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# Effects of urban pollution on hematological parameters and defensive capacity of rainbow trout from the Chalhuanca River

*Efeitos da poluição urbana nos parâmetros hematológicos e na capacidade defensiva da truta-arco-íris do Rio Chalhuanca*

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**RESUMO**

A truta arco-íris é essencial na dieta andina, especialmente em comunidades onde a qualidade da água é fundamental para a sua saúde e crescimento. Nossa pesquisa se concentra em como as condições ambientais do rio Chalhuanca afetam a saúde física, o estresse crônico e a capacidade imunológica da truta arco-íris, e qual é a relação desses efeitos com a segurança alimentar nas comunidades locais. Foram examinadas trutas juvenis do rio Chalhuanca, coletando 36 amostras em três pontos: antes de Cotaruse, depois de Caraibamba e passando por Chalhuanca. Os peixes foram medidos, pesados e amostras de sangue foram coletadas para avaliar componentes como glóbulos vermelhos, brancos, hemoglobina, hematócrito, glicose e cortisol. Também foi analisada a capacidade dos peixes de combater a bactéria *E. coli* e foram medidos parâmetros físico-químicos e microbiológicos da água. Os resultados mostraram diferenças significativas na saúde das trutas entre as áreas. As trutas de Chalhuanca apresentaram pior condição corporal e anemia microcítica hipocrômica (hemoglobina: 6.1 g/dL, hematócrito: 29%). Além disso, observou-se uma alta proporção de estresse crônico nesta área. A qualidade da água também mostrou variações, com maiores concentrações de coliformes fecais (1200 UFC/100 mL) e menores níveis de oxigênio dissolvido (4.5 mg/L) em Chalhuanca, indicando um maior grau de contaminação.

***PALAVRAS-CHAVE:*** Antropogênico; salmão; estresse.

***ABSTRACT***

Rainbow trout is essential in the Andean diet, especially in communities where water quality is essential for their health and growth. Our research focuses on how the environmental conditions of the Chalhuanca River affect the physical health, chronic stress, and immunological capacity of rainbow trout, and what the relationship of these effects is to food security in local communities. Juvenile trout from the Chalhuanca River were examined, collecting 36 samples at three points: before Cotaruse, after Caraibamba and passing Chalhuanca. The fish were measured, weighed and blood was drawn to evaluate components such as red and white blood cells, hemoglobin, hematocrit, glucose and cortisol. The ability of the fish to combat the *E. coli* bacteria was also analyzed and physicochemical and microbiological parameters of the water were measured. The results showed significant differences in trout health between areas. Chalhuanca trout presented worse body condition and hypochromic microcytic anemia (hemoglobin: 6.1 g/dL, hematocrit: 29%). Furthermore, a high proportion of chronic stress was observed in this area. Water quality also showed variations, with higher concentrations of fecal coliforms (1200 CFU/100 mL) and lower levels of dissolved oxygen (4.5 mg/L) in Chalhuanca, indicating a higher degree of contamination.

**KEYWORDS:** Anthropogenic; salmon; stress.

## INTRODUCTION

Rainbow trout, introduced to Peru from North America in 1925, plays a vital role in Andean nutrition, serving as a crucial protein source for local communities (VILLA 2021, PUCUHUARANGA 2019). The survival of this species is closely linked to water quality, with critical environmental parameters such as temperature, dissolved oxygen, and pH playing fundamental roles in its health and growth (PUCUHUARANGA 2019). Hematological parameters, including red blood cells, white blood cells, hemoglobin, and hematocrit, as well as cortisol levels, are essential for assessing rainbow trout health and welfare. These parameters reflect fish adaptability to their environment and their response to water quality changes, such as contamination and physicochemical fluctuations, with implications for assessing trout safety as a food source for local communities (CORRÊA et al. 2013, ZUTSHI et al. 2010).

