Chapter

Integrated Culture of *Oncorhynchus mykiss* (Rainbow Trout) in Pre-Cordilleran Sector under a Recirculation System in Northern Chile

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Abstract

An experience of integral farming of *Oncorhynchus mykiss* (rainbow trout) is carried out in Copaquilla, 90 kilometers inland from the city of Arica at 3,000 mamsl. The system used was the Recirculating Aquaculture System (RAS), which had six ponds of 40 mt^3 each, two decanters with a capacity of 3.5 mt^3 and a biofilter of 3.5 mt^3 with substrate for the fixation of ammonium and nitrite transforming bacteria. The three latter ponds were buried below the lowest level of the fattening ponds. Three pumps, two running and one 1.5 hp. backup, plus a 1 hp. blower, were the water and air equipment utilized in the system. Each pump had a flow capacity of 450 lt min^{-1}. This water was sucked from the biofilter and transferred to the accumulator tank with a capacity of 10 mt^3. From there it was distributed by gravity to the fattening ponds. In addition, the juvenile system had a particular SAR with a 0.5 hp. pump, a small 0.2 hp. blower and an 80 watt UV lamp. The grow-out SAR received 6,000 trout with an average weight of 15 grams. The group reached approximately 1,200 grams over a year. Thirty fish were selected for reproduction. Eggs were obtained, followed by fry, juveniles and adults. This initiative demonstrated the effectiveness of producing trout in the foothills of the interior city of Arica, Chile.

Keywords: indigenous communities, water quality, rainbow trout transport, spawning, rainbow trout eggs and larvae

1. Introduction

The lack of opportunities, technological improvements and diversification are issues to be resolved in mountain and foothill sectors and the reason for the insertion of new productive and sustainable alternatives. The unbeatable environmental conditions that many of the sectors of this territory present, such as the existence of good quality water sources, availability of space and microclimatic environments, are clear elements of potential. In such conditions, aquaculture shows favorable prospects for development.

Aquaculture, as indicated by Pepe-Victoriano et al. [1], is presented as a real productive alternative, by identifying crops of freshwater species of commercial value as a way to take advantage of the capacities installed in the agricultural activity of the area. Storage ponds for irrigation, greenhouses, hydraulic systems, among others, are some examples of facilities that add to the possibility of reusing water as many times as necessary before being derived as a final destination for irrigation of plants and vegetables.

The utilization of technology such as recirculation in aquaculture systems is presented as a powerful alternative for pre-cordillera areas [2]. A recirculation system allows more precise control of the main environmental parameters [3]. Water temperature, to name one, is a critical parameter for most poikilothermic organisms, such as fish, and can be controlled much more economically in a recirculating system than in an open flow one. Controlling this and other environmental parameters allows for faster growth and more efficient use of feed due to reduced stress on the farmed organisms.

The quality of the water in Copaquilla, as the main variable for trout farming, is within the optimal range for the development of this initiative. In previous studies to the three existing springs that would feed the farm, elements such as arsenic, copper, zinc, iron, and manganese are within the limits allowed by Chilean standards for drinking water consumption and therefore for freshwater crops.

Moreover, it is worth mentioning that trout production in recirculation systems would make it possible to increase the supply of good quality fish for local and regional population. Per capita fish consumption in Chile averages 13 kilos per year, which keeps us quite far from the world average of 20 kilos established by the Food and Agriculture Organization of the United Nations [4]. It is estimated that the unmet demand for fresh fish for human consumption in our region is around 1,000 tons per year. In addition to this, there is a growing consumption with clear preferences for salmonid fish species over red meat and poultry, mainly in the young segment of the population.

The implementation of this fish farm generates clear potential and economic benefits for the communities in the foothills of the Andes that might develop such initiative further into the future. This development is mainly projected as a diversification alternative, adding value to the aquifer resource, and improving the income of the territory by including other economic activities such as tourism and gastronomy, among others. The rich landscape and heritage of the area would also be benefited. In addition, these farming provides useful information to be used as a basis for other farming initiatives in the region. Aquaculture is expected to be boosted and to become one of the main strategic axes of regional development in the short term.

