Title:

Assessing Inputs of Aquaculture-derived Nutrients to Streams using Dissolved Organic Matter Fluorescence

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Abstract

Salmon aquaculture is an important economic activity globally where local freshwater supplies permit land-based salmon aquaculture facilities to cultivate early life stage salmon. Nitrogen, phosphorus and organic matter in aquaculture effluents contribute to the eutrophication of adjacent and downstream rivers and lakes. This study quantifies the enrichment of nutrients in land-based salmon aquaculture facility effluents compared to receiving waters. We measured nutrient concentrations and dissolved organic matter (DOM) quantity and quality via fluorescence spectroscopy in streams and effluent waters associated with 27 facilities in Chile. We found that facilities added on average 0.9 (s.d.=2.0) mg-C L^{-1}, 542 (s.d.=637) µg-total N L^{-1}, and 104 (s.d.=104) µg-total P L^{-1} to effluents compared to stream waters. DOM in stream water was enriched in humic-like fluorescence, while aquaculture effluents were enriched in protein-like DOM fluorophores. Principal component and correlation analysis revealed that tryptophan-like fluorescence was a good predictor of total N and P in effluents, but the strength of significant linear relationships varied among individual facilities (r^2: 0.2 to 0.9). Agreement between laboratory fluorescence and a portable fluorometer indicates the utility of in-situ sensors for monitoring of both tryptophan-like fluorescence and covarying nutrients in effluents. Thus, continuous in-situ sensors are likely to improve industry management and allow more robust estimates of aquaculture-derived nutrients delivered to receiving waters.

Keywords

salmon aquaculture, effluent, eutrophication, streams, water pollution, fluorescence sensors

1. Introduction

Aquaculture forms an important component of the global food system where the rapid expansion of the aquaculture industry in recent decades has been driven in part by limitations in wild-capture fisheries (Naylor et al. 2021). Chile is the second largest producer of farmed salmonids worldwide harvesting nearly two million tons of salmonids in 2019 (SERNAPESCA 2019). Abundant freshwater in Andean watersheds within Chile's North Patagonian Region (S˚39 to ˚41) is well-suited for diadromous salmon cultivation due to its low temperature (<15 ˚C), adequate dissolved oxygen (>5 mg L^{-1}), low dissolved and particle content and proximity to coastal waters (Soto et al. 2006). Salmon aquaculture accommodates the freshwater life stage of salmon using covered, above ground tanks near freshwater lakes and rivers before transporting smolts to marine facilities for further growth and harvesting. Land-based aquaculture facilities typically divert stream water into flow through systems where particulate (feces and unconsumed food) and dissolved (metabolic by-products, antibiotics, and salt) wastes accumulate in effluent waters (Quiñones et al. 2019, Tello et al. 2010). Sedimentation basins and in-line rotary filters are used to remove a fraction of solid waste. However, wastewater treatment is generally not effective for the removal of dissolved organic and inorganic nutrients which are delivered directly to adjacent rivers where they contribute to eutrophication (Rosa et al. 2013).

Allowable nitrogen and phosphorus concentrations in land-based aquaculture effluents range from 10-75 mg-N L^{-1} and 2-15 mg-P L^{-1} depending on the size and type of receiving water body (MINSEGPRES 2001). However, monitoring and enforcement of these limits are lacking in Chile and other countries (Quiñones et al. 2019). In addition, aquaculture operators and regulations do not monitor dissolved organic matter (DOM), a complex mixture of biologically-derived organic molecules (Dittmar and Stubbins 2014). DOM generated from fish feces, fish

food, and fish mucus associated with salmon aquaculture adds to the naturally derived DOM present in stream water (Molina and Fernandez 2020). Efforts to evaluate the impact of land-based aquaculture facilities on lakes and rivers are hampered by the lack of data describing the amounts and variability of nutrients and DOM emanating from these facilities. The rapid development of the salmon aquaculture industry has occurred amidst a legal framework for permitting and regulation that has not evolved with the scale of industrial activity and may ultimately threaten the long-term sustainability of the industry (Cid Aguayo and Barriga 2016, Little et al. 2015). Thus, novel cost-effective monitoring strategies for land-based aquaculture facility effluents and their impacts on downstream ecosystems are needed to support regulatory decisions based on robust scientific data.

Land-based salmon aquaculture effluents are point sources of pollution similar to other industrial liquid wastes where the application of fluorescence spectroscopy has been applied to industrial wastewater monitoring (Carstea et al. 2016, Ulliman et al. 2020). Fluorescent DOM (FDOM) is the fraction of chromophoric DOM that fluoresces due to the presence of numerous fluorophores that typically fall within humic-like or amino acid-like classifications (Dittmar and Stubbins 2014). Humic-like FDOM is characterized by broad emission peaks greater than 350 nm, while protein-like FDOM presents narrower emission bands less than 350 nm. Protein-like FDOM is similar to the fluorescence peaks of known aromatic amino acids tryptophan and tyrosine (Coble 1996). Both protein-like and humic-like FDOM are associated with wastewaters such as human sewage (Shi et al. 2021) and paper mill effluent (Baker 2002). FDOM in US wastewaters has been shown to correlate with dissolved organic carbon (DOC) and chemical and biological oxygen demand (Christian et al. 2017). The use of DOM fluorescence for water quality

monitoring is an active area of research where increasing availability of portable FDOM sensors has broad application potential (Carstea et al. 2016, Carstea et al. 2020).

In natural settings, DOM fluorescence associated with salmon spawning resembles that of the amino acid tryptophan and other protein-like material (Hood et al. 2007b). In addition to influencing DOM fluorescence quality, natural salmon runs provide important marine-sourced carbon and nutrients to freshwater and terrestrial ecosystems which alter stream metabolism by promoting heterotrophy (Naiman et al. 2002, Tiegs et al. 2009). In aquaculture, DOM fluorescence has been described for small, recirculating tank systems cultivating rainbow trout (Hambly et al. 2015) and Nile tilapia (Yamin et al. 2017), where fluorescent DOM accumulates during recirculation. High-resolution mass spectrometry indicated DOM molecular composition changes according to feed type in Atlantic salmon recirculating aquaculture systems in Norway which also impacts wastewater treatment performance (Aguilar-Alarcon et al. 2020). In non-recirculating, flow-through trout farms, Jean-Marc et al. (2018) used ^{13}C and ^{15}N stable isotope signatures in mixing models to estimate that 40 to 88% of aquaculture feed contributed to the diet of downstream organisms in France. On Jeju Island, South Korea, Kim et. al. (2021) reported significant organic carbon and nitrogen enrichment emanating from land-based halibut aquaculture facilities discharging directly into the coastal ocean which likely stimulated coastal autochthonous primary production.

