

Parasites as possible cause of mass mortalities of the critically endangered clam *Mesodesma mactroides* on the Atlantic coast of Argentina

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Abstract

A survey of the health status of the yellow clam *Mesodesma mactroides* was performed to contribute to establish the causes of mass mortality episodes. A sample of 32 clams was collected from Isla del Jabalí (40°32'S- 62°21'W, Buenos Aires province, Argentina), a relict population by 1999, that two years later suffered a mass mortality. Microscopical examination of the histological sections revealed the presence of four parasitic or commensal taxa: *Trichodina* sp. ciliates, coccidian, gregarines and turbellaria. Specimens of *Trichodina* sp. were found on the gills of 58% of clams with moderate intensities without apparent cell damage. Coccidian were present in the nephridial tubules with both high prevalence (93%) and intensity of infection. Macrogamonts and meronts containing merozoites were the more frequently observed stages, obtruding the lumen of the nephridial tubules and associated with hypertrophying epithelial cells. Gregarines were observed inside the digestive epithelial cells and in the adjacent connective tissue in high intensities, with a prevalence of 64%. They were present alone or in groups of several individuals, in which case the epithelial cells were lysed. Turbellaria specimens were found inhabiting the lumen of the digestive tract, parasitising 30% of examined clams. Additionally, histological preparations from 36 dying clams from Monte Hermoso (40°32'S- 62°21'W, Buenos Aires province, Argentina, collected during mass mortality episode of November 1995) were re-examined. Despite the bad-condition of tissues, the same parasites found in Isla del Jabalí were observed, with the exception of gregarines: *Trichodina* sp. (100%), coccidians (100% from the five histological sections which included nephridia), turbellaria (14%). To support the hypothesis of a diseases-caused epidemic it is necessary to examine samples from other clam populations and to examine for virus infection.

Introduction

The yellow clam, *Mesodesma mactroides* (Mesodesmatidae), is distributed on sandy beaches from the southern part of Brazil to the southern part of the Buenos Aires province in Argentina (Castellanos, 1970). The yellow clam is one of the most studied bivalves in Argentina, because it is the dominant or-

ganism of mesolittoral communities and constitutes an important economic resource (e.g., Christiansen 1971, Bastida et al., 1991, Defeo et al., 1992, Castilla & Defeo 2001). Its commercial harvesting was prohibited in 1958 after the population drastically declined due to overexploitation (Coscarón 1959). The populations never recovered completely



probably due to the impact of the tourism activity and illegal exploitation (Bastida et al., 1991).

Since the first mass mortality occurred in 1993 in Brazil (Odebrecht et al., 1995), successive episodes were reported from north to south, reaching Uruguay and Argentina (Fiori & Cazzaniga, 1999). Mortalities covered the entire area of distribution of the yellow clam in about three years (Fiori & Cazzaniga, 1999). By 1999, the only population with high clam density was Isla del Jabalí (F. Cremonte, pers. obs.), located in the southern limit of its distribution, but it suffered a mass mortality two years later (S. Fiori, pers. comm.). At present, *M. mactroides* could be considered "critically endangered" (Fiori & Cazzaniga, 1999).

Fiori and Cazzaniga (1999) studied a massive mortality event occurring in November 1995 that eliminated a population in about 10 days. This episode was in Monte Hermoso (Buenos Aires province, Argentina), a beach known to have high clam density. In this study, simultaneous samples of sediments, seawater, and clams did not reveal the probable cause of this phenomenon; even protozoan tissue-parasite analyses gave negative results. These authors only registered unidentified protozoans on gills, without mentioning the number of examined specimens and how they were processed (Fiori & Cazzaniga, 1999).

The only report of parasites or commensals previous to these massive mortalities is that by Coscarón (1959). This author mentioned the presence of two unidentified peritrichian ciliates and a rhabdocoelan turbellaria from the branchial cavity of the yellow clam.

The aim of this work was to perform a survey to assess the health status of the yellow clam *M. mactroides* to contribute to establishing the causes of the mass mortality episodes.

Materials and methods

In December 1999, 32 specimens of *M. mactroides* were collected manually during low tide in Isla del Jabalí (40°32'S, 62°21'W), Buenos Aires province, Argentina. Clams were maintained in an aquarium with aerated seawater for approximately 24 hours until their processing. The maximum length of each individual was measured with a calliper. The soft parts of these clam specimens were carefully removed from their shells and fixed in Davidson's solution (Shaw & Battle, 1957) for 24 hours. Oblique transverse sections, approximately 5 mm thick, were taken from each specimen including mantle, gills, gonad, digestive gland, nephridia and foot. Tissue samples were embedded in paraffin and 5 µm sections were stained with haematoxylin and eosin. Histological sections were examined under a light microscopy for the presence of parasites and pathological alterations. Sexes were recorded. The intensity of protozoan infections was estimated by using the following relative scale: 1= light, 2 = moderate, and 3 = heavy and the intensity of metazoans parasites by counting the number of individuals per histological section.

