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# Effect of fish farming on sediments and the proliferation of nitrifying bacterial communities in Lake Guamuez, Colombia

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 Elizabeth Burbano-Gallardo<sup>1\*</sup>,  Guillermo Duque-Nivia<sup>1</sup>,  
 Marco Imues-Figueroa<sup>2</sup>,  Edgar Gonzalez-Legarda<sup>1</sup>,  Mario Delgado-Gómez<sup>2</sup>  
 Jonny Pantoja-Díaz<sup>2</sup>

<sup>1</sup> Universidad Nacional de Colombia. Sede Palmira. Palmira, Colombia.

<sup>2</sup> Universidad de Nariño. Pasto, Colombia.

\*Corresponding author: Universidad Nacional de Colombia - Sede Palmira. Carrera 32 # 12 - 00, Palmira. Valle del Cauca, Colombia. [eburbanog@unal.edu.co](mailto:eburbanog@unal.edu.co)

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## Abstract

Fish farming modifies the sediments and the microbiota in water bodies due to supplementary feed residues, chemical substances, and wastes from the metabolism of animals under production. The current study determined the effect of extensive, semi-intensive, and intensive production of rainbow trout (*Oncorhynchus mykiss*) in sediments, and the proliferation of nitrifying bacterial communities. Total nitrogen, total phosphorus, total organic carbon, and organic matter, and their relationship with nitrifying bacteria (ammonia-oxidizing bacteria-AOB and nitrite-oxidizing bacteria-NOB) were analyzed in the sediment of Lake Guamuez, located in the department of Nariño, Colombia. The data collected were subjected to analyses of variance (ANOVA) and multiple correlation using the Statgraphics Centurion XV software. The variables evaluated showed significant differences ( $p < 0.05$ ) between the three fish farming systems and the control point. A high and positive correlation was found between nitrifying bacteria and nitrogen compounds, organic matter, and phosphorous in the sediment. This study concluded that the presence of nitrifying bacteria depends on the amount of existing nitrogen compounds and organic matter in the sediment, and increases as the production system intensifies.

**Keywords:** nitrifying bacteria, nitrogenous compounds, *Oncorhynchus mykiss*, pisciculture, sediments

## Efecto de cultivos piscícolas en los sedimentos y la proliferación de comunidades bacterianas nitrificantes en el lago Guamuez, Colombia

### Resumen

La piscicultura altera los sedimentos y la microbiota en cuerpos de agua debido a los residuos de concentrados, sustancias químicas y desechos provenientes del metabolismo de los animales en producción. El presente estudio determinó el efecto de la producción extensiva, semi-intensiva e intensiva de trucha arcoíris (*Oncorhynchus mykiss*) en los sedimentos, y la proliferación de comunidades bacterianas nitrificantes. Se analizó nitrógeno total, fósforo total, carbono orgánico total y materia orgánica, y su relación con la presencia de bacterias nitrificantes (bacterias oxidantes de amonio [BOA] y bacterias oxidantes de nitritos [BON]) en el sedimento del lago Guamuez, ubicado en el departamento de Nariño, Colombia. Los datos recolectados fueron sometidos a un análisis de varianza (ANOVA) y de correlación múltiple, usando el *software* Statgraphics Centurion XV. Las variables evaluadas presentaron diferencias significativas ( $p < 0,05$ ) entre los tres sistemas de cultivo y el punto control. Se encontró una correlación alta y positiva entre las bacterias nitrificantes y los compuestos nitrogenados, la materia orgánica y el fósforo medidos en el sedimento. Se concluyó que la presencia de bacterias nitrificantes depende de las cantidades de compuestos nitrogenados y materia orgánica existentes en el sedimento e incrementa a medida que se intensifica el sistema productivo.

**Palabras clave:** bacterias nitrificantes, compuestos nitrogenados, *Oncorhynchus mykiss*, piscicultura, sedimentos

## Introduction

Much of the biodiversity of an ecosystem depends on lake system functioning (Esquivel, 2014). However, lakes suffer continuous contamination that affects their fragility due to the disposal of different organic and inorganic elements originating from foreign activities in the basins (Margalef, 1983; Reques, 2005). Lake Guamuez (La Cocha lagoon) is located in the south of Colombia, Department of Nariño, whose glacial origin highlights its hydric and ecological importance and classifies it in the category of wetlands of international importance according to the Ramsar Convention (Merino et al., 2013). Despite being a strategic ecosystem protected by local authorities, it shows levels of contamination by anthropogenic factors such as rainbow trout culture; the reception of waste, domestic, and agricultural water; illegal coal exploitation; deforestation; agricultural crops, and fuel spillage from boats used for tourism (López & Madroño, 2015).

