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Tracing dissolved organic matter (DOM) from land-based aquaculture systems in North Patagonian streams

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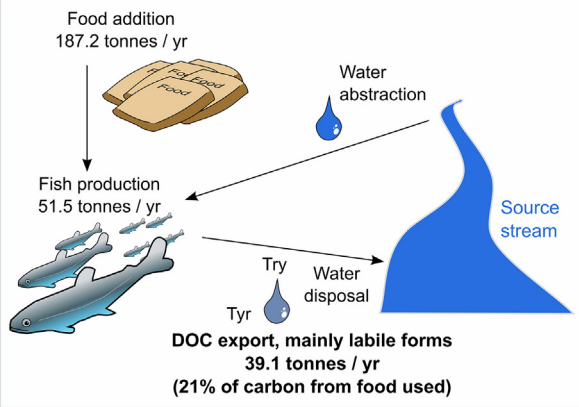
HIGHLIGHTS

- DOM from land-based aquaculture mainly consists of protein-like fluorescence.
- DOM from the fish farm has high potential of impairing stream metabolism.
- DOC export from the fish farm amounted to 21% of food and 76% of fish production.

GRAPHICAL ABSTRACT



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ABSTRACT

Chile is the second largest producer of salmonids worldwide. The first step in the production of salmonids takes place in land-based aquacultures. However, the effects of the discharge from these aquacultures on stream dissolved organic matter (DOM) content, molecular composition and degradability are unknown. The aim of this study was thus to investigate the inputs of anthropogenic DOM from land-based aquaculture to the predominantly pristine river systems of North Patagonia. We hypothesized, that i) DOM exported from land-based aquaculture mainly consists of protein-like fluorescence (tyrosine and tryptophan) released from fish feces and food remains, and that ii) this DOM is highly degradable and therefore rapidly turned-over within the receiving

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Fish farms

streams. In the North Patagonian region we conducted a screening of ten land-based aquacultures and an intensive sampling campaign for one aquaculture. This was combined with longitudinal transects and a degradation experiment in order to couple the composition of DOM exported from land-based aquacultures to its degradability in streams. We measured dissolved organic carbon (DOC) concentration by high-temperature catalytic oxidation and DOM composition by fluorescence spectroscopy and parallel factor analysis. In the effluent of the ten screened aquacultures and in the repeated sampling of one aquaculture, we consistently found an increase of DOC concentrations and a dominance of protein-like fluorescence. The protein-like fluorescence rapidly disappeared downstream of the aquacultures, and in the degradation experiment. 21% of the DOC export from the repeatedly sampled aquaculture resulted from food addition and 76% from fish production. We conclude that large amounts of degradable DOM are exported from land-based aquacultures. This probably has strong effects on the ecological structure and function of North Patagonian streams, and similarly affected streams worldwide.

1. Introduction

Chile is the second largest producer of salmonids worldwide, with an annual production of 818 thousand tons in 2012 (FAO, 2014). The older and larger stages of salmonids are grown in anchored cages in lakes and the ocean, whereas the youngest salmonid stages (fry) are grown in tanks in land-based aquaculture flow-through systems. Discharge from these aquacultures may be responsible for large inputs of dissolved organic matter (DOM) to the mostly pristine river systems of North Patagonia. This may potentially cause a significant decline in water quality not only in the streams themselves, but also in the lakes and sea in close proximity to contaminated stream waters.

The North Patagonian Region in Chile (S39°–41°) possesses ca. 25% of the freshwater resources of the country originated by precipitation (CAPP, 2010). The water quality of freshwater streams in this region is highly suitable for the development of aquaculture activities, as physico-chemical and bacteriological characteristics of these water courses are perfect for the development of this activity (Aatland and Bjerknes, 2009; Castillo and Peña-Cortés, 2012). During the last two decades the annual salmonid production rose from 62,000 tons in 1992 to 818,000 tons in 2012 (FAO, 2014). In 2012, Chilean salmonid production had a value of 4.87 billion USD, which represented 32% of the total worldwide value of salmonid production (FAO, 2014). At present, 70% of the total production of Chilean fry is located in the North Patagonian region, as its pristine waters are highly suitable for the early stage life cycle of salmonids. There are currently 252 land-based aquacultures active in North Patagonia which amounts to 68% of the total number of active land-based aquacultures in Chile (SERNAPESCA, 2014), and new aquaculture activities are pending or projected for 2020. However the decrease of this pristine water quality of North Patagonian streams due to discharge of waste waters in terms of organic loads originated by flow-through and/or recirculating aquaculture systems (RAS) remains unknown.

The main problem of land-based aquaculture sited at streams is the generation of solid and liquid waste (Tello et al., 2010; Rosa et al., 2013). In Chile, wastewater generated by land-based aquaculture is insufficiently treated (i.e. primary treatment only, hence with solid-waste removal), and thus might be categorized as a biological stressor for the receiving stream (e.g. Cripps and Bergheim, 2000; Buschmann and Pizarro, 2001; Ruiz-Zarzuela et al., 2009; Tello et al., 2010). The main pollutants of land-based aquacultures are feces, unconsumed food waste and metabolic sub-products, which enter the rivers as DOM and inorganic matter (i.e. mainly nitrogen, phosphorus and carbon) (Wang et al., 2012). Nitrogen and phosphorus from land-based aquaculture may directly contribute to eutrophication downstream of the outfall, and carbon inputs may alter stream metabolism (Rosa et al., 2013). It has been shown that after passing land-based aquaculture facilities, effluents show elevated levels of ammonium and phosphate, originating from direct excretion and nutrient leaching processes from organic matter and suspended solids (Green et al., 2002; Brinker et al., 2005; Sindilariu, 2007). Xenobiotics such as antibiotics, medical disinfectants and anesthetics are also widely used and are consequently present in the effluent from land-based aquaculture (Tello et al., 2010).

