STUDY OF THE MORPHOLOGY AND PARASITIC INFECTION OF THE CUTTLEFISH, *SEPIA PHARAONIS* EHRENBERG, 1831 (CEPHALOPODA: SEPIIDAE) IN THE MARINE WATER OFF KUWAIT

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ABSTRACT

In the present study, 200 adult specimens of the cuttlefish, *Sepia pharaonis* (119 male and 81 female) were obtained from a Fish Market (Souq Sharaq, Capital –Governorate) in the State of Kuwait, during the period from January to June 2006. They were examined for external and internal parasites as well as their morphology and anatomy of the male and female specimens.

No adult or larval stages of parasites were detected externally nor internally in the studied samples. The absence of parasitic infection in the studied cuttlefish was discussed.

This is the first study so far dealing with the cuttlefish *Sepia pharaonis* morphology, anatomy and posible infection with parasites in the marine waters off Kuwait.

INTRODUCTION

Cephalopods play an important role in the marine ecosystem and are valuable to man as food and for biomedical utilization (Forsythe and Han1on, 1980; Forsythe, 1984; Hanlon and Forsythe, 1985). Although they are usually regarded as non-conventional resources, cephalopod stocks are of recent importance as international commercial fiheries (Boy1e, 1990). Total world catch reached 2.56 million tons in 1991. (F A O 1991). Besides, the value of cephalopods as important and reliable research animals, additional interest has also been developed in their mariculture potential (Hanlon *et al*., 1991).

Cephalopods have attracted considerable interest because they represent the most highly developed invertebrates and because they show numerous adaptations that have evolved convergently with vertebrates (Packard, 1972).Cousteau and Diole (1973) stated that cephalopods have the most developed eyes of any invertebrate group, as they are as complex as those of mammals.Hanlon and Messenger (1996) as well as Ellis (1998) reported that cephalopod eyes are adapted to see at great depths, where there is a little light.

All cephalopods are dioecious and many exhibit external sexual dimorphism, either in structural or size differences. They are active predators that prey upon shrimps, crabs, fishes and other cephalopods (FAO 1983).

Commercial fisheries for cephalopods have grown rapidly in many parts of the world, in some places even bringing in larger catches than "conventional" fisheries operations for fish (Rathjen and Voss, 1983). Several studies have been carried out with cephalopod behaviour and life cycle (Van Heukelem, 1976; Calow, 1987; Forsythe and Heukelem, 1987; Forsythe *et al.* 1994; Hanlon and Messenger, 1996).

Trematode worms have been shown to occur in some squids of the genus *Dosidicus* (Nesis, 1983; O'Dor, 1983; Summers, 1983; Van Heukelem, 1983).

As stated by Bustamante *et al.* (2002), the cuttlefishes are nectobenthos and they spend most of their times on the bottom sediments. Cannibalism seems to be rather common and has been interpreted as an efficient "strategy" to overcome temporary shortage of suitable sized food items (Caddy, 1979). They are opportunistic, subdominant predators thriving upon the depiction of their major finfish. The Cuttlefish, when hunting a shrimp or a fish, it first notices the prey and then yaw on its vertical axis until it has subject within its sight, then it moves either forward or backward until it attains a proper "attack distance", at this point, it ejects its tentacles towards the prey (Hanlon and Messenger, 1996).

In France, Pinczon *et al.* (2000) investigated the diet of *Sepia officinalis*. during its life cycle. Their results showed that amphipods are the main prey for the first three months. During the growth period, fish occupied the first place in the diet. They stated that in animals returning to coastal waters for reproduction, the diet changed again and crustaceans became the most important prey.

Chichery and Chichery (1987) demonstrated the heterogenous function of the anterior basal lobe and its complex role in the control of predatory behaviour of the cuttlefish *Sepia officinalis.*

Boletzky (2003) stated that the cephalopod buccal mass includes the radial and the horny beaks that are fully differentiated by the time of hatching. He reported that the strong buccal musculature enables the beaks to bite into prey items and to ingest the pieces severed from a prey by the radula.

Gonzalez *et al.* (2003) analyzed the cephalopod parasite systems taking into account the abiotic and biotic factors that may affect the host risk of infection (contact) with parasites and stated that the ecological niche of a cephalopod species is more important in determining its risk of parasitic infection than its phylogeny.

