White spot syndrome virus (WSSV) prevalence in wild and aquaculture crustacean populations from Mozambique, assessed by molecular diagnosis

Amélia Mondlane-Milisse[1,*], Olívia Pedro [1,2] , Denise R. A. Brito[1],
Fernando Chanisso Mulandane[1], Lucinda De-Araújo[1], Joelma Leão-Buchir[1], Jussa Falique[1],
Artimísia Monjane-Mabuié[1], Elvira Penina[3], Maria Isabel Virgílio Omar[3,4],
Saquibibi Valgy Ibraimo[5], Sónia Esperança Gemo[5], Latifo Maembo[5], Dácia Correia[1,2],
Luis Neves[1,6], Elisa Taviani[1,7]

[1] Centro de Biotecnologia, Universidade Eduardo Mondlane, Maputo, Mozambique

[2] Faculdade de Veterinária, Universidade Eduardo Mondlane, Maputo, Mozambique

[3] Instituto de Desenvolvimento de Pesca E Aquacultura, Maputo, Mozambique

[4] Fundo de Desenvolvimento da Economia Azul-ProAzul, Maputo, Mozambique

[5] Instituto Nacional de Inspecção de Pescado, Maputo, Mozambique

[6] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa 7 Dipartimento Scienze del Territorio Dell'Ambiente E Della Vita, University of Genoa, Genoa, Italy

*Correspondence to Amélia Mondlane-Milisse. Email: halilamondlane@gmail.com

Abstract

White spot syndrome virus (WSSV) is still one of the most dangerous viral pathogens in crustacean aquaculture since its first identification in 1992, especially for tropical and subtropical countries. In Mozambique, WSSV was first detected in 2011 in cultured shrimp after mass mortality of shrimp in ponds. To control the virus, disease surveillance is the most recommended approach. The aim of this study was to report WSSV infection in crustaceans from wild stock and farms in Mozambique. Frozen and fresh samples of pleopods, larvae, and muscle from shrimp and crabs collected in seven provinces of Mozambique between 2011 and 2013, and in 2018, were tested for WSSV using the commercial IQ2000™ kit. A total of 946 out of 3480 tested samples were WSSV positive and it corresponds to a total prevalence of 27.2% in crustaceans. The infection rate was 31.6% (723samples) in wild shrimps, 21.8% (152) in wild crabs, and 14.4% (71) in aquaculture shrimps. The positivity infection rate varied within the sampling provinces. The highest infection rate was reported in Gaza (61.43%) followed by Inhambane and Zambezia (45.79 and 40%, respectively). The lowest prevalence of WSSV was reported in Cabo Delgado (2.99%). This study demonstrated the presence of WSSV in Mozambique in high prevalence in both wildlife and aquaculture crustaceans, demonstrating the need for constant monitoring and implementation of preventive measures to decrease the rates of positive infectivity both in the wild and aquaculture crustaceans.

Keywords: WSSV; Wild crustaceans; Shrimp; Aquaculture, PCR

1 Introduction

White spot syndrome virus (WSSV) is the causative agent of white spot disease (WSD), a disease with high economic impact on the shrimp aquaculture industry, and one of the main causes of massive loss of shrimp production in several countries (Stentiford et al. 2012; Bir et al. 2017). Since its first identification in 1992, the costs related to WSSV worldwide, are estimated to be 10 billion USD (Stentiford et al. 2012).

In Mozambique, the first WSSV cases were reported in 2011, after a mass mortality of shrimp in aquaculture ponds, which resulted in the closing of several shrimp farms and workers dismissed (FAO 2013). Before the 2011 outbreak, shrimp aquaculture represented 79% of the national shrimp production (www.biofund.org). Commercial marine fisheries in the country targets shallow prawns and deep-water shrimps, and explores the central region (known as Sofala Bank) and Maputo Bay in the South (Afonso 2006).

WSSV is a double-stranded DNA virus belonging to the genus Whispovirus family Nimaviridae (Mayo 2002). It has a very stable genome of 300 kbp with approximately 98% sequence identity between strains (Marks et al. 2004). The WSSV affects not only farmed shrimp but also wild crustaceans (Flegel 2006; Jang et al. 2009; Meng et al. 2009), from larvae to adult stages, and the infection can result in death that occurs within 3–10 days (Lo et al. 1997). It has been proved that WSSV can be transmitted horizontally through water, by cannibalism of infected shrimps and via carrier organisms (Chou et al. 1998). Additionally, it can be transmitted vertically through infected broodstock (Lo et al. 1997). Mud crabs and seabirds can act as intermediate hosts and play a role in the transmission of the virus (Kanchanaphum et al. 1998; Vanpatten et al. 2004).

WSSV is a wide host range virus that comprises at least 98 species, including decapods and other crustaceans such as copepods. This is considered as a key reason for the quick and widespread spread of the disease around the world, which is worsened by the high volume of international shrimp trade (Stentiford et al. 2009; Escobedo-Bonilla et al. 2008; Bir et al. 2017). It is also considered as a lethal virus to all commercially important penaeid shrimp species (Pradeep et al. 2012). Moreover, the transmission characteristics of WSSV, such as its presence in frozen shrimp products (Reville et al. 2005 and Reyes-López et al. 2009) and viability after freezing (Reddy et al. 2010), play an important role in the spread of the disease (Escobedo-Bonilla et al. 2008).

