

Effect of low salinity on the yellow clam *Mesodesma mactroides*

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(With 4 figures)

Abstract

The aim of this study was to determine the lethal salinity (LC₅₀) for the yellow clam *Mesodesma mactroides* (Bivalvia: Mesodesmatidae) and identify histopathological alterations that could be used to diagnose structural changes in clam tissue. Clams in two size classes (adults and juveniles) were placed in 10 L chambers and exposed to salinities of 35, 30, 25, 20, 15, 10, and 5 g/L. There were triplicate chambers with seven clams each for each salinity. The LC₅₀ values for a 48 h exposure were 6.5 g/L and 5.7 g/L for adults and juveniles, respectively. For a 96 h exposure, the LC₅₀ values were 10.5 g/L for adults and 8.8 g/L for juveniles. The histological examination of yellow clams exposed to 10 g/L for 96 h showed intercellular oedema and necrotic foci in the epithelium of the digestive gland and occlusion of the lumen of the digestive gland. In conclusion, *M. mactroides* can be characterised as a moderately euryhaline species, tolerating salinities from 35 to 15 g/L.

Keywords: yellow clam, lethal salinity, LC₅₀, histology, extreme southern Brazil.

Efeito da salinidade reduzida no marisco branco *Mesodesma mactroides*

Resumo

O objetivo deste estudo foi determinar a salinidade letal (CL₅₀) para o marisco branco *Mesodesma mactroides* (Bivalvia: Mesodesmatidae) e as alterações histopatológicas que poderiam ser úteis para o diagnóstico de mudanças estruturais no tecido dos bivalves. Mariscos de duas classes etárias de tamanha (juvenis e adultos) foram colocados em recipientes de 10 L e expostos a salinidades de 35, 30, 25, 20, 15, 10 e 5 g/L. Os tratamentos foram realizados em triplicata com sete bivalves em cada recipiente, a CL₅₀ para 48 h de exposição foi 6,5 g/L e 5,7 g/L para adultos e juvenis, respectivamente. Para 96 h de exposição, a CL₅₀ foi 10,5 g/L para adultos e 8,8 g/L para juvenis. O exame histológico dos mariscos expostos à salinidade de 10 g/L por 96 h revelou edema intracelular e focos necróticos no epitélio da glândula digestiva e oclusão da luz da glândula digestiva. Em conclusão, *M. mactroides* pode ser considerada uma espécie eurialina moderada, tolerando salinidades de 35 até 15 g/L.

Palavras-chave: marisco branco, salinidade letal, CL₅₀, histologia, extremo sul do Brasil.

1. Introduction

The yellow clam *Mesodesma mactroides* (Deshayes, 1854) is an intertidal sandy beach bivalve that is distributed along the Atlantic coast of South America from Brazil to Argentina (Rios, 2009). Historically, *M. mactroides* had been considered an important economic resource that is commercially exploited by fishermen using shovels in Brazil, Uruguay, and Argentina (Coscarón, 1959; Gianuca, 1985; Bergonci and Thomé, 2008). However, yellow clam populations collapsed as a result of overfishing, which was associated with cyclic mass mortalities due to unknown causes (Odebrecht et al., 1995; Fiori and Cazzaniga, 1999; Cremonte and Figueras, 2004). Some of the largest numbers of mortalities occurred near the influence of the Patos Lagoon and River Plate, which can affect the salinity of the coastal zone in extreme situations. *M. mactroides*

has been identified as a threatened species with a critically endangered status (Herrmann et al., 2011).

According to Manzi and Castagna (1989), the salinity tolerance range of the species is one of the most fundamental biological information required for assessing its environmental suitability for culture purposes. Studies claim that reduced salinities in regions near freshwater streams or rivers are unfavourable environments for the development of *M. mactroides* (Olivier et al., 1971; Defeo et al., 1992; Marins and Levy, 2000). However, these studies did not determine the minimum tolerable levels of salinity for the yellow clam. Therefore, the objective of this study was to investigate the influence of salinity on *M. mactroides* survival. The lethal effects of salinity were examined in juvenile and adult clams in the laboratory through short-

term exposure bioassays. In addition, histological changes that could be used to diagnose the exposure of yellow clams to low salinities were analysed.

