Cytochemical Investigation of the Digestive Gland of Two Strombidae Species (*Strombus gigas* and *Strombus pugilis*) in Relation to the Nutrition

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ABSTRACT   *Strombus gigas* and *Strombus pugilis* are threatened species and aquaculture represents a good alternative solution to the fishing. In this study, we highlighted the intracellular digestion process in the digestive gland of two Strombidae species, *S. gigas* and *Strombus pugilis*, by the cytochemical characterization of two lysosomal enzymes: acid phosphatase and arylsulfatase. In order to check the efficiency of artificial food digestion, we conducted the characterization on freshly collected, starved and artificially fed individuals of *S. pugilis*. TEM observations of digestive gland sections from freshly collected individuals of both species revealed the presence of acid phosphatase and arylsulfatase activity mostly located in the apical third of digestive cells. Both enzymes were also detected in artificially fed individuals. In response to the starvation, acid phosphatase is not produced anymore by digestive cells, while arylsulfatase is still present. To our knowledge, this is the first cytochemical validation of intracellular digestion of artificial food in Strombidae. This study highlights the intracellular digestion of artificial food developed for Strombidae aquaculture. Moreover, we have shown that the lysosomal activity could be used as a feed index. *Microsc. Res. Tech. 75:1353–1360, 2012.*   © 2012 Wiley Periodicals, Inc.

INTRODUCTION

Strombidae are marine benthic Gastropods which represent an important economic resource in the Caribbean. At least four species represent a staple food including *Strombus gigas* LINNAEUS, 1758 and *Strombus pugilis* LINNAEUS, 1758. In 2001, for example, 3,132 tones of *S. gigas* have been fished, which represent more than 30 million USD (Aldana Aranda, 2003). Due to an important fishing pressure, some species as *S. gigas* are threatened and some populations have already completely disappeared like in the Yucatan (Mexico) for example. Natural stocks decrease rapidly and international authorities have taken protection measures (Adams, 1970; Aldana Aranda, 2003; Appeldoorn, 1987): *S. gigas* is included in the annex II of the Convention on International Trade in Endangered Species (CITES) and also in the red list of the International Union for Conservation of Nature (IUCN). For these reasons, aquaculture represents a good alternative solution to the high pressure on Strombidae (Brito Manzano et al., 1998). While studies report the development in laboratory of fertilized eggs from the sea (Brito Manzano et al., 1999; Brito Manzano and Aldana Aranda, 2004), to our knowledge no studies report a complete reproduction cycle of Strombidae in the laboratory. Veliger larvae are obtained and grown up to the metamorphosis by feeding with unicellular algae. Then juveniles are bred with artificial food provided by small pellets. Few studies have investigated the efficiency of unicellular algae and pellets for Strombidae breeding (Aldana Aranda and Suárez, 1998; Aldana Aranda et al., 1997, 2007). In these publications, the authors have studied the digestive gland structure by histological observations and the quantification of the growing rate of individuals. However, to our knowledge, no study reports an ultrastuctural investigation of the digestive gland of Strombidae except our previous works (Gros et al., 2009; Volland et al., 2010, 2012). Strombidae are among the few Gastropods which present a crystalline style. Such structure is more common in Bivalvia and is often associated to a continuous microphagous nutrition (Fretter and Graham, 1962). The few studies which focused on the digestion physiology of Strombidae reported the nature of enzymes of the crystalline style (Alyakrinskaya, 2001; Horiuchi and Lane, 1965, 1966). To our knowledge, no cytochemical investigation of the digestive gland of Strombidae has been conducted to date. Such organ has a key role in the digestion process and represents with the stomach the most complex part of the digestive tract (Owen, 1966). In this study, we highlighted the intracellular digestion process in the digestive gland of two Strombidae species, *S. gigas* and *Strombus pugilis*, by the cytochemical characterization

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of two lysosomal enzymes: acid phosphatase and aryl-sulfatase. In order to check the efficiency of artificial food digestion, we conducted the characterization on freshly collected, starved and artificially fed individuals of *Strombus pugilis*.

