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Research Article

Characterization of the welfare conditions of salmonids reared in freshwater in southern Chile

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ABSTRACT. A study was conducted to collect information on the welfare status of salmonids reared in freshwater in southern Chile and to identify the most suitable operational welfare indicators (OWIs) for application in hatcheries. The study was carried out with the voluntary participation of 54 hatcheries that represent 36% of the total number of freshwater hatcheries operating in Chile, which were classified according to the production stage and comprised 12 hatcheries with recirculation system (RAS); 32 hatcheries with flow-through system (FTS); 7 hatcheries with water reuse system (REU) and 3 hatcheries with mixed system (FTS+RAS). Thirty OWIs were selected as the most suitable from the consulted literature and from a questionnaire administered to the fish farmers participating in the study. Three aspects were considered: animal welfare, feasibility, and economic cost, which were grouped into indirect (15) and direct OWIs (15). The OWIs were validated in 16 hatcheries where incubation, fingerling, and smoltification steps were covered. The results enabled the identification of critical conditions for the welfare of farmed fish, both in flow-through hatcheries and recirculation hatcheries, for the three salmonid species reared in Chile (Atlantic salmon *Salmo salar*, coho salmon *Oncorhynchus kisutch*, and rainbow trout *Oncorhynchus mykiss*). Water quality was considered the most critical issue for smolt production in freshwater, followed by precocious maturation in Atlantic salmon males, observed primarily in RAS hatcheries, and mortality classified as non-infectious. The latter included the culling of fish that are considered not suitable for commercial production.

Keywords: fish welfare; operational welfare indicators; water quality, freshwater salmon production; Chilean salmon farming

INTRODUCTION

The concept of animal welfare, as it pertains to farmed fish, has gained increasing relevance on a global scale, with a growing awareness among consumers regarding the rearing conditions of the farmed fish, based on the knowledge that fish are sentient, conscious, and have the capacity to suffer (Olesen et al. 2010, Bshary et al. 2014, Barreto et al. 2021). Thus, fish farmers have come to understand that the best productive results, in both biological and economic terms, are achieved when fish are farmed considering their physiological, environmental, and behavioral needs. In contrast, fish farmed under suboptimal conditions exhibit a low growth

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rate and incur significant expenditures on disease control. The quality of the final product is precarious, resulting in poor economic returns, which has led to the development and implementation of codes of practice and protocols aimed at enhancing the quality of life for farmed fish and reducing the stress and pain caused by human actions (Read 2008).

The initial regulations and recommendations on fish welfare primarily focused on slaughter operations and subsequently extended to encompass the transport of live fish. In 2005, the European Food Safety Authority (EFSA 2005) adopted a recommendation on the welfare of farmed fish, and in 2008, the World Organization for Animal Health (WOAH) followed suit. The concept of fish welfare is now considered across the entire production cycle, with particular attention paid to the operations identified as being particularly stressful.

The freshwater phase is considered a key stage for the salmon industry worldwide. The quality of eggs and smolts depends on the quality of the broodstock. The productive performance of salmon during the on-growing stage at sea depends on the quality of smolts, particularly in terms of physiological processes and health status. Fish that have not smoltified or have only partially smoltified will experience problems with osmoregulation and growth, and in the worst case, they may not survive (Noble et al. 2018, Khaw et al. 2021). It has been well-documented that the stress of captivity and inadequate environmental conditions can impact the immune system, growth, and reproduction in fish, altering the endocrine system, gamete development and quality, and the development and survival of eggs and larvae (Schreck et al. 2001, Wagner et al. 2002). Thus, the production of robust and well-adapted smolts is vital to success in the post-transfer period and in performance in seawater.

According to the Chilean Fisheries and Aquaculture Service (SERNAPESCA, by its Spanish acronym, 2024), a total of 257 million smolts were transferred to seawater in 2023, with 65% corresponding to Atlantic salmon (*Salmo salar*), 27% coho salmon (*Oncorhynchus kisutch*), and the remaining 8% to rainbow trout (*Oncorhynchus mykiss*). Even though the average monthly mortality in seawater was 0.89% in that year, the average monthly mortality in freshwater was higher (4.77%), where 4.5% corresponded to Atlantic salmon, 5.3% to coho salmon, and 5.5% to rainbow trout. The most significant causes of mortality in Atlantic salmon were culling (49.7%), infectious diseases (12.5%), and emaciation (10.1%). Mortality of coho salmon was culling (69.2%), infectious (6.9%), and embryonic (6.8%), and for rainbow trout these classifications were culling (38.7%), infectious (19.7%), and emaciation (19.1%) (SERNAPESCA 2024). The criteria defined in the General Sanitary Program of Mortality Management (PSGM-1468), established in 2012, classify mortality as follows: environmental, embryonic, deformity, mature, mechanical damage, emaciation, predators, culling, others, and without apparent cause.

Although regulations on fish welfare have not been implemented in Chile to date, recommendations on fish welfare standards and certification schemes for farmed salmonid species have emerged over the last two decades (e.g. Royal Society for the Prevention of Cruelty to Animals (RSPCA) welfare standards for farmed Atlantic salmon and rainbow trout, RSPCA 2018a,b). However, one of the significant challenges has been how to assess fish welfare status on farms by fish farmers (Huntingford et al. 2006, Ashley 2007, Martins et al. 2012, Segner et al. 2012). Noble et al. (2018) proposed operational welfare indicators (OWIs) to assess the overall welfare status of farmed fish, which can be either direct, animal-based (i.e. on the fish), or indirect, resource-based (i.e. on the environment). On the other hand, Barreto et al. (2021) conducted a review of emerging indicators for fish welfare assessment in aquaculture, categorizing them into two categories: non-invasive and invasive methods. The non-invasive methods avoid any direct physical contact, including handling, capture, and restraint.