Due to the lethality of WSSV, several and different vaccines were developed but currently there is a lack of effective immunization programs and the route of administration is also a challenge (Feng et al. 2018). Despite the wide range of hosts, the worldwide distribution and as vaccines are still not completely effective, WSD effects can still be managed by surveillance and it is still the major recommended measure to control the spread of WSD. Molecular methods are widely used to prevent and control diseases through differential diagnosis (Lightner and Redman 1998). The methods recommended for WSSV detection include PCR, DNA sequencing, real time PCR, in situ hybridization and Loop-mediated Isothermal Amplification (World organisation for animal health 2018; Lightner and Redman 1998). To address the lack of knowledge about the status of WSSV in Mozambique, and as part of a surveillance program of the Ministry of Fisheries, a cross-sectional study was conducted in the country between 2011 and 2013, and in 2018, to report the WSSV events in crustaceans in wild stocks and in aquaculture, and compare the prevalence of WSSV in crustacean populations in Zambezia province within the study period in the country.

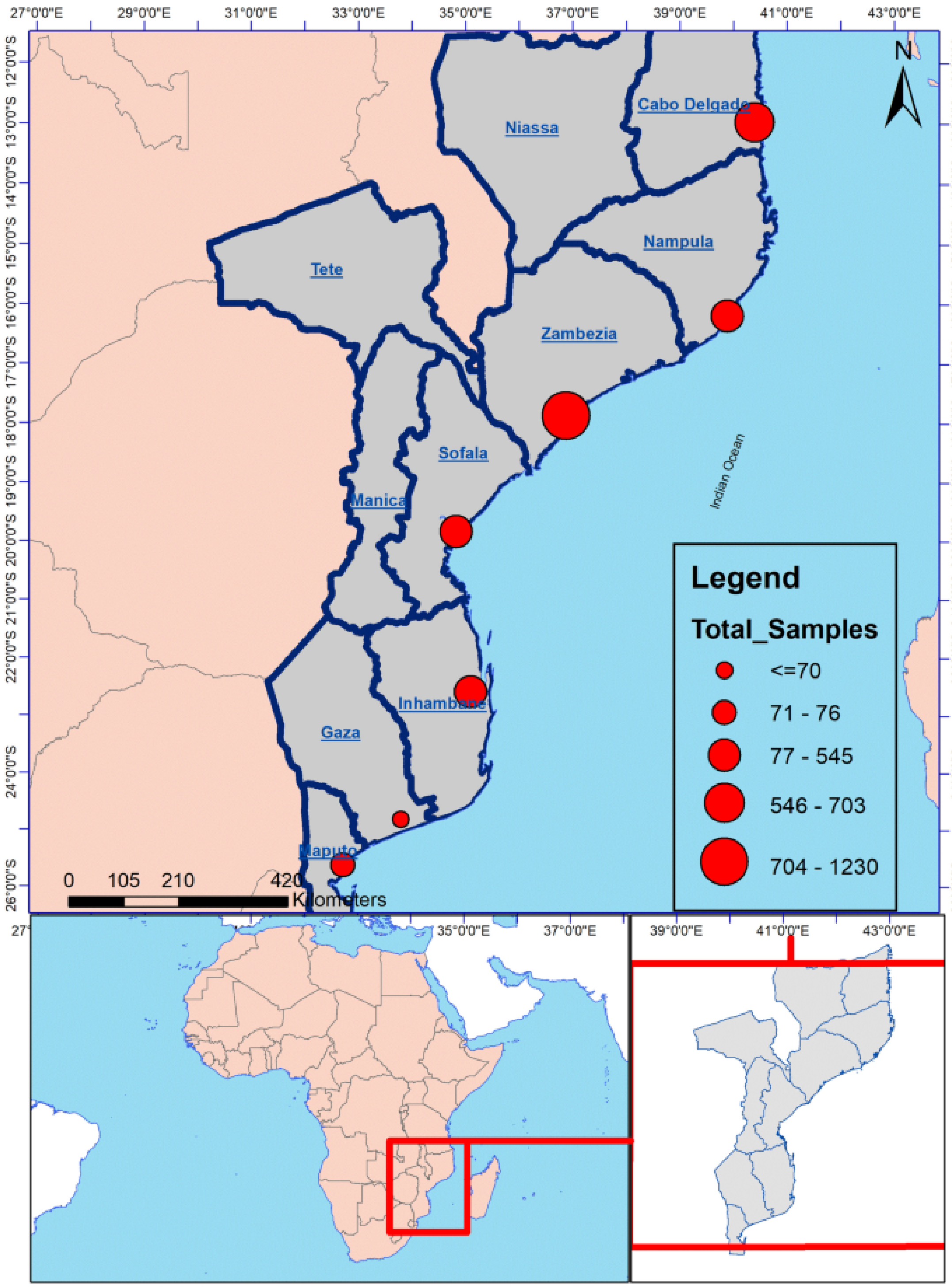

Fig. 1. Map of Mozambique showing the size of samples collected by provinces

2 Methods

2.1 Sample collection

From 2011 to 2013 and in 2018, in seven coastal provinces of Mozambique, namely Maputo, Gaza, Inhambane, Sofala, Zambezia, Nampula, and Cabo Delgado (Fig. 1), samples from larvae to adult shrimps from aquaculture, wild environments, and crabs from wild environments were collected in triplicates and placed into 2 ml microcentrifuge tubes containing 99% ethanol. Shrimp samples of larvae and post-larvae were pooled, while for adult shrimps, samples of pleopods and muscles were collected. Crab samples were collected from muscle and gills. Wild samples were collected from semi-industrial fishery boats (frozen shrimp), artisanal fisherman (fish landing centers), and shrimp aquaculture samples were collected from shrimp farms (Zambezia and Sofala provinces), hatcheries (at Sofala and Nampula), and broodstock (at Inhambane). A total of 3480 samples of crustaceans from aquaculture (493) and wild environments (2987) from 7 provinces of Mozambique were collected in 2011 (624 samples), 2012 (1553 samples), 2013 (454 samples), and 2018 (849 samples) to screen for the presence of WSSV positive infections (Table 1). Wild samples consisted of 2289 shrimp and 698 crab samples.