Hochberg (1990) noted that about 150 species of protistan and metazoan parasites are found on and in a total of 650 cephalopod species. Hulet *et al*. (1979), Kelly (1984), Hanlon *et al*. (1984), Ford *et al*. (1986), Forsythe *et al*. (1987), Forsythe *et al*. (1990) and Forsythe and Hanlon (1991), have also investigated the disease etiology and potential treatment protocols for cephalopods maintained or reared in the laboratory. Despite this, our present knowledge on the diseases of cephalopod molluscs is still limited and characterized by an almost complete lack of information on etiologies (Kinne, 1990).

Hanlon and Messenger (1996) discussed the morphological adaptations in cephalopods for feeding and predation and stated that by moving up and down, a marine animal can use the water currents at different depths to move effectively without spending much energy.

Kusugai (2001) observed the feeding behavior of the Japanese pygmy cuttlefish, *Idiosepius paradoxus*, inhabiting sea grass beds in captivity and concluded that it preferred to feed on crustaceans and the feeding behavior consisted of two phases: attacking and eating.

Like other cephalopods, the sexes in *Sepia* sp. are separate, where the differentiation into female ovary or male testis begins at hatching and is not hormone driven (Mangold, 1987).

Male *Sepia* sp. produces their elaborated spermatogonia in a complicated system of spermatophoric glands and transfer them with their modified fourth arm into the female during mating. Spermatophores are produced later and accumulate within the Needham's sac until they are transferred at mating. In older animals the testis start to degenerate while degeneration of the spermatophoric glands starts (Nigmatullin *et al.* 2003).

In female, the nidamental gland is a huge paired organ located in the ventral mantle. They are contractile and produce a gelatinous coat which covers the egg as a second egg capsule as it is released into the mantle cavity by the oviduct (Zatylny *et al.*, 2000).

Rajasekharan *et al.* (2005) reported that, the gland nidamental of *Sepia pharaonis* has certain structural features of a secretory organ containing a number of tubules filled with dense population of coccoid and rod shaped bacteria. The authors concluded that, it may be playing a secretory role in reproductive cycle, by coating the ovulated eggs with a layer of symbiotic bacteria having potent antibacterial property to ward off the egg pathogen.

Gabr *et al.* (1998) studied the maturation, fecundity and seasonality of reproduction of two commercially valuable cuttlefish, *Sepia pharaonis* and *S. dollfusi* in the Suez Canal. Gabr *et al.* (1999) studied the reproductive versus somatic tissue growth during the life cycle of the cuttlefish, *Sepia pharaonis*.

MATERIALS AND METHODS

I- Collection of data on the studied samples

Collection of the cephalopods morphology, anatomy and relationships to parasites data were developed from published information on the cephalopods.

Moreover personal communication with "Kuwait Institute for Scientific Research" (KISR) was helpful in the collection of information dealing with *Sepia* sp. classification and morphology.

II- Collection of the studied samples

Cephalopods samples were collected weekly from the Fish-market (Souq Sharaq) from January to June 2005. The samples were kept in iceboxes till arrival to laboratory. 119 males and 81 females cuttlefish were collected and examined.

III- Laboratory work

a. The samples were washed in running water for 10-15 minutes to get rid of ink.
b. The samples were examined for external parasites.
c. The mantle was incised from the dorsal side of the animal.
d. The samples were dissected and the respiratory and digestive organs were examined for parasites. The mantle cavity was opened by

making an incision, which run almy the entire length of the posterior surface from siphon to apex. Dissection was done with care as not to disturb the internal organs. The mantle edges were turned laterally and pinned out to expose the internal organs clearly.

e. The gills and gastrointestinal tract were inspected for endoparasites. The stomachs and the intestine were removed and some stomach compartments were opened and washed on a sieve of 1mm mesh to detect any helminthes (Andrade *et al.,* 2001) and the food items were identified using a light microscope.

RESULTS

The studied cuttlefish was identified as *Sepia pharaonis* Ehrenberg. Identification was based on Carpenter *et al.* (1997) and on personal communication with Kuwait Institute for Scientific Research (KISR) in addition to Jones (1986). It belongs to Family Sepiidae Keferslein, 1866, Order Sepiida Zittel, 1895, phylum Mollusca.

Morphology of the studied samples (Figs. 1, 2, 3, 4, 5)

The body is mainly divided into a distinct head and a conical visceral hump, separated from each other by a short neck. The head carries two large eyes and bears anteriorly the mouth opening surrounded by outer and inner circular lips and two large horny jaws projecting from it. The two lateral sides of the visceral hump are extended into two thin lateral fins.