The main objective of this initiative was to develop the integral culture of *Oncorhynchus mykiss* under recirculation conditions, as a productive development alternative for communities in the pre-cordillera of the Arica and Parinacota Region.

2. Why grow trout at 3,000 meters above mean sea level?

The productive development of the communities located in the foothills of the Arica and Parinacota Region, especially in the Copaquilla sector (**Figure 1**), is based mainly on agriculture, livestock and to a lesser extent, tourism. Most recently, activities such as agriculture and livestock have suffered a clear deterioration and abandonment, mainly due to the lack of diversifying alternatives that encourage and avoid one of the main problems of the territory; the migration of young population to the city.

Figure 1.
Geographical location of the Pukara de Copaquilla Cultivation Center (CCPC).

3. Biological characteristics of trout

The rainbow trout (**Figure 2**) is a teleost fish belonging to the salmonid family (Salmonidae), whose distribution range covers cold waters of North America, Asia and Europe. It tolerates temperatures going from 0 to 25° C, with an optimum range of 10 to 14° C to remain healthy. In order to ensure excellent growth, however, temperatures between 15 and 20° C are preferred [6] in good water quality conditions.

The life cycle of rainbow trout is highly variable in terms of migratory patterns. Out in the wild, they spawn in rivers or streams, and many complete their life cycle in freshwater. Some varieties, nevertheless, migrate and spend their adult life in the ocean. They only return to the river where they were born to spawn, completing the cycle. This behavior is known as anadromous reproduction [7]. Anadromous forms migrate to the sea as juveniles and can travel long distances in the ocean. Freshwater forms (non-anadromous) move between affluent and main river, between river and lake, or spend their entire lives in a particular stream or river [7]. The growth and sexual maturation of these organisms can occur in freshwater or seawater. This last phase can last between 1.5 and 3 years of the fish's life. Spawning generally occurs in water flows of both rivers or affluents of the main channel, on gravel beds of rivers or lake shores, where water seeps through it. The gravel provides protection for the eggs until they emerge as fry ready to eat and migrate [7].

Rainbow trout are highly adaptable to their environment, which is why they have achieved a wide distribution [8]. According to studies carried out by other authors, trout born from the same progeny can adapt to totally different habitats. Some can grow, reproduce and live in a small stream, with a few centimeters of water above their bodies. Meanwhile, others can travel many kilometers to the ocean to feed and grow much larger than the first [9]. This is why resident trout, migratory trout from rivers and lakes, or anadromous trout can be found in the same watercourse [9].

Figure 2.
Adult specimen of Oncorhynchus mykiss (Rainbow trout). Image extracted from Cornejo-Ponce et al. [5].

4. Justification for the usage of a recirculation system

Recirculating Aquaculture Systems (RAS) are one of the emerging production technologies that are being applied in the national and global aquaculture sector. This is especially true when considering the concept of efficient improvement in production by minimizing the usage of water resources and increasing environmental responsibility. Thus, concentrating and treating the waste generated during the production process.

This technology is ideal for utilization in production systems that involve fish farming and the implementation of wastewater for agricultural irrigation, since the latter improves productivity and profitability in economic terms. In Chile, this technology is mainly used in the early stages of the salmon production process and is associated with fish farming centers where the incubation phase is carried out until smoltification. In the northern zone of Chile, there are no experiences or farms in operation that involve the application of recirculation technology in production processes with trout or other freshwater species.

The implementation of this type of technology (SAR) in aquaculture has enormous potential, especially in desert areas such as the Arica and Parinacota Region, where water resources are scarce. It has been demonstrated that 90 to 98 percent of water can be reused, compared to traditional open flow farming systems. It also has other advantages, such as: energy savings, maximization of production under water and space limitations, minimization of effluent problems by reducing waste discharges to the environment and controlling and regulating water quality parameters of the crop, among others. On the one hand, experiences in farming demonstrate that these systems can intensively produce up to 25–30 kg/m^3 of rainbow trout and about 80 kg/m^3 of salmon. On the other hand, in semi-closed aquaculture systems with water recirculation, rainbow trout productions of 6,257 kg per year (120 kg per week) have been reported, with a maximum biomass of 66 to 74.6 kg/m^3.

