

## MASS MORTALITIES AFFECTING POPULATIONS OF THE YELLOW CLAM *AMARILLADESMA MACTROIDES* ALONG ITS GEOGRAPHIC RANGE

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**ABSTRACT** The yellow clam *Amarilladesma mactroides* (Deshayes, 1854) is considered a vulnerable species since the mid-1990s. Populations have experienced mass mortalities throughout their entire range of distribution (23–41°S) along exposed sandy beaches of Atlantic South America. Detrimental anthropogenic impacts have further contributed to failure of populations to make a recovery. To determine the factors involved in these events, density prior to a mortality event was calculated and live yellow clams encompassing most of its geographic range distribution were analyzed histologically to describe parasites and pathologies. Moreover, moribund specimens were analyzed by molecular techniques to test for the presence of the virus OsHV-1. A mortality event was recorded after a maximum density of 127 clams/m<sup>2</sup> was attained. No clear pattern was found between the prevalence and intensity of infection and localities, mortality events, or sampling season. Although OsHV-1 was not observed in any of the yellow clams tested, the possibility that another viral agent was implicated cannot be ruled out. The presence of bacteria of the genus *Vibrio* in combination with stress caused by a relatively high population density is suggested as the likely cause of these episodic mass mortalities.

**KEY WORDS:** mass mortalities, viral susceptibility, *Amarilladesma mactroides*, pathologies

### INTRODUCTION

Bivalve populations commonly suffer mass natural mortalities and it is often difficult to unequivocally assign a cause to these events (Burdon et al. 2014). Such outbreaks are of particular concern when a species either supports a commercial fishery or is of conservation importance (Parada & Morales 2008). An improved understanding of possible causes of such episodic mass mortality events will thus aid in management of commercial fisheries and natural conservation.

The yellow clam *Amarilladesma mactroides* (Mesodesmatidae) is a dioecious species characterized by two reproductive events per year, one in winter and another in spring in Argentinean populations (Herrmann et al. 2009), and an extended or quasi-continuous recruitment season in more northern populations in South America (Fiori & Defeo 2006). It is endemic to the Atlantic Coast of South America, and is distributed from San Pablo, in southern Brazil (23°S), to the mouth of the Negro River, in Argentina (41°S, Fig. 1) (Coscarón 1959). This species was dominant in mesolittoral communities and constituted an important economic resource in Argentina during the 1940s and 1950s (Coscarón 1959). Commercial harvesting was prohibited in 1958 after populations experienced a marked decline due to over-exploitation (Coscarón 1959), resulting in establishment of a ban such that only recreational (tourist) capture was allowed. Stocks continued to decline gradually, however, and the species disappeared from many beaches due to tourist harvesting, poaching, and coastal urbanization (Dadon et al. 2001). The declining status of yellow clam populations was aggravated by mass natural

mortalities that have occurred since 1993 throughout its geographic distributional range. These mortality events occurred mainly between late spring and early summer, followed a sequential north to south direction, and did not affect other macroinvertebrate species. Mortalities first occurred in Brazil in March 1993, where in less than 1-mo yellow clams were virtually eliminated along 350 km of the southern Brazilian coast (Odebrecht et al. 1995). In December 1994, similar phenomena were recorded both in a 12-km-long beach near Hermenegildo (southern Brazil) and in Barra del Chuy (a 22-km-long beach in northern Uruguay), where losses were estimated at 9 tons (Méndez 1995). Finally, in November 1995, mass mortalities reached Argentina (Fiori & Cazzaniga 1999), where the last mortality event was reported during September 2004 (Thompson & Sánchez de Bock 2007). These outbreaks have been occurring annually each spring–summer (Fiori et al. 2004) and have prevented the rehabilitation of yellow clam populations (Defeo 2003). As a result of these serial events, the status of the species changed from being the most important economic resource of the intertidal fauna of southwestern Atlantic sandy beaches, to becoming almost extinct [“critically endangered,” according to the International Union of Conservation of Nature (IUCN 1994) criteria] (Fiori & Cazzaniga 1999). Previous studies have suggested possible causes of these episodes (summarized in Table 1), although research was performed only in one specific *A. mactroides* locality. Therefore, the reasons underlying these large-scale events remain uncertain. Different lines of evidence based on the environmental and demographic and health features of the Uruguayan yellow clam population suggest that climate change may be considered the main causative agent of the population changes observed in the last decades (Ortega et al. 2016). Similar findings were described in the congeneric clam *Mesodesma donacium*; in this species, a lower body

