Good aquaculture production practices and detection of pathogens in rainbow trout alevins

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ABSTRACT

Objective: To evaluate the compliance with Good Aquaculture Practices (GAPs) in the Health Compliance Units (UPAs) during the incubation of rainbow trout eggs and to identify the presence of *Aeromonas salmonicida* and *Aeromonas hydrophila* using the Polymerase Chain Reaction (PCR) technique.

Design/Methodology/Approach: Seven and three egg incubation UPAs were identified in Puebla and Veracruz, respectively. An structured questionnaire was applied to evaluate the compliance with the GAPs. Samples were collected from the trout avelin batches and were analyzed in search of *A. salmonicida* and *A. hydrophila* using the PCR technique. The results were compared with the health characteristics of the fish of each batch.

Results: The GAP-certified UPAs comply with the recommendations made by the authorities and do not show morbidity. UPAs that comply with less GAP points have health issues. *A. hydrophila* was detected in batches with a lower compliance with the GAPs. The presence of *A. salmonicida* was not identified.

Study Limitations/Implications: The lack of compliance with the GAPs can lead to infection by other trout pathogens (not taken into consideration in this study).

Findings/Conclusions: Compliance with the GAPs reduces the health risk at the rainbow trout egg incubation UPAs.

Keywords: *Aeromonas salmonicida*, *Aeromonas hydrophila*, PCR, trout incubation, aquaculture health.

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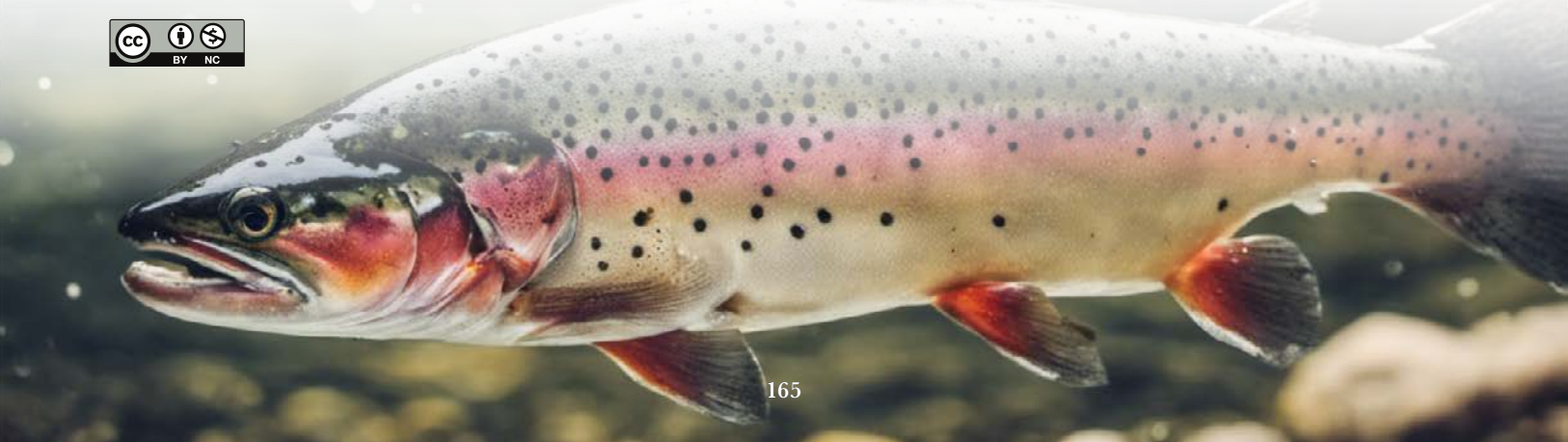
INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) is a fish species with high importance in Mexico. It is raised by small-scale producers in the mountainous regions of Mexico. This is the first species to be introduced as a freshwater species to repopulate rivers and for its commercial development. Small producers have successfully adopted management practices and technology in the fattening process; these producers obtain economic and social benefit from this practice. However, since this species is not native to the tropics, it faces constant health problems (INAPESCA, 2018), which have intensified in recent years



as a consequence of global warming and the vulnerability of the open production system (Carrillo-Longoria *et al.*, 2018).