In this innovative technology, some advantages stand out: a) flexibility in the selection of the farming site related to the possibility of using a small amount of water; retaining waste; manipulating the farm medium (especially temperature) and avoiding the entry of organisms from the natural environment or their exit to it, b) biosafety, since it avoids the entry and exit of pathogenic organisms or the exit of specimens of the cultivated species to the natural environment. Moreover, by having direct control over the growing conditions, optimal growing conditions can be kept independent of environmental variations, thus reducing the risk of diseases and ensuring productive yields, c) expedite treatment of certain diseases as it is simpler to handle and minimize the amount of product needed for treatments by immersion, in conditions of relatively high farm density, d) reduction in water consumption, e) reduction in the amount of waste and the possibility of treating it, thus avoiding possible impacts on the environment, f) scaling and replicability in other sectors of the foothills of the region and the rest of the northern side of the country, and g) possibility of integrating renewable energy systems. As of today, there are no viable renewable energy systems due to the volumes and consumption required, but which are perfectly compatible, such as solar energy.

5. Proposed recirculation system for trout farming in the pre-cordillera sector

The closed water recirculation system installed at the CCPC (**Figure 3**) consists of 6 circular Australian-type ponds for intensive trout production. It is provided with a central drainage system and hydraulic connections for water, air and oxygen

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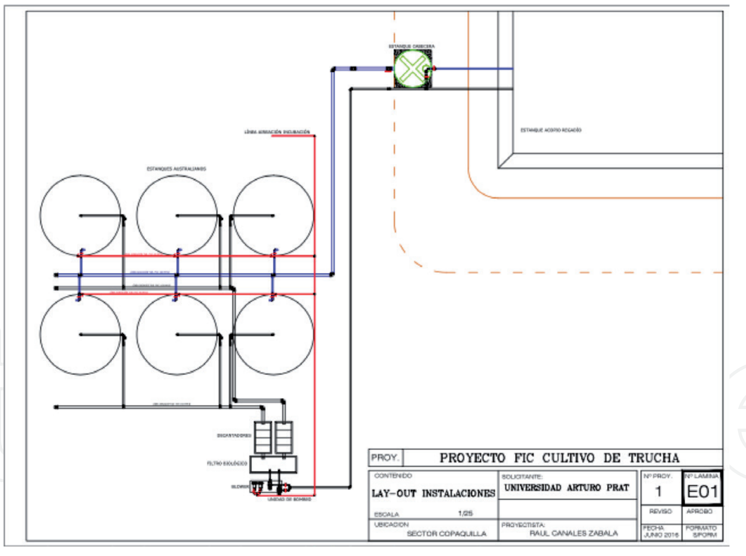

Figure 3.
Schematic of the recirculation system employed in the Rainbow trout farming at the CCPC.

supply; a system of fiberglass ponds located below ground level, including two sedimentation ponds and a pond with biofilters. In addition, two 1.5 hp. water suction pumps, two 1 hp. high pressure blower and an emergency oxygen generator.

The ponds (Australian type) for intensive trout production are made of corrugated galvanized steel with a diameter of 5.4 m and a nominal height of 1.76 m, with a maximum water volume of 40 m^3. The water reaches each pond through a central distribution pipe and lateral outlets, which supply water to each farming unit. The flow is controlled by PVC valves, which, due to their arrangement, generate a circular water movement.

Each pond is internally covered with a 1.0 mm thick black non-toxic liner or geomembrane, which acts as a waterproofing layer. In the center of each pond there is a drain with a 110 mm diameter outlet and a drainage system with 5 mm diameter openings.

In addition, there is an air distribution pipeline that, by means of a blower, allows continuous aeration and oxygenation of the water column to ensure high levels of dissolved oxygen.

Furthermore, there is an oxygen pipeline distributed to all the ponds and that, by means of an oxygen generator will supply a high concentration of this gas, in case of emergency.

The water leaving the six ponds passes through a PVC pipe below ground level until it reaches the sedimentation ponds. They feature an internal division to facilitate the sedimentation of suspended solids.

