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RESEARCH NOTE

Screening for WSSV in crustacean from marine areas of Buenos Aires, Argentina

Examen para WSSV en crustáceos de áreas marinas de Buenos Aires, Argentina

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Abstract.- In total 374 specimens corresponding to four species of shrimp: *Artemesia longinaris*, *Pleoticus muelleri*, *Peisos petrunkevitchii*, *Palaemon macrodactylus*, and two species of crabs: *Neohelice granulata* and *Cyrtograpsus angulatus* were studied in the search of white spot syndrome virus (WSSV). The crustaceans were collected in the estuary of Bahia Blanca, the port of Mar del Plata and the Samborombón Bay, Argentina, between 2010 and 2014. A polymerase chain reaction (PCR) analysis in all of them has not detected any virus presence. These new results suggest that the discovery of infected shrimp in 2008-09 in the Bahia Blanca estuary may have been a one-time local event, promoted by special conditions of seawater temperature inside the estuary, coincident with the presence of a virus carrier or transporter. To determine if the virus was finally installed in the estuary of Bahia Blanca more screening are needed coming from a larger number of shrimp samples. These studies will be necessary mainly in *A. longinaris* (Penaeidae) since this family of shrimps is most susceptible to be affected by WSSV.

Key words: Shrimps, crabs, WSSV, Argentina

INTRODUCTION

Since 1992, in the Laboratory of Parasites of Fishes, Crustaceans and Mollusks, macro and micro parasites of crustaceans from the South Atlantic Ocean have been investigated. Different parasites were reported in several species of crustaceans (shrimps and prawns) from freshwater, marine and estuarine environments (Martorelli 1989, 1992; Martorelli & Schuldt 1990, Martorelli & Higgins 2004, Martorelli *et al.* 1994, 1999, 2002, 2012; Sardella & Martorelli 1997). Recently, Martorelli *et al*. (2010) reported the viral pathogens WSSV from the shrimps *Artemesia longinaris*, *Palaemon macrodactylus* and the crab *Cyrtograpsus angulatus* in the estuary of Bahia Blanca, Argentina. That report was the most austral occurrence for the virus in the South Atlantic Ocean. In relation with the more commercially important argentine red shrimp *Pleoticus muelleri*, Martorelli *et al.* (2010), Roccamo *et al.* (2010) and Costagliola *et al.* (2011) did not find specimens infected by WSSV.

It is also important to consider that in some nearby water areas of Brazil, several reports of the virus in natural populations of crustaceans were published (Marques-Muller 2011, Cavalli *et al.* 2010, 2011). The report of WSSV in Argentina was coincident with a very slight increase of seawater temperature this in relation to previous periods (IADO 2008, 2009; Freije & Marcovecchio 2004). According to Beigt & Piccolo (2003) and Beigt (2007), the increase of temperature could have been related to a slow warming of the estuary system. On the other hand, it is known that WSSV is extremely dependent on temperature, which is crucial for their proliferation (Gao *et al.* 2011, Moser *et al.* 2012). The virus replicates faster at high temperatures (Jiravanichpaisal *et al*. 2004). Generally between 25 °C and 27 °C is the optimum temperature for its proliferation, while it is fully suspended at 4 °C (Jiravanichpaisal *et al.* 2004, Wongmaneeprateep *et al.* 2010). Thi-Lua & Hirono (2015) working with the shrimp *Marsupenaeus japonicus* showed that WSSV infection is temperature-dependent and shrimp are highly susceptible to infection at 25 ºC. Low temperatures (15 ºC) reduce the replication rate of the virus and the hosts remain as carriers that could disperse the infection when the water temperature increases. Du *et al.* (2006) studied the effect of the hyperthermia in *Procambarus clarkia* and observed no mortality when crayfish were at 32 ± 1 °C, and 100% mortality at 24 ± 1 °C. The authors reported viral charges of 10^5 copies mg^{-1} tissue at 32 ± 1 °C instead 10^4 to 10^{10} copies mg^{-1} tissue at 24 ± 1 °C. At higher temperatures the virus is inactivated in 120 min at 50 °C and 1 min at 60 °C (Lo 2012).