A large conical muscular funnel is found on the ventral side of the head and has a narrow anterior opening and a broad open base projecting backwards into the mantle cavity. The visceral hump is covered all over by the mantle, which terminates anteriorly in a free ridge surrounding the neck. The mantle has a pronounced anterior central point, reaching dorsally over the head with the anterior edges sharply cut a way laterally, nearly forming right angles. The dorsal side of the hump is hard due to the presence of an internal shell (Cuttlebone). On the ventral side of the hump, the mantle forms a thick muscular wall, which covers the large mantle cavity, with a wide anterior mantle opening.

This species of cuttlefish is characterized by its brown colour with white streaks dorsally and paler colour ventrally. As with other cuttlefish species, the present species has eight muscular arms and two long prehensile tentacles, the latter providing for the initial prey capture. The tentacles have small suction cups (suckers) that are used for clinging to the substrate or to objects, including potential prey. The suckers, which are present in the distal part of the tentacles, are cup-like, stalked and have different sizes and are arranged in oblique transverse rows. They are larger than arm suckers. The arms ventral surfaces have suckers and some structures, which look like stalked papillae along all the arm length. The web between arms is not deep. Mantle length is nearly 1.5 times width.

Fins are arising shortly posterior to anterior mantle margin. These are relatively broad and nearly uniting posteriorly, arising posterior to mantle margin.

Regarding external features, male *Sepia* sp. can't be distinguished from females.The shell (Cuttlebone) (Fig. 6) is represented by a flattened feather–shaped light plate buried under the mantle of the dorsal surface. It is bilaterally symmetrical and has a rounded anterior end and narrow posterior end which projects like a spine or rostrum and laterally bordered by thin wing-like ridges. The cuttlebone provides the cuttlefish with buoyancy.

Anatomy of internal organs (Figs. 6-8)

- Respiratory organs

A single pair of gills (Figs. 7 & 8) are long, feather–shaped highly vascular organs that are attached to the mantle and extend along the anterior half of the mantle. Their central axis contains the main afferent and efferent blood vessels and flattened gill filaments containing capillary buds.

- Reproductive system

The internal anatomy reveals quite some differences between the male and female reproductive systems (Figs. 7& 8). The genital opening in both sexes, is found on the left side, close to the base of the left gill.

The complex male reproductive glands consist of a sperm duct (proximal canal), several glands and a sperm reservoir (Needham's sac). The spermatophoric glands (Seminal vesicles I and II and III, appendage of seminal vesicle II and accessory glands and appendages of accessory gland) are sac or tube – like structures that lead to the Needham's sac, a sperm reservoir (Fig. 7).

The huge paired nidamental glands lie in the ventral mantle cavity of the female *Sepia*, while the accessory nidamental glands are located next to the nidamental glands (Fig. 8).

- Digestive tract

The oesophagus is thin walled tube surrounded by the liver and leads into the thick–walled stomach, which extends to the tip of the visceral mass. The intestine runs forwards from the stomach towards a diverticulum (ink sac) and terminates in the rectum. The rectum extends along the middle line and ends anteriorly by the anus, carried at the end of long anal papillae.

The stomach contents of the *Sepia pharaonis* revealed highly varied items: including remains of crabs, fishes scales and bones.

A remarkable peculiarity of the colloid digestive system is the specialization of the rectal gland into an ink-sac. The ink-sac is a large, ovoid, thin walled sac, projecting posteriorly in the mantle cavity and leading forwards by a long duct into the rectum, a short distance behind the anus. The dark-pigmented fluid secreted by the ink sac may be discharged deliberately through the anus forming a cloud that presumably confuses potential predators and that also may act as a mild narcotic.

Parasitic infection

` No adult or larval stages parasites were detected infecting the body surface, gills or digestive tract of *Sepia pharaonis*. However, possible infection could not excluded.

DISCUSSION

This study is, so far, the first to investigate the morphology, anatomy and possible parasite infection of *Sepia pharaonis* in Kuwait marine waters. The present cuttlefish was identified as *Sepia pharaonis* according to the distinctive features given by Jones (1986).

The absence of parasites from the present samples could be correlated with the used method of collection from a fish market where they were thoroughly washed and frozen. The interaction between cephalopods and their parasites is still poorly understood, and almost nothing is known about parasitism in cephalopod paralarvae juveniles (Vecchione, 1987).