Within Chile, DOM fluorescence and molecular composition has been previously characterized in relatively few places where the fraction of biolabile DOM increased in streams receiving aquaculture effluent (Kamjunke et al. 2017, Nimptsch et al. 2015). Chile is an apt location for the study of salmon aquaculture given that 287 land-based facilities are permitted to operate in the country (SERNAPESCA 2021). Given the prevalence of these facilities in Chile and elsewhere

in the world, the quantification and characterization of aquaculture-derived DOM and nutrients is necessary to predict downstream water quality in waters receiving aquaculture effluents (Boyd 2003, Sindilariu 2007). Findings in Chile are applicable to other waters receiving aquaculture effluents globally where similar trends and concerns related to intensive salmon aquaculture exist in Canada, Norway, and the United Kingdom (Iversen et al. 2020, Verdegem 2013).

This study aimed to address the persistent problem of water quality data scarcity associated with the impact of land-based salmon aquaculture facilities on adjacent and downstream freshwaters. Our objectives were to characterize the inorganic chemistry, DOC concentration, and optical properties of DOM in land-based salmon aquaculture effluent, and to identify potentially useful strategies for improved monitoring of aquaculture-derived nutrients to receiving waters. We hypothesized that (1) effluent waters would be enriched in DOM and inorganic nutrients compared to natural streams, (2) effluent DOM would be characterized by protein-like fluorescence indicating recent biological production from fish, and (3) DOM fluorescence quality would covary with nutrient concentrations in aquaculture effluents. To test these hypotheses, we collected water samples from 27 land-based salmon aquaculture facilities and assessed relationships among water quality variables. To our knowledge this is the first study to quantify the relationships between DOM fluorescence intensity and inorganic nutrient concentrations in land-based salmon aquaculture facilities.

2. Materials and Methods

2.1 Study Sites

The 27 land-based aquaculture facilities sampled for this study were permitted after the year 2005 and varied in their location within Chile (Fig. 1; Table S1). Each facility allowed continuous freshwater input to flow by gravity through covered, aboveground tanks. Most facilities diverted stream water directly into their systems, however, two facilities used groundwater springs for water input. One facility used partial recirculation within the system. Rotary filters and sedimentation ponds, or a combination of the two, were the most common solid removal treatment strategies. Treatment of dissolved water composition was employed at only three facilities using ultraviolet radiation. Maximum annual permitted production ranged from 60 to ~1,000 wet tons of salmon, while maximum allowed effluent water discharge ranged from ~100 to ~3,000 L sec^{-1}. Maximum annual facility production efficiencies, estimated as the maximum annual production tons of salmon per liter of effluent were less than 34 x 10^{-9} wet tons L^{-1}.

2.2 Sampling and Field Measurements

Each facility was sampled on one to 15 separate days between 2014 and 2018. The effluents of three facilities were sampled again in 2021 along with the effluents of four additional facilities not sampled previously. During each sampling visit, water samples were collected from the facility effluent outlet channel and from the associated stream upstream of the facility. All bottles were pre-cleaned with acidified ultrapure water (MilliQ) and rinsed with sample water three times prior to filling. Water for dissolved organic carbon (DOC) and DOM fluorescence analyses was immediately filtered (0.22 µm; Whatman) into amber glass bottles in the field. Water for DOC concentration analysis was further acidified to pH 2 using 37% HCl (Merck). DOC concentrations and DOM fluorescence were measured within 72 hours after collection. Filtered and unfiltered water was collected for further chemical analyses. All water samples were immediately stored at 6˚C on ice until analysis. A portion of unfiltered samples were stored frozen as an archive. Select archive samples were allowed to warm to room temperature and agitated prior to further analysis.

A WTW 3420 water quality multi-probe sonde (Weilheim, Germany) was used to measure water temperature (precision = ±0.1˚C), pH (±0.01 standard units), and specific conductivity (±0.3 µS cm^{-1}) in-situ. Dissolved oxygen was measured in-situ with a WTW FDO 925 (Weilheim, Germany) optical sensor (±0.1 mg L^{-1}). Turbidity was measured in the field using a AL250T-IR (AGUALYTIC, Germany) turbidimeter in Nephelometric turbidity units (±0.1 NTU).

2.3 Chemical Analysis

DOC was measured on filtered samples using high temperature catalytic oxidation using HighTOC Elementar Systems (Hanau, Germany). The limit of detection for DOC was 0.2 mg-C L^{-1}. Dissolved nutrients were measured colorimetrically on filtered samples with a segmented flow analysis model SKALAR (Holand) following standard methods for nitrate (method 4500-NO_3 – F), nitrite (method 4500-NO_2 – B), ammonia (method 4500-NH_3 – F), and soluble reactive phosphorus (method 4500-P – E). Total phosphorus (TP) (Koroleff (1983) and method 4500-P – B/5) and total nitrogen (TN) (Koroleff (1983), method 4500-N/C, and method 4500-NO_3 – F) (Standard Methods APHA 2005) were measured for unfiltered samples. The limits of detection were 2 µg-N/P L^{-1} for nitrate, nitrite, and phosphate, 5 µg-N/P L^{-1} for ammonia and TP, and 15 µg-N L^{-1} for TN. Dissolved inorganic nitrogen (DIN) was calculated as the sum of dissolved nitrogen species (N-NH_4, N-NO_3, N-NO_2).

