

***Mercenaria mercenaria* (Linn.): Gonadal Tissue Response to Man-Induced Environmental Changes in Cataño Lagoon, Puerto Rico**

V. JUSTE and R. CORTÉS
Department of Marine Sciences
University of Puerto Rico
P.O. Box 5000
Mayaguez, Puerto Rico 00709-5000

ABSTRACT

This study was conducted in Cataño Lagoon, on the north coast of Puerto Rico. The study area is polluted with industrial and domestic discharges.

Mercenaria mercenaria were collected in October, 1985 and April, 1986 with hand shovels. Clams were manipulated to induce spawning but no results were obtained. Histology was done to determine if *M. mercenaria* was following its reproductive cycle normally. Analysis of gonads indicate abnormal development of germ cells concomitant with leukocytic infiltrations. Also, a generalized hemocytic response was observed in the vesicular connective tissues. The authors believe the abnormal gametogenesis observed in sampled clams from Cataño Lagoon may be due to the toxic effects of a PCB 1260 discharge which occurred one month before the study, or due to synergistic or additive actions of disease, other pollutants, and PCBs. Control clams were collected from Torrecilla Lagoon, on the north coast of Puerto Rico, and treated in the same manner for comparison to Cataño Lagoon clams.

INTRODUCTION

The bivalve mollusc *Mercenaria mercenaria* was found in various localities of Puerto Rico (Juste and Cortés, 1991; Juste, 1987). Its presence in the Caribbean established an ecological record of the distribution for the species. The population of *M. mercenaria* found in Cataño Lagoon, which was chosen for this study, has been significantly reduced by overfishing as well as by high mortalities (Juste, 1987). The study area is a semi-enclosed lagoon, located inside of San Juan Bay, on the north coast of Puerto Rico. It is surrounded by dredged deposits. Its water is brownish in color, and contains a high content of particulate matter. Its average depth is two meters. The maximum temperature and salinity recorded in a previous study (Juste, 1987) at the location were 33°C and 31 ppt, respectively. The sediment in this area is sandy on the shoreline changing to muddy towards the center, with a strong smell of hydrogen sulphide (Juste and Cortés, 1991; Juste, 1987).

The control population chosen for this study is located in Torrecilla Lagoon, on the north coast of Puerto Rico, between Isla Verde and Loiza. It is surrounded by mangroves, and communicates directly with the open ocean. The

sediment is soft, even though it is primarily composed of fine sand (Juste and Cortés, 1991).

The presence of polychlorinated biphenyls in the marine environment and in individual marine organisms has been documented by different authors (Zitko, 1971; Jensen *et al.*, 1969). In oysters from Escambia Bay, Florida, it was found after exposure to PCB, shell growth in young organisms was significantly inhibited (Duke *et al.*, 1970). Exposure to PCBs done under laboratory conditions caused atrophy of digestive diverticular epithelium and degeneration of vesicular connective tissue with leukocytic infiltration in *Crassostrea virginica* (Lowe *et al.*, 1972).

The purpose of this study was to determine if *Mercenaria mercenaria* was following its reproductive cycle normally in Cataño Lagoon, after being exposed to such a carcinogenic contaminant as PCB 1260, and to compare those animals to a control population from an area not exposed to contaminants.

METHODS AND MATERIALS

This study was conducted in Cataño Lagoon, on the north coast of Puerto Rico. Clams were collected in October, 1985 and April, 1986 with hand-shovels. The sediment extracted was passed through a metal sieve (1 cm mesh) in order to facilitate the collection of clams. Clams were placed in previously marked plastic bags, containing sufficient water to maintain moisture. The bags were sealed and placed in a cooler with ice for their transportation to the laboratory.

Once in the lab, the clams collected in October, 1985, were placed in an aquarium, at a controlled temperature of 26°C. For two weeks, temperature was increased gradually to induce spawning. No results were obtained. The clams were then placed in trays, one by one, inside a thermal bath. Controlled variations of temperature were carried out (from 20°C to 28°C and vice versa) to induce spawning, for a total of three weeks. No results were obtained. Sperm was extracted from control clams with a syringe and injected into the trays containing females from Cataño Lagoon. The females did not react to this stimulus either. Clams were then dissected to estimate gonadal development.