Rainbow trout culture in Lake Guamuez began in 1980 when the floating cage farming system was adopted, and extensive, semi-intensive and intensive production systems were developed. The estimated production of this activity is 2,585 t/year (Autoridad Nacional de Acuicultura y Pesca, 2013), and its growth is important for the region. However, there is little research on the adverse effects that fish farming has in this lake environment.

Fish farming provides different pollutants that come from uneaten food, feces, and metabolic waste of fish, and various chemical and pharmaceutical substances used for the treatment of diseases, and cleaning and disinfecting facilities. These pollutants cause an excessive supply of nutrients, including nitrogen in water and sediments (Wang et al., 2012). The nitrogen that reaches aquatic organisms in the form of ammonium, nitrate, and nitrites is assimilated by nitrifying bacteria, being their abundance an indicator of water quality. If the ideal oxygen, pH, and temperature conditions are present, these bacteria carry out the nitrification process in these ecosystems (Infante, 2005).

Physical and chemical variables are indicators of water quality that can be altered due to the decomposition of products resulting from inputs and raw materials that promote the proliferation of bacteria, whose food source is organic matter. This process affects the chemical, physical and structural interaction of the water-sediment relationship in aquatic ecosystems (Brown et al., 1987; Tsutsumi & Kikuchi, 1983). Consequently, excess nitrogen compounds in the environment could inhibit nitrogen fixation by bacterial communities, causing a change in their abundance, structure, biomass, and density. This, in turn, leads to adverse environmental problems such as acidification and eutrophication of water bodies (Olsen et al., 2014). Cornel and Whoriskey (1993) and Buschmann (2001) reported eutrophication cases in salmonid fish cultures of intensive floating cage systems, generating not only the liquidation of the companies, but also severe environmental problems. So far, this has not been the case in Lake Guamuez.

This study aimed to analyze the environmental effects that the levels of extensive (300 t/year), semi-intensive (120 t/year), and intensive (21t/year) production systems of three companies culturing rainbow trout in floating cages cause on the presence of nitrifying bacterial communities that carry out the oxidation of ammonium and nitrites. Furthermore, also a relationship with the nitrogenous compounds

present in the sediment of Lake Guamuez. The importance of this research lies in the management that producers carry out and the application of good aquaculture production practices. Failure to perform proper procedures in the production of rainbow trout in floating cages can lead to the accumulation of nitrogen compounds that cause anoxic zones in the sediment, eutrophication problems, and poor water quality.

## Materials and methods

### Location

Lake Guamuez is located in the department of Nariño in southern Colombia, at the coordinates 00°53'28" and 01°20'36" N latitude, and 76°50'50" and 77°14'17" W longitude. It has an area of 41.62 km<sup>2</sup>, a volume of 1,668,567,000 m<sup>3</sup>, and an average depth of 40.57 m. Moreover, it has a load capacity of 930 t/year and hydraulic retention of 6.8 years. Therefore, it is classified as oligotrophic (Corporación Autónoma Regional de Nariño, 2014).

### Collection and analysis of sediments in fish farming stations

Sediment samples were collected during the low rainy season with a 250 cm<sup>3</sup> Eckman dredge. Three sediment samples were taken from each fish production level: extensive (21 t/year), semi-intensive (120 t/year), and intensive (300 t/year) at an average depth of 20 m. Besides, samples were also collected in an area with no fish farming intervention, referred to in the study as a control point.

The collected sediment was deposited in hermetically sealed bags, covered with aluminum foil, and kept refrigerated until its analysis in the laboratory, following the NTC-ISO/IEC 17025 standard of 2005. Organic matter (mathematical formula), total nitrogen (ISO 11261 of 1995), total phosphorus (Andersen ignition [1979], SM-4500-PE), and total organic carbon (ISO 14235 of 1998) were determined in each sample. Then, to establish the count of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), 10 g of each sediment sample was taken. A volume of 90 mL of physiological saline solution was added and homogenized in a vortex shaker for 5 min.

