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Indirect effects of brook trout (*Salvelinus fontinalis*) on the structure of epilithic algal communities in an oligotrophic boreal forest stream

José A. Bechara*, Dolors Planas and **Serge Paquet**[1]

With 4 figures and 4 tables

Abstract: The impact of fish on epilithic algae community structure was studied in a replicated series of artificial outdoor channels fed by a boreal forest stream (Canada) to test the hypotheses that brook trout (*Salvelinus fontinalis*) induce a three-trophic chain top-down change in epilithic community structure, at both taxonomic and physiognomic levels. Fish were introduced into five of ten channels and the epilithic algae growing on tiles were sampled four times during summer to determine biomass, algal physiognomy and species composition in relation to presence of fish and invertebrate community structure. Diatom biomass showed distinctive development patterns, increasing at the beginning of fish introduction and decreasing towards the end of the experiment. The responses were the reverse in the absence of fish. Chrysophyceae increased in the absence of fish, particularly at the end of the experiment. From the seven algal physiognomic types considered, epilithon overstory, attached erected algae and loosely attached algae were generally more abundant in the presence of fish. Species' biomass was markedly different in both treatments and followed contrasted temporal patterns. In the presence of fish, attached erected diatoms species rapidly reached a complex spatial overstory structure, while in the absence of fish the epilithic community remained at a younger successional stage. Partial redundancy analysis showed that those changes were mediated by large grazing invertebrates, which had lower abundance over the bottom surfaces in the presence of fish. Variance partitioning analysis reinforced the hypothesis that most of the variability in epilithic community structure was explained by fish-invertebrate interactions and that the effect of fish presence alone was not significant. The results show that reduction in total epilithic algal biomass was a transient top-down effect, but the three-trophic level control on major taxa, species composition and community physiognomy remained along the summer experiment.

Key words: epilithon, three trophic effects, top-down control, community structure, invertebrate grazing.

Introduction

Effects of top predators transmitted through the food webs in terrestrial and aquatic environments have been widely studied, generating a large amount of field and theoretical work under the designation of "top-down-bottom-up controls", or "cascading trophic interactions" (Paine 1980, Oksanen et al. 1981, Carpenter et al. 1985, Fretwell 1987, McQueen et al. 1989, Power 1992, Polis et al. 2000). While variations in the relative importance of top-down and bottom-up controls in different environments have been object of considerable debate (Strong 1992, Morin 1999, Shurin et al. 2006), there is overwhelming evidence about the importance of grazers top-down effects in determining and controlling periphytic algae biomass, size-structure and assemblage composition in aquatic ecosystems (Feminella et al. 1989, Feminella & Hawkins

1995, Biggs et al. 1998, Bourassa & Cattaneo 1998, Morin et al. 2001, Polis et al. 2000, Shurin et al. 2002). Some of these studies have found that, under certain circumstances, grazers may override any nutrient or hydrological effects in the regulation of periphyton community structure in unshaded streams.

Fish, being the common top predators in many aquatic ecosystems, may play a fundamental role in top-down control of primary producers' community structure. Their effects can even expand to terrestrial habitats by indirectly affecting plant pollination through trophic cascades impacting both aquatic and terrestrial invertebrates (Knight et al. 2005). In addition, fish can produce a top-down effect by intimidation, forcing grazers to hide or drift (Bechara et al. 1993, Flecker 1992, Forrester et al. 1999). However, most research on top-down control in flowing waters has focused on coarse biomass responses of the periphytic community such as chlorophyll-*a* (Bechara et al. 1992, Flecker & Townsend 1994, McIntosh & Townsend 1996, Forrester et al. 1999, Rosenfeld 2000, Meissner & Muotka 2006), ash-free dry matter (Bechara et al. 1992, McIntosh & Townsend 1996) or more rarely physiognomy (Hill & Harvey 1990, Power 1990). Very few studies have analyzed the indirect effect of fish on the composition of epilithic assemblages at different taxonomic levels. In a previous paper Bechara et al. (1992) found that the top-down effects of fish on epilithon chlorophyll-*a* and AFDW appeared only on the first weeks, being undetectable the rest of the summer experiment. However, fish control on the abundance of a particular grazer species could also result in modifications in epilithic community structure at different taxonomic and functional levels, generating compensations within the algal assemblage that hide top-down effects at the total biomass level (Tessier & Woodruff 2002).

Brook trout (*Salvelinus fontinalis* Mitchill) is the only fish species inhabiting the Montmorency Basin, in the Eastern boreal Canadian Shield. A system with one fish species represents an ideal condition to validate the top-down hypothesis because the confounding effects of several interacting fish species are absent. In spite of not being herbivorous, brook trout can also affect periphyton community structure by bioturbation of the substrate when swimming close to the bottom, by chasing invertebrates hidden among algal mats, or even by releasing nutrients that enhance algal growth, such as in some lacustrine food chains (Vanni & Findlay 1990, Attayde & Hansson 2001). These fish-induced modifications can be confounded with the classical top-down cascading interaction, but direct and indirect effects can be partitioned

out when analyzing community structure (Borcard et al. 1992).

