



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

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### ABSTRACT

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.

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### 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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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 rain-forest. 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 Correas 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 (6000MS, 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<sub>3</sub>–N), and ammonium nitrogen (NH<sub>4</sub>–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<sup>2</sup>, 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 ( $F_{med}^{200}$ ) (Runkel, 2002).

### 2.6. Nitrate uptake

In parallel with NaCl injections, known concentrations of NaNO<sub>3</sub> 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 ( $\lambda$ , in s<sup>-1</sup>) were estimated from the obtained nitrate breakthrough curves using the reactive transport mode of OTIS-P (Runkel, 1998). Spatial decay coefficients ( $k$ , in m<sup>-1</sup>) were then calculated by dividing  $\lambda$  values by the median current velocities ( $v_{med}$ , in m s<sup>-1</sup>) 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<sup>-2</sup> h<sup>-1</sup>) 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<sup>-1</sup>) 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

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