In April, 1986, new specimens from Cataño Lagoon were collected for histological analysis only, in order to determine the state of gonadal development. Clams were placed inside a concrete tank with continuous flow for a week, and then dissected. Control clams were collected from Torrecilla Lagoon in the same manner and processed for histological analysis.

The method used for histology (Gray, 1964) consisted of four parts. The first was dissection, which was performed by making two transversal incisions on the body mass to extract the central section of the clams, where the gonads are located. Secondly, gonad tissues were cleaned and fixed in Bouin's solution for 24 hours, then washed with 70% ethanol. Tissues were embedded in paraffin, cut at ten microns, and mounted on albuminized slides. The staining

routine used was Harris' hematoxylin and Eosin (HHE₂) regressive stain (Howard and Smith, 1983). All material analyzed was photographed using an Olympus BH-2 microscope with a CANON AE-I camera.

RESULTS

During the samplings in Cataño Lagoon, no *Mercenaria mercenaria* juveniles were found. The population density of four clams/m², reported in 1980 (J.G. González, pers. comm.), has declined to less than 1/m² (Juste, 1987). All efforts to induce spawning in *M. mercenaria* specimens, collected in October, 1985, were unsuccessful. The dissections subsequently done on those specimens did not show any gonadal development.

Females collected in April, 1986, when dissected, presented differences in their gonadal development. Figure 1. I shows a female with poor gonadal development. There are cells undergoing meiosis, and primary oocytes are clearly distinguishable along the follicle walls, which are thick in appearance (A). The follicles are semi-contracted, and in some, non-sexual cells are present inside the lumen cavity (B). Figure 1. II shows a female with less advanced gonadal development. Cells are undergoing meiosis (A), and are clustered on the internal wall of the follicles. The nuclear membrane is pronounced. The follicles are also semi-contracted. This type of development is not normally found in *Mercenaria mercenaria* at this time of the year. Figure 2.I shows a normal female from Torrecilla Lagoon. Follicles are expanded, and the follicle walls are thin. Many oocytes are free in the lumen of the follicles.

Another type of development found in specimens dissected in April is shown in Figure 1. III. In some follicles there are some well developed but immature eggs (A). There are also less developed secondary oocytes (B) still adhered to the wall. Some follicles contain primary oocytes (C), and some sexual cells undergoing meiosis (D).

Abnormalities in the gonadal development of the males were found in all the specimens collected in April, 1986. Figure 1. IV illustrates a male with mature sperm (A), but the number is very small compared to the control male (Figure 2. II). The control males from Torrecilla Lagoon showed the presence of spermatocytes and spermatids in the follicles, with more than 50% of the follicles containing spermatozoa, and they were all in the late developmental stage.

Closer observation demonstrates tissue irregularities. In all males and females from Torrecilla Lagoon, the normal compact vesicular pattern was lost, and vesicular cells were replaced by a heavy infiltration of leukocytes. The degenerative changes of these tissues were very extensive in most clams. Figure 1. V illustrates a heavy infiltration in the vesicular connective tissue, with apparent degeneration of the tissue. Figure 3 shows the normal compact pattern of the connective tissue found in control clams from Torrecilla Lagoon.

