

## THE DIGESTIVE SYSTEM OF RHYNCHOTEUTHION PARALARVAE (CEPHALOPODA: OMMASTREPHIDAE)

*Eve Boucaud-Camou and Clyde F. E. Roper*

### ABSTRACT

The anatomy of the digestive system and the digestive system enzymes of three types of rhyngoteuthion paralarvae A', B', and C' (*Illex*) were studied. No differences among the three were found. The main features of the digestive system of these species of paralarval Ommastrephidae are described. The buccal mass is comprised of a beak and radula with conspicuous teeth. The well developed posterior salivary glands contain glandular tissue with two cell types: A, goblet cell, and B, granular cell. The esophagus is lined with a thin cuticle, but the stomach lacks cuticle and has a more strongly developed muscular wall than the esophagus. The large vestibule is lined with ciliated and glandular cells. Some primary folds of ciliated epithelial cells begin to develop in the caecum. The digestive gland is compact, round, red-pigmented and enclosed in a thick elastic capsule; it contains conspicuous inclusions: large boules, typical brown-body vacuoles and numerous lipid droplets. Thirteen hydrolases involved in digestive processes were examined. High proteasic activity and histochemically undetectable amylasic activity suggest a carnivorous diet. The occurrence of typical lysosomal enzymes in the digestive gland reveals a high intracellular digestive activity. The digestive system appeared to be developed and functional in the smallest specimens examined (ML: mantle length 1-2 mm). Typically juvenile features include the anterior part of the digestive system which is more highly developed than the posterior part, thus it is more functionally important than the posterior part. The muscular wall of the posterior part of the digestive tract is very thin: tractus with long cilia probably move food. Because the caecal leaflets are not yet fully developed, the digestive gland probably assumes the greatest part of the digestive and absorptive functions in paralarvae.

Oegopsid squids of the family Ommastrephidae are extremely important in commercial fisheries on a world-wide basis. In particular, *Todarodes pacificus*, *Ommastrephes bartrami*, *Illex illecebrosus* and *I. argentinus* are fished extensively.

In these short-lived oceanic animals (1-2 yr lifespan, Boyle, 1987) the success of annual recruitment has a dramatic and direct impact on the exploited stocks. The paralarval stage is the most critical time in the life history of cephalopods. It is a period of high mortality that could be related, in part, to the transition from embryonic feeding (yolk absorption) to the ability to capture and digest prey (Vecchione, 1987). Therefore, it is important to learn about the digestive capability (development and maturation of the digestive tract and appearance of the digestive enzymes) during this critical transitional stage. In spite of this need for information, however, few data are available, especially on oceanic squids. This research was conducted at the Smithsonian Marine Station in Fort Pierce, Florida.

### MATERIAL AND METHODS

**ANIMALS.** Live specimens were collected during six sampling trips of the R.V. SUNBURST (15/10, 24/10, 16/11, 26/11, 11/12 /1990 and 08/01/1991) off Fort Pierce in the Florida Current, a component of the Gulf Stream. During each cruise five to seven 15-min, oblique, open net plankton tows were made with a 1-m diameter 500 mm mesh plankton net. Details of the sampling program are presented in Boucaud-Camou and Roper (1995). Sixty-three specimens (1-4.6 mm ML) of three types of rhyngoteuthions, A', B' and C' (*Illex*) (Roper and Lu, 1979), were collected during the six cruises. Catches reached a peak of 44 specimens in January, which agrees with the observed spawning period of Ommastrephidae in this area.