Trouts are raised in 19 Mexican states and its production amounts to $876 millon pesos and 19,118 t. The states of Puebla and Veracruz produce 2,785.82 and 1,012.48 megagrams (Mg), respectively (Ontiveros-Córdova, 2022). Single sex alevin raising has not been successfully established in Mexico; therefore, production depends on the introduction of eyed eggs (Ortega *et al.*, 2011). Eggs are incubated in UPAs known as "incubators". Alevins are sold to fattening farms once they have reached a length of 5 cm. Three-hundred-and-five fattening farms have been registered in Puebla, while 186 have been reported in Veracruz (DOF, 2021). This is evidence of a reduced traceability and of the risk of distribution of water parasites.

Fish diseases are visually identified through changes in their behaviour and their physical characteristics, as well as through an increase in mortality. Nevertheless, high sensititivy techiques, such as the polymerase chain reaction (PCR), can be used to improve the accuracy of pathogen identification (Tufiño-Loza *et al.*, 2020).

In Mexico, the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) is in charge of following up epizootic diseases in aquaculture. However, scarce information has been published about the introduction or outbreak of pathogen bacteria in trouts (Alcántara-Jauregui *et al.*, 2022).

SENASICA encourages the use of Good Aquaculture Practices (GPAs). The purpose of these practices is to reduce health risk through the careful selection of the growing site, the supply of water and the control of its quality, the source of eggs or alevins, the reception and storage of the food, health criteria, use of chemicals and drugs, and harvest operations (García-Ortega and Calvario-Martínez, 2003).

Therefore, the compliance with GPAs in the UPAs where rainbow trout eggs are incubated is an important measure, along with the identification of the presence of *Aeromonas salmonicida* and *Aeromonas hydrophila*, using the Polymerase Chain Reaction (PCR) technique.

MATERIALS Y METHODS

Identification of the UPAs

Ten UPAs were identified in the states of Veracruz and Puebla, with the help of the Sistemas Producto Trucha (Table 1).

Sample collection

Avelin batches with <5-cm long specimens were visually inspected to detect integument and behavior anomalies. Productions logs were reviewed and the operators were subjected to non-structured questionnaires. Finally, samples were collected from each batch and put in 90% ethanol.

Evaluation of Good Aquaculture Practices

A structured questionnaire was applied to the operators of the UPAs, in order to obtain information regarding the management of the GAPs in each farm; additionally, in the case

Table 1. Aquaculture Production Units evaluated in the states of Puebla (P) and Veracruz (V).

Aquaculture Production Units	V1	V2	V3	P1	P2	P3	P4	P5	P6	P7
Municipality	X	X	TN	TH	TH	TH	H	H	H	TH
CBPA						*	*			
Origin of eggs	MEX	MEX	USA	MEX	MEX	MEX	USA	MEX	MEX	MEX

X=Xico, TN=Tlalnelhuayocan, H=Huauchinango, TH=Tlahuapan, GAPC=Good Aquaculture Practices Certificate, (*)=observed parameter, MEX=Mexico, USA=United States of America.

of GAP-certified units, the corresponding documentary evidence was requested. Following the proposal of García-Ortega and Calvario-Martínez (2003), a discriminatory table was developed to define the biosafey status of the sample UPAs. The operations were subjected to a visual evaluation. A value was assigned based on the compliance percentage, using a measurement unit called Health Compliance Unit (UCS). To obtain an overall assessment of the processes of each UPA, the UCSs of each processes were added together.

Sample treatment

A. salmonicida and *A. hydrophila* strains were requested from the Centro de Investigación en Alimentación y Desarrollo (CIAD), with reference keys CAIM 674 and CAIM 675, respectively. After their DNA was extracted; these strains were used as positives. Freeze-dried oligonucleotides were acquired (Table 2) and hydrated with ultrapure water, in order to adjust the concentration to 100 μMo.l.