Waste accumulated at the bottom of the sedimentation ponds is removed periodically through a submersible pump. There, fresh water is added daily to maintain the volume of the pond, which represents approximately 1 to 2% of the total volume of the system. In addition, it has the purpose of removing the final product of the nitrification process (nitrate). Thus, it maintains the water level and compensates for evaporation and handling losses.

Water coming from settling ponds passes into a fluidized submerged biofilter, which contains an ample spectrum plastic substrate for the attachment of nitrifying bacteria. Such bacteria convert the ammonia nitrogen discarded by the fish to nitrites and subsequently to nitrates; molecules that are less harmful to aquatic organisms and, conversely, the main nutrient for most plants.

The nitrification process requires the addition of dissolved oxygen. Reason why this system takes advantage of the volume of air generated by the blower. The diffusion hoses are arranged in such a way that they allow a uniform movement of the biofiltration substrates. By doing so, ammonia nitrogen and oxygen are distributed over the entire surface of the biofilter avoiding the accumulation of solids in the biofilter.

The water from this pond will finally pass to the water accumulator pond, which has a volume of 10 m^3. In this pond the water is aerated before being sent by gravity to the six production ponds.

All power for the recirculation system (two 1.5 hp. pumps, one 1 hp. blower, one 0.5 hp. pump for the juvenile system) was provided by a 7 kw/hour photovoltaic plant, which had 28 photovoltaic panels of 250 watts each, two inverters and 24 batteries. The batteries operated 18 to 20 hours per day, occupying the oxygen generator the rest of the hours.

6. Water quality

The chemical compounds dissolved in the water, as well as other physical factors that affect the water, merge together to form what is known as "water quality". In aquaculture systems, changes in water characteristics that improve the production of a crop should be considered as improvements in water quality,

Parameters	Prior to fish entry into farming ponds	After fish are introduced into the farming ponds	Unit	Methodology
Ammonia	< 0,1	0,12–0,48	mg/L	SMWW 4500-NH3F
Alkalinity	35–43	36–38	mg CaCO3/L	SMWW 2320B
Arsenic	0,24–0,29	0,07–0,16	mg/L	SMWW 3114C
Chloride	65–110	10–70	mg/L	SMWW 4500Cl-
True color	6–8	11–28	Pt-Co	SMWW 2120C
Electrical conductivity	572–631	412–513	µS/cm	SMWW 2510B
Hardness	148–185	101–181	mg CaCO3/L	SMWW 2340C
Nitrate (N-NO3-)	1,63–4,79	21–224	mg/L	SMWW 4500-N B
Organic Nitrogen (N-org)	< 0,01	2–42	mg/L	SMWW 4500-NorgB
Nitrite	< 0,1	< 0,1	mg/L	SMWW 4500-NO_2^-B
Dissolved Oxygen	6,02–7,48	5,78–6,32	mg/L	SMWW 4500-O G
P-H2PO4-	7,25–8,99	21,20–43,19	mg/L	SMWW 4500P-C
pH	6,5–6,6	6,9–8,2	—	SMWW 4500B-H + B
Potassium	7,50–9,00	7,10–8,21	mg/L	SMWW 3111B
Total Dissolved Solids	476–515	257–409	mg/L	SMWW 2540B
Temperature	7–18	7–20	∘C	SMWW 2550B
Salinity	0,28–0,30	0,20–0,25	PSU	SMWW 2520B

Table 1.
Physical and chemical parameters of farming water, before and after trout were introduced.

while those changes that reduce production are a consequence of a degradation of said water quality.

This is given by the combination of physical and chemical properties and their interaction with living beings. With respect to the farming of aquatic organisms, any water characteristic that affects in one way or another the behavior, reproduction, growth, yields per unit area, primary productivity and management of aquatic species is a water quality variable.

Since one of the main objectives of aquaculture is to obtain the best yields possible, it is necessary to be thoroughly aware of ecological conditions in the ponds and the processes carried out there.