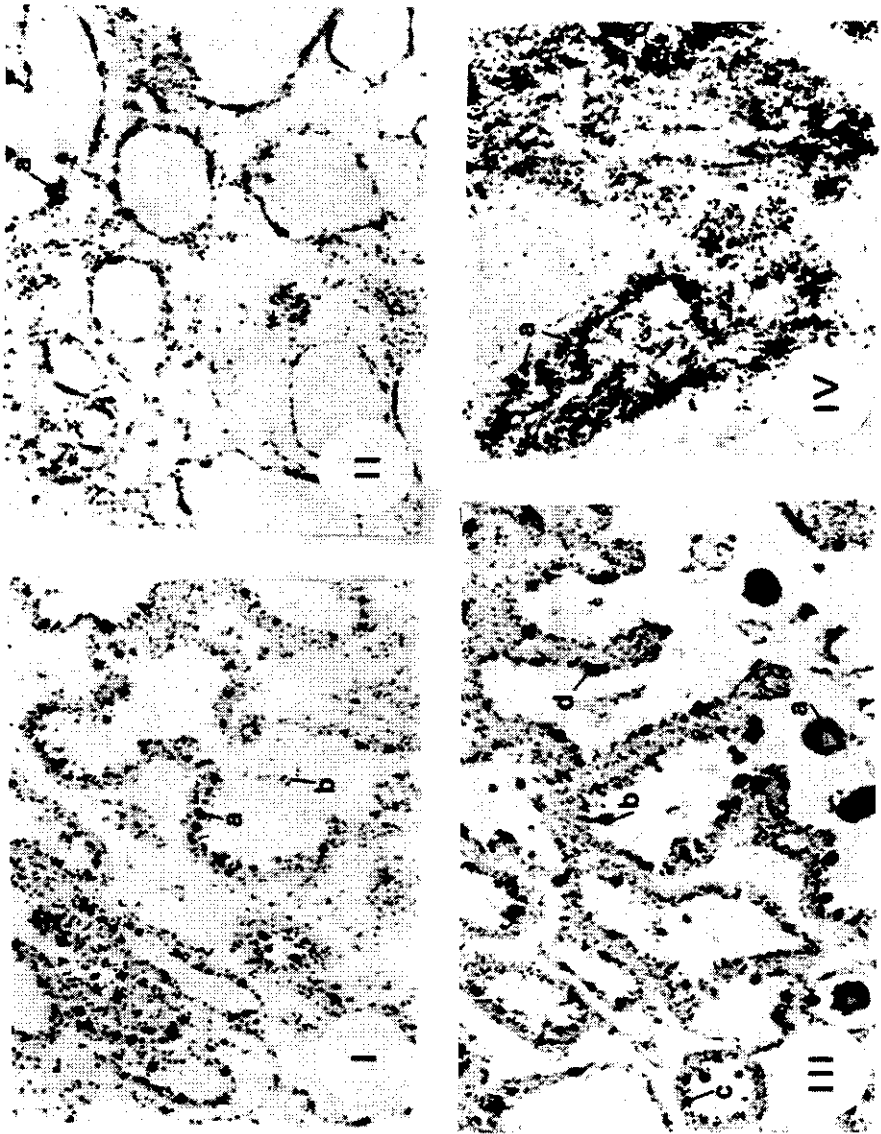


Figure 1. *Mercenaria mercenaria* from Cataño Lagoon. I: Section of a female clam showing primary oocytes along the follicle walls (A), and non-sexual cells inside the lumen (B) (100X); II: female undergoing meiosis (A), less advanced gonadal development (100X); III: section of a female showing some developed but immature eggs (A), and secondary oocytes (B) still adhered to the follicle walls. Primary oocytes (C) and some sexual cells undergoing meiosis (D) also present (100X); IV: section of a male clam with mature sperm (100X).