2. Material and Methods

Experiments to determine the lethal effects of salinity were carried out with different-sized *M. mactroides* clams at the Marine Aquaculture Station of the Federal University of Rio Grande-FURG, Southern Brazil. The experiments were carried out during summer–early autumn 2012, and the clams were obtained from Cassino Beach (Figure 1) (latitude 32° 24' S and longitude 52° 20' W). Experimental procedures were based on standard methods for static acute bioassays with aquatic invertebrates (Rand and Petrocelli, 1985).

The clams were acclimated to laboratory conditions for one week. Juveniles (with no functional gonads, mean shell length = 29.4 ± 2.9 mm) and adults (with functional gonads, mean shell length = 62.2 ± 2.8 mm) were maintained at ambient temperature (25 °C) in 200 L tanks with aerated seawater (35 g/L) without sand. The clams were fed daily with 1 L of *Nannochloropsis oculata*. Only clams showing

healthy signs and normal behaviour (normal shell gape and protrusion of siphons and foot) were used in the bioassays.

The clams of two size classes were placed in 10 L tanks at 25 °C. For each treatment, 21 juvenile clams (7 for each replica) were exposed to salinities of 35, 30, 25, 20, 15, 10, and 5 g/L. The same procedure was performed for adult clams. The different salinities were obtained by diluting seawater (35 g/L) with fresh water. The clams were not fed during the experiment. Mortality was recorded at 12, 24, 36, 48, 72, and 96 h. Test subjects with a permanent wide valve gape with extended siphons and a foot that was not responsive to touch were considered dead.

For each treatment, the percentage survival of clams was plotted against the exposure period. The acute lethal effects of low salinities on different-sized clams were analysed by determining the median lethal concentration (LC_{50}), which represents the salinity estimated to cause 50% mortality of a test population over a specific period (Rand and Petrocelli, 1985). A trimmed Spearman Karber was used to calculate the LC_{50} for each exposure time.

To analyse possible histological changes resulting from osmotic stress, clams that survived at the end of the experiment were fixed in Davidson's solution (Shaw and

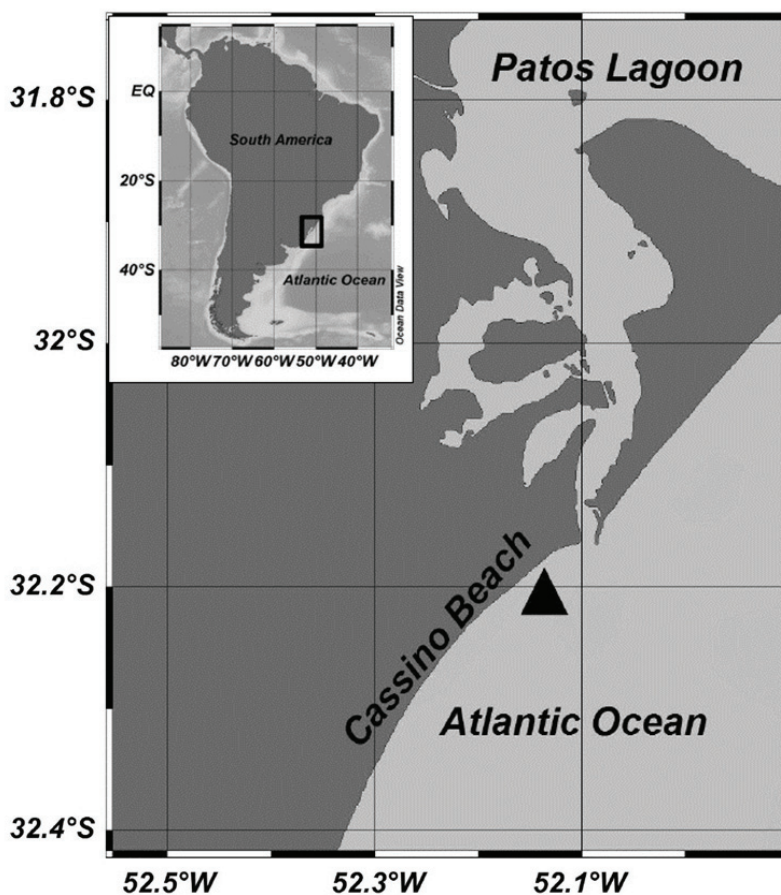


Figure 1. Map of South America depicting Rio Grande do Sul State in southern Brazil. The star indicates the sampling location of *Mesodesma mactroides* in Cassino Beach.