Within pisciculture parameters, water quality is of upmost importance. It must have adequate characteristics in terms of quantity (flow) and quality (physical, chemical and biological factors). Physical properties, such as temperature, pH, oxygen, transparency, turbidity, among others, may be subject to sudden variations due to the influence of external factors —mainly atmospheric and climatic changes. Chemical properties, however, are much more stable and their variations are minimal. Only in exceptional cases contamination can produce irreversible effects. From a biological stand, water quality is conditioned by the absence or presence of living organisms in the aquatic ecosystem, as well as by the greater or lesser presence of pathogenic agents.

Water quality in the Copaquilla trout farm (**Table 1**) is within normal ranges. Exceptional were some parameters in specific conditions, outside the optimal range, with no result in harming the crop itself.

In general, water used for trout farming complies within Chilean standard NCh1333.Of78 Mod. 1987, regarding the maximum limits allowed for water used in aquatic life farming.

7. Acquisition and transport of rainbow trout specimens

Trout were purchased at Rio Blanco fish farm, a department of Universidad Católica de Valparaíso, located in the city of Los Andes, Valparaíso Region.

The truck used to transport the trout was equipped with a thermos that facilitated the regulation of the internal environmental temperature during transport. A support vehicle was guarding it in case of emergency. These vehicles were disinfected (**Figure 4**) before entering Rio Blanco fish farm, in accordance with the fish transport procedures established by Servicio Nacional de Pesca y Acuicultura (SERNAPESCA) in Chile (or National Fisheries and Aquaculture Service).

Once the truck arrived at Rio Blanco fish farm, 8 ponds of 1 m3 each inside of it were filled with water (**Figure 5**). The ponds housed the fish during the transfer, being loaded at a rate of 625 fish per tank. Once the loading was completed, the outlet temperature and oxygen were recorded, and the ponds were carefully closed.

Parameters mentioned previously were taken (**Figure 6**) during the first 6 hours of the trip and visual evaluation was carried out by a technical team in charge of the transport. Behavior of the animals was evaluated, mainly swimming and opercular movement. During the following 12 hours, parameters were measured, and visual evaluation was carried out every two hours. Twelve more hours later, parameters were taken every three hours. For the last 15 hours they were measured every 5 hours. Thus, completing a total of 45 hours of travel.

Once the fish arrived at the top of Copaquilla, they were transferred to 800-liter water ponds on a pickup truck that carry them to the farm. Eight trips were made to empty the eight ponds. Once all the fish were in the farming center, they were distributed in three 40 m^3 ponds in equivalent number of fish.

Figure 4.
Disinfection of truck and support vehicle, for rainbow trout transport.

Figure 5.
Filling of water and fish to be transported into the ponds.

Figure 6.
Measurement of parameters during the transport of rainbow trout.

8. Development of the trout farming system

8.1 Trout fattening

A large part of the productive efficiency of a commercial fish farm is determined by feed management. For it to be successful, it is essential that farms develop a database where the indispensable parameters for a correct management

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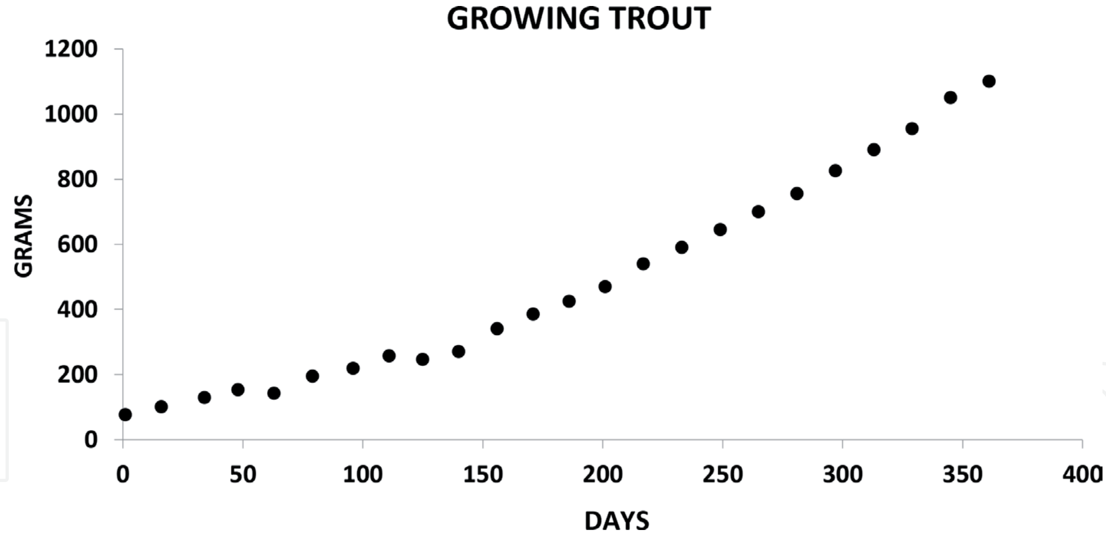