Water contamination, characterized by toxic substances, inadequate oxygen levels, pH fluctuations and extreme temperatures, can cause significant changes in the hematological parameters of fish, reflecting a stress response that affects their immunological capacity and resistance to diseases (ZHELEV et al. 2016, JENTOFT et al. 2005)

Anthropogenic pollution severely impacts aquatic biodiversity, as observed in the Ganges River in India, where domestic effluents and pesticides affect fish (KHANNA et al. 2007). In Brazil, fish health deterioration in the Tramandaí and Mampituba rivers has been linked to population growth in adjacent areas, attributed to hydrocarbon and metal discharge, along with alterations in water pH and temperature (DE-ANDRADE et al. 2004). Toxic substances found in Lake Mamacocha (Cajamarca, Peru) caused detrimental effects on rainbow trout health, including gill structure alterations, liver tissue damage and necrosis, and fluid accumulation in striated skeletal muscle (HUANCARÉ 2014).

Given the critical importance of rainbow trout in Andean diets and the documented impacts of pollution on aquatic ecosystems, our research examines how environmental conditions in the Chalhuanca River affect the physical health, chronic stress, and immune response of rainbow trout, and how these effects relate to food security in local communities.

## MATERIALS AND METHODS

### Sampling Strategy and Sample Size Determination

Wild rainbow trout (*Oncorhynchus mykiss*) specimens from the Chalhuanca River were examined, measuring approximately 15 cm in length, in juvenile stage and exhibiting fair to poor body condition. The sample size was determined using the formula for infinite populations, considering a prevalence of 2% according to the aquatic animal disease surveillance guidelines of the Aquatic Animal Health Code (WOAH 2022). The sampling design required 31 specimens, but 36 fish were analyzed. The data were collected at three sampling sites along the river: upstream from Cotaruse, downstream from Caraibamba, and near Chalhuanca. The animal sampling formula: $n = ((Z\hat{}2 * p * q)/d\hat{}2\ )$

### Ethical considerations

During the study, fish were returned to the river after weighing, measuring, and blood collection, with no specimens sacrificed. Furthermore, anesthetic agents were not used in fish due to their effects on hematocrit (HABIB et al. 2023).

### Data collection

*Body condition and blood components of rainbow trout from the Chalhuanca River*

The assessment of trout body condition was performed by capturing specimens with a fishing net and transferring them to a holding tank. A precision balance submerged in water was used for weighing. In addition, an ichthyometer was used to measure each trout from the tip of the snout to the junction of the caudal peduncle, following the formula: $K = (100xW(g))/L\hat{}3$ (CIFUENTES et al. 2012).

3 ml of blood were collected from the tail vein of rainbow trout using Vacutainer tubes with EDTA to evaluate their blood components, including red blood cells, white blood cells, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (SUEIRO & PALACIOS 2016). Furthermore, the concentration of glucose and cortisol was determined from the plasma obtained (MARTINEZ et al. 2009).

Standardized methods were used to measure blood values of rainbow trout. A commercial glucose kit was used according to the manufacturer's instructions, where a standard glucose solution was prepared and absorbance was measured at 505 nm using a spectrophotometer (NABI et al. 2022). Hematocrit was determined by microcentrifugation of blood samples in heparinized capillary tubes at 12,000 rpm for 5 minutes, followed by measurement of packed red blood cell volume using a hematocrit reader (NABI et al.). 2022).

Hemoglobin levels were measured using the HemoCue® Hb 201 analyzer; blood samples were diluted in hemolysis solution, placed in the HemoCue® device, and hemoglobin concentrations were recorded (HARTER et al. 2015). White blood cell counts were performed using a hemocytometer; blood was diluted in Turk's solution, which stains the nuclei of white blood cells, and cells were counted under a microscope (MESHKINI et al. 2012, NABI et al. 2022). Plasma cortisol levels were measured using an ELISA kit (Neogen Corporation). A 100-µL plasma aliquot was mixed with 1 mL of ethyl ether in a glass tube, vortexed for one minute, and frozen at -80 °C for one hour to obtain the liquid phase. The ether was evaporated in Eppendorf tubes. Subsequently, 100 µL of extraction buffer (diluted 1:5) was added, and a second dilution (1:100) was performed by adding 10 µL into 990 µL of diluted extraction buffer. Finally, the solution was stirred with a vortex. On the ELISA plate, 50 µL of the dilution was placed for reading, which was performed at 620 nm. Seven standard samples were prepared to confirm the quality of the kit (SAMARAS & PAVLIDIS 2022). Cortisol concentrations were quantified using the standard curve equation ($X = e^{[(52.589-y)/15.13]}$).

