

Can the early hatched cuttlefish *Sepia savignyi* cope with the environmental conditions in the Red Sea by the help of eyes and optic lobes?

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ABSTRACT

The Red Sea is characterized by its high temperature, pH and salinity in summary which affect greatly the eggs development and early hatched embryos of cephalopods. Therefore, the present work aims to study the structure of eyes and optic lobes of the hatchlings of the cuttlefish *Sepia savignyi* to know how these structures can help these juveniles to live in their habitats in the Red Sea. Also, the structures of these organs were compared with that in other hatchlings of cephalopods in other ecological niches. The results indicated that the anatomical structure of eyes and the optic lobes is related to their functions and the different needs of the lifestyle of hatchling of this cuttlefish in its environment in the Red Sea for swimming, sensation and foraging in its habitats.

Key words: Eyes, optic lobes, hatchling, *Sepia savignyi*, cephalopods, Red Sea.

INTRODUCTION

Most of the previous research works were on the adaptive plasticity and functional relevance of the eye and optic lobes structures of adult cephalopods (e.g. Fishelson *et al.* (2004), Scharpf *et al.* (2008). Warrant and Locket (2004) found that the size of eyes and density of their photoreceptors have great impacts on the sensitivity of vision in adult cephalopods living in the deep sea. The distribution of visual cell nuclei in the retina in relation to the habitat of five species of Decapods cephalopods had been studied by Makino and Miyazaki (2010). The fine structure of the visual cells and the retinal plexiform layer of adult *Octopus vulgaris* and *Octopus macropus* have been investigated by the electron microscopy by Tonosaki (1965) and Ali (2018), respectively.

The post-hatching development of the brain was studied by Yamazaki *et al.* (2002) in *Octopus ocellatus*. Also, the close relation between morphology and function of the visual system in the hatchlings cephalopods have been investigated by

Bozzano *et al.* (2009) in the squid *Sepioteuthis australis* who indicated the importance of this visual system for development and survival of hatchlings in their habitats. The size of eyes and brain neuropil was found to be correlated to differences in six hatchlings of cephalopods which were attributed to the differences in species-specific habitats and habits (Wild *et al.*, 2015). In the present study, the macro- and microanatomy of the eyes and optic lobes of the early post hatchling *Sepia savignyi* from the Red Sea have been investigated to know how this animal can cope with life in its environment. Also, the results were compared with those of hatchlings of other groups of cephalopods from the same and different families, habitats and behavior. This will provide the necessary information for inter and intra-specific comparison between this species and different ones. Also, it will indicate the eco-adaptive interpretation of cephalopod nervous systems in hatchlings and their adaptive radiation to survive in their habitats.

MATERIALS AND METHODS

Sit of Study:

This study was conducted in Magawish Resort at Hurghada city on the Red Sea. It is located between the kilometer

mark (10) and kilometer (12.5) on the Hurghada / Safaga road and within the city of Hurghada on the Red Sea coast in front of the Great Magawish Island (Fig. 1).



Fig. 1. Site of study.

Physicochemical parameters:

The surface seawater Temperature, pH and salinity in the eggs collection site at the sea shore of Magawish Resort (Fig. 1) were determined by using the multi-parameter meter (Hanna Instrument, HI 9829) during April 2021.

Eggs collection:

Egg mass of *Sepia savignyi* were collected by hand net from the shore of Magawish resort where sea grass mass is found.

Hatching of embryos:

The collected eggs were kept in plastic tank (50cm L X 50cm W X 20cm Depth) filled with seawater and with continuous aeration using air pump till eggs hatched. Water was changed every day. Eggs were hatched after 10 days of their collection.

Preparation of Sections:

Hatchlings *S. Savignyi* were fixed in Bouin's solution for 12 hr and then preserved in 70% ethanol till sectioning. For preparing sections, the preserved hatchlings were embedded in Paraplast wax.

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Transverse sections (5 μm thick) were cut and prepared for staining with Mayer's hematoxylin and eosin. The direction of cutting sections was shown in Figure (2).

Terminology: The current terms are those used Wild *et al.* (2015) for hatchlings cephalopods.

