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Evaluating the Bioremediation Capacity of the Polychaete *Perinereis gualpensis* (Jeldes, 1963) for Atlantic Salmon Aquaculture Sludge

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Abstract: The potential of polychaetes for the bioremediation of aquaculture sludge gained more attention in recent years. These organisms can reduce organic matter and nutrients contained in the sludge of several aquaculture species, improving the sustainability of these activities. The aim of this study was to evaluate the removal performance of *Perinereis gualpensis* (Jeldes, 1963) being fed with aquaculture sludge produced by a recirculating system cultivating Atlantic salmon. The experiment involved adding different amounts of sludge (10% and 20% with respect to total substrate) at a density of 300 organisms m^{-2} during 30 days. A treatment without sludge served as a control, using natural substrate. The highest removal rate of total organic matter (TOM) (23.95 $\pm$ 13.19 g m^{-2} day^{-1}) was achieved by *P. gualpensis* with 20% sludge addition, a reduction of about 36% compared to the total amount added at the beginning of the trials. The organisms fed with aquaculture sludge presented higher nitrogen (8–9%) and carbon (40–43%) contents, with a maximum organic carbon assimilation of 32% in relation to the total content in the sludge. The high survival (88–95%) and positive growth rates (0.28% day^{-1}) achieved by *P. gualpensis* indicated that this species can be sustained with salmon sludge as the only source of food. These results indicate that *P. gualpensis* is a promising candidate for removing nutrients from salmon effluents. Moreover, the protein contents achieved by the organisms (52–58%) meet the dietary protein requirements of several aquaculture species. Further research is needed to determine the maximum bioremediation capacity of this species and to evaluate the lipid content and fatty acid profiles of *P. gualpensis* to determine its potential application in aquaculture feed.

Keywords: bioremediation; aquaculture sludge; polychaete; organic matter; nutrients

Key Contribution: The bioremediation potential of *Perinereis gualpensis* for aquaculture sludge was assessed for the first time. The results indicate significant TOM removal, nutrient assimilation, and high survival during the trials. Additionally, the protein levels in the polychaetes' biomass meet the nutritional requirements of aquaculture species, suggesting its potential use in aquafeeds.

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1. Introduction

In the past few years, the need to transition towards circular production systems became paramount. Aquaculture, which plays a key role in the global food supply, experienced a remarkable growth in production over the last several decades that also resulted in a corresponding increase in waste generation [1].

Aquaculture sludge, the main organic waste product derived from organisms feeding, is a significant concern for aquatic ecosystems. The discharge of organic matter, including uneaten feed and faecal matter, can lead to nutrient accumulation in the water, which can promote eutrophication and growth of algae and undesirable microorganisms [2–4]. Organic matter releases can cause a decrease in water quality, oxygen depletion, and disrupt food chains, negatively affecting the health of aquatic organisms and ecosystem function [5].

Recirculating aquaculture systems (RAS) gained popularity in recent years due to their ability to operate independently of aquatic environments. Although these systems are designed to concentrate waste for easier handling, organic sludge generation and management remains a major challenge for aquaculture production in RAS [6,7]. Additionally, and despite significant advances in aquaculture nutrition research, the amount of sludge produced continues to be a substantial issue. For example, commercial diets for Atlantic salmon generated approximately 100 g of sludge (dry matter) per kg of salmon produced in 2010 [8]. The management practices in aquaculture systems can influence the amount of feed left unconsumed by the fish, which can range from 3% to 20%, and some of the nutrients provided in the feed may not be fully digested, ending up in the sludge [9]. Some of these nutrients have high nutritional value, particularly protein and lipids, which are essential components of animal feed, making their release to the environment also a significant loss of valuable components [10].

The potential of polychaetes for bioremediation of aquaculture sludge was increasingly recognized in recent years. Several species of polychaetes demonstrated their ability to reduce the organic matter and nutrient content of different aquaculture wastes [11–18]. Many of these studies reported organic matter removal by the Nereididae and Arenicolidae families, which are the most commonly studied for this purpose. For example, *Hediste diversicolor* was found to effectively remove total organic matter (TOM) from fish and shrimp effluents, also exhibiting notable growth and survival rates in several investigations [9,10,16,19]. Similarly, *Perinereis aibuhitensis* and *Abarenicola pusilla* reduce carbon and nitrogen levels by consuming organic matter from fish sludge [20–22].

Polychaetes are a valuable source of protein and lipids, often used in the diets of fish and shrimp broodstock to enhance their nutritional value, particularly during the maturation phase [23–27]. Incorporating polychaetes in bioremediation processes can potentially recycle proteins and polyunsaturated fatty acids present in aquaculture sludge. Several studies showed that certain species of polychaetes, such as *Perinereis nuntia vallata*, *Abarenicola pusilla*, and *Hediste diversicolor* can assimilate nitrogen, organic carbon, and increase their lipid content when fed with specific types of aquaculture sludge [21,28–33]. These appreciated components can be incorporated into aquaculture feeds, thus reducing dependence on scarcer traditional ingredients such as fishmeal and fish oil.