Cephalopods, as stated by Vidal (1999), play an important role in the transfer of parasites through the food web, and some act as second and third intermediate hosts for larval stages of digenea, cestodes and nematodes.

Pascual *et al.* (1995a) surveyed parasites in 600 short finned squid, *Illex condetti* taken from two locations off the northwestern Iberian

Peninsula and they concluded that parasite infection showed close positive correlation with host life-cycle, often with the greatest number of parasite among the largest and highest maturity individuals.

In the present study, only adults *Sepia* sp. were collected and examined. So, further studies are still needed for detecting parasites in these cephalopods, by examining different stages in their life cycles.

Bustamante *et al.* (2002) stated that the digestive gland in the cuttlefish *Sepia officinalis*, plays a major role in the subsequent storage and presumed defoliation of the elements, Zinc and Cadmium, regardless of the uptake pathways and that food is the likely primary pathway for such elements. This may explain the absence of parasitic infection of digestive tract in the present samples.

Gonzalez *et al.* (2003) analyzed the cephalopod–parasite system taking into account the abiotic and biotic factors that may affect the host risk of infection with parasites. The authors made an attempt to find an association between parasite relative species diversity and cephalopod life cycle and their results suggest that the ecological niche of a cephalopod species is more important in determining its risk of parasites than the phylogeny. The authors stated that the occurrence of a given marine parasite in any cephalopod host species anywhere will depend on the presence of a suitable definitive host, a suitable intermediate host and complex abiotic and biotic factors hierarchically arrayed and dynamically interactive. This may explain the absence of parasite infection in the present samples since the absence of definitive and intermediate hosts for the parasites lead to incomplete life cycle. The results presented by Gonzalez *et al.* (2003) suggested that environmental factors (i.e. host external factors) are not important determinants of eukaryotic parasitic disease risk in terms of parasitic species. The authors stated that tropic ecology might have a significant impact on the intestinal parasitic fauna of cephalopods so that continued transmission ensures a spatial and temporal connection between parasites and cephalopod host population.The authors stated that the ecological and behavioral features of the cephalopod life cycle (namely: deep range, bathymetry, mantle size, potential fecundity, and vagility) appear to be extremely important as determinants of parasite relative species diversity.

Pascual *et al.* (1998) stated that economic importance of diseases caused by parasites in cephalopods argues that the ecology in exploited systems should be studied in depth.

The stomach contents of the present cuttlefish include crustaceans as previously reported in other species by Carpenter *et al.* (1997) and Pinczon *et al.* (2000).

The ecological factors of the marine waters which are considered as the surrounding environment for the cutlefish, were not included in the present work, so further ecological studies are needed to determine the factors affecting the parasite infection in the cuttlefish, *Sepia pharaonis*, in Kuwaiti waters.

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EXPLANATION OF FIGURES

Fig. 1: Photograph of *Sepia pharaonis* male dorsal view, A: arms, E: eye, SF: swimming fin, Su: suckers, T: tentacle.

Fig. 2: Photograph of *S. pharaonis* male ventral view, A: arm, BF: buccal funnel, F: funnel, LRA: lateral ridge of ventral arm, SU: suckers, T: tentacle.

Fig. 3: Photograph of *S. pharaonis* male top view A: arm, I W: interbranchial web, IL: inner circular lip, OL: outer circular lip, SU: suckers, T: tentacle.

Fig. 4: *S. pharaonis* distal end of the arm showing the stalked appendages (SA).

Fig: 5: Male *S. pharaonis* distal end of the tentacle. Su: suckers, T: tentacle.

Fig. 6: Cuttlebone (shell) of male *S. pharaonis*. Ro: rostrum (Spine).

Fig. 7: Male *S. pharaonis* dissected mantle cavity. An: anus, B:branchia,. Go: genital orifice, Fc: funnel cartilage, Mc: mantle cartilage, Np: Needham's pocket, SF:swimming fin, Spg: speramtophoric gland, Te: testis.

Fig. 8: *S. pharaonis* female dissected mantle cavity. Acng: accessory nidumental gland, Ang: aperture of nidumental gland, An: anus, B: branchia, Dg: digestive gland, Go: genital opening, God: glands of oviduct, Is: ink sac, M: mantle, N: nidamental gland, O: ovary, Sf: swimming fin.