Evaluation of environmental and management risk factors effective in the occurrence of certain viral diseases in the production center of SFP rainbow trout in the Iran

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Abstract

One of the most important main challenges in the production of rainbow trout trout (*Oncorhynchus mykiss*) in Iran is the strong dependence of production on the import of eyed eggs and losses due to the occurrence of dangerous viral diseases which, while creating dependence on foreign countries, damages billions of Tomans annually to producers. This study was done aims to monitor and evaluation of environmental and management risk factors effective in the occurrence of certain viral diseases (IPN, IHN and VHS) in the production center of Specific Pathogens Free (SPF) rainbow trout in the Iran in order to achieve management guidelines and reduction of assessed risks. For this purpose, some physical, chemical and microbial factors of SPF center's incoming water were measured twice a month for 6 months from May to October 2017 based on standard methods. The results showed that the mean temperature, dissolved oxygen, pH, ammonia and total count were 17±1.55°C, 8.32±0.65 mg/l, 8.98±0.37 mg/l, 0.08±0.09 mg/l and 90.50±86.58 colonies per ml, respectively. The result of the examination of the contamination of the center fish with IPN, IHN and VHS viruses was also negative. In general, the results showed that in the management of the production of SPF rainbow trout, the introduction of IPN, IHN and VHS viruses has been avoided. In general, the results showed that in the management of the production of rainbow salmon free of specific pathogens, there has been an avoidance of the introduction of IPN, IHN and VHS viruses.

Keywords: Risk factors, environmental and managerial, viral diseases, rainbow trout, SPF

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Introduction

The relationship between aquatic organisms and the surrounding water environment is a vital relationship and water quality directly affects the health and growth of cultivated organisms (Boyd and Green, 2002). The water used in aquaculture contains organic and inorganic substances, and dissolved mineral ions, dissolved gases, suspended substances, dissolved organic compounds and microorganisms have an effect on water quality (Van Wyk *et al.,* 1999). Water quality changes under the influence of biological processes such as photosynthesis, respiration, metabolic waste disposal and physical factors such as temperature and management factors (Boyd, 1990). Industrial advances, development programs and infrastructure projects, despite all the advantages and benefits they have brought to humans, have been the source of many hazards, risks and significant failures in the environment. Therefore, the idea of preventing and controlling accidents and risks and maintaining the safety of people and the environment has been raised as the most important issue in development projects in recent years (Mirjalili and Mirjalili, 2009). Risk assessment is a logical method for determining the quantitative and qualitative size of risks and examining the potential consequences of possible accidents on people, materials, equipment and the environment. In fact, in this way, the effectiveness of the existing control methods is determined and valuable data is provided for making decisions in the field of risk reduction, hazards, improvement of control systems and planning to respond to them (Ghahramani, 2005). Establishing biosecurity is one of the important ways to prevent the occurrence of diseases in aquaculture facilities (Pruder, 2004). Biosecurity includes a set of measures that are taken to prevent the entry of a pathogen into a farm and also to reduce or prevent the spread of a disease within a farm or an area. The biosecurity program in fish propagation and breeding complexes includes regular monitoring and care of diseases, preventive measures, effective management during the outbreak of diseases, disinfection and cleaning between breeding periods and general protective measures. Biosafety includes a set of actions taken to prevent a pathogen from entering a farm, as well as to reduce or prevent the spread of a disease within a farm or area. The biosafety program in fish breeding complexes includes regular monitoring and supervision of disease, preventive actions, effective management during disease outbreaks, disinfection, cleaning between farming periods, and general protection actions (Horowitz and Horowitz, 2003). The main strategy and recommendation of FAO1 and OIE2 to compensate for the lack of production, increase biosecurity and health management in farms based on HACCP3 and GAP4 (FAO) (2008). The stages of risk assessment of a set have three main elements, including hazard identification, risk assessment of identified hazards, and providing suggestions for safety measures. Risk management planning is a process during which decisions are made for risk management planning regarding the activities of a project. Planning for risk management processes is important and it should be ensured that the level, type and transparency of the applied management is proportional to the risk