Fluorescence spectroscopic characterization of wastewaters from urban sewage and different farm wastes has been carried out extensively by several authors (Baker, 2001, 2003; Ma et al., 2001; Baker and Inverarity, 2004; and reviewed by Hudson et al. (2007)). Protein-like fluorescence, especially tryptophan-like components, is suggested as pollution tracers in river systems at catchment scale. More recently, Williams et al. (2013) recommended fluorescent DOM as a non-selective tracer for tracking wastewater discharges in freshwater systems. However, the amount and composition of DOM exported from land-based aquaculture to the receiving stream is mostly unknown. To our knowledge there is only one study which measured DOM composition by group classification for the in- and outflow of a single land-based aquaculture and found an increase in tryptophan-like fluorescence DOM (Stedmon et al., 2003). Furthermore it was found that during salmon migration, ecological effects are not only generated due to nutrient enrichment and changes in river metabolism (Tiegs et al., 2009), but that salmon also release mainly tyrosine-like fluorescence DOM when decaying after migrating upstream (Fellman et al., 2008, 2009a). Due to the production of dissolved feces, unconsumed food waste derivatives and metabolic sub-products in aquaculture, it is likely that the increase of tryptophan-like and tyrosine-like fluorescence is a common effect of land-based aquaculture on DOM in receiving streams. Based on studies on the biodegradability of protein-like DOM in streams it is very likely that such DOM output from aquaculture to streams is highly bioavailable (Fellman et al., 2009b). If this is the case, this DOM will strongly impair stream metabolism by increasing bacterial growth and respiration, with further consequences for the stream food web and community composition of species within the food web. However, no comprehensive information on the effect of land-based aquaculture on DOM exists yet in the scientific literature. The effects of land-based aquaculture on in-stream DOM thus remain largely speculative, which effectively prevents the prediction of ecological consequences and any kind of regulation of this environmental pollution.

The aim of this study was to investigate the inputs of anthropogenic DOM from land-based aquaculture to the predominantly pristine river systems of North Patagonia. We hypothesized, that i) the DOM exported from land-based aquaculture mainly consists of protein detectable as (tyrosine and tryptophan)-like fluorescence, due to residual dissolved feces, unconsumed food waste derivatives and metabolic sub-products in the effluent from aquaculture systems, and that ii) this DOM is highly degradable and therefore rapidly decreases within receiving streams.

1.1. Materials & methods

1.1.1. Screening of different salmon aquaculture systems

Ten aquacultures were screened from 13th to 17th of January 2014 in order to assess the general effect of land-based aquaculture on stream DOC concentration and DOM composition. At each fish farm, water samples were taken upstream of the effluent as a control, as well as directly from the effluent. The ten aquaculture systems are situated in the IX and

X region (38°50′32.8″S–71°40′10.6″W to 41°55′30.3″S–72°26′21.8″W) in Southern Chile (Fig. 1, Table S1) and characterized by an annual production of around 80 to 650 tons of salmonids per year, low conductivity and low to moderate nutrient content (Tables 1, S2).

1.1.2. Seasonal DOM variability in aquaculture wastewater

One land-based aquaculture system situated at Molco River was investigated in more detail during the study. The watershed of Molco River is situated near Villarica city (IX Region of Chile) and has a catchment area of 35.52 km^2 (Fig. 1c). It is mainly composed of native forest and presents low anthropogenic influences. The region is characterized by a wet season in winter (~15 rain days/month – April to October) and a dry season in summer (~6 rain days/month – November to March). Molco River is a second order stream with low nutrient content (Table 1) and low to moderate discharges (control site: 0.2–1.1 m^3/s; 4300 m downstream of the effluent: 0.7–3.6 m^3/s). The Molco River has supplied two salmon aquaculture systems for the last decade (Molco Aquaculture and Chosco Aquaculture) (Fig. 1c). Production of salmonid fish in Molco Aquaculture increased by nearly a factor of seven in the last seven years, from 69 t/y (2006) to 460 t/y (2013). During the sampling campaigns there was no production at Chosco Aquaculture, which discharges into Chosco River (0.2–0.3 m^3/s) reaching Molco River 1800 m upstream of the river outfall (Fig. 1c). At Molco Aquaculture, wastewater (discharge: 0.3–0.5 m^3/s) enters Molco River ~4800 m upstream of the river outfall into Lake Villarica (Fig. 1c). Wastewater is pre-treated by a rotation filter followed by flocculation treatment and is finally discharged back into Molco stream.

Repeated sampling was conducted at Molco Aquaculture in order to assess the temporal effects of land-based aquaculture on the DOM in streams. For DOC concentration, samples were measured on six occasions from 15th October 2013 to 26th June 2014, and for DOM composition, samples were measured on eight occasions from 1st August 2013 to 26th June 2014. On two occasions, DOC concentration could not be measured as the DOC analyzer was not available. Samples were taken upstream of the effluent from Molco Aquaculture, directly from the effluent and 4300 m downstream, close to the outflow of Molco stream to Villarica Lake.

1.1.3. Tracking of aquaculture effluent in receiving waters (Molco River)

Water samples were taken at different distances downstream of the sewage outfall from Molco Aquaculture into Molco River on two occasions, in order to investigate changes in DOC concentration and DOM composition along the longitudinal stream profile. The discharge of the control and the effluent was almost similar resulting in dilution factor of the effluent of about 2.1–2.2 (Table 5). Complete mixing of the effluent and the stream water was observed after 60–65 m (see conductivity in Table 1). On the 15th October 2013, eight additional samples were taken from Molco stream, starting 65 m below the effluent of Molco fish farm to 2700 m downstream, and on 24th January 2014 seven additional samples were taken from Molco stream, starting 60 m downstream of the effluent to 2700 m downstream. The dilution factor over the studied stretches (60 to 2700 m) was 1.13 and 1.28 (Table 5).

1.1.4. Field sample processing

On site, three replicates were taken for DOM composition analysis by fluorescence spectroscopy, and two replicates were taken for DOC concentration analysis by high-temperature catalytic oxidation (HTCO) HighTOC Elementar Systems (Hanau, Germany): For the classification of DOM, each sample was taken three times with a 0.5 l conditioned glass beaker and filtered through 0.22 μm Millex sterile PES syringe filters (Merck Millipore, Billerica MA, USA) into three 40 ml amber glass containers (I-Chem 100 Thermo Scientific, Waltham MA, USA). For the DOC analysis, three samples were filtered through pre-conditioned 0.22 μm Millex sterile PES syringe filters (Merck Millipore, Billerica MA, USA) inside the amber glass containers and acidified to pH ~ 2 by addition of 100 μL HCl 37% (Merck). For transport to the laboratory, all samples were immediately cooled with ice water and stored at max.