*Ability of rainbow trout to fight E. coli bacteria (ATCC 25922)*

A working solution containing approximately 200 colony-forming units (CFU) of *Escherichia coli* (ATCC 25922) was prepared in sterile phosphate-buffered saline (PBS). Plasma samples were diluted 1:10 in sterile PBS. The reactions were performed by mixing 10 µL of bacterial solution with 90 µL of diluted plasma samples and incubating at 18 °C for 4 hours. As controls, 10 µL of the bacterial solution were mixed with 90 µL of PBS, seeding samples before, during and after the process. All reactions and controls were plated in duplicate (50 µL aliquots) on 4% tryptic soy agar and incubated overnight at 25°C. Finally, the number of bacterial colonies on each plate was counted and the percentage of colonies was calculated based on the average number of colonies in the controls (MATSON et al. 2006, SUEIRO & PALACIOS 2016).

*Assessment of river water quality*

Water quality parameters (temperature, dissolved oxygen, conductivity, turbidity, pH, total alkalinity, total hardness, calcium, and magnesium) were measured in situ using a Hanna multiparameter probe (Hi 9829). For microbiological analysis (total and thermotolerant coliforms), water samples were collected in 250-mL sterile bottles and transported under refrigeration to the Environmental Sanitation Laboratory (ESL) of Apurímac, where they were processed within 24 hours (PALAMULENI & AKOTH 2015). Coliform counts were determined using the Most Probable Number (MPN) method with multiple tube technique. Samples were incubated in Lauryl Tryptose Broth and EC Broth at specific temperatures, with gas production indicating coliform presence. Results were compared against water quality standards established by regulatory guidelines. No. 002-2008-MINAM.

**Data processing and analysis**

Statistical analysis was performed using SPSS version 26. To compare the different sampling locations, Tukey's statistical test was applied.

## RESULTS

**Body condition and blood components of rainbow trout from the Chalhuanca River**

The study revealed that rainbow trout from Chalhuanca exhibited less developed body conformation compared to specimens from Caraibamba and Cotaruse.

Hematocrit values in Caraibamba and Chalhuanca were outside the reference ranges. Hemoglobin levels indicated anemia across all sampling sites, and Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were used to determine the type of anemia. In all locations, MCV values were below normal ranges, indicating microcytosis, while low MCH counts suggested a hypochromic state (Table 1).

An increase in the percentage of chronically stressed rainbow trout was observed towards the mouth of the river: 50% in Cotaruse, 75% in Caraibamba and 91.7% in Chalhuanca. The microcytic and hypochromic anemia observed in these trout may be attributed to chronic stress (Table 2).

Table 1. Variables studied to analyze the health status of rainbow trout (*Oncorhynchus mykiss*) in the Chalhuanca River.

