The digestive tract of *Nautilus pompilius* (Cephalopoda, Tetrabranchiata): an X-ray analytical and computational tomography study on the living animal

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Summary

Using X-ray analytical studies and computational tomography, the position of the digestive tract of the tetrabranchiate cephalopod *Nautilus pompilius* L. was demonstrated in a living animal. For the first time, a detailed analysis of the rate of digestion and the duration of the different phases of a digestive cycle has been made using these *in vivo* methods. At 20 min after food intake, the food has entered the stomach, where it is reduced to small pieces; most is stored in the crop, which is enlarged to approximately four times its original size. The chyme reaches the midgut gland 3 h and the rectal loop 5 h after food intake. The time between food intake and elimination is 12 h. Thus, in *Nautilus pompilius*, digestion takes approximately the same time as described for necto-benthic sepioids and benthic octopods but is approximately 6 h longer than in loliginids, which have a pelagic mode of life. Furthermore, the three-dimensional structure of the digestive tract of a living *Nautilus pompilius* L. was demonstrated using computational tomography.

Key words: digestive organ, morphology, histology, three-dimensional reproduction, X-ray examination, computational tomography, *Nautilus pompilius*, Cephalopoda.

Introduction

Few studies have investigated the complex digestive tract of *Nautilus pompilius* and *Nautilus macromphalus*, the only remaining externally shelled chambered cephalopods (Keferstein, 1866; Haller, 1895; Griffin, 1900; Bidder, 1966; Fukuda, 1980; Ruth et al., 1988, 1999/2000). In recent years, several experimental studies have contributed to a better understanding of the dynamics of digestion in coleoid cephalopods (Boucaud-Camou et al., 1976; Andrews and Tansey, 1983). Bidder (1950, 1957) and Boucher-Rodoni and Mangold (1977) studied the digestive processes in European squids (*Loligo vulgaris*, *Loligo forbesi*, *Alloteuthis media* and *Alloteuthis subualata*) and *Octopus vulgaris* both histologically and experimentally. Histological changes during digestion and the sites of absorption were analysed by autoradiography in *Sepia officinalis* (Boucaud-Camou, 1974, 1976, 1977; Boucaud-Camou and Péquignat, 1973). The dynamics of digestion were investigated in *Eledone cirrhosa* and *Illex illecebrosus* (Boucher-Rodoni, 1975, 1976). Boucher-Rodoni and Boucaud-Camou (1987) showed that the midgut gland is the main organ of absorption of nutrients in loliginids.

In contrast to the predatory coleoids, nautiloids are largely scavengers, using their strongly developed calcified jaw to grind food. Analyses of the contents of the digestive tract of the benthic-living *Nautilus macromphalus* showed, however, that hermit crabs and brachyuran crabs are their most important food sources (Ward and Wicksten, 1980).

