



White Spot Syndrome Virus (WSSV) risk factors associated with managerial shrimp farming practices through expert opinion in monoculture farms in Iran

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Abstract

The objective of this study was to investigate the relationship of managerial factors with incidence WSSV severity in shrimp farms located in Golestan province of Iran. This research was carried out in cooperation with Iran Fisheries Org- Office of Shrimp, General directorate of Golestan Province, in cooperation with the Fisheries Science Research Institute and the Stock Assessment of Inland Water Research Center, on the shrimp farms of Gomishan site in Golestan, in 2019. The data were designed using a questionnaire addressing thirteen variables such as educational level, temperature fluctuation, Mortality in shrimp farms, removing the pond bottom sludge, stocking density of PLs and pre-stocking test. According to the opinion of shrimp farmers, Pearson's Chi-square showed a significant relation between severity of mortality and weather temperature ($p=0.049$). effect of types of monthly salary on the severity of WSSV occurrence has been significant. shrimp PLs stocking in ponds showed a significant effect on the severity of white spot disease WSD. It is concluded that a few managerial factors such as PLs origination, lowering the temperature, pre-stocking test and stocking density are critical indicators, which can effect on severity of mortality in WSSV outbreak.

Keywords: Cultured shrimp, WSSD, Managerial factors, Gowmishan, Golestan

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Introduction

The rapid expansion of aquaculture and the growth of domestic and international demand for shrimp production have created the conditions for intensifying shrimp farming by increasing production capacity and stock density (Ebnetorab *et al.*, 2020). Thus, the prevalence of diseases such as shrimp white spot disease and their spread within the borders and outside the geography of countries has developed. Shrimp white spot disease accounts for a large share of the \$ 20 billion in economic losses suffered by the shrimp farming industry in Asia (Davies, 2016). In most shrimp producing countries, the system used is the semi-intensive system to prevent diseases, but some problems especially WSSV still cause huge mortality in shrimp farms in most countries (Hasan *et al.*, 2020). Shrimp farming has expanded throughout the world, especially in East, Southeast Asia, and in Latin America, so that the global production of farmed shrimp increased from 50,000 tons in 1975 to more than 4.5 million tons in 2018 (FAO, 2018).

During the last three decades that the shrimp industry in Iran has reached its peak, the white spot syndrome virus (WSSV) caused great force to farmers (Bokaie *et al.*, 2012). This has led to the fact that during the past years especially in the last two decades, sometimes the predictions made in the five-year development plans did not come down (Radkhah *et al.*, 2014). The amount of fishery production in the country in 2020 was about 1,200,000 MT of which the total aquaculture production has reached

approximate 550,000 MT. Out of this amount, 49,000 MT were related to shrimp production (IFO, 2021). According to the same report, the active area of shrimp farms in Iran until 2021 was about 12,000 ha and the capacity of the studied cultivable area is estimated at nearly 200,000 ha. There are more than 35 hatcheries in Bushehr and Hormozgan provinces addition to two centers in Sistan and Baluchestan province. Khuzestan and Golestan provinces did not have active hatcheries units in 2021 (Madani, 2021). Accordingly, the rate of farm involvement with white spot disease (WSD) has been 10-14% of all farms in the country. With this calculation, approximate 5,000 MT of the Iran shrimp production is removed from the country's production circuit annually due to mainly shrimp diseases. The results of research projects conducted in Iran during the last two decades indicate the role of managerial factors, climate, knowledge and educational level of farm staffs in shrimp production in the country (Radkhah *et al.*, 2014).

In a study in the Delta Mekong area of Vietnam (Duc *et al.*, 2015), it was suggested that larger shrimp farms have poorer management potential, especially in water supply and sanitation, and therefore disease is more likely to occur. Similarly, it was found that smaller pools produce more than larger pools (Muniesa *et al.*, 2017). In addition, depending on the characteristics of each pond, the rate of disease transmission in larger ponds can be higher (Ruiz-Velazco *et al.*, 2010). The higher the