In the present paper, we test the hypothesis that brook trout predation or intimidation on invertebrates induce a three-trophic level top-down change in epilithic community structure, at both taxonomic and physiognomy levels. We designed a replicated field experimental set-up, where the presence and absence of fish was controlled and the bottom-up variables that could influence periphyton growth or species composition were kept constant among treatments. We analyzed changes in epilithon communities on several occasions along a summer season and explored the relative importance of direct and indirect fish effects using variance partitioning analysis. Our main predictions were that i) the effects of fish on algal community structure will be indirect, through control of large invertebrate abundance over exposed surfaces, and ii) fish effects on large invertebrate grazers would provoke a modification in epilithic assemblage structure and physiognomy, by favoring the growth of the more grazing-vulnerable algae.

Methods

Study site

The experiments were conducted in an outdoor, flow-trough channel system fed by a second order stream on the Eastern Canadian Shield (Ruisseau des Cascades stream, Forêt Montmorency, Quebec, Canada, 47°17′ N, 71° 07′ W). The stream was naturally inhabited by a low density of brook trout, presumably due to the abundance of cascades that blocked upstream migration. The drainage basin lies at an altitude of 800 m and is covered by a boreal forest dominated by balsam fir (*Abies balsamea* L.). During the study period (June – September), the water temperature of the stream varied between 8 and 19 °C, the pH was slightly acidic to neutral (6.7–7.2), and the conductivity was low (18–24 μS/cm). Reactive soluble phosphorous concentrations varied between 2.5 and 8.1 μg/l, and nitrates between 63.0–68.6 μg/l (Bechara et al. 1992).

Experimental system

The set-up was located besides the stream, and consisted of five plasticized wooden channels measuring 6 m length, 40 cm in width and 40 cm in height. Each channel was completely divided into two 20-cm wide halves by a plasticized wood, which prevented water exchange between each half. The system was fed by gravity from a pool in the stream through a PCV pipe to a wooden plasticized head box. Water entered the experimental system through the head box reservoir which distributed the water evenly among the channels so as to maintain a similar current speed through the system (6–7 cm/s). Water depth was maintained at about 35 cm and discharge from each channel averaged 3.5–4.5 l/s. The bottom of the channels was covered with smooth granite pebbles (2–4 cm diameter) placed within

plastic mesh baskets (10 × 10 × 7 cm). Granite pebbles were obtained from a quarry located in the same river basin. Unglazed ceramic tiles (2 × 5 cm), placed vertically on the sides of each channel to prevent sediment accumulation, were used as substrate and sampling units for periphyton. The position of the tiles did not affect the results of the experiments, since tiles placed at a 45° angle over the bottom of the channels showed total chlorophyll-a responses similar to those placed vertically (Bechara et al. 1992). Moreover, vertical tiles emulate epilithon growing on sides of large boulders, which are typical of Canadian Shield streams. Experiments were carried out in the ice free season, from early June to early September.

Channels were colonized by drifting algae and stream invertebrates over a period of about 3 weeks prior to the introduction of fish. At the end of this period, eight brook trout collected from a nearby stream, were introduced into one halve of each of the five channels following a randomized block experimental design, producing five replicate "No Fish" and five replicate "Fish" treatments. Brook trout, the only fish inhabiting streams in the region, reach high densities but in populations of stunted individuals that attain small maximum size (c. 18 cm). The fish density used (6.5 individuals/m^2, 70 g/m^2) was close to that measured in pools of a neighboring stream (Ruisseau des Eaux Volées Stream, Richard Lacelle, unpubl. data). This density was chosen to more closely imitate natural conditions since environmental traits and macroinvertebrate community composition was similar to that of beaver pools that were common in the area streams (Bechara et al. 1993). To prevent fish from escaping and to eliminate predation by terrestrial vertebrates, movable wire mesh screens were placed at both ends of the channel and over the entire experimental system. Trout number was monitored weekly and occasional losses were replaced as soon as possible so as to maintain densities between 4.4 and 6.5 individuals/m^2 over the entire study period. Scarce losses occurred after capture and manipulation of fish for stomach content analysis by stomach flushing (Bechara et al. 1992). Further details about trout responses to experimental manipulations can be found in Bechara et al. (1993).

Field sampling and biological analyses

Three ceramic tiles were randomly sampled in each of the channels 4 d before and 21, 50, and 65 d after fish introduction. Each tile was carefully washed with a soft brush and preserved in a 1 % Lugol's solution (Wetzel & Likens 1991). Samples from each half of the channel were pooled and a subsample was sedimented in an Utermöhl counting chamber. The algae with intact protoplasm-containing cells were identified and counted with an inverted microscope (Olympus IMT-2) at the species level using two different magnifications; 600× was used for the smaller and more common algae and 150× for the larger and rarer ones. For each taxon, cell dimensions were measured in a minimum of 20 cells and the biomass, in wet weight (µg/m^2), was estimated by multiplying the cell abundance to the specific biomass (pg per cell) calculated by applying average dimensions to a geometric form that best approximated the cell shape, to get a specific biovolume (µm^3, Table 1) and using 1 pg/cm^3 as a density value (Proulx et al. 1996).

The species were placed into seven physiognomic categories according to position, attachment, growth structure and architecture (Table 1). The classification system adopted by Wellnitz & Ward (2000) was employed with some modifications to fit the particular traits of algae colonizing our experimental system. According to the degree of attachment to the substrate, four categories were recognized: attached erect algae (attached by a mucilaginous pad at one end); attached filamentous algae

Table 1. Physiognomy type classification according to attachment modes and canopy position of the 15 main algae species found colonizing tiles. Attachment: AE = attached erect; P = prostrate; LA = loosely attached; AF = attached filamentous. Canopy: O = overstory, U = understory, T = tychoplankton.

