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Feeding the river: The fate of feed-pellet-derived material escaping from land-based trout farms

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ABSTRACT

Environmental impacts of intensive fish cultivation on receiving food webs have been investigated mainly for cage culture systems in lake and marine ecosystems; however, the potential influence of land-based salmonid farms on rivers is less documented. Stable C and N isotopes were used to examine the influence of aquaculture waste on aquatic organisms in three streams with different discharge rates (1–5 $m^3 \cdot s^{-1}$) that receive effluents from rainbow trout (*Oncorhynchus mykiss*) farms with different rearing capacities (100–600 t wet weight per year). Feed pellets from each farm were significantly ^{13}C-enriched (up to +8‰ $\delta^{13}C$) compared to the isotopic backgrounds of the receiving streams. Benthic invertebrates (detritivores and predators) and small-bodied fish were consistently ^{13}C-enriched downstream the fish farm effluents. This pattern was stronger for low stream discharges and high-production farms. The trophic niche breadths of these organisms, estimated by the spread of their isotopic values. decreased in downstream these farms, suggesting a reduction in the diversity of dietary sources. Upstream-downstream comparisons based on multiple-source mixing models revealed high contributions (40–88%) of pellet-derived material in their diet, which indicates that waste from land-based salmonid farms enhanced the detritus-based food chain via this particulate route.

1. Introduction

The “green” and “brown” pathways, refering to algae-based and detritus-based food chains, respectively, co-exist and contribute to aquatic food webs to varying degrees. Primary producers are fuelled by nutrients and dissolved inorganic carbon (C) (Elser et al., 2007; Bumpers et al., 2017), resulting in “green” C available to primary consumers and higher trophic levels. In addition, bacterial and invertebrate communities are primary consumers that feed on dissolved and detritic particulate organic matter, and through this detrital energy pathway, “brown” C rises to higher trophic levels in aquatic food webs. Detrital resources may dominate certain ecosystems that receive abundant allochtonous inputs such as terrestrial plant litter (Vannote et al., 1980). However, algae usually provide high-quality food resources to primary consumers (Guo et al., 2016a; Brett et al., 2017) and enhance dietary use and assimilation of detrital resources (Guo et al., 2016b). Therefore, the green and brown pathways are closely related, and the origin of C flowing from basal sources to higher trophic levels in aquatic food webs is usually intricate.

Human activities can modify the balance between green- and brown-derived C in aquatic food webs. Anthropogenic nitrogen (N) and phosphorus inputs greatly affect contemporary freshwater ecosystems (Galloway et al., 2004; Penuelas et al., 2013), where they stimulate the algae-based pathway. Excessive nutrient loads can induce the proliferation of damaging macro- and micro-algal blooms (Diaz and Rosenberg, 2008; Paerl et al., 2011) and ultimately change C cycling in aquatic environments (Brenner et al., 1999; Roussel et al., 2014). Anthropogenic nutrients can alter the decomposition and assimilation of detrital resources via microbial activity and consumer feeding (Rosemond et al., 2015; Guo et al., 2016b); thus, they also indirectly influence the brown pathway. Anthropogenic inputs of particulate and dissolved organic matter can directly fuel the detritus-based pathway as well, providing primary consumers with alternative resources of potentially high nutritional value and availability. This is especially true for intensive cultivation of fish in cage culture systems, in which a large amount of organic matter is released as either wasted food pellets or fish excreta. Several studies have described how aquaculture waste is assimilated and transferred into the receiving food webs in ponds (Xia et al., 2013), lakes (Grey et al., 2004; Kullman et al., 2009; Gondwe et al., 2012) and marine environments (Mazzola and Sarà, 2001; Vizzini et al., 2005; Dolenec et al., 2007; Fernandez-Jover et al., 2011; Wai et al., 2011; Handå et al., 2012), and how this waste contributes to the

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diet of local invertebrate and vertebrate fauna. Similar patterns are expected for river biota that receive aquaculture waste, but to date they have not been clearly quantified.

Intensive cultivation of salmonids on land-based farms requires cool water of fairly good quality; consequently, they are usually connected to low-order headwater streams. Because terrestrial plant litter is abundant in these relatively small and light-limited streams, the detrital-based pathway is expected to dominate (Vannote et al., 1980). Gravity usually supplies water from the stream, which flows into fish rearing ponds, then back to the stream after settling treament. Depending on farm capacity and the effectiveness of settling, however, some faeces and uningested and dissolved feed pellets escape into the stream. Large amounts of dissolved organic matter downstream from fish farms decrease the abundance of primary producers and increase bacterial production (Nimptsch et al., 2015; Kamjunke et al., 2017), while benthic invertebrate biomass usually increases (Tello et al., 2010; Camargo et al., 2011; Guilpart et al., 2012; Stojanović et al., 2017). However, the assimilation of aquaculture waste and energy transfer within riverine food webs downstream from land-based salmonid farms have not yet been explored, notably how the balance between green and brown pathways could be affected nearby fish farm outlets.

In the present study, stable C and N isotopes were used to examine the influence of aquaculture waste on three streams with different discharge rates (1–5 $m^3 \cdot s^{-1}$) that receive effluents from rainbow trout (*Oncorhynchus mykiss*) farms with different rearing capacities (100–600 t wet weight per year). We expected that 1) trout-farm effluents would contrast with the isotopic background of stream biotia, allowing stable isotope ratios to track the fate of aquaculture waste in receiving stream food webs; 2) pellet-derived particles (both faeces and uningested food) would enhance the detritus-based pathway downstream from the fish farm; and 3) this pattern would be positively linked to farm size but less evident in high-flow rivers.