This study aimed to identify and validate the most suitable OWIs in terms of ease of use, reliability, and accuracy for application in farmed salmonids reared in freshwater in southern Chile.

MATERIALS AND METHODS

The study was conducted between 2018 and 2019, within the framework of a project funded by the Undersecretary of Fisheries and Aquaculture in Chile (SUBPESCA, by its Spanish acronym) through the Fund for Research in Fisheries and Aquaculture (FIPA, by its Spanish acronym).

Study site and classification of the hatcheries

Information on the welfare status of the salmonids reared in freshwater was collected from 54 hatcheries that voluntarily agreed to participate in this study. These hatcheries, belong to 17 salmon companies, representing 36% of all freshwater hatcheries, distributed in southern Chile between 38°43' and 46°30'S. Of this total, 5.8% are located in the Bio-Bio

Region; 28.8% in La Araucanía Region; 53.8% in Los Lagos Region; 1.9% in Aysén Region; 3.9% in Magallanes Region and 5.8% in Los Ríos Region.

Hatcheries were classified according to production stage in two categories: (i) broodstock and egg production facilities, and (ii) parr and smolt production facilities. The hatcheries were also classified according to the production system, which comprised 12 hatcheries with a recirculation system (RAS), 32 hatcheries with a flow-through system (FTS), 7 hatcheries with a water reuse system (REU), and 3 hatcheries with a mixed system (FTS+RAS) (Table 1). FTS was declared to be used throughout the freshwater production cycle for coho salmon and rainbow trout, while RAS was only used for Atlantic salmon (Table 2). In comparison with the RAS, where water is recycled at 95.0 to 99.5%, hatcheries with a REU recycle up to 50% of the freshwater and do not use a biofilter.

Characterization of the management applied to the farmed salmonids in freshwater.

The objective was to collect information about the welfare conditions of farmed salmonids reared in freshwater. Two questionnaires were prepared for this study: one with closed-ended and the other with open-ended questions. The purpose of these questionnaires was to characterize the hatcheries operating in Chile methodologically and to understand the management practices applied by salmon companies during the freshwater phase, with a focus on fish welfare. One questionnaire was administered to hatcheries with broodstock and egg production, and the other was administered to hatcheries with smolt production.

The questionnaires were designed using bibliographical information and supplemented with information collected directly from visits to fish farms. Table 3 shows the aspects covered by the questionnaires.

Selection of potential operational welfare indicators (OWIs)

With the indicators mentioned in the literature, those considered in international standards, and those collected from the questionnaires applied to professionals working in the 54 hatcheries participating in the study, a list of 42 potential OWIs was developed. These were organized according to the OWIs proposed by Noble et al. (2018) in the Fishwell salmon handbook, taking into account the welfare needs of salmon.

Through the "panel of experts" methodology, judgments and opinions were gathered from professionals working for the participating companies and from academic researchers. The 30 OWIs selected as the most suitable were considered within three criteria: animal welfare, feasibility, and economic cost, which were grouped into indirect (15) and direct OWIs (15).

Validation of the potential OWIs

The OWIs selected by the experts in two rounds of work were incorporated into a descriptive protocol to be applied in the field, which was categorized into three groups: environment-based, production-based, and fish-based. The visual material, included in the file of the protocol, corresponding to scores, to assess the damage to the fin, eye, mouth, and skin lesions, followed the authorship of Noble et al. (2018), and was authorized by Dr. Chris Noble.

To validate the protocol with the selected OWIs, the sample size was calculated to be a minimum of 15 hatcheries, considering a sensitivity of 99%, an error of 5%, and a 95% confidence interval. Finally, the protocol was validated on 35 occasions in 16 fish hatcheries (29.6% of the hatcheries that collaborated in the study), located in the Los Lagos Region, encompassing the incubation, fingerling, and smoltification stages.

In addition, the vaccine operation was assessed in two hatcheries, the grading operation in one hatchery, and the precocious maturation selection was assessed in another hatchery.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics 27.0 and Microsoft Excel for Microsoft 365 MSO software. Descriptive statistical analysis was performed using tables and case numbers. A comparison of cumulative mortality by species and the eyed-egg ratio by smolt production system was performed using the Kruskal-Wallis test. In cases where significant differences were identified, multiple pairwise comparisons were performed using the Bonferroni correction method to control the type I error associated with multiple tests. Significance was set as $P < 0.05$.

RESULTS

In a survey of hatcheries, 51.7% stated that they had animal welfare protocols in their fish farming, 30% did not have protocols, and 18.3% did not respond. In addition, 81.7% of the hatcheries surveyed stated that they had received training in good farming practices, 83.3% indicated that they had received training in animal welfare, and 60% of the fish farms surveyed

Table 1. Classification of hatcheries surveyed in this study according to the production stage, source of water, and production system. RAS: recirculation system; FTS: flow through system; REU: water reuse system; and FTS-RAS: mixed composed of flow through and recirculation system.