It is crucial to consider using native organisms for bioremediation of aquaculture waste in order to mitigate the risks associated with introducing exotic species that can act as disease vectors and compete with native populations [34]. Chile is the second largest producer of farmed Atlantic salmon globally, with an annual production of over 700,000 tons [35]. Salmon cultivation caused harmful environmental effects on channels and fjords, the main ecosystems where it is grown in its fattening phase. In addition, the release of organic waste from pre-smolt culture into lakes and river streams is a major concern for the salmon industry [15,36].

Perinereis gualpensis (Jeldes, 1963) is an endemic polychaete species in Chile that exhibits remarkable tolerance to varying physicochemical conditions. This species can tolerate salinities ranging from 2.4 to 34 g L^{-1} and temperatures between 7 and 21 °C [37,38]. It is commonly found in benthic macroinfauna inhabiting intertidal and subtidal areas of muddy sand and mudflats with high organic matter content [39,40]. As a keystone species in these ecosystems, *P. gualpensis* serves as a vital food source for other species and plays a crucial role in biogeochemical processes as a nutrient recycler [40]. Furthermore, it is used in toxicological experiments and as a pollution bioindicator due to its high capacity to tolerate adverse environmental conditions [40,41]. Due to its feeding behaviour, which includes both subsurface detritivorous feeding and surface deposit feeding, *P. gualpensis* can effectively utilise organic matter, making it a promising species for aquaculture sludge bioremediation.

The aim of this study was to investigate the efficiency of using the detritivorous polychaete *Perinereis gualpensis* for removing organic matter from aquaculture sludge produced by Atlantic salmon farming. The study assessed the removal of total organic matter (TOM) and the assimilation of protein, total nitrogen (N), and total carbon (C) content by the polychaetes. Furthermore, the survival and growth rates of the organisms being cultured were evaluated to determine their overall efficiency in bioremediation of aquaculture sludge.

2. Materials and Methods

2.1. *Perinereis gualpensis*

Wild polychaetes were collected at low tide in the Queule estuary, Araucanía, Chile (39°23′27″ S 73°11′50″ W). Upon arrival at the Laboratorio Costero de Calfuco (UACh), the polychaetes were immediately transferred to flow-through tanks and kept at a constant temperature of 15 °C until the start of the experiments. Throughout this acclimation period, the polychaetes were fed twice a week with commercial fish feed.

Before the beginning of the experiment, the polychaetes were starved for 4 h to remove any potential intestinal contents [19]. The initial and final wet weights of the polychaetes were determined with a precision scale (Jadever SNUG II-300, Diprolab, Concepción, Chile) after removing excess water. Dry weight and water content were determined after dehydration in a muffle furnace (60 °C until constant value). The survival of the polychaetes during the experiment was calculated as the percentage of organisms remaining alive at the end of the trials. Mortality was recorded daily by collecting dead organisms from the sediment surface.

Growth performance was calculated using the specific growth rate (SGR) according to the following formula:

$$\mathrm{SGR}\ (\%\ \mathrm{day}^{-1}) = [(\ln \mathrm{W_t} - \ln \mathrm{W_0})/\mathrm{t}] \times 100 \quad (1)$$

where W_t and W_0 represent the final and initial weights of the polychaetes in each period, respectively, and t represents the time interval in days between weight measurements [42]. The absolute growth rate (AGR) was calculated per unit area according to [43]:

$$\mathrm{AGR}\ (\mathrm{g\ m^{-2}}) = (\mathrm{W_t} - \mathrm{W_0})/\mathrm{m}^2 \quad (2)$$

The change in individual weight of the organisms (g) was also calculated as:

$$\text{Change individual weight (g)} = [(\text{final (g)}/\mathrm{q}) - (\text{initial (g)}/\mathrm{n})] \quad (3)$$

where final (g) denotes the total wet weight of the remaining polychaetes and q represents the number of polychaetes remaining, while initial (g) refers to the total wet weight of the polychaetes at the beginning of the experiment and n indicates the number of polychaetes at the start of the experiment. This involved comparing the initial average wet weight of individuals with the final average wet weight, considering the population size of the remaining polychaetes [16]. Additionally, the percentage of weight gain (%) was calculated as [44]:

$$\text{Weight gain (\%)} = [(\mathrm{W_f} - \mathrm{W_0})/\mathrm{W_0}] \times 100 \quad (4)$$

where W_f and W_0 represent the final and initial average wet weight of the organisms (g).

2.2. Salmon Aquaculture Sludge

The aquaculture sludge used in this study was obtained from a recirculating system stocked with Atlantic salmon smolts operated using freshwater. To maintain its original state, no dewatering or drying processes were conducted. In order to prevent degradation by bacterial remineralisation, the sludge was collected and promptly transported to the laboratory and used within 72 h [9,45].

