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Brown trout density effects on the macroinvertebrate and biofilm communities of headwater streams: a cage experiment

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ABSTRACT

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Salmo trutta is a key species of headwater streams that is able to influence the structure of these ecosystems through predation. Populations of this species are being threatened by climate change and hydrological alteration. In order to understand how their disappearance would affect the ecosystem structure of headwater streams, we used cage experiments to analyse different biofilm and macroinvertebrate structural parameters and the biodiversity of their communities under different trout densities. Due to a heavy rain event, our experiment was destroyed on the 10th day, but the role of trout as stream ecosystemic structure drivers was observed, as multiple parameters were modulated by their presence or absence. In the high-density treatments, a significant reduction in the biomass of macroinvertebrate families that fed on biofilms was observed due to direct predation on them, which caused the biofilms to be more autotrophic. A decrease in brown trout population densities could cause cascading trophic effects on the ecosystem structure of headwater streams.

Key words: *Salmo trutta*, ecosystem structure, dam, climate change, Pyrenees, predation, biofilms, macroinvertebrates, headwater streams

RESUMEN

Efectos de la densidad de truchas en las comunidades de macroinvertebrados y biofilms de los ríos de montaña: un experimento con jaulas

La trucha de río es una especie clave de los ríos de montaña ya que puede influenciar la estructura de estos ecosistemas a través de su papel como depredadora. El cambio climático y las alteraciones hidrológicas amenazan muchas de las poblaciones de esta especie. Para determinar cómo su desaparición podría afectar a la estructura del ecosistema, hemos utilizado experimentos con jaulas para analizar diferentes parámetros estructurales de los biofilms y los macroinvertebrados, así como la biodiversidad de sus comunidades, bajo diferentes densidades de truchas. Debido a un fuerte evento de lluvias, nuestro experimento fue destruido tras diez días, pero se observó el papel de la trucha en la estructura del ecosistema, ya que múltiples parámetros variaban en función de su presencia o ausencia. En los tratamientos de alta densidad de truchas se observó una disminución de la biomasa de familias que se alimentan de biofilms debido a su depredación directa por parte de la trucha, lo que causó que los biofilms fueran más autotróficos. Una disminución en las densidades de las poblaciones de trucha podría tener consecuencias tróficas en cascada en la estructura de los ecosistemas de ríos de montaña.

Palabras clave: Salmo trutta*, estructura ecosistémica, presa, cambio climático, Pirineos, depredación, biofilms, macroinvertebrados, ríos de montaña*

INTRODUCTION

Biotic interactions, such as predation and competition, have major influences on the organization of biological communities at local scales in both time and space (Biggs et al., 2005). Fish are key predators of freshwater ecosystems that are often linked to significant effects on benthic and littoral macroinvertebrates, including their abundance, community composition and size structure (Morin, 1984; Nasmith et al., 2012). The effects of fish predation on the macroinvertebrate community are especially observed when the predation regime is qualitatively (Tonn et al. 2004; Venturelli & Tonn 2005) and quantitatively (Morin, 1984) altered. Aquatic food webs have provided unquestionable evidence of strong, community-wide effects determined by predator-driven constraints (Mancinelli et al., 2002). Variations on natural levels of predator abundance and habitat complexity, among other important factors, are responsible for different responses in a community (Crowder & Cooper, 1982). Consequently, experimental exclusions of fish can alter the abundance and dominance of other species, highlighting the role of fish predation on the structuring of freshwater communities (Morin, 1984). Fish have the potential to influence other components of ecosystems by controlling the abundance of important basal resources, affecting the periphyton community structure (Hill et al., 2000) and, consequently, the macroinvertebrate community (Rubio-Gracia et al., 2017).

Properly designed and controlled field experiments are one of the best ways to determine cause and effect relationships among organisms (Kennelly, 1991), and cage experiments with fish have been proven to be effective and provide reliable results (Power, 1990; Rubio-Gracia et al., 2017). Cage experiments may cause artefacts that controls cannot always detect, confounding the results and causing secondary outcomes associated with the cage, like altered algal growth, sedimentation changes due to alterations in hydrodynamics, and the confusion of experimental effects with local-scale patchiness of predators (Connel, 1997). Despite this, cage mesocosms experiments tend to be a simplification of the natural world that allows us to isolate and measure processes but cannot be disconnected from the scale at which ecological phenomena occur (Ghedini et al., 2015).