Table 1. WSSV prevalence (%) by province, year and origin of crustaceans collected in Mozambique

Year	Origin	Cabo Delgado	Gaza	Inhambane	Maputo	Nampula	Sofala	Zambézia
2011	Aquaculture			100.0				62.5
	Wild	9.3		26.5		50.0	1.2	34.2
2012	Aquaculture	23.9		73.1		0.0		0.0
	Wild	0.0	97.1	44.9	33.9	29.1	8.4	76.2
2013	Aquaculture						42.5	5.3
	Wild	0.0	25.7	78.2	0.0	54.4	0.0	48.1
2018	Aquaculture							0.0
	Wild							44.7

2.2 DNA extraction

The DNA was isolated by a lysis buffer provided in the IQ2000™ WSSV Detection and Prevention kit following the manufacturer's instructions (GeneReach Biotechnology Corp. Taiwan). The DNA was eluted with 100 µl ultrapure water.

2.3 PCR amplification

The PCR amplification reactions were performed using the kit IQ2000™ WSSV Detection and Prevention according to manufacturer's instructions, and all reagents were provided by the kit. Amplicons were run on agarose gel (1.5%) using TBE buffer at 100 V for 40 min and visualized using a UV light. The IQ2000 kit distinguishes four levels of infection: very light, light (about 20 copies/µl), moderate (about 200 copies/µl) and severe (more than 2000 copies/µl). A negative result means no infection or lower than the limit of detection (10 copies/µl). Negative control (ultrapure water) and 3 positive standards consisting of 10^3, 10^2 and 10^1, were included. To determine the size of the amplicons, a molecular marker provided by the kit (848 bp, 630 bp and 333 bp) was loaded onto the gel together with samples. The kit was designed to generate 3 amplicons from WSSV genome (one above 848 bp, one at 550 bp, and one at 296 bp) and one from the host crustacean DNA (848 bp). Based on the interpretation of the results described in the instructions manual, severe infections present the 3 amplicons of the WSSV genome (above 848 bp, 550 bp, and 296 bp), moderate infections consist of 2 bands of 550 bp and 296 bp, light infection consists of one band at 296 bp and very light infection consists of 2 bands of 848 bp and 296 bp.

2.4 Data analysis

Data were collected using the data collection form and entered into Excel (Microsoft Corporation, 2018) and all statistical analyses were done using Statistica 13 v13.3 (TIBCO Software Inc, 2017). A Kruskal–Wallis ANOVA was conducted to compare data between years, provinces, and species, with a Multiple Comparisons post-hoc test. A Mann–Whitney *U* test was performed to compare data on the origin of the samples (wild vs aquaculture) and type of crustacean.

3 Results

A total of 3480 wild and aquaculture crustaceans were analyzed to screen for the WSSV infections using the IQ2000™ WSSV Detection and Prevention kit. From the tested samples, 946 were WSSV positive, of which 723 (31.6%) samples were wild shrimps, 152 (21.8%) wild crabs, and 71 (14.4%) aquaculture shrimps (Table 1). Overall, the total prevalence of WSSV in crustaceans observed in Mozambique was 27%.
3.1 WSSV prevalence of crustacean populations among the coastal provinces in Mozambique
The presence of WSSV estimation was performed through the IQ2000™ WSSV nested PCR kit using samples of crustaceans. Table S1 (supplementary data) shows the prevalence of WSSV detected in the seven coastal provinces throughout the sampling years.

Analyzing each individual province (Table S1, Fig. 2), it was observed that the prevalence of WSSV reported from 2011 to 2013, decreased significantly in Cabo Delgado (from 9.3 to 0%), Gaza (from 97.1 to 25%) and Maputo (from 33.9 to 0%) while in Sofala the prevalence increased significantly from 1.2% in 2011 to 34% in 2013 (Kruskal–Wallis test: $H_{(3, 3480)} = 113.03$, $p = 0.00$. post-hoc: $p = 0.00$). Different trend was observed in Inhambane and Zambezia where we observed an increase of the prevalence from 2011 to 2012 (from 29.5 to 50.4% and from 37 to 66.7% for Inhambane and Zambezia respectively) followed by a significant drop of the prevalence up to 7.8 and 39.6% in 2013 respectively (Kruskal–Wallis test: $H_{(3, 3480)} = 113.03$, $p = 0.00$. post-hoc: $p = 0.00$). Five years later, in 2018, the prevalence in Zambezia had a slight decrease to 36.9% (Fig. 3). Moreover, in Nampula, a reduction in the prevalence of WSSV was verified from 2011 to 2012 (from 50 to 18.2%) then a significant increase up to 54.4% was verified in 2013 (Kruskal–Wallis test: $H_{(3, 3480)} = 113.03$, $p = 0.00$. post-hoc: $p = 0.00$).