Additionally, those histological preparations studied by M. Kroeck that allowed to determine the health condition of *M. mactroides* from Monte Hermoso (38° 59'S, 61° 41'W) population (Fiori & Cazzaniga 1999), were re-examined. Those clams collected were either dead or moribund in November 1995, during

a mortality episode and fixed in 10% formaline (S. Fiori, pers. comm.).

Histological sections of parasitised clams from Isla del Jabalí were deposited in the Protozoological Collection of the Museo de La Plata (MLP015 to MLP017), Museo de La Plata, La Plata, Argentina, and some bi-valves of the examined clams in the Malacological Collection (MLP6492) of the same institution.

Results

The mean of the maximum shell length of the clam sample from Isla del Jabalí was 65.56 mm, varying from 56 to 78 mm. The 48 % of individuals were male and the 52 % females. Microscopical examination of these histological sections revealed the presence of four parasitic or commensal taxa: *Trichodina* sp. ciliates, coccidian, gregarines and turbellaria. Ciliates belonging to the genera *Trichodina* were found on the gills of the 58% of clams with moderate intensities (1.94 of mean intensity), without apparent cell damage (Figure 1). Unidentified coccidian were present in nephridial tubules with both high prevalence and intensity of infection (93% and 2 respectively). Macrogamonts and meronts containing merozoites were the more frequently observed stages. They were hypertrophying epithelial cells and protruding the lumen of the nephridial tubules (Figures 2 and 3). Gregarines were found inside the digestive epithelial cells and in the adjacent connective tissue (Figure 4). The prevalence was 64 % and the estimated mean intensity 1.64. They were present alone or forming groups of several individuals, in which case the epithelial cells were lysed. Turbellaria were the only metazoan present in the sample. Specimens were

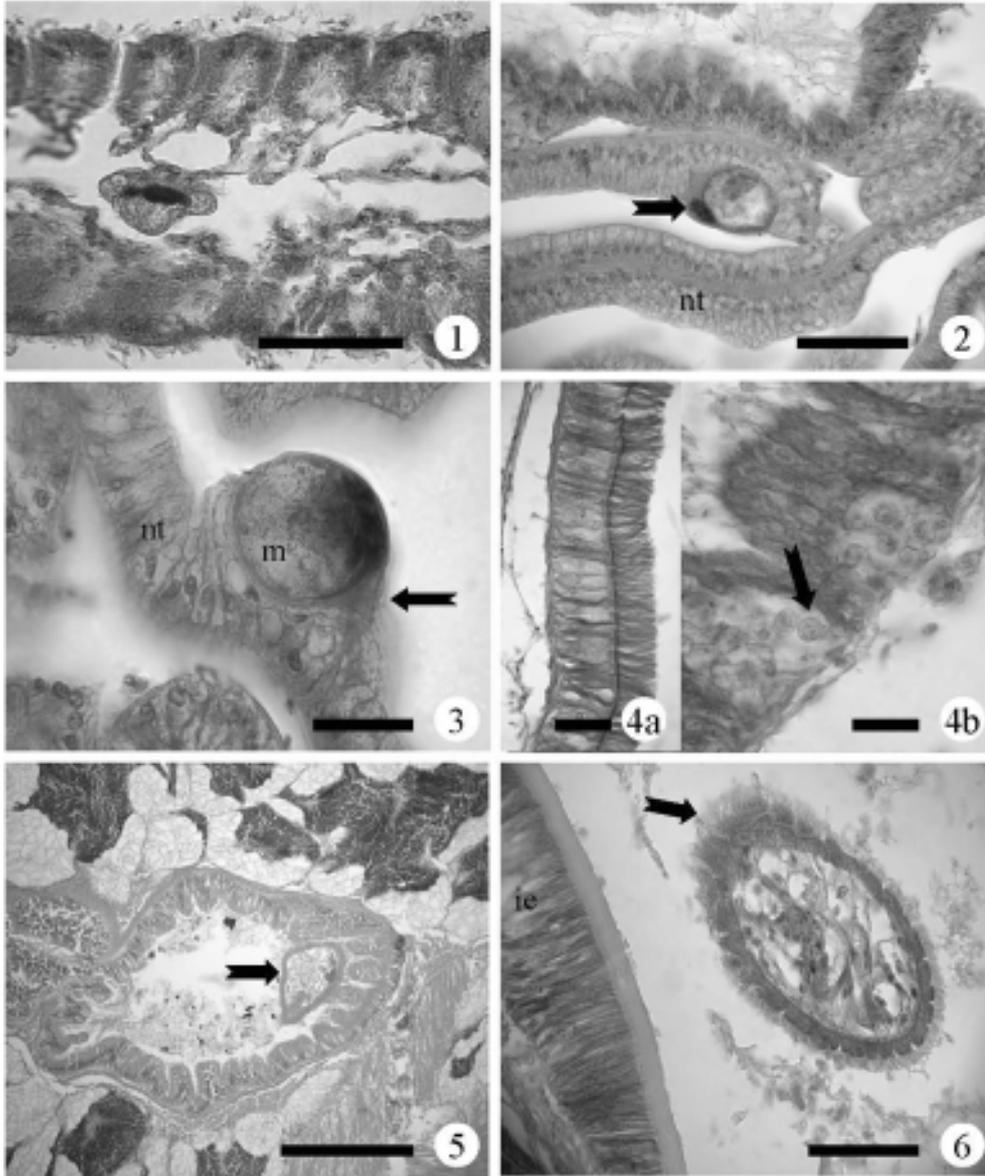
found inhabiting the digestive tract lumen (Figures 5 and 6). Thirty % of the clams were parasitised, with 1 to 8 individuals (mean intensity 2.63).