MATERIAL AND METHODS
Experimental Conditions

Individuals of *S. gigas* were collected during the authorized fishing period by professional fishermen on or near *Thalassia testudinum* sea grass beds in Le Gosier, Guadeloupe (French West Indies). *Strombus pugilis* samples were collected by hands on sand area in Saint-François, Guadeloupe. Living materials were rapidly brought to the laboratory. Three *S. gigas* and three *Strombus pugilis* (batch $B_{control}$) individuals were directly processed for cytochemical detection of lysosomal enzymes. Six other *Strombus pugilis* were placed in two 400 L raceway filled with sand-filtered sea water (pH = 8; temperature = 26°C; psu = 36; photoperiod = 12 h) which was continuously oxygenated using an air pump. Sea water was renewed twice a week and raceway was cleaned to avoid development of microalgae and biofilm which could be used as food source. One batch named B_{fed} was fed ad libitum during 4 months with artificial food developed and provided by the CINVESTAV-IPN of Mérida (Nutrition and Aquaculture of Molluscs Laboratories). It has been controlled visually that food pellets were ingested by animals. The other batch named $B_{starved}$ was kept 5 months in starvation. After their respective experimental maintenance, individuals from B_{fed} and $B_{starved}$ were sacrificed and processed for cytochemistry.

Characterization of Lysosomal Activity

Acid Phosphatase. Small digestive gland pieces were dissected and fixed for 2 h in 2% glutaraldehyde and 1% paraformaldehyde in cacodylate buffer (0.1 M; 1,100 mOsm; pH = 7.2). Pieces were then washed in the same buffer and thick sections (50–100 µm) were cut in refrigerated cacodylate buffer using an OCT Slicer®. The Gomori (1952) acid phosphatase detection method modified by Pasteels (1971) was used. Tissues slices were incubated in a 3.3 mg mL^{-1} sodium ß-glycrophosphate solution saturated with lead nitrate. Incubation was realized at 37°C during 25 min. Slices were then rinsed in acetate buffer (sodium acetate 50 mM, acetic acid 15 mM; pH = 5) and in 3.5% acetic acid before dehydratation through an ascending ethanol series and embedded in a resin mixture composed of Epon (63.2%) and Araldite (36.8%). Controls were also prepared by omitting sodium ß-glycrophosphate in the incubation medium.

Arylsulfatase. We used a method adapted from Hopsu-Havu et al. (1965) described in Lewis and Knight (1992). Thick section (100–150 µm) of prefixed digestive gland were obtained as described above and incubated 45 min at 37°C in the reactive media (*p*-nitrocatechol sulfate 8 mg mL^{-1}, acetate buffer 60 mM, barium chloride 60 mM; pH = 5.5). Slices were then washed in cacodylate buffer overnight before dehydratation and embedding in the same resin as described above. Control slices were incubated without *p*-nitrocatechol sulfate.

Light and Transmission Electron Microscopy

Semithin sections (0.5 µm thick) were cut from the resin-embedded samples and stained with 0.5% toluidine blue in 1% borax for light microscopy observation. Ultrathin sections (60 nm) were obtained from resin blocks and observed without supplementary contrast with Energy Filtered Transmission Electron Microscopy (EFTEM). Acquisitions in spectra mode (EELS, Electron Energy Loss Spectroscopy) were performed using a LEO 912 Omega transmission electron microscope (LEO Electron Optics GmbH, Oberkochen, Germany) at 120 kV. Observation on image mode (ESI, Electron Spectroscopic Imaging) was accomplished with the ESIvision program (version 3.0 Soft-Imaging Software, SIS, GmbH, 48153 Münster). For the elemental cartography, the subtractive method of the "three windows" was used (Jeanguillaume et al., 1978; Reimer et al., 1992).

Histochemical Detection of Lipids

Digestive gland samples of *S. gigas* and *Strombus pugilis* were fixed for 6 h in 6% paraformaldehyde in sea water. Samples were then washed in sea water and transferred in 30% sucrose in sea water. They were then rapidly frozen in isopentane cooled at −35°C with liquid nitrogen. Cryosections of 10 µm realized with a Cryo-cut® (American Optical Corporation) were stained with black Soudan B as described by Gabe (1968).