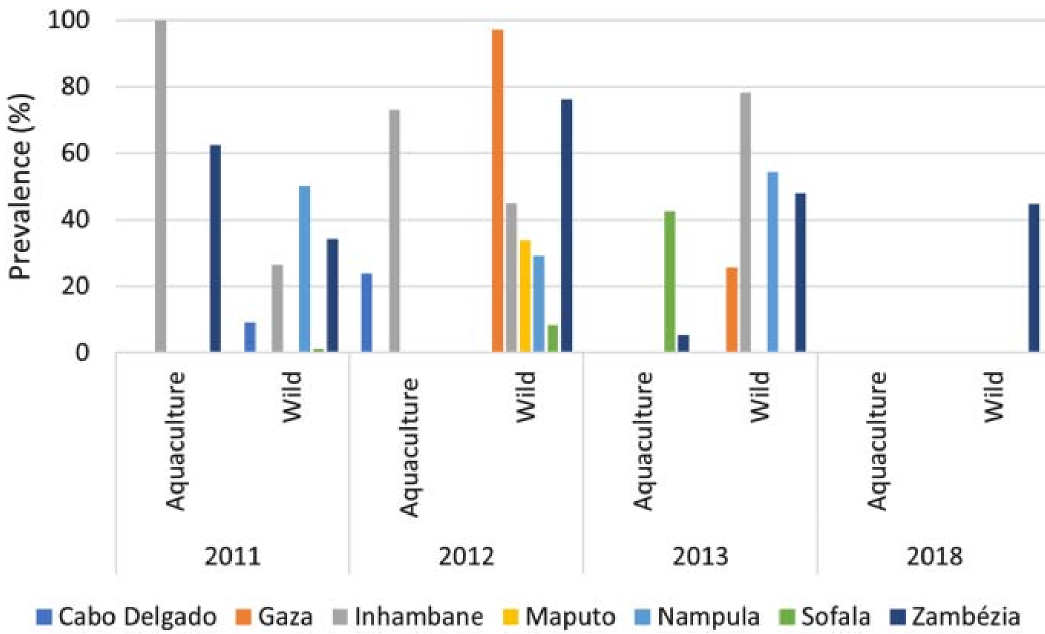

Fig. 2. WSSV prevalence in the seven coastal provinces by year and type of crustaceans

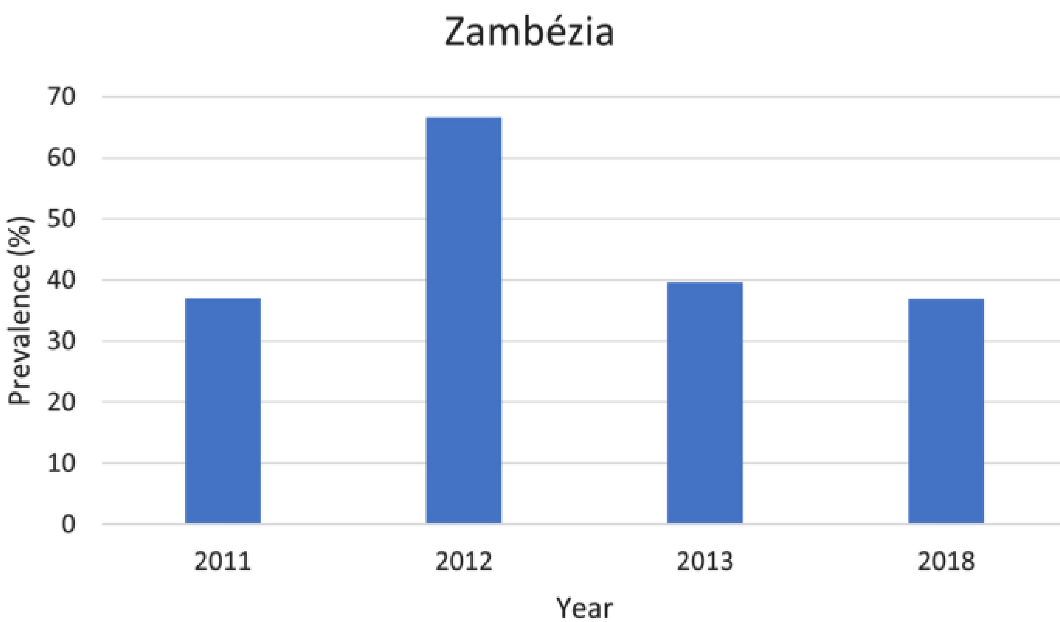

Fig. 3. WSSV prevalence in Zambezia province for 2011, 2012, 2013, and 2018

Comparing the results from Zambezia province during the whole sampling period it was observed that the WSSV prevalence increased from 37% in 2011 to 66.7% in 2012, however it decreased to 39% in 2013 and then 36% in 2018 (Fig. 3, Kruskal–Wallis test: $H_{(3, 1230)} = 39.63$, $p = 0.00$. post-hoc: $p = 0.00$). The same trend was revealed when analyzing wild crustaceans from Zambezia (Table S1), while farmed shrimps revealed a pronounced decrease from 62.2% in 2011 to 0% in 2012 and then a slight increase to 5.3% was noticed in 2013 followed by a reduction to 0% in 2018. Crab populations had an increasing prevalence from

2011 to 2012 and 2013 (33.1, 40.3 and 60.5%), whereas the lowest prevalence (10.3%) was detected in Zambezia (2018).