Figure 7.
Rainbow trout growth in the CCPC.

of feeding are recorded. Among these it is worth mentioning; number of individuals corresponding to each pond, mortality, average body weight, growth evolution, feed supply and water temperature. Once the numbers are in, it is possible to calculate the ration to be supplied to each pond as well as the feeding efficiency of each one.

The fish were sampled every 15 days. Between 5 and 7% of the total fish per pond were sampled, in this way, it is possible to correct the variables used to calculate the feed ration to be supplied month by month. The number of fish in the ponds was calculated as the difference between the initial number of the crop minus the number of dead individuals. Mortality was recorded daily to keep numerical control of the fish. With the updated number of individuals per pond and the average body weight records, the total biomass of the crop could be determined (**Figure 7**). After several samplings, the growth rate of the fish could be calculated by the difference in average weights, allowing for definition of trout harvest.

For an optimized determination of the feed ration supplied to the trout, the biomass, water temperature and average individual body weight in the pond must be known. Initially, the commonly disseminated feeding tables for the species can be used as a guide. However, we elaborate our own feeding table under specific conditions of the crop. Under this concept the fish were fed four times a day at a rate of 3 to 4% of the fish biomass in the pond. The conversion factor fluctuated between 1.2 and 1.35.

According to general results obtained in the present study, it is not recommended to apply any type of food restriction when the trout are growing within the optimum temperature range for the species, i.e., they were fed *ad-libitum*.

8.2 Conditioning of breeding specimens at CCPC

After one year of farming, 30 specimens were selected out of the 6,000 15-gram fish that initially arrived. These were separated in a 40 m^3 pond as potential broodstock. A density of 1 kilo of fish per m^3 was considered, feeding was supplied three times per week, twice with trout broodstock pellet and once with normal fattening pellet. Water flow was 120 liters per minute.

Fish were selected mainly for their phenotypic appearance, rapid growth rate and presenting no deformities. After one year they had an average weight ranging from 1,300 to 1,500 grams.

8.3 First trout spawning and hatching at the CCPC

In August 2017, the trout had their first spawning. This process was carried out through an abdominal massage on the female, which expelled her oocytes over a dry stainless-steel container. Already having the females spawn, the males were similarly massaged (two to three males per female). Once the gametes were in the container, they were activated by introducing clean water, which was also used as a mean to wash the eggs. This process was carried out several times until the water turned completely transparent. The eggs were then placed in trays adapted for incubation (**Figure 8A**) in raceway-type ponds of approximately 550 liters. After the first 48 hours, the eggs in the trays were cleaned, removing dead eggs in the process (**Figure 8B**) and finally leaving them unhandled until the eyed stage [10].

About 15,000 eggs were fertilized and hatched, reaching approximately 10,500 eggs to larvae (**Figure 9**), 6,500 to juveniles and 2,400 to 160-gram adults as of June 2018. It should be noted that a large percentage of animals were removed at each stage due to the limited space available at the CCPC.

Water temperature in the hatchery trays varied between 7 and 10 degrees at night and between 12 and 15 degrees during the day. The water flow in the raceway-type ponds was 5 liters per minute. For each pond there were four trays that held the eggs. Each tray was provided with a wire mesh in front of the water flow and at the bottom of the tray, which allowed water circulation and oxygenation.

Once the eggs hatched in the trays and the larvae absorbed the yolk sac, they were transferred directly to the raceway ponds, where they were kept for 20 to 25 days (**Figure 10**).