[1] Food and Agriculture Organization of the United Nations
[2] International Office of Epidemic Diseases
[3] Hazard Analysis and Critical Control Points
[4] Good Aquaculture Practices

and importance of the project for the stakeholders (Masoudi Ashtiani *et al.,* 2014). The most important goals and necessity of conducting risk assessment in the environment include creating awareness of various environmental hazards and risks, identifying sources of risk, identifying environments or species at risk, assessing the severity and scope of incompatible effects and possible risks, planning for control measures, Management and prevention, reducing the severity and recurrence of accidents, minimizing damage to ecosystems, providing safe conditions for the environment (humans, plants and animals), preparing and compiling related laws and regulations, and planning regular monitoring of ecosystems and their organisms and the evaluation of the effectiveness of the measures has been done (Monavvari, 2005; Ghale *et al.,* 2012; Shenavar *et al.,* 2009). One of the most important main challenges in the production of rainbow trout in the country is the strong dependence of production on the import of eyed eggs and losses due to the occurrence of dangerous viral diseases such as IPN1, VHS2 and IHN3, which, while creating dependence on foreign countries, damages billions of Tomans annually to producers (Wertheim *et al.,* 2009). One of the most important ways to increase production and productivity in this industry is to provide the possibility of producing and distributing healthy and pathogen free breeders, fry and eyed eggs in the country. The alarming current situation has demanded that the Iranian Fisheries Science Research Institute prepare and present the master plan to production of SPF4 rainbow trout in the country. The purpose of this project was to acquire and transfer technical knowledge of mass production of specific pathogens free rainbow trout in the country and to stop dependence on foreign products, which includes all components and stages of fish production, including breeder production and production of pathogen free lines, Propagation and breeding, pathology, genetic identification, monitoring of pathogens, water health management and improvement of biosecurity level based on the requirements set by OIE in SPF trout production center. Many studies have been conducted on the assessment of environmental and management risk factors effective in the occurrence of some specific viral diseases in Iran, which can be monitored and evaluated in selected farms of West Azarbaijan province (Nekuifard *et al.,* 2018), risk assessment and analysis the stages of the production of SPF Pa sfid shrimp (Litopenaeus vannamei) (Aein Jamshid *et al.,* 2016), monitoring the health status, pollutants and water quality (biotic and non-biotic factors) in the production of shrimp at the Bandargah Research Station (Ghaednia *et al.,* 2016) and the production of SPF shrimp in the Iranian shrimp research institute (Nourinejad *et al.,* 2016). Therefore, this study was done aims to monitor and evaluation of environmental and management risk factors effective in the occurrence of certain viral diseases (IPN, IHN and VHS) in the production center of Specific Pathogens Free (SPF) rainbow trout in the Iran in order to achieve management guidelines and reduction of assessed risks.

Materials and methods

This study was conducted from May to October 2017 in the location of the quarantine hall of the master plan to production of SPF rainbow trout located

[1] Infectious Pancreatic Necrosis
[2] Viral Hemorrhagic Septicemia
[3] Infectious Hematopoietic Necrosis
[4] Specific Pathogens Free

at the Coldwater Fishes Research Center- Tonekabon (Mazandaran Province, Iran) (Figure 1). The center's quarantine hall with an area of about 1250 m^2 has 60 concrete pools of 2 m^3 with separate entrances and exits and is equipped with an aeration and an automatic feeding mechanisms. This center has 2 water wells with a maximum flow rate of 14 l/s, a water storage tank, an ozone generator, an aeration tower, and a cooling system (chiller model R407C made by ISISO, Turkey). Pre-breeders of Rainbow trout from 7 farms (three farms in Mazandaran province, three farms in West Azerbaijan province, and one farm in Kohgiluyeh and Boyer-Ahmad Province) selected by the Iranian Fisheries Organization were transported to the project implementation site after obtaining health approval from the Iranian Veterinary Organization and Virology Laboratory of the Iranian Inland Water Aquaculture Center- Bandar Anzali (Guilan Province, Iran) according to the loading requirements and kept separately in separate ponds.