2.1 Measurement of DOM Spectroscopic Composition

DOM absorbance and fluorescence were measured in triplicate for each sample at room temperature (~21˚C) and blank corrected using ultrapure water (Merck). DOM absorbance (A) was measured from 240 nm to 600 nm (1 nm steps) with a spectrophotometer (Merck Pharo Spectroquant 300, Darmstadt, Germany) and converted to Naperian absorbance coefficients (m^{-1}) according to Eq. 1:

$$a = 2.303 \times \frac{A}{l} \quad \text{Equation 1}$$

where A is absorbance and *l* is the path length in meters (Kirk 1994). Fluorescent DOM was measured with a fluorescence spectrometer (Varian Eclipse). Excitation-Emission-Matrices (EEM) were generated by recording fluorescence emission from 300 to 600 nm (1 nm steps) during excitation from 240 to 450 nm (5 nm steps).

A portable ultraviolet fluorometer sensitive to dissolved tryptophan-like aromatic organic molecules (UviLux Tryptophan, Chelsea Technologies, United Kingdom) was acquired in 2021 and calibrated to report fluorescence in tryptophan equivalents (µg L^{-1}) (Fig. S1). The UviLux sensor central excitation wavelength is 280 nm with a 30 nm full width half maximum window and the emission detection range is centered at 360 nm by 50 nm full width half maximum. The UviLux sensor uses a stabilized ultraviolet LED light source and a photomultiplier detector to achieve higher sensitivity and lower detection limits than other commercially available portable fluorometers. The UviLux sensor was not available for in-situ use during field campaigns for samples collected prior to 2021, however, the portable sensor was used to measure fluorescence in available archived samples (frozen unfiltered). To compare the EEMs results to the UviLux sensor, the processed EEM fluorescence in Raman units was summed within the excitation (265 to 295 nm) and emission (335 to 385 nm) range corresponding the UviLux sensor. The sum was then converted to tryptophan equivalents via a calibration curve from 1 to 1000 µg L^{-1} using 98% pure HPLC grade tryptophan (Sigma-Aldrich Lot BCBZ9255) in ultrapure MilliQ water (Fig. S2).

2.5 Data Analysis

Absorbance spectra and EEMs were processed in R using the *eemR* and *staRdom* R packages (Murphy et al. 2013, Pucher et al. 2019, R Core Team 2020). Briefly, EEMs were corrected for inner filter effects using sample absorbance spectra and for instrument-specific lamp intensity bias prior to subtracting the EEM of the ultrapure water blank. Each EEM was normalized to the area of the pure water Raman fluorescence peak at 350 nm (Lawaetz and Stedmon 2009, Murphy et al. 2013). Data within fluorescence bands influenced by Rayleigh and Raman scattering were removed from the EEMs and not interpolated prior to further analysis.

The *staRdom* R package was used to generate a parallel factor analysis (PARAFAC) model of principal fluorescence components contributing to EEMs across samples and sites (Pucher et al. 2019). Each EEM was normalized to maximum fluorescence prior to running the model to minimize concentration effects on model results. Laboratory replicates (3 per sample) were not combined prior to PARAFAC model building. The model was fitted using random initialization (100 starts) under a non-negativity constraint. The final PARAFAC model was validated using split-half validation (Fig. S3) and Tuckers Congruence Coefficients (TCC > 0.99). The sample-specific maximum fluorescence (Fmax) for each model component in Raman units (RU) was averaged across laboratory replicates of each sample prior to further data analysis. PARAFAC model components (C1/HumA, C2/HumC, C3/Tryp, C4/Prot) were compared to relevant fluorophores reported in the OpenFluor database (Murphy et al. 2014).

Statistical summaries and tests were completed using the *rstatix* R package (Kassambara 2020). The difference in variable response between the effluent and upstream samples was calculated by subtracting the upstream value from the effluent value for a given sampling day. Normal distributions of the paired differences were confirmed by visually inspecting the quantile-

quantile plots of each parameter. A two-sided paired t-test was used to assess whether effluent water quality variables were significantly different from upstream samples across all aquaculture facility sites. The direction and spread of the paired differences were assessed by calculating the coefficient of variation (CV) for each water quality parameter across all facilities.

The percent enrichment of each analyte in the effluent compared to the upstream site for each sampling event was calculated to compare relative differences among the measured parameters (Eq. 2),

$$\text{Percent Enrichment} = \frac{C_{efl} - C_{us}}{C_{us}} \times 100 \quad \text{Equation 2}$$

where C_{efl} and C_{us} represent the variable for the effluent and upstream samples, respectively.

To explore the relative variability among possible indicators of water quality, a principal components analysis (PCA) was completed using the *FactoMineR* R package (Lê et al. 2008). Variables were log transformed to satisfy the assumption of normal distribution during PCA. To ascertain the general strength and direction of individual relationships among variables of interest, Pearson's correlation coefficients (r), p-values and coefficients of determination (r^2) were calculated for variables across all sites. P-values were adjusted for type I errors due to multiple testing using the false-detection rate approach (Benjamini and Hochberg 1995). To test the efficacy of individual parameters to predict nutrient and DOC concentrations, linear regression statistics for selected measured parameters and nutrients and DOC were calculated for individual sites and across all sites.

3. Results

3.1 Physiochemical Water Quality and Solute Concentrations

A total of 236 samples were collected across all 23 sites and sample locations (Table S1). The number of effluent-upstream sample pairs ranged from 1 to 13 for each site and for each water quality parameter (Table S2). All physiochemical parameters in effluents were significantly different from upstream samples (Table 1; paired t-test; $p<0.01$). Across all sites on average, effluents were elevated in water temperature, specific conductivity, and turbidity while pH and dissolved oxygen decreased on average. DOC and nutrient concentrations were significantly enriched in effluents compared to upstream samples (Table 1; paired t-test; $p<0.001$). Although DOC concentrations of individual samples varied among sites, no site exceeded 11 mg-C L^{-1}. On average across all sites, aquaculture facilities increased nutrient concentrations in effluents by 208 (sd=230) µg-N-NH_4 L^{-1}, 98±265 µg-N-NO_3 L^{-1}, and 67±78 µg-P-PO_4 L^{-1} (Table 1).