Most specimens were sorted onboard from the plankton samples and immediately preserved or

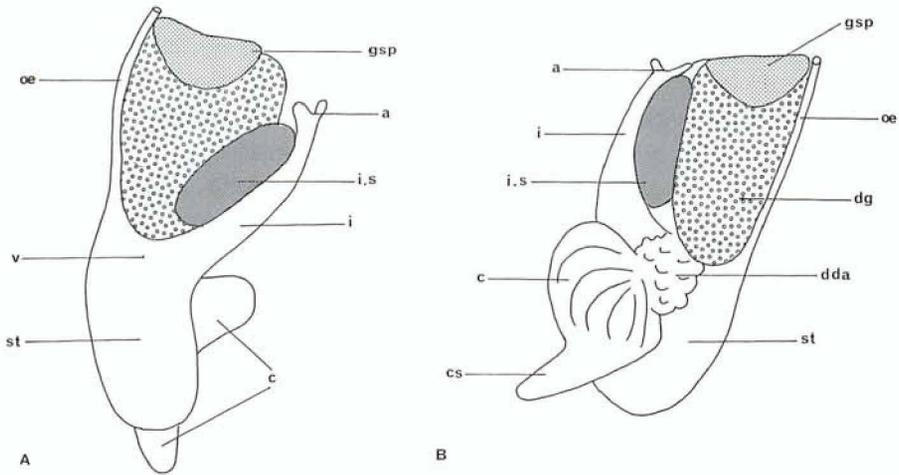


Figure 1. Anatomy of the digestive system of a rhynchoteuthion. A: right side. B: left side. a, anus; c, caecum; cs, caecal sac; dda, digestive (gland) duct appendages; dg, digestive gland; gsp, posterior salivary glands; i, intestine; is, ink sac; oe, esophagus; st, stomach; v, vestibule.

frozen for histology, enzyme localization or electron microscopy (see below). At the laboratory, further specimens were sorted in the chilled plankton samples, 2 to 4 h after capture, and preserved for histology and enzyme localization.

**ANATOMY AND HISTOLOGY.** Onboard, the specimens were fixed in cold 4% formaldehyde (freshly prepared from paraformaldehyde) in pH 7.4 0.4 M cacodylate buffer with 10% sucrose ("PFA" fixation). At the laboratory, they were fixed in 10% neutralized formalin in seawater for at least several days ("F" fixation). Some specimens were micro-dissected under a dissecting microscope to study the gross anatomy of the digestive tract. For histological study, preserved specimens were dehydrated in ethanol, then treated several days in iso-butanol prior to being embedded in paraffin. Serial sections were made at 6  $\mu\text{m}$  and stained with Azan of Romeis.

**ENZYME LOCALIZATION.** The specimens were frozen on dry ice in a drop of 12% sucrose 0.4 M cacodylate buffer pH 7.4 and stored later at  $-18^{\circ}\text{C}$ . ("D" fixation for substrate film methods) or preserved in cold PFA fixative, 2 to 3 h, then rinsed in the cacodylate buffer with 12% sucrose ("L" fixation for precipitation methods). The method of Shear and Pearse (1963) was used to detect amylasic activity and the method of Chrétien (1965) was used for proteasic activity. A precipitation method was used for testing all other enzymes: simultaneous coupling with naphthylamide or naphthol substrates and a diazonium salt. The following enzymes were researched according to the methods of the authors cited: Non-specific esterases, Dipeptidyl aminopeptidases I (DAP I), II (DAP II), IV (DAP IV); glucuronidase (Lojda et al., 1979). Alkaline phosphatase (Pearse, 1953). Acid phosphatase (Grogg and Pearse, 1953). Acetylglucosaminidase (Lojda et al., 1979). Chymotrypsin (Lagunoff, 1967). Trypsin (Gossrau, 1981). Aminopeptidase (Moore et al., 1980).

**CYTOLOGICAL STUDY OF THE DIGESTIVE GLAND.** All the specimens for cytological study were preserved on board in ice-cold fixative. The whole animals were fixed for one to several days at  $5^{\circ}\text{C}$  in 4% glutaraldehyde in 0.4 M cacodylate buffer pH 7.3 with 10% sucrose ("A" fixation). The digestive gland was then dissected out, cut into small pieces in the same fixative, rinsed in the same cacodylic buffer with 12% sucrose and post-fixed in 2% osmium tetroxide in 8% sucrose in the same cacodylic buffer. The pieces were then dehydrated and embedded in epon. Semi-thin (1.5  $\mu\text{m}$ ) sections were cut and stained by toluidine blue. Thin sections were contrasted with uranyl acetate and lead citrate.

