Environmental Pollution 174 (2013) 204–213

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Impacts of fish farm pollution on ecosystem structure and function of tropical headwater streams

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A R T I C L E I N F O

Article history:
Received 25 July 2012
Received in revised form
3 November 2012
Accepted 28 November 2012

Keywords:
Aquaculture
Eutrophication
Nutrient spiraling
Gross primary production
Hydrodynamics
OTIS-P

A B S T R A C T

We investigated the impacts of effluent discharge from small flow-through fish farms on stream water characteristics, the benthic invertebrate community, whole-system nitrate uptake, and ecosystem metabolism of three tropical headwater streams in southeastern Brazil. Effluents were moderately, i.e. up to 20-fold enriched in particulate organic matter (POM) and inorganic nutrients in comparison to stream water at reference sites. Due to high dilution with stream water, effluent discharge resulted in up to 2.0-fold increases in stream water POM and up to 1.8-fold increases in inorganic nutrients only. Moderate impacts on the benthic invertebrate community were detected at one stream only. There was no consistent pattern of effluent impact on whole-stream nitrate uptake. Ecosystem metabolism, however, was clearly affected by effluent discharge. Stream reaches impacted by effluents exhibited significantly increased community respiration and primary productivity, stressing the importance of ecologically sound best management practices for small fish farms in the tropics.

1. Introduction

River biodiversity and human water security are highly threatened in heavily populated regions of the world (Dudgeon et al., 2006; Vörösmarty et al., 2010). While threats to water security are more severe in developing countries due to lower investments in water infrastructure, river biodiversity is globally threatened as a result of limited or ineffective conservation efforts (Vörösmarty et al., 2010). In addition to human water security and biodiversity, modern river restoration and conservation should focus on ecosystem processes that generate ecosystem services, i.e. the benefits humans derive from ecosystems (Palmer and Filoso, 2009), such as gas regulation, nutrient cycling, natural waste treatment, food production and recreation (Costanza et al., 1997).

A variety of human pressures related to catchment disturbance, pollution, water resource development and direct biotic factors, such as invasive species, fishing and aquaculture, poses threats to the integrity of running waters (Allan, 2004; Paul and Meyer, 2001; Vörösmarty et al., 2010). Stressors related to catchment disturbance, pollution, and water resource development are often spatially correlated and their spatial concordance causes the severest impacts on a global scale (Vörösmarty et al., 2010). Direct biotic stressors, however, tend to be spatially decoupled from human population density, and thus from the aforementioned stressor combinations. Accordingly, direct biotic stressors, including aquaculture, are more likely to affect pristine running waters. Indeed, aquaculture impacts on the water quality of pristine streams have been reported (Beveridge and Phillips, 1993; Boaventura et al., 1997).

The production of food fish from aquaculture has increased at a mean annual rate of 8.3% between 1970 and 2008 and has reached a production of 30.5 million tons per year in 2008 (FAO, 2010). Latin America and the Caribbean have exhibited the highest annual growth rates of all regions, averaging about 21%. With 55% of the produced quantity and 41% of the produced value, freshwater fish were the most important group of aquaculture products in 2008 (FAO, 2010). Freshwater fish are commonly produced in fish ponds and tanks, or fish cages located in the surface waters of larger water bodies (Naylor et al., 2000). However, freshwater fish production may lead to environmental impacts in adjacent waters, such as eutrophication and organic carbon pollution (Schindler, 2006; Smith, 2003), the introduction of exotic species, parasites and pathogens (McVicar, 1997; Pérez et al., 2003), as well as the introduction of harmful substances, such as antibiotics and pesticides (Gravningen, 2007).

Albeit increases in fish farm pollution can be expected as results of foreseeable increases in freshwater fish production in tropical countries (FAO, 2010), little is known about the structural and functional consequences of this development for tropical lake and

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http://dx.doi.org/10.1016/j.envpol.2012.11.034

stream ecosystems. Both, aquatic ecosystem structural characteristics, such as water quality and invertebrate community structure, and important ecosystem processes (including primary productivity, community respiration rates, and whole-system nutrient cycling) are known to respond to a wide range of anthropogenic impacts (Allan, 2004; Paul and Meyer, 2001) and may thus be key players in the understanding of fish farm pollution impacts. In this study, we evaluated the impacts of rural fish farm effluents on the water quality, the invertebrate community and key ecosystem processes in three tropical headwater streams. We hypothesized that fish farms discharge substantial amounts of inorganic nutrients and organic matter into adjacent streams, leading to changes in the invertebrate community, and increases in whole-ecosystem rates of primary productivity, community respiration and nutrient uptake.

2. Materials and methods

2.1. Study sites

The three studied headwater streams were situated in the Rio das Mortes catchment (Upper Paraná basin) in the Federal State of Minas Gerais, in southeastern Brazil, in the transition zone between the Cerrado savanna and the Atlantic rainforest. Acidic soils, rich in iron and manganese, and poor in nutrients are typical for Rio das Mortes catchment. The climate is tropical and with warm, rainy summers (September–March) and mild, dry winters (March–September). Small rural flow-through fish farms that abstract stream water and discharge water from fish ponds into the streams were located in the riparian zones of the streams. These fish farms produced mainly Nile tilapia (*Oreochromis niloticus* L.), common carp (*Cyprinus carpio* L.), and Paraná pacú (*Piaractus mesopotamicus* Holmberg) in three to five fish ponds, but did not have any effluent treatment. Catchment land use upstream of the investigated fish farms on these rural streams was dominated by cattle pasture (35–54% of total catchment area) (MMA, 2008) and natural semi-deciduous Atlantic forest and Cerrado savanna (37–50%). Crop plantations contributed only small fractions to total catchment land use (9–15%). Fish farming has become an important sideline for local farmers in recent years. According to the local fisheries association, there were 33 registered small to medium sized fish farms in the middle Rio das Mortes catchment only.

2.2. Study design

All measurements were performed following a spatial control-impact design. At each stream, sampling stations and stream reaches directly upstream and downstream of fish sewage outfalls – henceforth referred to as reference and impacted stations and reaches – were investigated in parallel in short sampling campaigns (three to four days) in June and September 2010 (Água Limpa and Correias streams, respectively), and in November 2011 (Chaparrals stream). For the evaluation of fish farm impacts on stream ecosystem metabolism, three reference reach systems, i.e. pairs of stream reaches upstream of the control-impact reach systems and thus not impacted by effluents, were additionally sampled at each stream in order to test whether the responses found fell outside the range of natural spatial variability. Sampling occurred on cloudless days under stable baseflow conditions, with no bed-moving floods having occurred in the previous two weeks, in order to avoid succession-related variability in invertebrate community structure (Matthaei et al., 1997), ecosystem metabolism (Uehlinger, 2006) and nutrient uptake (Martí et al., 1997). The three investigated streams were 2nd and 3rd order headwater streams with an average wet channel width between 2.1 and 3.5 m and an average water depth between 0.18 and 0.32 m. The streams were chosen from a larger number of candidate streams impacted by fish farm effluents, because they exhibited upstream and downstream reaches that were very similar in terms of channel morphology, sediment structure and riparian vegetation.