The dry matter content of the sludge was determined using the same method as the polychaetes analysis (60 °C until constant value). Ash content was calculated after dry samples were incinerated (550 °C for 6 h) [46]. Total organic matter (TOM) was calculated based on the weight ratio between dry and incinerated samples, according to the following formula:

$$\text{TOM}\ (\%) = (m_{\text{sample+cruc. bef.}} - m_{\text{cruc.}}) - (m_{\text{sample+cruc. aft.}} - m_{\text{cruc.}})/(m_{\text{sample+cruc. bef.}} - m_{\text{cruc.}}) \times 100 \quad (5)$$

where $m_{\text{sample+cruc. bef.}}$ = mass of crucible + sample before combustion (g); $m_{\text{cruc.}}$ = mass of the crucible (g); and $m_{\text{sample+cruc. aft.}}$ = mass of crucible + sample (ash) after combustion (g) [16]. Temperature and pH were measured with a portable multiparameter probe (Hach HQ40D), and salinity was determined using a refractometer (RHS-10ATC, Veto, Santiago, Chile).

2.3. Experimental Design

The experimental design involved randomly distributing polychaetes of each treatment in 3 independent experimental units, each with a 0.5 m^2 area. These units consisted of plastic containers with a volume of 68 L. A total of 9 units were used (3 per treatment). The density was set in all treatments at 300 organisms m^{-2}, a level at which other polychaete species exhibited higher feed intake, growth, and survival rates compared to higher densities (400, 600, 1000, and 3000 organisms m^{-2}) [22,34,47–49].

To provide a suitable habitat for the organisms, a 70–80 mm layer of natural substrate was implemented in each experimental unit. The substrate, obtained from the collection site of the organisms, was washed and sieved to 1 mm to remove coarse materials. The substrate was not incinerated to preserve its original composition [16,19,22,50]. The sludge was incorporated into the units at different percentages relative to the volume of natural substrate. The treatments included: (A) 10% aquaculture sludge; (B) 20% aquaculture sludge; and (C) control with only natural substrate. In the 10% and 20% treatments, the sludge was added and thoroughly mixed with the natural substrate in the subsurface layers. Subsequently, three random samples were collected from the entire sediment layer to determine the initial concentration of organic matter in all treatments.

To simulate the natural spring habitat of the organisms, the units were filled with a 100 mm layer of water and maintained at a temperature of 15 °C and a salinity of 15 g L^{-1} [40]. To maintain water quality and simulate low tide discharge in intertidal estuaries, a water replacement was performed every 48 h throughout the 30 day experiment. This procedure was focused exclusively on replacing the surface water and did not involve the interstitial water present in the sediment layers [28,51]. The surface water in the units was permanently aerated with an oxygen diffuser. The photoperiod was maintained under natural conditions (approx. 14 h of day light).

2.4. Organic Compounds Analysis

The total organic matter (TOM) content in the substrate was analysed at the beginning and at the end of the experiments to assess the removal capacity of *P. gualpensis* [16]. To evaluate the flow and transfer of organic matter to the organisms, total nitrogen (N), total carbon (C), and stable isotope profiles (δ13C and δ15N) were determined using an isotope ratio mass spectrometer (IRMS Thermo Delta Advantage, Thermo Fisher Scientific, Waltham, MA, USA) coupled to an Elemental Flash EA2000 analyser (Thermo Fisher

Scientific, Waltham, MA, USA). The associated isotopic error was 0.26‰ y 0.38‰ for nitrogen and carbon, respectively.

The assimilation of organic carbon (OC) by isotopes was analysed using the Bianchi's model with the formula:

$$\text{OC Assim. (\%)} = \{(\delta 13\text{C } P.\ gualpensis - \delta 13\text{C stand.})/(\delta 13\text{C initial} - \delta 13\text{C stand.})\} \times 100 \quad (6)$$

where δ13C *P. gualpensis* represents the final isotopic composition of organisms, δ13C initial represents the isotopic composition of aquaculture sludge, and δ13C standard represents the isotopic composition of wild *Perinereis vallata* in marine environments of the sub-Antarctic Magellan Strait [52,53].

The protein content of aquaculture sludge was determined by the Kjeldahl method (AOAC 928.08), while the protein content of the organisms was obtained from the nitrogen content by multiplying by 6.25 [20].

2.5. Statistical Analysis

Data were analysed using GraphPad-Prism version 8.4.3. The Shapiro–Wilk test was used to test for normality and homogeneity of variance. The TOM removal and weight gain of the organisms were determined with independent samples *t*-test ($p < 0.05$). Data of isotopic profiles, TOM removal, survival, and growth performance of *P. gualpensis* between treatments were determined using one-way ANOVA analysis. The significant differences were analysed using the Tukey test ($p < 0.05$).