Production stage	Water source	Production system				
		RAS	FTS	REU	FTS+RAS	Total
Broodstock & egg production	Groundwater well	1				1
	Groundwater well/spring water				1	1
	River		5			5
	spring-water		4			4
	Groundwater well/river	1	2	2	1	6
	Groundwater well/river/spring water		1			1
Sub-total		2	12	2	2	18
Smolt production	Groundwater well	8			1	9
	Groundwater well/spring water	1				1
	River	1	16	4		21
	Spring-water		2	1		3
	River/spring-water		2			2
Sub-total		10	20	5	1	36
Total		**12**	**32**	**7**	**3**	**54**

Table 2. Hatcheries used for breeding and egg production (n = 18) and smolt production (n = 36), by salmon species and rearing system. RAS: recirculation system; FTS: flow through system; REU: water reuse system; and FTS-RAS: mixed composed of flow through and recirculation system.

Production system	Salmonid species	Production stage		
		Broodstock & eggs	Smolt	Total
FTS	Coho salmon	3		3
	Atlantic salmon-coho salmon	2	3	5
	Atlantic salmon	3	8	11
	Atlantic salmon-rainbow trout	1		1
	Atlantic salmon, coho salmon, rainbow trout		4	4
	Atlantic salmon-rainbow trout	3	5	8
FTS-RAS	Atlantic salmon-coho salmon		1	1
	Atlantic salmon	1		1
	Atlantic salmon-rainbow trout	1		1
RAS	Atlantic salmon	2	10	12
REU	Atlantic salmon-coho salmon -		1	1
	Coho salmon-rainbow trout	1		1
	Atlantic salmon		3	3
	Atlantic salmon-rainbow trout		1	1
	Rainbow trout	1		1
Total		**18**	**36**	**54**

reported having a professional dedicated exclusively to animal welfare.

The OWIs obtained from the questionnaires administered to the 54 fish farmers were organized at the environmental, group, and individual levels.

OWIs based on the environment

Water quality

According to the answers generated by the questionnaires, the hatcheries with water recirculation (27.8%) have the strictest control of the water quality (24 parameters), in comparison with the hatcheries with

Table 3. Aspects included in the questionnaires applied to fish farmers working in hatcheries.

Farmed species	- Atlantic salmon
	- coho salmon
	- rainbow trout
Production system	- flow through system (FTS)
	- recirculation system (RAS)
	- water reuse system (REU)
Environment OWIs	- water supply
	- water quality
	- lighting
	- stocking density
Fish group OWIs	- fish management
	- fish behaviour
	- feeding strategies
	- growth
	- mortality
	- diseases
Individual fish OWIs	- condition factor
	- smoltification state
	- precocious maturation
	- abnormalities
	- vaccination
Staff training	- animal welfare
	- good farming practices

flow-through water (59.2%) and reuse water (13.0%), which control 15 parameters (Table 4).

Water current speed

All reporting hatcheries did not measure the water current speed in relation to the fish body size (body length s^{-1}). However, they all declared that they aimed to control the water velocity and the rate of water change.

Lighting (photoperiod)

All fish farmers reported that artificial light is used to control photoperiod. During the first feeding and fingerling stages, aerial white light is applied for 24 h to encourage feeding. Submerged green light LED lamps are used in the smoltification stage to prevent early maturation.

Coho salmon and rainbow trout are exposed to 24 h of artificial light in the summer and a regime of 8 h of light and 16 h of darkness in the winter. For Atlantic salmon, the most common strategy for the summer regime was 24 h of artificial light. In contrast, for the winter regime, a wide range of photoperiod strategies was reported, with 8 h of light and 16 h of darkness being the most prevalent, followed by 12 h of light and 12 h of darkness (Table 5).

Table 4. Water analysis reported for the parr-smolt step in hatcheries with flow-through system (FTS), recirculation system (RAS), and reuse (REU) (n = 54 hatcheries). X: reported, -: no reported.

Quality parameters	FTS	RAS	REU
Oxygen	x	x	x
Temperature °C	x	x	x
Ph	x	x	x
Carbon dioxide	x	x	x
Alkalinity	x	x	x
Hardness	x	x	x
Alkalinity + hardness	-	x	-
Nitrite	x	x	x
Nitrate	x	x	x
Total nitrogen	x	x	x
Ammonium	x	x	x
Non-ionized ammonium	-	x	-
Ammonia	-	x	-
Total suspended solids	x	x	x
Aluminium	x	x	x
Copper	x	x	x
Iron	x	x	x
Phosphorus	x	x	x
Zinc	-	x	-
Manganese	-	x	-
Hydrogen sulphide	-	x	-
Ozone	-	x	-
Total gas pressure	-	x	-
Salinity	-	x	-

Table 5. Photoperiod regime reported for farmed Atlantic salmon in freshwater (n = 24 hatcheries).

n	Summer	Winter
8	24:0	8:16
6	24:0	12:12
5	24:0	10:14
3	24:4	6:18
1	24:0	14:10
1	16:8	12:12

Stocking density

Table 6 shows the percentage of hatcheries (%) that declared stocking density, by salmon species and size distribution. For fish ≤ 30 g, the stocking density was higher than the RSPCA recommendations, while for fish > 30 g, the stocking density was in concordance with the RSPCA recommendations.

OWIs based on the fish group

Grading

For Atlantic salmon, 88.9% of hatcheries reported two size gradings in the production cycle, with a third and

Table 6. Stocking density by salmon species and fish weight in comparison with the RSPCA recommendations (n = 54 hatcheries).

