastal waters of Wimereux (France). Deposition of the eggs took place in the seawater aquaria of the "Station Marine". The oxygen consumption of *S. officinalis* was measured during embryonic and juvenile development. Aerobic metabolism occurs as soon as the early embryonic Stage 21. Oxygen diffuses through the initially thick egg shell; the oxygen level in the perivitelline liquid reaches a maximal value just before hatching (116.7 ± 6.9 mm Hg). Hatchlings display only a slight increase in oxygen consumption compared to embryos in the last stage of development. Respiration experiments with 40 d old juveniles showed that oxygen consumption increases with temperature, but is not affected by photoperiod. Experiments under increasing hypoxia revealed that *S. officinalis* juveniles are good regulators and maintain a constant oxygen consumption in the range of 4 to 7 mg O_2 l^{-1}. Juveniles successfully recover from an hypoxic stress of 2 mg O_2 l^{-1} maintained for 1 h. This suggests that the respiratory pigments (pre-hemocyanins) of 40 d-old juveniles have a high oxygen affinity and/or that these juveniles have the ability to adapt to anaerobic conditions.

Introduction

Most cephalopods display a high aerobic metabolism which surpasses that of similar-sized aquatic invertebrates and many fast-swimming fishes (Altman and Ditmer, 1971). The respiration of adult cephalopods (*Sepia officinalis, Octopus vulgaris, Loligo* spp., etc.) has already been investigated (Johansen *et al.*, 1982; O'Dor, 1982; Wells *et al.*, 1983), but as far as we know no data have been published on the respiration of embryonic and juvenile life-stages of *S. officinalis.*

Sepia officinalis embryos develop in eggs. Initially the egg shell has a thickness of ± 1.5 mm (Lemaire, 1971; and present Fig. 1), which becomes thinner during embryonic development, being almost transparent at the moment of eclosion. It is likely that the developing embryo is provided with oxygen by diffusion of this gas through the egg shell. This diffusion will be most efficient at the end of embryonic life, when the egg shell is very thin (Fig. 2). It has been calculated that the diffusion rate of oxygen through most kinds of living substances (even vertebrate connective tissue which consumes practically no oxygen) is in the order of 0.1 μl cm^2 atm h^{-1} (Florey, 1969). However, if the barrier exceeds 1 mm, a special oxygen transport system is required, or the organism must be able to survive by the use of anaerobic pathways, reduced metabolism, presence of respiratory pigments, etc. Such mechanism(s) probably exist(s) in *S. officinalis* since, for example, the blood circulation including respiratory pigments is already developed at Stage 18 of the embryonic life (Lemaire, 1970). During development the embryo is surrounded by the perivitelline liquid (Fig. 2); the volume of this liquid, which is hypertonic to seawater (De Leersnijder and Lemaire, 1972),

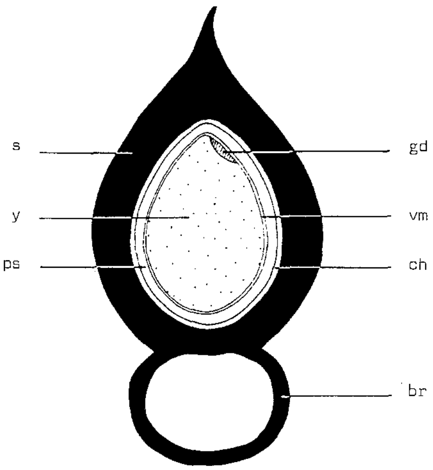

Fig. 1. *Sepia officinalis.* Schematic view of freshly laid egg. br: basal ring, ch: chorion, gd: germinal disc, ps: perivitelline space, s: egg shell, vm: vitelline membrane, y: yolk