Species	Attachment	Canopy	Mean biovolume (µm^3)
Cyanobacteria			
Lyngbya taylorie (Drouet)	LA	T	144.5
Diatoms			
Achnnantes linearis (W. Smith)	P	U	366.6
Achnnantes minutissima (Kützing)	P	U	75.0
Eunotia pectinalis (Müller)	AE	O	5389.9
Eunotia pectinalis undulata (Rabenhorst)	AE	O	674.7
Fragillaria intermedia (Grunow)	AE	O	662.3
Gomphonema acuminatum (Ehrenberg)	AE	O	3455.8
Gomphonema constrictum (Ehrenberg)	AE	O	29565.5
Meridion circulare (Agardh)	P	U	3180.7
Tabellaria fenestrata (Lyngbye)	AE	O	4318.3
Tabellaria flocculosa (Roth)	AE	O	2411.6
Chlorophyta			
Mougeotia sp.	LA	O	25447.0
Oedogonium sp.	AF	O	8815.1
Pseudendoclonium sp.	AE	U	170.4
Chrysophyceae			
Stichogloea doederleinii (Schmidle)	LA	T	139.7

(mainly Chlorophyta); non-filamentous prostrate algae (non motile or motile such as raphid diatoms) and loosely attached algae (mainly tychoplankton; i.e., epilithic algae that can be easily dislodged and occasionally being a component of phytoplankton communities). Following the relative position respective to the substrate, three canopy development categories were established: overstory, understory and tychoplankton (Table 1). Algae of the overstory and loosely attached forms are presumed to be more susceptible to grazing (Steinman 1996). There was some degree of redundancy between the groups formed according to the two physiognomy classification criteria (attachment and relative position). For example, attached erect and overstory algae shared about 75 % of the species (19 out of 25), and it would be expected that both groups exhibit similar responses to experimental treatments.

Random samples of epibenthic invertebrates were obtained during daylight from the walls of each channel by covering a 10×10 cm quadrate with a transparent Plexiglas box. All attached organisms were removed by siphoning the wall surface with a brush attached to a plastic tube. Samples were filtered with a 250 μm-mesh screen. Three replicate samples from each channel half were collected 22, 51 and 66 days after fish introduction. The density of the large trichopteran *Psychoglypha subborealis* (Banks) was estimated by counting all the larvae crawling on the channel walls.

Invertebrates were preserved in 4 % formaldehyde and later separated and identified to the genus or family level (Bechara 1992). All organisms were measured to the nearest 0.1 mm using a dissecting microscope fitted with a drawing tube and a digitizing tablet connected to a Sigma-Scan Scientific Measurement System (R). Biomass was estimated from regression relationships that we developed, between body length or head width and body weight, for each taxon (Bechara 1992).

Data analyses

Algae and invertebrate biomass data were first tested for normality and homogeneity of variance using the Kolmogorov-Smirnov test and the Cochran test, respectively (Sokal & Rohlf 1995). Whenever data did not conform to the assumptions of normality and homogeneity of the variance they were transformed using the Box-Cox algorithm or the Box-Cox-Bartlett algorithm (Sokal & Rohlf 1995). To test for indirect effects of fish on major taxa and physiognomic groups of the epilithon, the normally distributed data was analyzed using repeated measures factorial ANOVA, with FISH (presence-absence) and TIME (0, 21, 50 and 65 days after fish introduction) as main treatments. To test for significant differences in temporal patterns between the two FISH treatments, orthogonal polynomial trend analyses were applied, considering linear, quadratic and cubic components. Although the experiments were originally set-up using randomized block design, block effects were never significant in ANOVA tests. For that reason we excluded block effects from the ANOVAs to increase test power.

To test for the indirect top-down effects of fish on epilithic algal community structure at the species level, the significance of FISH and TIME on taxonomic composition were analyzed using canonical discriminant analysis (Tabachnick & Fidell 2001). Eight groups were formed by pooling together the two fish treatments with the four different sampling times. Forward stepwise selection was applied to obtain an uncorrelated set of species, representative of the whole community variations, using a 15 % significance level cut-off point.

To test our first hypothesis concerning the relative importance of invertebrates and FISH×TIME in structuring epilithic algal community, the variability was partitioned by combining a series of redundancy analysis and partial redundancy analysis (RDA and PRDA, respectively, Ter Braak & Smilauer 1998). If most of the variability in epilithon community structure is explained by the shared effect of fish and invertebrates, the trophic cascade hypothesis will be supported. Scores of the first and second discriminant axes were employed as dependent variables (excluding samples obtained four days before fish introduction). Invertebrate data were log-transformed biomass values. FISH presence was coded as a dummy variable (0 = absence, 1 = presence) and TIME was expressed in days after fish introduction (21, 50 and 65). To reduce the chances of an artificial increase in explained variance, independent variables (invertebrates, FISH and TIME) that contributed most to explanation of relative biomass were selected using stepwise forward selection based on a Monte Carlo permutation test for the sum of all eigenvalues, as implemented in CANOCO 4 (Ter Braak & Smilauer 1998). A 25 % significance level was used as cut-off point since it allowed keeping a representative subset of the whole invertebrate community, while the variance inflation factors in final models were always inferior to 2.0, indicating low correlation among independent variables.