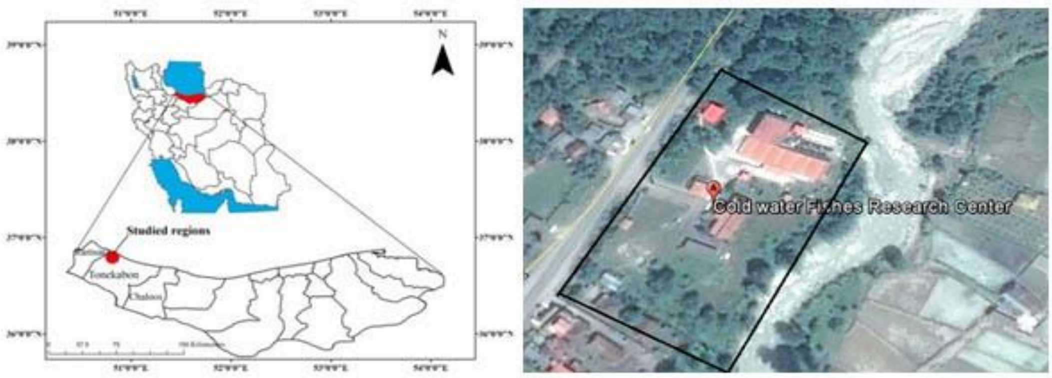

Figure 1: Aerial map and geographical location of the SPF rainbow trout production master plan.

Measurement of physical and chemical factors of water

Physical and chemical factors of water were measured monthly for 6 months a based on the existing conditions of Iranian Coldwater Fisheries Research Center- Tonekabon. Temperature, DO[1] and pH of the water entering and leaving of the quarantine hall were measured by the HACH HQ40d portable multimeter. Then, the inlet and outlet water samples were transported to the center's ecology laboratory to measure nitrate, nitrite, ammonia, and ammonium factors in the vicinity of the ice and were measured by colorimetry by Palintest 7500 portable spectrophotometer according to the standard method (Standard method, 1998).

Measurement of total water bacteria

Water sampling containers (500 ml glass bottles) were sterilized at 121.5°C in an autoclave for 15 min to measure the bacterial load of water. The inlet and outlet water of the quarantine hall was sampled with 3 repetitions monthly for 6 months according to the national standard 4208 of Iran. For this purpose, we opened the bottle under water so that water enters it but does not fill it completely so that the aerobic bacteria are not destroyed. Then, the water bottles in the ice-containing unolith

[1] Dissolved Oxygen

were transferred to the laboratory and were tested in the shortest possible time. Total water bacteria were counted by pour plate method (APHA) (1998).

Virology test

Health status of rainbow trout pre-breeders of selected farms with IPN, IHN, and VHS viral diseases were inquired from the General Veterinary Administration of the respective provinces and the Virology Laboratory of the Research Institute of the of the Iranian Inland Water Aquaculture Research Center- Bandar Anzali to investigate their possible infection before transfer to the quarantine hall in Coldwater Fishes Research Center- Tonekabon. Then, larvae, juveniles, and kidney tissue, spleen, gill, egg, and spermatozoa of the breeders of each of the selected farms were sampled separately according to the OIE protocol to measure the breeders' virus up to the reproductive stage. After preparing supernatant from interior tissues, dark cell EPC proliferation was prepared in a 25cm^2 cell culture flask and appropriate cell monolayer was used on 24-cell plates. Moreover, PCR and serological methods were used for diagnosis of the disease (Radonic *et al.*, 2004).

Data analysis

After data normalization using Kolmogorov-Smirnov test, pre and post t-test was used to analyze the data using SPSS 20 at 95% confidence level. In addition, Excel 2018 was employed to draw the diagrams.

Results

Table 1 presents the results of the investigation of physical, chemical, and microbial factors of inlet and outlet water of the quarantine hall during May to October.

Table 1: Standard deviation ± average physical, chemical, and microbial parameters of inlet and outlet water of the quarantine hall during 6 months.

Parameters	Inlet water	Outlet water
Temperature (°C)	17.00±1.55*	17.59±1.46*
DO (mg/L)	8.32±0.65	8.20±0.62
pH	7.98±0.37**	8.18±0.35**
Nitrite (mg/L)	0.003±0.000*	0.04±0.04*
Nitrate (mg/L)	3.10±0.71**	3.74±1.14*
Ammonium (mg/L)	0.09±0.10	0.09±0.07
Ammonia (mg/L)	0.08±0.09	0.08±0.07
Total bacteria (CFU/mL)	90.50±86.58	784.50±1329.95

* Significance level up to 0.05 **Significance level up to 0.01.

In general, the average inlet and outlet water temperatures were 17.00±1.55 and 17.59±1.46°C, respectively, which was statistically significant (p<0.05) (Fig. 2).