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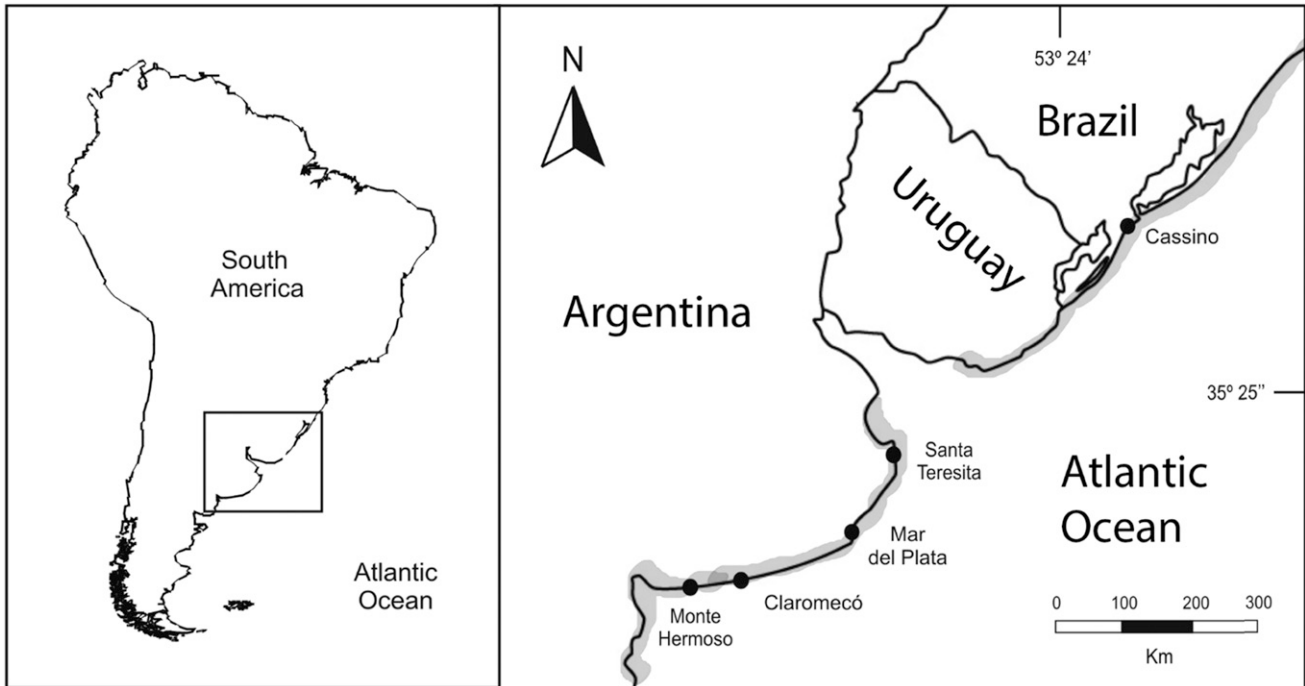


Figure 1. Right: sampling localities of the yellow clam *Amarillodesma mactroides* and its distributional range (gray shading). Left: study region within South America.

condition index was related to the level of infestation by a parasitic polychaete that increased its prevalence under increasing temperatures during a warm El Niño phase (Riascos et al. 2008).

In this study, the density of a single population of *Amarillodesma mactroides* in the province of Buenos Aires was monitored to allow identification of the potential causes of mortality outbreaks. Sampling of both moribund and apparently healthy clams was carried out monthly and during two mortality events to determine the parasites and pathologies present. Complemen-

tary molecular techniques were used to test for the presence of OsHV-1, a virus known to affect several bivalve species including oysters, clams and scallops in several countries around the world (Renault & Novoa 2004).

#### MATERIALS AND METHODS

Over the period 2008–2010, the population abundance (density) of the yellow clam was estimated in Monte Hermoso

TABLE 1.

Documented mortality events of *Amarillodesma mactroides* populations and their studied causes along its range of distribution in South America.

