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GUT CONTENT ANALYSIS OF *Anadara tuberculosa* (SOWERBY, 1833) THROUGH HISTOLOGICAL SECTIONS

Análisis del contenido intestinal de *Anadara tuberculosa* (Sowerby, 1833) a través de cortes histológicos

RESUMEN. Se examinó el contenido intestinal de 30 especímenes de *Anadara tuberculosa* recolectados en la costa pacífica de la Península de Baja California en abril, mayo, junio y octubre de 2000 y se hicieron cortes histológicos de intestino y estómago; se encontraron 1002 estructuras. De éstas, 917 correspondieron a diatomeas (91.5%), de las cuales, la diatomea bentónica *Paralia sulcata* (45.3%) y las diatomeas planctónicas *Thalassiosira* spp. (42.9%) y *Thalassionema nitzschioides* (2.7%) fueron las más frecuentes. El silicoflagelado *Dictyocha* sp. (1.6%) fue poco abundante. Asimismo, se encontraron 85 estructuras (8.5%) que consistieron en huevos de bivalvos (1.8%), espículas de esponjas (0.4%) y material no identificado (6.3%). Los cortes histológicos representan una nueva alternativa para el estudio del contenido estomacal e intestinal para este bivalvo.

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Muñetón-Gómez, M. S., M. Villalejo-Fuerte & I.Gárate-Lizárraga. Gut content analysis of *Anadara tuberculosa* (Sowerby, 1833) through histological sections. *CICIMAR Oceánides*, 25(2): 143-148.

Anadara tuberculosa is an abundant bivalve mollusk, distributed from Baja California, Mexico to Southern Peru (Squires *et al.,* 1975). In Baja California Sur this species is found on both the Pacific and the Gulf of California coasts, living 1 to 4 meters deep among the roots of the mangrove trees *Avicennia germinans* and *Laguncularia racemosa*. Early accounts of the food of the bivalve filter feeders were the result of gut content analysis of commercially important species. McCrady (1874) reported that the gut content of *Crassostrea virginica* included diatoms. Morse (1944) noted that the gut content in marine bivalves might be different in each season. Studies about the feeding habits of benthic species may provide a unique opportunity to determine not only their role in energy transformation between benthic and pelagic environments, but also the relationship between these two layers in terms of suspended particles (Shumway *et al.,* 1987; Muñetón-Gómez *et al*., 2001). *Anadara tuberculosa* and *A. grandis*, also commonly known in Mexico as "pata de mula" are mostly filter-feeding organisms that feed on benthic and tychoplanktonic diatoms, and may also ingest some zooplanktonic organisms. In southern Baja California, "pata de mula" banks are intensively exploited and when a bank is depleted the fishery moves on to other areas. Most investigations on *A. tuberculosa* have focused on anthropological subjects (Flores-Mendoza, 1971). Because there is no information regarding the feeding habits of *A. tuberculosa*, this study aims at providing information on the gut content of *A. tuberculosa*. Additionally, a histological technique is proposed as an alternative method to conduct investigations on the gut and intestinal content of bivalve and other mollusks.

Bahia Magdalena is a shallow lagoon located on the western coast of Baja California Sur (24°15´ -25°20´ N, 111°30´-112°12´ W). This lagoon system may be divided into three zones. The northern zone consists of a series of channels with a mean depth of 3.5 m. The central part is known as Bahia Magdalena and is connected to the ocean by a wide opening. The southeastern zone, Bahia Almejas, is connected to Bahia Magdalena by a narrow mouth. The system is considered an anti-estuary with irregular bathymetry (Gárate-Lizárraga & Siqueiros-Beltrones, 1998). A total of 30 specimens of *A. tuberculosa* were collected during April, May, June and October 2000 from two traditional clam-fishing areas, Bahia Magdalena and Bahia Almejas (Figure 1). Specimens were collected from the mud, between the roots of mangroves *Avicennia germinans,*