In a next step, and following Borcard et al. (1992), four different RDA and PRDA were performed in order to partial-out the total variance of the epilithic community structure explained by independent variables: (1) epilithic variation related to benthic invertebrate taxa alone, estimated as the variation explained by these variables after statistical removal, by PRDA, of FISH×TIME variables; (2) epilithic variation related to FISH×TIME variables alone, estimated as the variation explained after statistical removal, by PRDA, of invertebrates variables; (3) epilithic variation explained by both FISH×TIME and invertebrates, estimated as total variation explained by invertebrate variables minus quantity (1) above, or total variation explained by FISH×TIME variables minus quantity (2) above; (4) unexplained variation and stochastic fluctuation, estimated as the remaining variation after the removal of the three previously calculated percentages.

Results

Variations in major algal taxa

The algal community was largely dominated by diatoms (74 %, all dates pooled). There were significant differences in the temporal pattern for both total and diatom biomass, in the presence and absence of fish (Fig. 1, Table 2). In the absence of fish, total algal and diatom biomass first declined, and then increased steadily, until the end of the experiment. In the presence of fish, the trend was reversed. Only the TIME effect was significant in Cyanobacteria and Chlorophyta epilithon biomass (Fig. 1, Table 2). In contrast, FISH but not TIME had a significant effect on Chrysophyceae biomass, which increased in the absence of fish (Table 2).

Table 2. Repeated measures ANOVA results (*F* values) for biomass of large taxa and physiognomy types in the presence and in the absence of fish along the experiment. Significant differences are denoted by asterisks (* = P < 0.05; ** = P < 0.01). Biomass data are shown in Figs. 1 and 2. Results for understory were not significant and are not shown.

| Dependent Variable (large taxa or physiognomy types)	Treatments			Polynomial trend analysis		
	Fish (d.f. = 1,8)	Time (d.f. = 3,24)	Fish × Time (d.f. = 3,24)	Linear (d.f. = 1,8)	Quadratic (d.f. = 1,8)	Cubic (d.f. = 1,8)
Total Algae	0.34	2.99	4.31*	0.32	8.73*	4.51
Diatoms	1.62	2.09	4.26*	0.04	9.54*	4.26
Cyanobacteria	1.41	5.31**	1.97	0.38	2.81	0.06
Chlorophyta	1.58	5.48**	1.91	0.04	3.85	1.24
Chrysophyceae	6.42*	5.14	2.02	4.37	1.13	1.14
Tychoplankton	3.12	0.88	2.09	1.27	5.21	0.21
Overstory	1.70	3.92*	4.53*	0.14	14.81**	3.59
Attached erect	0.92	3.53**	5.24*	0.02	30.85**	2.20
Attached filamentous	0.25	4.12*	3.35*	3.56	0.28	5.04
Prostrate	0.01	1.99	1.85	0.12	0.73	8.76*
Loosely attached	1.72	1.60	2.26	0.29	6.33*	0.08
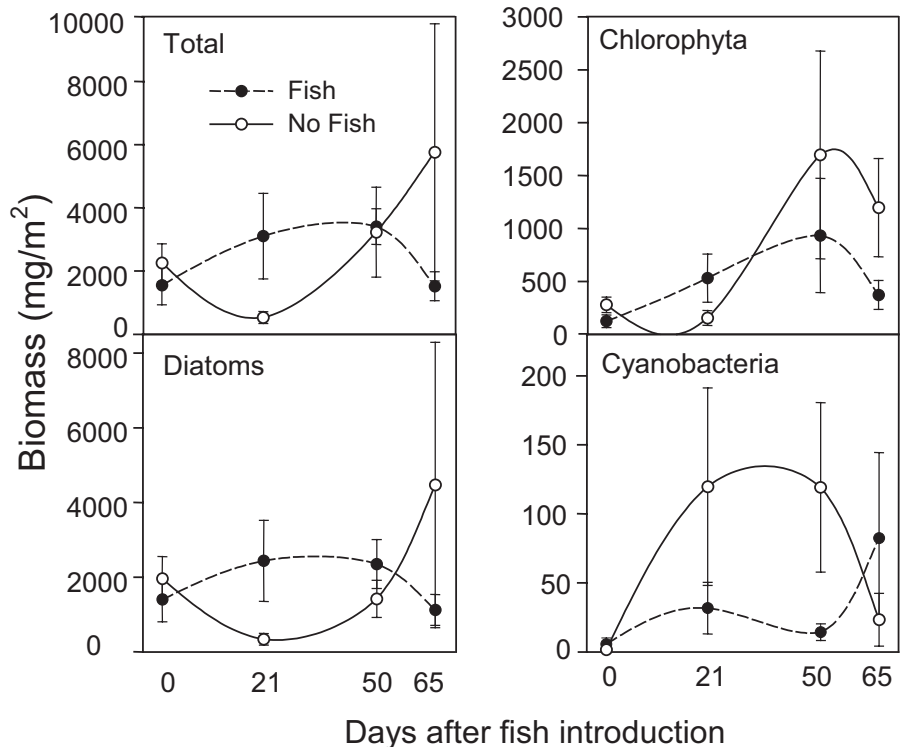
Fig. 1. Biomass (mean ±1 SE) of major algal taxa in the presence and absence of fish on the four sampling dates. Scaling ranges for biomass are different.