Subsequently, the serial dilution method was used, making decimal dilutions of the previously homogenized samples under sterile conditions. Then, the bacterial culture was carried out in a Petri dish, using agar or selective and differential culture media for ammonium-reducing bacteria —calcium chloride (CaCl<sub>2</sub>): 7 mg; iron chloride (FeCl<sub>2</sub>): 1 mg; potassium chloride (KCl): 7 mg; monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>): 11 mg; magnesium sulfate (MgSO<sub>4</sub>): 5 mg; disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>): 29 mg; sodium bicarbonate (NaHCO<sub>3</sub>): 6,000 mg; ammonium chloride (NH<sub>4</sub>Cl): 500 mg; distilled water: 1,000 ml, and agar: 15 g— and nitrites — sodium nitrite (NaNO<sub>2</sub>): 1 g; calcium carbonate (CaCO<sub>3</sub>): 1 g; 0.85 % standard saline solution: 50 ml; distilled water: 950 ml, and agar: 15 g.— Bacterial cultures were incubated for 24 hours, and finally, colony-forming units were counted. This procedure was established under the standardized guidelines of analytical methods for soils (Motta et al., 1990).

## Statistical analysis

Given that the data collected from nitrifying bacteria did not comply with the statistical assumption of normality (Shapiro-Wilk test = 0.0001;  $p \leq 0.05$ ), the Kruskal-Wallis test was applied. This is a non-parametric method to test the existence of significant differences ( $\alpha = 0.05$ ) and the variances of the samples. Subsequently, the Student's t-test was applied to compare the means of the AOB/NOB proportions and determine if there were significant differences.

The nutrient data measured in the sediments complied with all the statistical assumptions, allowing the application of an analysis of variances (ANOVA), a parametric test to compare and demonstrate the presence of significant differences ( $\alpha = 0.05$ ) between the studied areas. Finally, using Pearson's correlation coefficient, a multivariate analysis was performed to observe the correlation between the different variables evaluated and determine the association relationships between the nutrient content and the bacterial populations of AOB and NOB. The statistical software used for the analyses was Statgraphics Centurion XV.

## Results and discussion

### Nutrient content in sediments according to production intensity

The ANOVA showed significant differences ( $p < 0.05$ ) for organic matter, total phosphorus, total nitrogen, and organic carbon measured in the sediments of the areas with fish cages and the control point (table 1). The areas with fish farming have higher values compared to the control point, and the value of the variables increases in the areas whose productions are intensified. As expected, the fish farming processes in the cultivation areas generate contamination in the benthic zones of the lake. On the contrary, the values recorded in the control point are the most favorable for the natural conditions of the lake bottom.

**Table 1.** Values (mean  $\pm$  standard deviation) of sediment nutrients measured in the four sampling zones

Parameter	Total nitrogen mg N/kg	Total phosphorus mg P/kg	Total organic carbon mg/kg	Organic material (%)
Zone 1 (extensive fish culture)	7,622.12 $\pm$ 37.32 <sup>c</sup>	171.61 $\pm$ 6 <sup>c</sup>	29,293.42 $\pm$ 492 <sup>c</sup>	5.05 $\pm$ 0.09 <sup>b</sup>
Zone 2 (semi-intensive fish culture)	9,532.44 $\pm$ 314.16 <sup>b</sup>	246.57 $\pm$ 21 <sup>b</sup>	32,577.98 $\pm$ 226 <sup>b</sup>	5.65 $\pm$ 0.09 <sup>a</sup>
Zone 3 (intensive fish culture)	11,506.40 $\pm$ 148.69 <sup>a</sup>	1,030.02 $\pm$ 39.54 <sup>a</sup>	33,433.33 $\pm$ 131.48 <sup>a</sup>	5.78 $\pm$ .05 <sup>a</sup>
Zone 4 (control)	6,598.72 $\pm$ 248.23 <sup>d</sup>	155.49 $\pm$ 2 <sup>c</sup>	27,953.28 $\pm$ 753 <sup>d</sup>	4.72 $\pm$ 0.17 <sup>c</sup>

Note: Different letters indicate significant differences, Tukey's test ( $p < 0.05$ ).

Source: Elaborated by the authors

Previous studies demonstrated the degradation of the aquatic environment caused by trout farming in floating cages by reporting nitrogen values higher than 12,030 mg/kg in the sediment (Temminck & Schlegel, 1843, cited by Molina & Vergara, 2004). Likewise, amounts of total phosphorus of 950.62 mg/kg have been reported in the lagoon Los Patos (Sucre state, Venezuela) associated with domestic wastewater (Márquez et al., 2007).