## RESULTS

**ANATOMY OF THE DIGESTIVE SYSTEM (Fig. 1).** The main features of the digestive system of teuthoids were recognized in all the young ommastrephids examined. The digestive tract is U-shaped: the esophagus runs posteriorly dorsally from the buccal mass to the stomach, and the intestine runs anteriorly ventrally from the caecum opening to the anus (appended by two anal flaps) in the mantle cavity. The space between the esophagus and intestine is occupied by the digestive gland.

The ink sac lies on the ventral side of the digestive gland along the intestine. The paired, well developed posterior salivary glands are situated in an apical anterior depression of the digestive gland. The stomach and caecum form the posterior bend of the U. A well developed vestibule connects the esophagus, the stomach and the intestine. The stomach is a large pouch. The caecum consists of two parts, the proximal coiled part (caecum sensu stricto) and a small distal caecal sac. The digestive gland ducts (= digestive ducts, Bidder, 1976) surrounded by the digestive duct appendages (= digestive appendages, Bidder, 1976) extend from the digestive gland into the caecum.

**HISTOLOGICAL STRUCTURE OF THE DIGESTIVE ORGANS.** The buccal mass (Fig. 2A) is well developed, even in the smallest specimens. It consists of a muscular bulb enveloping two beaks which are chitinous, sclerotized structures. The center of the buccal mass is occupied by the salivary papilla and the odontophore, which carries the radula which originates in the radula sac and bears conspicuous teeth. Along the two lateral lobes run the ducts from the anterior salivary glands. Several glands are found in the buccal mass: the submandibular gland, lying ventral to the odontophore; the anterior salivary glands, incorporated into the basal part of the buccal mass. In addition, numerous glandular cells occur in the epithelium of the buccal mass. These cells are of two types: typical goblet cells (A) and granular cells (B). The posterior salivary glands discharge their secretion via ducts through pores near the opening of the mouth. They are formed by glandular tubules that contain the same cell types (A and B) as found in the buccal mass (Fig. 2B).

The wall of the esophagus is thin and the epithelium is covered with a thin cuticle (Fig. 2C). The stomach has a thicker muscular wall. No cuticle is detectable, but cytoplasmic expansions of the epithelial cells towards the lumen are visible (Figs. 2C,D). The muscular wall is thicker in the posterior part of the stomach (Fig. 2D).

The caecum has a thin muscular wall comprised of connective tissue encompassing muscular fibers. The mucosa of the caecum forms primary folds that increase the epithelial surface and are perceptible through the thin wall (Fig. 2E). The vestibule connects the stomach, the caecum and the intestine. It also is comprised of folds lined by a ciliated, glandular epithelium (Fig. 2F). The intestine comprises a main groove which originates in the vestibule (Fig. 2F) and is formed by two typhlosoles composed of high epithelial cells that bear very long cilia.

The digestive gland connects via the digestive (gland) ducts into the caecum. The digestive gland, enclosed in a thick elastic capsule, is compact, round, unilobed, and red-pigmented. It consists of closed-ended tubules which open into a central main lumen (Fig. 2B). The tubules are formed by the glandular (digestive) cells that lie on a thin layer of connective and muscular tissue. The lumen of the digestive gland is continuous with the digestive gland ducts which are surrounded by the glandular tubules, the digestive (gland) duct appendages (Fig. 2B) that have striated cells that contain no conspicuous inclusions (Figs. 2B,D). The gastric ganglion is present on the vestibule at the level of the opening of the digestive gland ducts (Fig. 2C).