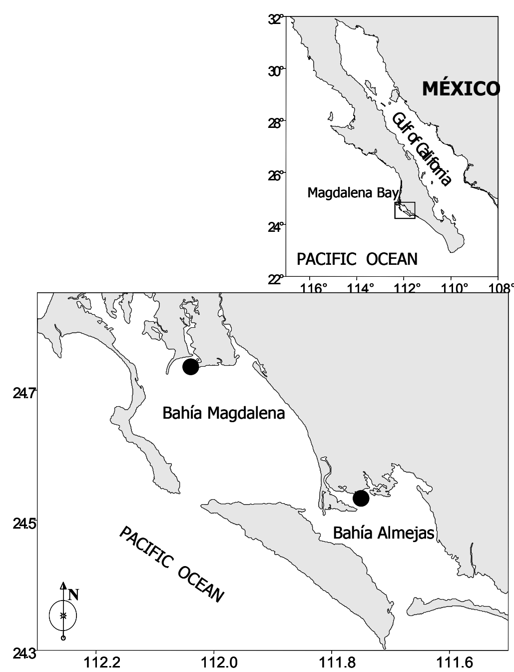

Figure 1. Sampling sites in two clam-fishing grounds in the Bahía Magdalena-Bahía Almejas lagoon system in the west coast of Baja California Sur, Mexico.

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Laguncularia racemosa and *Rhizophora mangle*. In the field, specimens were fixed in 10% formalin, and the shell dimensions and meat weight were recorded in the laboratory.

To carry out the histological study the visceral mass was processed through the histological technique, embedding specimens in paraffin, then locating the gut middle portion and obtaining frontal-dorsal 7µm-thick sections including the stomach and intestine. Sections were stained with hematoxylin-eosin and mounted in synthetic resin (Muñetón-Gómez *et al.,* 2000). Histological sections were observed under a phase contrast microscope, gut and intestine contents were identified to genus or species using the taxonomic keys by Cupp (1943), Tomas (1996) and Siqueiros-Beltrones (2000). The number of phytoplankton cells was determined, and the result was expressed as percentage.

The *Anadara tuberculosa* specimens collected exhibited an average size of 58 mm and an average meat weight of 15 g (with no shell). One thousand two items were counted, 917 of which corresponded to diatoms (91.5%), the most abundant being *Paralia sulcata* (46.3%), *Thalassiosira* spp. (42.9%), *Thalassionema nitzschioides* (2.7%), *Diploneis* sp. (1.2%). Different aspects of gut and intestinal content in *Anadara tuberculosa* are observed in Figure 2. Some species are easily recognized at genus or species level (Fig. 3). The silicoflagellate *Dictyocha* sp. (1.6%) appeared in low quantity. Additionally, another 85 non-diatom structures were found (8.5%), including bivalve eggs (1.8%), sponge spicules (0.4%) and unidentified materials (6.3%). Oocytes of this same species were found in the gut content, even in males (Table 1).

Through direct observations Siqueiros-Beltrones (2000) reported that the benthic diatom genera *Amphora, Navicula* and *Nitzschia* can be food sources for the abalone *Haliotis* spp. However, histological sections of the bivalve gut and intestine represent an alternative to study gut content, although only hard structures prevail, such as diatom frustules