| Parameters | Reference values | Cotaruse | Caraibamba | Chalhuanca | P |
|---|---|---|---|---|---|
| Weight (g) | - | 67.8 ± 15.4 $^{a}$ | 52.5 ± 11.8 $^{a}$ | 57.3 ± 24.1 $^{a}$ | 0.117 |
| Size (cm) | - | 174.1 ± 13.7 $^{a}$ | 163.8 ± 10.1 $^{a}$ | 166.9 ± 20.8 $^{a}$ | 0.232 |
| *K factor* | 1.2 – 2.0 (SHARMA & BHAT 2015) | 1.27 ± 0.1 $^{a}$ | 1.19 ± 0.1 $^{ab}$ | 1.17 ± 0.1 $^{b}$ | 0.048 |
| Hemoglobin (g/dL) | 5.6 – 9.4 (CLARK et al. 2008, JALALI et al. 2009) | 4.4 ± 0.9 $^{a}$ | 4.3 ± 0.9 $^{a}$ | 4.1 ± 0.6 $^{a}$ | 0.671 |
| Hematocrit (%) | 35 – 44 (JALALI et al. 2009, REINOSO 2017) | 36.0 ± 3.8 $^{a}$ | 33.8 ± 2.9 $^{a}$ | 29.9 ± 4.3 $^{b}$ | 0.001 |
| RBC ($10^{6}$) | 1.30 – 1.58 (REINOSO 2017) | 1.35 ± 0.1 $^{a}$ | 1.35 ± 0.1 $^{a}$ | 1.37 ± 0.1 $^{a}$ | 0.528 |
| VCM (fl) | 275 – 369 (JALALI et al. 2009) | 266.3 ± 33.8 $^{a}$ | 249.5 ± 28.7 $^{a}$ | 216.9 ± 29.7 $^{b}$ | 0.002 |
| HCM (pg) | 69 – 102 (JALALI et al. 2009) | 32.5 ± 6.9 $^{a}$ | 31.3 ± 6.3 $^{a}$ | 29.8 ± 4.3 $^{a}$ | 0.546 |
| MCHC (g/dL) | 12 – 22 (JALALI et al. 2009) | 12.3 ± 2.3 $^{a}$ | 12.7 ± 2.9 $^{a}$ | 13.9 ± 2.0 $^{a}$ | 0.278 |
| Glucose (mmol/L) | 3.89 – 4.14 (MARTINEZ et al. 2009, ROJAS 2005) | 4.5 ± 2.2 $^{a}$ | 3.9 ± 1.8 $^{a}$ | 3.6 ± 1.5 $^{a}$ | 0.532 |
| Cortisol (ng/ml) | 6 – 14.5 (MARTINEZ et al. 2009) | 6.3 ± 2.8 $^{a}$ | 6.1 ± 2.2 $^{a}$ | 3.4 ± 2.1 $^{b}$ | 0.007 |

P-values were used to assess differences in parameters between cities, and means and standard deviations were reported for each city. Means that did not share letters were significantly different between cities (a, b). Reference values were indicated with superscripts to show the sources from which they were obtained (MARTINEZ et al. 2009) (SHARMA & BHAT 2015) (CLARK et al. 2008) (JALALI et al. 2009) (REINOSO 2017) (ROJAS 2005).

Table 2. Anemia and stress condition in rainbow trout in the Chalhuanca River.

| | Variable | Cotaruse | Caraibamba | Chalhuanca | Average (*x*) |
|---|---|---|---|---|---|
| Stress | No stress | 50.0% | 25.0% | 8.3% | 27.8% |
| | Chronic stress | 50.0% | 75.0% | 91.7% | 72.2% |
| | Total | 100.0% | 100.0% | 100.0% | 100.0% |
| Anemic state | No anemia | 8.4% | 8.3% | 0.0% | 5.6% |
| | Hypochromic anemia | 33.3% | 16.7% | 0.0% | 16.7% |
| | Hypochromic microcytic anemia | 58.3% | 75.0% | 100.0% | 77.8% |
| | Total | 100.0% | 100.0% | 100.0% | 100.0% |

**Ability of rainbow trout to fight *E. coli* bacteria (ATCC 25922)**

The bactericidal activity of blood plasma was used to assess the rainbow trout immune response across three distinct regions. Significant variation in immune responses was observed among the three groups; however, no significant differences were found in bactericidal activity against *E. coli*. Furthermore, a moderately negative relationship was observed between cortisol and hematocrit concentrations and fecal coliform levels in water, with no significant differences in this parameter among the three cities (Table 3).