The screening presented in this paper was conducted to increase the still low numbers of crustaceans tested in the studied area after the mentioned report (Martorelli *et al.* 2010). In addition, some comments about the possible relationship between water temperatures in the Bahia Blanca Estuary and the WSSV are presented.

MATERIALS AND METHODS

Crustaceans used in this study, more than 600, were collected from late 2010 to 2014, usually in February and March of each year. In the laboratory all the crustaceans were observed under a binocular microscope to determine the existence of spots on the carapace (Martorelli *et al.* 2010). Subsequently, a subsample of specimens corresponding to four species of shrimp: *Artemesia longinaris* (n= 187), *Pleoticus muelleri* (n= 85), *Peisos petrunkevitchii* (n= 15), *Palaemon macrodactylus* (n= 64), and two species of crabs: *Neohelice granulata* (n= 7) and *Cyrtograpsus angulatus* (n= 16) were studied in the search of the virus (Table 1). Those crustaceans were obtained in various ways. Some were purchased fresh at the Ingeniero White fishing port (38º47'26''S; 62º16'12''W), after commercial vessels trawling channels of the Bahia Blanca estuary captured them. Others were collected using cast nets or crab-traps in Puerto Cuatreros, General Daniel Cerri (38º45'05''S; 62º22'46''W), in Mar del Plata coast (38º00'26''S; 57º32'17''W), in Samborombon Bay (35º44'46''S; 57º22'52''W). Finally, other shrimps were captured by an oceanographic survey in the Vessel ARA Puerto Deseado from 'el Rincon' area (39º44'24''S; 64º16'00''W). Sampling sites were chosen mainly considering the site where the virus was found in 2008-2009 (Bahia Blanca Estuary). In addition, in this study specimens of *P. macrodactylus* from Samborombon Bay were included, a new place reported for this alien shrimp (Martorelli *et al.* 2012), and also specimens of *Pleoticus muelleri* from Mar del Plata because of the commercial importance of this crustacean in Argentina.

Whole crustaceans were immediately fixed in 95º molecular grade ethanol. DNA was extracted mainly from the gills (main tissue where the virus was detected in the crustaceans of the Bahia Blanca Estuary), but also from skeletal muscle and hepatopancreas, using the DNeasy kits (Quiagen) methodology. Extracted DNA was quantified by an Ampliquant AQ07 spectrophotometer. PCR-amplification was conducted on the extracted DNA using GoTaq® Green Master Mix (Promega) kit. For virus detection 3 different methods were used: A nested PCR reaction using the primer set WS800/ WS500 (Martorelli *et al.* 2010), a modified one step PCR, which can detect WSSV virions from different geographical regions (Nunan *et al.* 2011) and the commercial primer set IQ2000 (Farming IntelliGene Tech, Taipei, Taiwan) that could discriminate different levels of infection. The controls included in the commercial kit IQ2000 and the extracted DNA from infected *Litopenaeus vannamei* were used as positive controls. These mentioned infected shrimps were collected from a WSSV outbreak in Costa Rica and were provided by the Laboratorio de Patologías y Parasitología de Crustáceos, Nicoya, Guanacaste, Costa Rica.

Table 1. Species of shrimps and crabs screening, tissue examined, number of specimens analyzed, locality, and year of capture / Detalle de los camarones y cangrejos estudiados, tejido examinado, número de especímenes, localidad y año de captura