3. Results

3.1. Aquaculture Sludge

The details of the sludge analysis are shown in Table 1. The Atlantic salmon sludge presented a protein content of 17.06 ± 0.13% and a TOM content of 845.84 ± 14.73 mg g dry matter^{-1}. The total N and C contents were 3.20 ± 0.23% and 25.41 ± 1.71%, respectively, with a C/N ratio of 7.95 ± 0.05. The aquaculture sludge contained about 95% water.

Table 1. Aquaculture sludge characterisation (mean values ± SD).

Water content (%)	**95.67 ± 0.74**
Dry matter (mg/g WW)	43.31 ± 7.39
Total organic matter (mg/g DM)	845.84 ± 14.73
T (°C)	16.77 ± 0.06
Salinity (g L^{-1})	0.00 ± 0.00
pH	5.98 ± 0.02
Ash content (%)	15.42 ± 1.47
N total (%)	3.20 ± 0.23
C total (%)	25.41 ± 1.71
C/N ratio	7.95 ± 0.05
Protein content (g/100 g)	17.06 ± 0.13

Values are mean ± SD of 3 determinations. WW: wet weight; and DM: dry matter.

3.2. Growth and Survival of Perinereis gualpensis

The initial wet weights of *P. gualpensis* were similar in all treatments: 0.60 ± 0.25 g (treatment 10%), 0.61 ± 0.23 g (treatment 20%), and 0.59 ± 0.23 g (control).

The details of the growth and survival data are shown in Table 2. *Perinereis gualpensis* obtained significantly higher survival (88–95%) when fed with aquaculture sludge compared to those grown on natural substrate (55%) used as a control.

Perinereis gualpensis achieved the highest growth performance in the 20% sludge treatment. The absolute growth rate (AGR) and specific growth rate (SGR) in this treatment were 0.11 ± 0.04 g m^{-2} and 0.28 ± 0.10% day^{-1}, respectively. These rates resulted in a change in individual weight of 0.05 g and a weight gain of almost 9%. There were no significant differences in growth rates between treatments with addition of aquaculture sludge. The control organisms experienced negative growth rates during the tests, indicating weight loss.

Table 2. Survival and growth parameters of *Perinereis gualpensis* in all treatments (mean values ± SD, n = 450, 30 days of trials).

	10% Sludge	20% Sludge	Control
Absolute growth rate (g m^{-2})	0.03 ± 0.01 ^{a}	0.11 ± 0.04 ^{a}	−0.42 ± 0.05 ^{b}
Specific growth rate (μ) (% day^{-1})	0.08 ± 0.04 ^{a}	0.28 ± 0.10 ^{a}	−1.50 ± 0.28 ^{b}
Survival (%)	88.00 ± 7.21 ^{a}	95.33 ± 1.15 ^{a}	55.33 ± 8.08 ^{b}
Change in individual weight (g)	0.02 ± 0.01 ^{a}	0.05 ± 0.02 ^{a}	−0.21 ± 0.03 ^{b}
Weight gain (%)	2.56 ± 1.23 ^{a}	8.76 ± 3.32 ^{a}	−36.13 ± 5.24 ^{b}

Values with different letters are significantly different from each other ($p < 0.05$).

3.3. Organic and Inorganic Components Analysis

3.3.1. Total Organic Matter

Perinereis gualpensis evidenced a significant removal of total organic matter (TOM) in all treatments ($p < 0.05$). The 20% aquaculture sludge treatment resulted in the highest TOM removal rate (23.95 ± 13.19 g m^{-2} day^{-1}), with a reduction of 35.92 ± 19.78% compared to the total recorded at the beginning of the trials. Significant differences in TOM removal were found between the control and the 20% sludge treatment.

Figure 1 shows the differences in TOM content between treatments and time periods. Table 3 provides details of the TOM removal rates achieved by *P. gualpensis* per unit area and day for all treatments.