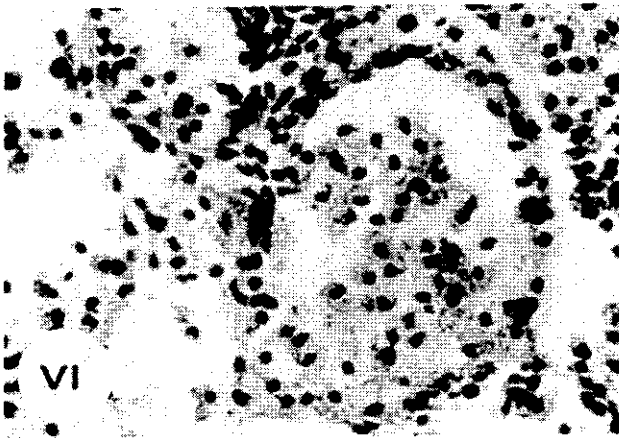
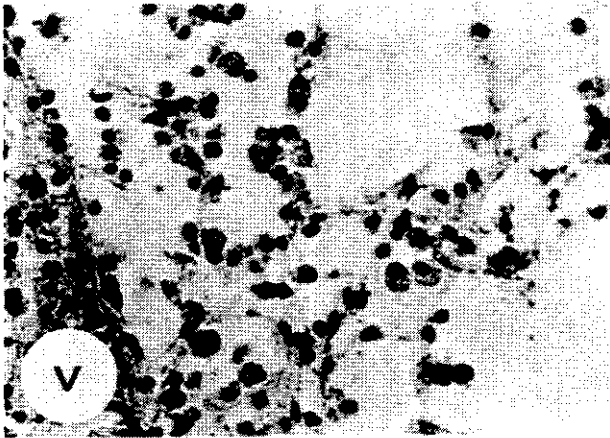


Figure 1. V: detail of heavy infiltration of leukocytes in the vesicular connective tissue, showing tissue degeneration. Note the absence of the normal compact pattern of the connective tissue (400X); VI: detail of heavy infiltration of leukocytes inside the gonads (400X).

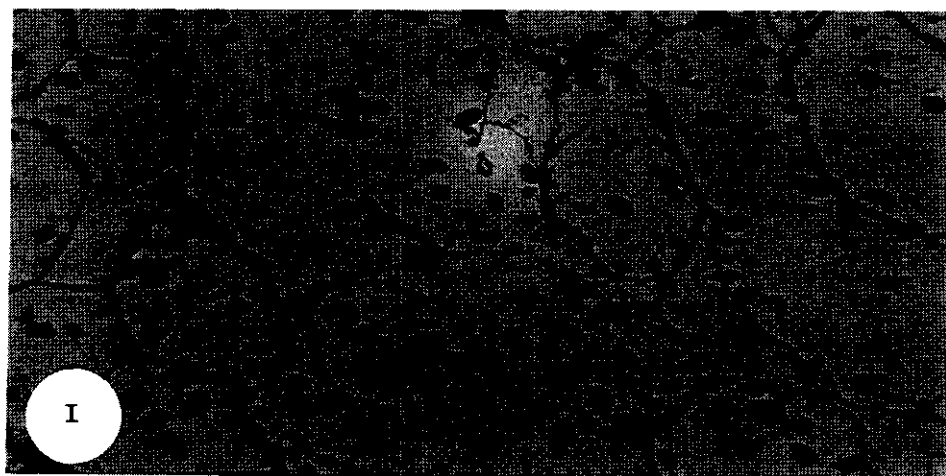


Figure 2. *Mercenaria mercenaria* from Torrecilla Lagoon. I: Section of a female clam showing follicles expanding, many oocytes free in the lumen, and some still attached to the follicle walls (400X); II: Section of a male clam in the late developmental stage, showing spermatids and spermatocytes in the follicles; more than 50% of the follicles contain spermatozoa (400X).