2.3. Stream water and effluent characteristics

Specific conductivity, pH, temperature, and dissolved oxygen concentration of stream water and fish farm effluent were measured using multiparameter probes at 1 min intervals for 24 h (600OMS, 6920 and 556MPS, Yellow Springs Instruments, OH, USA). Effluent characteristics were measured directly at the outfall pipes in triplicate and stream water characteristics were measured at three reference sampling stations upstream and three impacted stations downstream of the fish farm outfalls at each stream. These sampling stations coincided with those used for hydrodynamic tracer experiments (see Subsection 2.5) and were between 29 and 186 m away from each other, depending on water travel times and hydrodynamic characteristics. Triplicate samples for stream water particulate organic matter (POM) and nutrient concentrations, i.e. soluble reactive phosphorus (SRP), nitrate nitrogen (NO_3–N), and ammonium nitrogen (NH_4–N) were taken at each station and the outfall pipes prior to tracer experiments. Nutrient concentrations were determined using flow injection analysis (FIALab 2500, FIALab, Bellevue, WA, USA) and standard spectrophotometric methods (APHA, 1995). Stream water POM was determined as the ash-free dry mass (AFDM) of material filtered onto pre-combusted GF/F filters (Whatman, Maidstone, UK, 0.7 μm nominal pore size) and then incinerated at 550 °C for 2 h. We used two-sample *t*-tests, after confirming normality and homoscedasticity of data, to test for differences in stream water characteristics between upstream and downstream reaches.

2.4. Benthic invertebrates

Benthic invertebrates from surface sediments were sampled at the end of each sampling campaign using a Surber sampler (sampled area 0.09 m^2, 250 μm mesh; Limnotec, São Carlos, Brazil). Six replicate samples were taken at equidistant sampling sites distributed along 200 m long reference and impacted reaches. Invertebrates were preserved in 70% ethanol for later taxonomic identification and were processed in the laboratory by counting and identifying all invertebrates present to the lowest possible taxonomic level using stereo-dissecting microscopes (Zeiss, Germany). We used two-sample Welch tests, after confirming normality and heteroscedasticity of data, to test for differences in invertebrate density and taxa richness between upstream and downstream reaches. We used a two-way permutation multivariate analysis of variance (PerMANOVA) on the original density data matrix with the fixed factors 'stream' and 'impact' (reference or impacted) to test for differences in invertebrate community composition. All statistical tests were calculated using the 'R' software (RCoreTeam, 2012).

2.5. Hydrodynamic characteristics

For the estimation of stream hydrodynamics, constant-rate conservative tracer (NaCl) addition experiments were performed on each stream. A NaCl solution of known concentration was injected into the stream with a peristaltic pump, sufficiently upstream of the first reference stream reach to guarantee complete lateral mixing at the start of this reach. We recorded conductivity breakthrough curves at a resolution of 30 s at the start and the end of two reference and two impacted stream reaches using conductivity loggers equipped with temperature probes for temperature compensation (μS-Log540, Driesen + Kern GmbH, Bad Bramstedt, Germany). Increases in NaCl concentration were later calculated using NaCl-specific conductance response curves established in the laboratory with stream water and the used NaCl salt. We used the dilution discharge equation (Kilpatrick et al., 1989) to calculate discharge and tracer dilution from the breakthrough curves. Advective velocity, longitudinal dispersion, main channel and storage zone cross-sectional area and storage rate were estimated from the breakthrough curves with the one-dimensional solute transport model OTIS-P (Runkel, 1998). We used the breakthrough curve at the start of the first reference reach as an upstream boundary condition and a zero gradient downstream boundary condition located downstream of the end of the last impacted reach in the modeled system. We used the parameter estimates from transport modeling to calculate the fractions of median residence time due to transient storage (F^{200}_{med}) (Runkel, 2002).

2.6. Nitrate uptake

In parallel with NaCl injections, known concentrations of $NaNO_3$ were injected into the studied streams with a peristaltic pump. Smaller than 5-fold experimental increases of ambient nitrate concentrations were planned in order to meet the model assumption of first-order decay (Dodds et al., 2002; Mulholland et al., 2002). To obtain nitrate breakthrough curves, stream water samples were taken at flexible intervals for 180–250 min at each of the sampling stations previously established for conservative tracer experiments (Subsection 2.5), filtered, stored on ice and analyzed for nitrate concentration as described previously. Based on the hydrodynamic characterization of the stream reaches obtained by conservative transport modeling, first-order temporal nitrate decay coefficients (λ, in s^{-1}) were estimated from the obtained nitrate breakthrough curves using the reactive transport mode of OTIS-P (Runkel, 1998). Spatial decay coefficients (k, in m^{-1}) were then calculated by dividing λ values by the median current velocities (v_{med}, in m s^{-1}) obtained from the conservative tracer injections. We calculated nutrient-uptake length (S_W, in m) as the inverse of k. We calculated areal ambient uptake rate (U, in mg m^{-2} h^{-1}) as

$$U = \frac{QC_A}{wS_W},$$

where Q is the discharge, C_A is the ambient nitrate concentration of stream water, and w is the mean wetted-channel width. Last, the nitrate uptake velocity (V_f, in mm s^{-1}) was calculated by dividing U by C_A.

2.7. Ecosystem metabolism

We used the open-channel two-station diel dissolved oxygen (DO) change technique (Marzolf et al., 1994; Young and Huryn, 1998) to estimate community

Table 1
Discharge (*Q*), daily water temperature (*T*), specific conductance (SC), pH, and water-chemistry variables of the three investigated streams and fish farm effluents. Reported values are means of three sampling stations per reach and three replicate measurements of effluent. NH_4–N = ammonium nitrogen, NO_3–N = nitrate nitrogen, SRP = soluble reactive phosphorus, POM = particulate organic matter measured as ash-free dry mass (AFDM) and DO = dissolved oxygen. Significant differences between reference and impacted reaches are indicated after the mean of the impacted reach (*$p < 0.05$, $n = 6$), according to *t*-tests. If no indication is given, tests were not significant.