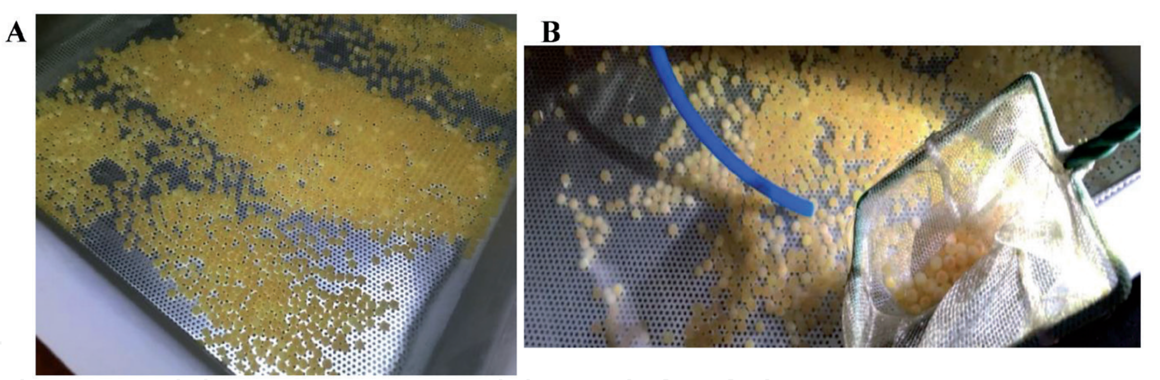

Figure 8.
Trout eggs (A), extraction of dead eggs (B).

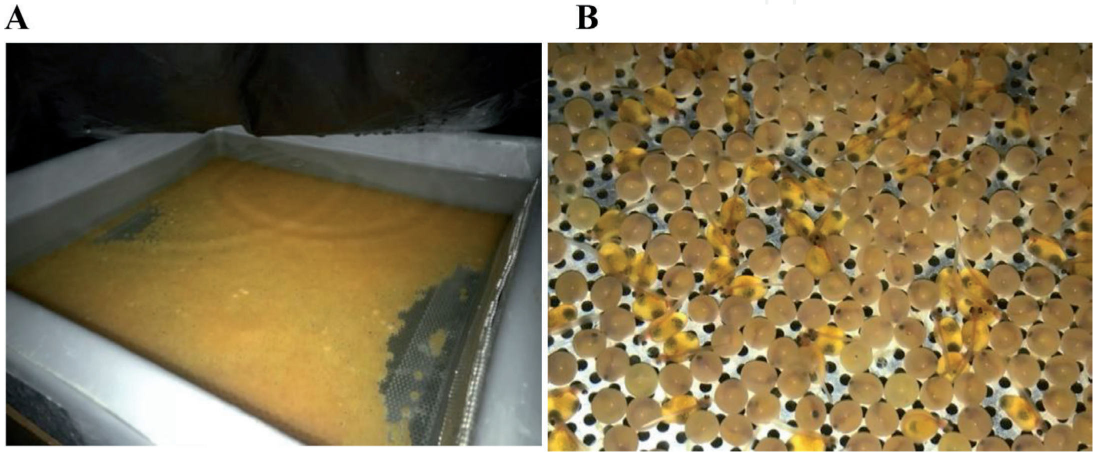

Figure 9.
Eggs in hatchery trays (A), rainbow trout larvae in the CCPC (B).