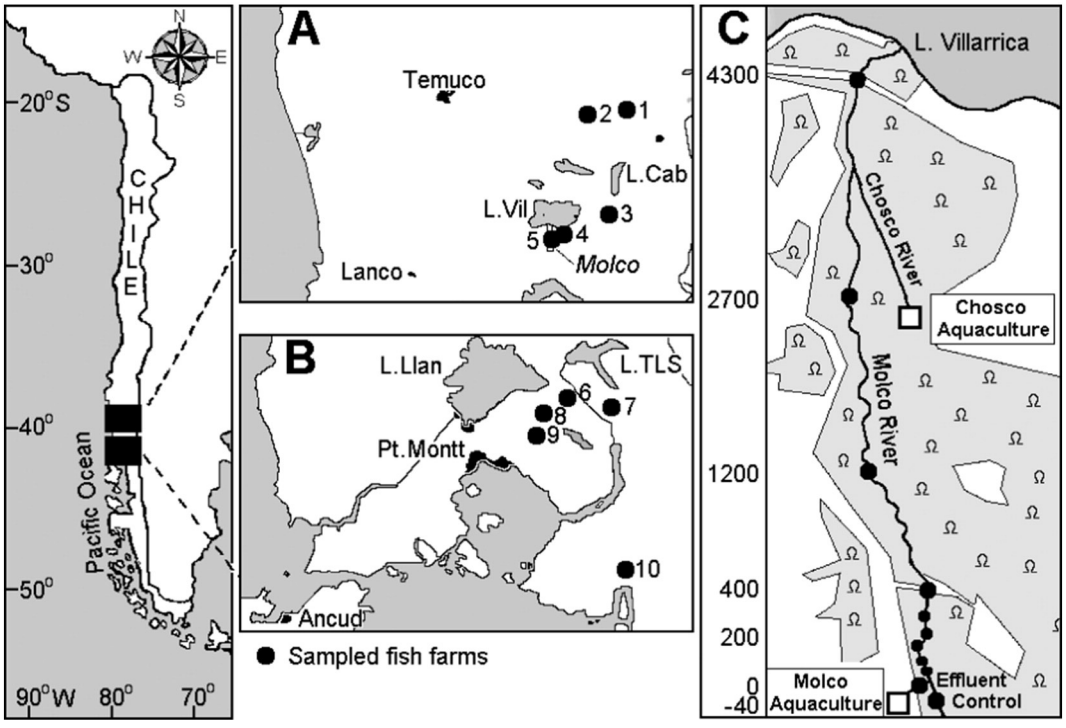

Fig. 1. Location of ten sampled land-based aquaculture systems in Southern Chile. A: IX Región de la Araucania; B: X Región de los Lagos. Important towns and lakes are also indicated: L.Vil = Lake Villarrica; L.Cab = Lake Caburgua; L.Llan = Lake Llanquihue; L.TLS = Lake Todos Los Santos. Corresponding names of land based aquaculture systems: Melipeuco (1); Confluencia (2); Caburgua II (3); Los Chilcos (4); Molco (5); Copihue (6); Lago Verde (7); Cuyamco (8); Hornohuinco (9); Chaqueihua (10). Geographical locations and average annual production are indicated in Table S1. C: Sampling sites (.) at River Molco, numbers correspond to distance from the Molco Aquaculture effluent outflow.

Table 1
DOC, discharge and water quality data at different sample stations (control: −40 m; effluent: 0 m; 1200 and 2700 m distance from effluent) in Molco river. SRP: soluble reactive phosphorous, TP: total phosphorous, TN: total nitrogen; n.d.: not determined.

Date	Distance from effluent [m]	DOC (mg/L)	Discharge (L/s)	T [°C]	Conductivity [μS/cm]	pH	Oxygen [mg/L]	Turbidity [ntu]	SRP μg/L	TP μg/L	N-NH4 μg/L	N-NO2 μg/L	N-NO3 μg/L	TN μg/L
01-08-2013	0	5.4	318	9.2	790.0	7.1	11.4	n.d.	295.3	578.4	453.4	3.9	445.4	1996
15-10-2013	−40	0.5	371	8.5	51.5	6.9	11.3	0.4	51.7	58.9	5.6	<2	46.3	79.4
	0	5.2	332	11.1	164.7	6.6	10.4	2.0	350.2	461.8	439.8	4.5	237.3	1993
	60	3.3	n.d.	n.d.	88.7	7.3.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	400	1.7	633	9.5	87.4	7.4	11.0	1.0	194.0	195.0	127.9	3.5	237.3	610.1
	1200	1.1	n.d.	9.9	93.7	7.1	10.7	0.5	147.8	200.9	184.0	10.4	135.0	698
	2700	1.6	n.d.	10.2	92.7	7.1	10.8	1.2	133.8	144.6	52.6	11.1	162.3	442
05-11-2013	−40	0.5	n.d.	13.9	50.0	6.9	9.2	0.5	46.1	52.9	7.7	<2	41.3	66.3
	0	2.1	440	16.1	136.8	6.6	8.4	1.1	242.3	296.3	846.4	7.8	553.5	1563
26-11-2013	−40	0.5	621	7.9	49.9	6.6	11.3	0.1	61.1	48.1	9.9	<2	35.3	62.1
	0	7.2	151	9.6	311.0	6.5	10.7	5.5	411.9	435.6	902.6	15.3	972	3270
12-12-2013	−40	0.5	244	8.7	71.0	7.3	11.0	0.6	55.9	56.1	12.0	<2	35.9	64.2
	0	5.4	177	13.0	210.5	7.5	0.0	1.7	381.2	459.2	1640.5	10.0	864	3243
23-01-2014	−40	0.9	191	8.1	51.3	6.7	11.1	0.7	47.1	90.0	3.1	<2	<2	62.2
	0	1.6	193	8.4	206.3	6.7	10.9	1.2	184.5	220.9	583.5	17.9	1487	2559
24-01-2014	−40	0.9	197	10.2	52.1	6.8	10.5	0.3	53.4	63.5	<2	<2	<2	59.8
	0	3.8	164	11.2	1070.0	6.9	10.5	7.9	536.3	806.5	930.0	53.3	8528	11,236
	65	n.d.	n.d.	10.0.	533	7.06	9.57	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	400	1.7	445	10.0	486.0	7.4	10.8	1.5	179.4	217.4	402.9	23.6	4148	4824
	2700	1.3	n.d.	10.9	399.0	7.4	10.7	0.4	162.9	190.9	163.4	55.7	3120	3396
26-06-2014	−40	0.9	1069	7.3	57.8	6.3	11.6	0.4	57.1	58.7	<2	<2	74.8	109.0
	0	10.8	508	7.5	160.7	6.2	11.7	4.9	333.9	486.6	365.3	10.9	712	2061

4 °C. DOC concentration measurements and DOM spectroscopy were performed within 48 h of sampling.

1.1.5. DOM degradation experiment

For the DOM degradation experiment, 400 ml of an unfiltered water sample from the Molco Aquaculture effluent was taken and incubated at 10 °C in the laboratory in a covered beaker. Sub-samples were taken at the start, hourly for the first six hours and subsequently at 24 and 48 h. At each sampling time three sampling replicates were taken separately and filtered with 0.22 μm Millex sterile PES syringe filters (Merck Millipore, Billerica, MA, USA). Fluorescence measurements were performed immediately afterwards.