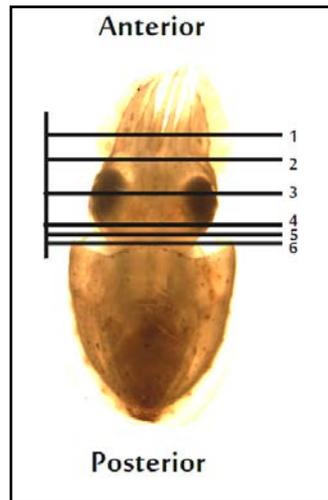


Fig. 2. Drawing of early hatched *Sepia savignyi* DV indicating plans of cutting sections

RESULTS AND DISCUSSION

Physicochemical study:

In the present study, the average values of the surface water temperature, pH and salinity at Magawish resort were 22.5 ± 0.12 °C, 8.1 ± 0.01 and 40.56‰ , respectively during April 2021. The relatively high water temperature is attributed to solar radiation and seasonal changes in air temperature during this period of the year (spring). The surface water temperature recorded for seawater near the study area was ranged from 22.77 ± 0.11 to 22.14 ± 0.03 °C by Abdelmongy and Meselhy (2015). The pH value recorded in this study lies within the preferred pH levels (7.0 - 8.5) for biological productivity (Abowei, 2010). Salinity is an important for some chemical processes, and it has a strong effect on

nutrients levels at the coastal areas. The high salinity value at the investigated current site ($40.65 \pm 0.01\text{‰}$) agreed with that recorded by Abdelmongy and El-Moselhy (2015) at spring (40.23 ± 0.1 - $41.33 \pm 0.09\text{‰}$) near the present site.

Morphology of hatchling of *S. savignyi*:

The hatchling of *S. savignyi* has horizontal head with 3.7 mm diameter, big eyes and eight arms arranged in a circle surrounding the buccal mass (Fig. 3), in addition it has two tentacles between arm pairs 3 and 4 on each side (Figs. 4,5). Funnel extends from mantle opening in the ventral side. The mantle surrounds the internal organs and has two lateral fins (Fig. 3). The nervous system is prototypic in the arms and tentacles of hatchling *S. savignyi* (Figs. 4,5).

The eyes:

Eyes of *S. savignyi* hatchling are big and slightly dorsal to the head's horizontal midline (Fig. 3). Each has an outer lid that poorly developed and behind the cornea there is a thickened integument (Fig. 6A, B). There is a quite voluminous anterior eye chambers that lead backward halfway ventral of each optic lobe (Fig. 6A). The horizontal diameter of each eye is about 1.4 mm. The eyes "float" in the anterior eye chambers and are attached to the optic lobes through their axon bundles. The eye space is filled with the white body and anterior chamber organ (Fig. 5B). The vitreous body has a diameter of about 450 μ m.

The retina of hatchling *S. savignyi* appears comparatively narrow and the retinal pigment in the central parts of the eye cup is dark and thin, while the central part of retina is connected to the optic lobe, and it is flattened (Fig 6B). Retina is formed of two layers; an outer layer that contains the visual cells with photoreceptors and an inner proximal layer that appear darkly stained. There is a second retinal layer or the retinal plexus (Fig. 7).

The structure of retina of the adult *Octopus macropus* is formed of four types of visual cells and cornea which is formed of three layers by using TEM (Ali, 2018). This may indicate the differences in *Octopus* rather than that in decapods cephalopods and this also indicates the variation in their habitats.

The internal yolk reservoirs provided the nutrient sources for embryos of cephalopods during their development (Boletzky, 2003) and it is almost completely depleted before hatching, and their visual systems are adapted to match various conditions of light in the aquatic environment and to see preys to catch them for survival (Villanueva *et al.*, 1996). This explains the big eyes of the hatchling of *S. savignyi* as well as in hatchling of *Sepia*

officinalis described by Wild *et al.* (2015). Groeger *et al.* (2005 & 2006) indicated that vision of the hatchlings *Sepia officinalis* improves slowly with growth, while the growth rate of eyes is much slower than the rest of the body.