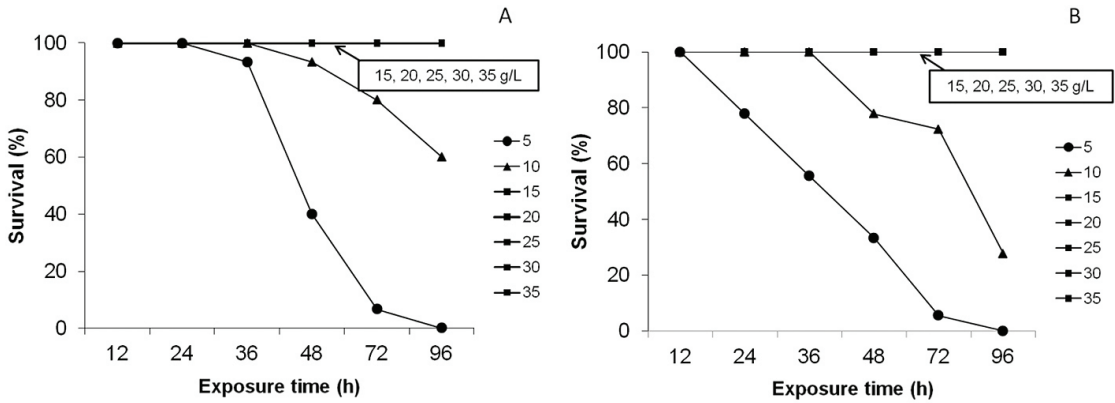


Figure 2. Percentage survival of *Mesodesma mactroides* exposed to different salinities (g/L) at an ambient temperature of 25 °C. (A) juveniles, (B) adults.

Battle, 1957). Tissue samples (especially the digestive gland, which appeared sensitive to low salinity exposure in our preliminary experiments) were embedded in Paraplast®, and 5 µm sections were stained with haematoxylin and eosin.

3. Results

M. mactroides clams of all size classes were tolerant to low salinities. Mortality was recorded at salinities ≤ 10 g/L (Figure 2). All clams succumbed within 96 h of exposure to 5 g/L salinity. The survival after 96 h of exposure to a salinity of 10 g/L was 60% and 27% in juveniles and adults, respectively. At salinities ≥ 15 g/L, all animals tested survived.

The median lethal salinity (LC_{50}) of a 48 h exposure was 6.5 g/L and 5.7 g/L, respectively, for adults and juveniles. For a 96 h exposure, the LC_{50} was 10.5 g/L for adults and 8.8 g/L for juveniles.

The histological evaluation revealed clear trends that could be useful in the presumptive diagnosis of low salinity exposure. Figure 3 shows that in yellow clams exposed to salinities ≥ 15 g/L, the structure of the digestive gland remained normal, whereas structural changes were detected in the digestive gland of clams exposed to a salinity of 10 g/L for 96 h. The pathological signs observed were occlusion of the digestive tubular lumina, necrotic foci, and intracellular oedema in the epithelium of the digestive glands (Figure 4).

4. Discussion

Knowledge of the minimum salinity tolerance of commercially important bivalves, such as the yellow clam *M. mactroides*, will be of prime importance to determine causes of mortality in the environment. According to Kinne (1970), the greater tolerance of juveniles to low salinities than adults could be explained by the additional metabolic demand due to the onset of gonad maturation. Marins and Levy (2000) reported that only juvenile yellow clams lived near the Patos Lagoon outflow because adult clams

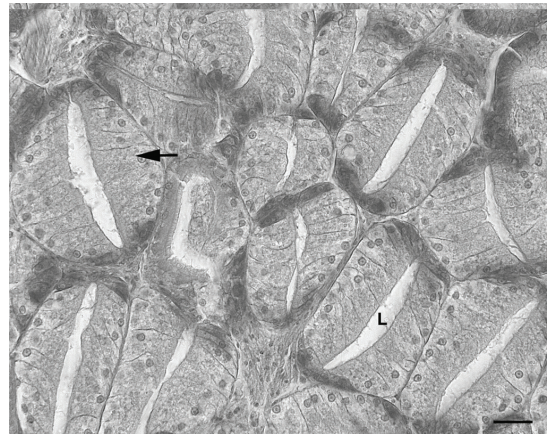


Figure 3. Histological section of a normal *Mesodesma mactroides* digestive gland showing high absorptive epithelial cell height (arrow) and the open lumen of the digestive gland (L). Haematoxylin and eosin staining. Bar, 20 µm.