2. Materials and methods

2.1. Study sites and sample collection

Three French rivers that receive effluents from outdoor rainbow trout farms producing 250 g to 2 kg fish were studied in spring 2010. Annual production and river discharges varied considerably among farms (Table 1). Fish were fed extruded pellets containing fish meal, fish oil and plant-based exogenous ingredients according to formulations developed by fish-feed companies. The mean isotopic signal of the food provided to fish was obtained by collecting 6–20 samples of feed pellets (PEL) per farm over a one-year period to encompass possible changes in ingredients (Table 1). In a preliminary experiment, stable isotope analyses were run on faeces- and food-pellet samples (n = 12) collected in Farm 1. Very small differences between faeces and food-pellet samples were obtained, on average 0.4‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. We therefore focused on food-pellets only in the present study, for convenience. In addition, two sites approximately 50 m long were selected in each receiving river. UP (control) and DW (impact) were located immediately upstream and 100 m downstream from the fish-farm outlet, respectively. For each river, all samples were collected on the same day.

Three 2 L water samples were collected at each site (UP and DW. 3 rivers) and kept in a cooler until filtered on fiberglass Whatman® GF/F (0.7 μm) in the laboratory to obtain suspended particulate organic matter (SPOM). Three samples of sedimented organic matter (SOM) were collected at each site using a syringe (100 mL) to suction the sediment surface. Samples were filtered in the laboratory following same procedure as that used for SPOM. The most abundant species of primary producers (PP) were collected at each site. i.e. *Myriophyllum spicatum* (River 1). *Cladophora* sp. (River 2). and *Ranunculus penicillatus* (River 3). Three samples of each species per site were kept in a cooler until reaching the laboratory.

Primary and secondary consumers were sampled from the river bottom at each site. A standard 0.05 m^2 Surber® stream bottom sampler (mesh size 500 μm) was used to collect invertebrates from several microhabitats. At field, a magnifying glass was used to identify invertebrates to the family level. They were then sorted into three major trophic groups (grazers GRA, detritivores DET, and predatory invertebrates PRI) based on the guilds of Usseglio-Polatera et al. (2000), then stored in microtubes and kept in a cooler. The most common taxa were Baetidae and Ephemerellidae (GRA); Asellidae, Ephemeridae, Similidae and Hydropsychidae (DET); and Rhyacophilidae, Calopterygidae and Perlodidae (PRI). Dipnetting was used to collect small fish (FISH), considered as invertivorous (*Cottus gobio*, *Barbatula barbatula*, *Phoxinus phoxinus*, and *Gobio gobio*), at each site. They were anaesthetized in a benzocaine bath, then euthanized and kept in a cooler.

2.2. Sample preparation and stable isotope analysis

In the laboratory, a stereomicroscope was used to carefully remove sand particles and animal and plant fragments from the GF/F filters; then, the 36 filters (SPOM and SOM) were dried in an oven at 60 °C for 48 h. PP samples were rinsed in distilled water and inspected using a stereomicroscope to remove microinvertebrates. Similarly, GRA, DET and PRI were carefully examined to remove detritus fragments. Following the recommendation of Jardine et al. (2005), we did not remove the guts of benthic invertebrates before stable isotope analysis. FISH were carefully dissected to obtain dorsal muscle samples, which were individually stored in microtubes. At the end of this process, 18 plant and 169 animal samples, as well as 32 PEL samples from the fish farms, were freeze-dried (lyophiliser CHRIST, ALPHA 1-4 model, −85 °C for 48 h) and ground into a homogeneous powder using a mixer mill (RetschMM 200). Samples were weighed to the nearest 0.01 mg into tin cups (0.3 mg for animal samples and 1 mg for plant samples) using a precision balance (Mettler Toledo UMX 2).

Ratios of ^{13}C:^{12}C, ^{15}N:^{14}N, and C:N were obtained from continuous-flow isotope-ratio mass spectrometry using Thermo Finnigan mass spectrometers (Delta Plus XP or Delta V Plus) interfaced elemental analysers (Carlo Erba NC2500 or Costech 4010), depending on the sample type (plant or animal tissue). Ratios were reported in conventional delta (δ) notations as parts per mil (‰) relative to the international standard for Peedee Belemnite Carbonate and atmospheric N. Samples were run in separate batches from September 2010 to June 2011. Repeat analyses of 8–13 standards (certified by the International

Table 1
Annual production of the three trout farms and a brief description of the rivers receiving their effluents.

Farm	Annual trout farm production ($t \cdot year^{-1}$)	Isotopic signatures of feed pellets (‰ ± 1 SD)	Annual river discharge ($m^3 \cdot s^{-1}$)	River pH	River substratum	Catchment
1	615	$\delta^{13}C = -24.4 \pm 0.9$ $\delta^{15}N = 4.6 \pm 1.5$	3.0	6.7	Sand	Forest
2	250	$\delta^{13}C = -24.8 \pm 0.8$ $\delta^{15}N = 4.0 \pm 0.8$	1.0	7.9	Sand, Gravel	Crop and livestock agriculture
3	130	$\delta^{13}C = -22.2 \pm 0.2$ $\delta^{15}N = 9.3 \pm 0.4$	4.9	6.9	Granite, Gravel	Crop and livestock agriculture