Headwater streams are unique environments because of their cold waters, conspicuous seasonal and daily variations in flow, oligosalinity, and particular hydrological and morphological conditions (Bona et al., 2008). The brown trout, *Salmo trutta*, is found in this kind of habitat due to its requirements for cold and well-oxygenated waters. It is a generalistic-opportunistic visual predator (Klemetsen et al., 2003; Bylak, 2017) that focuses on capturing the most abundant prey, which makes it an important predator in headwater streams from the Pyrenees mountain range (Alonso et al., 2012), with an estimated general abundance between 0.355 ind/m^2 and 0,898 ind/m^2 (García de Jalón et al., 1986). Its main diet consists of Chironomidae and other Diptera, Ephemeroptera, and, to a lesser extent, Plecoptera (Sánchez, 2009) invertebrates. This species modulates its diet, adapting it to the seasonal variability, the differences between rivers and between different microhabitats (Greenberg & Dahl, 1998; Nislow et al., 1998). This fact allows *Salmo trutta* to use many different resources (Alonso et al., 2012), making it a good model for ecological experimentation.

The Pyrenees are facing, by the end of the century, a decrease in precipitation and an increase in air and water temperature (López-Moreno et al., 2008; López-Moreno et al., 2009). Within this context and considering the increasing demand for water and the rising presence of flow regulation structures like dams (Zarfl et al., 2019), trout populations in Pyrenean headwater streams might be severely affected and could potentially disappear. In order to assess how this hypothetical loss of trout, a key predator of the study site, would affect the structure and function of Pyrenean headwater streams, we will perform an exclusion experiment. Taking into consideration this theoretical framework, the objective for this experiment is to assess how fish (1) absence and (2) high density affects the stream ecosystem structure, measured as algal structural parameters (chlorophyll-*a*, ash-free dry mass, community structure, and autotrophic index) and invertebrate structural parameters (biomass, abundance, and diversity). As the brown trout is a visual and generalist predator, we hypothesize that (1) the high

density of trout in the inclusion treatment will decrease the biomass of macroinvertebrate families that feed on biofilms, and thus are more exposed to the trout and that (2) the macroinvertebrate biomass reduction of these groups will increase the production and the biodiversity of the biofilm communities in the inclusion treatments.

MATERIALS AND METHODS

Study area

The study area is a small Pyrenean headwater stream named Catllar, an influent of the Ter River, located in Vilallonga del Ter, Catalonia (NE Iberian Peninsula), with a basin located between 1200 and 1600 m a.s.l. The stream is divided by a hydroelectric dam that deviates most of the upstream water to a hydroelectric power station, so downstream water comes mainly from the adjacent *Clot de la Jaceta* stream. The experiment was set up in the downstream part of the stream.

Mesocosms setting

We set up a mesocosms experiment with characteristics that allowed us to control the fish density and quantify the effects of different treatments.

We constructed cages with a wood structure and a plastic mesh that enclosed a volume of 0.45 m^3 (1·1·0.45 m) (Fig. 1). The bottom of the cages was filled with 30 cobbles (of 200-300 mm in diameter), gravel, and sand from the riverbed that allowed the macroinvertebrate community to establish inside the cages. Cobbles were carefully collected to minimize the disruption to biotic compartments. Three treatments with three replicas per treatment were established by varying the fish density inside each cage: (1) control cages with open sides, thus exposed to actual fish densities and used to account for potential artefacts of the cages, (2) exclusion cages without trout simulating the future possibility of their disappearance, and (3) inclusion high-density cages with 2 trout. Cages were separated by a minimum of 10 m between them to avoid interferences. Trout were caught from the same stream by electrofishing (LR-24 Smith-Root Ltd. 120V DC-0.6 A), meeting the standardized European normative (CEN, 2003). The fish caught were weighed and measured (standard length), and the ones with the previously determined normal size distribution for that stream (6-12 cm standard length) were used for the mesocosms setting. This size was also chosen in order to avoid cannibalism, as this behavior can begin after they surpass 12