pool water level, the less likely it is that the water temperature will fluctuate during the day or night, resulting in less stress on the shrimp (Tendencia *et al.*, 2011). Timely change of water can positively cause the release of uneaten foods, waste products in the ponds, soluble and insoluble nitrogen from water. Lack of timely discharging of these substances can stimulate and encourage pathogens to grow and multiply (Duc *et al.*, 2015). Shrimp stocking has been considered as a risk factor in shrimp ponds (Tendencia *et al.*, 2011; Duc *et al.*, 2015). In a study conducted in the Philippines (Tendencia *et al.*, 2011), managerial indicators were used as a model. Climatic conditions, size of ponds, removal of black soil, pre-stocking test of post-larvae (PLs), etc. were studied. Water reservoir pond or in other words, sedimentation pond plays as an effective role in reducing the entry of organic suspended solids into ponds and decreasing the incidence of diseases in pool shrimp (Tendencia *et al.*, 2012). season can regulate the prevalence of WSSV (Withyachumnarnkul *et al.*, 2003). several environmental stresses such as temperature fluctuation, salinity reduction and pH variability can be the main predisposing factors for WSSV (Millard *et al.*, 2020). In addition, cannibalisms of infected and dead shrimp could encourage further WSSV infection (Lotz and Soto, 2002).

According to aforementioned studies, the objective of this study was to determine the correlation of managerial characteristics with incidence WSSV

severity in shrimp farms located in Golestan province of Iran.

Materials and methods

This research was carried out in cooperation with the Iran Fisheries Org-Office of shrimp, General directorate of Golestan Province, in cooperation with the Fisheries Science Research Institute and the Inland Water Resources Assessment Research Center, on the shrimp farms of Gomishan site in Golestan, in 2019.

Definition of terms

Due to the mortality was observed in all farms of the Gomishan site, severity of WSSV incidence means that whether in time of incidence the severity was moderate or severe. Monoculture means that no species in addition to *Penaeus vannamei* was co-cultured in shrimp ponds. Education level refers to a degree of education. Temperature fluctuation refers to the remarkable changes of weather temperature measured on time of mortality. Salary refers to types of wage of each worker gained after each month working in farms. Pond bottom preparation refers to pond preparation before next crop including removal black soil (sludge), liming, plowing and leveling the pond bottom. Stocking density means individuals stocked in the pond per m². Aerators refers to paddle wheels used in the ponds. Post larvae refers to Origination of PLs bought by farmers. Pre-stocking test refers to salinity or formalin test was carried out for PLs before stocking. Water filtration refers to water filtration system installed

in the water entry of farm. Feed-stock refers to an independent room kept shrimp feeds. sharing refers to devices and workers shared among farms.

Therefore, independent variable (severity of mortality), dependent variable with their categories and dedicated scores were given in Table 1.

Table 1: Risk assessment for severity of outbreak of white spot disease in shrimp ponds: managerial variables at the farm level.

#	Risk factor and categories	score
	Educational level	
	Under diploma	1
	Diploma	2
1.1	Technician & Bachelor	3
	Master & PhD	4
	Mix but used three former categories as more	5
	Mix but used Master and PhD as more	6
	Mortality	
1.2	Mild	1
	Moderate	2
	Severe	3
	Temperature fluctuation	
	No	0
1.3	Low	1
	Mild	2
	severe	3
	Type of salary	
1.4	Fixed	1
	Fixed and monthly tip	2
	Fixed and crop sharing	3
	Pond preparation	
1.5	No	0
	Yes	1
	Stocking density	
	20	1
1.6	21-30	2
	31-40	3
	41 and more	4
	PLs origination	
	Mh.gr	1
1.7	Am.Ja	2
	Gh.Le	3
	Am.Gh	4
	Pre-stocking test	
1.8	No	0
	Yes	1
	Filter installation	
1.9	No	0
	Yes	1
	Feed-stock	
1.10	No	0
	Yes	1
	Probiotic	
1.11	No	0
	Yes	1
	Aerator	
1.12	No	0
	Yes	1
	Sharing	
1.13	No	0
	Yes	1

Data collection

The data were designed using a questionnaire addressing thirteen variables which explained in the Table 1. The selection of these criteria were based on farming conditions, techniques employed by shrimp farmers and on the measures were probable associated with WSSV occurrence and prevention mentioned in the Table 1.