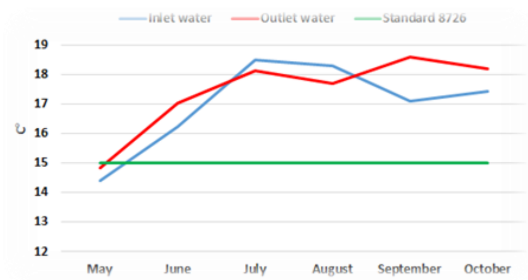

Figure 2: Average temperature of inlet and outlet water.

The average DO of the inlet and outlet water of the hall was 8.32 ± 65 0.65 and 8.20 ± 0.62 mg/l, respectively, which was not statistically significant ($P>0.05$) (Fig. 3).

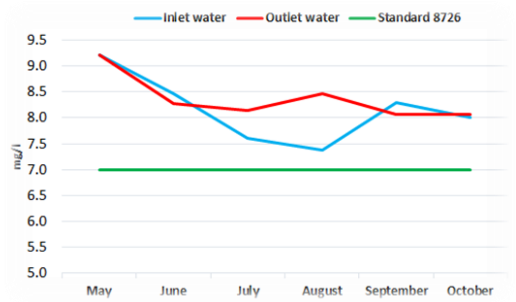

Figure 3: Average dissolved oxygen of inlet and outlet water.

The average value of pH of the inlet and outlet water of the hall was 8.98±0.37 and 8.18±0.35, respectively, which was statistically significant ($p<0.05$) (Fig. 4).

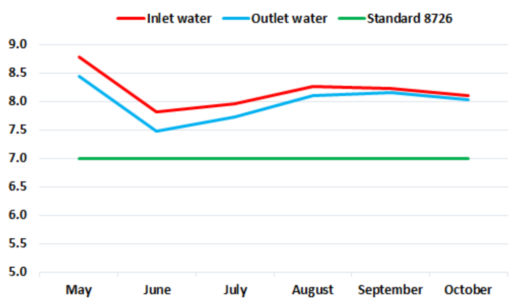

Figure 4: Average pH of the inlet and outlet water.

Furthermore, the average nitrite of the inlet and outlet water of the hall was 0.003±0.000 and 0.040±0.040 mg/L, respectively, which was statistically significant ($p<0.05$) (Fig. 5).

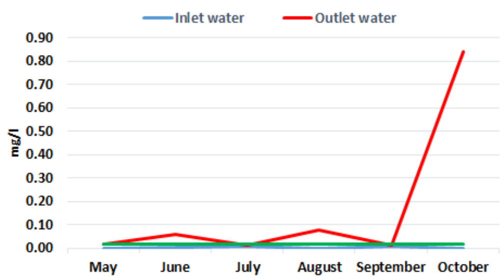

Figure 5: Average nitrite of the inlet and outlet water.

The average nitrate of the inlet and outlet water of the hall was 3.10±0.71 and 3.74±1.14 mg/L, respectively, which was statistically significant ($p<0.05$) (Fig. 6).

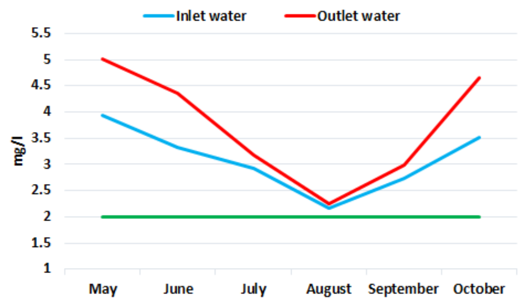

Figure 6: Average nitrate of the inlet and outlet water.

The average ammonium of inlet and outlet water of the hall was 0.09±0.10 and 0.09±0.07 mg/L, respectively, which was not statistically significant ($p>0.05$) (Fig. 7).

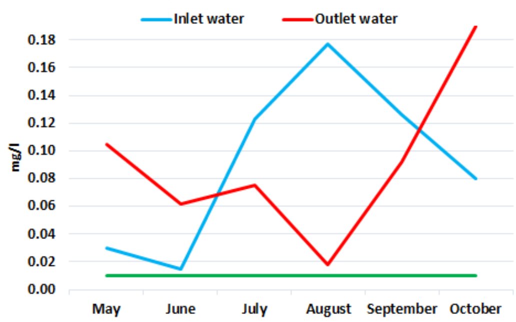

Figure 7: Average ammonium of the inlet and outlet water.

The average ammonia of inlet and outlet water of the hall was 0.08±0.09 and 0.08±0.07 mg/L, respectively, which was not statistically significant (p>0.05) (Fig. 8).