RESULTS
Ultrastructure of the Digestive Cells

According to Gros et al. (2009), the digestive gland of Strombidae is composed of an assemblage of digestive tubules and ducts. Digestive tubules are composed by three cell types: short pyramidal crypt cells, vacuolated cells, and the predominant long columnar digestive cells (Figs. 1C and 2A). This last cell type is the most important in terms of number and volume in the digestive tubules. They form a unistratified epithelium and are regularly aligned (Fig. 1A). Digestive cells were on average 90 µm long in *S. gigas* and 80 µm long in *Strombus pugilis*. They present a basal nucleus and three compartments can be distinguished in the cytoplasm: the basal third, the median third, and the apical third (Figs. 1D, 2B, and 2C). The basal third is characterized by the presence of vesicles from 2 to 5 µm in diameter. Such vesicles appear empty on resin section, due to the sample preparation. Histochemical staining with black Soudan B of cryosections revealed that these structures are lipid droplets (data not shown). The median third of the cell is characterized by the presence of one or few large granules (Figs. 1A, 1B, 1D and 2B–2D). Such granules have a diameter of 4 to more than 10 µm and their average diameter is 7 µm for both species. Finally, the apical third of the cell is composed by two areas (Figs. 1D and 1E). The area 1, adjacent to the median third, present various vesicles with a diameter of 0.5 to 5 µm. The area 2 presents small vesicles (less than 2 µm).The apical pole of the digestive cells, in contact with the tubule's lumen, is bordered by short microvilli of 2 µm on average (Fig. 1D).
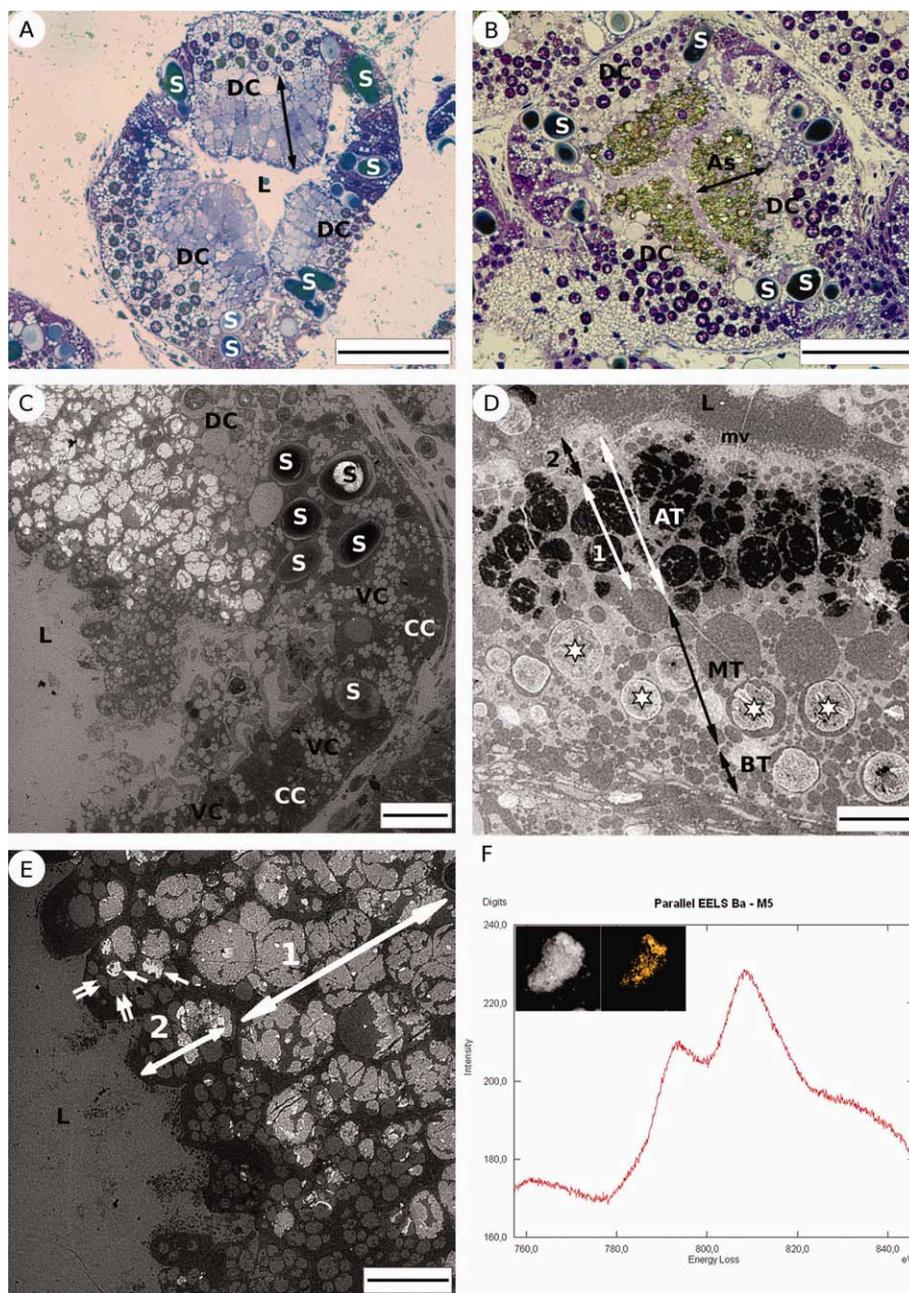
Fig. 1. Arylsulfatase activity in the digestive gland of *S. gigas* observed in photonic and transmission electron microscopy. **A**, **B**. Semithin sections of a digestive tubule following tissues incubation in the control media (A) and the reacting media (B). Yellow-green precipitates reflecting an arylsulfatase activity (As) are clearly visible in the upper half of digestive cells (DC) incubated in the reacting media (B). The same area in digestive cells of control samples does not present any precipitates (A). Scale bars: 50 μm. **C–E**. TEM observations of samples incubated in the arylsulfatase revealing medium. C. General view of a digestive tubule showing the three cell types: crypt cells (CC), vacuolated cells (VC), and digestive cells (DC). This last cell type is the only one which presents electron dense precipitates reflecting an arylsulfatase activity. On this observation, contrast is inversed and electron dense precipitates appear in white. Scale bar: 20 μm. D. Higher magnification of digestive cells. Here, the contrast has been restored in order to show better electron dense precipitates in black. The three regions of digestive cells are clearly visible. The basal third (BT) is