Stream	*Q* ($L\ s^{-1}$)	*T* (°C)	SC ($\mu S\ cm^{-1}$)	pH	Water chemistry variable ($mg\ L^{-1}$)				
					NH_4–N	NO_3–N	SRP	POM (AFDM)	DO
Água Limpa									
Reference	78	16.5	13.7	6.5	0.017	0.022	0.006	1.7	8.6
Fish farm effluent	3	18.2	15.9	7.3	0.189	0.239	0.060	12.4	9.3
Impacted	85*	16.2	13.9	6.6	0.035*	0.044*	0.011*	2.7*	8.4*
Correias									
Reference	132	14.9	28.9	6.7	0.174	0.041	0.012	4.2	9.0
Fish farm effluent	2	13.8	16.4	6.9	0.061	0.299	0.129	18.5	8.9
Impacted	135*	14.8	28.3*	6.8	0.170	0.052*	0.017*	5.7*	8.7*
Chaparrals									
Reference	138	19.1	15.5	7.1	0.023	0.021	0.019	4.1	7.6
Fish farm effluent	3	21.4	28.0	6.2	0.471	0.006	< 0.005	13.3	8.4
Impacted	141*	19.0	16.6*	6.4*	0.031*	0.020	0.014*	5.8*	7.4*

respiration (CR) and gross primary production (GPP). Dissolved oxygen and water temperature were measured every minute for a minimum of 48 h at the upstream and downstream ends of one reference stream reach and one impacted reach on each stream using multiparameter probes with data loggers (O_2-Log550, Driesen + Kern GmbH, Bad Bramstedt, Germany and 600OMS, Yellow Springs Instruments, OH, USA). The reference and impacted reaches for metabolism measurements encompassed the combined length of the two reference and two impacted reaches, respectively, used for hydrodynamic and nitrate uptake measurements. Additionally, DO and water temperature were measured at the upstream and downstream ends of two reference reaches located upstream of each control-impact reach system. Prior to field measurements, we calibrated the DO probes in water-saturated air at known atmospheric pressures and placed them for a 2 h comparison period in the thalweg at the end of the investigated stream reach. Using data from this cross-comparison, diel DO data were corrected for small differences between probes when calculating CR and GPP according to the two-station approach. In parallel to DO and temperature measurements, atmospheric pressure was measured using a digital quartz resonator precision barometer (DPI 740; Omega Engineering, Stamford, CT, U.S.A.). Barometric pressure and temperature data were used to calculate oxygen saturation. Reaeration (K_{oxy}^{20}; i.e., the reaeration coefficient standardized for 20 °C) was estimated based on DO change rates and DO deficits at night (Young and Huryn, 1996) using DO data from the day of measurement or the night before. Reaeration coefficients obtained by this method ranged between 22 and 57 d^{-1} and corresponded well with coefficients obtained by empirical equations (Melching and Flores, 1999) for the same reaches (Pearson correlation, $p < 0.05$, $R^2 = 0.72$, $n = 12$). Subsequently, we computed CR and GPP as detailed elsewhere (Marzolf et al., 1994; Young and Huryn, 1998). Based on the assumption that lateral water influx obtained from conservative tracer experiments and DO concentrations in inflowing water were constant over the measurement period, we corrected the metabolic rates of one of the investigated streams that exhibited significant lateral water influx (i.e., the Água Limpa stream) as described elsewhere (Hall and Tank, 2005; McCutchan et al., 2002).

3. Results

3.1. Stream water and effluent characteristics

Fish farm effluents exhibited higher values in several variables compared to stream water upstream of the outfall sites (Table 1).

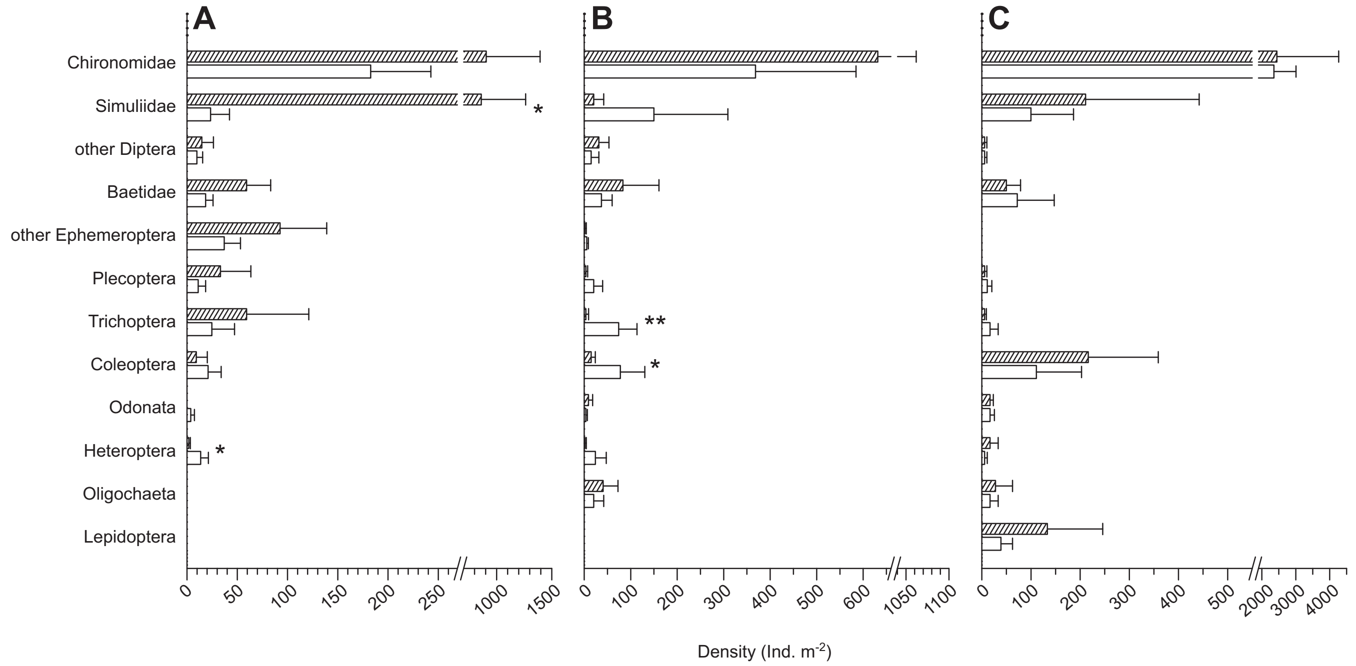

Fig. 1. Mean densities (+1 SD) of benthic invertebrate taxa at reference (empty bars) and impacted sites (hatched bars) on the streams Água Limpa (A), Correias (B) and Chaparrals (C). Significant differences between reference and impacted reaches are indicated beneath the bars (*$p < 0.05$, **$p < 0.01$, $n = 6$), according to Welch tests. If no indication is given, tests were not significant.