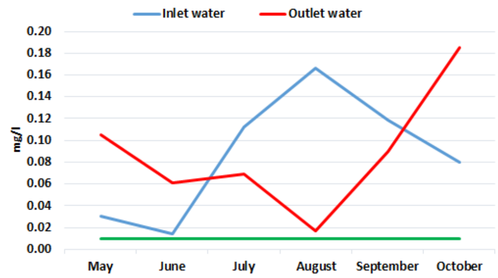

Figure 8: Average ammonia of the inlet and outlet water.

The average of total inlet and outlet bacteria of the hall was 90.50±86.58 and 1329.95±784.50 CFU/mL, respectively; however, this difference was not statistically significant (p>0.05) (Fig. 9).

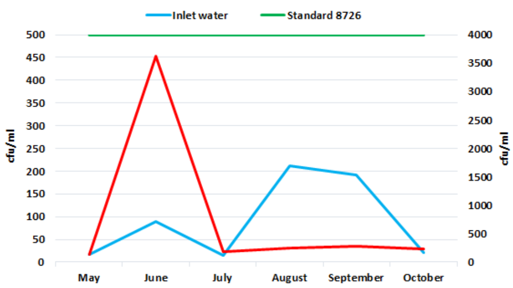

Figure 9: Average total bacteria of the inlet and outlet water.

Sampling was performed in June and July under conditions without ozone injection to evaluate the efficiency of ozone injection and aeration mechanism. The results of viral tests also indicated that the fish of the selected farms were free of IPN, IHN, and VHS viruses before transfer and during the farming process in the quarantine hall.