Figure 1. Setting of the mesocosms experiment in the stream, showing the three fish treatments: control treatment (open cages), exclusion treatment (closed cages without trouts) and inclusion treatment (closed cages with trouts). *Estructura del experimento de mesocosmos en el río, mostrando los tres tratamientos con peces: tratamiento de control (cajas abiertas), tratamiento de exclusión (cajas cerradas sin truchas) y tratamiento de inclusión (cajas cerradas con truchas).*

cm in standard length (Sánchez, 2009). Feeding intensity in *S. trutta* increases with temperature, the highest being in spring and summer (Alonso et al., 2012). The experiment started in July 2020, with the objective of running it for two weeks, removing leaves, branches, and detritus from the mesh every two days in order to avoid artefacts. This period has been demonstrated to be sufficient to allow the detection of fish effects on periphyton and invertebrate assemblages (Rubio-Gracia et al., 2017). Unfortunately, after 10 days of experimentation, an intense rain event caused the water flow of the stream to rise, which destroyed most of the cages and the experiment had to be suspended, having only been able to sample the cages once, after 7 days.

Sample collection

Biofilm and macroinvertebrate samples were planned to have been taken 7 and 14 days after the start of the experiment, but due to the cages being destroyed on the tenth day, only the first sampling after 7 days was performed. Information on the biofilm and macroinvertebrate community of the stream was previously obtained during a two-year span and was used in order to understand the initial conditions of the experiment (López-de Sancha et al., 2022).

Biofilm samplings and analyses

Biofilm samples were taken by scraping 25 cm^2 of the surface of random cobbles in the cage and stored in 30 ml vials with stream water. Two repetitions for each sampling were performed in order to obtain samples for the algal community, chlorophyll-*a* (Chl-*a*), and Ash-Free Dry Mass (AFDM) analyses. Chl-*a* and AFDM samples were frozen until their analysis. For the algal community analysis, a 15 µl drop of 4 % formalin was added to the fresh sample in order to preserve it and was stored cold until its analysis.

To analyse the Chl-*a* and AFDM concentration of the samples, we used 47 mm diameter glass-fiber filter papers to void-filter them. The solid residue was put together with the paper in a glass container with 20 ml of 90 % acetone in order to extract its Chl-*a*, and was preserved in

cold storage, covered from light, for 24 hours. After that period, it was sonicated (Ultrasonic bath, J.P. Selecta) for 2 minutes to promote cell lysis and improve the Chl-*a* extraction, and then, using another pre-dried and weighed filter paper, it was filtered again. Extra acetone was used to wash all the periphyton, and the total volume was considered for the calculations. The filtered acetone contained the Chl-*a* of the sample, which was measured by using a spectrophotometer (Shimadzu UV-1800) following the methodology described in Jeffrey and Humphrey (1975). We dried the residue on the paper at 50 ºC for one week and weighed it in order to measure the dry mass of the biofilm. Afterwards, it was dehydrated at 450 ºC and weighed again to measure its ash-free dry mass.

Using the absorbance lectures of the spectrophotometer and the biomass measurements, we calculated different extra indexes. Firstly, the 430/665 index, which is calculated by dividing the absorbance of light at 430 nm and 665 nm. Secondly, the percentage between organic and inorganic matter, which is the result of dividing the dry mass weight by the ash weight. Lastly, the autotrophic index, obtained by dividing the AFDM by the Chl-*a* content.

In order to analyse the algal community structure of the biofilms, a 15 µl drop of the scraped sample was mounted under a 22x22 mm slide cover and observed with an optic microscope (Nikon E600, Tokyo, Japan) at ×400 to identify all algal species found (mainly diatoms) to a genera taxonomic level, counting until 300 cells. 25 cells per genera found were measured in different dimensions in order to estimate its cellular biovolume from the shape equations proposed by Hillebrand et al. (1999). Total algal biovolume per sample was calculated by multiplying the cell abundance by the calculated biovolume for each genus and then expressed as density ($\mu m^3/cm^2$) considering all the surface and volume measurements.

Macroinvertebrate samplings and analyses

Macroinvertebrate samplings were performed on each cage by taking out 5 cobbles and using a hand net to remove 0.0625 m^2 of sand and gravel (corresponding to the surface of the hand net)