At the Água Limpa stream, effluents had 11-fold higher NH_4–N and NO_3–N concentrations and 10-fold higher SRP concentration than reference stream water. At the Correias stream, effluents were enriched in NO_3–N (7-fold) and SRP (11-fold), but depleted in NH_4–N. Effluents of the fish farm at the Chaparrals stream were only enriched in NH_4–N (20-fold). Due to high, i.e. 26- to 66-fold, dilution of effluent with stream water (Table 1), effluents enriched in nutrients only caused 1.3–2.1-fold increases in those nutrients in impacted stream reaches. There was no clear pattern of effluent effect on stream water temperature, pH, and specific conductance, but effluents were consistently enriched in POM (3–7-fold) compared to stream water of reference sites, leading to 1.4–1.6-fold increases in stream water POM concentrations at impacted sites (Table 1). Dissolved oxygen concentrations were similar or higher in effluents than in reference stream water. However, impacted sites had lower stream water DO concentrations than reference sites, pointing to increased heterotrophy at those sites.

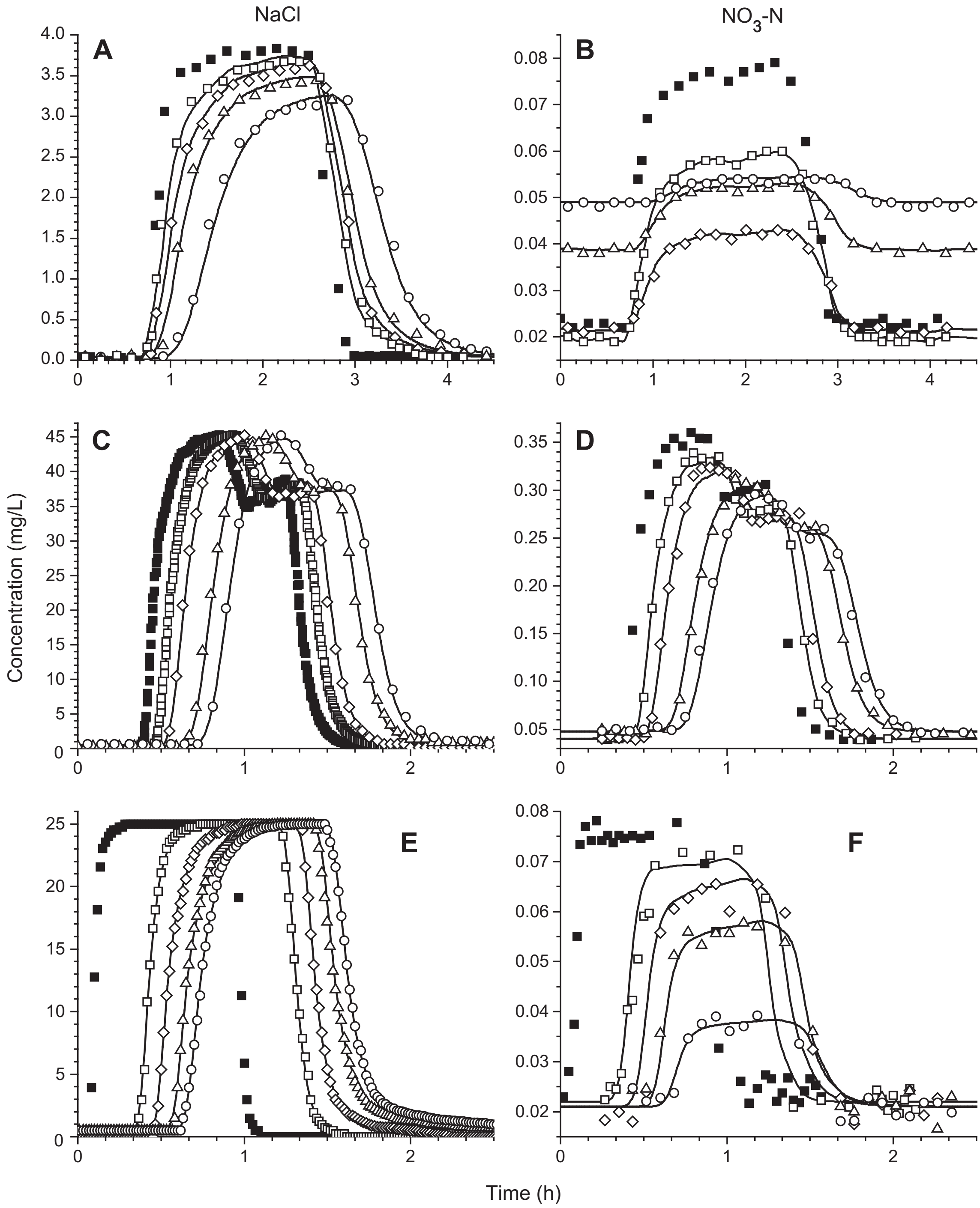

Fig. 2. Constant rate NaCl and nitrate addition experiments in the investigated streams Água Limpa (A, B), Correias (C, D) and Chaparrals (E, F). Upstream boundary condition (USBC, filled squares) and observed concentrations at sampling stations 1 (empty squares), 2 (diamonds), 3 (triangles) and 4 (circles) downstream of the USBC, and simulated concentrations based on final parameter estimates with OTIS-P (solid lines). The reach between the USBC and station 1 corresponds with reach reference 1 (see Table 2), between stations 1 and 2 with reach reference 2, between stations 2 and 3 with reach impacted 1 and between stations 4 and 5 with reach impacted 2. Please note different y-axis scales.

3.2. Benthic invertebrates

A total of 34 benthic invertebrate taxa were identified in this study. Diptera, Ephemeroptera, Coleoptera, Trichoptera and Plecoptera were the groups occurring in highest densities (Fig. 1). There were no significant differences in taxa richness between upstream reference sites and downstream impacted sites at any of the investigated streams (Welch tests, $p > 0.1$, $n = 6$). There were also no significant differences in total invertebrate density between reference and impacted sites at the streams Correias and Chaparrals (Welch tests, $p > 0.1$, $n = 6$). However, at the stream Água Limpa, impacted sites (1963 ± 1350 individuals m^{-2}, mean ± 1 SD) had significantly higher invertebrate densities than reference sites (328 ± 58 individuals m^{-2}) (Welch test, $p < 0.05$, $n = 6$). This difference was due to significantly higher densities of Simuliidae at impacted sites than at reference sites (Fig. 1). According to two-way PerMANOVA on taxa densities, neither location of sampling sites in different streams (factor stream, $p > 0.1$, $R^2 = 0.59$) and location in reference or impacted reaches (factor impact, $p > 0.1$, $R^2 = 0.17$), nor the interaction of both factors (stream × impact, $p > 0.1$, $R^2 = 0.24$) caused any significant difference in invertebrate community composition.