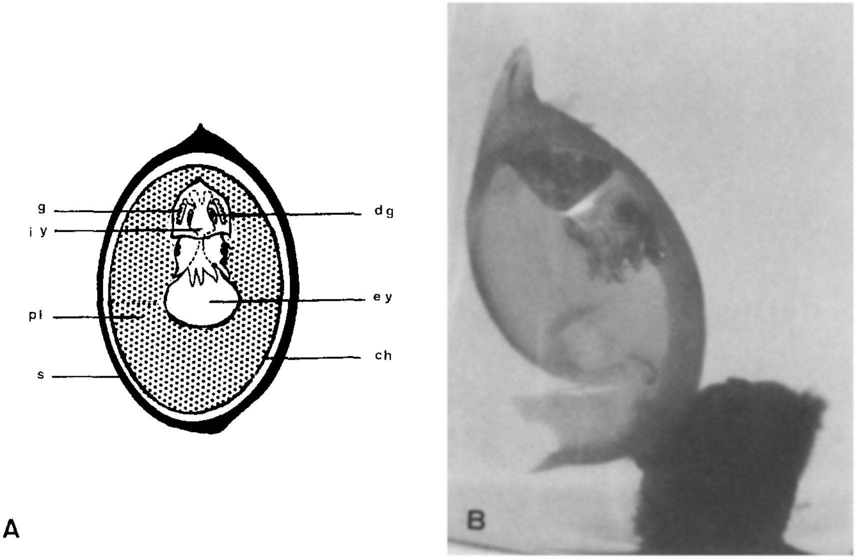

Fig. 2. *Sepia officinalis.* (A) Schematic view of developing embryo, Stage 26; ch: chorion, dg: digestive gland, ey: external yolk sac, g: gills, iy: inner yolk sac, pl: perivitelline liquid, s: egg shell. (B) Embryo at Stage 29; egg shell has become very thin; chorion is dilated by the perivitelline liquid

increases and dilates the chorion. This transparant aquatic medium is suspected to fulfil several functions, and undoubtedly it is involved in the availability of oxygen to the developing embryo.

Embryonic life can be divided into 30 stages. After segmentation (from Stages 1 to 9) and gastrulation (from Stages 10 to 17), organogenesis begins. Embryonic development from Stages 18 to 30 occupies more than two-thirds of the the total time of embryonic development (Lemaire, 1971).

In earlier studies it has been demonstrated with electrophoretic and immunological procedures that the blood of embryos contains respiratory pigments, the so called pre-hemocyanins (pre-Hcns) (Decleir *et al.,* 1971; Wolf *et al.,* 1980) from at least Stage 18. Many of these embryonic pre-Hcns are still present in juveniles, but they are replaced by the adult molecule within ± 70 d after eclosion.

During the present study, the partial oxygen pressure (pO_2) of the perivitelline liquid of three embryonic life stages was measured. We also studied the respiration of three early embryonic stages (within the egg shell) as well as the oldest stage (Stage 29) within and manually removed from the egg shell. Juvenile stages were studied under various conditions of temperature, inactive or active swimming, hypoxia and photoperiod, and throughout a 24 h respiration cycle.

Materials and methods

Adult specimens of *Sepia officinalis* were caught in the coastal waters of Wimereux, France (June 1984) and kept at the "Station Marine", where egg deposition took place. The eggs were collected and placed in well-aerated 100-litre aquaria provided with a flow-through system of fresh seawater. The temperature of the seawater gradually increased during development from 14 ° to 21 °C. Immediately after eclosion (Stage 30), the hatchlings were placed in breeding aquaria and fed with *Artemia* sp., *Gammarus* spp. and/or *Crangon vulgaris* (Richard, 1975).

Respiration of the eggs and juveniles was measured with a Warburg apparatus (Braun-Melsungen). The vessels (± 14 ml) each contained two eggs or two juveniles, 4.5 ml of freshly filtered seawater (0.45 μm – Millipore), and 0.3 ml of 20% KOH in the centre well. Before each experiment the eggs were washed for 10 min in filtered seawater containing penicillin and streptomycin (600 and 768 mg l^{-1}, respectively), to eliminate gas-exchange due to bacterial growth. Larger vessels (± 145 ml) were used for experiments with actively swimming individuals. They contained three juveniles, 12 ml of washed, sterilized and dried seasand, 40 ml of filtered seawater, and 1 ml of 20% KOH in the centre well.