Discussion

Most pathogens in fish are opportunistic (Moksness *et al.*, 2004). Maintaining the health of fish in farms and preventing any harmful factors play important roles in increasing production and reducing costs. Complexity of diseases, diversity and abundance of infectious pathogens (parasites, fungi, bacteria, and viruses) and non-infectious pathogens (environmental, nutritional, genetic, physical harms, ...) are the main concerns of the fish farmers, which lead to harmful effects in the form of death, growth reduction, fertility reduction, decreased feed conversion ratio, reduction in the production quality, loss of production time, increased costs, and restrictions on the use of fish eggs, sales, marketing and so forth in the production cycle (Zorrieh Zahra *et al.*, 2016). The main problems of the cold water fish farming industry in Iran are caused by non-compliance with the health management principles and issues such as unsuitable place and improper design of the farming centers of rainbow trout , proximity to each other, lack of proper water supply, influence of upstream centers on downstream centers, temperature changes due to undesirable climates, lack of infrastructure, unscientific expansion of CCTV farms, non-compliance with hygienic standards when buying and transporting eggs, juveniles, and breeders of rainbow trout, lack of breeding programs, non-compliance with the quarantine principles and disinfection and hygienic instructions, and existence of stressors and outdated technical knowledge (Zorrieh Zahra *et al.*, 2013). In the farming stages, any severe changes in the physical, chemical, and biological properties of water act as debilitating environmental conditions and weakens the fish's immune system by disrupting their biological balance and ultimately leads to disease or death (Wedemeyer, 1996; Leatherland and Woo, 1998). Water temperature is a key predisposing factor in fish. Disease depends not only on the growth and potency of the pathogen, but also on the fish's immune system, which is dependent on ambient temperature (Morvan *et al.,* 1998). The preferential temperature range of water for farming and propagation of rainbow trout is 9-17 and 7-10 °C, respectively (Esmaeili Sari, 2003) and the temperature changes measured at the entrance and exit of the quarantine hall during 12 stages range from 14.40-18.50 and 14.85-18.70 °C, respectively. According to the results, the average temperature of inlet water in July, August, September, and October is higher than the normal range of rainbow trout farming. Therefore, temperature is considered as a risk factor in reducing dissolved oxygen. The preferential dissolved oxygen in rainbow trout ranges from 9-11.5 mg/l (Esmaeili Sari, 2003) and changes in dissolved oxygen measured at the entrance and exit of the quarantine hall during 12 stages range 7.61-9.22 and 7.45-9.22 mg/l, respectively. According to the results, the average dissolved oxygen in the inlet and outlet water in different months was higher than the normal range of rainbow trout farming, which can be caused by aeration, regular water change, and sudden decrease in water temperature changes. The pH of water ranged 6.4-8.8 (Abdollah Mashaei, 2002) and the pH changes measured at the entrance and exit of the quarantine hall during 12 stages was 7.47-8.44 and 7.47-8.44, respectively. According to the results, the average pH of the inlet water in the months under study was normal and was not considered as a risk factor in this study. Farming and maintenance of aquatic animals, including fish, is always associated with the production of large amounts of nutrients, ammonium, nitrate, nitrite, and so forth, the increase of which in the environment directly affects aquatic health. In addition, these factors provide a suitable habitat for the growth of undesirable microorganisms that can indirectly influence the health of fish. The amount of preferential nitrite of water in rainbow trout farming system is less than 0.05 mg/l (Abdollah Mashaei, 2002) and the nitrite changes measured at the entrance and exit of the quarantine hall during 12 stages range 0.009-0.001 and 0.014-0.840 mg/l, respectively. According to the results, the average nitrite of inlet water in the months under study was within the normal range of rainbow trout farming and was not considered as a risk factor in this study. The amount of preferential nitrate of water in rainbow trout is 0.1 mg/l (Esmaeili Sari, 2003) and its changes measured at the entrance and exit of the quarantine hall during 12 stages was 3.94-2.15 and 2.26-5.01 mg/l, respectively. According to the results, the average nitrate of inlet water in the months under study was more than the normal range of rainbow trout farming, which can be caused by the high water temperature in the mentioned months as well as deep water well problems. Hence, nitrate is considered as a risk factor. Ammonia toxicity is dependent on ammonium nitrogen content, pH, and temperature (Colt *et al.,* 2009). The preferential ammonia range of water in rainbow trout is 0.01-0.02 mg/l (Esmaeili Sari, 2003) and the ammonia changes measured at the entrance and exit of the quarantine hall during 12 stages was 0.014-0.166 and 0.017-0.185 mg/l, respectively. According to the results, the average ammonia of inlet water in May, July, August, September, and October was higher than the acceptable level of rainbow trout farming, which can be caused by the high amount of the water temperature in the mentioned months and the problems of deep wells. Therefore, ammonia is considered as a risk factor. Microorganisms in aquatic environments, including gram-negative bacteria, are naturally found in the water, sludge, and body surface of fish and have been identified as potential pathogens for different kinds of fish, causing major diseases such as fin, gills, and jaws rot, Columnaris, BGD[1], saddle-back disease, and larva mortality syndrome cause problems in the farmed fish (Zorrieh Zahra *et al.*, 2016). It should be mentioned that 15-20 g/h Ozone (10.1 mg/l) was used in the Iranian Coldwater Fishes Research Center to remove microorganisms in the inlet water of the quarantine hall. The total amount of preferential bacteria of water in rainbow trout is 500 CFU/ml and changes in all water bacteria measured at the entrance and exit of the quarantine hall during 12 stages range from 14-212 and 138-3630, respectively. According to the results, the average of total bacteria of inlet water in the mentioned months was within the acceptable range of rainbow trout farming and was not considered as a risk factor in this study. To evaluate the correct operation of the ozone generator during the sampling process, it was not used for 2 months and the results were compared with other months. When using the ozone generator, total water bacteria reached from 110 to 223 CFU/ml at the entrance and exit of the quarantine hall, but it reached from 52 to 1907 CFU/ml when the ozone generator was not used, which indicated the importance and position of the ozone generator in controlling the total amount of water bacteria. Also, the negative results of viral tests performed on rainbow trout pre-breeders during different stages of sampling can indicate compliance with biosecurity principles in the quarantine hall.

[1] Bacterial Gill Disease

Conclusion

The results of measuring the physical, chemical, and microbial parameters of water and virology of fish from the pre-breeding stage to the breeding stage in the production center of SPF rainbow trout showed that are prevented from the entry of any pathogens in the center due to proper and systematic management at all stages of disinfection, aeration, regular water change, and feeding. Changes in physical and chemical factors of water during the farming period, lack of oxygen generator, aeration system problems and low flow rate of inlet water were also identified as risk factors.

Recommendations

According to the analysis of the results, experts in the field should follow these instructions: prevention of unnecessary traffic, non-use of common tools, regular cleaning of ponds, control of inlet water flow rate, supply of suitable and disinfected return water, regular control of physical, chemical, and microbial factors of water, regular evaluation of fish health status, use of special clothing and boots by the personnel, installation of water cooling systems, use of spare electric motor, use of oxygen generator, and installation of warning systems to establish complete biosafety and improve aquaculture management.

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