**CYTOLOGY OF THE DIGESTIVE GLAND.** The cells of the digestive gland (digestive cells) contain numerous inclusions. The most typical are the brown bodies and the "boules." The brown bodies are large pigmented inclusions characterized by crystals, and generally they are enclosed in large vacuoles (Fig. 2G). The boules are large, spherical, proteinaceous inclusions (Figs. 2G,H) that stain red with the azan dye (Figs. 2B,C). Most boules are secretory granules, although some of the largest appear to be lysosomes. Numerous lipid droplets also are present. Mitotic cells are common. In the apical part of the cell, endocytotic or exocytotic pro-

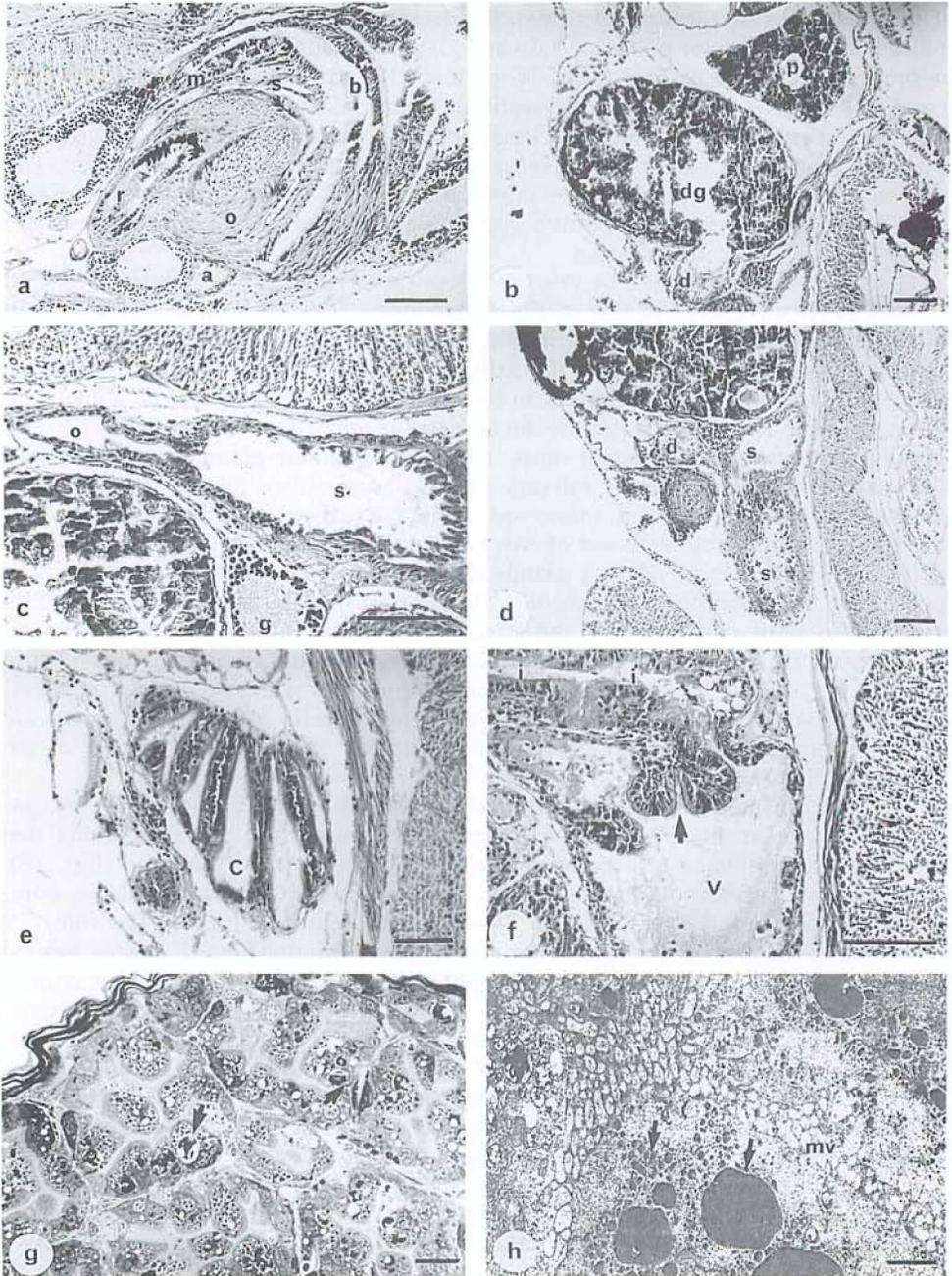


Figure 2A-F Histology of the digestive system of rhynchoteuthions. Paraffin sections, azan of Romo-  
eis (scale bar: 0.1 mm ). a: longitudinal section of the buccal mass; (a, anterior salivary glands; b,  
beak; m, submandibular gland; o, odontophore; r, radula sac; s, salivary papilla); b: section of the  
posterior salivary glands (p) and of the digestive gland (dg). The digestive (gland) ducts, surrounded  