from the cages. Macroinvertebrates were washed from the cobbles and sediment and stored in alcohol until their identification. Individuals were identified using a stereoscopic microscope (Optika SZR-10) by means of identification keys (Campaioli, 1999; Malicky, 2004; Tachet et al., 2010; Oscoz et al., 2011). Taxonomic resolution was based on the IBMWP biological quality index (Alba-Tercedor et al., 2002) in which taxa were identified up to family level, except for Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Hydracarina, and Collembola. For each sample, all individuals found were counted. Taxon abundance was calculated by extrapolating the abundance of all individuals of each taxon present on the analysed fraction plus the number of individuals on the rest of the sample. Considering the sampled area, results were expressed in density (ind/m^2). Dry mass for each taxon in the samples was assessed using a representative sub-sample of each taxon dried at 60 ºC, until constant weight, using a drying oven (Raypa DOD-20) and weighed using an analytical scale (Sartorius Practum 124-1S). For biomass calculations, sub-samples that did not reach the detection limit (DL = 1 mg) had an assigned value of 0.7·DL. Samples that had 30 % of the taxa under the detection limit were not considered (Gough et al., 1998; Bennet et al., 2000). Total weight of each sub-sample was divided by their total number of individuals. These values were multiplied by the abundance, and the biomass was expressed as mg of dry mass/m^2 for each taxon. Finally, the Iberian Biological Quality Index for macroinvertebrates (IBMWP) was calculated (Alba-Tercedor et al., 2002).

Table 1. Biofilm and macroinvertebrate analysed variables after 7 days of the experiment, with their average and standard deviation values, plus the results of the ANOVA analysis, with the F value and its significance (*p*-value). Variables include Chlorophyll-*a* (Chl-*a*) concentration, ash-free dry mass concentration (AFDM), index 430/665, autotrophic index (A.I.), inorganic matter concentration (I.M.), organic matter percentage (% O.M.), algal biovolume, macroinvertebrate biomass, macroinvertebrate density, and IBMWP biodiversity index. Significant values are in bold. *Variables analizadas de biofilms y macroinvertebrados después de 7 días de experimento, con sus valores promedio y de desviación estándar, más los resultados del análisis ANOVA, con su valor F y significación (*p-valor). Las variables incluyen la concentración de Clorofila-a (Chl-a), concentración de biomasa seca sin ceniza de biofilms (AFDM), índice 430/665, índice autotrófico (A.I.), concentración de materia inorgánica (I.M), porcentaje de materia orgánica (% O.M.), biovolumen algal, biomasa de macroinvertebrados, densidad de macroinvertebrados e índice de biodiversidad IBMWP. Los valores estadísticamente significativos se muestran en negrita.*

Variables	Control	Exclusion	Inclusion	F value	*p*-value
Chl-a (mg/m²)	28.81 ± 16.58	19.52 ± 6.94	25.60 ± 7.07	0.536	0.610
AFDM (mg/m²)	801.18 ± 233.77	610.27 ± 50.95	598.28 ± 105.95	1.704	0.259
430/665	2.04 ± 0.07	2.04 ± 0.03	2.01 ± 0.02	0.540	0.609
A.I.	31.19 ± 11.68	34.72 ± 14.63	25.14 ± 9.59	0.478	0.642
I.M. (mg/m²)	1477.46 ± 356.73	1109.25 ± 211.86	1558.76 ± 336.10	1.811	0.242
%O.M.	35.00 ± 6.22	35.78 ± 3.19	27.86 ± 1.68	3.319	0.107
Biovolume (µm³/cm²)	9.22x10^6 ± 4.88x10^6	1.08x10^7 ± 7.23x10^6	1.36x10^7 ± 6.79x10^6	0.370	0.705
Macro. Biomass (g/m²)	0.85 ± 0.26	0.79 ± 0.54	0.73 ± 0.36	0.068	0.935
Macro. Density (ind /m²)	1398.53 ± 313.99	1113.57 ± 123.72	2236.87 ± 981.26	3.885	0.083
IBMWP	124.00 ± 26.06	118.67 ± 2.52	108.33 ± 20.84	0.510	0.624

Statistical analyses

All statistical analyses were performed using the R software version 1.2.1335. The metrics used for biofilm, macroinvertebrate, and abiotic parameters analyses were checked for normality using the Shapiro test and for homoscedasticity using the Levene test. If data was not normal, it was transformed using logarithms in order to reach data normality. All the variables were compared between treatments using ANOVA analyses for dependent variables.

RESULTS

None of the analysed general biofilm and macroinvertebrate structural variables (Table 1) presented statistically significant differences between treatments after 7 days of experimentation. Despite this, a qualitative impact of the trout inclusion was observed, in which there was a reduction in the organic matter proportion and promotion of the autotrophy of the biofilm (relation between AFDM and Chl-*a*). Considering the algal community of the biofilms (Table 2), we only found an effect of the treatment with the *Cymbella* genus, since no individuals were found in the inclu-

sion treatment. Qualitatively, it was observed that the algal genera *Gomphonema* was favoured by the trout inclusion, while *Cocconeis* density diminished.