Overall, wild shrimp populations seemed to have a greater prevalence of WSSV during the sampling periods compared to aquaculture populations (Fig. 4, Mann–Whitney test: U (N_{Wild} = 2987, $N_{Aquaculture}$ = 493) = 467,070, z = 6.52, p = 0.00). The prevalence of WSSV in wild crustaceans was 29.3% and in farmed ones was 14.4%. Although crab samples were not collected from every sampling province and dates, the analyzed data revealed low prevalence of WSSV in crabs (21.8%, 698 out of 2987 total wild crustaceans) compared to the total prevalence of WSSV in wild shrimps (31.6%, 2289 of 2987 total wild crustaceans) (Fig. 4). The highest WSSV positivity was detected in the wet season, specifically in November and December of each year (Table 2).

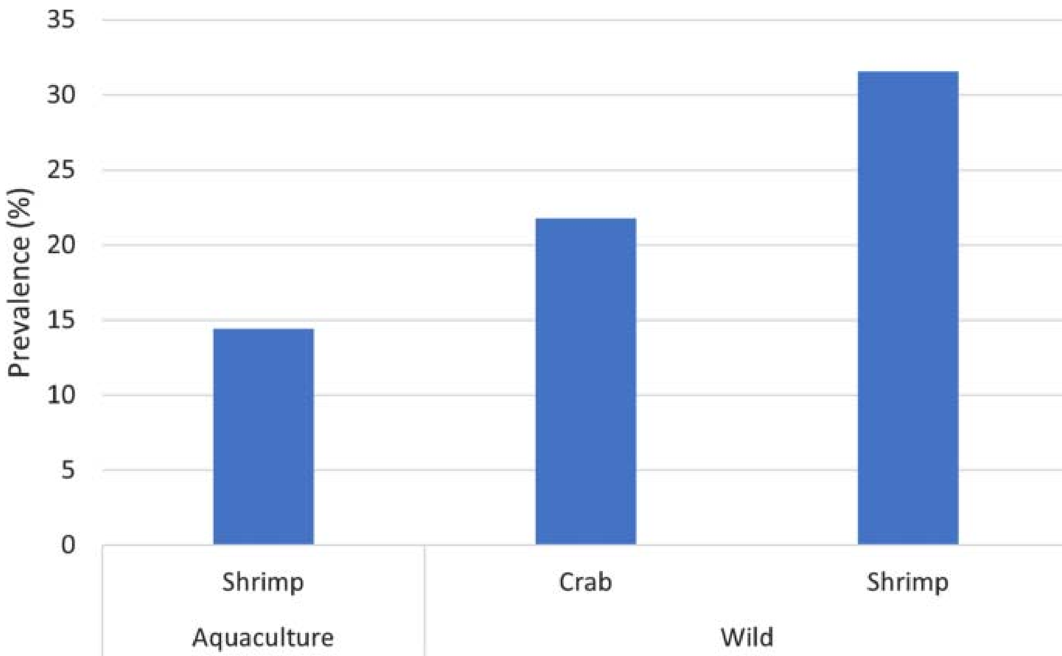

Fig. 4. WSSV prevalence in crustaceans from aquaculture and wild environments collected in Mozambique

Table 2. WSSV prevalence (%) by season, origin and species collected in Mozambique

	2011		2012		2013	2018	
	Dry	Wet	Dry	Wet	Wet	Dry	Wet
Aquaculture	**62.5**	**100.0**	**12.8**	**14.0**	**30.5**	**0.0**	
Shrimp	62.5	100.0	12.8	14.0	30.5	0.0	
Penaeus indicus					8.0		
Penaeus monodon	62.5	100.0	12.8	14.0	5.3	0.0	
Penaeus sp					100.0		
Wild	**47.2**	**19.4**	**0.0**	**24.1**	**39.5**		**44.7**
Crab	51.7	29.3		40.3	60.5		10.3
Not identified		33.3					
Portunus pelagicus		35.1		33.3	60.5		
Scylla serrata	73.3	27.3		100.0			10.3
Uca sp	28.6	8.7					
Shrimp	41.7	16.0	0.0	23.1	36.9		96.1
Acetes indicus	50.0	100.0					
Exopalaemon styliferus					100.0		
Macrobrachium rosenbergii	25.0	41.7					
Metapenaeus monoceros		33.3					
Not identified		53.7					
Penaeus indicus	33.3	9.6			46.3		96.1
Penaeus monodon	100.0	33.3	0.0	2.3	83.8		
Penaeus sp		0.0		56.8	25.2		
Grand total	**50.7**	**20.5**	**5.8**	**23.1**	**38.3**	**0.0**	**44.7**

Bold numbers refer to the total prevalence per group

Over the years, Gaza, Inhambane and Zambézia had significantly higher WSSV prevalence when compared to other provinces (Table 1 and Fig. 2, Kruskal–Wallis test: $H_{(6, 3480)} = 500.82$, $p = 0.00$). In general, higher WSSV infections were observed in wild samples from all sampling areas from 2012 to 2018, except for Cabo Delgado and Inhambane (2012), and Sofala (2013) where higher infections were reported in aquaculture samples (Table S1).