Algal physiognomy responses

In the presence of fish epilithic algae growing at the overstory first increased and then decreased, describing a unimodal response curve in function of time. These responses were significantly different in the absence of fish (Fig. 2, Table 2). The understory (not

shown) and the tychoplankton (Fig. 2, Table 2) did not reveal significant effects respective to the treatments applied.

All of the different degrees of attachment were significantly affected by the FISH treatment or the FISH × TIME interaction (Table 2). As expected, attached erect algae showed a temporal pattern fairly similar to
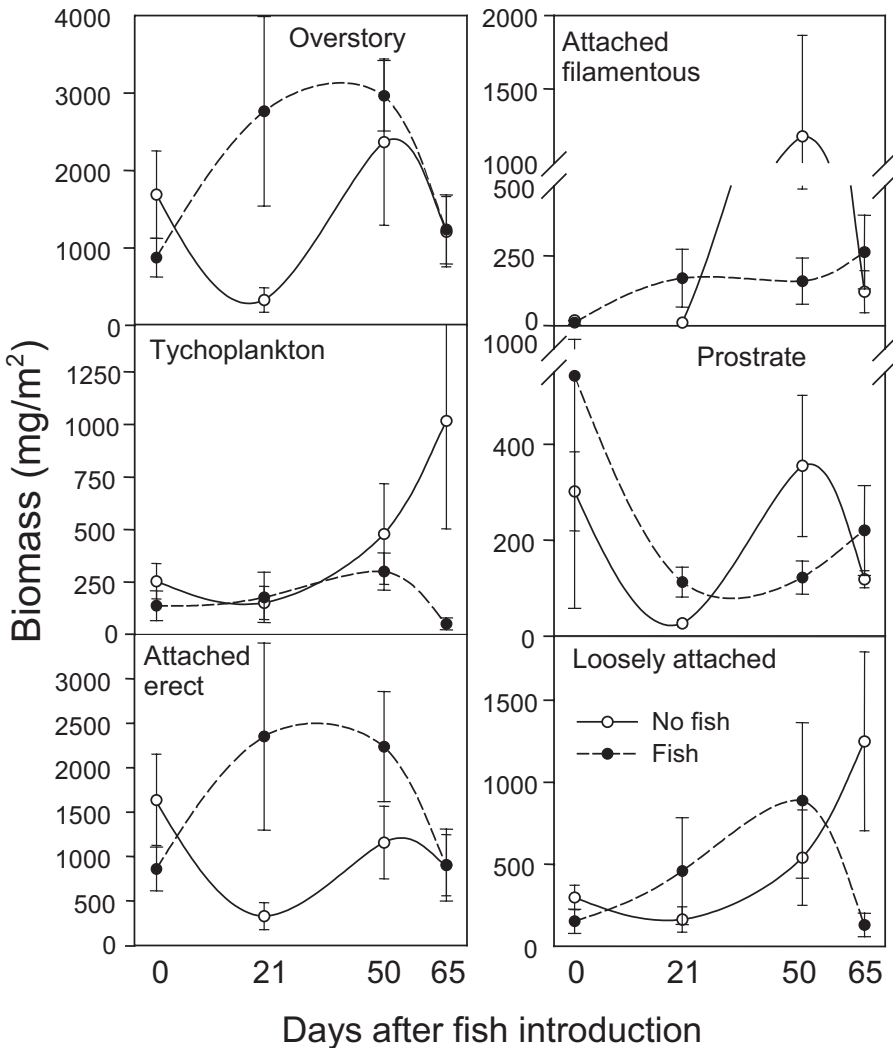
Fig. 2. Biomass (mean ± 1 SE) of the main algal physiognomy types according in the presence and absence of fish on the four sampling dates. Scaling ranges for biomass are different.

that of the overstory with an increase in the presence of fish on days 21 and 50 (Fig. 2). In the absence of fish, prostrate and attached filamentous algae increased on day 50 and loosely attached algae increased at the end of study period.

Patterns in epilithic species composition among treatments

From a total of 114 recorded species, a set of 15 was extracted using forward stepwise discriminant analysis (Table 3). This sub-set of species explained a large proportion of the community structure variation among the eight different FISH×TIME combinations (2 FISH and 4 TIME), and contributed on average for about 53.8 % of the total algal biomass. A direct discriminant function analysis was performed using the selected species as predictors of the different FISH×TIME combinations. The overall test was highly significant (Wilks' Lambda = 0.0001048, F =

2.1459, P < 0.0001, d.f. = 126, 89). From the seven discriminant functions extracted, only the first two were significant (P < 0.05) and accounted for 72.2 % (47.4 and 24.8 %, respectively), of the total variation in algal community structure. Thus, axes I and II were only retained for subsequent analyses.