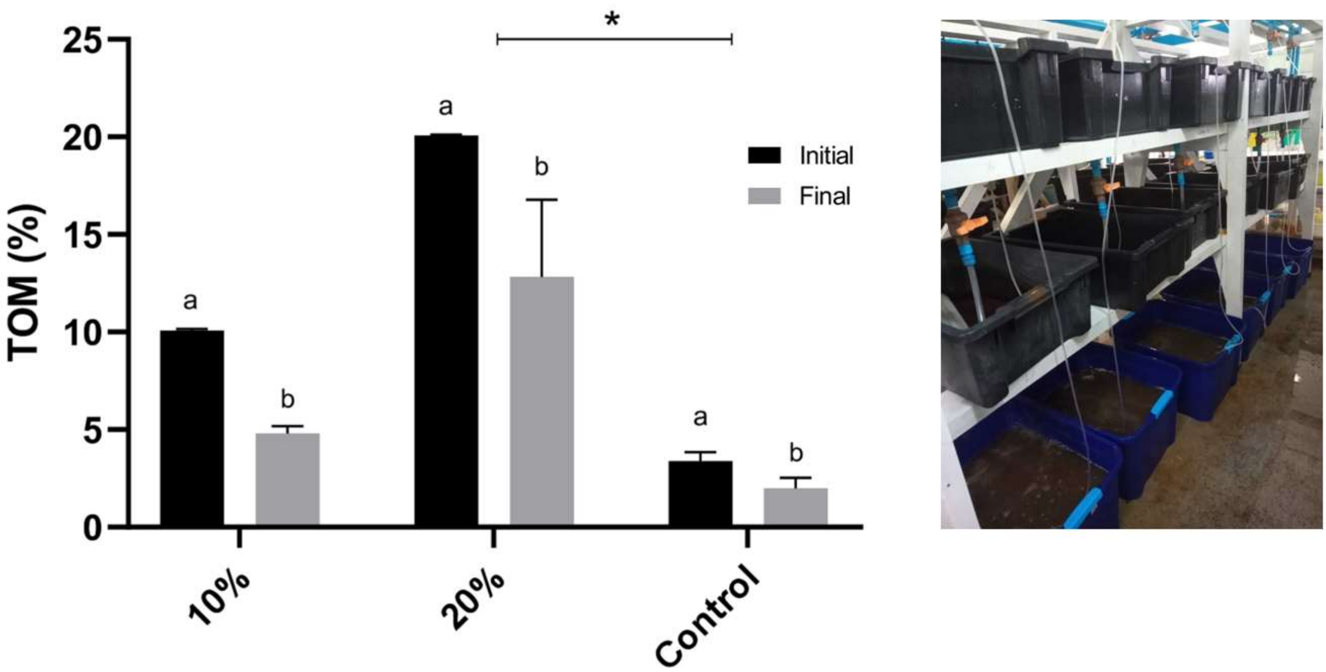

Figure 1. Total organic matter (TOM) removal achieved by *Perinereis gualpensis* in the different treatments. Mean values ± SD (n = 3 per experimental unit, 30 days). Significant differences ($p < 0.05$) are expressed with different letters. Differences in TOM removal among treatments are expressed with (*).

Table 3. Total organic matter removal rates achieved by *P. gualpensis*.

	10% Sludge	20% Sludge	Control
Density (org m^{-2})	300	300	300
Biomass (g m^{-2})	59.85 ± 2.71 [a]	60.72 ± 5.30 [a]	58.71 ± 4.28 [a]
TOM RR (g m^{-2} day^{-1})	17.30 ± 1.20 [ab]	23.95 ± 13.19 [a]	4.69 ± 0.47 [b]
TOM removal (%)	51.90 ± 3.61	35.92 ± 19.78	42.13 ± 8.09

TOM RR: Total organic matter removal rate. Values are mean ± SD of 3 determinations. Significant differences ($p < 0.05$) are expressed with different letters.

3.3.2. Total Nitrogen (N) and Total Carbon (C)

The nitrogen (N) and carbon (C) contents of *P. gualpensis* at the end of the trials are shown in Table 4. The biomass of the organisms fed with aquaculture sludge exhibited higher N (8–9%) and C (40–43%) contents compared to the control organisms, resulting in a C/N ratio of about 4.7–4.9% in the treatments with sludge addition.

Table 4. Analyses of organic and inorganic components of *P. gualpensis* in the different treatments (mean values ± SD).

	10% Sludge	20% Sludge	Control
N total (%)	9.29 ± 0.88	8.28 ± 1.38	5.51 ± 2.86
C total (%)	43.13 ± 1.20	40.52 ± 5.17	32.94 ± 9.49
C/N ratio	4.66 ± 0.30	4.92 ± 0.22	6.71 ± 2.17
Protein content (g/100 g)	58.03 ± 5.47	51.74 ± 8.62	34.45 ± 17.90
Water content (%)	82.05 ± 1.42	82.12 ± 1.70	81.59 ± 2.66
Dry matter (mg/g WW)	179.53 ± 14.22	178.75 ± 17.03	184.08 ± 26.60

Values are mean ± SD of $n = 3$ determinations. WW: wet weight.

Perinereis gualpensis demonstrated significant organic carbon (OC) assimilation, with consumption rates of 21.89 ± 4.40% (10% sludge) and 31.85 ± 16.21% (20% sludge) in relation to the total content present in the aquaculture sludge.

The aquaculture sludge had an isotopic profile of −23.10 ± 0.28 (δ13C) and 15.96 ± 0.35 (δ15N). At the end of the experiments, no differences were found in the isotopic profile of the organisms fed with aquaculture sludge in the different treatments. The δ13C values were −19.51 ± 0.20 (10% sludge) and −19.97 ± 0.75 (20% sludge). The δ15N values were 11.13 ± 0.54 and 11.24 ± 0.37, respectively. The control organisms exhibited an isotopic profile of −20.25 ± 0.70 (δ13C) and 11.53 ± 0.24 (δ15N).