Overall WSSV prevalence was higher in wet seasons, except for 2011 (Table 1). In aquaculture shrimp, it was registered a fluctuating prevalence. In the wet season in 2011 the prevalence was 100% just after the first detection of WSSV and it dropped in 2012 to 14% and increased again in 2013 to 30.5%. The average lowest prevalence in aquaculture shrimp was 0% in the dry season in 2018 and the highest was 100% in the wet season in 2011 with more contribution of *Penaeus monodon*. On the contrary, wild populations had an increasing trend with a pronounced prevalence in the wet season that ranged from 19.4% (2011), 24.1% (2012), 39.5% (2013) and 44.7% in 2018. The highest prevalence was registered in 2011 in the dry season (47.2%).

3.2 WSSV severity levels in crustaceans

Cumulative severity infection levels of WSSV in crustacean samples were estimated through the IQ2000™ WSSV Detection and Prevention kit nested PCR. The degree of infection severity was qualified as very light, light, moderate, and severe following the band patterns described in the instructions manual. Figs. 5, 6 show the rates of the degree of infection by WSSV in wild and aquaculture crustaceans. Aquaculture shrimp had a higher moderate to severe infection rate by WSSV than wild shrimp (Fig. 5). Furthermore, comparing the two tested samples it was reported a higher moderate and severe infection rate in shrimp than in crabs (Fig. 6). Comparing the degree of the severity between species (Fig. 6) it varied from higher moderate to severe, with higher severity in *Penaeus indicus* in both farmed and wild shrimps.

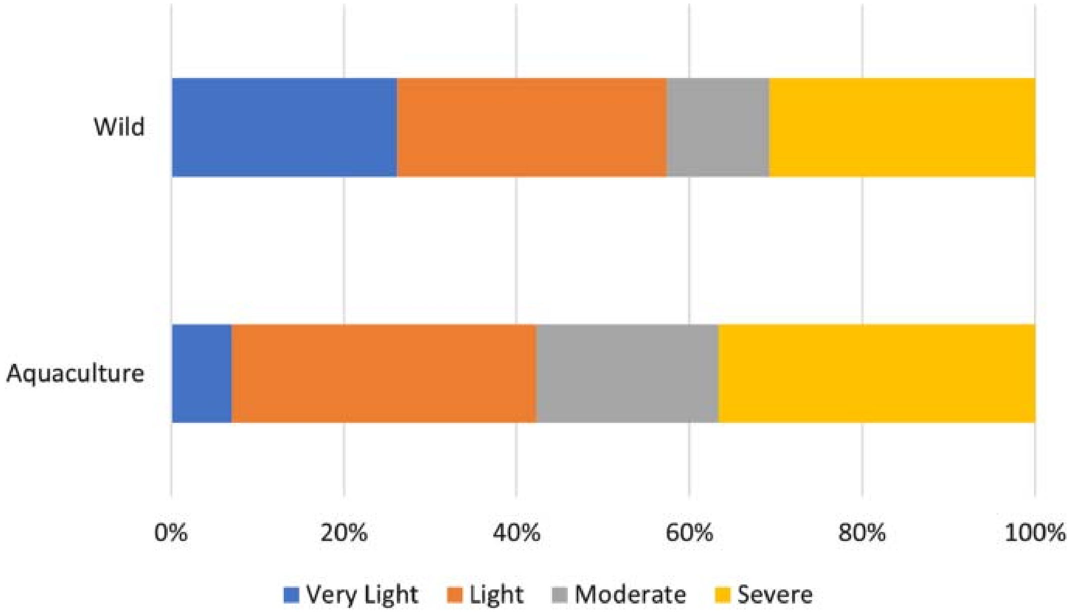

Fig. 5. WSSV degree of infection in wild and farmed shrimp collected in Mozambique

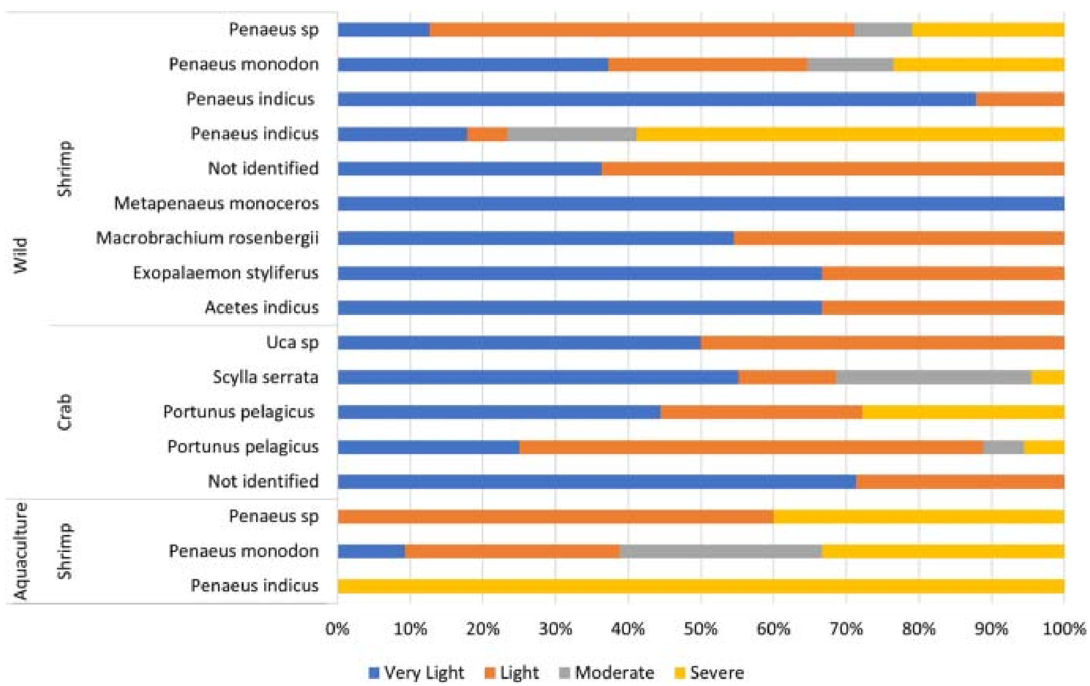

Fig. 6. WSSV degree of infection in crustaceans by species collected in Mozambique