Nitrogen values (11,506.40 mg N/kg) in the intensive fish farming area are close to those reported in the literature, and total phosphorus values (1,030.02 mg P/kg) demonstrate the negative impact on the water-sediment interaction of the lake. This is because both are the result of the contribution of phosphorus and nitrogen from the physiological processes of fish (excretion, respiration), the excess of commercial concentrate supplied, and other fish farming processes (González, 2012) that can progressively deteriorate the lake.

Limiting nutrients in the ecosystem, such as nitrogen and phosphorus, are essential for the development and functioning of organisms. However, aquaculture productive activities have altered the behavior of these elements, modified the balance of lentic aquatic systems, altered nitrifying bacterial communities, and generated diseases in farmed animals (Diemer et al., 2010). However, these compounds can be assimilated by the phytoplankton present in lake bodies, so when nitrogen and phosphorus increase, the growth of algae also increases, generating eutrophication problems (Ramírez et al., 2012).

Similarly, nitrogen compounds and, above all, phosphates can be assimilated and converted to chlorophyll levels present in algae and aquatic vegetation. López and Madroño (2015) evaluated chlorophyll A levels in relation to nitrogen and phosphorus levels in different areas of Lake Guamuez. The highest value found (2.58 µg/L) corresponds to the trout production area in culture cages belonging to Universidad de Nariño. Similarly, López et al. (2008) reported chlorophyll A concentrations of 2.47 and 7.3 µg/L in different zones of the fish farming stations; the highest values were recorded in higher intensity fish farms.

These alterations of phosphate and nitrogen compounds can be controlled by adequate management of the productive variables, applying good aquaculture practices, cleaning and disinfecting farming units, and the moderate use of disinfection products such as detergents (González-Legarda et al., 2018).

With regard to total organic carbon (TOC), values of  $33,433 \pm 144.86$  mg/kg were obtained in intensive fish farming systems,  $32,578 \pm 144.86$  mg/kg in semi-intensive, and  $29,293 \pm 144.86$  mg/kg in extensive. The lowest value was found in the control point with  $27,953 \pm 144.86$  mg/kg. These amounts can be observed to increase according to the intensity of the fish farming system.

In the highest trout production areas, a higher quantity of commercial feed is used —reflected in higher TOC values— and animal biomass in the harvest (Yuvanatemiya & Boyd, 2006). In this way, the carbon assimilated by fish and, subsequently excreted as metabolic waste has important effects on the quality of water and sediments. The proper and assertive management of feeding tables is of utmost importance in the large companies that use them, and their implementation in extensive fish farming, which, in general, are not managed in Lake Guamuez.

Avnimelech and Ritvo (2003) reported that farmed fish assimilate only 13 % of the carbon present in commercial feed; the remaining 87 % is discharged to the natural environment or accumulated in sediments (Boyd & Green, 1998). In lentic systems where freshwater crops are implemented, the highest contributor of foreign carbon compounds is concentrate feed, which also contributes with high amounts of lipids, proteins, and carbohydrates (Vásquez, 2004). All these elements affect the carrying capacity of lakes and lagoons, leading to the accumulation of organic compounds and the degradation of water and sediment in aquatic systems (Wang et al., 2016).

One of the major environmental problems generated by aquaculture is organic matter (OM) accumulation (Organización de las Naciones Unidas para la Alimentación y la Agricultura, 2007). As the results show, zones 2 and 3, corresponding to semi-intensive and intensive fish farming, recorded the highest OM values, unlike zone 1 and the control point, whose values are lower (table 1).

In the Tota lagoon —located in Boyacá (Colombia)—, with similar climatic and morphological characteristics as Lake Guamuez, Torres Barrera and Grandas Rincón (2017) reported OM values in sediments between 13-16 % and a projection of nitrogen and phosphorous waste contribution to the lake of 5,000 t in the last 10 years. This is because OM recycling in the sediments produces hypoxia and anoxia, decreasing the diversity of benthic species, including bacterial communities where the most tolerant predominate. On the other hand, the values found in this study do not exceed 5.78 % in the intensive systems and 4.72 % in the control point. This is why these values are much lower than those reported in the Tota lagoon and are within the typical ranges of high mountain lakes classified as oligotrophic.