3.3.3. Protein Content

The protein contents of *P. gualpensis* in the different treatments are shown in Table 4. Polychaetes fed with aquaculture sludge presented higher protein contents (52–58%) compared to control organisms (34%) after 30 days of trials.

3.3.4. Water Content

Table 4 also presents the water content and dry matter values for *P. gualpensis*. The average water content of the organisms for all treatments was 81.92 ± 1.75%, while the dry matter content was 180.79 ± 17.50 mg g wet weight^{-1} ($n = 15$). No significant differences were found between treatments.

4. Discussion

The findings of this study indicate that *P. gualpensis* is a promising species for reducing the organic waste content in salmon aquaculture sludge.

4.1. TOM Removal Rates

Perinereis gualpensis can effectively remove total organic matter (TOM) in bioremediation systems with the addition of salmon sludge as the only feed. The highest TOM removal achieved by the organisms (≈24 g m^{-2} day^{-1}) in 20% sludge treatment resulted in a 36% reduction after 30 days of trials.

It is difficult to make comparisons between TOM bioremediation potential by polychaetes among different studies, as this depends on various factors, such as the sludge nutrient content, the extractive species itself, and the conditions and design of the bioremediation systems [15,30,54,55]. Despite this, the results obtained in this research are similar to other bioremediation studies with polychaetes. For example, in a longer study (45 days), *A. pusilla* achieved a maximum TOM removal of 35.77 g m^{-2} day^{-1} when fed with *S. lalandi* aquaculture sludge [21]. In contrast, *Perinereis nuntia* and *Perinereis helleri* removed approximately 50% of the TOM present in aquaculture sludge from *P. monodon* during a 16-week trial with much higher polychaete densities [56]. Other studies found growth but no TOM removal by *H. diversicolor* when fed with *O. mykiss* sludge over a 60-day period [48].

Perinereis gualpensis achieved better TOM removal rates by increasing the amount of sludge in the experimental system. This is in line with other investigations using *H. diversicolor* and *A. pusilla* as extractive species in aquaculture sludge [16,17,21]. The TOM removal rate in the control (about 4.7 g m^{-2} day^{-1}) was significantly lower than in treatments where aquaculture sludge was added. Nevertheless, the control organisms also fed on the organic matter contained in the natural substrate, but this was not enough to sustain the growth of the organisms. Similar results were reported in other studies with *A. marina* and *H. diversicolor* due to poor food availability [11,16,30].

There are, however, other factors that could potentially influence TOM removal. For example, increasing the density of organisms [21,56], using a sludge richer in organic components, or complementing the diet provided to the polychaetes with fish feed could improve the results obtained [15,30,57]. On the other hand, sludge remineralisation processes can negatively affect TOM removal by polychaetes [9,20,55]. Conducting further investigations to determine the maximum capacity of a remediation system with *P. gualpensis* by increasing the density of polychaetes, the ratio and quality of sludge added could provide further insights on this topic.

4.2. Total N and Total C

The polychaetes fed with aquaculture sludge displayed higher contents of total nitrogen (8–9%) and total carbon (40–43%) after 30 days of trial compared to the control organisms. The accumulation of total N and C in the biomass of the organisms is a crucial factor to define the bioremediation potential of a species [45]. The gain of N and C during the feeding period suggests that *P. gualpensis* can play a significant role in the bioremediation processes of salmon aquaculture sludge. Similar N (8–9%) and C (42–45%) contents were found in *P. aibuhitensis* fed on aquaculture fish sludge in a multitrophic experimental system and in *H. diversicolor* after feeding smolt sludge over a 30-day period [19,22].

The assimilation of organic carbon (OC) was significant in the treatments with sludge addition, with a maximum of 32% compared to that contained in the salmon sludge. This assimilation is higher than that achieved by *P. aibuhitensis* (≈20%) fed with *P. olivaceus* sludge during a 35-day feeding period [20] and by *A. pusilla* (≈25%) after 45 days being cultured using *S. lalandi* sludge [21].

The absence of significant differences in the isotopic profiles of the organisms among the different treatments at the end of the trials indicated the presence of similar organic matter in the substrate across all three conditions [58,59]. This suggests that the lack of detectable δ13C uptake from the aquaculture sludge in the polychaetes may be attributed to their access to alternative carbon sources present in the substrate.

In order to enhance the δ13C and δ15N uptake signals, future investigations could explore higher nutrient concentrations from aquaculture sludge compared to those used in this study [60]. Additionally, the analysis conducted using discrete measurements taken at the beginning and end of the experiments possibly contributed to the lack of significant differences in the isotopic profiles of the organisms [61]. Future studies would benefit from increased amounts and/or quality of aquaculture sludge and detailed sampling to capture the potential variability and finer-scale dynamics of isotopic compositions in *Perinereis gualpensis* feeding on aquaculture sludge.