In the case of *A. hydrophila*, the PCR test was carried out following the methodology proposed by Lee *et al.* (2000). The following conditions were established: a thermal cycler at 94 °C for 2 min, 35 amplification cycles (denaturation at 94 °C for 40 s, recooking at 60 °C for 40 s, and one extension at 72 °C for 60 s), and a final 5-min elongation period at 72 °C. In the case of *A. salmonicida*, the methodology proposed by Del Cerro *et al.* (2002) was used. The following conditions were established: thermal cycler with one 40-cycle amplification, 94 °C for 1 min, 35 °C for 2 min, and a 2-min elongación at 72 °C. The cycle was extended to 20 min.

Data analysis

The GAP results of the UPAs are shown as descriptive statistics, using the UCS as the measurement unit. The results of the PCR tests were expressed as the presence or abscence of the studied bacteria. Finally, the visual results of the batches are shown in a descriptive style, for their correlation with the results of previous tests.

Table 2. Oligonucleotides used to test the Polymerase Chain Reaction.

Sequence description	Base pairs	Sequence	Bacteria	Reference
AH-F	23	GAA AGG TTG ATG CCT AAT ACG TA	h (*Aeromonas hydrophila*)	Lee *et al.* (2000)
AH-R	21	CGT GCT GGC AAC AAA GGA CAG	h (*Aeromonas hydrophila*)	
PAAS1	19	CGT TGG ATA TGG CTC TTC T	S (*Aeromonas salmonicida*)	Del Cerro *et al.* (2002)

RESULTS AND DISCUSSION

Evaluation of Good Aquaculture Practices

The UPAs that show the highest compliance with the recommendations made by García-Ortega and Calvario-Martínez (2003) also have a GPA certification (Table 3). Farms V1, V2, P3, and P7 face high health risks, given their failure to comply with the expected health criteria.

All the UPAs under study are located in growing sites (GS) with the adequate altitude and temperature for the species. However, the supply and control of water quality (SCWQ) was inadequate, as a consequence of the sediments and the lack of filtration treaments. Sediments can damage gill cells, which may cause stress and be an entry point for pathogens (David-Ruales and Vásquez-Torres, 2010).

Pathogens such as *A. salmonicida* may enter the facilities through eyed eggs (Zepeda-Velázquez, 2015). The V1, V2, P3, and P7 UPAs produce eggs from their reproducers. However, they fail to provide the minimum health care to the incubation area and mortality reaches up to 100% of the batches.

A proper feeding program diminishes the vulnerability of fish against oportunistic pathogens (Velasco-Garzón and Gutiérrez-Espinoza, 2019). Although all UPAs use quality commercial food, not all of them have an adequate program for the productive stages. Farms V1, V2, and P3 do not protect the food from light, humidity, or pests, which may diminish its nutritional quality and pollute it.

The UPAs with the highest GPA compliance face a lower risk of pathogens entering and scattering in their production unit, consequently avoiding stress in the organisms and improving the response capacity during disease treaments.

Consequently, they diminish their losses, while also contributing to public health and environmwental wellbeing (Figueredo *et al.*, 2020). Biosafety criteria diminish the need to apply control chemicals (Fajer-Ávila *et al.*, 2017). The infrastructure and equipmen of farms P4 and P5 favours health; their staff is trained, and they have their biosafety protocols in writing.

Table 3. Result of the evaluation of the Good Aquaculture Practices, expressed in Health Compliance Units (UCS).

Activity	Veracruz (V)			Puebla (P)						
Aquaculture Production Unit	**V1**	**V2**	**V3**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**
Cultivation site	100	100	100	100	100	100	100	100	100	100
Water supply and quality control	38	38	50	40	45	48	88	88	45	45
Origin of eggs	0	0	60	33	10	0	100	95	18	0
Reception and supply of food	53	53	75	67	67	50	100	100	67	67
Health criteria	0	0	50	64	0	0	100	100	14	0
Chemicals and drugs	0	0	25	85	25	0	85	85	0	0
Harvest process	30	30	75	100	60	60	100	100	30	30
Total UCS	51	51	110	138	67	53	192	190	72	58
Certified farm							*	*		

(*)=observed parameter.