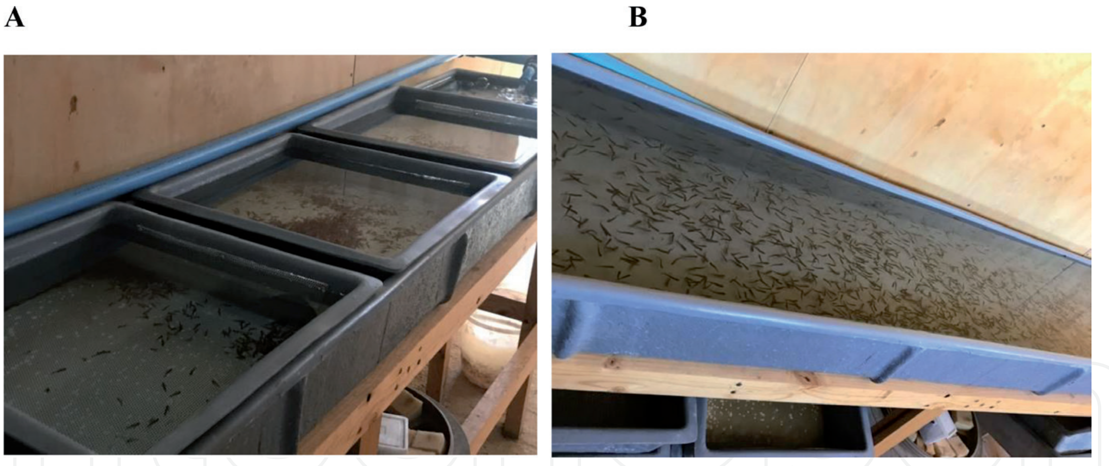

Figure 10.
Raceway pond, hatchery trays A, juveniles B.

Figure 11.
Trout fry ponds.

Figure 12.
Trout fattening ponds of 40 mt^3.

Temperature and water parameters were maintained during the time juveniles were in these ponds. Once the stage in the raceway ponds was completed, juveniles were transferred to the 450-liter fry tanks (**Figure 11**). They remained there for a month, approximately.

This system had a completely independent SAR, with a 0.5 hp. pump, 0.2 hp. blower and 80 w UV lamp. Temperature parameters fluctuated between 9 and 12°C at night and 12 and 16°C during the day. Water flow was 8 to 10 lt m^{-1}. After thirty days, the fish were transferred to a 40 m^3 tank (**Figure 12**). They were to remain there until they reached 160 grams, at which point the study was completed and splitting and grading were performed.

9. Problems addressed in trout farming

Trout farming in the Copaquilla sector was not exempt from problems inherent to fish farming and the climatic conditions of a sector located at 3,000 mamls.

Immature biofilter, massive proliferation of microalgae and accumulation of organic matter at the bottom of the water storage pond, were recurrent problems at the beginning of the culture.

They led to rapid responses in order to ensure the survival of the fish. One of the most worrying natural factors were summer rains, known as "the altiplanic winter". The natural phenomenon causes rains and cloudy days that affect the efficient operation of the solar panels, a system on which the energetic exercise of the equipment (pumps, blower, oxygen generator, etc.) depends.

10. Prospects for viability and sustainability of the Copaquilla Pukara farming center

The implementation of an aquaculture system in the pre-Cordilleran region made it possible to disseminate knowledge about it and to predict its viability under the surrounding conditions (quality of the water). In order to implement this type of technology on a large scale in the future, both for trout farming systems and for other species, the first step had to be made. Therefore, it is a means by which companies and entrepreneurs can obtain investment resources that allow for the beginning of new companies. This is based off the need to diversify aquaculture and the region's need to promote it; a strategic axis for the region of Arica and Parinacota. In addition, this aquaculture technology is highly suitable for coupling it with solar energy, as it has been demonstrated at the CCPC.

This initiative also poses a potential for entrepreneurship and innovation through the implementation of aquaculture and training of personnel in the region. Newly trained personnel would be capable of operating the crops, analyzing water quality, applying growth rates, among other activities. This, in turn, will allow new studies to be carried out, complementing the ones related to economic activities in which aquaculture plays an important role. The implemented equipment will allow the follow through continuum of the procedures, generating positive externalities such as internships for high school and university students.

Finally, the CCPC is an important business opportunity for small companies in the region that consider aquaculture in surrounding communities and for the sustainability and permanent operation of aquaculture production. In general, the CCPC will generate a preponderant added value that will help to promote and strengthen small businesses. Regarding environmental mitigation measures and risk prevention, the project is entirely sustainable from an environmental stand, as it allows water to be continually reused. Furthermore, this productive activity is intended to prevent the migration of young people from their native villages, providing them with a work alternative and specialization in aquaculture. Lastly, the implementation of the farm and other equipment does not create a significant visual impact as it is installed in a small area similar to local structures.

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