Considering the macroinvertebrate families, we observed that three of the families (Baetidae, Glossosomatidae, and Simuliidae) presented differences in their densities between treatments, but only Glossomatidae and Simuliidae were statistically significant (Table 3). By comparing the inclusion and exclusion treatments, we observed that, qualitatively, there was a predation pressure from the trout on the macroinvertebrate community (Fig. 2). Trout consumed preferably heptagenids, ephemeropterans, different tricopteran families (Glossosomatidae; Odontoceridae, Philopotamidae and Goeridae), Ancylidae molluscs, Leuctridae plecopterans, Elmidae coleopterans, and dipterans (Empididae, Athericidae and Ceratopogonidae). The lack of consumption of the most abundant families (Baetidae, Hydracarina, Chironomidae and Planaridae) when the trout was present was remarkable.

Previous information about the biofilm and macroinvertebrate community of this stream (López-de Sancha et al., 2022) has shown that, in summer, the seasonal production of the

Table 2. Averaged algal biovolumes ($\mu m^3/cm^2$), with standard deviation, of different algal genera found in the biofilms under different treatments after 7 days of experiment. Also, results for the ANOVA analyses checking for differences in the biovolume of each genus between treatments. Significant values are in bold. *Valores promedio de biovolumen algal ($\mu m^3/cm^2$), con desviación estándar, de diferentes géneros algales encontrados en los biofilms bajo diferentes tratamientos tras 7 días de experimento. Además, resultados de los análisis ANOVA que comparan diferencias en el biovolumen de cada género entre tratamientos. Los valores estadísticamente significativos se muestran en negrita.*

Algae genera	Control	Exclusion	Inclusion	F value	*p*-value
Achnanthidium	4475.07 ± 3936.41	4378.39 ± 1388.15	4903.24 ± 1680.24	0.406	0.683
Cocconeis	154167.35 ± 146875.65	116667.18 ± 45869.03	71528.09 ± 44296.55	2.986	0.126
Cymbella	24093.43 ± 13801.25	27105.11 ± 9035.04	0.00 ± 0.00	7.300	**0.024**
Fragilaria	1376.76 ± 1577.27	344.19 ± 596.15	688.38 ± 1192.30	0.583	0.587
Gomphonema	69039.46 ± 21568.62	67658.67 ± 18576.62	128873.67 ± 67366.86	2.504	0.162
Oscillatoria	122.93 ± 212.92	546.35 ± 328.66	375.62 ± 515.20	0.975	0.430

Table 3. Averaged densities (ind/m^2), with standard deviation, of different macroinvertebrate families (Baetidae, Glossosomatidae and Simuliidae) under different treatments after 7 days of the experiment. Also, results for the ANOVA analyses checking for differences in the density of each family between experimental treatments. Significant values are in bold. *Densidades promedio (ind/m^2), con desviación estándar, de diferentes familias de macroinvertebrados (Baetidae, Glossomatidae y Simuliidae) bajo diferentes tratamientos tras 7 días de experimentación. También, resultados de los análisis ANOVA que buscan diferencias en la densidad de cada una de estas familias entre los distintos tratamientos experimentales. Los valores estadísticamente significativos se muestran en negrita.*

Families	Control	Exclusion	Inclusion	F value	p-value
Baetidae	709.90 ± 287.30	435.47 ± 152.12	1394.07 ± 640.06	4.256	0.071
Glossosomatidae	38.97 ± 19.57	28.83 ± 9.92	5.10 ± 4.52	5.419	**0.045**
Simuliidae	6.60 ± 6.32	5.03 ± 8.72	30.77 ± 5.46	12.850	**0.007**

biofilm community of this stream is the lowest and presents a low level of biodiversity, with a dominance of just 3 to 6 genera, and is highly heterotrophic. Despite this, the macroinvertebrate community in summer is not the lowest in this season and presents high biodiversity values, with a dominance of filter feeder macroinvertebrates. Glossomatidae and Simuliidae macroinvertebrates present similar densities in this season.

DISCUSSION

Due to the short duration of the experiment, it was not possible to obtain robust and statistically significant results, but tendencies and statistically non-significant results were observed. Consequently, we consider the observed effects of the different treatments to have a qualitative interpretation.