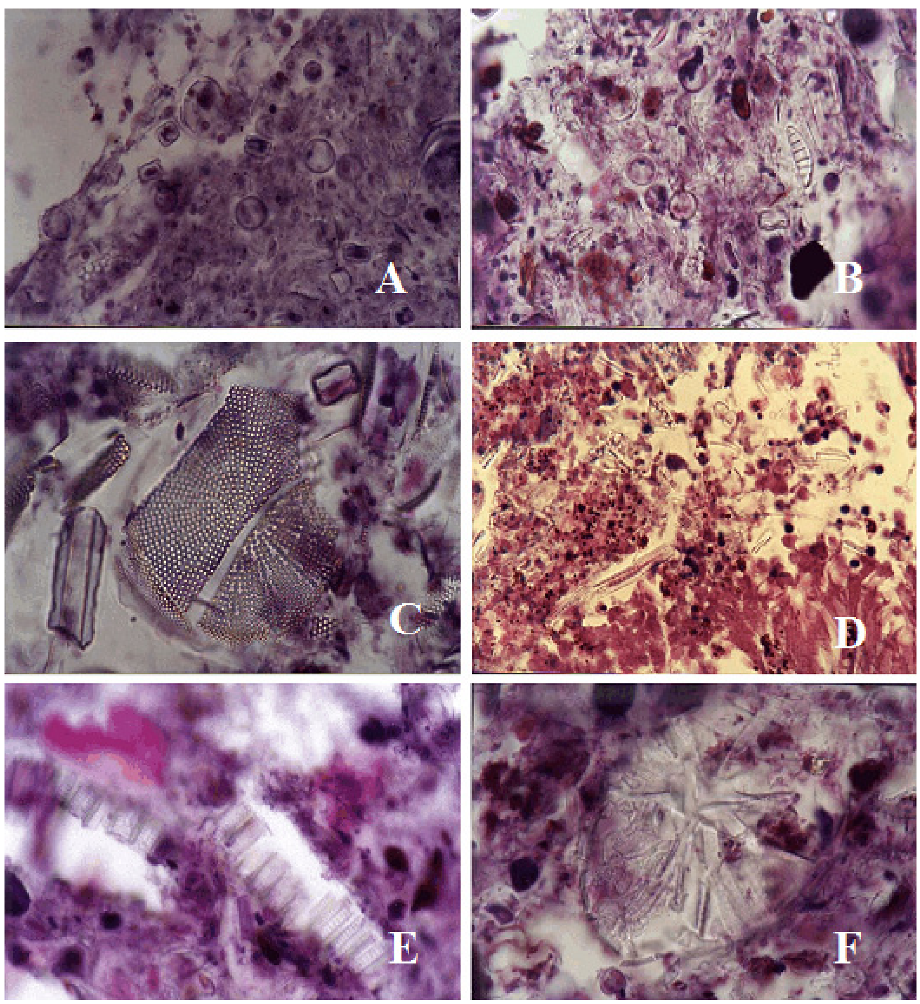

Fig. 2. A-F. Different aspects of gut and intestine content in *Anadara tuberculosa*.