The questionnaire was developed in Farsi language and distributed among 15 farmers. It was pre-tested to realize and validate it with 5 shrimp disease specialists and farmers. Therefore, the study questions were retrieved based on the feedback of them. The final questionnaire was finalized by the main author and implemented by co-researchers from the provincial and central units or from the local cooperatives. It was emphasized to farmers that responses and names of aquaculture companies were confidential therefore, accuracy of the answers was needed for the appropriate analysis of the results.

Due to quarantine measures were implemented in the Gowmishan site, interviewers were not permitted to attend farms in most cases but directly linked to farm owners. This made the authentication of the gathered data.

Statistical analysis

A total of 13 variables (please refer to column 1 of Table 1) were applied for the analysis. Data collected were mostly binary (yes/no) except for PLs origination, temperature fluctuation, education level, types of salary, stocking

density, pond bottom preparation and severity of mortality. Pearson's chi-squared, likelihood and Fisher's exact tests were used to find the correlation of the selected independent variables with severity of WSSV occurrence in multiple and 2×2 cross-tabulations, respectively. All the statistical analyses were done using SPSS®26.

Results

Table 2 shows the relation between severity of mortality due to WSSV occurred in Golestan province located in North-east of Iran and independent variables at farm level.

Educational level of workers and experts of shrimp farms had no effective association with severity of the outbreak ($p>0.05$). According to the opinion of shrimp farmers, Pearson's Chi-square showed a significant relation between severity of mortality and weather temperature ($p=0.049$) (Table 2). This finding confirmed by the weekly and daily temperature data presented in Figure 1.

Based on the results, effect of types of monthly salary on the severity of WSSV occurrence has been significant (Pearson-Chi-square=10.90, $p=0.004$, Likelihood ratio=12.39, $p=0.002$). All of farms that suffered severe losses had a fixed monthly salary for their employees, in contrast to only 9.1% of farms that suffered mild-moderate losses paid fixed salaries to their employees.

Table 2: Statistical assessment risk factors for evaluation the severity of outbreak of white spot disease at the farm level.

Risk factor	Pearson Chi ² -(Sig.)	Likelihood (Sig.)	Fisher's exact (Sig.)
	value	value	
Educational level	0.32	0.27	-
	3.49	3.90	
Temperature fluctuation	-	-	0.049
Type of Salary	0.004	0.002	-
	10.91	12.39	
Pond preparation	-	-	0.476
Stocking density	0.001	0.00	-
	15.00	17.40	
PLs origination	-	-	0.004
Pre-stocking test	-	-	0.011
Filter installation	-	-	0.270
Feed-stock	-	-	0.057
Probiotic	-	-	1.00
Aerator	-	-	0.103
Sharing	devices	-	0.267
	workers	-	0.095

