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Physicochemical and biological changes downstream from a trout farm outlet: Comparing 1986 and 2006 sampling surveys

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ABSTRACT

Physicochemical and biological changes downstream from a trout farm outlet: Comparing 1986 and 2006 sampling surveys

In this investigation we compare the results obtained in the 1986 and 2006 sampling surveys regarding physicochemical and biological changes caused by a trout farm effluent in the upper Tajuña River (Guadalajara, Spain). For this comparison three sampling sites were selected: S-1, placed upstream from the trout farm, was used as a reference station; S-2 and S-3 were respectively placed about 10 m and 1000 m downriver from the trout farm outlet. In both surveys, the concentration of nutrients (ammonia, nitrite, nitrate, phosphate) increased downstream from the trout farm, whereas the concentration of dissolved oxygen decreased. *Gammaridae*, *Heptageniidae*, *Elmidae*, *Perlidae*, *Ancylidae*, *Hydrobiidae*, *Glossosomatidae* and *Sericostomatidae* significantly decreased their abundances downstream from the trout farm outlet. On the contrary, *Tubificidae*, *Erpobdellidae*, *Glossiphoniidae*, *Planorbidae*, *Sphaeridae* and *Chironomidae* were clearly favoured. The water quality in ecological terms, as assessed by biotic indices, was markedly reduced, with average scores (ASPT, ASPT′, a-BMWQ) showing a better indicator value than total scores (BMWP, BMWP′, BMWQ). Shredders and scrapers were the functional feeding groups most severely affected, whereas collectors were clearly favoured. We conclude that the wastewater depuration system of the trout farm must be improved since, during the last twenty years, it has been insufficient to prevent significant alterations in the recipient river.

Key words: Trout farm pollution, physicochemical and biological changes, 1986 and 2006 sampling surveys, comparison.

RESUMEN

Cambios fisicoquímicos y biológicos río abajo del vertido de un criadero de truchas: Comparando las campañas de muestreo de 1986 y 2006

En esta investigación comparamos los resultados obtenidos durante las campañas de muestreo de 1986 y 2006 para los cambios físico-químicos y biológicos producidos por el vertido de una piscifactoría en el tramo alto del río Tajuña (Guadalajara, España). Para ello se seleccionaron tres puntos de muestreo: S-1, aguas arriba de la piscifactoría, sirvió de punto control; S-2 y S-3 se emplazaron, respectivamente, a 10 m y 1000 m aguas abajo del punto de vertido. En ambas campañas, la concentración de nutrientes (amoniaco, nitrito, nitrato, fosfato) aumentó río abajo de la piscifactoría, mientras que la concentración de oxígeno disuelto disminuyó. Gammaridae, Heptageniidae, Elmidae, Perlidae, Ancylidae, Hydrobiidae, Glossosomatidae *y* Sericostomatidae *redujeron significativamente sus abundancias aguas abajo del punto de vertido. Por el contrario,* Tubificidae, Erpobdellidae, Glossiphoniidae, Planorbidae, Sphaeridae *y* Chironomidae *fueron claramente favorecidos. La calidad del agua en términos ecológicos, evaluada por medio de índices bióticos, empeoró marcadamente, con las puntuaciones medias (ASPT, ASPT′, a-BMWQ) mostrando un mejor valor indicador que las puntuaciones totales (BMWP, BMWP′, BMWQ). Desmenuzadores y raspadores fueron los grupos tróficos más severamente afectados, mientras que los recolectores fueron claramente favorecidos. Concluimos que el sistema de depuración de las aguas residuales de la piscifactoría tiene que ser mejorado ya que, durante los últimos veinte años, ha sido insuficiente para prevenir alteraciones significativas en el río receptor.*

Palabras clave: *Contaminación por una piscifactoría, cambios fisicoquímicos y biológicos, campañas de muestreo de 1986 y 2006, comparación.*

INTRODUCTION

According to Alabaster (1982) and Jones (1990), effluents of inland fish farms can contain three different types of pollutants: (1) pathogenic bacteria, viruses and parasites; (2) drugs and disinfectants for disease and parasite control; (3) residual food and faecal materials. The third type of pollutants appear to be most important in generating physicochemical and biological changes downstream from fish farm outlets, primarily when artificial diets with dry pellets are used (Alabaster, 1982; Jones, 1990).