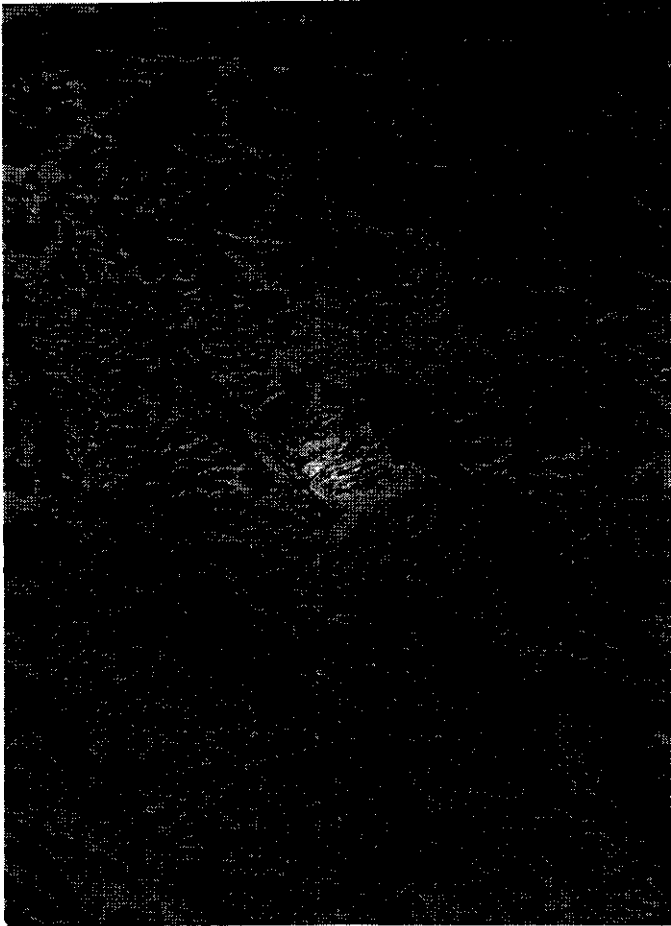


Figure 3. Section of normal connective tissue of a control clam from Torrecilla Lagoon. Note the compact pattern of the connective tissue (400X).

Infiltration of Leukocytes in the gonads (Figure 1. VI) in females from Cataño Lagoon was also found. In some follicles there are no sexual cells present, only high numbers of leukocytes.

DISCUSSION

Histological analysis of the gonads of *Mercenaria mercenaria* from Cataño Lagoon suggests that there is a factor interfering with their development. According to Loosanoff (1937 b), females of this species (in temperate waters) contain a great number of large oocytes (66 - 68 microns) in April. They also have smaller oocytes which should continue their development during May. In Delaware Bay, spawning begins at the beginning of June, and continues until October, with two major peaks occurring in August and September (Keck *et al.*, 1975). The population of *Mercenaria mercenaria* from Core Sound (North Carolina) had the same reproductive peaks as the Delaware populations (Porter, 1964). In Clark Sound, South Carolina, the spawning period begins in May, and has a major peak in October (Manzi *et al.*, 1985). In Wassaw Sound, Georgia, clams of this species exhibit a continuous gametogenic cycle, with three major peaks in spring, fall, and winter (Heffernan *et al.*, 1988). In the Indian River Lagoon, Florida, spawning of *M. mercenaria* is bimodal, with peak periods occurring from September to December and from March to June (Hesselman *et al.*, 1989). In Puerto Rico, there are two major peaks, occurring in May and October (Juste, unpubl. data). Clams from Cataño Lagoon did not show the kind of reproductive development needed to follow a normal cycle. The control clams from Torrecilla Lagoon did show the late developmental stage in the gonads, which would have allowed them to spawn in May.

In some specimens from Cataño Lagoon, large oocytes were found, but were few. The majority of the female clams showed immature gametes, primary oocytes, and great infiltration of leukocytes in the gonads and the connective tissue. Males showed mature sperm, but were few in number compared to the control clams, and tissues were greatly infiltrated with leukocytes. The clams from Cataño Lagoon could have spawned earlier in the season than the ones from Torrecilla Lagoon, but the histological evidence of leukocyte infiltration, and tissue degeneration, suggest that some factor is inhibiting the normal reproduction of the former. Also, Cataño and Torrecilla Lagoons are close together and similar in conditions, so habitat/climate differences were ruled out as a possible source.

Another possible explanation for these irregularities could be stress suffered by the clams during their transportation and acclimation to the laboratory. However, control clams from Torrecilla Lagoon were exposed to the same conditions, and did not show the same symptoms, such as infiltration of leukocytes. Starvation was ruled out as a cause, as clams were fed *Isochrysis galvana*, in addition to the natural food in the continuously flushing water.