Previous morphological studies showed that the digestive tract of *Nautilus pompilius* is divided into the buccal complex with the anterior salivary glands, the radula, jaw, crop, oesophagus, stomach, vestibulum, caecum, midgut gland, midgut and rectum (Fig. 1). The digestive tract of the Nautiloidea differs from that of the Coleoidea. It has no posterior salivary glands and the pancreatic appendages are missing. Within the proximal part of the midgut, two longitudinal folds, the so-called typhlosolis major and minor, separate this organ into an inferior and superior part (Griffin, 1900; Naef, 1913; Bidder, 1966; Westermann and Schipp, 1998b). Cytological and enzyme-histochemical investigations and tracer experiments indicate that the midgut gland and the caecum are involved in the absorption of nutrients (Westerman and Schipp, 1998a, 1999; Westermann et al., 2000). However, there is no information about the duration and sequence of the different phases of digestion. In addition, an analysis of the precise
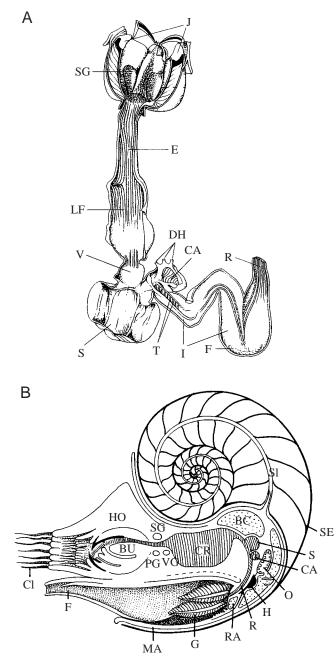
Fig. 1. (A) Diagram of the digestive tract of *Nautilus pompilius* according to Griffin (1900). CA, caecum; DH, ductus hepatopancreas; E, oesophagus, F, longitudinal fold of the rectum; J, jaw; I, intestine; LF, longitudinal folds; R, rectum; S, stomach; SG, salivary glands; T, typhlosolis; V, vestibulum. (B) Median section of *Nautilus pompilius* according to Naef (1913). BC, body cavity; BU, buccal complex; CR, crop; CA, caecum; CI cirrus; F, funnel; G, gills; HO, hood; H, heart; MA, mantle; O, ovary; PG, pedal ganglion; R, rectum; RA, renal appendages; S, stomach; SE, septum; SI, siphuncle; SG, supraoesphageal ganglion; VG, visceral ganglion.

topography of the digestive tract in dead animals is insufficient because the soft parts do not retain their exact positions after removal of the shell. To determine the topography of this organ

complex and the duration and dynamics of a digestive cycle, *Nautilus pompilius* was investigated using histological methods and *in vivo* using X-ray analytical studies and computational tomography. This approach makes experimental observations possible without killing the animal.

Materials and methods
Histology

Histological investigations were carried out on the digestive organs of two *Nautilus pompilius* L. (shell diameter 8 cm and 17 cm, net body mass 65 g and 527 g, respectively) from Philippine coastal waters. The animals were anaesthetised in 2 % ethanol in sea water, and the foregut, stomach, caecum, midgut and rectum were removed and fixed in Bouin's solution or in 4 % formaldehyde in sea water (Robertson, 1989). After embedding in paraffin, serial sections with a thickness of 7 μm were made and stained according to Masson (modified by Goldner, 1938) using Azocarmine Aniline Blue staining (Romeis, 1989). Micrographs were taken with a Zeiss Photomikroskop II. Current guidelines for laboratory animal care were followed.

X-ray analyses

For the X-ray analytical studies, three juvenile *Nautilus pompilius* L. (shell diameter 10–12 cm) from Philippine coastal waters were used. The animals were kept without food in a closed seawater system for 5 days to stimulate their appetite. The contrast medium barium sulphate was used to visualize the digestive tract. The medium has a particle size of 1–3 μm and is not water-soluble (Elke et al., 1982). Each animal was fed with four medium-sized barium-sulphate-labelled shrimps (*Crangon crangon*) together containing 1 ml of concentrated contrast medium. For these investigations, a special acrylic aquarium (40 cm×25 cm×20 cm, length×width×height) was designed and constructed. At 5, 6, 7, 8 and 9 cm along the aquarium length, a partition could be fixed to constrain the animal to the front of the aquarium. A plastic stick was placed in the aquarium for the animal to grip. As the nautiloids usually remained motionless during the day, adhering to the wall of the aquarium, no anaesthesia was necessary. During the X-ray analyses, the sea water was maintained at 18–19 °C.