The highest OM value reported in this study was recorded in intensive fish farming systems (5.78 %). However, this value is within the acceptable ranges in natural waters, so it does not represent a danger to the sediment quality of the lake so far. This is confirmed by González-Legarda et al. (2018), who assures that OM and carbonate values higher than 10 % and 5 %, respectively, correspond to lakes with organic sediments of the Gytja type, while lower percentages correspond to lakes with mineral sediments.

However, the eutrophication and nutrient accumulation state in lentic aquatic systems receiving aquaculture processes can vary easily, thus, disturbing the ecology of the lake and causing adverse effects on the economy of the producer whose income depends directly on the resources that these lake systems provide (Márquez et al., 2007). Lake Guamuez houses around 94 producers, who, according to the latest census from Autoridad Nacional de Acuicultura y Pesca (2013) and Corporación Autónoma Regional de Nariño (2014), have estimated in 912 the number of floating cages dedicated to rainbows trout production (545 are currently producing), occupying 0.49 % of the total area of the lake.

### **Proliferation of nitrifying bacteria in sediments according to fish farming intensity**

The results show a positive relationship between the colony-forming units (CFU) of nitrifying bacteria and the productive intensity, being higher in zone 3 (intensive fish farming) with  $417.8 \times 101 \pm 18.1$  CFU/g of AOB and  $293.9 \times 103 \pm 20.5$  CFU/g of NOB. This relationship decreases in zones 1



(extensive fish farming) and 2 (semi-intensive fish farming), and the lowest concentration is recorded in the control point (table 2).

**Table 2.** Colony-forming units of nitrifying bacteria in the sediments of Lake Guamuez (mean  $\pm$  standard deviation)

Nitrifying bacteria	AOB (CFU/g)	NOB (CFU/g)
Zone 1 (extensive fish farming)	$33.0 \times 10^1 \pm 6.8$	$109.3 \times 10^3 \pm 16.8$
Zone 2 (semi-intensive fish farming)	$128.4 \times 10^1 \pm 6.3$	$228.8 \times 10^3 \pm 17.3$
Zone 3 (intensive fish farming)	$417.8 \times 10^1 \pm 18.1$	$293.9 \times 10^3 \pm 20.5$
Zone 4 (control)	$30.0 \times 10^1 \pm 13.1$	$58.9 \times 10^3 \pm 17.6$

Source: Elaborated by the authors

Because Guamuez is an oligotrophic lake, it shows optimal oxygen levels higher than 7 mg/L, with an average temperature of 13 °C, and a saturation percentage of 140.24, favorable conditions that allow the development of nitrifying bacteria. Masmela-Mendoza et al. (2019) reported that high mountain lakes have a high amount of nitrifying bacteria than other aquatic ecosystems, according to alkaline pH and high oxygen concentrations, distinctive characteristics of the water of this lake (González-Legarda et al., 2018).

Lake Guamuez shows physical and chemical conditions at the water-sediment interface favorable for the growth of nitrifying bacteria, and demonstrated in the control point results. However, anthropic processes such as trout production significantly increase the presence of OM, and nitrogen and phosphate compounds, hindering the biodegradation process as no environmental control occurs (Morata et al., 2012).

According to the results of the current study, this class of bacteria still has the ability to preserve the natural conditions of this water system. However, it is essential to implement adequate management of organic and inorganic substances discharged into this water body, since nitrifying bacteria are highly susceptible to various toxic compounds that slow down their growth. In the worst case, these bacterial colonies can decrease significantly, and thus, generate eutrophication processes (González et al., 2010).

On the other hand, significant differences were found between the areas studied by calculating the AOB/NOB ratios; lower proportion values were identified for AOB, and higher values for NOB (table 3).

**Table 3.** Relationship between ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (mean  $\pm$  standard deviation)

Proportion of bacteria	AOB/NOB (%)
Zone 1 (extensive fish farming)	0.31 $\pm$ 0.04 <sup>a</sup>
Zone 2 (semi-intensive fish farming)	0.56 $\pm$ 0.04 <sup>b</sup>
Zone 3 (intensive fish farming)	1.43 $\pm$ 0.62 <sup>c</sup>
Zone 4 (control)	0.56 $\pm$ 0.22 <sup>b</sup>

Note: Different letters indicate significant differences, Tukey's test ( $p < 0.05$ ).

Source: Elaborated by the authors

This is possibly because in ecosystems at higher altitudes, the proportion of AOB bacteria is inhibited by some parameters that do not meet the environmental conditions for their growth, such as the presence of heavy metals, pH alteration, temperature, or concentrations of nitrogen compounds (Ye & Thomas, 2001).