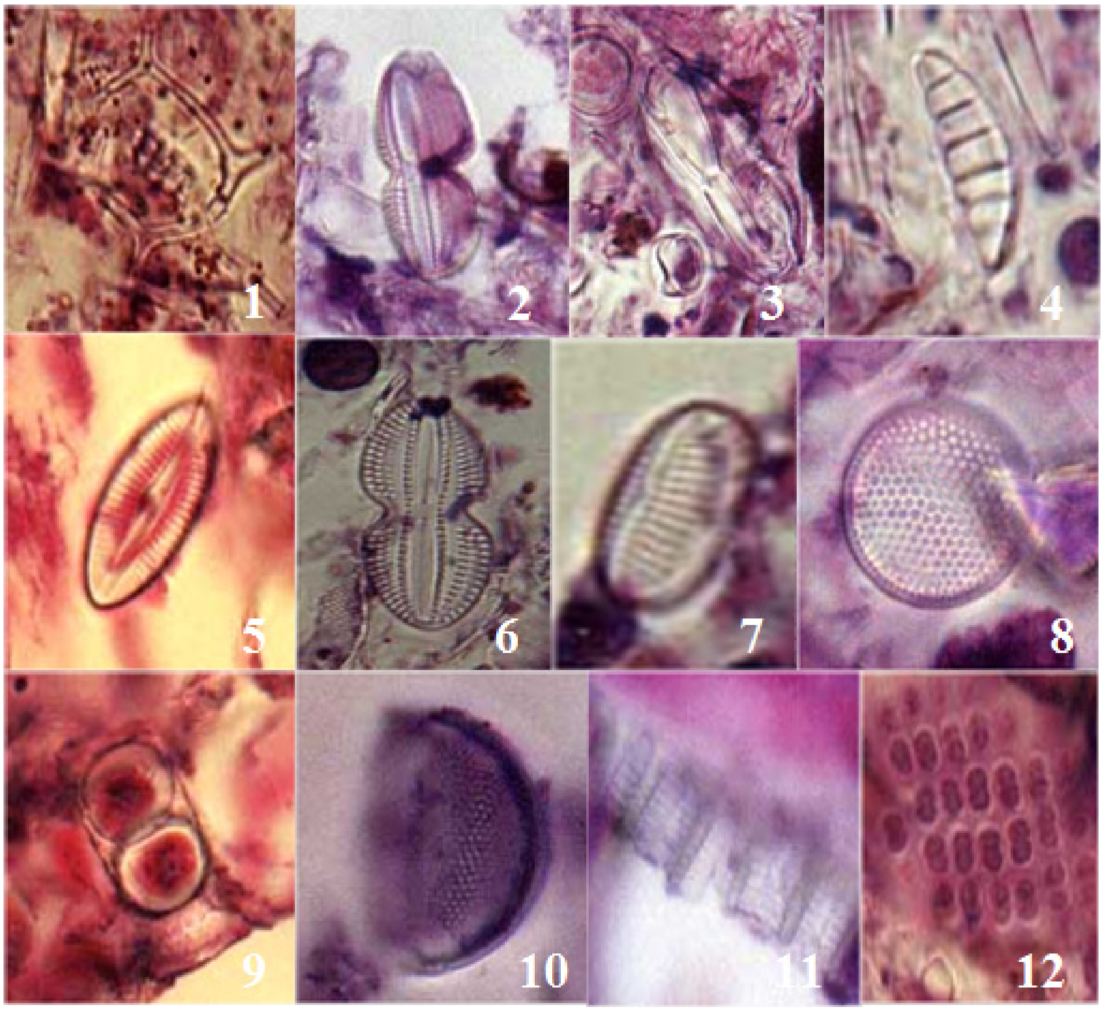

Figure 3. Phytoplankton species of gut and intestine contents in *Anadara tuberculosa*. 1) *Dictyocha* sp. 2) *Diploneis gruendleri* 3) *Amphora* sp., 4) *Eunotogramma laevis*, 5) *Diploneis smithii*, 6) *Diploneis gruendleri*, 7) *Diploneis* sp., 8) *Thalassiosira* sp., 9) *Paralia* sp., 10) *Thalassiosira* sp., 11) *Paralia* sp. 12) Unidentified organism.

and some recently ingested soft structures. Studying transversal histological sections of *A. tuberculosa* guts, Flores-Mendoza (1971) observed fragments of the diatoms *Coscinodiscus* sp., *Navicula* spp., and *Fragilaria* sp. adjacent to the ciliated pseudo-stratified cylindrical epithelium covering the gut walls.

Dietary components vary depending on geographic location. Sea scallops in coastal areas and bays encounter more seaweed and seagrass detritus and may be periodically exposed to significant amounts of resuspended inorganic material. Offshore scallops feed mainly on phytoplankton and resuspended organic material (Grant & Cranford 1991). Thus, phytoplankton appears necessary to meet scallop energetic demands, although seaweed detritus may be an important food supplement in near shore environments (Grant & Cranford 1991). In our study bivalves were collected in a shallow mangrove area, and diatoms were found to be the main component in the gut content of *A. tuberculosa*. A similar finding was reported for the oyster *Hyotissa hyotis*, where the gut content included mostly diatoms (86.5%) and dinoflagellates (13.5%), coinciding with the phytoplankton composition in the area (Villalejo-Fuerte *et al.,* 2005). This is not surprising since pelagic and tychoplanktonic diatoms are the main phytoplankton group in Bahía Magdalena (Gárate-Lizárraga & Siqueiros-Beltrones 1998; Gárate-Lizárraga *et al*., 2003; Siqueiros-Beltrones *et al*., 2005). Likewise Siqueiros-Beltrones *et al.* (2005) found that benthic diatoms are substantial component of benthic communities.