Table 3. Rainbow trout's ability to cope with and combat *E. coli* (ATCC 25922)

| Parameter | Control | Cotaruse | Caraibamba | Chalhuanca | P |
|---|---|---|---|---|---|
| Amount of *E. coli* (CFU/plate) | 201 | 142 ± 51 | 140 ± 30 | 139 ± 37 | 0.9 |
| Bactericidal capacity | 60 (SUEIRO & PALACIOS 2016) | 29.2 ± 25 | 30.2 ± 14 | 31.0 ± 18 | 0.9 |

Results are expressed as mean ± standard deviation (x±S). A control value was used to evaluate bactericidal activity, as extracted from Sueiro and Palacios (2016), and the P-value indicates whether there are significant differences between the analyzed cities.

**Physical, chemical and microbiological parameters of the Chalhuanca River**

Table 4 shows the water quality assessment results across three sites for multiple parameters.

Table 4. Physical, chemical and microbiological parameters of the Chalhuanca River.

| | Parameters | Reference values | Cotaruse | Caraibamba | Chalhuanca |
|---|---|---|---|---|---|
| Physical-chemical analysis | Temperature (°C) | 10 to 16 | 11 | 13 | 14 |
| | dissolved oxygen | >5 | 5.4 | 4.6 | 4.1 |
| | Conductivity (µS/cm) | <2000 | 241 | 243 | 235 |
| | Turbidity (NTU) | <25 | 5.07 | 5.8 | 4.91 |
| | pH | 6.5 to 8.5 | 8.32 | 8.34 | 8.31 |
| | Total alkalinity (mg/l $CaCO_3$) | 20 to 200 | 113.4 | 109.2 | 105 |
| | Chlorides | <200 | 30 | 78 | 88 |
| | Total hardness (mg/L $CaCO_3$) | 5 to 200 | 121.8 | 128.1 | 113.4 |
| | Calcium (mg/L) | <52 | 31.08 | 42.84 | 41.16 |
| | Magnesium (mg/L) | <150 | 10.73 | 5.11 | 2.56 |
| Coliforms /100ml | Total (35 °C) | <5000 | 14000 | 16530 | 17000 |
| | Thermotolerant (44.5 °C) | <1000 | 6000 | 7500 | 9000 |

**DISCUSSION**

Rainbow trout health was assessed using the K-condition index, which provides information about their reproduction, nutrition, growth and general health. The findings suggest that fish from Cotaruse exhibit superior body conformation compared to those from Caraibamba and Chalhuanca, with the latter showing the poorest health conditions. Previous studies have shown that poor body condition is common in contaminated environments (HANANA et al. 2021). This phenomenon has also been observed in other fish species in different parts of the world, such as in Lake Llanquihue in Chile (ARISMENDI et al. 2011), in Lake Temenggor in Malaysia (ABD-HAMID et al. 2015), in Lake Wular in India (MUSHTAQ et al. 2016), in the Gomti River in India and in the Niger River in Nigeria (GUPTA et al. 2011). These studies indicate that pollutant discharge significantly impacts fish health and body condition.

The hemoglobin concentration in fish is crucial to assess their adaptability to the environment (NABI et al. 2022). In the Chalhuanca River, hemoglobin concentrations were comparable across studied cities but remained below reference values. This may be attributed to pollution from wastewater and industrial waste, which has been shown to reduce hemoglobin levels in fish, as observed in Labeo rohita in Bangalore lakes (TABREZ et al. 2022, ZUTSHI et al. 2010). Furthermore, hypoxia and anemia are factors that affect the hemoglobin concentration in fish. This was observed in rainbow trout kept under hypoxic conditions (GARCÍA et al. 2022, SIMONOT & FARRELL 2007). Although temperature can also influence hemoglobin concentrations, in the case of the Chalhuanca River, the low hemoglobin level appears to be due primarily to the presence of wastewater and industrial residues, rather than to temperature variations (RAVICHANDRAN et al. 2016).