For the study of juveniles under hypoxia, a polyvalent polarographic respirometer was used, as described by Verheyen *et al.* (1985). Ten individuals were placed in the respiration chamber (1 litre, 20 °C); the system was then closed, and the oxygen decrease monitored. The pO_2 of the perivitelline liquid was measured with a gas and acid-base analyzer (A. V. L. 983, Schweiz), using 50 μl samples.

Results

The respiration of Embryonic Stages 21, 24 and 29 (in the egg shell), and of Embryonic Stage 29 (out of the egg shell) of *Sepia officinalis* is shown in Fig. 3. The oxygen consumption of Stage 29 individuals inside the egg shell was only slightly lower than for those removed from the shell. Table 1 shows the relationship between embryonic respiration and oxygen availability. The pO_2 of the perivitelline liquid increased gradually during development and seemed to be the result of diffusion of oxygen into the eggs. The eggs were kept in water oversaturated with oxygen (164.3 mm Hg) but, despite this, the maximum oxygen

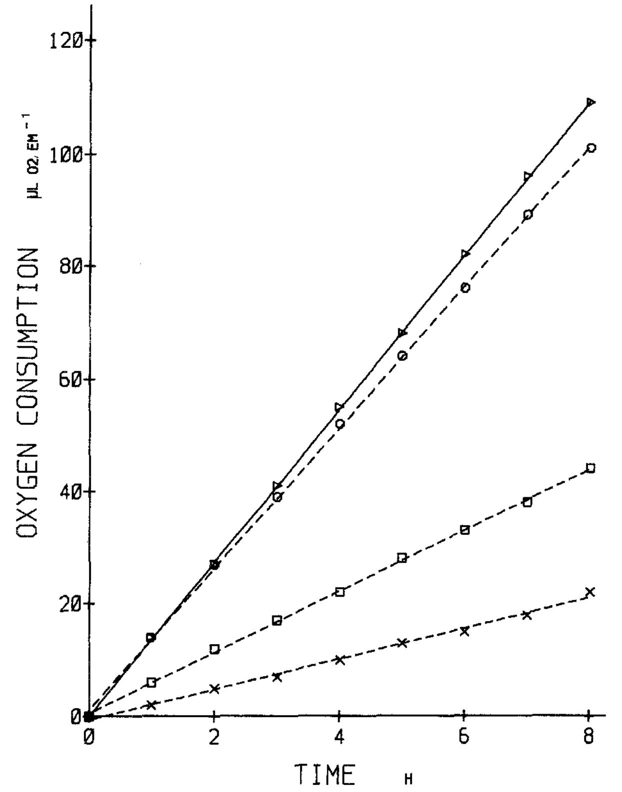

Fig. 3. *Sepia officinalis.* Oxygen consumption (µl O_2 embryo^{-1}) of Embryonic Stages 21 (×), 24 (□) and 29 (○) in the shell at 20°C. △: Stage 29 out of the egg shell, showing only a slight increase compared to Stage 29 in the shell

Table 1. *Sepia officinalis.* Relationship between embryonic respiration and oxygen content of the perivitelline liquid. nd: no data

Embryonic stage	O_2 uptake of embryo		pO_2 content of perivitelline liquid	
	µl h^{-1}	*n*	mm Hg	*n*
21	2.75 (± 0.71)	8	nd	
24	5.88 (± 0.45)	8	91.5 (± 4.2)	4
27	nd		102.7 (± 2.2)	4
29	12.64 (± 0.09)	8	116.7 (± 6.9)	4
29 (out of egg shell)	13.66 (± 0.04)	8	–	
Seawater			164.3 (± 1.0)[a]	6