4 Discussion

WSSV is a cosmopolitan disease responsible for high mortality and huge economic loss in the crustacean industry in more than 30 countries (Yan et al. 2007; Hoa et al. 2011; Gonçalves-Soares et al. 2012). The results from the present study on the prevalence of WSSV on crustaceans revealed the evidence that the disease is widespread in the seven studied coastal provinces from Mozambique. Gaza, Inhambane and Zambézia provinces had significantly higher WSSV prevalence when compared to other provinces. Variation on the prevalence was observed between provinces and dates, where in Cabo Delgado, Gaza and Maputo the prevalence decreased progressively from 2011 to 2013, while in Inhambane and Gaza the prevalence first increased from 2011 to 2012 and then reduced to low levels in 2013. In general higher prevalence was observed in the first 2 years of surveillance. These results were expected, since the first outbreak of WSSV in Mozambique occurred in September 2011 (Brummett 2014) and nothing was known regarding the WSD and no strategy to control the disease was in place. After the first report of WSSV in Mozambique, measures were discussed and efforts were put in place to reduce and/or eradicate the disease.

WSSV was detected both in shrimp and crabs and also in wild and farmed crustaceans. Results shown in table S1 revealed a consistent higher prevalence in the wild crustaceans. As described by Chakraborty et al. (2002) and Walker et al. (2011) the extreme complexity and the wide range of hosts of the virus contribute to the rapid spread as well as the infection of several species from different sources (Chakraborty et al. 2002; Walker et al. 2011). The lower prevalence observed in farmed crustaceans can be explained by the fact that they are farmed under a controlled environment with minimum biosafety measures in place, therefore the disease control is more effective than the wild environment. In addition to that, Otta et al. (1999) and Chamberlain et al. (2013), reported that wild crustaceans can be the reservoir of WSSV and the source of infection for aquaculture. Furthermore, Rozenberg et al. (2015) and Mijangos-Alquisires et al. (2006), supported the idea that WSSV might have originated from certain wild species in natural waters, and then dispersaed of WSSV from infected shrimp farms to the marine environment. On the other hand, this result contrasts with the findings from Vaseeharan et al. (2003) in India, Cavalli et al. (2008) in Brazil and Walker et al. (2011) in Thailand, where the farmed shrimp presented higher infection rates than the wild crustaceans.

WSSV prevalence in crabs was 21.8% (152 WSSV positive samples out of 698 total analyzed crab samples), a relatively low prevalence compared with the 31.6% of wild shrimps (723 out of 2289 total analyzed shrimp samples). The prevalence was high in 2011 (33.1%), 2012 (40.3%) and 2013 (60.5%) and low in 2018 (10.5%) (Table S1). Higher prevalence observed from 2011 to 2013 may be related to the period of high infection rate verified in Mozambique immediately after the first outbreak. WSSV in crabs has been reported in other studies with relatively low (Sethi et al. 2011) to high prevalence reaching 60% (Chen et al. 2000). These findings are similar with the ones reported in India (8% prevalence in mud crabs) and Taiwan (60% prevalence in larvae from mud crabs) respectively.

The general decrease of the WSSV prevalence in wild and aquaculture samples was observed in Cabo Delgado, Gaza, Inhambane and Maputo from 2012 to 2013, and up to 2018 in Zambezia. These findings may be related to the implementation of the specific pathogen-free

(SPF) system adopted recently by some shrimp farms to control WSSV infections and recovery from the losses caused by the disease, with promising results as reported by one of the farm technicians. This system has been implemented by many asian countries impacted by the WSSV, that stopped the use of wild broodstock and instead implemented the specific pathogen-free system, resulting in quick recovery of shrimp farms and effective control of the disease (Alday-Sanz 2018). At contrary, a consistent increase was observed in the same period in Sofala and Nampula. However it is important to notice that in these two provinces relatively low number of samples were collected in 2013 compared to 2012 (Table S1), which may have influenced the results.

According to the results of this study, the initial objective of filling the gap in knowledge regarding the WSSV situation in Mozambique was achieved, but little can be said regarding the evolution of the disease from 2011 to 2013 for all seven provinces and the seasonality of the disease. This is due to missing data for some provinces from 2011 until 2018. Unfortunately it was not possible to collect samples from every sampling area and seasons. However, from the available data it is possible to infer that higher prevalence of WSSV was observed in the wet season (Table 1). Furthermore, additional studies have to be conducted in the seasonality of the disease to confirm our findings.