The percentage enrichment of each variable between the effluent and upstream waters was calculated for each independent site visit (Eq. 2; Fig. 3). Among solute concentrations, the mean enrichment for individual sites was highest for ammonium (2500±2100%) and lowest for DOC (270±400%; range = -51 to 1400%). Mean enrichment for individual sites indicated a depletion of DOC for two of the 23 sites (-21 and -51%). Mean percent enrichment of TN and TP across all sites was 770±970% and 380±460%, respectively.

3.2 DOM spectroscopic composition

Mean Napierian absorbance coefficients at 254 nm (a_{254}) in upstream samples (7.7±7.9 m^{-1}) were not statistically different from effluent samples (7.6±5.2 m^{-1}) across all sites (Table 1; paired t-test; p=0.8). Mean a_{254} for effluent and upstream samples within individual sites ranged from <1.0 to 23 m^{-1}.

A single, four-component PARAFAC model was fit to determine the principal DOM fluorescence components across all samples (Fig. 2; Table S3). Separate PARAFAC models for the upstream and effluent sample groups revealed neither additional nor distinct components. Components C1/HumA (peak excitation= 245 and 325 nm; peak emission = 424 nm) and C2/HumC (peak excitation= 245 and 365 nm; peak emission = 483 nm) were named according to classical interpretation of ultraviolet A and UVC humic-like fluorescence (Fellman et al. 2010) (Table S3). Components C3/Tryp (peak excitation = 275 nm; peak emission = 326 nm) and C4/Prot (peak excitation= 245 and 290 nm; peak emission = 338 nm) were each named for their tryptophan-like and protein-like fluorescence, respectively, as previously described for a broad range of aquatic systems (Fellman et al. 2010). The complete PARAFAC model results were compared to the OpenFluor online database (Table S3) (Murphy et al. 2014).

Fluorescence magnitudes are reported as the sample-specific fluorescence maximum (Fmax) for each component in Raman units (RU). Component C3/Tryp was significantly higher in effluent samples (0.2±0.3 RU) compared to upstream samples for all sites combined (0.02±0.02 RU; paired t-test; $p<10^{-4}$; Table 1). The second protein-like component C4/Prot was also enriched in effluent compared to stream water for all sites combined (paired t-test, p=0.06). One humic-like component, C2/HumC, was significantly lower in effluent samples compared to upstream (paired

295 t-test, $p<0.01$) while the second humic-like component, C1/HumA, was not significantly
296 different among the sampling locations for all sites combined (paired t-test; $p=0.4$).

297 The tryptophan-like component, C3/Tryp, had the greatest enrichment of fluorescence in effluent
298 of all fluorescence components. C3/Tryp also had to greatest percent enrichment of all optical
299 quality variables for all sites combined (median = 410%, Fig. 3).

300

301 **3.3 Analysis of Covariance between DOM Optical Quality and Water Quality**

302 Principal components analysis (PCA) was used to explore the covariance among potential in-situ
303 indicators of water quality. Specific conductance, a_{254}, and the PARAFAC model fluorescence
304 components were used as active variables in two separate PCAs for the upstream and effluent
305 samples. Additional available physiochemical parameters (temperature, pH, dissolved oxygen)
306 were excluded from the PCAs due to their limited overall variability (Table 1; Fig. 3). Turbidity
307 was excluded due to limited data (Table S2). Concentrations of DOC, total nitrogen, and total
308 phosphorus were added as supplementary variables overlain on the results of the PCAs (Fig.
309 4A&C).

310 Two principal components explained greater than 70% of the variation within each PCA (Fig.
311 4A&C; Fig. S4). In both PCAs, principal component one (PC1) aligned with positive variation in
312 a_{254} and humic-like fluorescence PARAFAC components (C1/HumA and C2/HumC), while
313 principal component two (PC2) aligned with positive variation in protein-like PARAFAC
314 components (C3/Tryp and C4/Prot). For the upstream samples, the DOC concentration
315 supplementary variable aligned positively with PC1 (humic-like fluorescence), but for effluent
316 samples DOC aligned positively with PC2 (protein-like fluorescence). Variability in TN and TP

did not align with either of the two primary principal components in the PCA for upstream samples but did align with the third principal component driven by specific conductance (Fig. 4A; Fig. S5). In the effluent PCA, the TN and TP concentration supplementary variables aligned with PC2, the tryptophan-like component (C3/Tryp), and specific conductivity.

The covariance between the optical and specific conductance parameters and the measured nutrients were further explored through Pearson correlation matrices of the coefficients of determination (r^2) between each set of variables (Fig. 4B&D). Within the upstream samples, significant linear relationships ($p<0.05$; $r^2 > 0.7$) were found between DOC concentration and the optical variables a_{254}, C1/HumA and C2/HumC. Additional significant linear relationships were found between specific conductivity and all nutrient concentrations except ammonium with r^2 ranging from 0.17 to 0.56. Within the effluent samples, significant linear relationships were found between all nutrient concentrations and the C3/Tryp fluorescence component (Fig. 4D). The highest overall coefficient of determination within the effluents was between ammonium concentration and C3/Tryp intensity ($r^2 = 0.67$). Significant relationships between specific conductivity and all nutrient concentrations were observed, although r^2 ranged from 0.24 to 0.47 suggesting weaker relationships than for those observed with C3/Tryp.

3.4 Linear Relationships for Individual Aquaculture Facilities

Covariance among variables across all sites indicated significant positive relationships (Fig. 4B&D), however, the strength and slopes of linear regressions among variables at individual sites varied greatly. Linear relationships among all variables at all individual sites were explored, although only variables with the potential for improved aquaculture monitoring were of

339 particular interest in this study. Significant linear relationships for selected variables of interest
340 across individual sites are shown in Figures 5 and 6.