Weight (g)	RSPCA	Atlantic salmon		Coho salmon		Rainbow trout	
	(kg m^{-3})	(kg m^{-3})	%	(kg m^{-3})	%	(kg m^{-3})	%
<1	10	≤ 10	0	≤ 10	0	≤ 10	0
		13-45	100	13-45	100	13-25	100
1-5	20	≤ 20	33.3	≤ 20	60	≤ 20	42.9
		35-50	66.7	21-35	40	25-35	57.1
5-30	30	≤ 30	12.5	≤ 30	16.7	≤ 30	0
		35-50	87.5	35-45	83.3	> 30	100
30-50	50	≤ 50	100	≤ 50	100	≤ 50	100
>50	60	≤ 60	91.3	≤ 60	100	≤ 60	100
		>60	8.3				

fourth grading conducted only if the fish had a high size dispersion after the second and third gradings. For coho salmon, 83.3% of hatcheries reported three size gradings in the production cycle, while for rainbow trout, 50% reported two size gradings and the other 50% reported three size gradings.

According to the answers given in the survey, the first grading is carried out when the fish weigh between 1.0 and 10.0 g, and the last grading is performed before the fish are transferred to the sea, aiming to minimize size variation during the growing phase at sea. In sea cages, fish are not graded to avoid stress from handling and to minimize the risk of disease outbreaks. Two hatcheries reported that the first grading was carried out when the fish reached 1.0 g (5%), while most of the hatcheries reported that the first grading was carried out on fish weighing more than 5.0 g (Table 7). The RSPCA does not recommend grading fry weighing less than 1.3 g.

For Atlantic salmon, 63% of hatcheries declared the last graduation when fish weighed <80 g; 18.5% when fish weighed 80-100 g; and 18.5% when fish weighed over 100 g. For coho salmon, 66.7% declared the last graduation when fish weighed <80g and 33.3% when fish weighed 80-100 g. For rainbow trout, 66.7% of hatcheries declared the last graduation when fish weighed <80 g; 6.6% when fish weighed 80-100 g, and 26.7% when fish weighed >100 g.

Growth

Quantified as the specific growth rate (SGR). The sampling frequency for weight was declared as either weekly (58%) or biweekly (42%), depending on the fish weight. All hatcheries sampled contained between 100 and 120 fish that had been previously anesthetized to evaluate the SGR. The SGR obtained from the monthly sampling is compared with the SGR projected for each fish group to assess stock performance and identify potential problems.

Mortality

From the information generated by the questionnaires, the mortality rates reported for the green eggs, which include mortality post-shocking, were 24% for rainbow trout, 18% for Atlantic salmon, and 22.0% for coho salmon, with no statistically significant differences between species (*P* = 0.244). For the eyed-eggs, 10% mortality was declared in Atlantic salmon and rainbow trout, and 6% for coho salmon, with no statistically significant differences between species (*P* = 0.338).

The mortality reported for the parr-smolt stage in Atlantic salmon reared in RAS was 20 and 22% in FTS hatcheries. Mortality in rainbow trout was 18 and 14.0% in coho salmon, with statistically significant differences between species (*P* = 0.013).

Diseases

According to the sanitary regulations established by SERNAPESCA (2003a) (Res. Ex. N°70; PSGR), the broodstock must be individually analyzed (screened) for infectious salmon anemia (ISA), infectious pancreatic necrosis (IPN), and bacterial kidney disease (BKD) after spawning. Furthermore, the screening process includes piscirickettsiosis (SRS). The hatcheries involved in the study reported that polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) were the screening methods used.

The most prevalent problems reported by fish farmers during the incubation and first feeding stages were white eggs for the three salmonid species, yolk sac constrictions for Atlantic salmon, and microphthalmia for rainbow trout (Table 8). The 36 hatcheries that

Table 7. Number of gradings in the parr-smolt stage, according to the weight (g) and salmonid species (n = 41).

Salmonid species	Number of gradings	Weight of grading (g)			
		1°	2°	3°	4°
Atlantic salmon	2	1 - 10	25 - 80		
		20 - 40	80 - 100		
		60 - 90	110 - 160		
	3	1.5 - 2	3 - 10	30 - 40	
		1 - 10	50	100	
	4	1.8 - 2	15 - 28	40 - 40	80 - 100
Coho salmon	2	30	100		
	3	1 - 10	30 - 70	90 - 120	
	4	1.8 - 2	15 - 28	40	80 - 100
Rainbow trout	2	5 - 6	30 - 160		
	3	1 - 10	20 - 45	95 - 120	

Table 8. Number of cases by disease, declared in the incubation-first feeding period (n = 18 hatcheries).

Diseases	Atlantic salmon	Rainbow trout	Coho salmon
White egg	3	4	5
Soft egg	1	0	0
White spot	2	1	1
Yolk sac constrictions	4	1	2
Blue sac disease	1	1	1
Microphthalmia	1	3	0

produce smolts, as well as five hatcheries that maintain broodstock in captivity throughout the entire life cycle, reported that Saprolegnia and Flavobacteria are the most prevalent diseases affecting the parr-smolt stage in the three salmonid species. Atlantic salmon reared in FTS showed a greater diversity of diseases than in a RAS hatchery, with the highest report of BKD (Table 9). All fish farms indicated that they had the assistance of a veterinarian who inspected the health status of the farmed fish. A weekly inspection periodicity was declared by 65% of fish farmers, while 23.3% declared a biweekly inspection by the veterinarian assigned to freshwater farms, and the remaining fraction corresponded to statutory visits.