The absence of juvenile clams in Cataño Lagoon strongly suggests abnormalities in the population dynamics. This area is subjected to pollution by sewage and industrial wastes. On September 8 (1985), a month before the first sampling was done in Cataño, there was an oil spill contaminated with Polychlorinated Biphenyl (PCB) 1260 from an exploded transformer, belonging to the Electrical Company of Palo Seco. The areas affected were Palo Seco, San Juan Bay, and Cataño Lagoon (Junta de Calidad Ambiental, pers. comm.). No studies of the ecological impact on the fauna and flora were made by any of the agencies in charge of pollution control in the environment. However, the histological analysis of the clams, collected after the spill, strongly suggests that the irregularities in reproduction, and the heavy infiltration of leukocytes could have been induced by the spill. Clams from Cataño Lagoon showed the same symptoms as *Crassostrea virginica* when exposed to PCB 1254 (Lowe *et al.*, 1972). These symptoms are characteristic of organisms that have been exposed to pesticides and other toxic agents.

The presence of bacteria (coccos) in the tissues of *Mercenaria mercenaria* from Cataño Lagoon was also observed. This cannot be appreciated in the photographs due to the refractive index. The control clams from Torrecilla Lagoon did not show presence of bacteria. The presence of these bacteria are probably due to sewage discharges in the area of Cataño. Therefore, possible synergistic or additive actions of disease, other pollutants, and PCBs might have been the cause of the irregularities found in the tissues and reproductive cycle of *Mercenaria mercenaria* from Cataño Lagoon.

ACKNOWLEDGEMENTS

The authors would like to thank Shawna Reed and Peter Rocafort for the preparation of the photographs and slides for this presentation. This work was supported by the Department of Marine Sciences of the University of Puerto Rico.

LITERATURE CITED

- Duke, T. W., J.I. Lowe and A.J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254) in the water, sediment and biota of Escambia Bay, Florida. *Bull. Envir. Contam. Toxicol.* 5: 171-180.
- Gray, P. 1964. *Handbook of Basic Microtechniques*. 3rd Ed. MacGraw-Hill Book Company, N.Y., 302 pp.
- Hefferman, P.B., R.L. Walker, and J.L. Carr. 1988. The reproductive cycle of the hard clam, *Mercenaria mercenaria*, in Wassau Sound, Georgia. *J. Shellfish Res.* 8(1): 51-61.
- Hesselman, D.M., B.J. Barber, and N.J. Blake. 1989. The reproductive cycle of adult clams, *Mercenaria* spp., in the Indian River, Florida. *J. Shellfish Res.* 8(1): 43-49.

- Howard, D.W. and C.S. Smith. 1983. Histological Techniques for Marine Bivalve Mollusks. U.S. Dept. of Comm., National Ocean and Atmospheric Adm., Woods Hole, Mass.; NOAA Tech. Memor. NMFS-F/NEC-25.
- Juste, V. 1987. Estudio preliminar sobre la viabilidad del cultivo de la almeja *Mercenaria mercenaria* en el suroeste de Puerto Rico. M. Sc. Thesis, University of Puerto Rico, 156 pp
- Juste, V. and R. Cortés. 1990. Distribution and biological aspects of the hard clam *Mercenaria mercenaria* (Linnaeus), *M. mercenaria notata* (Say), and *M. campechiensis* (Gmelin) in Puerto Rico. *Caribb. J. of Sci.* 26 (3-4): 136-140.
- Keck, R. T., D. Maurer and H. Lind. 1975. A comparative study of the hard clam gonad development cycle. *Biol. Bull.* 148: 243-258.
- Loosanoff, V. L. 1976. Spawning of *Venus mercenaria* Linnaeus. *Ecology*. 18: 506-515.
- Lowe, J. I., P.R. Parrish, J.M. Patrick and J. Forester. 1972. Effects of the Polychlorinated Biphenyl Aroclor 1254 on the American oyster *Crassostrea virginica*. *Marine Biol.* 17: 209-214.
- Manzi, J.J., M.Y. Bobo, and V.G. Burrell. 1985. Gametogenesis in a population of the hard clam *Mercenaria mercenaria* (L.) in the North Santee Bay, South Carolina. *Veliger*. 28(2): 186-194.