1.1.6. DOM measurements

DOM composition in the sampling replicates was measured by fluorescence spectroscopy with a Varian Eclipse fluorescence spectrometer (Santa Clara, CA, USA) and a Merck Pharo Spectroquant 300 (Darmstadt, Germany) absorbance spectrometer. Briefly, Excitation-Emission-Matrices (EEM) fluorescence scanning was achieved with excitation from 240 to 450 nm (5 nm steps) and emission from 300 to 600 nm (2 nm steps) with a slit width of 5 nm, and absorbance was scanned from 190 to 800 nm. Both absorbance and fluorescence were measured at room temperature (25 °C) and corrected by CHROMASOLV LC–MS grade (MERCK, Germany) water blanks. The absorbance measurements were done to correct for the inner-filter effect (Lawaetz and Stedmon, 2009) but were not used for further data analysis, further details are described in Nimptsch et al. (2014). Two of the sampling replicates of each sample were used to determine the DOC concentration by HTCO (HighTOC Elementar Systems, Hanau, Germany) in Valdivia and one was kept as backup. Samples from Molco River from one date (26th November 2013) were measured by HTCO HighTOC Elementar Systems (Hanau, Germany) at the Helmholtz Centre of Environmental Research (Germany) as the instrument in Valdivia was damaged at this time. Parallel measurements with different sample storage times revealed a good agreement between the two instruments (12% deviation). The limits of detection (LOD) for the HTCO in Valdivia and Germany were 0.1 and 0.5 mg C L^{-1} respectively.

1.1.7. Data processing

Before the fluorescence data was modeled by parallel factor analysis, it was pre-treated with the FDOMcorr toolbox to account for the inner-filter effect and wavelength dependent sensitivity of the fluorescence (Murphy et al., 2010). The fluorescence was normalized by the area under the Raman peak at 350 nm and is therefore expressed in Raman units (Lawaetz and Stedmon, 2009). We interpreted the component spectra based on the existing literature and entries in the OpenFluor database (Murphy et al., 2014).

Two parallel factor analysis (PARAFAC) models were generated with the DOMFluor toolbox (Stedmon and Bro, 2008) from the data: 1) a PARAFAC model for the screening of all ten aquacultures and 2) a PARAFAC model exclusively for the data from Molco River. This was done in order to investigate whether the same or similar components would be found in the two models. The sampling replicates were used separately in the generation of the PARAFAC models. This resulted in 60 samples for the PARAFAC model of the ten aquacultures, which in addition to the 6 unregularly measured sites, resulted in 272 samples for the PARAFAC model of Molco River. Due to the irregularity of sampling, samples from the additional sites at Molco River were not used for assessment of the effect of Molco Aquaculture. The PARAFAC models were validated by assessing randomness of the residual fluorescence, split-half validation and random initialization. Based on the PARAFAC models and the pre-treatment steps, the sample-specific maximum fluorescence (Fmax) of each component in Raman units was used for further data analysis.

1.1.8. Data analysis

In all data analyses the averages of the sampling replicates were used for each sample for both the field sampling campaigns and the DOM degradation experiment.

In order to assess the effect of the aquaculture effluent on DOC concentration and the Fmax of the PARAFAC components in the screening of the ten aquacultures, the control and the aquaculture effluent were compared by a Monte Carlo test with the aquaculture system as a block, and with 9999 iterations (oneway_test function, coin package, Hothorn et al., 2006; Hollander et al., 2013) in R (version 3.1.2, R Core Team, 2014). A Nemenyi test was used to test if the differences of the effluent to the control differ between the PARAFAC components (adapted oneway_test function, Hollander et al., 2013).

In order to assess the effect of the aquaculture effluent on DOC concentration and the Fmax of the PARAFAC components for Molco Aquaculture, the control and the aquaculture effluent were compared

by a Monte Carlo test with the sampling occasion as a block, and with 9999 iterations (oneway_test function, coin package) in R.

In both of the above cases Monte Carlo tests were applied as the data were not normally distributed.

A Spearman rank correlation (cor.test function in R, Hollander et al., 2013) with the distance from the effluent site was carried out in order to prove that changes in DOC concentration or the Fmax of the PARAFAC components occurred along the flow path of Molco River. A linear model could not be applied due to missing variance homogeneity of the residuals.

A linear model (lm function in R) was applied to the measurements from hour 0 to hour 6 ($n = 7$) for each of the PARAFAC components in order to investigate if significant disappearance over time occurred for the Fmax signal of the PARAFAC components during the DOM degradation experiment.

1.1.9. Estimation of the release of DOC from the Molco Aquaculture

The maximum annual fish production of 460 t wet weight per year for Molco Aquaculture was converted into carbon units by multiplying with the fish dry weight:wet weight ratio (0.236), and the fish carbon:dry weight ratio (0.475; Table 2). Food addition in carbon units was based on the dry weight of fish production (calculated as fish production per year multiplied by the fish dry weight:wet weight ratio), divided by the growth efficiency (0.292) and converted to carbon (0.504). The DOC export per year from Molco Aquaculture was calculated based on the increase in DOC concentration in the effluent relative to the control and multiplied by the average effluent discharge. The resulting DOC export was converted from mg/s to t/y. In addition to expressing the estimated DOC export from Molco Aquaculture to the stream per year in total numbers, it was also expressed in percentages relative to the fish produced or relative to the food used for fish production for the same period of time. In order to estimate the uncertainties of the budget all steps were calculated by a Monte Carlo simulation in R (R core team, 2015) using the literature-based standard deviations given in Table 2 and applying 10,000,000 iterations. We used the 25th and 75th percentiles of the final distributions from the Monte Carlo simulations in order to calculate the interquartile range as an estimate of the uncertainties in the budget due to the uncertainties in the input data.

2. Results

2.1. PARAFAC models

A three-component model was established based on the data from the ten aquacultures, and a five-component model was established based on the samples from Molco River. The 3-component model revealed components generally similar to the 5-component model, except for component 5 of the 5-component model (Table 3). The model components were named based on their spectral characteristics and similarity (Table 3). Component 1 from the 3-component model, and components 1 and 3 from the 5-component model resemble tryptophan-like fluorescence (Try/Try2, Tables 3 and 4). Component 2 from the 3-component and the 5-component model resembles tyrosine-like fluorescence (Tyr, Tables 3 and 4). Component 3 from the 3-component model and component 4 from the 5-component model resemble a humic-like fluorescence peak (HS, Tables 3 and 4). Finally, component 5 of the 5-component model resembles another humic-like fluorescence peak (HS2, Tables 3 and 4), and there is no corresponding component in the 3-component model. The given abbreviations will be used throughout the results for better comparability of the two models and easier assessment of the data. Further details on results of OpenFluor database query results are given in Table 4.