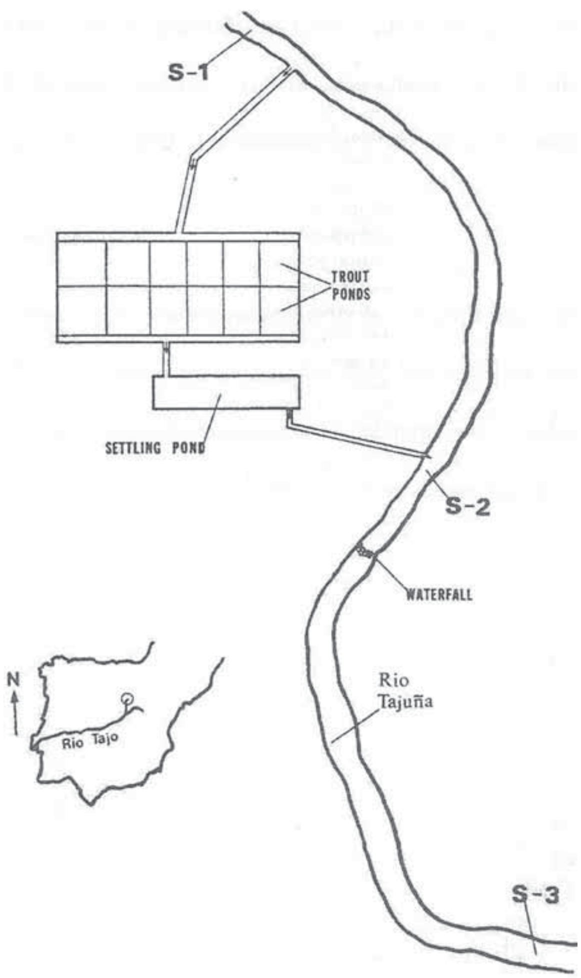

Figure 1. General diagram of the trout farm showing the location of sampling sites along the Tajuña River (Guadalajara, Spain). *Diagrama general de la piscifactoría mostrando la situación de los sitios de muestreo a lo largo del río Tajuña (Guadalajara, España).*

Physicochemical alterations caused by residual food and faecal materials downriver from fish farm effluents often are high in inorganic nutrients (ammonia, nitrate, phosphate) and suspended solids, a decrease in dissolved oxygen, and a settlement of suspended solids on the river bottom (Alabaster, 1982; Solbé, 1982; Kaspar *et al.*, 1988; Jones, 1990; Garcia-Ruiz & Hall, 1996; Nordvarg & Johansson, 2002). The biological alterations have been less studied and may depend on fish farm management, fish farm location along the river, and particular ecological characteristics of each recipient river (Camargo, 1992). A significant increase in the abundance of primary producers is expected to occur as a consequence of nutrient enrichment (Carr & Goulder, 1990; Villanueva *et al.*, 2000; Daniel *et al.*, 2005). Regarding aquatic animals, benthic macroinvertebrates can result more adversedly affected than fish because of siltation, (Mantle, 1982; Domezain *et al.*, 1987; Camargo, 1994; Oscoz *et al.*, 1999; Kirkagac *et al.*, 2004).

The main purpose of this investigation was to compare 1986 and 2006 sampling surveys regarding physicochemical and biological changes caused by a trout farm outlet in the upper Tajuña River. The community of benthic macroinvertebrates was sampled in both surveys. Additionally, we estimate the values of different biotic indices to compare their suitability for assessing the impact of fish-farming pollution on fluvial ecosystems.

THE STUDY AREA AND SAMPLING SITES

Field studies were conducted in the Tajuña River (Guadalajara province, Central Spain), a small river within the Tajo River Basin. The watershed of this river is mainly underlain by calcareous rocks such as limestone, which induce the formation of hard waters with high ionic content. The natural flow regime of the Tajuña River is characterized by maximum flows during winter and spring and minimum flows during summer and fall.

The trout farm is situated about 13 km downriver from the river source, in the upper

Tajuña River (Fig. 1). Current annual production of this inland fish farm is about 35 tons of rainbow trout (*Onchorhynchus mykiss*), using dry pellets as artificial diet, but twenty years ago the annual production was about 75 tons. Apparently, waste treatment has always involved simple settlement in a small sedimentation pond without the addition of chemicals.

For the purpose of our comparison, three sampling sites were selected along the study area (Fig. 1). A sampling site (S-1) placed upstream from the trout farm was used as a reference station. Sampling sites S-2 and S-3 were placed about 10 m and 1000 m downriver from the trout farm outlet respectively.

The riverbed was about 2-4 m wide along the study area. The river bottom was mainly stony with cobbles and pebbles at S-1 and S-3, but at S-2 it was covered by a thick layer of organic sediment.

MATERIALS AND METHODS

Water sampling and analysis

The sampling surveys were conducted during the summer (June and July) of 1986, and during the winter (December, January, February, and March) of 2006. Water temperature, conductivity, pH, and dissolved oxygen were measured *in situ* according to standard methods (American Public Health Association, 1980, 1992). Additionally, water samples for analysis of inorganic nutrients were collected using clean polyethylene containers, chilled to 1-4°C in the dark, and transported to the laboratory within 24 hours. In the laboratory, water samples were used to determine concentrations of nitrate (as NO_3-N), nitrite (as NO_2-N), total ammonia (as NH_4-N) and phosphate (as PO_4-P) according to standard methods described by the American Public Health Association (1980, 1992). Because unionized ammonia is much more toxic to aquatic animals than other inorganic nutrients (Camargo & Alonso, 2006), we also estimated concentrations of unionized ammonia (as NH_3-N) using total ammonia concentrations and Emerson *et al.*'s (1975) formulas.