Fish hematocrit levels serve as a vital indicator of oxygen transport capacity and hematopoietic function (RUIZ et al. 2024). In the Chalhuanca River, rainbow trout hematocrit levels were significantly lower compared to other locations and fell below reference values. This finding suggests that environmental pollution, particularly the discharge of wastewater and other residues, may be negatively affecting fish health in this area (GARCÍA et al. 2022, RUIZ et al. 2024) The decrease in hematocrit may reflect an adaptive response of fish to adverse conditions, where pollution affects the ability of fish to utilize their blood cell reserves and may even lead to the deterioration of their spawning habitats (THOMAS et al. 2019).

Although temperature can also influence hematocrit levels, changes within the range of 5 to 20 °C do not usually cause significant changes in this parameter (TABREZ et al. 2022, ZUTSHI et al. 2010). Furthermore, previous studies have shown that chronic exposure to pollutants can reduce hematocrit levels in different fish species, reinforcing the idea that pollutants are a likely cause of the low levels observed in the Chalhuanca River (NABI et al. 2022, SHAH 2006, TABREZ et al. 2022).

Red blood cell count in fish can serve as a valuable indicator for assessing pollution impact, as chemical substances may induce hypoxic conditions that trigger blood cell production in response to low oxygen levels (PALA & DEY 2016, RUIZ et al. 2024). In the study of rainbow trout in the Chalhuanca River, red blood cell counts showed no significant differences among sampling sites, with all values remaining within normal ranges. These findings suggest that environmental pollutants did not significantly impact erythrocyte production in these trout specimens.

These findings corroborate previous studies on other fish species exposed to pollutants. For example, it has been observed that at low levels of pollutants, there may be an increase in red blood cell count due to induced hypoxia (GARCÍA et al. 2022, RUIZ et al. 2024). However, at higher pollution levels, a decrease in red blood cell count may occur due to more severe adverse effects on fish health (RAVICHANDRAN et al. 2016). In our study, the absence of variation in red blood cell counts suggests that pollution levels in the Chalhuanca River did not reach a threshold sufficient to induce significant changes in this hematological parameter.

The analysis of red blood cell indices, including MCH (Mean Corpuscular Hemoglobin), MCV (Mean Corpuscular Volume), and MCHC (Mean Corpuscular Hemoglobin Concentration), provides crucial information about cardiac function, respiratory capacity, and osmotic balance in fish erythrocytes (STOSIK et al. 2020).

In our study, we found that while MCH and MCHC values in rainbow trout were similar across Chalhuanca, Cotaruse, and Caraibamba, MCV concentrations showed significant variations among these locations. Furthermore, all MCV, MCH, and MCHC values were below reference limits, suggesting microcytosis in the studied trout.

The findings indicate that MCV and MCH parameters are sensitive to changes in water quality, which may be influenced by physicochemical factors such as temperature, conductivity, total dissolved solids, dissolved oxygen, and the presence of chemical contaminants (CORRÊA et al. 2013, ESPOSITO et al. 2024). In particular, the observed microcytosis may be linked to environmental contaminant exposure, as documented in studies examining the effects of toxic substances such as paraquat and malathion on various fish species, where decreased Hb, MCH, MCHC, and MCV values were reported (AMAEZE et al. 2020, CHOWDHURY et al. 2013, SALAZAR et al. 2009).

Evidence from previous studies supports the association between environmental contaminants and alterations in erythrocyte indices. For example, it was observed that domestic waste, heavy metals, textile industry waste and compounds such as zinc and lead can cause low values in these indices, reflecting adverse effects on fish health (KATALAY & PARLAK 2004, TABREZ et al. 2022, ZHELEV et al. 2016). The reduction in MCH, MCHC, and MCV may represent an adaptive response to pollution, suggesting potential adverse effects on the overall health status of trout across all three sampling sites.