341 Predictor variables a_{254} and C2/HumC were positively correlated with DOC in upstream samples
342 at 13 sites ($p<0.1$; r^2: 0.41 to 0.95) (Fig. 5A&B). Specific conductivity was negatively correlated
343 with DOC concentration in upstream samples at 4 sites ($p<0.1$; r^2: 0.29 to 0.58). Specific
344 conductivity was positively correlated with soluble reactive phosphorus in upstream samples at 8
345 sites ($p<0.1$; r^2: 0.39 to 0.97). Mean slopes for selected linear models indicated 0.12 mg-C L^{-1}
346 increase per unit a_{254} (m^{-1}) and 1.3 µg-P-PO_4 L^{-1} increase per unit specific conductivity (µS cm^{-1}
347).

348 Within effluent samples, the tryptophan-like fluorescence component C3/Tryp was positively
349 correlated with DOC concentration at only two sites ($p<0.1$; r^2: 0.40, 0.49) (Fig. 6). A single site
350 (#123) had a significant negative correlation between specific conductivity and DOC
351 concentration ($p=0.04$; $r^2=0.40$). C3/Tryp was positively correlated with TN, ammonia, and TP
352 in effluent samples at 4 sites ($p<0.1$; r^2: 0.30 to 0.97; Fig. 6C, E, & G), although the significant
353 relationships did not always occur for the same aquaculture facilities. C3/Tryp was negatively
354 correlated with ammonium in effluent samples at one site (#113; $p<0.01$; $r^2=0.59$). Specific
355 conductivity was positively correlated with TN, ammonium, and TP in effluent samples at 4, 3,
356 and 3 sites, respectively ($p<0.1$; r^2: 0.59 to 0.90). Overall, analysis of covariance of all sites
357 combined indicated positive linear relationships between protein-like DOM fluorescence and
358 nutrient concentrations in aquaculture effluent.

359

360

3.5 Relationship of Aquaculture Effluent Fluorescence with In-Situ Sensors

To further assess the potential for in-situ monitoring of aquaculture effluents using DOM quality, the tryptophan-like fluorescence observed in the effluent EEMs was compared to the UviLux sensor output (Fig. 7). The intensity of C3/Tryp in effluent samples was positively correlated with the sum of the total fluorescence in the EEM corresponding to the wavelengths utilized by the UviLux sensor (excitation = 265 to 295 nm; emission = 335 to 385 nm; Fig. S6; r^2=0.99). The sensor proxy derived from the EEMs was also positively correlated with the UviLux sensor output for unfiltered archive and field samples measured in 2021 (Fig. 7, adjusted r^2=0.67). Tryptophan equivalents measured using the UviLux sensor ranged from below detection (<2 µg-tryptophan L^{-1}) to 43 µg-tryptophan L^{-1}. Mean turbidity measured for the archived samples was less than 10 NTU.

4 Discussion

4.1 Physiochemical Water Quality and Solute Concentrations

Physiochemical results and solute concentrations in upstream samples were representative of typical oligotrophic Patagonian freshwaters (Garcia et al. 2015). Within effluent samples, the physiochemical results for water temperature, pH, and dissolved oxygen did not indicate conditions immediately harmful to aquatic ecosystems. While specific conductivity and turbidity were elevated in effluents compared to the upstream samples, they did not indicate significant water quality impairment for freshwater biota (mean < 0.2 mS cm^{-1}; mean < 2 NTU) (Canedo-Arguelles et al. 2013, Newcombe 2003).

Similarly, despite the consistent enrichment of DOC and nutrients in effluents, no effluent was found to exceed current Chilean water quality standards (10-75 mg-N L^{-1}, 2-15 mg-P L^{-1}) (MINSEGPRES 2001). DOC concentrations in effluents (< ~5 mg-C L^{-1}) were lower than typical treated human wastewaters (~9 mg-C L^{-1}) (Worrall et al. 2019). Particulate carbon may be an important contributor to the total carbon loads in effluents however particulate carbon was not measured in this study. Although the retention time of stream water increases within facilities due to the increased tank volume compared to the stream channel, the consistent enrichment of DOC and nutrients in effluents (Fig. 3) suggests water quality changes in effluents are due to aquaculture activity rather than increased residence time in the tanks.

Increased nutrient concentrations in effluents contribute to the eutrophication potential of receiving streams and downstream aquatic ecosystems (van der Struijk and Kroeze 2010). Increasing prevalence of algae blooms in North Patagonian lakes may be influenced in part by aquaculture-derived nutrients, although other nutrient sources exist (volcanic soils, human waste, agriculture, urban development) (MMA 2018). The degradation of water quality and the alteration of freshwater biotic communities downstream of land-based flow through salmon aquaculture effluents has been documented (Encina-Montoya et al. 2020, Kamjunke et al. 2017, Nimptsch et al. 2015), indicating the current Chilean water quality permitting standards are not adequate to protect downstream ecosystems. Improved monitoring strategies are required to aid management of aquaculture wastes to reduce the impact on sensitive freshwater systems.

The high range of the coefficients of variation (CV) of the paired differences of nutrient concentrations across all sites (100 to 269%, Table 1) suggest that facility size and operation type influence effluent chemistry. Within sites, the CV of paired differences for solute concentrations

404 ranged widely (ca. -100 to 1,000%) suggesting that occasional grab samples do not adequately
405 capture the dynamic concentration ranges occurring in effluents.

406

407 **4.2 DOM Optical Quality**

408 Mean Napierian absorbance coefficients at 254 nm (a_{254}) for both upstream and effluents across
409 all sites (8 m^{-1}) were similar to other Andean streams where absorbance coefficients at 350 nm
410 were reported to be less than 3 m^{-1} (Garcia et al. 2015). These oligotrophic Andean streams have
411 lower chromophoric DOM content than rivers in temperate North America (a_{254} > 10 m^{-1})
412 (Spencer et al. 2012). The prevalence of humic-like fluorescence PARAFAC components
413 C1/HumA and C2/HumC in upstream samples is consistent with fluorescence in natural streams
414 draining forested catchments broadly where natural terrestrial vegetation sources contribute the
415 bulk of DOM (Fellman et al. 2010). PARAFAC component C1/HumA was similar to a
416 component identified in Andean streams (Garcia et al. 2015). The C3/Tryp component
417 contributed least to DOM fluorescence in upstream samples, indicating fresh tryptophan-like
418 material is not naturally abundant in Chilean streams. However, the presence of the more general
419 protein-like component (C4/Prot) in upstream samples (Table 1) suggests there exists some
420 background of protein-like fluorescence from groundwater or freshly produced autochthonous
421 microbial DOM.