reduced and characterized by the presence of lipid droplet. The median third (MT) contains its characteristic large granules (stars) and the apical third (AT) presents an important arylsulfatase activity. Precipitates are mostly located in vesicles of the area 1 of the apical third. Scale bar: 10 μm. E. Higher magnification of the apical third of digestive cells. The area 1 presents vesicles from 3 to 7 μm in diameter with an important arylsulfatase activity which appears in white. In the area 2, smaller vesicles are present (1–3 μm). Such vesicles are mostly negative to the enzyme detection. Scale bar: 10 μm. F. Spectrum obtained focusing on an electron dense precipitates after background subtraction for barium. Moreover, observation by electron spectroscopic imaging (ESI) using the three windows method, confirmed that barium is detected in the whole precipitate and only inside (element cartography is given in the right insert). Such EFTEM analysis confirms that precipitates are characteristic of the arylsulfatase activity. L: lumen; mv: microvilli; S: symbiont. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
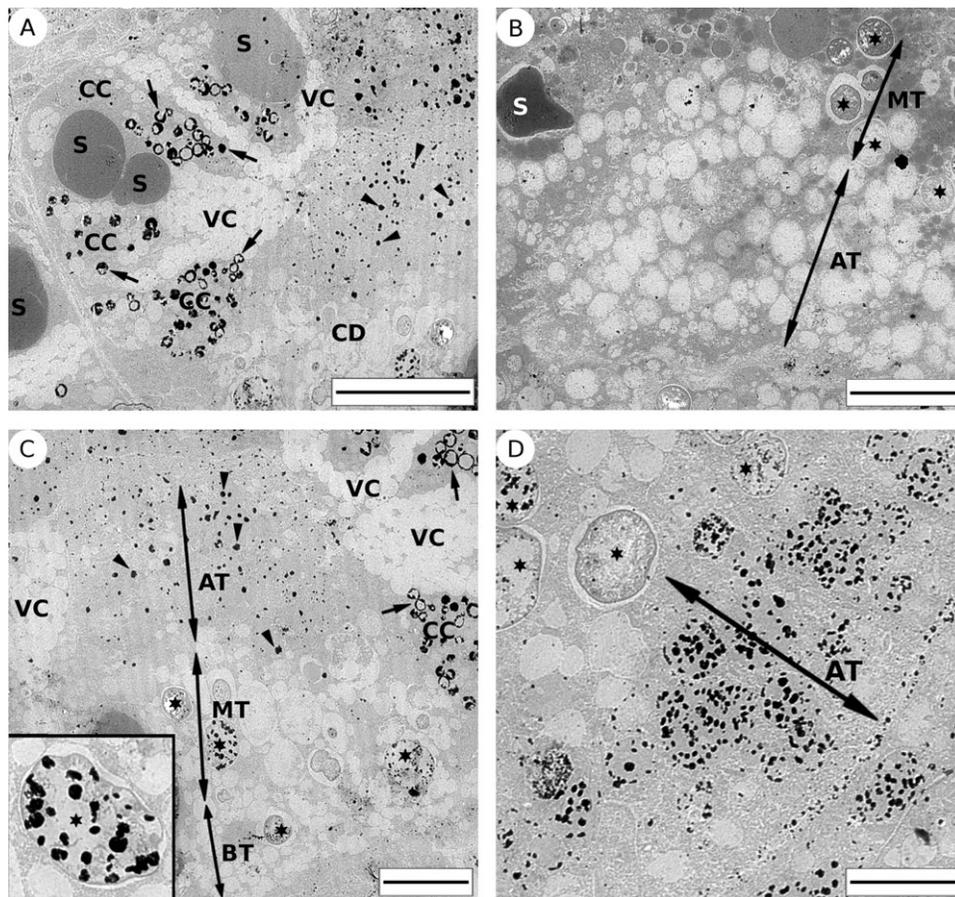
Fig. 2. Acid phosphatase activity in the digestive gland of *S. pugilis* observed from thin sections. **A**, **B**. Semithin sections of a digestive tubule following tissues incubation in the reacting media (A) and the control media (B) which does not contain the enzyme substrate. On the (A), a partial view of a digestive tubule with the three cell types is visible. In vacuolated cell (VC), which hosts the symbionts (S), no precipitates are detected. In crypt cells (CC), spherocrystals (arrows) present electron dense precipitates reflecting an acid phosphatase activity. A group of digestive cells (DC) is also visible and present an important acid phosphatase activity mostly located in the apical third of cells (arrow heads). Scale bar: 20 μm. In contrast, on the control (B), no precipitates are detected. Scale bar: 20 μm. **C**. Observations focusing on digestive cells. Electron dense precipitates (arrow heads) reflecting acid phosphatase activity are mostly detected in the apical third (AT) of cells. Precipitates are also presents in the large granules (stars) of the median third (MT). The detail of a granule is showed in the insert of (C). In the basal third (BT) lipid droplet are present but no enzyme activity is detectable. Small scattered precipitates (0.1–0.5 μm) are also present in the whole cytoplasm of digestive cells. An acid phosphatase activity is also detected in spherocrystals (arrows) of crypt cells. Precipitates form a ring in the periphery of spherocrystals. Scale bar: 10 μm. **D**. Detail of the apical and median thirds of digestive cells showing the important acid phosphatase activity in the apical third. In some large granules an enzyme activity is present as small numerous precipitates. Scale bar: 5 μm.