[a] Oversaturated

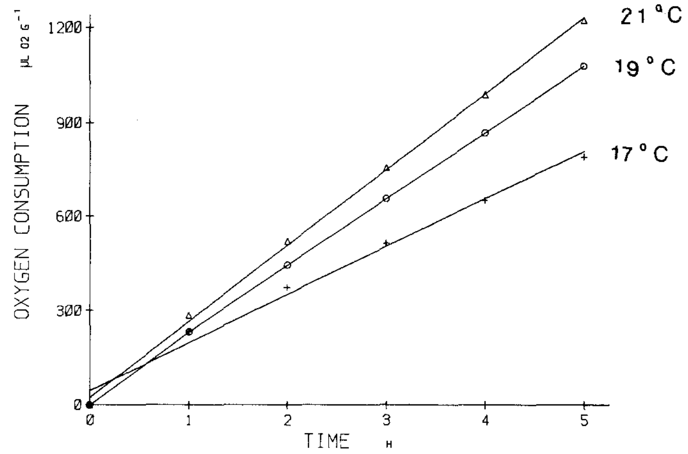

Fig. 4. *Sepia officinalis.* Influence of increased temperature on oxygen consumption (µl O_2 g^{-1} wet weight) of 40 d-old inactive juveniles (*n* = 4)

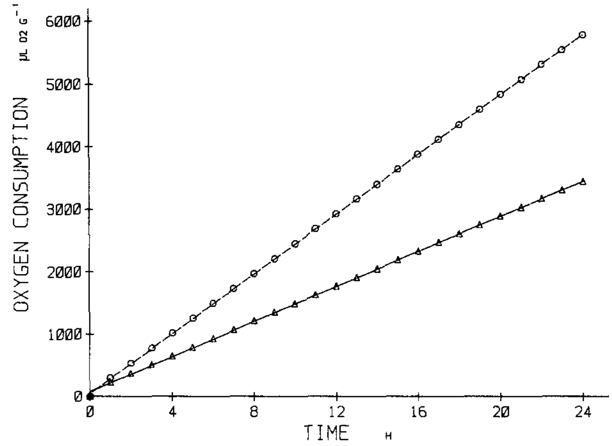

Fig. 5. *Sepia officinalis.* Influence of photoperiod on oxygen consumption (µl O_2 g^{-1} wet weight) of actively swimming (○) and inactive (△) juveniles during a 24 h respiratory cycle (40 d old, 20 °C; *n* = 4). Active individuals consumed 1.7 times more oxygen than inactive ones. Sunset and sunrise were at 10 and 17 h, respectively

content of the perivitelline liquid at Stage 29 was only about 116.7 ± 6.9 mm Hg.

The influence of increased temperature on the respiration of juveniles (40 d-old and inactive) is presented in Fig. 4. Oxygen consumption increased with increasing temperature. To ascertain the possibility of a biological rhythm (oxygen uptake being influenced by photoperiod) a 24 h respiration cycle was followed, using active and inactive individuals. During this cycle a period of 7 h was considered to be totally dark. The results showed that oxygen uptake by juvenile *Sepia officinalis* is in no way influenced by photoperiod (Fig. 5). They also indicated that active swimming specimens consume 1.7 times more oxygen than inactive individuals (measured at 20 °C).

The results obtained with the respirometer, where progressively hypoxic conditions were created, indicated that, at environmental oxygen levels ranging between 4 and 7 mg l^{-1}, juveniles maintain a constant oxygen consumption of 238 µl g^{-1}h^{-1} (20 °C); this corresponds to the values registered with the Warburg respirometer (245 µl g^{-1}h^{-1} at 21 °C). Furthermore, these tests demonstrated that, under conditions of increasing hypoxia, oxygen consumption declines markedly below the critical oxygen level of 4 mg O_2 l^{-1} (Fig. 6). The pO_2 at which recovery is still possible is 2 mg O_2 l^{-1} for a period of at least 1 h.