Year and geographical area of mortality event	Studied associated factors to mortality events	Reference
1993: Praia do Cassino, Brazil	Harmful algal bloom*	Odebrecht et al. (1995)
1994: Barra del Chuy, Uruguay	Harmful algal bloom*	Méndez (1995)
1995: Monte Hermoso, Argentina	Heavy metal contamination, poor water quality, phycotoxins, abnormal phytoplankton composition, protozoan tissue parasites	Fiori and Cazzaniga (1999)
1995: Monte Hermoso, Argentina	Three parasitic or commensal taxa ( <i>Trichodina</i> sp. ciliates, coccidians, and turbellarians)	Cremonte and Figueras (2004)
1999: Isla Jabalí, Argentina (healthy population)	Four parasitic or commensal taxa ( <i>Trichodina</i> sp. ciliates, coccidians, gregarines*, and turbellarians)	Cremonte and Figueras (2004) Cremonte and Figueras (2004)
2002: Isla Jabalí, Argentina	Unidentified coccidian parasite*	Fiori et al. (2004)
2004: Partido de la Costa, Argentina	Heavy metal contamination*	Thompson and Sánchez de Bock (2007)
2011: Praia do Cassino, Brazil	<i>Rickettsia</i> infection*	Carvalho et al. (2013)
2013: state of Rio Grande do Sul, Brazil (healthy populations)	OIE-listed pathogens and OsHV-1 $\mu$ -var virus	Carvalho et al. (2016)

OIE, World Organization for Animal Health.

\* Suggested as possible cause of mortality.

Beach (Fig. 1), province of Buenos Aires. Five transects were established equidistant from each other (5 m apart) and perpendicular to the tide line extending from the upper to the lower intertidal zone (swash zone), during daytime and at low tide. Each transect was divided into levels, located every 10 m in the middle and upper intertidal, and every 5 m in the lower intertidal. At each level, one sample was taken per transect with a plastic core 16 cm in diameter and to a 40 cm depth (sampling area = 201 cm<sup>2</sup>), and sieved through a 1-mm plastic mesh; all retained clams were counted and measured. In addition, sea surface temperature was obtained from the Toms and OMI, NASA website.

Between October 2003 and 2011, a total of 335 yellow clams were collected manually during low tide from five locations: Praia do Cassino (32° 12'S, 52° 11'W) in Brazil; and four locations in Argentina, that is, Claromecó (38° 54'S, 60° 03'W), Mar del Plata (38° 00'S, 57° 33'W), Santa Teresita (36° 32'S, 56° 51'W), and Monte Hermoso (38° 59'S, 61° 06'W) (Fig. 1, Table 2). In addition, yellow clams from Monte Hermoso were sampled seasonally (2009 and 2010) and those from Santa Teresita were collected during two mortality events (October 2010 and 2011), when fishermen noticed large numbers of dead or moribund clams on the sediment surface.

Samples were transported within 24 h to the Laboratory of Parasitology, where the maximum shell length of each individual was measured with a calliper to the nearest millimeter and soft tissues were carefully removed from the shell and fixed in Davidson's solution (Shaw & Battle 1957) for 24 h until histological processing. Oblique, transverse, 5-mm-thick sections, containing gills, digestive gland, mantle, nephridia, gonad, and foot were taken from each clam. Tissue samples were embedded in paraffin and then 7- $\mu$ m-thick sections were stained with hematoxylin and eosin. Histological sections (one per individual) were examined under a light microscope with  $\times 40$  and  $\times 100$  of magnification for the presence of parasites and pathological condition. For each yellow clam, sex and gonad stage were recorded; for each gametogenic stage was given a numerical score from 1 to 6 based on the maturity of the follicles and gametes; that is, 1: proliferation, 2: early ripe, 3: ripe, 4: spawning, 5: spent, and 6: repose (sex undefined). Prevalence (P) and mean intensity (MI) of parasites were calculated according to Bush et al. (1997). The intensity of infection was estimated by counting the number of parasites in each histological section. A special Gram staining procedure according to Brown and Hopps (1973) was performed in 10 yellow clams suspected to be infected by *Rickettsia*-like organisms (RLO), based on previous observations under the light microscope. In addition, 15 yellow clams from a single mortality event (Santa Teresita, October 2011) were preserved in 100% ethanol for subsequent DNA extraction. The presence of OsHV-1 (Oyster Herpesvirus type 1) was tested by real-time polymerase chain reaction ([http://www.eurlmollusc.eu/content/download/42545/578238/file/OsHV-1%20RTPCR\\_1.pdf](http://www.eurlmollusc.eu/content/download/42545/578238/file/OsHV-1%20RTPCR_1.pdf)) of extracted DNA. Furthermore, paraffin blocks prepared from these 15 yellow clams were also tested by *in situ* hybridization according to Lipart and Renault (2002).