The eye structure of hatchling *S. savignyi* is quite similar to that in other cephalopods that have cornea (Budelmann *et al.*, 1997; Young and Vecchione 2004). However, the size of the anterior eye chamber is large in hatchlings of *S. savignyi*. Similar eye size was reported by Wild *et al.* (2015) in hatchlings of *S. officinalis* and *Rossia macrosoma*. The same authors detected medium eye size in hatchling of *O. vulgaris*, and smaller in hatchlings of *Sepietta obscura*, *Idiosepius notoides* and *Loligo vulgaris*. The large lens diameter of hatchling *S. savignyi* increases resolution and sensitivity than smaller ones as described for *S. officinalis* by Wild *et al.*, (2015).

The development of cornea to enclose the eye occurs at different stages according to species, it occurs at stage 25 in *S. pharaonis* (Lee *et al.* (2016), 28 in the *Sepia officinalis* (Lemaire 1970), 38 in *Sepiella japonica* (Yamamoto 1982), and after 2 days of post-hatching in *Sepioteuthis australis* (Bozzano *et al.*, 2009). In the present study, the hatchling *S. savignyi* (stage 30 of embryonic development) has cornea enclosed the eye. However, this must be occurred before this stage.

The retina of hatchling *S. savignyi* is similar to that of *S. officinalis* hatchling described by Wild *et al.* (2015), whereas the proportions of each retinal single layer may be designed different. In hatchling of *Sepioteuthis australis* (Bozzano *et al.* 2009) the distal visual cells of retina are comparatively short, which may result in a low photon yield at hatching time. The high amount of pigment granules in both the inner and outer granular layers of retina of

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hatchling *S. savignyi* may suggest the ability of retina for diurnal activity and light-adapted state. Similar suggestion was given to hatchling of *L. vulgaris* (Wild *et al.*, 2915). The habitat and feeding behavior

may play a role in determination the enclosure time of eyes in hatchling *Sepia savignyi*, however this role cannot be detected by Lee *et al.* (2016) for *Sepia pharaonis*, therefore it needs further studies.

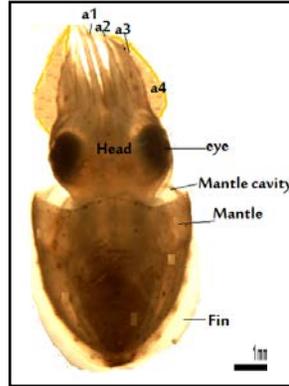


Fig. 3. Photomicrograph of DV of hatchling *Sepia savignyi*

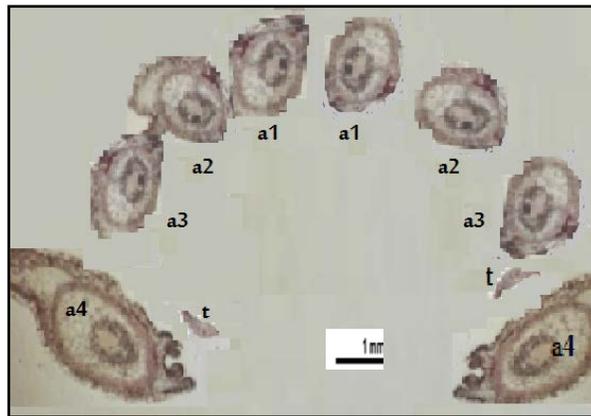


Fig. 4. Photomicrograph of TS of hatchling *S. savignyi* at brachial crown with arms and tentacles at Plan 1 showing 8 arms and tentacles. a1-a4=arms 1-4, t=tentacle.

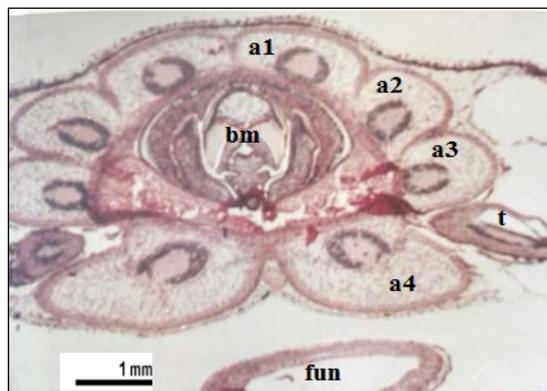


Fig. 5. Photomicrograph of TS of hatchling *S. savignyi* at the level of buccal mass at Plan 2 showing 8 arms and tentacles. Also, funnel. A1-a4=arms 1-4; bm= buccal mass; fun=funnel; t=tentacle.