422 Although DOC concentrations (mean DOC < 2 mg-C L^{-1}) and chromophoric DOM (mean a_{254} <
423 10 m^{-1}) in effluents were also low compared to US rivers, the DOM quality was altered by
424 aquaculture facilities. Specifically, effluents were enriched in tryptophan-like fluorescence
425 component C3/Tryp which has been previously described in waters associated with natural

426 salmon runs (Hood et al. 2007a) and salmon aquaculture production (Kamjunke et al. 2017,
427 Nimptsch et al. 2015). This protein-like fluorescence is generally present in wastewaters where
428 its biodegradability increases stream heterotrophy (Carstea et al. 2016).

429

430

431 **4.3 Relationships between Nutrients and DOM Optical Quality**

432 In upstream samples, the linear increase of a_{254} and C2/HumC with DOC concentration is
433 consistent with observations across a wide range of natural aquatic systems (Spencer et al. 2012),
434 including Patagonian streams (Garcia et al. 2015), where increases in chromophoric and humic-
435 like DOM are interpreted to be driven by the input of natural, terrestrially derived DOM sources.
436 The slope of the linear relationship of a_{254} versus DOC concentration in upstream samples (mean
437 = 8.3 L mg-C^{-1} m^{-1}) was within the range of relationships within US rivers (~5 to 10 L mg-C^{-1} m^{-1}) (Spencer et al. 2012).

439 Similarly, a linear decrease in DOC concentration with specific conductivity at individual
440 upstream samples (Fig. 5C) is commonly observed and suggests mobilization of natural DOC
441 sources during higher flow conditions (Bieroza et al. 2018). The positive linear relationships
442 between phosphate and specific conductivity in upstream samples (Fig. 5F) suggest geologic
443 sources of phosphate are present in Chile's volcanic soils (Borie and Rubio 2003).

444 The significant covariance between C3/Tryp and all solute concentrations across all effluent sites
445 combined (Fig. 4D) suggests tryptophan-like fluorescence is a useful indicator of effluent
446 quality. Significant positive relationships between tryptophan-like fluorescence and dissolved
447 nutrients, such as ammonium and soluble phosphorus, have been reported in the River Thames,

448 England (Old et al. 2019) and for inland water bodies in central China (He et al. 2021), although
449 relationships are site specific. Similarly, Cohen et al. (2014) found that humic-like fluorescence
450 in human sewage wastewater treatment plants significantly correlated with total nitrogen. Within
451 freshwater systems impacted by aquaculture, increased protein-like fluorescence of particulate
452 organic matter was observed in a large freshwater reservoir in China (Wang et al. 2020) and in a
453 freshwater reservoir in southeast Brazil (Chaves et al. 2020, Chaves et al. 2021). Chaves et al.
454 (2020) found Nile tilapia aquaculture in a tropical reservoir had minimal impact on DOC
455 concentrations but significant impact on DOM fluorescence quality where the proportion of
456 protein-like fluorescence was elevated within 100 m of the net cages. Protein-like fluorescence
457 from commercial feed pellets for Nile tilapia aquaculture dissolved in distilled water increased
458 linearly with the mass of pellets added (Figueiró et al. 2018). On Jeju Island, South Korea, Kim
459 et. al. (2021) reported elevated protein-like fluorescence in halibut aquaculture discharge and
460 used a principal components analysis to suggest this fluorescence covaried with TDN. Thus,
461 while protein-like DOM fluorescence is a well-known characteristic of wastewater from biologic
462 processes, few studies have characterized fluorescence for salmon aquaculture, and no studies to
463 our knowledge have quantified relationships for nutrient concentrations and salmon aquaculture
464 DOM fluorescence indices to compare to this study.

465 The significant relationships between specific conductivity and all solutes among all effluent
466 samples also indicates potential monitoring utility. Specific conductivity is a widely used
467 indicator of water quality requiring minimal maintenance of in-situ sensors (Pellerin et al. 2008).
468 For example, Suresh et al. (2009) found specific conductivity was a good predictor of total N and
469 ammonia in swine slurry in South Korea. To our knowledge, no quantitative relationship among

specific conductivity and dissolved nutrients have been previously reported for aquaculture effluents.

Maximum annual facility production, treatment type, and production efficiency (wet tons produced L^{-1}; Table S1) did not explain variability of covariance among individual sites. While the permitted maximum annual production efficiency varied among sites, the maximum annual production and effluent discharge were related linearly (Pearson's $r = 0.6$, $p < 0.01$). Operators and regulators account for dilution during permitting, however, annual permitted production is a poor indictor of water quality on shorter time scales since facility characteristics change continuously throughout facility operation due to daily feeding cycles, growth cycles, harvesting, and facility maintenance (e.g., tank cleaning).

The production life cycle stage of facilities during each sampling event is a likely factor influencing our results where sites with many sampling events are more likely to capture a range in the operational stage of the aquaculture facility and a concomitant range in water quality. We note that 9 of the 23 sites were sampled on one occasion only in this study, precluding assessment of linear correlations due to lack of data for those sites (Table S2). Irregular or infrequent grab sampling for assessment of aquaculture loads has been shown to be of limited use (Hennessy et al. 1996). Temporally resolved monitoring of individual facilities is required to understand the influence of production life cycle stage on effluent quality as well as to empirically establish meaningful baselines of effluent nutrient concentrations and loads. Monitoring of land-based aquaculture facilities that divert stream water into flow-through systems must also consider natural variation in upstream water quality which can change frequently due to seasonal and hydrologic variability. The limitation of periodic grab sampling

and the need for more individual assessment of facilities can be alleviated through continuous monitoring using commercially available sensors.