Cytochemical Detection of Arylsulfatase

Observations of semithin digestive gland sections from freshly collected individuals of both species following cytochemical detection of arylsulfatase revealed the presence of yellow-green precipitates located in the digestive cells of the gland (Fig. 1B). Results presented below were observed identically on the three replicates for both species. TEM observations of ultra-thin sections confirmed that the arylsulfatase activity is only located in the digestive cells. No electron dense precipitates were observed in vacuolated and crypt cells (Fig. 1C). In digestive cells, arylsulfatase activity is mostly detected in the area 1 of apical third of cells (Figs. 1D and 1E). Most of small vesicles (0.5 to 2 μm) of the area 2 of the apical third are not positive to the enzyme detection (Figs. 1D and 1E). The arylsulfatase activity is the most important in large vesicles (average 7 μm)

of the apical third in which the precipitate occupied the whole vesicle (Figs. 1C–1E). While the large vesicles of the median third are not positive to the enzyme detection, some large granules of this compartment present a weak arylsulfatase activity. In such granules, the activity is limited to few restricted precipitates (Fig. 1D). The validity of the enzyme detection has been controlled twice, first, by observations of control slices (Fig. 1A) and secondly by EFTEM microanalysis of the precipitates. Such analysis in spectrum mode confirmed the presence of barium. In image mode, we also checked that barium was only located in the precipitates (Fig. 1F).