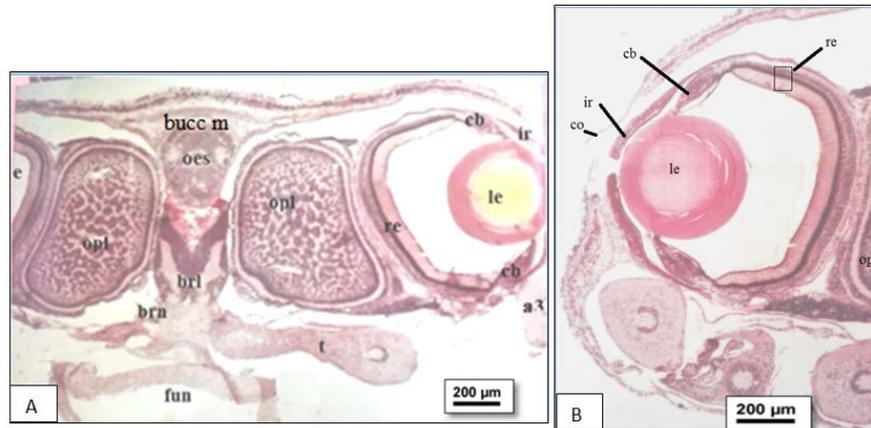


Fig. 6. Photomicrograph of TS of the eye of hatchling *Sepia savignyi* at Plan 3 as seen from ventral side of the animal. A-Eyes with optic lobes connected to them through optic nerve. Optic lobes are separated by brachial lobe and buccal mass and oesophagus. B- Right eye to show its structure. a3=arm3, brl=brachial lobe, bucc m=buccal mass, cb=ciliary body, co=cornea, e=eye, fun=funnel, oes=oesophagus, ir=iris, le=lens, opl=optic lobe, t=tentacle.

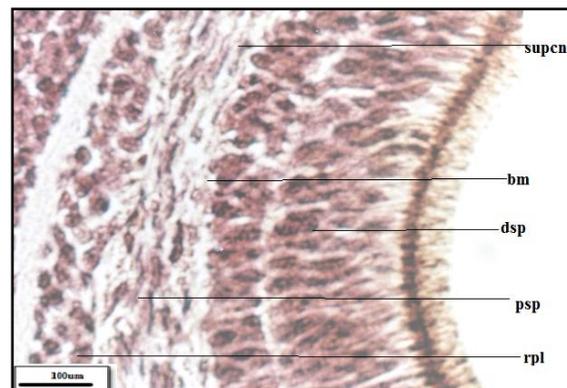


Fig. 7. Photomicrograph of TS of retina of hatchling *S. savignyi* showing its structure of photoreceptors, psp=proximal segments of photoreceptors, bm=basal membrane, dsp=distal segments, rpl=retinal plexus, supcn=supporting cell nuclei.

The Optic lobes:

The hatchling of *S. savignyi* have two apart optic lobes (minimal distance ca. 280 µm dorsally) and separated by the buccal and frontal lobes (Figs. 5A , 7). The esophagus, the inner yolk sac and the brachial lobes are found between the frontal portions of the two optic lobes. The thin ventral portion of the optic commissure passes across the esophagus under which a profile of the inner yolk sac is visible (Fig. 5A).

Each optic lobe consists from outside to inside of an outer cortex or deep retina

(retina profunda) and an inner medulla. The former consists of an outer granular cell layer, outer plexiform zone, inner granular cell layer and the inner plexiform zone. The medulla is continuous spongy structure without sharp divisions and contains a number of layers which are not clearly distinguished in hatchling *S. savignyi*. The neuropil in medulla has a complex spongy like structure and islands of numerous cell nuclei (Fig. 8). The optic commissure is well developed and connects the two optic lobes also it connects them with other supra and subesophageal lobes (Fig. 8).