4.4 Toward Improved Aquaculture Effluent Monitoring

Currently, continuous in-situ monitoring is not required for most flow through land-based aquaculture facilities in Chile. Elsewhere, physiochemical sensors have been used to monitor aquaculture waters (Danh et al. 2020) and sensors are frequently used within systems to monitor temperature and dissolved oxygen (Li and Liu 2019). Ion selective electrode sensors that directly measure specific solutes of interest, such as nitrate, vary in required maintenance, calibration and periodic replacement as membranes age (Alahi and Mukhopadhyay 2018, Crespo 2017). Specific conductivity and DOM fluorescence have been observed to covary in freshwaters (Zhu et al. 2020), although the influence of ionic strength on DOM fluorescence is an active area of research (Gao et al. 2015). In addition, the use of salt (NaCl) in aquaculture operations could confound or otherwise influence calibrations of specific conductivity with nutrients (Encina-Montoya et al. 2020).

The strong correlation between the commercially available portable UviLux fluorometer and the C3/Tryp PARAFAC component identified in effluent samples (Fig. S6 & 8) suggests that in-situ tryptophan-like fluorescence sensors may be useful in monitoring salmon aquaculture effluents. Deviation from a 1:1 relationship between the EEM sensor proxy (filtered, never frozen) and the UviLux sensor output (unfiltered, thawed) may be due to turbidity interference or DOM composition changes during sample storage, although turbidity of the unfiltered samples was low (<10 NTU) (Fig. 7). The low turbidity in these samples suggests minimal influence from

514 ruptured cells or other particles on fluorescence as a result of freezing. Although freezing
515 samples prior to analysis of DOM fluorescence is not generally recommended (Spencer et. al.,
516 2007), we expect negligible influence of freezing on DOM fluorescence in these samples due to
517 the low DOC concentrations, low absorbance and focus on protein-like fluorophores (Fellman et
518 al., 2008; Thieme et al., 2016). Most critically, the close correspondence in quantitative
519 tryptophan equivalents between the filtered, unfrozen lab-determined fluorescence and the
520 thawed, unfiltered archived samples reported in Fig. 7 suggests that freezing unfiltered samples
521 from these aquaculture effluents did not bias the data and that freezing may be an acceptable
522 sample storage practice with practical implications for effluent managers without ready access to
523 a laboratory seeking to increase sampling frequency. Nevertheless, future use of the in-situ
524 fluorescence monitoring should include careful calibration that accounts for temperature, inner
525 filter, and turbidity interferences as is standard for more widely available humic-like FDOM
526 sensors (Carstea et al. 2020, Khamis et al. 2015).

527 The current sporadic monitoring framework allows for potential draining and flushing of
528 aquaculture tanks to occur without knowledge of the associated solute loads. Continuous
529 monitoring using sensors can more fully quantify the loads delivered from flushing events, or at
530 minimum indicate the timing and duration of such events. Sensor records can capture baseline
531 variance in water quality to establish meaningful relationships between sensors and solutes.
532 Daily, seasonal, and various production life cycle stages should be targeted for calibration. In the
533 absence of a known, robust relationship between DOM fluorescence intensity and aquaculture-
534 derived solutes, periodic or continuous fluorescence monitoring can still help indicate key
535 moments when nutrient loads may be high. At such times standard grab samples should be
536 collected to determine accurate solute concentrations using traditional laboratory techniques.

An additional important advantage of continuous monitoring strategies is the opportunity for the simultaneous monitoring of discharge which would allow nutrient loads to be assessed. Importantly, solute concentration spikes may be dampened via dilution. For gravity flow-through systems such as those sampled in this study, operators may adjust water input in order to dilute concentrations in the effluent. However, diluting concentrations in effluents does not dilute concentrations in most downstream receiving streams which have a fixed overall discharge no matter how much water is diverted into flow-through aquaculture facilities. Thus, small streams receiving effluents from flow-through aquaculture facilities are more vulnerable to receiving high loads of nutrients even if concentrations remain low in effluents. Pulses of aquaculture effluents coinciding with natural DOM pulses during seasonal storms could also have strong impacts on downstream ecosystems. Thus, continuous monitoring of effluent and total downstream loads is necessary to more fully quantify the contributions of land-based aquaculture facilities to streams and downstream lakes.

Additional study is required to effectively integrate improved monitoring strategies into land-based aquaculture facility operation and regulation. The sensitivity of sensors should be assessed under various conditions to establish thresholds of sensor response that indicate pollution events. With respect to analytical sensitivity, only three effluent EEMs in this study indicated tryptophan equivalent concentrations lower than the 2 $\mu g\ L^{-1}$ detection limit for the UviLux sensor. Tryptophan equivalent concentrations observed in the 118 effluent samples in this study ranged from 2 to 200 $\mu g\ L^{-1}$ (mean = 20 $\mu g\ L^{-1}$) indicating nutrient concentrations covary within the dynamic range of field detectable tryptophan-like fluorescence. Changes in operation standards at the level of individual facilities are necessary to leverage new technologies for the improved management of aquaculture wastes (Boyd 2003, Verdegem 2013). Complimentary studies

560 tracing the fate of aquaculture derived DOM and nutrients in freshwater ecosystems will also
561 benefit from improved quantification of aquaculture effluent loads. Our results indicate that
562 current commercially available in-situ sensors are likely to improve monitoring of aquaculture
563 effluents, increasing the amount of relevant information operators and communities have to
564 better manage wastewaters for improved environmental conditions.

565 **5. Conclusions**

566 Salmon aquaculture effluents in Chile are enriched in nutrients compared to their receiving
567 streams, however concentrations of nitrogen and phosphorus in effluents sampled in this study
568 did not exceed current Chilean water quality standards. Tryptophan-like DOM fluorescence
569 measured on filtered samples in the lab covaried linearly with nutrient concentrations in salmon
570 aquaculture effluent suggesting in-situ fluorescence could be a useful indicator of aquaculture
571 effluent water quality. Specific conductivity also varied with effluent nutrient concentrations. A
572 single calibration for all sites between fluorescent DOM and aquaculture-derived nutrients could
573 not be developed suggesting there is no universal, mechanistic relationship between tryptophan-
574 like fluorescence and nutrients that can be applied to all systems. Linear regression statistics for
575 relationships between tryptophan-like DOM fluorescence and nutrients varied among sites likely
576 due to natural variation in stream chemistry and differences among facility design and operation.
577 Continuous fluorescence monitoring would alert operators and regulators to the release of solutes
578 which can occur on timescales not captured during occasional grab sampling. In-situ fluorescent
579 DOM monitoring is likely best supplemented by sampling for traditional laboratory analyses at
580 frequencies appropriate to capture a range of concentration dynamics. The use of continuous in-
581 situ sensors for specific conductivity and protein-like fluorescent DOM would improve effluent

582 monitoring efforts and estimates of the total solute loads contributed to receiving waters by
583 aquaculture facilities.