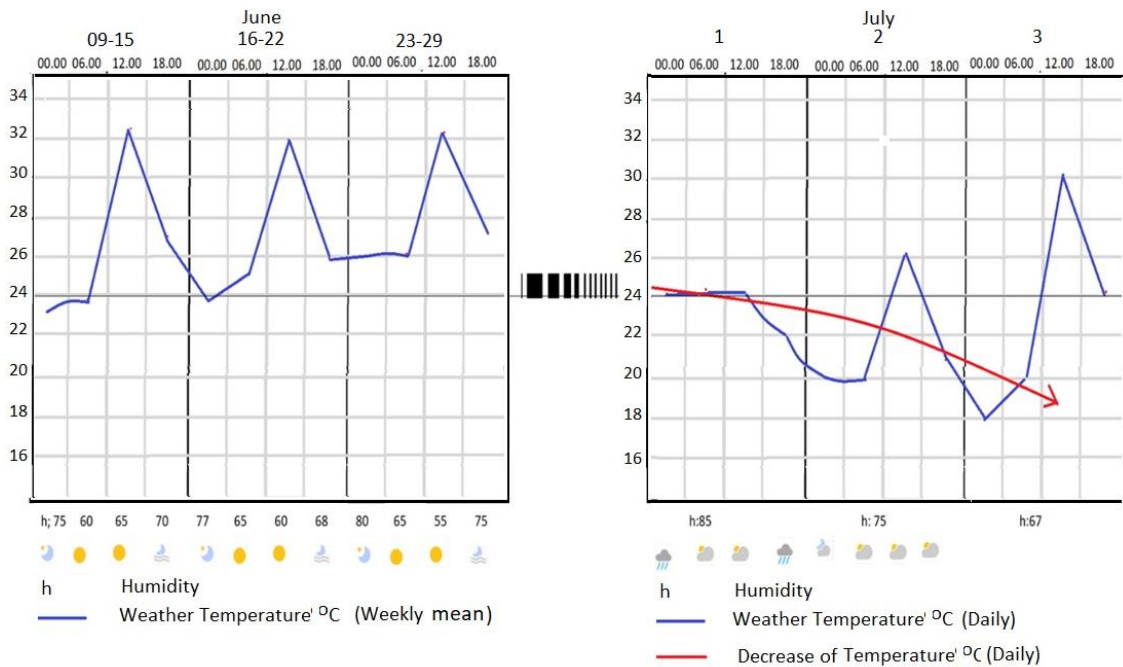


Figure 1: Weather fluctuation close to the outbreak of Gowmishan site-Golestan province in 2019.

81.8% and 9.1% of later farms, in addition to salaries, provided some rewards and part of the production to their employees, respectively. According to Fisher's exact test, the

effect of removing the bottom sludge on the severity of shrimp WSSV was not significant ($p=0.476$). Approximately 90.9% of farms with low to moderate mortality occurred during the disease,

removed the black soil from ponds, while 75% of farms that were severely affected by the disease, removed the black soil. Accordingly, there was no significant difference in the severity of losses in farms with black soil harvest and those did not ($p>0.05$). It is worth mentioning that all the studied ponds had been plowed ponds before the stocking. The effect of number of shrimp PLs stocking in ponds on the severity of white spot disease (WSD) was significant (Pearson- $\chi^2=15.00$, $p=0.001$, Likelihood ratio=17.39, $p=0.001$, Table 2). All farms that experienced mild-moderate losses stocked 21-30 shrimp/m² in their ponds, while 25% and 75% of farms that experienced severe losses in their farms stocked 31-40 and 20 PLs/m², respectively (Fig. 2).

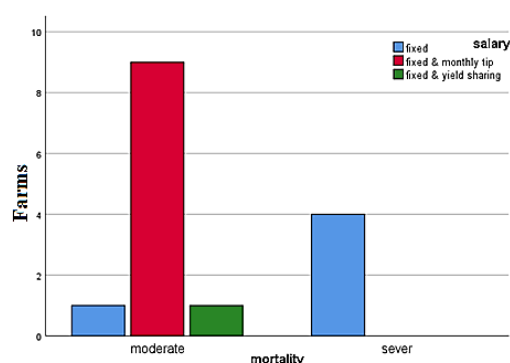


Figure 2: Variety of salaries of shrimp farms and its effect on the severity of disease losses.

Based on the results of Table 2, the effect of shrimp PLs origination on the severity of shrimp WSD losses was significant (Fisher's Exact test, $p=0.004$). About 90.9% and 9.1% of the farms that had low-moderate mortality in the time of occurrence of the disease, provided their

shrimp PLs from group 2 and 1 hatchery centers, respectively; while 100% of the farms that were severely affected by WSSV, farmers got their PLs from group 1 breeding centers (Fig. 3).

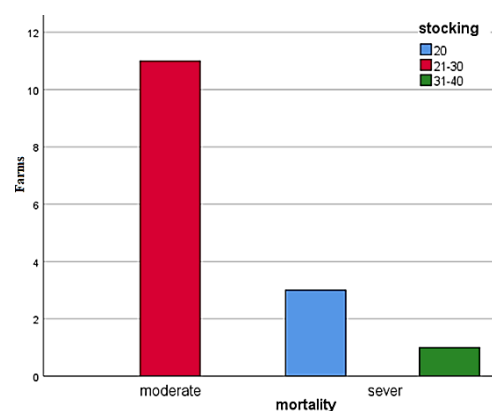


Figure 3: The amount of shrimp PLs stocked in ponds and its relation to the severity of disease losses in Gomishan farms.