For the X-ray pictures, the animals were restricted to the front of the aquarium using the partition. For lateral-view photographs, the X-ray film (Ektascan DNB for mammography from Kodak) was placed in the aquarium 80 cm from the X-ray unit (Siemens, series number 01120 S 02) behind the partition with a plastic film cover. For top-view photographs, the film was placed at the same distance from the X-ray unit below the aquarium, and the sea water was removed until the animal was just covered. The lateral-view X-ray exposures were taken at 63 kV and 12.6×10^{-3} C, those from above at 70 kV and 12.6×10^{-3} C approximately 5 min later. X-ray pictures were taken 20 min after food intake, and at 40 min and 60 min. Thereafter, photographs were taken at hourly intervals until the digestive cycle was finished (12 h).

For computational tomography, the acrylic aquarium was made smaller (20 cm×25 cm×20 cm, length×width×height) to reduce the volume of sea water causing random noise and beam-hardening effects. The *Nautilus pompilius* used in these studies were the same as in the X-ray analyses. To stimulate their appetite, the animals were kept in a closed seawater aquarium without food for 5 days. For these experiments, each animal was also fed with four medium-sized barium-sulphate-labelled shrimps (*Crangon crangon*) containing in total 1 ml of concentrated contrast medium. These investigations were carried out at a water temperature of 18–19 °C. Twelve hours after feeding, scans (slice thickness 1 mm, 94×10^{-3} A, 140 kV per kernel Ab 82, slice spacing contiguous, reconstruction interval 1 mm) in frontal and sagittal orientation were performed using a helical CT scanner (Somatom plus 4; Siemens, Erlangen). The animal's hard body regions such as the shell, siphuncle channel and jaw show up as high density. The density of the soft tissue is comparable with (although slightly higher than) that of the surrounding sea water. The digestive tract was easily discernible because of the orally administered contrast material. The image of the shell has to be removed digitally to isolate the digestive tract.

Using a post-processing tool (Magic view VA 31; Siemens, Erlangen), a region of interest was drawn manually that included the digestive tract and excluded the shell (program edited for three dimensions). Using this data set, a shaded surface display was generated using a surface threshold calculation (lower limit 100 Hounsfield units; upper limit 2500 Hounsfield units; see Fig. 4A–C). To transform the two-dimensional CT scans into the final three-dimensional presentation of the complete animal, for comparison with the X-ray photographs, a special post-processing software was used (Vitrea 2 2, version 2.2 for Windows NT; Vital images, Plymouth, MN, USA). A three-dimensional volume-rendered view in grayscale was obtained that enables the user to see through one structure to visualise another (program, bone CT; three-dimensional, see-through; contrast, see-through bone shading, 0.7; transparency, CT bone surface 50; colour, monochrome; slice thickness, 1 mm; Fig. 4D,E)