Species	Tissue	Nº	Locality	Date
Artemesia longinaris	gills-muscle	71	Ingeniero White	Feb/Mar-2010
Artemesia longinaris	gills-hepato	70	El Rincón	Ene-2011
Artemesia longinaris	gills	16	Ingeniero White	Feb-2011
Artemesia longinaris	gills-muscle	30	General D. Cerri	Mar-2014
Cyrtograpsus angulatus	gills	16	General Cerri	Feb-2010
Neohelice granulata	gills	7	General Cerri	Feb-2010
Palaemon macrodactylus	gills	15	General Cerri	Feb-2010
Palaemon macrodactylus	gills	19	Samborombon Bay	Mar-2011
Palaemon macrodactylus	gills	10	General D. Cerri	Mar-2012
Palaemon macrodactylus	gills	10	General Cerri	Feb-2013
Palaemon macrodactylus	gills	10	General Cerri	Feb-2014
Pleoticus muelleri	gills	35	Ingeniero White	Feb/Mar-2010
Pleoticus muelleri	gills	25	Mar del Plata	Ene-2012
Pleoticus muelleri	gills	25	Ingeniero White	Mar-2013
Peisos petrunkevitchii	gills	15	General Cerri	Feb-2010

Results and discussion

From 2010 to 2014 more than 600 shrimps and crabs were examined for external and histological signs of WSSV. The crustaceans were observed under a binocular microscope to determine the existence of spots on the carapace, same as they had been observed in a previous study (Martorelli *et al.* 2010). As a result all of specimens were negative. Subsequently, of the total of crustaceans more than 50% (374) were chosen at random and examined by PCR using the previously indicated primers. All these crustaceans analyzed were negative for WSSV (Table 1).

Martorelli *et al.* (2010) found, using Real Time Quantitative PCR (qPCR) a low viral copy number (7.3×10^1 to 3.96×10^4) in the infected crustaceans studied, and only one shrimp showed magnitude values close to 10^4. Normal values in outbreaks are near to 10^4-10^{10} (Ligthner 2003). These low viral copies found in the infected shrimp in 2008-2009 may have been one of the factors that conditioned the expansion of the virus in the Bahia Blanca Estuary. During the period 2008-2009, in which WSSV was detected, Bahia Blanca Estuary showed a significant variation in seawater surface temperature anomalies (GISTEMP Team 2016, Hansen *et al.* 2010). This could be related to the assertions of Tendencia & Verreth (2010), who exposed that a fluctuation of 3 °C or 4 °C in water temperature can trigger outbreaks of WSSV in culture conditions. According to the Goddard Institute for Space Studies (2008)[1], the year 2008 was one of the warmest years since the period of instrumental measurements.

In conclusion, since the original report of WSSV in Bahia Blanca Estuary (Martorelli *et al.* 2010) an annual screening was carried out on many crustaceans and positive crustaceans for WSSV were not found with the PCR methods used. These new results suggest that the discovery of infected shrimp in 2008-2009 in the Bahia Blanca Estuary may have been a one-time local event, promoted by special conditions of seawater temperature inside the estuary, coincident with the presence of a virus carrier or transporter. The number of crustaceans examined should be increased considering the dilution effect of the environment and the low prevalence of the virus in natural environments. Takur *et al.* (2002) suggest, in a study of evaluation of WSSV in postlarvae, analysis of at least 300 crustaceans in pools to reduce the probability of false negatives.

Until now only infected specimens were found in Argentina in samples taken during 2008-2009 (Martorelli *et al.* 2010). At that time, 11 of 25 specimens of the shrimp *Artemesia longinaris*, 4 of 6 crabs *Cyrtograpsus angulatus* and 1 of 5 shrimp *Palaemon macrodactylus*, were found infected with WSSV. Those specimens were analyzed by PCR and qPCR.

[1]<http://data.giss.nasa.gov/gistemp/2008/>

All the shrimp examined by PCR between 2010 and 2014 were negative for WSSV. In addition to the results presented here, it is important to take into account the negative results obtained by Roccamo *et al.* (2010) and Costagliola *et al.* (2011) for *Pleoticus muelleri*. To determine if the virus was finally installed in the Bahia Blanca Estuary more studies are needed coming from a larger number of shrimp samples. These studies will be necessary mainly in the species *Artemesia longinaris* (Penaeidae), because this family of shrimps is most susceptible to be affected by WSSV (Lightner 2003).

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