Although aquaculture is crucial for food production and economic growth, it may be associated with various fish diseases, including normochromic microcytic anemia. This condition can be caused by a number of stressful environmental factors, such as nitrite, ammonium and heavy metal poisoning, as well as chronic exposure to insecticides and parasites (BAKRIM et al. 2018, NABI et al. 2022).

Each of these factors can affect fish health and result in various forms of anemia. In particular, normochromic microcytic anemia, characterized by red blood cells smaller than normal with adequate hemoglobin content, can be caused by various factors. Exposure to high concentrations of nitrites and ammonium in water can alter hematopoietic function, causing changes in erythrocyte indices (BAKRIM et al. 2018). Heavy metals, such as lead and zinc, are also associated with anemia in fish, as they can interfere with red blood cell metabolism and hemoglobin synthesis (TASLIMA et al. 2022). Furthermore, insecticides and parasites can induce chronic stress in fish, contributing to the appearance of normochromic microcytic anemia (CURRIE et al. 2022).

It is essential to differentiate between hypochromic microcytic anemia and other conditions that may present similar symptoms. Thalassemia and anemia due to chronic diseases, for example, have different causes and treatments than anemia induced by environmental factors (CURRIE et al. 2022). Thalassemia is a genetic disease that affects hemoglobin production, while anemia due to chronic diseases is linked to prolonged pathological processes (FAJARDO et al. 2022). Lead poisoning can induce hypochromic microcytic anemia, which differs from the normochromic microcytic anemia observed under environmental stress conditions (BAKRIM et al. 2018, CURRIE et al. 2022).

Fish stress triggers physiological responses, including the release of catecholamines and cortisol, key hormones in adaptation to adverse conditions. These hormone levels can vary depending on factors such as changes in water, temperature, oxygen, salinity, nutritional status and presence of diseases (FAJARDO et al. 2022). The stress response is a natural component of the stress regulation system in fish, designed to maintain homeostatic balance. However, chronic or severe stress can significantly impact fish health and welfare.

During chronic stress exposure, fish may undergo adaptation to environmental toxicants, which can initially lead to reduced cortisol levels as a compensatory mechanism (LEMOS et al. 2023). However, chronic or severe stress can transform this adaptation into maladaptation, resulting in decreased overall fish performance and increased susceptibility to infections and diseases (LEMOS et al. 2023).

The inability of fish to properly regulate cortisol levels may increase their susceptibility to pathogens and other stressors. In mammals, it has been observed that a persistent deficiency of cortisol can contribute to an increased susceptibility to stress-related disorders, a concept that can also be applied to fish (LEMOS et al. 2023). In this context, both high and low cortisol levels can modulate fish immune response, affecting their ability to cope with infections. Elevated cortisol levels, often associated with intense stress, can suppress the immune response and increase the risk of disease, while persistently low levels may be an indicator of an ineffective adaptive response to stress (HANNIBAL & BISHOP 2014, LEMOS et al. 2023).

Fish serum, including that of *O. mykiss*, possesses non-specific antibacterial properties essential for defense against pathogenic microorganisms. This capacity is a valuable indicator of the immune response in fish, as detailed in Table 3, and its assessment is a reliable methodology in aquatic immunological studies (ALY et al. 2008, FIERRO et al. 2024).

The colony forming unit (CFU) count test is widely used to measure serum bactericidal activity and evaluate the efficacy of the immune response in several fish species, including *Oreochromis niloticus* and Labeo *rohita* (ALY et al. 2008, FIERRO et al. 2024). However, bacterial colony counts may be influenced by variations in testing methods and bacterial concentrations, potentially affecting the results.

In the present study, serum bactericidal activity of O. mykiss was evaluated across three locations, with fish from Cotaruse, Caraibamba, and Chalhuanca exhibiting comparable bactericidal activity of 30%. This result is lower than the reference value of 60% required to inhibit the colonization capacity of E. coli, indicating a lower bactericidal activity than expected (BOLEZA et al. 2001, MIZAEVA et al. 2023).