OWIs based on the individual fish

Condition factor

The Fulton index (100 × body weight (g) × body length $(cm)^{-3}$) is used to calculate this. Hatcheries reported that length and condition factor are preferably measured before the smolt transference at sea. In the parr-smolt stage, hatcheries stated that weight samplings are carried out at a frequency of once a week or every other week, while length samplings are carried out on a weekly or monthly basis.

Smoltification state

Fish farmers from 34 hatcheries reported that the ATPase methods, followed by histochemistry techniques for ATPase (IHQ-ATPase), are the most commonly used methods for assessing smoltification in Atlantic and coho salmon (73.5%). Four hatcheries (11.8%) reported assessing the seawater adaptation by measuring plasma Na^+Cl^- concentrations, while five hatcheries (14.7%) with access to seawater reported carrying out the smoltification process by increasing the salinity gradient. Additionally, all fish farmers reported using hatchery operational manuals and a smoltification score to assess the smoltification state visually.

Precocious maturation

All hatcheries reported that they assess sexual maturation during sampling, grading, and vaccination operations. Some fish farmers use ultrasound to remove precocious males in fish weighing more than 25 g.

Deformities

The declaration by fish farmers revealed that Atlantic salmon had the highest level of abnormalities, with deformed opercula being the most common, followed

Table 9. Number of cases by disease, reported in the parr-smolt step by salmon species (n = 41 hatcheries) RAS: recirculation system, FTS: flow-through system. BKD: bacterial kidney disease; IPN: infectious pancreatic necrosis; ISA-HPRO: non-pathogenic variant of infectious salmon anemia.

Disease	Atlantic salmon		Coho salmon	Rainbow trout
	RAS	FTS	FTS	
Saprolegnia	8	19	9	5
Flavobacteria	7	18	8	13
Yersiniosis	1	0	0	0
Furunculosis	1	0	0	0
Amebiasis	1	0	0	0
BKD	3	13	2	0
IPN	2	7	4	7
PRV	3	7	2	0
ISA (HPRO)	3	1	0	0

by vertebral caudal compression. Vertebral caudal compression was the most common in coho salmon, while the deformed opercula were the most common for rainbow trout (Table 10).

Nephrocalcinosis

This abnormality in Atlantic salmon was reported by nine hatcheries with flow-through systems and in one hatchery with recirculating water. Additionally, it was reported in coho salmon and rainbow trout reared in hatcheries with flow-through (Table 10).

Vaccination

All fish farmers reported vaccinating fish at 600 degree-days (°D) before they come into potential contact with the pathogen. External services administer injection vaccines, while oral and immersion vaccines are administered by company staff. They do this following the protocols established by the pharmaceutical companies that provide the vaccines and also supervise the vaccination operation. The most diverse range of vaccine formulations is used in Atlantic salmon. Oral vaccination against ISA was reported to be used in the three salmonid species. In contrast, an oral vaccine against BKD was only used in Atlantic salmon (Table 11).

Validation of the OWIs on the farms

Tables 12-13 show the OWIs measured by hatcheries (RAS and FTS) where the protocol was assessed. Two hatcheries reported the separation of males from females, intending to transfer them to sea cages separately, as males grow at a rate of 15 to 17% faster than when they share a cage with females.

Hatcheries reported that feed is withdrawn for 24 h before any operational management of fish. Grading, vaccination, and the selection of precocious maturation were identified as the most stressful operations due to the mechanical damage generated by the use of vacuum pumps, resulting in scale loss, followed by *Saprolegnia* infections, primarily in Atlantic salmon.

Assessment of indirect OWIs

The RAS hatcheries recorded the 15 indirect OWIs listed in Table 12, while only six indicators were assessed in the FTS hatcheries. An online sensor in the tanks continuously monitors oxygen, temperature, and pH, and CO_2 is controlled daily in the RAS. In both hatchery systems (RAS and FTS), the water flow ($L\ s^{-1}$) in the tanks is controlled to provide a water change rate of between 1 and 2 changes per hour, depending on the tank volume and fish size, to avoid high or too low speeds that could affect the fish's swimming behavior.

Most RAS hatcheries, apart from external services, have their own laboratories to control water quality. All of them declared that hydrogen sulphide is monitored daily, that baking soda is used to stabilize the pH, and the salinity is kept between 5 and 7 to neutralize the nitrite toxicity. Furthermore, aluminium and iron, which, depending on the season of the year, are present in the inlet-water sources in concentrations higher than the level established as safe for salmon, are strictly controlled to avoid mortality in the fry stage. This stage is highly susceptible to these minerals.

Fish farmers pointed out that 0.05% is the accepted normal daily mortality rate for the parr-smolt stage. Fish farmers conduct the daily collection and classification of moribund and dead fish in accordance with the criteria defined by SERNAPESCA (2012). If

Table 10. Number of abnormal cases declared in the parr-smolt stage according to salmonid species (n = 41 hatcheries).

Abnormality	Atlantic salmon	Coho salmon	Rainbow trout
Vertebral deformities	1	1	0
Embryonic	2	2	2
Lordosis - scoliosis	2	2	1
Deformed opercula	25	6	5
Vertebral caudal compression	15	10	4
Mouth/jaw deformities	14	4	1
Nephrocalcinosis	10	2	1

Table 11. Vaccines applied in the freshwater stage, by salmonid species (n = 41 hatcheries). BKD: bacterial kidney disease, IPN: infectious pancreatic necrosis, SRS: syndrome rickettsial of salmonids, ISA: infectious salmon anemia.