Farms P1, P4, and P5 record the chemical products and drugs they provide; nevertheless, their health diagnostics are not carried out by specialists. As as standarized procedure, all the UPAs apply antibiotics to the fish from day 10, until day 20 after the hatching. González-Salas *et al.* (2021) explain that antibiotics are used as prophylactics in aquaculture, although bacteria develop resistance and their control is consequently more difficult.

García-Ortega and Calvario-Martínez (2003) and other authors described the harvest process (HP) in trout farms, under the consideration that stress and handling can cause diseases during this stage. In the analyzed UPAs, HP consists of counting, extracting, and selling on the farm itself 4- to 6-cm long alvins. UPAs V1, V2, P6, and P7 lack spoon nets that prevent mechanical injuries to the integument and fail to disinfect their equipment.

PCR test for the detection of Aeromonas

All PCR tests in search of *A. salmonicida* were negative. This bacteria can cause skin lessions, intestinal inflamation, typical furunculosis, severe septicemia, and over 100% mortality in fish batches (Zepeda-Velázquez, 2015). Since it is a non-mobile pathogen, it is passed on from fish to fish or from the reproducer to the egg. Trout egg importation is a critical point for Mexico. Castro-Escarpulli *et al.* (2003) have reported *A. salmonicida* cases in the country, especifically in tilapia; meanwhile, Salgado-Miranda *et al.* (2010) identified this species in seven samples from trout farms in the state of Chihuahua.

A. hydrophila is a mobile bacteria with flagella; it is found in bodies of water (*e.g.*, rivers) and can be an oportunistic pathogen. It is distributed all over the world and attemps to control it have led to the indiscriminate application of antibiotics in aquaculture (Perretta *et al.*, 2019). In this study, *A. hydrophila* was detected in 70% of the UPAs (Table 4). This finding matches the resutls of Salgado-Miranda *et al.* (2010) who identified this parasite as the most common bacteria that affects trouts.

Batches with positive results to *A. hydrophila* showed coincidences with the symptomatology described ty Fuentes and Pérez (1998), who linked the presence of the bacteria to exophthalmos, darkening of the skin, altered behaviour pattern, 80% sickness rate, and a 52% death rate. Nevertheless, these visual symptoms match other pathogens that also attack trouts (Alcántara-Jauregui *et al.*, 2022). However, the PCR technique has a 97.5% accuracy and the results regarding the presence of this bacteria can be therefore considered trustable (Chapela *et al.*, 2018).

CONCLUSIONS

Good Aquaculture Practices are inadequately implemented in most of the production units evaluated. Those farms with highest compliance with the biosafety points have less health problems. The presence of *Aeromonas salmonicida* should be monitored in imported trouts alevins and eggs The presence of *Aeromonas hydrophila* could be related to a defficient application of the Good Aquaculture Practices. The *A. hydrophila* bacteria can generate economic losses in trout farms, as a result of fish mortality and the cost of the supplies required for its control.

Table 4. Detection of *A. hydrophila* through the Polymerase Chain Reaction and visual evaluation of the health of fish batches in the evaluted Aquaculture Production Units (UPAs).

Batch	UPA	*A. hydrophila*	Natación Errática	Oscurecimiento de la Piel	Puntos Rojos en Abdomen	Exoftalmia	Mortality
1V1T	V1	+		*		*	A
1V2T	V1	+		*		*	N
1V3T	V1	+		*		*	A
2V1T	V2	+	*	*			MA
3V1T	V3	-					N
1P1T	P1	+	*	*			A
1P2T	P1	+	*	*			MA
1P3T	P1	+	*	*			MA
2P1T	P2	+	*	*	*		A
2P2T	P2	+	*	*	*		MA
2P3T	P2	+	*	*	*		MA
3P1T	P3	+	*	*	*		MA
3P2T	P3	+	*	*	*		MA
4P1T	P4	-					N
4P2T	P4	-					N
5P1T	P5	-					N
5P2T	P5	-					N
6P1T	P6	+	*	*	*		MA
7P1T	P7	+	*	*	*		MA
7P2T	P7	+	*	*	*		MA

(+)=Positive test; (-)=Negative test; N=normal; MA=moderately high; =high; (*)=observed parameter.

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