3.3. Hydrodynamic characteristics

While impacted reaches at the streams Correias and Chaparrals did not exhibit any dilution in addition to the effluent discharge (Fig. 2B and C, Table 2), impacted reaches at the stream Água Limpa received considerable lateral dilution (Fig. 2A, Table 2). According to personal observations – i.e., no vertical hydraulic gradients in the bed sediments and water percolating from the right stream bank, where the fish farm was situated – this dilution appeared to be due to additional water from the fish ponds percolating through riparian soils into this stream. There were no obvious differences in hydrodynamic characteristics between reference and impacted reaches in the studied streams (Table 2). However, the first reference reach at the stream Água Limpa – the only investigated reach that exhibited a larger side pool – had considerably lower median current velocities, and higher dispersion coefficients, as well as higher cross-sectional areas of the main channel (A) and transient storage zones (A_S) than any other stream reach in this study (Table 2). In general, the gravel-bottom Água Limpa stream had considerably higher fraction of median residence time due to transient storage and relative transient storage zone sizes (A_S:A ratios) than the sand-bottom Correias and Chaparrals streams.

3.4. Nitrate uptake

There was no consistent effluent impact pattern in nitrate uptake metrics (Fig. 3). At the Água Limpa and Correias streams, nitrate uptake was slightly less efficient in impacted reaches – i.e. these reaches had longer uptake lengths and slower uptake velocities – than in reference reaches (Fig. 3). At the Chaparrals stream, nitrate uptake was much more efficient in impacted than in reference reaches. Impacted reaches had much shorter uptake lengths, faster uptake velocities and higher areal uptake rates than reference reaches. In general, nitrate uptake in the reference reaches of the gravel-bottom stream Água Limpa (Figs. 2A and 3) was much more efficient than in reference reaches of the sand-bottom Correias and Chaparrals streams (Figs. 2D and F and 3).

3.5. Ecosystem metabolism

Impacted stream reaches exhibited 1.9–2.5-fold higher rates of community respiration (CR) than reference reaches (Fig. 4, Table 3). Absolute increases in CR due to effluent discharge ranged between 4.6 and 5.9 g O_2 m^{-2} d^{-1} and fell outside the range of natural spatial variability in CR in the investigated streams (0.2–1.1 g O_2 m^{-2} d^{-1}, Table 3). Thus, increases in CR due to effluent discharge were higher than random natural upstream–downstream differences in reference systems (Table 3; Welch test, $p < 0.01$, $n = 9$). At the Água Limpa stream, the impacted reach had a 2.6–2.9-fold higher gross primary production (GPP) than the reference reach (Fig. 4, Table 3). At the Chaparrals stream, the reference reach exhibited no detectable GPP, but the impacted reach had a GPP of 2.1–2.7 g O_2 m^{-2} d^{-1}. At both streams, increases in GPP due to effluent discharge (2.1–3.4 g O_2 m^{-2} d^{-1}) fell outside the range of natural spatial variability (0.1–0.7 g O_2 m^{-2} d^{-1}, Table 3). In general, increases in GPP due to effluent discharge were higher than random natural upstream–downstream differences in reference systems (Table 3; Welch test, $p < 0.05$, $n = 9$). The relative increases in GPP due to fish farm effluents at these two streams were higher than those in CR, and accordingly, both streams had higher P:R ratios in impacted reaches than in reference reaches (Fig. 4). Differences in GPP between the impacted and the reference reach at the Correias stream were low and fell within the range of

Table 2
Hydrodynamic characteristics and nitrate uptake metrics of reference and impacted stream reaches. Q = discharge, v_{med} = median current velocity, D = dispersion coefficient, α = storage rate, A = cross-sectional area of the main channel, A_S = cross-sectional area of transient storage zones, F^{200}_{med} = fraction of median residence time due to transient storage, DaI = Damköhler number, U = uptake rate, V_f = uptake velocity, S_W = uptake length.

Stream reach	Hydrodynamic characteristics									Nitrate uptake		
	Q (L s^{-1})	v_{med} (m s^{-1})	D (m^2 s^{-1})	α (s^{-1})	A (m^2)	A_S (m^2)	A_S:A	F^{200}_{med} (%)	DaI	U (mg m^{-2} min^{-1})	V_f (mm s^{-1})	S_W (m)
Água Limpa												
Reference 1	77	0.05	2.79	1.3×10^{-3}	1.59	1.72	1.09	52	2.4	0.63	0.49	32
Reference 2	79	0.35	3.8×10^{-2}	2.0×10^{-3}	0.22	0.24	1.06	35	0.6	0.46	0.36	74
Impacted 1	82	0.19	9.3×10^{-2}	4.2×10^{-3}	0.43	0.54	1.27	55	1.1	0.61	0.33	89
Impacted 2	88	0.14	6.8×10^{-1}	9.7×10^{-3}	0.59	0.80	1.33	57	8.3	0.82	0.31	76
Correias												
Reference 1	130	0.12	2.3×10^{-1}	1.5×10^{-4}	1.08	5.1×10^{-2}	4.8×10^{-2}	1.02	1.2	0.17	6.5×10^{-2}	646
Reference 2	133	0.15	4.5×10^{-1}	2.1×10^{-4}	0.91	1.3×10^{-2}	1.5×10^{-2}	0.36	4.2	0.10	4.2×10^{-2}	1057
Impacted 1	135	0.16	5.3×10^{-1}	1.0×10^{-4}	0.82	1.4×10^{-2}	1.7×10^{-2}	0.20	3.4	4.3×10^{-2}	1.6×10^{-2}	2876
Impacted 2	135	0.14	4.6×10^{-1}	6.3×10^{-5}	1.00	4.1×10^{-3}	4.1×10^{-3}	3.7×10^{-2}	5.9	0.10	6.5×10^{-2}	1154
Chaparrals												
Reference 1	138	0.16	1.7×10^{-1}	4.6×10^{-4}	0.84	6.3×10^{-2}	7.5×10^{-2}	3.0	4.2	0.17	0.12	481
Reference 2	138	0.34	1.1×10^{-2}	6.2×10^{-4}	0.40	0.13	0.31	7.2	0.9	6.2×10^{-2}	4.2×10^{-2}	1814
Impacted 1	141	0.18	1.0×10^{-2}	4.5×10^{-4}	0.76	0.13	0.18	5.8	1.2	1.94	1.51	42
Impacted 2	141	0.32	1.02	3.0×10^{-3}	0.45	9.7×10^{-3}	2.2×10^{-2}	1.8	3.8	3.01	2.41	25