Considering the biofilms, we observed changes in the biovolumes of different algal genera, as well as in the biofilm production values, with an increase of the autotrophy of the biofilm in presence of the trout, but without a change in the total biomass. This could be caused by a substitution of species in each kind of treatment, which could be related to the different traits of the algal species found (for example, *Cymbella* being a pedunculated genus), since algal communities in biofilms are highly sensitive to changes in the environment (Berthon et al., 2011; Corcoll et al., 2012) and each type of trait provides different resistance and resilience strategies to multiple stres-

sors (Round et al., 1990; Leira et al., 2009). This fact would indicate an impact of the trout on the structure of the biofilm community.

This impact could be, on one hand, caused by the nutrient addition that trout excretions suppose in this oligotrophic environment, which would be promoting the autotrophy of the biofilm (Rüegg et al., 2011). It is known that fish are able to modify the biogeochemical activity of streams when they are aggregated by increasing the concentration of the dissolved nutrients with their nitrogen and phosphorous excretions (Capps & Flecker, 2013), altering the primary production and respiration rates of the ecosystem (Taylor et al., 2006). Al-
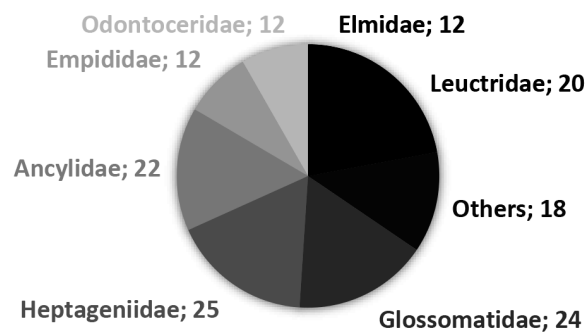
Figure 2. Number of individuals of each macroinvertebrate family that diminishes in presence of the trout: differences from the exclusion to the inclusion treatments. *Número de individuos de cada familia de macroinvertebrados que disminuye en presencia de truchas: diferencia entre los tratamientos de exclusión e inclusión.*

though, the fast water flow of headwater streams could impede this nutrient enrichment.

On the other hand, the changes to the biofilm community could be caused by the direct predatory role of trout over macroinvertebrates that feed on biofilms. Fish presence can cause trophic interactions via predation, in a cascade effect that modifies the biomass of the algae in the biofilms and its organic matter content (McIntosh & Townsend, 1996; McIntosh & Winterbourn, 2003; Winemiller et al., 2014). The trout presence could be causing a reduction in the density of certain macroinvertebrate families by selective predation, but also by causing the macroinvertebrates to escape the cage in presence of a predator. Multiple similar experiments (Schofield et al., 1988; Flecker & Townsend, 1994; Meissner & Muotka, 2006) have found this kind of trout to have an impact on the macroinvertebrate community.

The short duration of this experiment, plus the lack of replicas under different seasons, positions from the dam, and precipitation regimes, do not allow us to extrapolate robust conclusions for our hypotheses, but it allows us to understand that the brown trout has direct and indirect impacts on the trophic chain due to its predation on macroinvertebrates, and due to the nutrient enrichment through excretions that they imply. On one hand, the drastic decrease in Glossomatidae abundances in the presence of trout is evidence of this direct impact, as this group feeds on biofilms and is usually found on the surface of stream rocks, and builds a new protective case for each shedding, which makes them vulnerable to the trout predation. On the other hand, as an indirect impact, the nutrient increase that the trout excretions imply would cause a biofilm production increase that favors the Simuliidae, as this family, despite being mainly filter feeders, can feed on biofilms in situations and environments with a low concentration of particles in suspension (Tachet, 2010), which is a characteristic of headwater streams.

Hydrologic alterations such as damming, and the expected water heating and flow reduction due to climate change on headwater streams are expected to have negative consequences for the brown trout populations (Reyes-Gavilán et al., 1996; Mims & Olden, 2013; Muñoz-Mas et al., 2016; 2018). A decrease in the population densities of this species could cause cascading trophic effects on the whole ecosystem structure of headwater streams. Effective stream management requires a better understanding of the relationship between anthropogenic alterations and the biotic responses of the stream biota (Mims & Olden, 2013). More research on this topic is encouraged in order to effectively manage the populations of brown trout and other predators in headwater streams.

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