The first discriminant function separated samples mostly along a temporal gradient (TIME effect), while the second discriminant function clearly ordered samples according to the FISH treatment levels (Fig. 3). The distances among group centroïds suggest that temporal variability was higher in the absence than in the presence of fish. Differences between the FISH and NO FISH treatments tended to increase over TIME. The loading matrix of correlations between predictors and discriminant functions (Table 3) shows that the best species predicting TIME differences (discriminant function I) were *Gomphonema constrictum* and *Achnantes minutissima*. These species showed a tendency
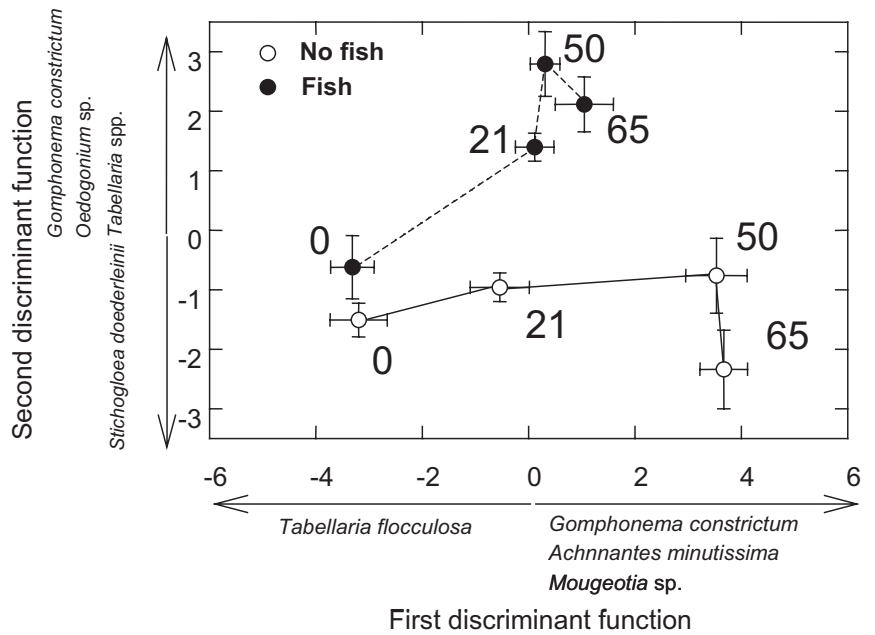
Fig. 3. Ordination diagram of the samples obtained in the presence and absence of fish on the four sampling dates in the discriminant space of the first two canonical axes. Arrows indicate direction of loadings for the most important variables (algal taxa). Vertical and Horizontal bars indicate one SE for each respective axis.

to be more abundant on latter sampling dates. *Tabellaria flocculosa* presented a reverse temporal trend. The best predictors for FISH effects (discriminant function II) were *G. constrictum*, as well as the two *Tabellaria* species, the three species being more abundant in the presence than in the absence of fish. In contrast, *Stichogloea doederleinii* had a high negative loading, being much more abundant in channels without fish (Table 3). *Pseudendoclonium* sp. also showed a negative loading on the second axis (Table 3).

Responses of epilithon to invertebrates and fish

In the redundancy analysis (RDA) using 11 invertebrate taxa as independent variables, stepwise forward selection allowed five to be retained (Table 4, A). In the RDA, using FISH and TIME as independent variables, both variables were retained (Table 4, C). When analyzed independently (RDA), both invertebrates and FISH×TIME significantly explained epilithon community structure (Table 4, A, C). However, when using invertebrates or FISH×TIME as covariables in partial redundancy analyses (PRDA), only invertebrates remained significant, while most of the apparent FISH×TIME explanation of community structure was removed by invertebrate covariables (Table 4, B, D).

The partitioning of the variance of epilithic algae demonstrated that 67.2 % was explained by epibenthic invertebrates and the experimental treatments (FISH×TIME), the remaining variance being related to sampling error or unknown factors. From the total explained variance, 34 % was related to inverte-

Table 3. Pooled within groups correlations between algae and canonical discriminant axis 1 and 2. Larger loadings appear underlined and were used in axis interpretation.

Species	Canonical axis 1	Canonical axis 2
Cyanobacteria		
Lyngbya taylorie	0.150	0.026
Diatoms		
Achnnantes linearis	0.112	−0.006
Achnnantes minutissima	0.424	0.179
Eunotia pectinalis	−0.094	0.034
Eunotia pectinalis undulata	0.090	−0.007
Fragillaria intermedia	0.171	0.179
Gomphonema acuminatum	0.198	0.132
Gomphonema constrictum	0.567	0.431
Meridion circulare	−0.108	0.013
Tabellaria fenestrata	−0.075	0.352
Tabellaria flocculosa	−0.374	0.300
Chlorophyta		
Mougeotia sp.	0.243	0.243
Oedogonium sp.	0.105	0.242
Pseudendoclonium sp.	0.024	−0.092
Chrysophyceae		
Stichogloea doederleinii	−0.098	−0.395

brate biomass alone, 55 % to both, invertebrate and FISH×TIME jointly (shared variance), and 11 % to FISH×TIME alone.