Table 2
Parameters used for the calculation of carbon fluxes in the Molco River Aquaculture System (Fig. 7) and ranges used for a Monte Carlo simulation. Fish-based increase in DOC concentration and effluent discharge are averages of 6 and 11 measurements, respectively. The ranges represent the standard deviation of the mean.

Item	Value ± SD	Source
Food carbon:dry weight ratio	0.504 ± 0.0057	Petersen et al. (2005); pellets 3.5 & 5 mm
Fish carbon:dry weight ratio	0.475 ± 0.0023	Lyle and Elliott (1998); pre-smolt
Fish dry weight:wet weight ratio	0.236 ± 0.0026	Lyle and Elliott (1998); pre-smolt
Fish growth efficiency	0.292 ± 0.002	Mundheim et al. (2004); diet 1 (high-quality diet)
Fish production ($t\ y^{-1}$)	460	Operating company
DOC increase ($mg\ L^{-1}$)	4.97 ± 1.20	This study
Discharge effluent ($L\ s^{-1}$)	277.4 ± 32.6	

Table 3
Spectral characteristics of the PARAFAC model components. Names in brackets are the component designations used throughout the results and discussion.

3 component model			5 component model		
Component	Excitation (nm)	Emission (nm)	Component	Excitation (nm)	Emission (nm)
C1 (Try)	280	350	C1 (Try)	280	346
			C3 (Try2)	270 (<240)	336
C2 (Tyr)	275	309	C2 (Tyr)	275	311
C3 (HS)	<240 (290, 335)	428	C4 (HS)	<240 (345)	434
–	–	–	C5 (HS2)	<240 (295–320)	384

Table 4
Results of Open Fluor data base query (www.openfluor.org, Murphy et al., 2014). Minimum similarity score 0.95.

Component	Excitation and emission (nm) maximum	Component author	Reference
3 component model			
C1 (Try)	Ex 280, Em 352.5	C7	Stedmon and Markager (2005a,b)
		C1	Brym et al. (2014)
C2 (Tyr)	Ex 275, Em 309.5	C7	Murphy et al. (2011)
		C4	Murphy et al. (2011)
		C4	Yamashita et al. (2011)
		C3	Yamashita et al. (2013)
C3 (HS)	Ex 240, Em 428.5	C1	Yamashita et al. (2011)
		C1	Walker et al. (2009)
		C1	Shutova et al. (2014)
		C1	Bianchi et al. (2014)
5 component model			
C1 (Try)	Ex 280, Em 346.5	C5	Cawley et al. (2012)
		C5	Osburn et al. (2012)
		C1	Brym et al. (2014)
C2 (Tyr)	Ex 275, Em 311	C7	Murphy et al. (2011)
		C4	Yamashita et al. (2011)
		C3	Yamashita et al. (2013)
C3 (Try2)	Ex 270, Em 336	C6	Murphy et al. (2006)
		C7	Yamashita et al. (2010a,b)
		C6	Kothawala et al. (2014)
		C5	Cawley et al. (2012)
		C6	Lapierre and del Giorgio (2014)
C4 (HS)	Ex 240, Em 434	C1	Murphy et al. (2014)
		C1	Yamashita et al. (2010a,b)
		C1	Stedmon et al. (2007)
		C1	Jørgensen et al. (2011)
		C4	Stedmon and Markager (2005a,b)
C5 (HS2)	Ex 240, Em 384.5	C2	Osburn et al. (2011)
		C4	Jørgensen et al. (2011)

2.2. General effect of land-based aquaculture systems on stream DOM

For the ten investigated land-based aquacultures DOC concentrations were significantly raised in the effluent compared to the upstream control (Fig. 2a). A significant increase was also found in two of the three PARAFAC components, only HS was not significantly increased (Fig. 2b). The differences between the effluent and the control were significantly higher for Tyr than for HS, and intermediate for Try (Fig. 2b).

The DOC concentration in the effluent was significantly increased relative to the control site for the repeated investigation of the Molco Aquaculture at Molco River (Fig. 3a). The same was true for all PARAFAC components (Fig. 3b). The increase differed however between the components and was significantly higher for Try than for Try2 or HS2 (Fig. 3b). Moreover, the effect was stronger for Tyr than for Try2, HS or HS2 (Fig. 3b).

2.3. Development of DOM along the Molco River flow path

DOC concentration declined significantly on both the 24th January 2014 (Spearman rank correlation, rho = −0.96, p = 0.003), and on 15th October 2013 (rho = −0.91, p = 0.003; Fig. 4) along the Molco River flow path. On both dates DOC concentrations below the effluent site were higher than the baseline (DOC concentration at the upstream control site, Fig. 4).

As for the DOC concentration, Try and Tyr declined significantly on both 15th October 2013 (Spearman rank correlation, Try: rho = −0.83, p = 0.015; Tyr: rho = −0.78, p = 0.028) and on 24th January 2014 (Try: rho = −1.00, p < 0.001; Tyr: rho = −0.96, p = 0.003; Fig. 5). None of the other PARAFAC components declined significantly on either of the sampling dates (rho < 0.62, p > 0.05; Fig. 5). At the end of the sampled river section, Try and Tyr approached the baseline (Fmax at the upstream control site) on 15th October 2013, whereas the other components kept an Fmax above the baseline (Fig. 5). On 24th January 2014 only Tyr decreased to the baseline whereas Try and the other compounds increased to higher values. Both the decrease of protein-like FDOM signals (Try and Tyr) and the DOC concentrations along the stream path were much higher than the theoretical estimates (Table 5). The protein-like signals amounted to only 5–24% of the expected value at 2700 m downstream from the mixing zone, whereas the humic-like signals and DOC decreased only to 74–93% and 67–74% of the expected value, respectively (Table 5).