Cytochemical Detection of Acid Phosphatase

TEM observations of digestive gland sections from freshly collected individuals of both species revealed

TABLE 1. Cytochemical detection of lysosomal enzymes activity in the digestive gland of S. pugilis

	$Batch_{control}$	$Batch_{fed}$	$Batch_{starved}$
Acid phosphatase	++	+	−
Arylsulfatase	++	+	+

Comparison of the batch of freshly collected individuals ($B_{control}$), the batch fed 4 months with artificial food (B_{fed}) and the batch starved 5 months ($B_{starved}$). The semiquantitative characterization was done visually by observation of the number and the size of precipitates detected on digestive glands section of the three replicates individuals.

the presence of electron dense precipitates after cytochemical detection of acid phosphatase. Results presented below were observed identically on the three replicates for both species. The enzyme activity has been detected mostly in the digestive cells but we also found precipitates in specific structures called spherocrystals located in the crypt cells (Figs. 2A and 2C). However, vacuolated cells did not show any acid phosphatase activity. In the digestive cells, the enzyme was mostly detected on the apical third of the cell, in small vesicles of 0.5 to 2 µm in diameter (Fig. 2D). Sometimes, acid phosphatase activity was also detected in the large granules of the median third of the digestive cells (Fig. 2C). The specificity of electron dense precipitates was confirmed by the observation of controls (Fig. 2B).

Starvation and Artificial Feeding

Preliminary starvation experiments of different period of time have been conducted and enzyme activity for both acid phosphatase and arylsulfatase was detected by photonic microscopy even following several weeks of experiments (data not shown). Consequently, we have chosen to use the oldest batches (i.e. 5 months starved *Strombus pugilis*) for the cytochemical detection of lysosomal activity. Concerning acid phosphatase, TEM observations of ultrathin section revealed the presence of precipitates in the digestive gland of $B_{control}$ as expected. On digestive gland of artificially fed individuals (B_{fed}) an acid phosphatase activity was also present. While no quantification were done, the observation of precipitates revealed that they are less numerous and more scattered, which means that enzyme activity is less important than in $B_{control}$. For both $B_{control}$ and B_{fed}, the enzyme activity was located as described earlier. However, in $B_{starved}$, we were unable to detect any acid phosphatase activity in the digestive gland of the three individuals (Table 1).

Concerning the arylsulfatase activity, even if it seemed stronger (precipitates more numerous) in the digestive cells of $B_{control}$ individuals, we could detect the enzyme activity on the three individuals of the three batches (Table 1). The enzyme was located as described earlier for the freshly collected individuals.

DISCUSSION

The digestive gland of both *Strombus gigas* and *S. pugilis* is composed by an assemblage of digestive tubules and ducts. Tubules are formed by an epithelium containing three cell types as described by Gros et al. (2009): crypt cells, vacuolated cells, and digestive cells. Digestive tubules of Caenogastropoda are known to be composed by, at least, two cell types: digestive cells and pyramidal crypt cells. This last cell type regroups secreting cells, excreting cells, basophile cells, and calcium cells (Boghen and Farley, 1974; Devi et al., 1981; Fretter and Graham, 1962; Lutfy and Demain, 1967; Merdsoy and Farley, 1973; Voltzow, 1994; Wigham, 1976). In the two Strombidae species, the most numerous cells are in the digestive tubules. They are also known to be the largest cell type in the digestive tubules of Mollusca in general (Lobo-Da-Cunha, 2000; Lutfy and Demain, 1967; Owen, 1966). Many studies have described their structure and ultrastructure in relation with their function in Bivalvia (Henry, 1984; Morse et al., 1997; Pal, 1972), in Cephalopoda (Boucaud-Camou and Roper, 1998; Semmens, 2002; Swift et al., 2005; Pernice et al., 2009) and in Polyplacophora (Lobo-da-Cunha, 1997). Among Gastropods many studies focused on the digestive cells in Opistobranchia (Coelho et al., 1998; Lobo-Da-Cunha, 2000; Taïeb, 2001), in Pulmonata (Luchtel et al., 1997; Walker, 1970, 1972), and in Prosobranchia (Boghen and Farley, 1974; Fretter and Graham, 1962; Lutfy and Demain, 1967; Merdsoy and Farley, 1973; Voltzow, 1994; Wigham, 1976). Such studies reported some general characteristics for the digestive cells of Mollusca because of the presence of microvilli and an important endocytic system at the apical pole. While the absorption and digestion process seems to be proven, the secreting function remains hypothetical, except for some Bivalves (Henry et al., 1991) and Cephalopods (Boucaud-Camou and Roper, 1995) where it has been confirmed. The long columnar shape and the presence of numerous vacuoles and large granules also belong to the general characteristics of these cells. Such characteristics are also observed in the digestive cells of Strombidae. However, the division of digestive cell into three regions seems to be more specific to Strombidae. Even if few studies reported lipid droplets in digestive cells of molluscs' digestive gland, they are not as important as in Strombidae in which a third of the cell is dedicated to such lipid storage (Dimitriadis and Andrews, 2000; Lobo-Da-Cunha, 2000). Otherwise, digestive cells have ever been suspected to have a storage function (Owen, 1966).