Results

Histological findings

The histological investigations reveal that all the organs of the digestive tract have fundamentally the same wall structure except for the midgut gland. The following terminology of the wall layers is the same as for vertebrates. The tunica mucosa is situated luminally and is subdivided into a lamina epithelialis mucosae and a lamina propria mucosae consisting of connective tissue. A large blood sinus can be found within this layer. Isolated muscle fibres are localised further within the lamina propria mucosae, but no complete lamina muscularis mucosae is developed. The lamina epithelialis mucosae is a columnar epithelium in all organs of the digestive tract; it is only in the stomach that a cuticle covers the epithelium. The
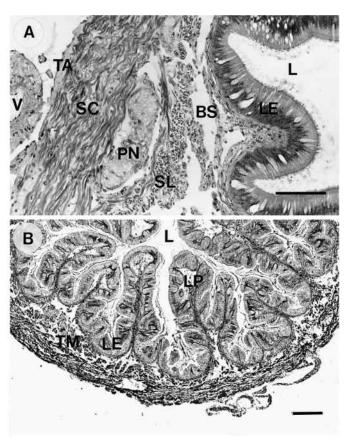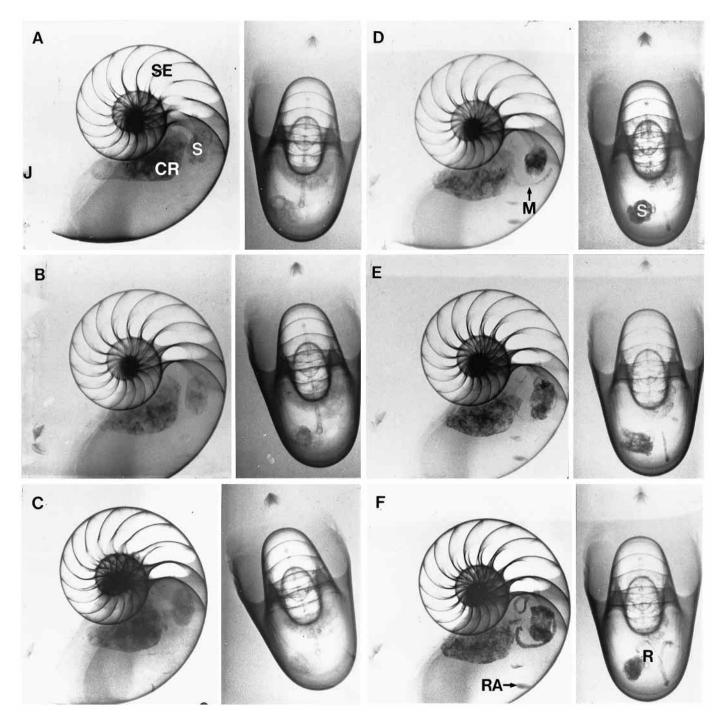

Fig. 2. Cross section of the oesophagus wall (A) and the crop (B) in the foregut of *Nautilus pompilius*. Masson staining after Goldner. Scale bars, 100 μm. Note the double folding of the tunica mucosa in B. BS, blood sinus; L, lumen; LE, lamina epithelialis mucosae; LP, lamina propria mucosae; SC, stratum circulare; SL, stratum longitudinale; PN, polyaxonal nerve fibres; TA, tunica adventitia; TM, tunica muscularis, V, vessel.

tunica muscularis surrounds the tract and is subdivided into a stratum circulare and a stratum longitudinale. This layer is covered by the tunica adventitia, consisting of connective tissue (Fig. 2A). The stomach, midgut and rectum are surrounded by a tunica serosa, which is an isoprismatic epithelium. In all the organs of the digestive tract, the tunica mucosa forms longitudinal folds, enlarging its surface. In the crop and caecum, there are secondary folds (Fig. 2B), which are better developed in the caecum.

X-ray analyses

Using X-ray photographs, the calcified organs of *Nautilus pompilius*, i.e. the shell, siphuncle channel, renal appendages and parts of the contrasted digestive tract, could be demonstrated in a living animal (Fig. 3). The X-ray photographs show the contrasted crop, stomach, midgut and rectum; the caecum and the midgut gland were not clearly identified by this method. The photographs show that the food has entered the stomach 20 min after food intake and has been reduced to small pieces (Fig. 3A). Most of the food is stored in the crop localised below the umbilical region and enlarged
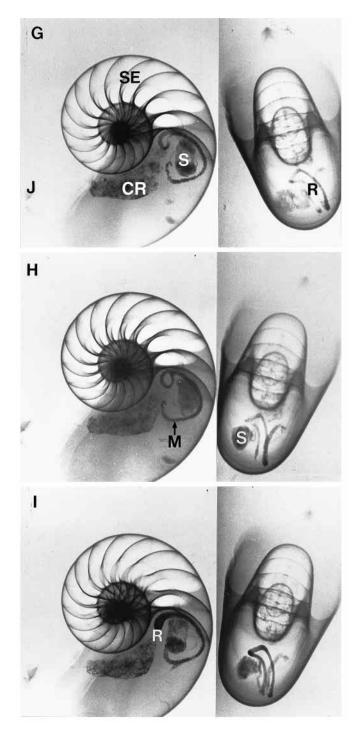
to approximately four times its original size. The chyme reaches the midgut, including the caecum and the midgut gland, 3 h later (Fig. 3D). Four hours after feeding, indigestible food enters the ascending branch of the rectum (Fig. 3E) and 1 h later the rectal loop. After 8 h, the excrement can be observed at the end of the descending branch of the rectum (Fig. 3G), and excretion into the mantle cavity occurs after 12 h (Fig. 3I). The excrement of these animals is in the form of red-brown threads, 2 cm in length and with no solid components, so that it disintegrates approximately 36 h after excretion. The X-ray pictures also show that the branches of the rectum are coplanar and adjoin the last septum (Fig. 3G–I). The crop and the stomach still contain food 12 h after food intake. In addition, the X-ray photographs reveal an enormously dynamic digestive tract. The stomach produces strong contractions and dilations, and undergoes changes in position during the digestive process depending on its volume (Fig. 3B,H).