These findings are consistent with previous research that reported a decrease in bactericidal activity in fish exposed to adverse environmental conditions such as hypoxia, changes in pH and contaminants (DENSMORE et al. 2004, MIZAEVA et al. 2023). Furthermore, studies have shown that exposure to heavy metals, pharmaceuticals, and chemical compounds in aquatic environments can significantly impair serum bactericidal activity (RIBAS et al. 2016, WANG et al. 2016). The presence of these contaminants in the aquatic habitat of O. mykiss can compromise the effectiveness of the fish's immune system, increasing their vulnerability to infections and affecting their overall health (MIZAEVA et al. 2023).

Fish stress response triggers complex physiological responses, with catecholamine and cortisol release being among the most prominent (LEMOS et al. 2023). In the present study, cortisol levels in O. mykiss were evaluated in three locations: Cotaruse, Caraibamba and Chalhuanca.

The results revealed that while cortisol levels in Cotaruse and Caraibamba remained within normal range (6 to 14.5 ng/mL), levels in Chalhuanca were significantly lower, measuring 3.4 ng/mL. This finding suggests that environmental factors in Chalhuanca may influence stress response differently compared to other locations.

Various physicochemical and microbiological water parameters, including temperature fluctuations, dissolved oxygen, salinity, time of day, light wavelength, tank bottom coloration, fish nutritional status, and disease occurrence, can modulate cortisol levels in fish. 2023, MARTINEZ et al. 2009, SAMARAS & PAVLIDIS 2022). These factors can trigger a stress response in fish, initially manifested through elevated cortisol levels. For example, studies have shown that exposure to a stressor for ten minutes can raise cortisol levels to over 60 ng/ml in O. mykiss (HABIB et al. 2023) and in Salmo salar (MADARO et al. 2023). However, chronic exposure to these factors may lead to toxic adaptation, reducing cortisol levels below normal (<6 ng/ml) after six days of continuous exposure (MADARO et al. 2023, OVERLI 2005).

In cases of severe or prolonged stress, fish may enter a state of maladaptation, resulting in decreased performance, pathological conditions or even death (MADARO et al. 2023, OVERLI 2005). This maladaptive state is characterized by elevated plasma cortisol levels, which may increase susceptibility to fungal, bacterial, and parasitic infections (MIZAEVA et al. 2023). On the other hand, low cortisol concentrations can also increase susceptibility to infections, as observed in O. mykiss in the Arlanza River (MIZAEVA et al. 2023, VERCAUTEREN et al. 2022).

In contrast to previous studies reporting elevated cortisol concentrations under stress conditions in Salmo trutta in Soria, Quintanar de la Sierra and Acera de la Vega (Spain) (VAL et al. 2006) our findings in Chalhuanca, characterized by remarkably low cortisol levels, suggest that local stressors might differ in nature or intensity compared to those in other sampled locations. Evidence indicates that both elevated and reduced cortisol levels may adversely affect fish health. High cortisol levels may be associated with increased vulnerability to infections and chronic stress (HANNIBAL & BISHOP 2014). While low levels may reflect an inadequate response to stress, potentially increasing susceptibility to stress-related disorders (VAL et al. 2006, VERCAUTEREN et al. 2022).

## CONCLUSION

The study shows that rainbow trout in the Chalhuanca River exhibit reduced body condition and hematological abnormalities, suggesting adverse effects of pollution on their growth and oxygen-carrying capacity. Furthermore, high levels of chronic stress and microcytic hypochromic anemia were identified, suggesting a correlation between environmental stress, derived from wastewater and industrial waste pollution, and fish health.

Reduced serum bactericidal activity compared to reference values suggests compromised pathogen defense mechanisms, with significant implications for food safety. Environmental, physicochemical, and microbiological factors influence stress response and may lead to maladaptation, increasing disease susceptibility and highlighting the need for continuous monitoring to ensure fish population health and welfare.

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