Salmon species	Vaccine formulation	Fish weight (g)	Application mode	n
Atlantic salmon	Furunculosis-Vibriosis-IPN-SRS-ISA	35-100	Injection	18
	BKD	20-100	Injection	11
		90	Oral	1
	Flavobacterium	1-7	Immersion	6
	Furunculosis	5	Immersion	1
	IPN	1-7	Immersion	16
		100	Injection	2
		3	Oral	1
	IPN-SRS	12-90	Injection	7
	IPN-SRS-ISA-Vibriosis	40	Injection	1
	IPN-SRS-Vibriosis	90	Injection	1
	ISA	24	Injection	1
	Livac SRS	35-100	Injection	9
	SRS	35-100	Injection	5
	Yersiniosis	1	Immersion	1
Coho salmon	Furunculosis -Vibriosis-IPN-SRS-ISA	35	Injection	1
	BKD	20-500	Injection	5
	Flavobacterium	1-5	Immersion	3
	IPN	1	Immersion	6
		2-30	Oral	5
	IPN-SRS	30-70	Injection	9
	IPN-SRS-ISA-Vibriosis	5	Immersion	1
	SRS	2-6	Immersion	1
		35	Injection	1
	Livac SRS	35	Injection	1
Rainbow trout	Furunculosis -Vibriosis-IPN-SRS-ISA	35	Injection	1
	BKD	35	Injection	2
	IPN	1-3	Immersion	7
		5-10	Injection	1
		2	Oral	2
	IPN-SRS	5-150	Injection	11
	IPN	35	Immersion	1

any pathological signs, abnormal mortality, or behavioral deviations are observed in the fish, this must be promptly communicated to veterinary staff to facilitate the identification of any potential issues that may affect the fish stock.

Farmers also reported discarding between 10 and 12% of the smallest fish in the population during the first grading, due to poor growth performance, which is categorized as non-infectious. Fish that are not suitable for intensive production are euthanized with an overdose of anaesthetic (humanitarian euthanasia). To

Table 12. Indirect operational welfare indicators (OWIs) measured according to the type of hatchery. X: measured OWI. RAS: recirculation system; FTS: flow-through system.

Indirect OWIs	RAS		FTS	
	Broodstock & eggs	Smolt production	Broodstock & eggs	Smolt production
Water quality				
Oxygen	x	x	x	x
Temperature (°C)	x	x	x	x
CO_2	x	x	x	x
pH	x	x	x	x
Alkalinity		x		
Ammonium	x	x		
Nitrite	x	x		
Total ammonia nitrogen	x	x		
Nitrate	x	x		
Salinity	x	x		
Aluminium	x	x		
Total suspended solids	x	x	x	x
Water flow and water change rate	x	x	x	x
Productive				
Stocking density (kg m^{-3})		x	x	x
Mortality (%)	x	x	x	x

increase stocking density, both hatchery systems (FTS and RAS) reported the use of oxygen supplementation.

Assessment of direct OWIs

In both RAS and FTS hatcheries, the 15 indicators listed in Table 13 were assessed in the field. Ten indicators at the individual level were assessed using the scores suggested by Noble et al. (2018). The smoltification state was assessed using the score recommended by the RSPCA (2018a-b).

The damage caused by the intraperitoneal vaccination was assessed using the Spielberg scoring scheme (Midtlyng et al. 1996). The assessment was applied to 134 fish, 33 (24.6%) of which had mild adhesions (grade 1 according to the Spielberg Scale). These damages were frequently located near the injection site, which is attributed to the bad handling of vaccinators.

At the group level, the three indicators were assessed directly in the tanks by visual monitoring. All fish farmers reported that fish behaviour and daily feeding are routinely controlled by their own trained staff working in the hatchery, which in turn allows any welfare problems to be addressed.

The first feeding was identified as the most critical issue, primarily for Atlantic salmon. Fry are fed under a satiation regime using a manual system, followed by micro-rations supplied for 24 h, which prevents the accumulation of food remains at the bottom of the tanks, thereby avoiding gill problems and infections caused by *Saprolegnia* and Flavobacteria.

Table 13. Direct operational welfare indicators (OWIs) measured according to the type of hatchery. X: measured OWI. RAS: recirculation system; FTS: flow-through system.

Direct OWIs	Smolt production	
	RAS	FTS
Individual level		
Smoltification state	x	x
Condition factor	x	x
Eye damage	x	x
Snout damage	x	x
Opercular damage	x	x
Caudal fin damage	x	x
Dorsal fin damage	x	x
Pectoral fin damage	x	x
Pelvic damage fin	x	x
Deformities	x	x
Scale loss	x	x
Vaccine side effects	x	x
Group level		
Saprolegnia	x	x
Flavobacteria	x	x
Behaviour	x	x

During the parr and pre-smolt periods, 24-h lighting is applied, and fish are fed using automated systems.

Smolts are fed only during the lighting hours, while for the broodstock, feeding stops one month before spawning.

DISCUSSION

Although there have been no regulations governing fish welfare in Chile, salmon farmers have developed their own protocols to assess fish welfare at various stages of the production cycle. In this way, it was possible to validate the operational indicators of animal welfare included in this study, which were based on the OWIs suggested by Noble et al. (2018).