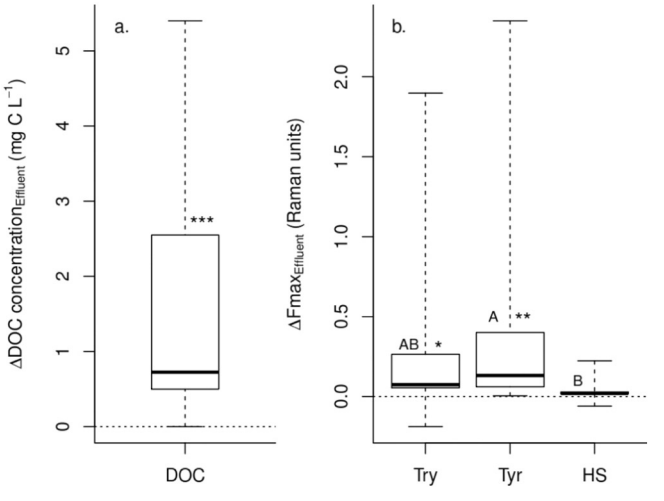

Fig. 2. DOC concentration (mg C L^{-1}) and the Fmax values (Raman units) of the PARAFAC components in the Molco Aquaculture effluent relative to the control. The horizontal dotted line indicates zero difference to the control, and the single measurements of effluent and control were taken at ten different aquacultures (n = 10). The asterisks indicate significant differences in either DOC concentration or PARAFAC component Fmax between effluent and control (Monte Carlo test, with aquaculture as a block, * p = 0.01–0.05, ** p = 0.001–0.01, *** p < 0.001). The capital letters indicate a significantly different group for the effect of the aquaculture on the Fmax of the PARAFAC components (Nemenyi test). The letters differ, if p < 0.05.

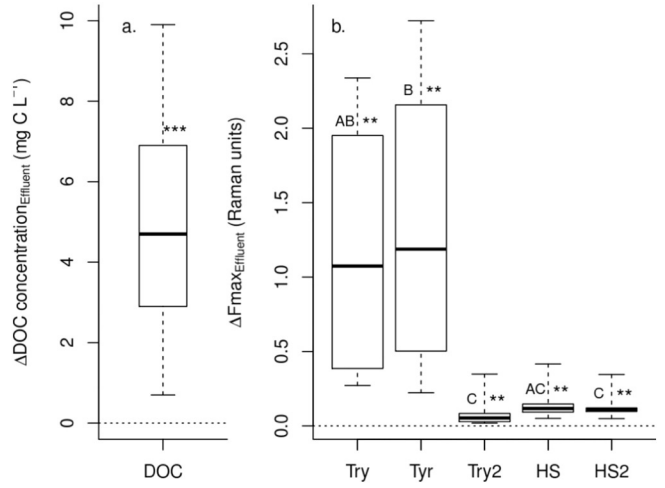

Fig. 3. DOC concentration (mg C L^{-1}) and the Fmax values (Raman units) of the PARAFAC components in Molco Aquaculture effluent relative to the control. The horizontal dotted line indicates zero difference to the control, and the single measurements are different sampling dates at the same sites (DOC concentration: n = 6; Fmax of the components: n = 8). The asterisks indicate significant differences of either DOC concentration or PARAFAC component Fmax values between effluent and control (Monte Carlo test, with sample date as block, * p = 0.01–0.05, ** p = 0.001–0.01, *** p < 0.001). The capital letters indicate a significantly different group for the effect of the aquaculture on the Fmax values of the PARAFAC components (Nemenyi test). The letters differ, if p < 0.05.

2.4. DOM degradation experiment

As a result of the DOM degradation experiment declines could be observed for both Try and Tyr but not for any other component (Fig. 6). This observed difference was supported by linear models for the first seven sampling times with a significant slope for Try (slope = −0.065 (±0.007 SE) Raman Units h^{-1}, R^2 = 0.94, p < 0.001) and Tyr (slope = −0.117 (±0.009 SE) Raman Units h^{-1}, R^2 = 0.97, p < 0.001), but with no significant slope for any other

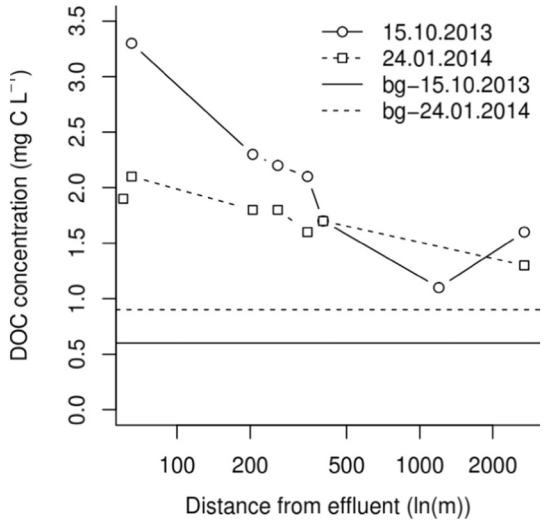

Fig. 4. DOC concentrations at different distances from the Molco Aquaculture effluent outflow for two sampling dates. The background (bg) is the DOC concentration at the control site above Molco Aquaculture effluent outflow for each of the two sampling dates.

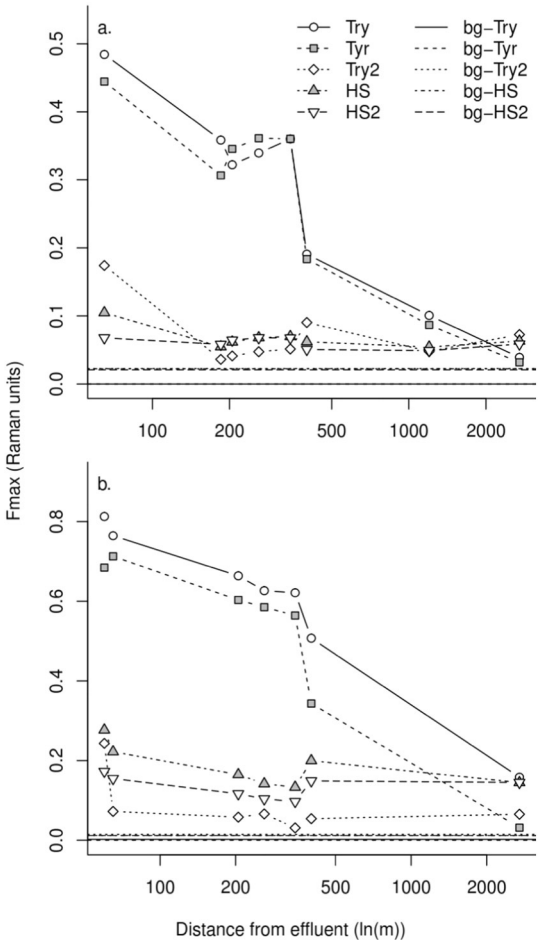

Fig. 5. Fmax values (Raman units) of the PARAFAC components at different distances to the effluent site at Molco Aquaculture for two sampling dates on 15th October 2013 (a) and 24th January 2014 (b). The background (bg) is the Fmax at the control site upstream of Molco Aquaculture for each of the two sampling dates.