The peduncle commissure lies slightly above the optic commissure. Figures (10 & 11) show the variation of optic lobe shape and size as the sections extend toward the posterior side of the head in hatchling *S. savignyi*. The different supra and subesophageal lobes are indicated in relation to the optic lobes.

The optic lobes tracks in adult cephalopod brain have been previously investigated (Wirz, 1959; Young, 1965a,b, 1971, 1976a,b, 1977 and 1979). The optic lobes in cephalopods are a conspicuous part of the highly developed visual system, and the big lens eyes can compete with the convergent developed vertebrate eyes (Packard, 1972). In contrast to vertebrates, the outer segments of ectodermal photoreceptors of cephalopods are directed toward the light, and in the optic lobes, the first synaptic connections occur and not in the retina. The retinal image from the eye in squid is sent to the optic lobe "retina profunda" (Young, 1974) where it undergoes visual information processing to help cephalopods to be adapted to environmental conditions. This explains why *S. savignyi* hatchlings are considered visual predators after hatching where their yolk is almost completely consumed, and they have to find and catch prey to survive (Villanueva *et al.*, 1996). Wild *et al.* (2015) mentioned that the hatchlings cephalopods are pre-adapted to the particular requirements of their habits, and this explain the inter alia, in morphological differences of eyes and optic lobes of their various species.

The idea of binocular vision in hatchlings of *S. savignyi* may be supported by the slight forward orientation of their optic axis, as it is done by former behavioral experiments carried out by Wells (1958) and Collewijn (1970). In the present study the structures of cortex with outer plexiform

layer and medulla with its spongy neuropil of the optic lobes of hatchlings *S. savignyi* are similar to those described by Wild *et al.* (2014) for hatchlings of *Sepia officinalis*, *Rossia macrosoma*, *Sepietta obscura*, *Idiosepius notoides*, and *Octopus vulgaris*. In *Loligo vulgaris*, an additional inner plexiform layer is present, and in outlines also visible in *S. officinalis* (Wild *et al.*, 2014), similar observation was recorded in the present study for hatchlings of *S. Savignyi*. Young (1974) mentioned that this layer is a decabrachian character and should be developed more or less pronounced. The structure of the medullar neuropil in hatchlings *S. savignyi* is found to be more developed than that in hatchlings of *O. vulgaris* (Wild *et al.*, 2014).

The optic lobes are widely separated in *S. savignyi* hatchlings which are similar to those in each of *S. officinalis*, *Rossia macrosoma*, *Sepietta obscura*, and *Octopus vulgaris*. However, they are less separated by the brain in *Idiosepius notoides* (Wild *et al.*, 2015).

Sepia savignyi hatchlings are greatly similar to those of *S. officinalis*, in structure of eyes and optic lobes and they have adult-like behavioral repertoire. Also, they are similar to those of *S. officinalis* in having a benthic lifestyle with self-entrenching behavior and visual hunting. This requires good vision, complex motoric competences, good swimming abilities and tentacle use (Hanlon and Messenger, 1996; Nixon and Mangold, 1998). Liu *et al.* (2017) found that the structure of the optic lobe is modified with the development of cuttlefish from late embryonic stage to adulthood and this is responsible for the continuous increasing in body complexity and visuomotor behavior of this animal. Sakurai and Ikeda (2022) reported that the eyes of the oval squid (*Sepioteuthis lessoniana*) grew faster than the optic lobes till age 120 days.

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In squids and cuttlefishes, mucosubstances secreted by nidamental glands served as an egg shell to protect the fertilized ova from infections (Cornet *et al.*, 2015). In the current study adult female *Sepia savignyi* lays eggs with protective thick envelopes near the coast at Hurghada and embryos are often exposed during low tide, therefore their sensory system may intervene to regulate stress regarding dehydration, and osmoregulation. The presence of thick envelope protects the embryos from the damage due to desiccation and can limit osmotic problems. With growing of embryos, their eggshell becomes thinner and permeable to seawater, allowing the supply with different ions and the required respiratory gas (Boletzky, 1993).