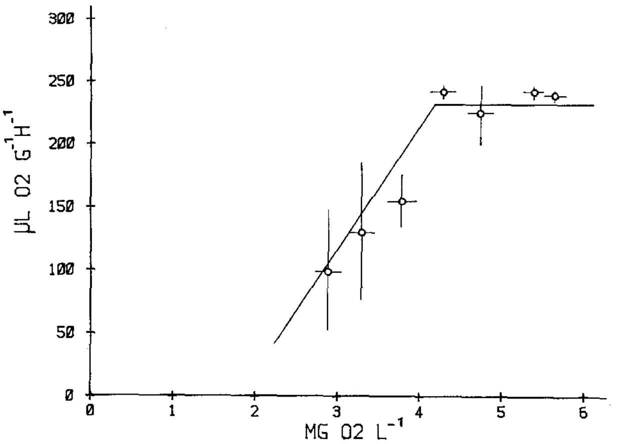

Fig. 6. *Sepia officinalis.* Relationship between oxygen concentration (mg O_2 l^{-1}), and oxygen uptake (μl O_2 $g^{-1}h^{-1}$) of 45 juveniles (40 d old, 20 °C); horizontal line is drawn on basis of mean values, and regression lines are calculated from class means (class = all observed measured values within oxygen intervals of 0.5 mg O_2 l^{-1}). Bars indicate standard deviation of class means (± SD)

Discussion

Embryonic stages of *Sepia officinalis* display aerobic respiration. In order to provide the embryo with sufficient oxygen, it is probable that some mechanism facilitating oxygen diffusion through the initially thick egg shell exists. As the egg shell becomes thinner during development, oxygen availability, which is reflected in the oxygen concentration of the perivitelline liquid, increases also. Nevertheless, the pO_2 of this liquid never attains the level of that of the external medium (164.3 ± 1.0 mm Hg). The results also clearly indicate that in early embryonic life (< Stage 24) *S. officinalis* is able to cope with environmental pO_2 values lower than 91.5 mm Hg, and that the embryonic hemocyanins (pre-Hcns) may contribute to this ability. Besides the suspected role of pre-Hcns for oxygen uptake and transport, Zandee and his group (University of Utrecht-Netherlands), demonstrated the presence of anaerobic end-products such as aspartate and glutamate in embryos < Stage 27 (D. Zandee, personal communication). Consequently, it seems likely that these embryos use both anaerobic and aerobic respiration to meet their energy requirements. From Embryonic Stage 27 until early juvenile life, respiration is predominantly supported by these pre-Hcns, indicating that these juveniles can adapt to relative low ambient pO_2 levels.

Our experiment to reveal a possible biological rhythm of respiration being affected by photoperiod indicated that aerobic metabolism is not influenced, despite the fact that in adult cephalopods light and/or darkness have a marked effect on sexual maturation (Wells and Wells, 1959; Richard, 1971; O'Dor and Wells, 1973). In other molluscs such as *Helix pomatia*, photoperiod affects the hemolymph composition (potassium concentration), and in most *Pulmonata* species it affects many aspects of somatic physiology (Meincke, 1972).

Adult cephalopods are very sensible to lack of oxygen (Ghiretti, 1966; Johansen, 1982). In our earlier work we have shown that hemocyanin of adult *Sepia officinalis* has a relative low oxygen affinity (Wolf and Decleir, 1981). However, our experiments with 40 d-old *S. officinalis* juveniles indicate that at this stage these cephalopods are quite resistant to hypoxia. After being submitted to hypoxic stress of 2 mg O_2 l^{-1} for 1 h, all tested individuals recovered completely, suggesting a considerable capacity for anaerobiosis and/or that pre-Hcns possess a high oxygen affinity.

In accordance with the results of Houlihan *et al.* (1982), who found that octopods (*Octopus vulgaris*) are regulators in the range between 4 and 7 mg O_2 l^{-1}, our results indicate that decapods such as *Sepia officinalis* are regulators in the same range from an early juvenile age. Below an oxygen concentration of 4 mg l^{-1}, a decline in oxygen consumption paralleling the decrease in the environmental oxygen level, was observed.

Further experiments with 40 d-old juveniles showed that a rise in temperature induces an increase in oxygen consumption. For molluscs such as *Pulmonata* spp., a similar temperature effect has been reported (McMahon and Russell-Hunter, 1977). Richard (1971) demonstrated the relationship between temperature and metabolism in *Sepia officinalis,* and showed that growth is accelerated by ambient water temperature.

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