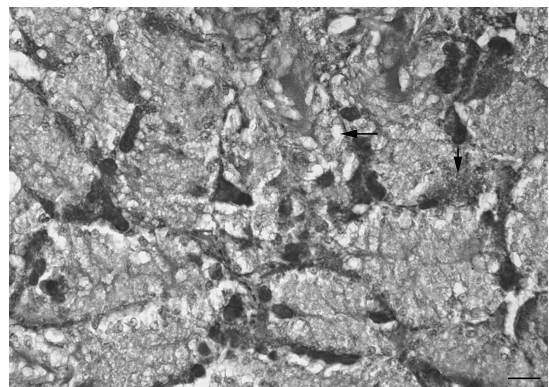


Figure 4. Histological section of a digestive gland from *Mesodesma mactroides* exposed to 10 g/L salinity for 96 h showing intercellular oedema (long arrow) and necrotic foci (short arrow). The absorptive cells appear to be massively swollen, occluding the lumen of the tubules. Haematoxylin and eosin staining. Bar, 20 µm.

were not able to survive the low salinities characteristic of this environment.

The coastal marine realms are affected by continental runoff during severe rain, resulting in periods of decreased salinity. Animals inhabiting such habitats adopt different mechanisms for survival (Kinne, 1970). Intertidal and estuarine bivalves are generally tolerant to sudden and large changes in salinity (Shumway et al., 1977). Sediment burial is a major mechanism used by clams to isolate themselves from unfavourable conditions in the water column. However, the yellow clams were maintained in an aquarium without sand in this study. If the clams were given the opportunity to burrow in the sand, they may have survived for a longer time in a low salinity environment.

On Cassino Beach on the coast of Rio Grande do Sul where the yellow clam *M. mactroides* is frequently found, the salinity ranges from 14 g/L to 38 g/L (mean = 28 g/L), with the minimum values related to El Niño events (Odebrecht et al., 2010). Thus, the lethal low salinities for *M. mactroides* were not far from the extreme values recorded by Odebrecht et al. (2010), and mortality might occur due to low salinities in some areas.

Most bivalves respond immediately to changes in the environmental salinity by closing their valves to isolate their soft body from the external environment (Dame, 2012). At the salinity of 5 g/L, the valves of *M. mactroides* were tightly closed until they died. In clams maintained in a salinity of 10 g/L, their valves were closed from the beginning of the experiment to 72 h of exposure.

After 72 h of exposure to 10 g/L salinity, siphons and the foot protruded out slightly and responded to external stimuli at a slow rate. No production of faeces or pseudo-faeces was observed, indicating that these clams were physiologically stressed.

At salinities ≥ 15 g/L, yellow clams were active from the beginning of the exposure to the end of the experiment, with the production of faeces and pseudo-faeces. The siphons and foot were withdrawn into the shell cavity at the slightest disturbance. Therefore, low salinities ≤ 15 g/L can be considered suitable for the yellow clam, at least for 96 h of exposure.

Histological changes observed in the digestive gland confirm that 10 g/L salinity is unsuitable for *M. mactroides*. Lesions and structural changes of the gastrointestinal epithelium are important indicators of bivalve health, and a significant loss of digestive gland absorptive cells is a pathological sign associated with mortality in bivalves (Elston, 1999). Syndromes that involve the digestive gland may result from changes in the environment, such as temperature and salinity (Elston, 1999)

The present study provided data that can be used for the diagnosis or forensic evaluation of yellow clams that are suspected of exposure to lethal or marginal low salinities. Similar results were obtained by Elston et al. (2003) in an experiment analysing the salinity tolerance of the Manila clam *Venerupis philippinarum* (Adams and Reeve, 1850). They concluded that the swelling of absorptive cells of the digestive glands of clams exposed to a salinity of 10 g/L

might be due the absorption of hypoosmotic seawater, followed by the sloughing of these cells into the lumen of the digestive gland.

In conclusion, the yellow clam *M. mactroides* can be considered a moderate euryhaline species that is able to tolerate salinities from 35 to 15 g/L, and populations, particularly the adults, that inhabit areas near the mouth of great rivers, such as River Plate or Patos Lagoon, can suffer mortality after several days of rainstorms that occur during strong El Niño events and are accompanied by an elevated discharge of fresh water into the coast.

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