According to the results of Table 2, the relationship between pre-stocking test (such as salinity or formalin test) and the severity of WSD was significant ($p=0.011$). One hundred percent of the farms that suffered severe losses did not perform the pre-stocking test for shrimp PLs. This is usually done on the farm pre-stocking, although it has been normally accomplished at the Shrimp hatchery center. In contrast, only 18.2% of the farms with mild to moderate losses did not perform the pre-stocking test but 81.8% of such farms had performed this test. According to Fisher's Exact test (Table 2), the relationship between filter installation and severity of WSD was not significant ($p=0.267$). Whole farms with mild to moderate losses and 75% of farms with severe losses have installed filtration. Therefore, it seems that the intensity of losses in farms was not

related to the filtration variable ($p>0.05$). Accordingly, the relationship between the existence of an independent warehouse in the field and the severity of shrimp WSD losses was not significant (Fisher's exact test, $p=0.057$). One-hundred percent of farms with low to moderate mortality occurred in the field, using independent on-farm warehouse, while 50% of farms that were severely affected by disease losses did not have an independent warehouse (Table 2).

Based on the Fisher's exact test, the relationship between shrimp feeding accompanied with probiotic and the severity of shrimp WSD was not significant ($p=1.00$). 90.9% of farms with low to moderate mortality and 100% of farms that were severely affected by WSD did not feed their shrimp with probiotics.

Statistical relationship between paddlewheel aeration and the severity of shrimp WSD outbreak of Gomishan site in 2019 (Fig. 4) was not significant (Fisher's exact test, $p=0.103$). In that year, 45% of farms with mild to moderate losses and 100% of farms with severe losses were deprived of aeration (Table 2). Therefore, it seems that the severity of losses in farms was not related to the applied the paddlewheel aerator ($p>0.05$). In this study, it was found that the sharing work-forces and equipment among farms has not been done in the production of 2019 in Gomishan site (Fig. 5).

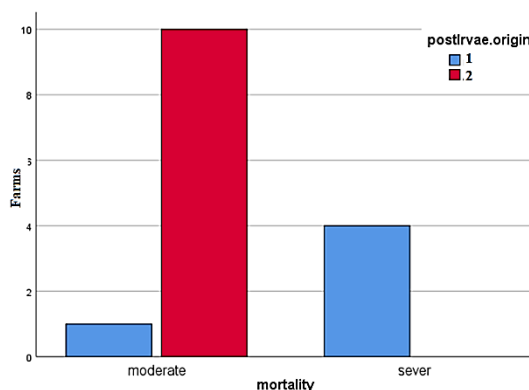


Figure 4: Shrimp PLs hatchery and its effect on WSD severity in shrimp farms of Gomishan.

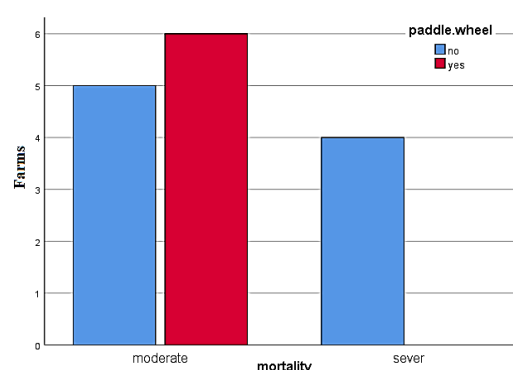


Figure 5: Pedal wheel aeration and its association with the disease severity.

Discussion

In this study (Table 2), it was found that the level of education of employees had no significant relationship with the severity of white spot shrimp disease in Gomishan Golestan in 2019. In a study (Karim *et al.*, 2014) it was found that the level of training of farm workers was not related to technology acceptance and possibly disease control. However, they believed that older people are better at accepting new content and training. The impact of quarantine-health management and foreman experience in the first stage of the epidemic suggests that farmers who did not comply biosecurity issues were the initiators of

the shrimp white spot epidemic (Bokaie *et al.*, 2012). As a result of this study, it was found that timely application of salinity or formalin tests before stocking shrimp PLs can prevent incurable diseases (Flegel *et al.*, 2004). Conversely, biosecurity measures such as crab fencing or bird scarecrows could not effectively prevent the virus from entering the farm (Tendencia *et al.*, 2011). The study exhibited that farms suffered casualties did not undergo pre-stocking test for shrimp PLs. This is usually done on the farm and before stocking, although it has been accomplished at the Shrimp Reproduction Center. Obviously, if no pre-stocking test is performed, the WSSV infected shrimp PLs may enter the ponds and cause the shrimp to become infected at appropriate intervals (Mirzaei *et al.*, 2021).