Davis and Marshall (1961) found that the benthic diatoms *Melosira, Licmophora* and *Cocconeis* were more abundant than planktonic diatoms in the gut content of the pectinid clam *Argopecten irradi-*

Table 1. Microalgae and other items found in the gut and intestine content in *Anadara tuberculosa*

SPECIES	APRIL	MAY	JUNE	OCTOBER	TOTAL	%
Amphora sp.	0	1	1	0	2	0.2
Amphora proteus	0	1	0	0	1	0.1
Chaetoceros spp.	0	6	4	0	3	0.3
Cyclotella sp.	0	4	0	0	4	0.4
Climacosphenia sp.	0	0	1	0	1	0.1
Cocconeis sp.	0	2	1	2	5	0.5
Coscinodiscus radiatus	0	0	1	0	1	0.1
Coscinodiscus perforatus	0	1	0	0	1	0.1
Coscinodiscus sp.	0	2	3	2	7	0.8
Diploneis smithii	0	4	0	3	7	0.8
Diploneis gruendleri	0	4	3	4	11	1.2
Dictyocha spp.	0	3	5	7	15	1.6
Eunotogramma laevis	0	1	0	0	1	0.1
Grammatophora sp.	0	0	1	0	1	0.1
Guinardia flaccida	0	3	0	0	3	0.3
Paralia sulcata	30	142	170	73	415	45.3
Navicula sp.	0	3	9	0	12	1.3
Thalassionema nitzschioides	2	0	11	12	25	2.7
Thalassiosira spp.	135	121	68	69	393	42.9
Triceratium favus	0	0	3	0	3	0.3
SUBTOTAL	167	297	281	172	917	91.5
OTHER STRUCTURES						
Sponge spicules	0	0	4	0	4	0.4
Unidentified structures	0	10	0	53	63	6.3
Eggs	0	4	0	14	18	1.8
SUBTOTAL	0	14	4	67	85	8.5
TOTAL	167	311	285	239	1002	100

ans. Additionally, Shumway *et al.* (1987) compared the gut content in *Placopecten magellanicus* populations in the Gulf of Maine, in shallow (20m) and deep (180m) water, and found that benthic microalgae like *Melosira, Navicula* and *Pleurosigma* were more abundant than pelagic species in the deep-water population; additionally, these authors pointed out that the gut content generally reflected the availability of organisms in the surrounding environment.

Vernet (1977, in MacDonald *et al*., 2006), determined the composition in the gut content of *Chlamys tehuelca* in the Patagonia, as well as the microflora in bottom sediments and plankton samples collected immediately above the scallop banks in the Gulf of San José, Argentina, finding that the diatoms *Synedra investiens, P. sulcata, Grammatophora marina* and *Navicula* spp. were dominant in the gut content. Whereas the diatoms *Glyphodesmia distans, Glyphodesmia* spp. and *Plagiogramma interruptus* although abundant in the sediment and were absent in the gut content, presumably because these are not resuspended. In our work, the benthic diatoms *P. sulcata* and the planktonic diatoms *Thalassiosira* spp. accounted for 88.2% of the gut content of *A. tuberculosa*. This suggests that the resuspension of bottom materials makes an important food source available for benthic bivalves, from which benthic diatoms are an important diet component of this mollusks

The presence of oocytes in the guts of *A. tuberculosa* was likely due to accidental ingestion after being expelled, as reported for *Crassostrea virginica* during the spawning season (Moreno-RuÍz *et al.,* 1994). This suggests that, although there is some extent of selectivity of ingested particles mainly in pectinid clams (Sakai 1979) this is seemingly not very strict. The work on *Spisula solidisima* in the Gulf of Maine by Shumway *et al.* (1994), and the review on a number of gut content studies in pectinids (Shumway ***et al.,*** 1987), show no evidence of particle selection based on size or type, and seems to confirm our point. On the other hand, studies about digestive enzyme activity show that bivalves possess the capability to ingest detritus (Wojtowicz, 1972). However, there are scarce references on the role of detritus as food source in the studies on bivalve gut content.

According to our findings we conclude that, for this bivalve and probably for other marine mollusks, histological sections represent a new alternative for studying the gut and stomach content, which allow the accurate identification of ingested diatoms. This method provides a wide spectrum of microalgae filtered by the bivalves. In order to have a better understanding of the food habits in these organism histological cuts may be done in the gut and stomach.

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