The PCR used in the present study to screen for the presence of WSSV infections is a sensitive method and has been extensively used in studies to detect the disease (East et al. 2004; Chapman et al. 2004; de La Peña et al. 2007; Walker et al. 2011). Moreover, the currently used PCR allows for the detection of infections at the early stage before disease is widespread in the pond. Therefore, a decision on whether to keep or discard the broodstock can be taken, helping to reduce the negative impacts of WSSV in the shrimp farming industry. The technique can also be used to explore the WSSV transmission routes and for epidemiological studies (Hoa et al. 2005). The comparison of infection degrees in this study showed that farmed shrimp had pronounced rates of moderate to severe infections than wild shrimp. This is probably due to the fact that in confined environments, WSSV infection can result in severe cases and mortalities of up to 100% (Pradeep et al. 2012).

Only crustaceans (shrimp and crabs) from both wild and aquaculture environments were screened in the study. However, it is known that the virus can be detected in other animal species (Vanpatten et al. 2004; Lo et al. 2006; Yan et al. 2007; OIE 2009). This fact is important when considering the source of contamination of shrimp aquaculture ponds in the country and should be further investigated.

The overall prevalence detected in the study varied from 25.4% (2011), 20% (2012) to 38.3% (2013) and then decreased to 36.8% (2018). Mozambique had the first outbreak of WSSV in 2011, and as we can see from the results, higher prevalence was observed in the first 2 years, but then decreased significantly in 2018. Relatively high WSSV prevalence was also reported in Japan (Mushiake et al. 1998; Maeda et al. 1998), and (Withyachumnarnkul et al. 2003), which detected 9, 25, and 18%, respectively. No clear explanation can be given to the variation between the years and between the provinces. WSSV prevalence in Zambezia Province is still remarkable seven years later, in samples from wild crustaceans. However, negative results observed in aquaculture shrimp in 2018 may be due to the implementation of the specific pathogen-free (SPF) system in the major sampled shrimp farms (Brummett et al. 2014). A few studies have shown the persistence ability of WSSV to be detected in soil ponds (over 10 months) and surrounding areas for long periods after an outbreak (Natividad et al. 2008; Quang et al. 2009). This ability might have a positive impact on WSSV transmission. Therefore, the increasing trend of WSSV infection noticed in most of the sampling sites throughout the sampling years could be attributed to the increase of the spillover and dispersal of WSSV from farm ponds to the sea and vice-versa. The high prevalence of WSSV in Mozambique can also be attributed to other factors such as water quality and environmental conditions such as fluctuation of temperature, salinity and presence of other pathogens that can cause stress to the animals (Usman et al. 2018, Tendencia and Verreth 2010). Contrary to the results of the present study, Xu et al. (2020) reported in a study conducted from 2016 to 2018 in the Bohai Sea, a downward trend of the WSSV prevalence year by year. The decreasing trend on the infection rate observed by Xu et al. (2020) was attributed to the effort that the government was making in guiding local farmers to use WSSV-free post-larvae of shrimp together with the strict quarantine policy of seedlings implemented. As the higher mortality of infected animals was attributed to the stressors, some studies have speculated the improvement of the surrounding environment along the seashore as the most effective way to prevent the negative impact of aquaculture in the wild environment (Zhu et al. 2019; Xu et al. 2020).

Understanding the epidemiology and distribution of WSSV is of paramount importance to manage the threat of the WSD. The first results of WSSV positive samples from 2011 (included in the present study), confirmed by the laboratory of reference at the University of Arizona (Brummett et al. 2014), served as baseline information to design and implement guidelines to control WSD in Mozambique. As for the aquaculture industry and Mozambican fishery in general, there were huge economic losses caused by the WSD. Therefore, the present survey will contribute to determining the extent and prevalence of WSSV, and identifying the most affected provinces in Mozambique. Moreover, the study demonstrated the need for constant monitoring of the presence of WSSV in aquaculture and wild environments to implement corrective measures to prevent contamination. The present study did not focus on the seasonal variation component therefore we recommend future studies to investigate how the season may affect the prevalence of WSSV in Mozambique.

5 Conclusions

Based on the results of the present study it is concluded that WSSV disease is present in Mozambique and affects both wild and aquaculture crustaceans. Higher WSSV prevalence was demonstrated to be present in wild crustaceans. Gaza, Inhambane and Zambézia provinces had significantly higher WSSV prevalence when compared to other provinces. Between different years, higher prevalence was reported in 2013. Therefore, it is crucial that more awareness is done and effective measures are adopted to control the trend of infection by WSSV observed in this study.

Data availability

Not applicable.

Code availability

Not applicable.

Acknowledgements

To the Aquaculture Institute from Mozambique (INAQUA), Fisheries Inspection Institute from Mozambique (INIP) and Aquapesca who provided samples to be analyzed in our lab. To Shrimp Farming Association (APCM) in Mozambique, who partially supported this research through the acquisition of reagents? To Italian Cooperation who supported the research through consumables acquisition and financed equipment maintenance. To CB-UEM where the analysis was performed, especially to Nuria Negrão.

Funding

None.

Contributions

The following authors have equally contributed to this work by analyzing samples in the laboratory, statistical analysis of the data, article writing: AMM, OP, DB, FM. JF and AM: analyzed the samples in the laboratory. AMM, LdeA, and JLB: had contributions in the sample analysis in the laboratory and in the article writing up. EP, IO, SV, SEG, LM: designed the sampling plan and collected the samples. DC, LN and ET: conceived the work, trained personnel and supervised project activities.

Ethics declarations

Conflicts of interest

None of the authors have any conflict of interest to declare.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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