Despite the bad condition of tissues from Monte Hermoso specimens, their re-examination revealed the presence of the same parasites or commensal taxa found in Isla del Jabalí population, with the exception of gregarines. Thus, *Trichodina* sp. was in 100% of the clams, generally with moderate to high intensities, coccidians were found in 100% of the histological section, which included nephridia (only in five specimens), and turbellaria were seen in 14% of the cases.

Discussion

Mortality causes of *M. mactroides* still remain unknown both in Argentina and in other countries (Fiori & Cazzaniga, 1999). However, it was neglected to investigate infectious diseases as a cause of these mortalities. Two of the four parasitic or commensal taxa registered in *M. mactroides* during the present study were previously recorded. Coscarón (1959) found in Buenos Aires province (Argentina) two undetermined ciliates, one of them looks like *Trichodina* sp. from the photograph (page 56), while the other was not observed in present specimens. Also belonging to the genera *Trichodina*, were those specimens mentioned by Fiori and Cazzaniga (1999) in Monte Hermoso (Buenos Aires province, Argentina).

Parasites in the badly preserved tissues from dying Monte Hermoso specimens could be detected only because they were seen first in specimens from Isla del Jabalí. The same parasites (excepting gregarines which were absent



Figures 1 to 6. Parasites of *Mesodesma mactroides* from Buenos Aires province, Argentina (H & E stain). **1.** *Trichodina* sp. ciliate on the gills, scale bar = 100 μ m. **2.** Unidentified coccidian in the nephridia tubules (nt), arrow indicates the displaced nuclei of the host cell, scale bar = 100 μ m. **3.** Detail of a macrogamont (m) of coccidian hypertrophying the epithelial cell of nephridia tubule (arrow), scale bar = 50 μ m. **4.a.** Unparasitised intestinal epithelia, scale bar = 200 μ m. **4.b.** Groups of gregarines (arrow) in the intestinal epithelial cells, note the lyses of the host cells, scale bar = 150 μ m. **5.** Turbellaria (arrow) in the intestine lumen, scale bar = 100 μ m. **6.** Detail of the turbellaria showing the ciliated epithelia (arrow) and undamaged intestinal epithelia (ie), scale bar = 20 μ m.

in Monte Hermoso sample) were detected in both populations, but with different prevalences. However, because gregarines were found parasitising digestive epithelia, and these tissues are the first to undergo autolyses, it is possible that they were lysed together with host tissues.

Prevalence and intensity of infection may vary with season and locality. The high prevalence of *Trichodina* from Monte Hermoso as compared to Isla del Jabalí (100% vs 58 %) could be because clams from the first mentioned locality were dying at the time of sampling. Most trichodines are bacterivorous and capable of ingesting bacteria present on bivalve gills (Lom 1995), thus, it is possible that in dying clams with more bacteria in branchia, trichodines could be more prevalent. While some controversy surrounds the pathogenic role of *Trichodina*, it is generally believed to cause large-scale mortality in many marine animals (Lauckner 1983). Although prevalences of *Trichodina* sp. can reach 100% in some clam populations, most infections are innocuous. Intensity of infection is usually low with only few ciliates attached to the gill surface. A heavy infestation with this ectoparasite can interfere with the respiratory function of the clam as a result of excessive mucus production (Lom, 1995). In this sense, mortalities attributed to *Trichodina* sp. have been reported in oysters from France (Boussaïd et al., 1999). Heavy infections in *Cerastoderma edule* less than one year old were associated with emaciation and mortalities in the German and Dutch Wadden Sea (Bower et al., 1994).

Heavy infections of coccidian parasites may cause kidney damage but associated

mortalities appear restricted to artificial growing conditions (Bower et al., 1994). An unidentified coccidian species has been observed to cause severe necrosis and chronic accumulation of haemocytes containing brownish pigment in *Scrobicularia plana* (Bower et al., 1994). Coccidian parasites vary in prevalence and intensity of infection but in the present case prevalence seems to be unusually high.

Turbellaria belonging to the order Rhabdozoela and Allocoela associate intimately with marine molluscs (Lauckner, 1983). Although they are sometimes considered commensals and on other occasions as parasites, they have to be regarded as harmless (Bower et al., 1994) as it was observed in *M. mactroides*.

It is not certain that the heavy infestations by gregarines and coccidians were responsible for the clams mortality in the present case. Usually the causes and mechanisms of mass mortalities in natural populations are difficult to ascertain, and they are probably due to a combination of factors. The population of Isla del Jabalí suffered mass mortality two years after the sampling for the present study, thus, the presence of parasites reported here would not be the only reason for mortality. To support the hypothesis of a disease etiology it is necessary to examine samples from other clam populations, and to examine for other microbiological agents.

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