Computational tomography

Using computational tomography, the exact position of the digestive tract of *Nautilus pompilius* could be reconstructed from a living animal (Fig. 4). This method enables a three-

Fig. 3. X-ray photographs of the digestive tract of *Nautilus pompilius*. Distribution of barium-sulphate-labelled food (A) 20 min, (B) 40 min, (C) 2 h, (D) 3 h, (E) 4 h, (F) 6 h, (G) 8 h, (H) 9 h and (I) 11 h after food intake. At each time point, a lateral (left) and top-view (right) image are shown. Shell length 11 cm. CR, crop; J, jaw; M, midgut; R, rectum; RA, renal appendages; S, stomach; SE, septum.

cavity, the foregut, which is widened to a crop, the stomach, the vestibulum, the caecum, the midgut gland, the midgut and the rectum (Fig. 5). The caecum connects to the midgut gland *via* the paired ductus hepatopancreas. The foregut narrows before entering the stomach. The view from the dorsal side of the animal shows that the stomach is situated on the left and the caecum on the right side, connected *via* the vestibulum. Before entering the mantle cavity, the rectum forms a loop that lies dorsal to the caecum and adjoins the last septum (Fig. 5).

Discussion
Topography of the digestive tract

This study represents the first demonstration of the position of the digestive tract in a living *Nautilus pompilius* using X-ray analyses and computational tomography. The oesophagus, the connection between the crop and the stomach, could not be depicted using either method, probably because rapid peristaltic movements of the tunica muscularis cause intermittent transport of food into the stomach. A feature of note is the enormous dilation of the crop, which extended to approximately four times its initial size following feeding; this was made possible by the highly folded tunica mucosa. Using our findings together with previous descriptions of the morphology of the digestive tract, it is now possible to derive a three-dimensional picture of this organ complex within the animal (Fig. 5).

Descriptions of the digestive tract by previous authors based only on morphological studies of dissected material contain some errors. Griffin (1900) describes the position of the vestibulum as between the oesophagus and the entrance to the stomach. Furthermore, he describes the ductus hepatopancreas as having three openings. In reports by Naef (1913), the digestive tract of *Nautilus pompilius* is represented as a short tube without the complex rectum. The caecum and the midgut gland were not visible in the X-ray photographs or the computational tomograms because the contrast medium, barium sulphate, cannot be absorbed and therefore did not penetrate the absorptive organs because it is not water-soluble (Elke et al., 1982). These results confirm previous investigations that identified the caecum and the midgut gland as the organs of nutrient absorption (Westermann and Schipp, 1999; Westermann et al., 2000).

The advantages of X-ray analysis and computational tomography compared with morphological and histological methods alone are that the digestive tract is visible in its unaltered position within the living animal. Furthermore, the different phases of digestion can be determined. Previous

dimensional image of this organ complex to be produced. The shell with the siphuncle was removed digitally and is therefore not visible in the three-dimensional reproduction (Fig. 4A–C). As in the X-ray photographs, the tomograms show the jaw, the renal appendages and parts of the contrasted digestive tract; the caecum and the midgut gland and other soft parts could not be depicted. The computational tomography results confirm the results of the X-ray study in which the crop and the stomach are still filled with food after 12 h (Fig. 4).