by the digestive (gland) duct appendages (d) are continuous with the central lumen of the digestive  
gland; c: longitudinal section of the esophagus (o) entering the anterior part of the stomach (s), (g,  
gastric ganglia); d: longitudinal section of the stomach (s), (d, digestive (gland) duct appendages); e:  
longitudinal sections of caecum (c) showing caecal leaflets; f: longitudinal sections of the vestibule  
(v) with the main groove (arrow) and of the anterior part of the intestine (I). g-h: Cytology of the

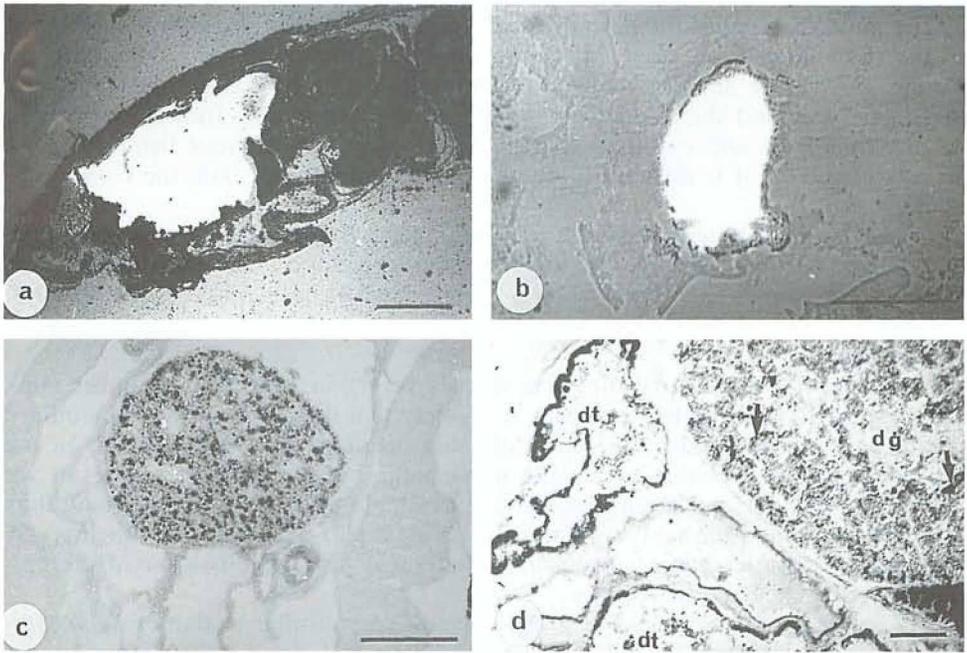


Figure 3. Localization of some hydrolytic enzymes (frozen sections). a: proteolytic activity in the digestive tract (scale bar: 0.5 mm); b: proteolytic activity in the digestive gland (scale bar: 0.5 mm); c: dipeptidyl aminopeptidase II activity in the digestive gland (scale bar: 0.25 mm); d: alkaline phosphatase in the epithelia of the digestive tract (dt) and in the digestive gland (dg); the activity is localized at the brush-border of microvilli of the digestive cells (arrows), (scale bar: 0.1 mm).

cesses are taking place (Fig. 2H). The luminal border is lined by a brush border of microvilli. (Fig. 2H).