## RESULTS

After a mortality event was recorded in October 2008, no more yellow clams were observed from the Monte Hermoso population. Between May 2009 and August 2010, the population density

remained relatively uniform (averaging 22 clams/m<sup>2</sup>), it reached a maximum of 127 clams/m<sup>2</sup> during September, and declined to zero in October (Fig. 2). There was no apparent relationship between temperature and densities of the yellow clam population at this study site (Fig. 2).

A summary of the data of the main characteristics of *Amarilladesma mactroides* localities and the results of histological examinations (P and MI of infection for all parasites) are presented in Table 2. The sex ratio remained relatively 1:1. No clear pattern between prevalence and intensity of infection was found with localities, shell length, sex, mortality events, or sampling season (Table 2). The most prevalent parasite was a *Trichodina*-like ciliate (Alveolata) located near the epithelial surface of the gills or attached to it, and was associated with no evident tissue damage or host response. The highest prevalence of 96% and the maximum intensity of infection of 477 ciliates were recorded in yellow clams from Monte Hermoso in May 2010. The second most prevalent parasites were RLO, observed as rounded, intracellular, basophilic inclusions in the digestive gland and gill epithelia, with no apparent host reaction. All Gram-stained samples revealed red-colored inclusions in the epithelial cells of the gills (Fig. 3), which supports the presence of Gram-negative colonies, likely RLOs. The highest prevalence of 59% and the maximum intensity of more than 200 colonies were recorded during the austral autumn season (May 2010) in Monte Hermoso (Table 2). The turbellarian *Paravortex mesodesma* (Graffillidae) was recorded in the lumen of the intestine of specimens from three of the five studied localities. The highest prevalence of 35% was observed in Praia do Cassino and the maximum intensity of four turbellarians was recorded in Mar del Plata. Gregarine and coccidian protozoans (Apicomplexa) found leading to hypertrophy of the digestive epithelial cells and nephridial tubules, respectively, were recorded only in the Mar del Plata population, both with a low prevalence and intensity of infection (Table 2). No hemocyte response was elicited by infection of these parasites.

Although positive controls (= plasmidic DNA) yielded positive results by real-time polymerase chain reaction for the OsH-1 virus, none of the tested yellow clams were positive using this technique. Similarly, positive controls (= the Pacific oyster *Crassostrea gigas* infected with OsHV-1) showed specific labeling in connective tissue by *in situ* hybridization, but none of the tested yellow clams yielded a positive signal. Moreover, no pathologies were observed in the tissues that could be attributed to a viral etiology.

## DISCUSSION

This study focused on episodic mass mortality events that affected most *Amarilladesma mactroides* populations, encompassing almost the entire geographic range of distribution of this species, and the identification of potentially associated pathogens or parasites by histological and molecular methods. Results obtained do not allow linking the presence of parasites or OsHV-1 as causative agents of the mass mortality events, because moribund yellow clams were devoid of previously known serious pathogens. The presence of bacteria of the genus *Vibrio*, and/or an unknown virus in combination with the stress caused by relatively high population densities added the spawning season, where the yellow clams would be depleted of energy, is thus suggested as the

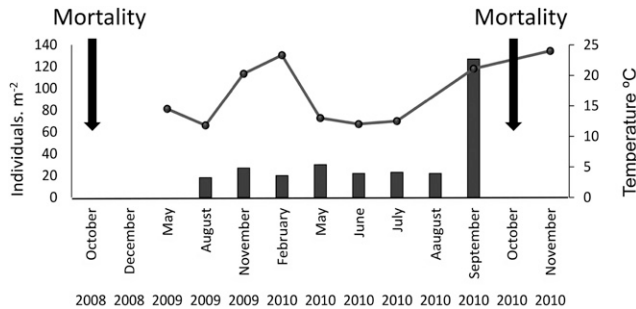
TABLE 2.  
Main characteristics and histological examination of *Amarilladesma mactroides* populations in this study.