A more detailed analysis of the PRDA results removing the not significant FISH×TIME variables reveals a clear pattern in community structure explained by fish top-down control on invertebrate grazers (Fig. 4, Table 4 B). Channels with and without fish ap-

Table 4. Inter-set correlations of environmental variables with axes of the canonical correspondence analyses for epilithon community structure as dependent variables (scores of the two first canonical axis of discriminant analysis). Invertebrates and FISH×TIME were employed as independent variables (A and C) and covariables (B and D). Only significant independent variables are presented.

| A) Invertebrates. Without covariables (RDA)			B) Invertebrates. Covariables: FISH × TIME (PRDA)	
Variables	Axis 1	Axis 2	Axis 1	Axis 2
Synorthocladius	0.376	0.499	0.343	0.406
Other Orthocladiinae	–0.407	0.572	–0.186	0.504
Tanytarsini	–0.497	0.052	–0.381	0.300
Baetis spp.	–0.237	–0.408	–0.354	–0.225
P. subborealis	0.633	–0.152	0.423	–0.077
	Overall Monte Carlos test: F-ratio = 18.56, P < 0.001		Overall Monte Carlo test: F-ratio = 2.64, P < 0.01	
C) FISH × TIME. Without covariables (RDA)			D) FISH × TIME. Covariables: Invertebrates (PRDA)	
Variables	Axis 1	Axis 2	Axis 1	Axis 2
FISH	0.650	0.186	–0.356	–0.176
TIME	–0.208	0.582	0.417	–0.128
	Overall Monte Carlo test: F-ratio = 9.55, P < 0.001		Overall Monte Carlo test: F-ratio = 2.16, P = 0.1	
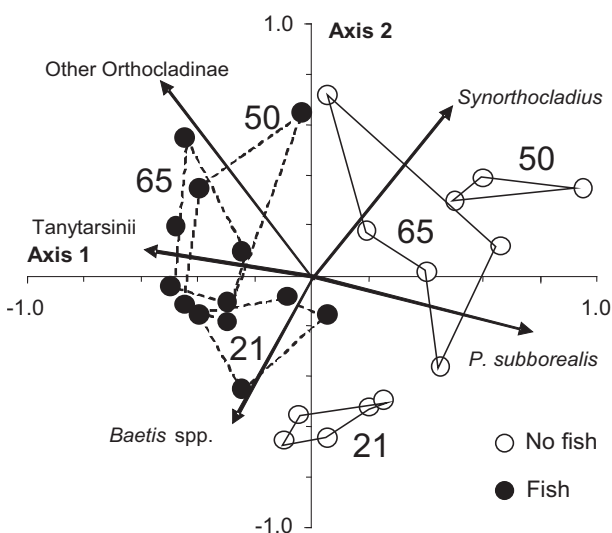
Fig. 4. PRDA ordination showing epilithic sample distribution (discriminant scores) in the presence and absence of fish in relation to invertebrates (arrows) in the space of the two first canonical axes. Line envelopes connect symbols representing scorers of the same sampling dates after fish introduction (21, 50 and 65). Covariables were presence or absence of fish, and times in days after fish introduction (See Table 4-B for further details).

peared separated mainly along the first axis (79.0 % of the explained variance), while changes with time were better expressed along the second axis (21.0 % of the explained variance). The first axis showed that the

trichopteran *P. subborealis* was positively related to epilithon communities of channels without fish, while Tanytarsini and Other Orthocladiinae (Chironomidae, Diptera) had larger biomass in the presence of fish (Fig. 4, Table 4 B). *Baetis* spp. was also important on the second axis, contributing to separate channels with and without fish on day 21 (Fig. 4).

Discussion

The results of the present study demonstrate a noticeable indirect effect of brook trout on epilithic algae communities. This indirect effect implied a modification of the temporal pattern of higher order taxa, species composition and physiognomy. The partial redundancy analyses (PRDA) and the partialling out of the variance reinforced our hypothesis that the impact of trout on species composition was largely mediated by invertebrate grazers, which in turn were affected by fish. This support is based on the 55% of the explained variation in epilithon community structure that was significantly related to the shared variability among FISH×TIME treatments and invertebrate biomass. The pure effect of FISH×TIME was not significant once the effect of invertebrates was removed, suggesting that fish did not have a noticeable impact on algal community structure through fertilization or physical

dislodging. In spite of the exploratory nature of this statistical method, it has been useful for highlighting the preeminence of top-down over bottom-up processes in large marine ecosystems (Halpern et al. 2006). Unlike that study, our experimental system allowed controlling the top predator presence, which strengthens the conclusions.

Previous studies showed the importance of fish in driving invertebrate community structure and biomass in the same experimental system (Bechara 1992, Bechara et al. 1992, 1993). In the present study, independently of the FISH treatment, invertebrate grazers affected epilithon community structure and algal succession. Furthermore, in the former studies the indirect effect of fish on total algal biomass was significant only at the beginning of the trials (Bechara et al. 1992), while in the present study community structure was affected all along the experiment. Therefore, these results suggest the existence of a top-down effect producing a non-transient three-level cascading trophic interaction. Our findings are in agreement with the study of Tessier & Woodruff (2002) conducted in lakes, which indicates that compensation within the algal assemblage may dissipate the magnitude of the trophic cascade on total biomass. An analysis of fish-invertebrate interactions helps clarify the complex mechanisms involved in the trophic cascade.