In the median and apical third of digestive cells, we highlighted a lysosomal activity by the cytochemical detection of acid phosphatase and arylsulfatase. These two lysosomal enzymes are known to be markers of the intracellular digestion process (Holtzman, 1989). Moreover, apical microvilli reflect an exchange process between the lumen and the digestive cell. Such observations confirm that the intracellular digestion process occurs in both apical and median third of digestive cells of Strombidae. Arylsulfatase activity has been detected in digestive cells of *Aplysia deplians* (Opistobranchia) (Lobo-Da-Cunha, 2000). This author reported an enzyme activity in the Golgi apparatus, in lysosomes, and a strong activity in heterolysosomes in the median and apical regions of the digestive cell. In the bivalve *Mytilus galloprovincialis* an arylsulfatase activity has also been detected, but limited to the Golgi apparatus (Dimitriadis et al., 2004). In the same species, another study reports an arylsulfatase activity in heterolysosomes of the apical pole and in some lysosomal residual bodies (Robledo et al., 2006). Finally a fourth study highlighted the presence of arylsulfatase in "cyto-

plasmic granules" of the digestive cells in the Bivalve *M. edulis* and in the pulmonate *Helix aspersa* (Sumner, 1969). In Strombidae, we observed an increasing size of arylsulfatase positive vesicles from the apical pole to the one-half of the cells. Such vesicles might represent heterolysosomes at different digestion stages.

Acid phosphatase has also been detected by cytochemistry in the digestive cells of several molluscs. In the prosobranch *Nucella lapillus* and in the bivalve *M. galloprovincialis*, it has been detected in heterolysosomes in the middle of the digestive cells, and, locally in some residual bodies (Dimitriadis and Andrews, 2000; Robledo et al., 2006). Sumner (1969) also reported it detection in "cytoplasmic granules" of *M. edulis* digestive cells and in the whole cells in *H. aspersa*. In *S. gigas* and *Strombus pugilis*, acid phosphatase localization is more or less the same as arylsulfatase localization. Even of different sizes, the vesicules from the apical third of digestive cells present an activity for both enzymes, confirming that such vesicles are lysosomes or heterolysosomes when their size exceeds 1 μm (Sumner, 1969). We also highlighted an acid phosphatase activity in the large granules of the digestive cells. Such granules are named "blue granules" by Gros et al. (2009) because of their affinity for alcian blue, reflecting their proteoglycan content. They seem similar to the large residual bodies described in the digestive cells of other molluscs (Dimitriadis et al., 2004; Henry, 1984; Lobo-Da-Cunha, 2000; Taïeb, 2001). Lobo-Da-Cuhna (2000) highlighted that such granules result from the fusion of every heterolysosomes and lysosomal residual bodies of the cell. Lipofushine pigment has been highlighted in such granules in *Aplysia punctata* and in *Maoricrypta monoxyla* and its function in a detoxification process has been proposed (Desouky, 2006; Dimitriadis et al., 2004; Marigomez et al., 1990; Nelson and Morton, 1979; Taïeb, 2001).