Locality	Date of collection	N	Shell length (range)	% Sex M/F	Gonad stage (%)	<i>Rickettsia</i> like organism									
						Digestive gland and gill epitheliums		<i>Trichodina</i> -like ciliates		Gregarine		Coccidian		<i>Paravortex mesodesma</i> (turbellarian)	
						P	I	P	I	P	I	P	I	P	I
Praia do Cassino	October 2003	17	32 (27–37)	35/24	4 (36)	0	–	0	–	0	–	0	–	35	1
Praia do Cassino	October 2009	16	22 (17–27)	Undefined	6 (100)	6	1	0	–	0	–	0	–	0	–
Claromecó	March 2010	27	42 (27–58)	30/48	4 (78)	0	–	40	2.7 (1–10)	0	–	0	–	4	1
Mar del Plata	October 2010	26	53 (35–66)	61/39	4 (89)	8	1	58	1	11	1	8	1	8	3 (2–4)
Santa Teresita*	October 2010	20	–	50/55	5 (70)	0	–	0	–	0	–	0	–	0	–
Santa Teresita**†	October 2011	26	60 (37–75)	42/58	4 (85)	4	3	50	36.5 (1–263)	0	–	0	–	0	–
Monte Hermoso	December 2008	30	40 (22–55)	53/47	–	0	–	77	2.9 (1–70)	0	–	0	–	0	–
Monte Hermoso	May 2009	32	47 (35–59)	48/52	5 (90)	0	–	80	1	0	–	0	–	0	–
Monte Hermoso	August 2009	31	50 (25–65)	43/57	2 (90)	0	–	83	1	0	–	0	–	0	–
Monte Hermoso	November 2009	25	52 (37–65)	68/32	4 (68)	0	–	58	1	0	–	0	–	0	–
Monte Hermoso	February 2010	25	59 (48–69)	50/50	4 (96)	8	7	92	46.1 (4–142)	0	–	0	–	0	–
Monte Hermoso	May 2010	30	37 (22–66)	41/44	5 (52)	59	>200	96	101.3 (1–477)	0	–	0	–	0	–
Monte Hermoso	August 2010	30	42 (32–56)	53/43	2 (70)	16	100	83	95.8 (2–385)	0	–	0	–	0	–

Shell length (mean followed by range in parenthesis) in mm; sex ratio (M: male; F: female); predominant gonad stage (percentage of yellow clams in the gonad stage in parenthesis): 1: proliferation, 2: early ripe, 3: ripe, 4: spawning, 5: spent, 6: repose (sex undefined); prevalence (P) (%) and intensity (I) (mean followed by range in parenthesis) of parasites and their sites of infection.

\* Samples collected during a mortality event.

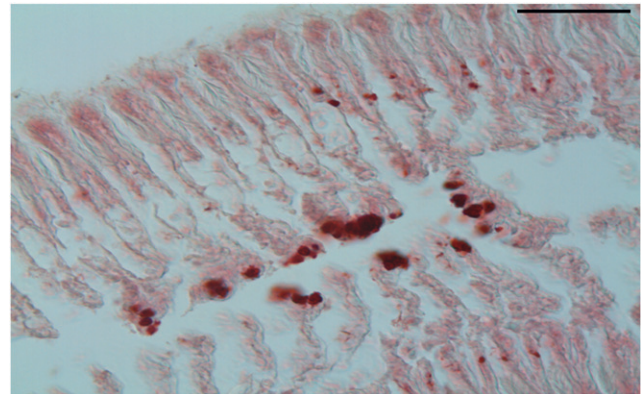
† Samples preserved for viral analysis. All sampling sites found in Argentina (province of Buenos Aires), except Praia do Cassino, found in southern Brazil (see Fig. 1).



**Figure 2.** Density of *Amarilladesma mactroides* (number of clams per square meter) versus sea surface temperature from October 2008 to 2010 in Monte Hermoso beach, Argentina.

probable cause of these episodic mass mortalities. Yellow clam mortalities could be the result of adverse extrinsic factors, including both physicochemical and biotic factors, such as temperature, in addition to population density. Intrinsic physiological factors, including the effects of disease or poor body condition, may also play a contributing role in these mortalities.