The presence of organic components in the natural substrate, which were not part of the aquaculture sludge, possibly influenced the obtained results [62]. To address this, further experiments could involve incinerating the substrate prior to its incorporation into the units, as this procedure eliminates any organic matter present. However, it should be noted that the absence of microorganisms, and micro and meiofauna that function as available organic matter could affect the results obtained. Previous studies conducted with *H. diversicolor* demonstrated that establishing microbiological activity in a non-inert substrate can enhance the survival and growth rates of polychaetes in bioremediation trials [50]. Additional investigations are needed to determine if the isotopic profiles of *P. gualpensis* show significant differences when fed salmon sludge in other types of studies.

4.3. Protein Content

The protein content in the polychaetes fed with sludge (≈52–58%) is suitable for the dietary protein requirements of several aquaculture species with commercial interest [15,16]. Similar protein contents were reported by *N. vexillosa* (58%) and *P. aibuhitensis* (61%) used as live food for shrimp culture [25]. Additionally, *H. diversicolor* showed 49–55% protein after being fed with *Huso huso* and salmon sludge during 8 weeks and 30 days, respectively [13,19]. In another study, the same species obtained 57–59% protein content after being fed with *O. mykiss* aquaculture sludge [48]. Other studies reported lower protein content in *H. diversicolor* (32–38% and 42%) after 30 days of feeding salmon sludge [15,16].

The aquaculture sludge used in this study showed a protein content of 17%, being consistent with the 18–19% reported in smolt sludge from Atlantic salmon [16]. However, other studies found higher protein content in salmon sludge (21–25%) [15,19]. Similarly, a protein content of 24% was reported in rainbow trout sludge [48].

Despite the low protein level in the sludge used in this research, an increase in protein content was achieved by the organisms. *Nereis virens* significantly increased its protein content when fed with a richer sludge (50% protein) from a recirculating system with Atlantic halibut [57]. However, other studies using sludge with a similar protein content (18%) did not observe a protein increase in the polychaetes [21]. These differences could be attributed to various factors, such as the efficiency of the extractive species used, the design of the remediation system, or the physicochemical conditions in the superficial water [50].

The polychaetes fed with aquaculture sludge obtained higher protein contents than the control organisms. However, the amount of sludge had little effect on the protein content of the polychaetes among 10% and 20% sludge treatments. This phenomenon was also observed in other studies and may be attributed to the standard nutritional profile in polychaete species [16].

The protein content achieved by *P. gualpensis* and the increase in total nitrogen and carbon suggest that exploring the nutritional value of this species could be promising for its use as an alternative ingredient in aquaculture feeds. It would be interesting to carry out more experiments to evaluate the lipid content and fatty acid profiles of *P. gualpensis* after bioremediation trials.

4.4. Growth and Survival of P. gualpensis

The salmon aquaculture sludge used in this study fulfilled the nutritional requirements of *P. gualpensis*. The high survival and positive growth rates obtained in bioremediation trials with polychaetes indicate that the organic matter used as food was nutritionally suitable and highlight the bioremediation potential of the species [10,11].

The highest survival achieved by *P. gualpensis* (95%) was obtained in the 20% sludge treatment, without significant differences with 10% sludge (88%). The lowest survivals were obtained in the control treatment (55%), where limited food availability likely led to starvation, competition, and predation [17,44,48].

Arenicola marina reported similar survival (93%) after being fed with fish waste during 55 days at a density of 150 organisms m^{-2} [63]. However, other studies with *H. diversicolor* reported lower survival (38–54%) when fed during 40 days with commercial fish feed at the same density used in this study (300 organisms m^{-2}) [49]. Additionally, lower survival (50–70%) was achieved by *P. nuntia vallata* fed on Japanese flounder sludge after 35 days of trials [28]. The sludge used in the latter study had higher protein content (≈21%) and a similar total N (3.2%) than the present study. The lower survival rates of *P. nuntia vallata* were possibly due to the experimental units being kept with no or a shallow surface water layer (0–5 mm) for 6 h per day. In contrast, in the present study, the experimental units were only deprived of surface water for 30 min every 48 h during water replacements. This possibly contributed to a higher survival due to improved water and habitat quality [50].

The highest specific growth rate (SGR) achieved by *P. gualpensis* (0.28% day^{-1}) was obtained in the 20% sludge treatment. Although the differences were not significant, the growth of *P. gualpensis* increased in relation to the amount of aquaculture sludge added to the bioremediation system. Other studies with polychaetes reported similar growth rates. *Arenicola loveni loveni* implemented in a *Haliotis midae* aquaculture effluent achieved 0.39% day^{-1} in a longer experiment (91 days) [11]. Additionally, *H. diversicolor* achieved 0.30% day^{-1} fed with halophyte detritus during 75 days [64], and *P. aibuhitensis* obtained 0.40% day^{-1} fed with aquaculture fish waste (*Hexagrammos otakii*) at a density of 600 org m^{-2} [22].