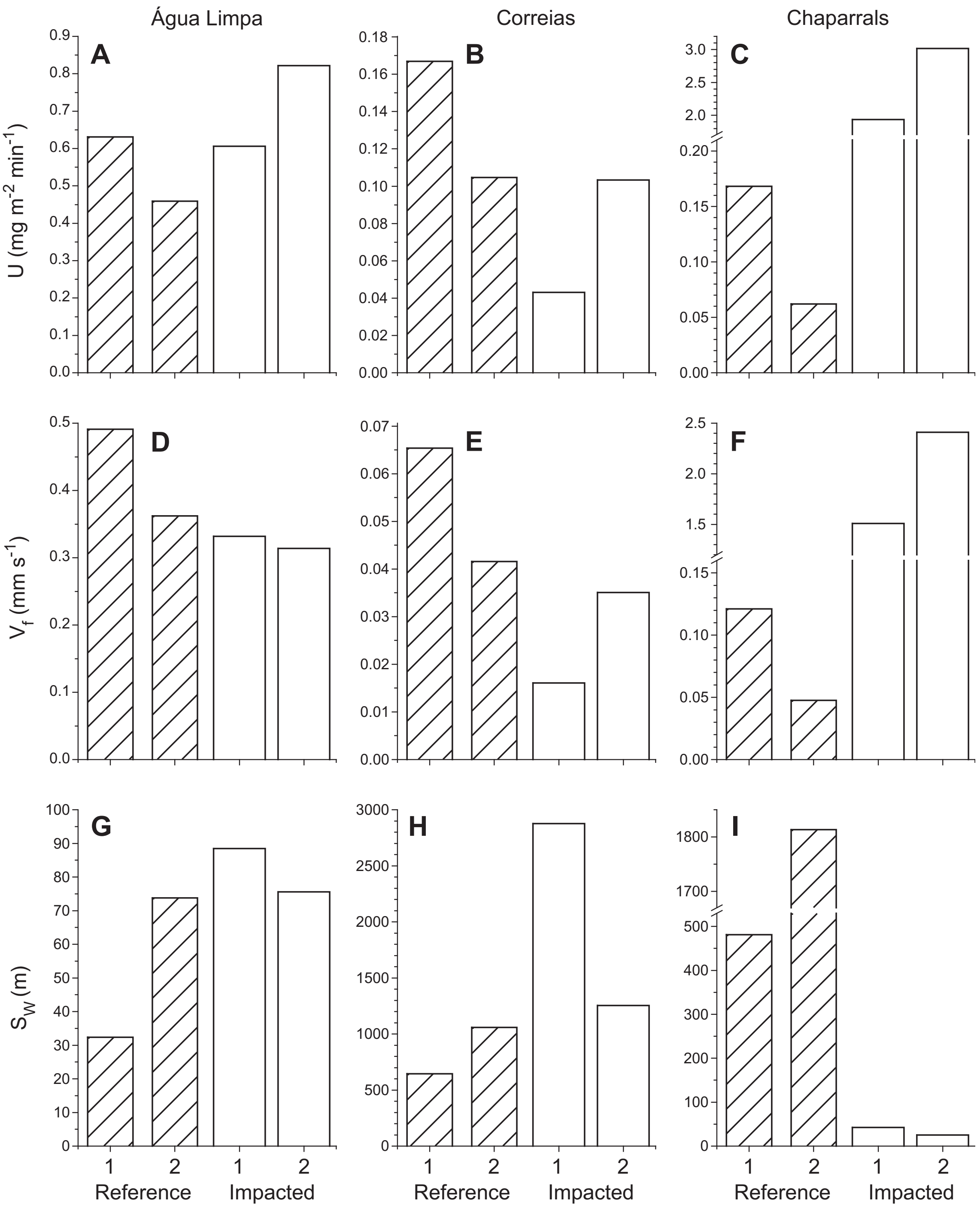

Fig. 3. Nitrate uptake rate (U), uptake velocity (V_f) and uptake length (S_W) of reference and impacted stream reaches. Values are calculated from the final parameter estimates of the reactive transport model OTIS-P (see Fig. 2). Please note different y-axis scales.

natural spatial variability in GPP observed in this streams (Table 3). Consequently, the 2.2–2.5-fold higher CR in the impacted reach of this stream caused a lower P:R ratio (Fig. 4).

4. Discussion

4.1. Fish farm effects on ecosystem structure

As land-based fish farming expands to meet the demands of a growing human population, detailed knowledge on its impacts is needed to guide efforts to prevent and mitigate adverse effects on ecosystems (Tello et al., 2010). Among a multitude of adverse effects on adjacent aquatic ecosystems, water pollution by fish pond effluents is probably the most important one, and also the most common public complaint (Boyd, 2003). Several studies have described adverse effects of land-based fish farming on the water quality of adjacent aquatic systems, in particular on the availability of organic carbon and organic and inorganic nutrients, and pointed out the need for efficient effluent treatment and the application of ecologically sound best management practices (Boaventura et al., 1997; Naylor et al., 2000; Tello et al., 2010). Nonetheless, impacts of small fish farms on water quality are often trivialized in the