**ENZYME LOCALIZATION.** No amylasic activity was detectable by substrate film method, whereas a high proteasic activity was found in the digestive tract (Fig. 3A), posterior salivary glands and digestive gland (Fig. 3B). In the digestive gland only, some of the proteolytic activity could be related to chymotrypsin. The digestive gland displayed several enzyme activities that are typically lysosomal and localized in the apical part of the digestive cell at the level of boules and brown-bodies: acid phosphatase activity, dipeptidyl-aminopeptidase II activity (Fig. 3C), acetylglycosaminidase activity (which also was found in the posterior salivary glands). Alkaline phosphatase, involved in active transport, was found in all the epithelia of the digestive tract and at the level of the brush border of the digestive gland cells (Fig. 3B).

#### DISCUSSION

No differences were observed among the three types studied (A', B', C' : *Illex*, Roper and Lu, 1979).

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digestive gland. g: glandular tubules, semi-thin section, toluidine blue (scale bar: 20  $\mu$ m). Notice the conspicuous inclusions of the digestive cells, especially brown-bodies (arrows) and the thick elastic capsule at the periphery of the gland; h: electron-micrography of the apical part of the digestive cell, showing the brush border of microvilli (mv) and large and small "boules" (arrows) (scale bar: 1  $\mu$ m).

The main features of the ommastrephid digestive system already are developed in the rhynchoteuthion stage: the large vestibule, the caecal sac (Mangold and Young, 1998), the straight tubular intestine, the single-lobe digestive gland and the digestive gland duct appendages without the "external" (renal) epithelium. As the histology and cytology of digestive systems throughout the family are largely unknown, it is difficult to compare rhynchoteuthions with the conspecific adults.

In all the rhynchoteuthions studied, the digestive system appeared functional and active. All the digestive organs were well developed, and the digestive enzymes were active. But some features were found to be juvenile characteristics: the anterior part of the digestive system is more extensively developed than the posterior part.

The muscular wall of the digestive tract is very thin, except in the more muscular stomach. In these tiny animals, it is likely that the food is moved by ciliary mechanisms; tractus with the long cilia that occur in the vestibule and in the intestinal groove probably create the movement. The caecal leaflets are not yet fully developed, so the absorptive surface available probably is insufficient; thus the digestive gland probably assumes the greatest part of absorptive functions. A high nucleoplasmic index appears to be a typical juvenile character of the epithelial cells of the digestive tract.

The cytology of the digestive gland appears very similar to that of *Sepia officinalis* (Boucaud-Camou, 1972; Boucaud-Camou and Yim, 1980). Actually, the digestive gland with large boules and typical brown bodies is more similar to the *Sepia-Octopus* type than to the loliginid type. Boules are associated with digestive activity (Boucaud-Camou, 1982; Boucaud-Camou et al., 1985; Best and Wells, 1983), thus confirming that the digestive system is already functional. High mitotic activity is also a juvenile character.

The high acid phosphatase activity, D.A.P. II and acetyl-glycosaminidase activities, typically lysosomal, reveal intracellular digestive processes in the digestive gland. The high alkaline phosphatase activity expressed in the brush border of the digestive cells and in the epithelia of the posterior digestive tract probably is involved in active absorption.

Because rhynchoteuthions (as well as other oegopsid hatchlings) do not readily accept food in captivity, it has been suggested that they could be suspension-feeders (O'Dor et al., 1985). But the presence of large beaks, the well-developed radular teeth, and the conspicuous posterior salivary glands (which are known to secrete toxins to poison prey) suggest active predatory habits. Moreover, the proteolytic activity found in the digestive gland, the stomach and the posterior salivary glands also suggest a carnivorous diet. Boletzky (1974) reported that cephalopod planktonic paralarvae are well equipped for prey capture with developed arms and tentacles. However, the role of the proboscis (fused tentacles) in rhynchoteuthion paralarvae is not yet understood.

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ADDRESSES: (E.B.-C.) *Laboratoire de Biologie et Biotechnologies Marines, Université de Caen, 14032 Caen Cedex, France*; (C.F.E.R.) *National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, and Smithsonian Marine Station, Ft. Pierce, Florida 34946.*