Environment-based welfare

Water quality

The risk profile of hatcheries with FTS, which are primarily supplied with water from rivers, was found to be lower than that of RAS hatcheries, which are mainly supplied with groundwater. The main problem observed in hatcheries with open flow (FTS) is the fluctuation in the temperature of the water, which is dependent on the prevailing environmental temperature, registering in summer the highest differences during the day, while the greatest risk in hatcheries with water recirculation (RAS) is the deterioration of the water quality, because the accumulation of metabolites such as ammonia and carbon dioxide (CO_2) as consequences of the water reuse, which can cause severe compromise on the fish welfare (Hjeltnes et al. 2012).

The CO_2 remediation is one of the most important factors in RAS hatcheries, where degasification equipment is used to maintain a CO_2 level of <15 mg L^{-1}. Diverse authors have stated that long-term exposure of fish to high CO_2 levels can lead to impaired growth performance (Mota et al. 2019), respiratory acidosis (Fivelstad et al. 2007), and ion mobilization in fish bones (Aslam et al. 2019), with consequences for fish health, welfare, and growth performance, and hence reduce production. Mota et al. (2019) reported that Atlantic salmon post-smolts cultured in brackish water RAS showed a maximum growth performance at CO_2 concentrations below 12 mg L^{-1}.

The minerals aluminium, iron, and copper present in the water sources, resulting from the geologic pattern and volcanic activity in southern Chile, pose a high risk to farmed salmonids, particularly in hatcheries with recirculation systems, where these metals tend to accumulate (Davison et al. 2009).

Kristensen et al. (2009) reported concentrations of aluminium >300 μg L^{-1} and up to 2,000 μg L^{-1} of iron in the inlet water used by hatcheries in Chile. In contrast, Atland & Bjerkness (2009) reported maximum concentrations of 1,160 μg L^{-1} of aluminum, 115.7 μg L^{-1} of iron, and 19.8 μg L^{-1} in natural freshwater courses feeding the fish farms' inflows in Chile.

High levels of aluminium, iron, and copper have been observed to cause adverse effects at the gill level, particularly when CO_2 levels are also elevated (Fivelstad et al. 2003). Aluminium is a metal frequently found in freshwater and has been demonstrated to cause gill damage in fish exposed to high concentrations (Kroglund et al. 2001). This results in respiratory alterations caused by plasma acidosis, hypoxia, and hypercapnia, which in turn lead to a loss of osmoregulation (Staurnes et al. 1993, Kroglund et al. 2001). Thus, fish farmers reported the addition of silica-lye to detoxify the water and prevent the mobilization of iron in hatcheries supplied with water containing elevated concentrations of iron and aluminium.

Lighting

The RSPCA's (2018a,b) position is that lighting levels must be maintained at a level suitable for each stage of development. In this study, all hatcheries reported utilizing lighting throughout the entire production cycle in freshwater, from the onset of the first feeding stage to the smoltification stage, employing a wide range of photoperiod strategies for Atlantic salmon. Ebbesson et al. (2007) reported that constant light has been found to have negative effects on the neurological development of parr. In contrast, Shulgina et al. (2021) reported that continuous lighting had a positive effect on the growth of juvenile Atlantic salmon. A similar outcome was reported by Nordgarden et al. (2003), who documented enhanced growth in conjunction with an improved FCR and an increased appetite in Atlantic salmon subjected to constant lighting, corroborated by Nordland et al. (2023), who reported that light conditions during early development did not affect the size of salmon at first feeding. However, there is a lack of information regarding the use of continuous lighting throughout the production process in freshwater aquaculture and its impact on fish welfare under Chilean conditions.

Stocking density

Stocking density is widely acknowledged as a crucial husbandry factor in intensive fish culture, given its potential to induce chronic stress, which may have detrimental effects on the physiological, health, and/or behavioural status of the individual fish involved (Ashley 2007). However, we found no significant differences in stocking density among the three salmonid species. The highest stocking density for Atlantic salmon was 70 kg m^{-3} for RAS systems, and 60 kg m^{-3} for the FTS systems. The highest stocking density was 60 kg m^{-3} for rainbow trout and coho salmon.

Although the stocking densities found in the present study are higher than the RSPCA standards for the small fish (<30 g), Hosfeld et al. (2009) reported that at densities between 21 and 86 kg m^{-3}, no negative effects of density were found if water quality and food rations are maintained according to the recommended standards. However, it is recommended that the stocking density in relation to water quality should be analyzed in future studies.

Group-based welfare

Behaviour

The feeding behaviour of fish and their appetite are subject to routine visual monitoring by trained personnel. Reduced appetite is one of the primary causes of growth reduction following stress exposure (Huntingford et al. 2006, Martins et al. 2012). The appetite of fish can be suppressed by several factors, including poor water quality and environmental conditions, as well as health issues affecting the fish (Noble et al. 2018).

Although behaviour is considered a non-invasive measure and one of the best indicators of welfare for farmed fish (Martins et al. 2012), it is difficult to assess and quantify. It requires trained staff to identify any deviations from the normal behaviour of fish. Relying solely on visual observation of behavior can also be time-consuming, subjective, and unreliable. The use of automatic systems to detect feed provision and consumption is highly recommended (Parra et al. 2018). However, visual observation during the parr-smolt stage is crucial for identifying any warning signs that may indicate a potential welfare issue.