In spite of the direct development and presence of protective envelopes, *S. savignyi* embryos are strongly have an early embryonic sensory nervous system, which is likely related to the perception of external signals as described for *Sepia officinalis* by Navet *et al.* (2014). Shigeno *et al.* (2001) mentioned that brain of cephalopod embryos is originated from 4 pairs of separately formed of ganglia; the optic, pedal, palliovisceral or visceral and the cerebral ganglia. This indicated the importance of optic lobes as main structures in the process of brain development in cephalopods. These ganglia are called here lobes and they are clearly visible in hatchling of *S. savignyi* as shown in Figures (10 & 11).

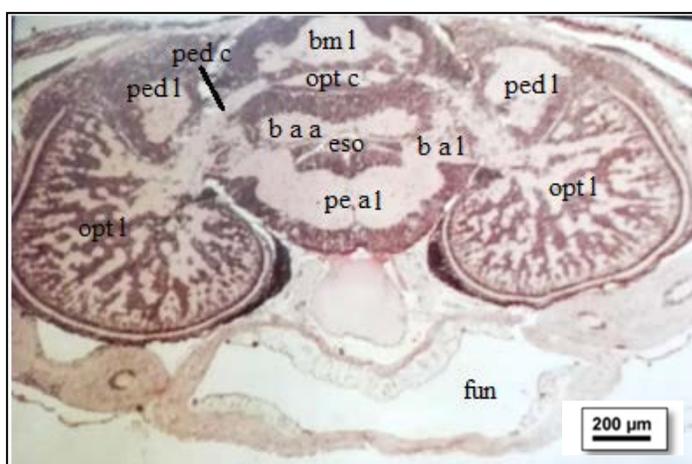


Fig. 8. Photomicrograph of TS through brain of hatchling *Sepia savignyi* at plan 4 showing optic lobes, superior frontal, basal and anterior pedal lobes. The optic commissure connects the optic lobes with the brain. b a a= anterior anterior basal lobe, b a l=lateral anterior basal lobe, b m l=median basal lobe, oes= oesophagus, pe a l= anterior pedal lobe, ped c= peduncle commissure, ped l= peduncle lobe, opt c= optic commissure, opt l= optic lobe.

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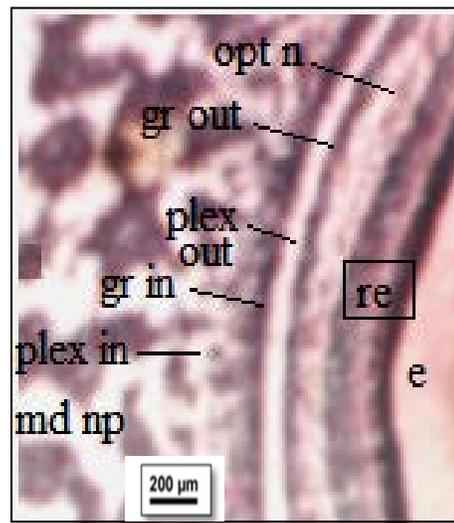


Fig. 9. Photomicrograph of TS through the optic lobe of hatchling *Sepia savignyi* showing its structure. e=eye, gr in=inner granular layer, gr out= outer granular layer, md np= medullar neuropil of optic lobe surrounded by pericaryal islands, opt n= optic nerve, plex in= inner plexiform layer, plex out= outer plexiform layer, re= retina.

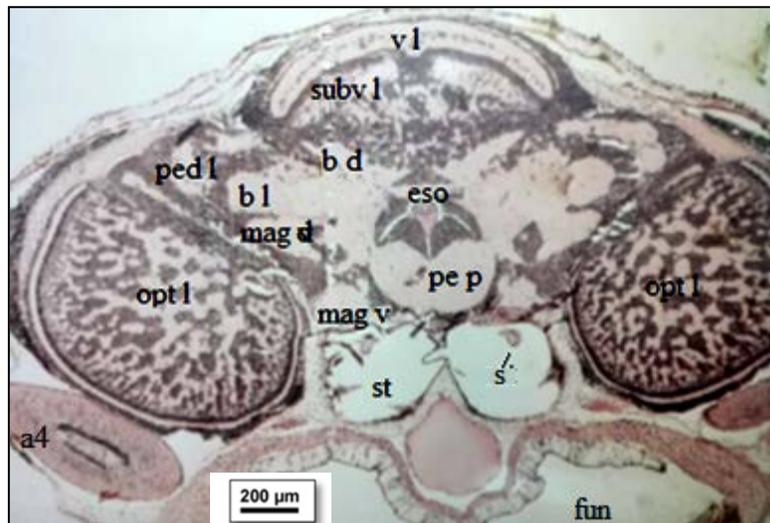


Fig. 10 . Photomicrograph of TS of hatchling *S. savignyi* at the posterior portion of optic lobes at Plan 5 showing different supra and subesophageal lobes, statocysts with statoliths.