The results of the morphological topography show that the digestive tract of *Nautilus pompilius* consists of the buccal
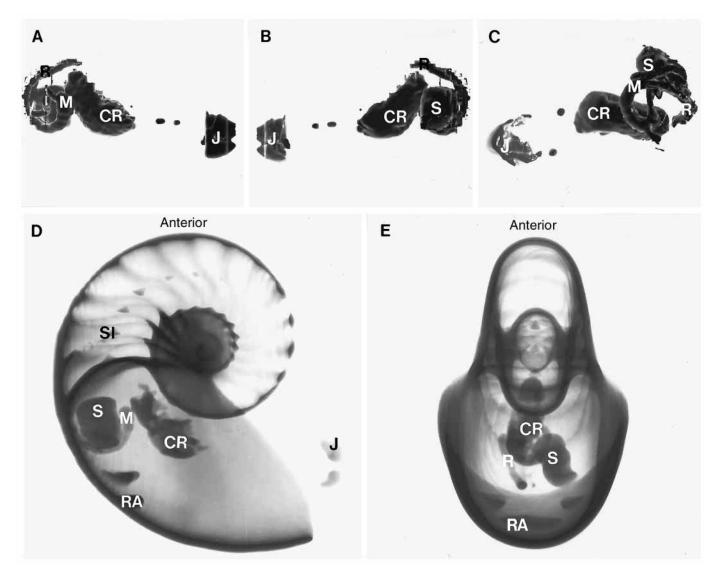
Fig. 4. Computer tomogram of the digestive tract of *Nautilus pompilius*. (A–C) Three-dimensional reproductions from the right side (A), from the left side (B) and in ventral view (C). (D,E) The digestive tract within the animal, from the left side (D) and the front (E). CR, crop; J, jaw; M, midgut; R, rectum; RA, renal appendages; S, stomach; SI, siphuncle.

studies on the digestive mechanisms of coleoid cephalopods were carried out on animals killed for this purpose at defined intervals after food intake (Bidder, 1950; Boucher-Rodoni, 1973; Boucher-Rodoni and Mangold, 1977). Using X-ray analyses and computational tomography, the exact topographic position of the renal appendages of *Nautilus pompilius* were also identified because of their high content of calcium compounds (Crick et al., 1985). These organs insert ventrally on the base of the four afferent branchial vessels (Kefersein, 1866; Vigelius, 1880; Griffin, 1900; Naef, 1913) and are described as a mineral-storage tissue found only in cephalopod species that have shells composed of calcium compounds (Schipp and Martin, 1981).

Duration of digestion

The present investigations show that the cycle of digestion in *Nautilus pompilius* takes 12 h at a water temperature of 18–19 °C, which is approximately the time reported for the necto-benthic sepioids and benthic octopods (Table 1). In *Octopus vulgaris*, digestion takes 12 h at the same temperature (Boucher-Rodoni and Mangold, 1977) and in *Sepia officinalis*, a necto-benthic species, the duration of digestion is 15 h at a water temperature of 20 °C (Boucaud-Camou, 1973). However, in the actively swimming predator *Loligo vulgaris*, the digestion cycle takes only 4–6 h at 18 °C and is thus faster than in sepioids, octopods and nautiloids (Bidder, 1950). These results suggest that the benthic mode of life of nautiloids influences their rate of digestion. Within a species, the duration of digestion is dependent upon temperature; e.g. in *Eledone cirrhosa*, the duration is 15 h at 20 °C, 20 h at 15 °C and 30 h at 10 °C (Boucher-Rodoni, 1973; Wells, 1978). Investigations on the digestive tract of coleoid cephalopods indicate that sex and stage of maturation can also influence the duration of digestion: the passage of chyme is faster in immature than in