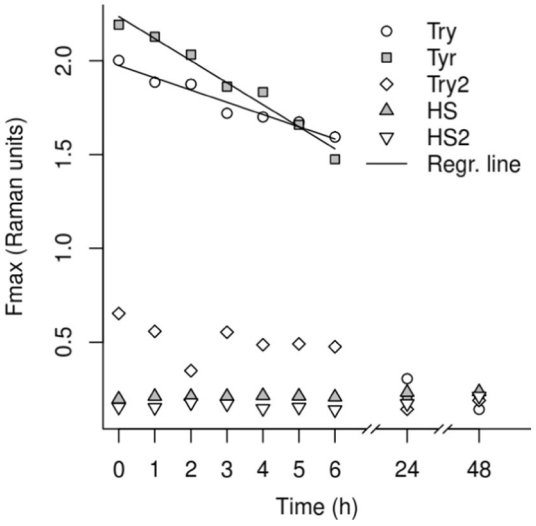

Fig. 6. The Fmax values (Raman units) of the PARAFAC components after different sampling times (hours) in the degradation experiment. Regression lines (Regr. line) are shown if the linear model of the regression exhibited a significant slope ($p < 0.05$, $n = 7$, see text for further details) for the first seven sampling times.

component ($R^2 < 0.10$, $p > 0.05$; Fig. 6). After 24 h, Tyr had already reached a final concentration whereas Try still decreased between 24 h and 48 h.

2.5. Quantitative importance of the DOC release from Molco Aquaculture

A quantitative estimation of carbon fluxes in the aquaculture of Molco River is given in Fig. 7. Fish production amounted to ~ 52 t C y^{-1} and showed low variability due to the low SD ranges of the ratios used for the calculation (Table 2). The food addition required to realize that production was 187 t C y^{-1} and varied little too. In contrast, the increase in DOC concentration in the river due to the fish farm and the discharge of effluent showed a higher variability (Table 2) resulting in a wide range of DOC export (20–62 t C y^{-1}). Consequently, ranges were also high when DOC export was related to food addition and fish production. The DOC export from the aquaculture amounted to 20.9% (10.8–33.2%; 25th and 75th percentile of the Monte Carlo distribution) from food addition and 75.9% (39.4–120.7%) from fish production.

3. Discussion

3.1. Interpretation of the PARAFAC components

Tryptophan-like (Try/Try2) and tyrosine-like fluorescence (Tyr) together dominated the fluorescence EEM's in all studied samples from the aquaculture effluents and river water downstream of the fish farm outflows. This finding is in accordance with literature to date on this topic (see review Fellman et al. (2010)). Peaks similar to Try/Try2 (Osburn et al., 2012) and to Tyr (Stedmon and Markager, 2005a,b) were also shown to be related to the aquatic production of DOM and were highly biodegradable (Fellman et al., 2009b).

In this study, we also found two different humic-like fluorescence components, HS and HS2 (Fellman et al., 2010). According to the literature these may have two different origins: HS is a more common component and is likely of terrestrial origin (Stedmon et al., 2007; Walker et al., 2009, Table 4). HS2 is less commonly found in studies on aquatic DOM and probably has a lower molecular weight, being of microbial origin (Fellman et al., 2010; Osburn et al., 2011, Table 4).

Table 5
Flowrate, theoretical values after mixing of effluent and stream water and measured values of electrical conductivity (EC), DOC and different FDOM components at control site, effluent and after 2700 m. The expected value after mixing was calculated according to the flow rate of the control site and the effluent and EC values. The percentage of measured values at 2700 m compared to expected value after mixing is given in parenthesis. n.d.: not determined.

Date	Site/value	Q (L/s)	EC (μS/cm)	DOC (mg/L)	FDOM Try (R.U.)	FDOM Tyr (R.U.)
15.10.2013	Control	371	52	0.5	0.00	0.00
	Effluent	332	165	5.2	0.92	1.03
	Expected value after mixing	703	105	2.7	0.44	0.49
	measured at 2700 m	n.d.	93 (89%)	1.6 (59%)	0.04 (10%)	0.03 (7%)
24.01.2014	Control	197	52	0.9	0.00	0.00
	Effluent	164	1070	3.9	1.86	1.74
	Expected value after mixing	361	515	2.26	0.84	0.79
	Measured at 2700 m	n.d.	399 (78%)	1.3 (58%)	0.16 (19%)	0.032 (4%)

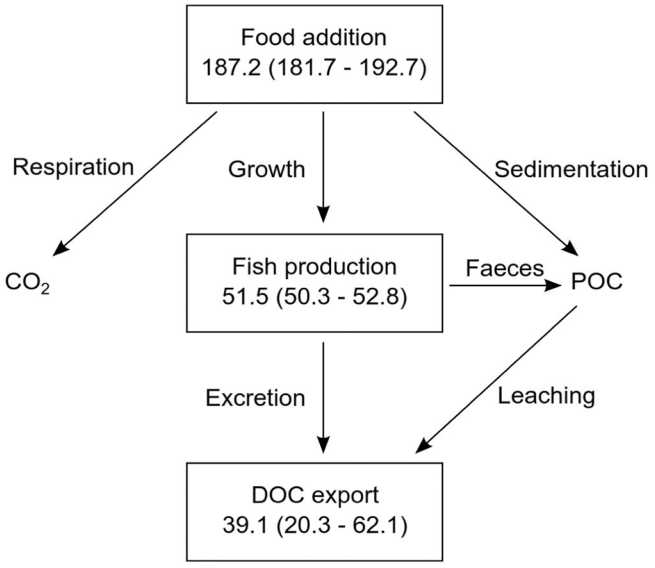

Fig. 7. Carbon fluxes (tons per year) in Molco Aquaculture on the Molco River. Ranges represent the 25% and 75% percentiles of the distributions of a Monte Carlo simulation with 10,000,000 iterations (see text).