Obviously, if severe stress such as a prolonged temperature drop within 24-48 hours with cloudy weather expose to shrimp, the virus multiplies rapidly in the shrimp body and causes severe losses in the pond (Cheng and Chen, 2000; Chen *et al.*, 2012; Kakoolaki *et al.*, 2015). At Golestan site, the air temperature and of course the water temperature dropped sharply and a lot of stress was entered to the shrimp for 72 hours before WSSV outbreak in 2019. In a study conducted in shrimp farms in Bangladesh (Hasan *et al.*, 2020), the main predisposing factor of WSD outbreak in Bangladesh in 2007 was considered to be high PLs stocking. WSD had then become endemic in traditional ponds in the extensive

system, spread to semi-intensive ponds, and when the stocking density of such these ponds increased, stress hit the shrimp and the virus was able to infect the system, overcoming immunity and cause disease or huge mortality. In a Philippine study, three managerial variables associated with shrimp white spot disease, but only one of them, stocking density, powerfully associated with WSSV (Tendencia *et al.*, 2011). In this study, stocking density showed that The higher the stocking density, the greater the chance of infection. Especially in areas where the workforces were not sufficiently experienced (Table 2), the use of high densities of shrimp PLS in rearing ponds could be considered as a risk factor and when other risk factors were added, WSD outbreak was heavily occurred. In another study (Talukder *et al.*, 2021), it was revealed that increased stocking and increased ammonia in pond water resulted in the incidence of shrimp WSD. They mentioned that the association of these indicators with the incidence of WSD are significant, especially after the monsoon and hurricanes. Therefore, the possibility that the occurrence of Golestan shrimp white spot disease in 2019 was related to floods of late 2018 was not far from the mind. According to shrimp farmers in the area, a large amount of flood-related stagnant water was observed in the farms at the beginning of the culture season in 2019 (Kakoolaki S., unpublished reports).

Water temperature affects the rate of virus replication. The optimal range for

this virus is 24-29 degrees Celsius. While above this range, temperatures play a protective role, and below this range, temperature increases the rate of virus replication (Muniesa *et al.*, 2017). It can be exhibited that one of the indicators of stress on shrimp in Gomishan Golestan in 2019 was the sudden change in water temperature, which occurred after raining and cooling the air on July 1, 2019 (Fig. 1). Climate change, especially during rainfall, which can cause changes in the aquatic environment of the rearing pond, has caused a change in salinity and the occurrence of shrimp white spot disease (Haque *et al.*, 2017). In a similar study (Tendencia *et al.*, 2011) only one factor in shrimp farming (climatic conditions) was associated with WSSV infection. The result was that the colder the weather in the area, the more likely it was to develop WSSV. However, climate was identified as a risk factor for WSSV only in co-cultivation or polyculture farms and was not identified as an effective disease predisposing factor in monoculture farms in that area. In this study (Figs. 1 and 2), the relationship between temperature and the incidence of shrimp white spot disease in Gomishan site was significant and a retrospective study of meteorological data showed that the air temperature has been cold during the 7 days before the disease occurrence and did become much colder and little rainy 3 days before WSD outbreak so that minimum temperature particularly at nights reached 18°C in the morning of July 3, 2018 (Fig. 1). This decrease in

temperature increases the proliferation of white spot virus in shrimp (Rahman *et al.*, 2007) and decreases shrimp immunity (Kakoolaki *et al.*, 2014). Virus loading was low at high water temperatures of 29-30 (Kakoolaki *et al.*, 2014) and 32°C (Granja *et al.*, 2006) compared to lower temperatures (25-22°C).

It is concluded that a few managerial factors such as PLs origination, lowering the temperature, pre-stocking test and stocking density are critical indicators, which can effect on severity of mortality in WSSV outbreak.

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