Table 1. *Duration of digestion in different cephalopods*

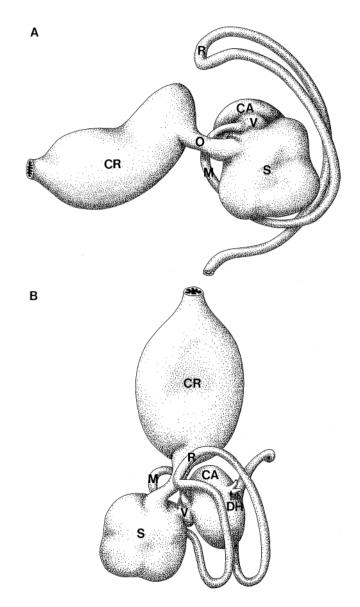| Species	Mode of life	Temperature (°C)	Duration of digestion (h)	Reference
Nautilus pompilius	Benthic	18–19	12	Present study
Octopus vulgaris	Benthic	14	16	Boucaud-Camou et al. (1976)
		18–19	12	Boucher-Rodoni and Mangold (1977)
		23	14–15	Bidder (1957)
Octopus cyanea	Benthic	30	12	Boucher-Rodoni (1973)
Eledone cirrhosa	Benthic	10	30	
		15	20	Boucher-Rodoni (1975)
		20	15	
Sepia officinalis	Necto-benthic	15	20	Boucaud-Camou (1973)
		20	15	
Loligo vulgaris	Pelagic	18	4–6	Bidder (1950)
Illex illecebrosus	Pelagic	10–11	12	Boucher-Rodoni (1975)

mature males and is faster in males than in females (Boucher-Rodoni and Mangold, 1977).

The present investigation provides no information of the efficiency of the digestive process. To obtain this information, the rate of digestion must be calculated as ingested food (as a percentage of body mass) divided by digestion duration (in h) (Wells, 1978). Wells showed that the rate of digestion in coleoid cephalopods was at least as efficient as in their fish competitors.

Course of digestion in nautiloids

Nautilus pompilius grinds its food into small pieces using its strongly developed jaw; the food is coated with mucus in the buccal cavity and then rapidly moved into the stomach by peristaltic movements of the foregut. In loliginids and octopods, the passage of food into the stomach is also very fast and slows down progressively further along the gut (Bidder, 1950; Boucher-Rodoni and Mangold, 1977). Nautiloids can store food in the crop while the first part of the meal is digested, as can octopods. In benthic species, storage of food in the digestive tract and the ability to fast for several weeks represent adaptations to extended periods of starvation, whereas nectonic species will migrate in search of food (Boucaud-Camou, 1987). The X-ray photographs and tomograms in the present study show that 12 h after food intake the crop and the stomach still contain food, indicating that the food moves along the gut in portions. In the stomach, the food is degraded by enzymes and by the mechanical activity of the organ itself. The chyme is then transported to the midgut gland, passing through the caecum.

Fig. 5. Diagram of the digestive tract of *Nautilus pompilius* based on the results of this study (A) from the left side and (B) from the top. CR, crop; CA, caecum; DH, ductus hepatopancreas; M, midgut; O, oesophagus; R, rectum; S, stomach; V, vestibulum.

These organs were identified as the sites of nutrient absorption by previous tracer studies (Westermann and Schipp, 1999; Westermann et al., 2000). Indigestible debris is transported directly from the stomach to the rectum *via* the division of the vestibulum and proximal midgut. The mechanism by which chyme and indigestible debris are separated in the vestibulum in *Nautilus pompilius* is still unclear. In coleoid cephalopods, sphincters enable the substances present in the stomach to enter the caecum or the intestine, but again the mechanism is still unclear (Boucaud-Camou and Boucher-Rodoni, 1983).

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