Fish reduced the densities of large insects, including *P. subborealis*, a species strongly associated with differences in algal composition between channels with and without fish. This species was heavily predated by trout due to their high size-selective feeding (Bechara et al. 1992), which suggest a direct fish induced suppression. In a cage experiment carried out in the stream feeding ours channels, Bechara et al. (1992) found that *P. subborealis* did not indirectly affect the total algal biomass, but significantly reduced chironomid density. This trichopteran is a relatively large-sized omnivorous feeder that crawls over the surface carrying a case (Winterbourn 1971, Merritt & Cummins 1996). Stomach content analysis showed that it consumed detritus, algae and small chironomid larvae (Bechara et al. 1992). In the same cage experiments, large *Baetis* nymphs reduced both epilithon biomass and chironomid density (Bechara et al. 1992). In the present study, the PRDA clearly showed the importance of this later genus in community structure differentiation 21 days after fish introduction. We could not rule out that the impact on epilithon structure of these two macroinvertebrates was a mixed effect of grazing and mechanical dislodging, as was demonstrated by Jacoby (1987) for a trichopteran of the same family (Limnephilidae), and

by Scrimgeour et al. (1991) for *Baetis* nymphs. In addition, reductions in invertebrate density on exposed surfaces may be also the consequence of behavioral changes of invertebrates in the presence of fish that induce grazers to hide in the bottom refuges (Flecker & Townsend 1994, Mcintosh & Peckarsky 1996). Those changes could have also occurred in channels without fish, due to the chemical cues from the trout naturally inhabiting the stream. But this chemical effect seems to be weak in our experimental set-up since invertebrate grazers were significantly more abundant on the epibenthos in the absence than in the presence of fish, suggesting that natural fish odor alone was not enough to prevent effective grazing on exposed surfaces (Bechara et al. 1993).

In our experiment the biomass of small Chironomidae such as Tanytarsini and Orthocladiinae larvae increased in the presence of fish, associated with dense epilithon development. Therefore, they apparently did not consume or dislodge algae as the larger invertebrates, and probably took some advantage of the abundant food and shelter. These findings are similar to those of Forrester et al. (1999), who described an increase in chironomid abundance in the presence of fish. Moreover, Meissner & Muotka (2006) in a meta-analysis of 24 studies on the impact of trout on benthic invertebrates found a positive impact of fish on chironomid larvae. Those results are similar to the results of studies in lake plankton communities, in which selective fish predation on larger invertebrate grazers allows the proliferation of more vulnerable algae, resulting in higher resource quality for the remaining grazers (Tessier & Woodruff 2002).

Other studies in different lotic ecosystems of the world have shown the indirect impact of fish on epilithon considering total biomass, major taxa, and some particular species or physiognomy types (Power 1990, Flecker 1992, Flecker & Townsend 1994, McIntosh & Townsend 1996, Rosenfeld 2000). In contrast, some authors found that fish had neither direct nor indirect impact on epilithic algal physiognomy (Hill & Harvey 1990), chlorophyll-a, or ash free dry mass (AFDM) (Meissner & Muotka 2006). However, as observed in our experiment, even if the effect of trout on total epilithon biomass disappears by the end of the summer, differences in physiognomy and community structure may remain significant. In our study, the temporal changes in the abundance of many epilithon taxa and physiognomy types in the presence and absence of fish presented a mirror image. Most taxa increased in the presence of fish at the beginning of the experiment and decreased towards the end of the experiment, while in

the absence of fish a steady recovery occurred following an initial decrease. Thus, in the absence of fish, the grazing effects seemed to keep epilithic community at a younger successional stage, while in the presence of fish, community rapidly attained a climax and became senescent afterwards. This fact was also reflected in the discriminant analysis, which showed a lesser temporal variability in the channels where fish depressed grazer abundance. Jacoby (1987) also found a healthier and most vigorous algal community in grazed periphyton mats, whereas the ungrazed community became senescent. One of the ecosystem-level consequences of grazers' algal consumption may be the enhancement of primary production as was suggested by the Lamberti & Resh (1983) model.

In the absence of fish, macroinvertebrates modified epilithon physiognomy by decreasing the biomass of the species for which growing architecture facilitates grazing or physical dislodging, such as *G. constrictum* or *T. flocculosa*. In contrast, algae growing in a single-cell layer over the bottom such as *Eunotia pectinalis undulata* were not affected by the presence or absence of fish or by invertebrate density. Some species that appeared favored by the presence of invertebrates were *S. doederleinii*, the cells of which are located within mucus masses, as well as *Pseudendoclonium* sp. that grows in prostrate colonies having short erect branches. Those adaptations probably preclude effective removal by grazers, allowing enhanced growth in the absence of a dense overstory. Similar results were found in lakes (McCollum et al. 1998, Tessier & Woodruff 2002) and streams (Peterson et al. 1998) where in the presence of grazers, the proportion of gelatinous or digestion resistant colonies increased, while large cells and filamentous diatoms decreased.

In summary, in this study we validated the hypothesis that trout induced a trophic cascade effect in a three-trophic levels stream community, by controlling the abundance of major taxa, species composition, physiognomy and successional trajectories. Those changes were mediated by large grazing invertebrates that were either removed by fish predation or avoided exposed substrates in the fish treatment. In contrast, small chironomid larvae proliferated in the presence of fish and were probably favored by higher food availability and quality. Invertebrates released from the fish effects fed on the most edible algal species thus allowing the growth of others more resistant to consumption. Therefore, reduction in total epilithic algal biomass was only a transient top-down effect, while community structure effects remained all along the summer experiment.

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