b d=dorsal basal lobe, b l= lateral basal lobe, eso=esophagus, mag d=dorsal magnocellular lobe, mag p=posterior magnocellular lobe, opt l=optic lobe, pe p=posterior pedal lobe, ped l=peduncle lobe, s=statolith, statocysts (st), subv l=subvertical lobe, v l=vertical lobe.

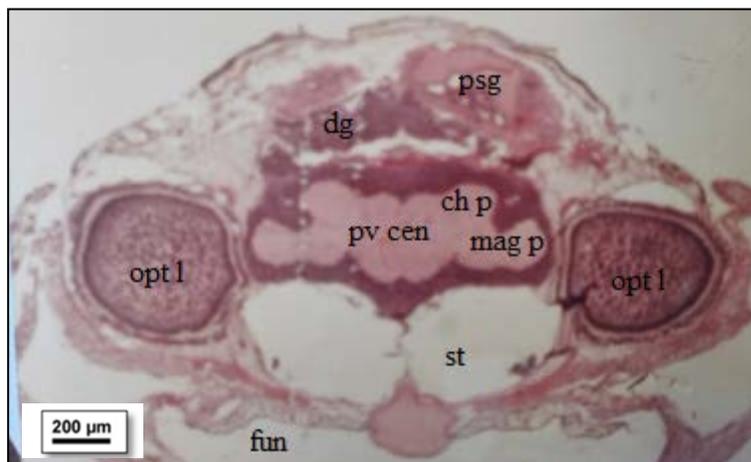


Fig. 11. Photomicrograph of TS of hatchling *S. savignyi* at the posterior portion of optic lobes (opt 1) at plan 6 showing central palliovisceral lobe (pv cen), posterior chromatophores lobe (ch p), posterior magnocellular lobe (mag p), posterior salivary glands (psg), digestive gland (dg), statocysts (st) and funnel (fun).

Conclusion

The structure of the eyes and optic lobes of hatchlings *S. savignyi* help them to pre-adapted to their lifestyle and the requirements to survive in their environment. These organs have the same basic bauplan in other coleoid cephalopods; however they vary interspecifically in size, proportions, and complexity.

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Can the early hatched cuttlefish *Sepia savignyi* cope with the environmental conditions in the Red Sea by the help of eyes and optic lobes?

هل يستطيع الحبار المبكر *Sepia savignyi* أن يتكيف مع الظروف البيئية في البحر الأحمر بمساعدة العيون والفصوص البصرية؟

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المستخلص

يتميز البحر الأحمر بارتفاع درجة حرارته ودرجة حموضته وملوحته باختصار مما يؤثر بشكل كبير على نمو البيض وعلى أجنة رأسيات الأرجل التي تفقس مبكرًا. لذلك ، يهدف العمل الحالي إلى دراسة بنية العيون والفصوص البصرية لصغار الحبار *Sepia savignyi* حديثي الفقس لمعرفة كيف يمكن لهذه التراكييب أن تساعد هؤلاء الصغار على العيش في بيئاتهم في البحر الأحمر. أيضًا ، تمت مقارنة تركيب هذه الأعضاء مع تلك الموجودة في صغار رأسيات الأرجل الأخرى في مناطق بيئية أخرى. أشارت النتائج إلى أن التركيب التشريحي للعينين والفصوص البصرية لهما علاقة وثيقة بوظائفهما والاحتياجات المختلفة لأسلوب حياة فقس هذا الحبار في بيئته في البحر الأحمر ومساعدته على السباحة والإحساس والبحث عن الطعام في بيئته.