Furthermore, we found an acid phosphatase activity in the spherocrystals of the crypt cells. Spherocrystals are implied in regulation and detoxification of essential trace metals (George et al., 1980, 1982; Volland et al., 2012). Note that an alkaline phosphatase activity has already been detected in midgut cell spherocrystals of the arthropod *Raillietiella* sp. (Pentostomida: Cephalobaenida) (Thomas et al., 1999). Among molluscs, a ß-glucuronidase activity has also been highlighted in spherocrystals of the basophile cells of the bivalve *M. galloprovincialis* (Dimitriadis et al., 2004). These authors have proposed that the enzyme is present under an inactive form up to spherocrystals are released in the lumen in order to participate to the extracellular digestion. In Strombidae, the acid phosphatase activity in crypt cells does not result of an intracellular digestion process since neither endocytic system nor lysosomal residual bodies have been detected in this cell type.

Concerning the lysosomal activity during starvation and feeding with artificial food, we found that arylsulfatase is detected whatever the nutritional status of *Strombus pugilis* individuals. Although we were not able to detect any acid phosphatase activity in digestive cells of starved individuals, we detected it in individuals artificially fed with pellets. Therefore, both enzymes present different profiles when individuals are starved for a long period. In response to the starvation, acid phosphatase is not produced anymore by digestive cells, while arylsulfatase is still present. A similar study on *H. aspersa* highlighted that individuals can survive 1 year in starvation and that the lysosomal enzyme activity is detected during all the experiment (Sumner, 1969). Among the five enzymes followed, only arylsulfatase and acid phosphatase activity decreased or stopped. Another study, in the same species, reported that during the first 6 months of starvation, the organism used the stored energetic resources (heterophagy), before beginning to use its own tissues as food source (autophagy) (Porcel et al., 1996). Autophagy of digestive cells has also been proposed as a response to a prolonged starvation in *M. edulis* (McVeigh et al., 2006). Such process could explain why an arylsulfatase activity is still detected after several months of starvation in *Strombus pugilis*. Moreover, Borges et al. (2004) have proposed that the digestive gland of *H. aspersa* can be used as principal food source during starvation.

Controlling the growth of Strombidae is a major issue for the aquaculture of this group. In this respect, it is essential to evaluate the nutritional status of individuals and it has been shown that the shell growth and weight are not precise indices of optimal growth and assimilation (Lucas and Beninger, 1985). Even if it is visually controlled that artificial food is eaten by individuals, it seems very important to confirm that the food is truly digested. The acid phosphatase activity detected in digestive cells of artificially fed *S. pugilis* highlights the fact that pellets are truly metabolized by individuals. To our knowledge, this is the first cytochemical validation of intracellular digestion of artificial food in Strombidae. Indeed, artificially fed *S. pugilis* present a lysosomal activity qualitatively similar to controls whereas starved individuals did not present acid phosphatase activity any more. This study highlights the intracellular digestion of artificial food developed for Strombidae aquaculture by the "Laboratorio de Biologia y Cultivo de Moluscos" (CINVESTAV, Unidad de Mérida, Mexico). Moreover, we have shown that the lysosomal activity could be used as a feed index. In a study focusing on *S. gigas* Aldana Aranda et al. (2011) investigated the potentiality of the digestive gland structure and more specifically the quantity of "blue granules" in digestive cells as a feed index. They found that such histochemical analysis of the digestive gland is a sensitive feed index that can be used as a tool to adapt the formulated feed and the feeding rate to the needs of individuals. They also pointed that "*the food pellets seems to be digested in the stomach and reduced to a fine homogeneous powder that is transported to the digestive gland tubules where it may be absorbed.*" Our cytochemical investigation of the Strombidae digestive gland has confirmed that such absorption occurs in digestive cells and induces an intracellular digestion process of the food. Optimization of Strombidae aquaculture requires a better understanding of intracellular digestion process. Cytochemistry proves to be an appropriate technique to investigate intracellular digestion in the digestive gland in relation to the nutrition status. Such cytochemical investigations could be conducted, for example, on individuals fed with different artificial food mix in order to determine the most efficient in terms of lysosomal digestion induction.

ACKNOWLEDGMENTS

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