In the Tota lagoon, this same relationship was found between AOB and NOB. Briceño et al. (2009) found that the highest contributors of nitrogen compounds came from onion crops in the rainy season due to soil runoff, affecting the number of nitrifying bacteria and the elimination of water nitrates in the lake. This same situation can occur in Lake Guamuez due to the constant supply of nitrogen and phosphorus from fish farming, and produced by the unconsumed concentrated feed and metabolic processes. Other agricultural activities around the lake also affect the growth rate of bacterial populations.

In the semi-intensive fish farming and control point areas, AOB/NOB percentages equal to 0.56 % were found, so no significant differences were identified. This may be because these bacterial groups found the same capacity to adapt to the environmental conditions of the evaluated areas (Infante, 2005). Thus, they managed to acquire the same growth rate, but at different CFU. The value of the semi-intensive fish farming was higher than that of the control point.

On the other hand, when comparing the AOB/NOB proportions in the evaluated areas, intensive fish farming recorded the highest proportion value with 1.43 % for AOB and 98.57 % for NOB. This may be because the available amount of nutrients inhibits cell synthesis or increases bacterial growth (Zhou et al., 2017). Moreover, in this area of intensive fish farming, the highest values of total organic carbon, total nitrogen, total phosphorus, and OM content were found. In this way, it can be affirmed that there is a positive relationship between the growth of AOB and NOB with the concentrations of these variables.

### Multiple correlation analysis between AOB/NOB proliferation and the variables measured in the sediment

This research shows the correlation of the CFU of NOB and AOB with the physicochemical variables measured in the sediments, considering that the interaction of nutrients at the water-sediment interface is of great importance for the behavior of the nitrogen cycle in natural water bodies. As shown in table 4, there is a high positive correlation of AOB and NOB with organic carbon, OM, total phosphorus, and total nitrogen present in sediments. The higher the amount of compounds with nitrogen, phosphorus, and OM, the higher the number of nitrifying bacterial colonies.

**Table 4.** Correlation between AOB/NOB and parameters of the sediment of Lake Guamuez

	Total nitrogen mg N/kg	Total phosphorus mg P/kg	Total organic carbon mg /kg	Organic Matter (%)
AOB	0.8112	0.8801	0.7169	0.6801
NOB	0.9741	0.7980	0.9616	0.9239

Source: Elaborated by the authors

As previously stated, concentrated feed is the highest contributor of nutrients given its composition: 51 % phosphorus, 78 % nitrogen, and 40 % carbon (Bueno et al., 2008). However, the percentage of assimilation by farmed animals ranges from 25-45 % nitrogen, 20-30 % phosphorus, and 10-15 % carbon (Boyd & Tucker, 1998). The excess and unconsumed quantities participate in the biogeochemical cycle of the lake, where a certain part is retained in the sediment, and the remainder is metabolized by bacteria (Krebs, 2003).

The accumulation of nitrogen, phosphate compounds, and OM from aquaculture cultures intensifies the density of nitrifying bacteria necessary for the degradation and decomposition of these compounds, and decreases the dissolved oxygen available in the lake system (Arana, 2004). On the other hand, if there are no ideal physicochemical variables (temperature, pH, dissolved oxygen, and toxic substances) for the proliferation of these bacteria, the large discharge of nutrients can cause an inverse relationship, and cause the eutrophication of any natural aquatic system (Briceño et al., 2009).

## Conclusions

There are significant differences between the variables evaluated in the sediment in terms of the contribution of organic matter, phosphorus, nitrogen, and organic carbon, whose values are higher in semi-intensive and intensive fish farming areas. This shows that the crops with the highest fish production generate more pollution in the benthic zones of the lake, due to the supply of commercial feed and the metabolic processes of fish.

There is a high positive correlation between the nitrifying bacteria evaluated with the nitrogenous compounds and the OM measured in the sediment, showing that these microorganisms increase their activity and growth rate to decompose these fish farming derivatives.

According to the results obtained in the control point, the sediment of Lake Guamuez shows adequate characteristics for the growth of nitrifying bacterial groups. However, fish farming can cause environmental and biological alterations if it is not carried out under proper management of the productive processes that contribute with nitrogen compounds.

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## Disclaimers

All authors made significant contributions to the document, agree with its publication, and state no conflicts of interest in this study.

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