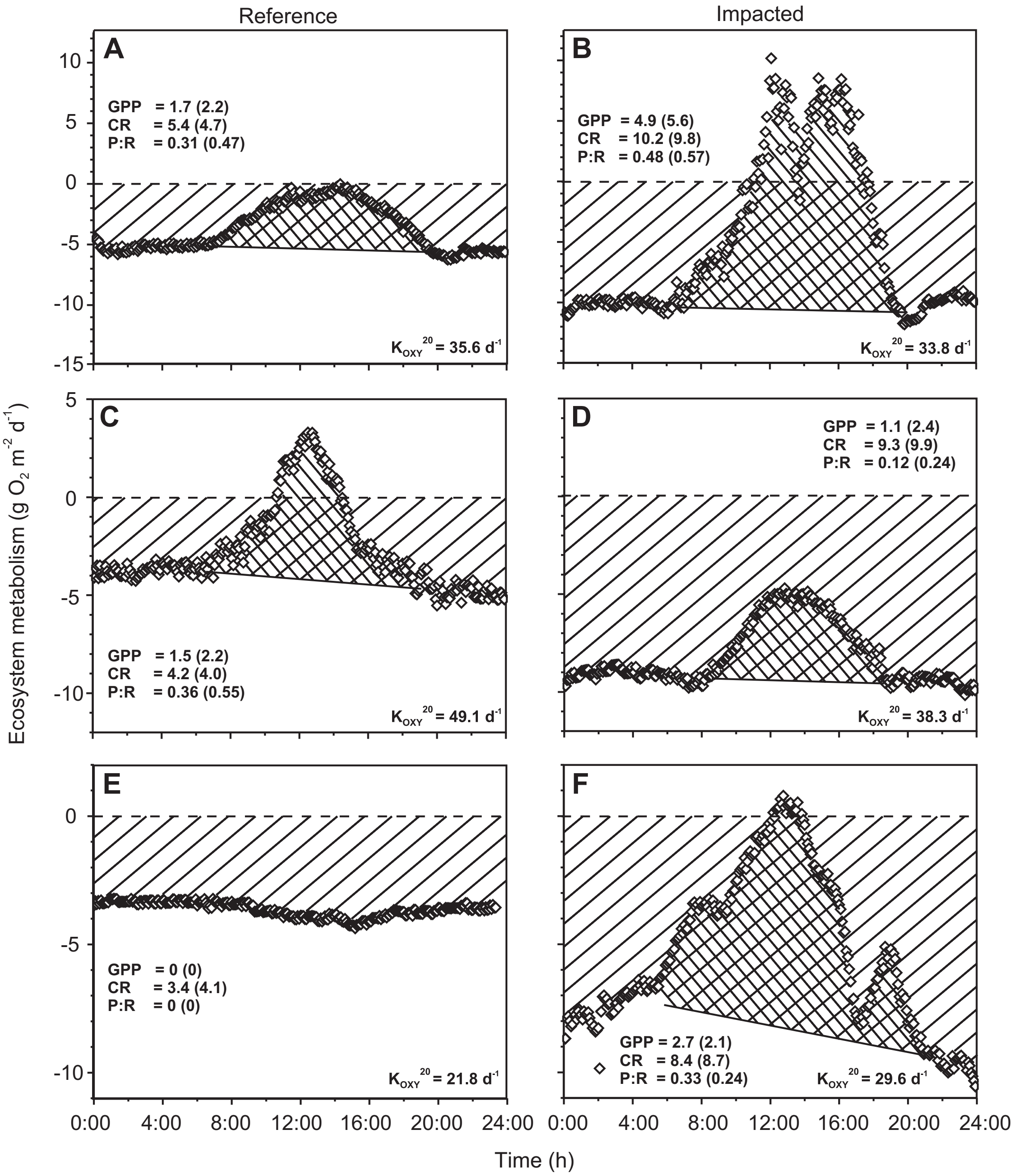

Fig. 4. Diurnal patterns of ecosystem metabolism (i.e., net O_2 change corrected for reaeration and dilution) in reference and impacted reaches on the investigated streams Água Limpa (A, B), Correias (C, D) and Chaparrals (E, F). The left-hatched area indicates gross primary production (GPP, in g O_2 m^{-2} d^{-1}). The right-hatched area represents community respiration (CR, in g O_2 m^{-2} d^{-1}). Values in parentheses are from a second sampling day (diurnal patterns not shown). The solid line extending from predawn to post sunset indicates the extrapolated rate of CR during the day. K^{20}_{oxy} is the reaeration coefficient standardized for 20 °C. P:R is the ratio between GPP and CR. Please note different *y*-axis scales.

aquaculture literature (Beveridge and Phillips, 1993; Boyd, 2006), e.g. by comparing impacts to those of massive urban sewage discharge, and fish farmers are often not willing to or not aware of the necessity to implement best management practices. In tropical developing countries, the awareness of potential pollution impacts may be especially low among rural fish farmers, while aquatic ecosystem responses to fish farm pollution may be more severe due to higher water temperatures than in temperate regions (Beveridge and Phillips, 1993).

In our study, we found significant, but relatively small increases in stream water POM and nutrient concentrations due to effluent discharge from three small tropical fish farms. Although small rural fish farms, operated by inexperienced personal and lacking settling ponds, are generally assumed to discharge effluents more enriched in POM and nutrients than larger farms (Boaventura et al., 1997; Boyd, 2003), the effluents of the investigated small fish farms exhibited relatively low POM and nutrient concentrations that were well below suggested international liquid effluent standards (Boyd, 2003). While the effluents of all studied fish farms were enriched in POM, enrichment in nutrients exhibited considerable variability, with NH_4–N, NO_3–N and SRP enrichment at the Água Limpa farm, NO_3–N and SRP enrichment at the Correias farm, and only NH_4–N enrichment at the Chaparrals farm. Accordingly, generalizations regarding dominant nutrient species in effluents of

Table 3
Gross primary production (GPP), community respiration (CR) and absolute upstream–downstream differences in reference systems, i.e. pairs of stream reaches not impacted by fish farms, and in the studied systems impacted by fish farms, i.e. a reference stream reach directly upstream of the fish farm outfall and an impacted reach downstream of the outfall. Values in parentheses are from a second sampling day.

	Reference systems			Fish farm impacted systems		
	Upstream rate ($g\ O_2\ m^{-2}\ d^{-1}$)	Downstream rate ($g\ O_2\ m^{-2}\ d^{-1}$)	|Difference| ($g\ O_2\ m^{-2}\ d^{-1}$)	Upstream rate ($g\ O_2\ m^{-2}\ d^{-1}$)	Downstream rate ($g\ O_2\ m^{-2}\ d^{-1}$)	|Difference| ($g\ O_2\ m^{-2}\ d^{-1}$)
GPP						
Água Limpa	1.5	2.2	0.7	1.7 (2.2)	4.9 (5.6)	3.2 (3.4)
Correias	1.7	1.2	0.5	1.5 (2.2)	1.1 (2.4)	0.4 (0.2)
Chaparrals	0.2	0.3	0.1	0.0 (0.0)	2.7 (2.1)	2.7 (2.1)
CR						
Água Limpa	4.8	5.9	1.1	5.4 (4.7)	10.2 (9.8)	4.8 (5.1)
Correias	3.5	4.1	0.6	4.2 (4.0)	9.3 (9.9)	5.1 (5.9)
Chaparrals	2.8	2.6	0.2	3.4 (4.1)	8.4 (8.7)	5.0 (4.6)

rural tropical fish farms may hardly be possible. As a result of relatively low effluent enrichment levels and considerable dilution of effluent with stream water, impacted stream reaches exhibited <2.0-fold increases in stream water POM and <1.8-fold and 1.6-fold increases in dissolved inorganic nitrogen and SRP, respectively. While such pollution levels are generally regarded as low and acceptable for aquaculture facilities (Boyd, 2003), they may be sufficiently high to cause profound changes in stream ecosystem functioning (Dodds et al., 2002; Gücker et al., 2009).