However, several studies reported higher growth rates in bioremediation trials with polychaetes than the present study. *Perinereis aibuhitensis* fed with *P. olivaceus* sludge obtained a SGR of 0.52–0.64% day^{-1} (35-day trial). In this study, the fish sludge used contained more protein (≈21%) and total C (≈30%) content than the present study [20]. Additionally, *N. virens* fed with *D. labrax* sludge exhibited a SGR of 0.9% day^{-1} in a 6-week trial [65]. Other experiments using salmon sludge also reported higher growth rates. *Hediste diversicolor* achieved a SGR of 1.0–2.1% after being fed during 30 days with smolt sludge [17,19,44]. On the other hand, the same species achieved negative growth results after 30 days of feeding with aquaculture sludge, only obtaining positive growth rates after being fed with commercial fish food [30].

It is difficult to make direct comparisons between the current study and previous research concerning polychaete growth due to the considerable differences in the composition and characteristics of aquaculture sludges. These variations are influenced by factors such as the cultured species, the production stage, the processing procedures, or the quality of the feed given to the fish [17].

The lower growth rates obtained in this study could be due to the low content in organic components in the salmon sludge used or the fact that the polychaetes were underfed [19,44,45]. *Hediste diversicolor* reported negative growth rates due to the lower protein content in the sludge (18–19%), but increasing the amount of sludge in the experimental system, this species achieved a SGR of 2.1% day^{-1} [16]. The high survival and positive growth rates achieved by *P. gualpensis* in this study are good results, meaning that the salmon sludge was suitable as a source of food [66,67]. However, further research with *P. gualpensis,* using a richer sludge or increasing the amount of salmon sludge added to the system, would be interesting.

The initial weight of the organisms (≈0.6 g) could also affect the growth results obtained. Growth rates generally decrease with animal size [19]. Smaller organisms (0.03–0.25 g) were reported to achieve higher growth rates (2.1–3.4% day^{-1}) in bioremediation trials [13,17,34]. Nevertheless, *P. aibuhitensis* achieved higher growth rates than this study (0.4% day^{-1}) using organisms of almost 2 g of initial weight [22]. The density of organisms implemented in bioremediation systems is another factor affecting growth rates. High densities cause depletion in feeding, competition, and cannibalism in other species of polychaetes [47,48,68,69]. Densities higher than 1000 organisms m^{-2} were reported to cause aggressive behaviour and lower survival, growth rates, and food consumption rates in *H. diversicolor* [34,49]. For *P. aibuhitensis*, these effects occur at densities higher than 400 organisms m^{-2}, being recommended to apply 200 organisms m^{-2} [22]. Additionally, the collection of the organisms during autumn could also have a negative effect on the growth rates obtained, as this is when organisms initiate maturation [19]. Further studies should be conducted using *P. gualpensis* with different initial sizes, densities, and collection periods to investigate these effects in this species.

The natural substrate used in the control treatment was not an adequate feed for *P. gualpensis*. In polychaetes culture, the most important factor influencing growth rates is food availability [44]. These organisms achieved negative growth and lower survival, probably due to the scarcity of organic components [20]. This effect was also reported in other studies with *A. loveni loveni* and *H. diversicolor* due to under feeding or starvation, decreasing SGR in -0.14% day^{-1} (30 days) and -0.19% day^{-1} (91 days), and resulting in a negative weight gain by 25% after 9 days of trials [11,16,17,44].

Most of the species within the family Nereididae are omnivorous and may change feeding methods depending on the food available [70,71]. In this trial, we added the salmon sludge in the subsurface layers of the substrate due to the ability of *Perinereis* species to feed on sediment [72]. However, *P. nuntia vallata* and *H. diversicolor* showed high growth rates with feeding aquaculture sludge and fish feed in the sediment surface [15,16,28,46]. Further studies are needed to evaluate the most suitable feeding strategy for *P. gualpensis* in bioremediation trials.

5. Conclusions

The results of this study indicate that *P. gualpensis* holds potential as a promising candidate for bioremediation of salmon sludge, which could contribute to reducing the environmental impact of aquaculture organic waste.

Perinereis gualpensis achieved a significant removal of organic components and accumulation in its biomass after the trials. The high protein content obtained by the polychaetes also suggests interesting alternatives for its use as a valuable component in aquafeeds.

Increasing the amount of sludge in the experimental system resulted in higher organic matter removal rates, with the addition of 20% sludge being recommended. However, the varying amounts of sludge used in this trial had no significant effect on the protein content of the polychaetes. Further investigation with higher sludge quantities may promote different results.

The high survival and positive growth rates indicate that *P. gualpensis* can be fed with salmon sludge as the only source of food. Further research should determine the maximum bioremediation capacity of this species and assess the lipid content and fatty acid profiles of *P. gualpensis* to evaluate its potential use as an additional ingredient to be used in the formulation of aquaculture feeds.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author upon request.

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