3.2. General effects and fate of DOM from land-based aquaculture systems in streams

Based on the screening of the ten land-based aquacultures and the repeated sampling campaign at Molco Aquaculture and Molco River, we consistently found a significant increase in DOC concentrations, mainly due to the significant increase of protein-like DOM in and after the outflow of the salmon farm. This fully supports our first hypothesis. With the two largely independent PARAFAC models, we could also prove that the DOM exported from the studied land-based aquaculture systems has consistently characteristic fluorescence spectra, implying a constant and similar source of DOM within the aquacultures. This finding is in accordance to literature where FDOM components, especially tryptophan, have been shown to be a suitable tracer of wastewater from anthropogenic sources (Baker, 2001; Baker et al., 2003; Williams et al., 2013). However, our results showed that the predominant protein-like FDOM in fish farm effluents corresponds to tyrosine-like signals. The source of this DOM can be multiple: derivatives of unconsumed feed, metabolic sub-products (Wang et al., 2012) and the effects of further waste water treatment. We assume a combination of these possible sources and that primary waste water treatment is likely insufficient for removing DOM from the effluent of aquacultures. With regards to the fate of the protein-like components, we observed a decrease along the longitudinal transects in the Molco River (see Table 5) and in the degradation experiment, whereas the humic-like components remained relatively unchanged, supporting our second hypothesis, that protein-like DOM from aquacultures is highly degradable and therefore rapidly processed within streams. In the degradation experiment, we did not observe the formation of flocs in unfiltered samples of the effluent over the experimental duration of 48 h (data not shown). Thus, the fluorescence signal was probably not affected by flocs formed during the incubation. Furthermore, the concentration of particulate organic carbon was much lower than that of DOC so that a small change in POC would have negligible consequences for DOC. Finally, we clearly observed the decrease of protein-like components whereas humic-like components did not changed significantly over time.

The high degradation of the protein-like components in comparison to humic-like components is supported by the literature (Fellman et al., 2009b). Furthermore, in nutrient spiraling experiments, DOM uptake velocities were positively related to the contribution of protein-like fluorescence to sample fluorescence (Fellman et al., 2009b). This is in good agreement with the development of the protein-like fluorescence and DOC concentration in our longitudinal transects and makes it likely that high uptake rates of DOC are a common phenomenon downstream of aquacultures.

The degradation of protein-like DOM in Molco River (Fig. 5) was much higher than in the degradation experiment (Fig. 6). Specifically, protein-like DOM was degraded within 2 h in the Molco River but within 24 h in the experiments. This might be explained by the fact that degradation in the stream was performed by both planktonic bacteria in stream water and by epilithic biofilm bacteria on the river bed, whereas in the degradation experiment DOM was only degraded by planktonic bacteria. Moreover, bacterial biomass production of biofilm bacteria was shown to be about tenfold higher than that of planktonic bacteria (Kamjunke et al. unpublished data). These results are supported by previous studies, where bacteria downstream of fish farm outflows showed much higher densities and heterotrophic activity due to the accumulation of generated organic matter (Boaventura et al., 1997; Carr and Goulder, 1990). Moreover, the presence of occasionally large mats of sewage fungus and bacteria downstream of the Molco Aquaculture effluent reinforces that the enhanced DOM concentration downstream of Molco Aquaculture (and the other studied fish farms) also increases the microbial respiration of the receiving stream, as clearly shown by Doughty and McPhail (1995) for similar Scottish aquaculture systems.

Photooxidation might also play an important role for DOM degradation. For example, Mostofa et al. (2007) suggest that fulvic acid such as FDOM is more photolabile than protein-like substances and Mayer et al. (1999) found tryptophan-like components being more susceptible to photo-degradation than tyrosine-like components. Osburn et al. (2014) also suggested that humic regions of fluorescent DOM are more susceptible to photo-degradation than other DOM components. However, Larson et al. (2007) in studies of DOM photo-degradation in forested streams, evidenced that photo-degradation of DOM was less important in streams than DOM-processing by heterotrophic uptake. This might be the case in the Molco River, where photo-degradation is likely of less importance, as dense forests and riparian vegetation cover and shade sunlight irradiation along the fluvial axis and the average resident time of the stream water is less than 2 h.

Taking the above studies into consideration, together with the fact that organic matter breakdown and ecosystem metabolism are intimately linked to ecosystem health (Young et al., 2008), we assume that this anthropogenic DOM input strongly impairs stream ecosystem health. It is also likely that the stream food web is no longer fuelled by a mixture of relatively refractory allochthonous and more labile autochthonous organic matter, instead being largely supported by highly labile allochthonous DOM from anthropogenic origin. This may result in a complete shift of species in all large groups when comparing stretches up- and downstream of land-based aquacultures. This biotic effect has been documented by Doughty and McPhail (1995), showing changes in benthic invertebrate communities downstream of aquaculture effluents in fluvial ecosystems.

Nutrient enrichment and subsidies generated by migrating pacific salmon in streams have been documented by Tiegs et al. (2009) and Levi et al. (2013) among others. By combining manipulative and observational studies a highly significant correlation between the abundance of salmon and dissolved nutrients was found. A significant disturbance to benthic biota was also suggested. However, the above-mentioned studies only show potential effects on stream disturbances during the migration of salmon where pulse effects are recorded (i.e. during the spawning period of fish). In contrast, our studies are focused on the continuous inputs of nutrients and organic matter into streams, and our results thus show similar but stronger effects in streams subjected to land-based aquaculture systems with an emphasis on subsidies of DOC and DOM.

3.3. Quantitative estimation of DOC release and its importance

A considerable amount of the food and fish production was exported to the stream as DOC. This implies that the retention of carbon in the aquaculture system is very limited and that measures such as secondary water treatment and recirculation of the water within the aquaculture can significantly reduce DOM loss. However, no studies have yet addressed the effects of such measures on the reduction of the DOM loss from land-based aquaculture, and these remain to be investigated. The budget sometimes included and exhibited large errors for the estimations, which were a result of uncertainties in the input data. Here the error was higher for the amount of DOC exported from Molco Aquaculture relative to food production than for the amount of DOC exported relative to fish production. The reason was that food production was calculated based on fish production, which made more calculation steps necessary and therefore resulted in a higher accumulated error. However, a considerable part of the fish and food production was transported as DOC to the stream, even when taking these errors into account. Considering the rapid increase in aquaculture industry in Chile and worldwide, the monitoring of aquaculture effluents and their impact on receiving water bodies is mandatory. In the case of Southern Chile, this is crucial as most streams discharge to oligotrophic lakes which are highly vulnerable to contaminants causing eutrophication (Castillo and Peña-Cortés, 2012).

4. Conclusions

We found clear evidence that DOM from land-based salmon aquaculture systems is an important source of organic matter in some North Patagonian streams. A strong increase of protein-like fluorescence was found at the discharge point, consisting mainly of highly degradable organic matter. Based on our results, it is likely that strong effects exist for stream ecosystems downstream of land-based aquacultures. We conclude that the adverse effects of land-based aquaculture systems can be strongly reduced by additional water treatment. This would reduce the negative effects on the ecosystem, while keeping the positive effects such as labor and economic welfare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.07.160.

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