584

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591 **Data availability**

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593 Stubbins, Aron; Woelfl, Stefan; Nimptsch, Jorge (2021), "Land-based Salmon Aquaculture
594 Effluent Chemistry in Chile", Mendeley Data, V1, doi: 10.17632/v4p2fpbbh3.1

595 Link to preview data release: https://data.mendeley.com/datasets/v4p2fpbbh3/draft?a=a84e70e0-
596 81d7-4e28-bd86-21bf7674ece5

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806 Fluoresc 30(6), 1383-1396.

Figure Captions:

Figure 1: Study Area. (A) Location of Chile in South America. (B) Location of permitted freshwater salmonid aquaculture facilities in Chile (SERNAPESCA 2021). (C) Location of sampled facilities in this study (37˚S to 42˚S). (D) Example of upstream and effluent sampling locations at each facility.

Figure 2. Excitation-emission matrices of PARAFAC model components C1/HumA, C2/HumC, C3/Tryp, and C4/Prot normalized to peak component fluorescence.

Figure 3. Boxplots and jitter plots of the percent enrichment between upstream and effluent waters calculated for each individual sampling event. Points are colored by site number. Boxes depict the median and first and third quartiles. Whiskers extend to the minimum or maximum value no further than 1.5 times the inner quartile range. Only parameters with positive mean paired differences are shown. Values greater than 100,000% enrichment not shown.

Figure 4. PCA and Correlations. Variable loadings of first and second principal components (Dim1 and Dim2) for DOM fluorescence components (C1/HumA, C2/HumC, C3/Tryp, C4/Prot), absorbance coefficient at 254 nm (a254) and specific conductivity (SC) variables included in principal component analysis for (A) upstream samples and (C) effluent samples. Dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) are added as ancillary variables to the PCAs. Matrices of coefficients of determination (r^2) for Pearson's correlations between selected optical and specific conductivity (SC) predictor variables and solute concentrations for (B) upstream samples and (D) effluent samples. Only values for which $r^2 > 0.1$ and $p < 0.05$ are shown. P-values were adjusted for type I errors due to multiple testing using the false-detection rate approach.

Figure 5. Upstream Site-specific Regressions. Linear regressions of dissolved organic carbon (DOC) and soluble reactive phosphorus (PO4) concentrations among selected variables (a254, C2/HumC, and specific conductivity) for upstream samples. Shapes and regression lines are shown only for significant correlations ($p < 0.05$ and $r^2 > 0.1$). Samples with no significant correlation within a site are shown as solid grey points. A linear regression line through all data is shown as a black solid line.

Figure 6. Effluent Site-specific Regressions. Correlations of dissolved organic carbon (DOC), total nitrogen (TN), ammonia (NH4), and total phosphorus (TN) concentration with selected variables (C3/Tryp and specific conductivity) for individual aquaculture facility effluent samples. Shapes and regression lines are shown only for significant correlations ($p < 0.05$ and r^2

> 0.1). Samples with no significant correlation within a site are shown as solid grey points. A linear regression through all data is shown as a black solid line. The x-axis is log-transformed to allow comparison of sites with varied response ranges.

Figure 7. Tryptophan sensor proxy derived from EEMs displayed as tryptophan concentration equivalents versus UviLux sensor tryptophan equivalents for unfiltered archive effluent samples (circles) and field measurements (squares). Black line depicts a 1:1 relationship. Dotted lines depict the least square linear regression.

Table 1. Water Quality Results. Table of water quality results for upstream and effluent samples, the paired differences (effluent - upstream), and the coefficient of variation (CV) of the paired differences across all sites. Results from the paired t-test applied across all sites are shown for each parameter.

	units	Upstream (mean ± standard deviation)	Effluent (mean ± standard deviation)	Paired Difference (mean ± standard deviation)	CV (%)	Paired t-test
T	˚C	12.0±3.0	12.5±3.0	0.5±1.0	194	****
pH	S.U.	7.0±0.6	7.0±0.6	-0.1±0.5	-320	**
DO	mg L^{-1}	10.3±1.1	9.9±1.3	-0.5±1.0	-241	****
Sp. Cond.	µS cm^{-1}	60±24	90±72	25±57	229	****
Turb.	NTU	1.0±0.6	1.5±0.8	0.5±1.0	227	***
DOC	mg-C L^{-1}	1.0±0.8	1.9±1.9	0.9±2.0	227	****
TN	µg-N L^{-1}	261±370	821±674	542±637	118	****
TP	µg-P L^{-1}	50±54	165±129	104±104	100	****
DIN	µg-N L^{-1}	163±277	474±501	312±445	142	****
$N\text{-}NH_4$	µg-N L^{-1}	10±14	228±246	208±230	111	****
$N\text{-}NO_2$	µg-N L^{-1}	1.4±1.6	5.0±6.5	3.5±6.7	191	****
$N\text{-}NO_3$	µg-N L^{-1}	148±274	245±353	98±265	269	***
$P\text{-}PO_4$	µg-P L^{-1}	35±42	106±96	67±78	116	****
a_{254}	m^{-1}	7.7±7.9	7.6±5.2	0.2±6.7	4441	ns
C1	Fmax RU	0.069±0.077	0.072±0.051	0.005±0.064	1280	ns
C2	Fmax RU	0.046±0.052	0.034±0.030	-0.010±0.039	-390	**
C3	Fmax RU	0.020±0.019	0.171±0.337	0.114±0.265	232	****
C4	Fmax RU	0.046±0.060	0.057±0.043	0.012±0.065	542	ns

ns = not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<10^{-4}$