Mortality

In the parr-smolt stage, mortality includes the culling of abnormal fish and those without potential growth, which are detected during grading and vaccination operations. As reported by SERNAPESCA (2024), in 2023, the mortality rate for non-infectious classifications corresponded to 87.5% of the total mortality in Atlantic salmon, 93% in coho salmon, and 80.3% in rainbow trout, which is high in comparison with the mortality caused by infectious diseases, which was less than 20% of the total. These values must draw attention to the welfare conditions to which fish are subjected during freshwater rearing.

Diseases

Saprolegnia, *Flavobacterium*, and *Renibacterium salmoninarum* (BKD) were the most frequently reported pathogens, primarily affecting Atlantic salmon reared in FTS hatcheries, which may be associated with the water source and the stress generated by natural fluctuations in water quality, which compares with the RAS hatcheries, where all parameters are under control and the water source is usually free of pathogens.

Developmental disorders recorded during the incubation and first feeding periods are mainly associated with hereditary, environmental, or nutritional factors that occurred during embryonic development (Valdebenito et al. 2021, Callet et al. 2022, Perez-Atehortúa et al. 2024). In this study, the main alterations were reported for Atlantic salmon, although microphthalmia was mainly reported for coho salmon. Few studies have focused on the embryonic alterations in salmonids reared in Chile. Perez-Atehortúa et al. (2024) recorded embryonic malformations and microphthalmia in Atlantic salmon eyed-eggs, while Valdebenito et al. (2021) reported chorion alterations in samples of embryonated eggs collected from different salmon and trout farms located in southern Chile over a period of 14 years. These were associated with changes in water conditions. These findings suggest that further studies are necessary to identify the critical factors that influence the survival of embryos, thereby improving the welfare conditions during the incubation process of salmonids produced in Chile.

Individual-based welfare

One of the important issues identified in this study was the precocious maturation in Atlantic salmon males, which was observed primarily in hatcheries utilizing RAS. Some authors have stated that the utilization of photoperiod and the increase in water temperature above optimal ranges, to facilitate fish growth, represent the most significant factors (Fjelldal et al. 2011, Good & Davidson 2016, Good et al. 2016). Pino-Martinez et al. (2025) declared that the body weight at the time photoperiod manipulation is initiated to induce smoltification may generate a high risk of early maturation. In this study, the temperature declared for the parr-smolt stage was in the range 13-16ºC, and a wide range of photoperiod strategies was used for the winter regime in Atlantic salmon, which could induce maturation.

No information was identified regarding the separation of males and females during the freshwater phase (pre-smoltification) and its implications for fish welfare, including the need for social contact once the fish are transferred to the sea cages, which are separated by sex.

Deformities and injuries are considered detrimental to fish welfare and production performance (Huntingford et al. 2006, Noble et al. 2012). In this study, deformities were primarily reported in Atlantic salmon during the parr-smolt stage, including deformed opercula, compression of the vertebral column, and mouth deformity. The cause of these deformities is often uncertain, although genetic, nutritional, and cultural factors have been suggested, associated with incubation temperatures and alterations in the stage of embryo development, among others (Ørnsrud et al. 2004, Eriksen et al. 2007, Noble et al. 2012).

The occurrence of nephrocalcinosis, included in the category "others" by SERNAPESCA, was reported by fish farmers in all three salmonid species, with a higher prevalence observed in Atlantic salmon reared in FTS. The etiology of nephrocalcinosis has been linked to a high concentration of CO_2 in the water, as well as nutritional aspects, including magnesium deficiency and selenium toxicity, in farmed salmonids (Bruno et al. 2013). Fish exposure to concentrations exceeding 15 mg L^{-1} of CO_2 has been associated with nephrocalcinosis (Fivelstad et al. 2003), which could explain the higher prevalence in Atlantic salmon reared in FTS, as opposed to RAS, where CO_2 is strictly controlled and a degasification equipment is used. Klykken et al. (2022) noted that nephrocalcinosis may be an indicator of disturbed osmoregulation and increased stress levels. They suggested that it is a common and serious welfare challenge in Atlantic salmon that warrants closer monitoring.

CONCLUSIONS

The results generated in this study, mandated by the SUBPESCA, allowed the management, environmental, and welfare conditions of salmon in the production cycle in freshwater to be characterized and classified in concordance with the indicators identified by Noble et al. (2018) for welfare assessment of farmed salmon.

The 30 OWIs selected as the most suitable were grouped into indirect (15) and direct OWIs (15) and successfully validated in the 16 hatcheries. Most of these are routinely assessed in the hatcheries, and their implementation does not require an increase in the operational costs.

Water quality was considered the most critical issue for smolt production in freshwater, followed by precocious maturation in male Atlantic salmon reared in RAS hatcheries. Mortality was classified as non-infectious, which included the culling of fish for disease control purposes.

The indirect OWIs, for which reference has been validated, were easier to measure than the direct OWIs, which require continued observation by trained staff.

Further studies are required to improve the welfare conditions at the freshwater production stage of salmon in Chile, to reduce mortality categorized as non-infectious.

Eighty-three point three percent of fish farmers indicated that they had training in animal welfare, and 60% surveyed indicated that they had a professional dedicated exclusively to animal welfare. Nevertheless, it is essential to have standardized protocols to enhance the assessment of welfare conditions in farmed salmonids in freshwater.

Credit author contribution

S. Bravo: funding acquisition, project administration, conceptualization, methodology, formal analysis, writing-original draft, supervision, review, and editing; N. Ponce: validation, methodology, data curation, and formal analysis; A. Strappini & G. Monti: methodology, validation, writing-original draft, review, and editing; M.T. Silva: methodology, data curation, and formal analysis. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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