Impacts of fish farm effluents on the benthic invertebrate community, such as decreases in total taxa richness and in densities of pollution-sensitive taxa, increases in densities of pollution-tolerant taxa, and changes in the trophic structure of the community have been widely reported (Camargo, 1992; Doughty and McPhail, 1995; Loch et al., 1996). In particular, increases in the densities of gatherers and filter feeders can be expected as a result of fish farm outfalls rich in suspended POM (Camargo, 1992). Interestingly, impacted sites of the Água Limpa stream, that exhibited the highest relative increase in stream water POM due to fish farm discharge among the studied streams, showed massive density increases of filter-feeding blackfly larvae (Diptera: Simuliidae) compared to reference sites. However, the absence of community and taxon responses to effluent outfalls at the other two investigated streams indicated that pollution levels were too small to exert substantial impacts on the benthic invertebrate community.

4.2. Fish farm effects on ecosystem function

Fish farm pollution impacts on stream ecosystem functioning have been conjectured (Tello et al., 2010). However, there is, to our knowledge, no study in the literature that has demonstrated such impacts. A multitude of stream ecosystem functions could be affected by fish farm effluents. As fish farms emit organic carbon and nutrients, they should cause increases in rates of GPP and CR, and thus augment both, the autotrophic and heterotrophic state of streams (Dodds and Cole, 2007). However, concomitant emissions of pharmaceutically active substances, such as medical disinfectants, parasiticides, antibiotics, and anesthetics, as well as heavy metals (Boyd and Massaut, 1999; Tello et al., 2010) that can accumulate in stream bed sediments may depress stream ecosystem metabolism. Similarly, emissions of pharmaceutically active substances and heavy metals may on the one hand adversely affect stream nutrient cycling, which, on the other hand, can be expected to be stimulated, or even saturated, by organically and nutrient enriched effluents (Dodds et al., 2002; Fellows et al., 2006; Gücker and Pusch, 2006). A series of more specific ecosystem processes that are closely related to carbon and nutrient cycling – such as nitrification, denitrification, extracellular enzyme activity, and litter decay – may also be depressed or stimulated by fish farm pollution (Tello et al., 2010).

In this study, we did not find a consistent impact pattern of fish farm effluents on stream nitrate uptake. In two of the investigated streams, whole-stream nitrate uptake efficiencies appeared to be diminished due to fish farm effluents. However, these decreases in impacted stream reaches were moderate and well within the expected natural variability in nitrate uptake (Ensign and Doyle, 2006), with the exception of the stream reach directly downstream of the fish farm outfall at the Correias stream that exhibited a considerably longer uptake length and a lower uptake rate and velocity than reference reaches. In contrast, impacted stream reaches on the Chaparrals stream had massively increased nitrate uptake efficiencies compared to reference reaches, despite comparable riparian vegetation, stream morphology and hydrodynamics. Absolute uptake rates and velocities in impacted reaches were amongst the highest values reported in the literature (Ensign and Doyle, 2006) which could be explained by high assimilative nitrogen demand due to high rates of GPP and CR, and possibly also by high rates of denitrification due to high organic carbon availability and quality in these reaches (Steinhart et al., 2001).

Interestingly, nitrate uptake in reference reaches exhibited a clearer pattern. Reference reaches at the investigated gravel-bottom stream had not only a higher importance of transient solute storage, but also much higher nitrate uptake efficiencies than those at the investigated sand-bottom streams. Among the reference reaches of the studied gravel-bottom stream, a reach that exhibited an additional side pool and the lowest current velocities had the most efficient nitrate uptake of all reference reaches. Accordingly, stream morphology, sediment characteristics and hydrodynamics appear to be important factors affecting nitrogen uptake in the investigated tropical streams (Gücker and Boëchat, 2004).

In contrast to nitrate uptake, stream ecosystem metabolism showed a consistent response to fish farm pollution. All stream reaches affected by effluents had higher rates of CR than reference reaches. A comparison between the amounts of these increases (1.9–2.5-fold increase in CR) and those in stream water POM concentrations (1.4–1.6-fold) may point to fish farm effects on both stream water organic matter quantity and quality. Alternatively, nutrient enrichment or potential increases in dissolved organic matter not quantified in this study may be responsible for increases in CR not explained by fish farm POM discharge alone (Bernhardt and Likens, 2002; Mulholland et al., 2001). Reaches impacted by effluents also had considerably higher GPP than reference reaches at the two more pristine of the investigated streams. However, the third stream, a rural stream that was already substantially enriched in NH_4–N prior to the effluent outfall (Cunha et al., 2011), did not exhibit increased GPP due to effluent discharge.

In conclusion, fish farm effluents with relatively low concentrations of POM and nutrients, and discharged at relatively low rates compared to stream flow, had considerable effects on the ecosystem metabolism of tropical headwater streams. As rural fish farms have become abundant in many tropical countries, these findings may have broad-scale relevance for the management of tropical headwater streams. Unlike urban sewage outfalls or wastewater treatment plants, that have to emit substantial nutrient and organic carbon loads in order to cause measurable effects on the already pre-impacted urban and agricultural streams they are often located at (Gücker et al., 2006), small rural fish farms can easily affect ecosystem functioning of nearby pristine running waters. Compliance with ecologically sound best management practices (Boyd, 2003; Naylor et al., 2000) and attempts to reduce effluent organic matter and nutrients with settling ponds or constructed wetlands (Naylor et al., 2003) are thus also important for small rural fish farms, and initiatives to exempt small aquaculture facilities from effluent regulations should be discussed critically.

Acknowledgments

We thank H.R.M. Aguiar, G.H. Almeida, M.S. Araújo, V.S. Belo, A.P.C. Carvalho, R.C. Chaves, A. Contin, F.L. Nascimento, O.C.C. Neto, A.B.M. Paiva, A.C.S. Prado, A.T.B. Santos, E.F. Silva Junior, G.C. Silva, M.N.C. Silva, and R.C.S. Silva for help with the field work. We thank the land owners of the studied fish farms, especially the Chaves family, for their assistance and for granting access to their properties. M. Brauns, T.P. Moulton, S.J. Ormerod and an anonymous reviewer provided helpful comments on earlier versions of the manuscript. R.S. Rosa and A.C.F. Aguiar were supported by fellowships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Universidade Federal de São João del-Rei (UFSJ). This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, CRA-APQ-02002-09) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 556953/2010-0).

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