Density may affect the growth and mortality of a bivalve population via intraspecific competition (for food and space), and its relationship to the incidence of predators (De Montaudouin & Bachelet 1996) and to increased infective pressure from pathogens or microbiological agents (Choi et al. 2002). In this context, Parada and Morales (2008) analyzed the relationship between cockle *Cerastoderma edule* mortality and the population density, precipitation and atmospheric temperature during a 7-y period in the Ria of Arousa (Spain) and they suggested that density levels may cause mass mortality as a result of intraspecific competition for food and space and the incidence of predators (after De Montaudouin & Bachelet 1996). In this study, the density of *Amarilladesma mactroides* appeared to be an important factor involved in mass mortalities, because a mortality outbreak occurred after the peak in the density of yellow clams was reached during the austral spring (October), coincident with the reproductive season. Environmental factors, such as the high density, and intrinsic conditions, such as the spawning stage of the yellow clams, may be a cause of stress and thus increase the susceptibility to disease and may also increase the probability of transmission of pathogens or microbial agents that can contribute to mass mortalities (Villalba et al. 1993, Petty 2014). Results of this study were unable to link the presence of a parasite as the etiological agent associated with these episodes, because most of the moribund yellow clams examined were devoid of serious parasites. Similar results were reported by Cremonte and Figueras (2004), who described the same parasites observed in this study, except for gregarines, in a healthy *A. mactroides* population, as in moribund specimens during a mortality event. In particular, RLO have been reported from different tissues of diverse bivalve species (Lauckner 1983). Recently, Carvalho et al. (2013) reported the infection of RLO as the cause of mass mortalities of *A. mactroides* population from Praia do Cassino (Brazil). These authors support this association because all moribund, juvenile yellow clams ( $n = 30$ ) showed positive infection by RLO based on transmission electron microscopy and molecular analysis, and showed no record of any other parasite. In this study, we discard this



**Figure 3.** Prokaryotic microorganism similar to *Rickettsia* stained dark red in the gill epithelium of *Amarilladesma mactroides* (Gram stain). Scale bar: 50  $\mu\text{m}$ .

possibility, because only a few cells from some moribund yellow clams were affected by RLO and occurred with a very low intensity and with no apparent host reaction or tissue damage. At present, these prokaryotes are considered to be of limited pathogenicity and significant pathology is only apparent during massive infections (Romalde & Prado 2011). With respect to the rest of parasites observed in this study, all have been previously reported in *A. mactroides*. In this clam species, *Trichodina*-like ciliates with a moderate intensity of infection were reported in prior studies (Fiori & Cazzaniga 1999, Cremonte & Figueras 2004). In this study, high prevalence and heavy infections of these ciliates were recorded in healthy yellow clams from Monte Hermoso during 2010, coincident with the highest intensity of RLO. This may be due to the fact that most trichodinids are bacterivorous, and as filter feeders are capable of ingesting bacteria present in the bivalve gill (Lom 1995). Gregarines and coccidians were suspected to be involved in the mass mortality events of *A. mactroides* Argentinean populations in prior studies (Cremonte & Figueras 2004, Fiori et al. 2004). Results obtained in this study, however, serve to discard this association, given that these organisms were absent from yellow clams collected during mortality events. Bower et al. (1994) reported the presence of these apicomplexan protozoans infecting several bivalve species, with no significant health effects, and neither gregarine infection nor mortalities were associated with coccidian parasitism in their study.

On the basis of the results of histological analyses and characteristics of the mortality events, such as the strong host specificity observed [mortalities did not affect other bivalve species, as *Donax hanleyanus*, that co-occurs with *Amarilladesma mactroides* (Defeo & de Alava 1995)] and the ability of these agents to cross zoogeographic barriers, the presence of a virus or a bacteria is here suspected as the etiological agent. Figueras and Novoa (2011) pointed out that most notifiable diseases of molluscs are caused by protozoan parasites due to the fact that they are more easily detected, whereas viruses and bacteria as causative agents of diseases have been neglected. In this study, although OsHV-1 was not detected in any of the tested yellow clams during the mortality event, the possibility that some other viral agent is implicated cannot be ruled out. Similar results were reported recently by Carvalho et al. (2016), where screening in *A. mactroides*

showed no evidence of the specific sequences of OsHV-1  $\mu$ -var.

Continuous studies to elucidate the causes of these mortality events and design effective management strategies to protect the intertidal system as a whole should be implemented. This is necessary given that these mortalities affect *Amarilladesma mactroides* throughout its distribution range, and populations of this species continue to suffer these episodes on an annual basis. In addition, detrimental anthropogenic impacts (i.e., of recreational fishing, growing beach tourism, coastal urbanization, and poaching) may increasingly contribute to the failure of recovery of populations of this species.

This justifies maintenance of the conservation status of this intertidal bivalve as vulnerable, according to IUCN criteria.

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