Macroinvertebrate sampling and analysis

Benthic macroinvertebrates were sampled in July 1986, and in February 2006. A hand net with a 250 μm mesh size was used in 1986 to collect 5 riffle bottom samples, with a sampling area of, approximately, 0.10 m^2 per sample, at each sampling site. A Surber sampler with a 250 μm mesh size was used in 2006 to collect 3 riffle bottom samples, with a sampling area of, approximately, 0.09 m^2 per sample, at each sampling site. All samples were preserved in 4 % formalin until laboratory analyses. In the laboratory, macroinvertebrates were identified to the family level (Tachet *et al.*, 1981, 2003) and counted. After identification and counting, macroinvertebrates were dried in an oven (60°C for 48 hours) and weighed in a precision scale to obtain total biomass (dry weight) for the whole macrobenthic community at each sampling site.

To examine changes in the trophic structure, benthic macroinvertebrates were allocated to five functional feeding groups in accordance with published literature (Merritt & Cummins, 1996, Tachet *et al.*, 2003): shredders basically feed on coarse particulate organic matter; scrapers mainly feed on periphyton and perilithon; collector-gatherers feed on fine organic detritus but many of them can also feed on periphyton and perilithon; collector-filterers basically feed on organic material suspended in the water column; and predators feed on animal preys. The contribution percentage of each functional feeding group was calculated on the basis of density estimates.

Biotic indices

All biotic indices estimated in this investigation are family indices, and only qualitative data (presence/absence of families) are required. Originally, the Biological Monitoring Working Party (BMWP) and the Average Score Per Taxon (ASPT) were developed to assess freshwater quality in rivers and streams of Great Britain (National Water Council, 1981; Armitage *et al.*, 1983), but they have been subsequently used and adapted to other European countries. In Spain's case, a first adaptation of the BMWP score system was carried out by Alba-Tercedor & Jiménez-Millán (1985, 1987), on the basis of the macroinvertebrate fauna in the Guadalfeo River Basin (Alba-Tercedor & Sánchez-Ortega, 1988): the well-known BMWP′ index and its average score per taxon, the ASPT′ index, with family scores ranging from 1 to 10.

A different adaptation of the BMWP score system for the Iberian Peninsula was performed by Camargo (1993): the biological monitoring water quality (BMWQ) score system. Its family scores (from 1 to 15) reflect the tolerances of Iberian macroinvertebrate families (mainly from rivers and streams of Northern and Central Spain) to freshwater pollution (organic pollution and nutrient enrichment, particularly). The total BMWQ index and the average BMWQ index are calculated by summing the individual scores of all families present at a sampling site (the t-BMWQ index), and dividing this total value by the number of families (the a-BMWQ index). For a better interpretation of a-BMWQ values, we used the following quality ratings (Camargo, 1993): ≥12 (excellent quality); <12-10 (good quality); <10-7 (moderate quality); <7-4 (poor quality); <4 (very poor quality).

RESULTS

Mean values of water physicochemical parameters at each sampling site are presented in Table 1. Differences between the reference site (S-1) and polluted sites (S-2 and S-3) were significant ($P < 0.01$; t-test) for dissolved oxygen, nitrate, nitrite, ammonia and phosphate in both sampling surveys: while the concentration of dissolved oxygen decreased (particularly at S-2), concentrations of inorganic nutrients increased downstream from the trout farm outlet.

Mean densities of macroinvertebrate families at each sampling site are presented in Table 2. *Tubificidae*, *Erpobdellidae*, *Glossiphoniidae*, *Planorbidae*, *Sphaeridae* and *Chironomidae* were significantly favoured by the trout farm outlet, ($P < 0.01$; t-test) increasing their abundances downstream. Other families, such as *Baetidae*, *Hydropsychidae*, *Hydroptilidae* and *Simuliidae*, significantly ($P < 0.01$; t-test) increased their abundances at S-3 with regard to S-1, but they were absent at S-2. *Sialidae*, *Empididae* and *Ceratopogonidae* were only found downstream from the trout farm outlet. On the contrary, *Ancylidae*, *Hydrobiidae*, *Gammaridae*, *Heptageniidae*, *Elmidae*, *Perlidae*, *Glossosomatidae*, and *Sericostomatidae* were either absent or significantly decreased their abundances downstream ($P < 0.01$; t-test) from the trout farm outlet. All these patterns of abundance were common to both sampling surveys. In contrast, *Ephemerellidae*, *Ephemeridae*, *Leuctridae* and *Lepidostomatidae* were found only during the 1986 sampling survey (at S-1), and *Calopterygidae*, *Cordulegasteridae*, and *Gyrinidae* were

Table 1. Mean (n = 3-9) values of water physicochemical parameters at each sampling site for the 1986 and 2006 surveys. *Valores medios* (n = *3-9) de los parámetros fisicoquímicos del agua en cada sitio de muestreo durante las campañas de 1986 y 2006.*

Physicochemical parameters	S-1 (1986)	S-2 (1986)	S-3 (1986)	S-1 (2006)	S-2 (2006)	S-3 (2006)
Water temperature (°C)	14.3	14.6	14.9	9.5	8.1	7.9
Conductivity (µmhos-µS/cm)	370	373	370	627	650	689
pH	8.3	8.1	8.3	8.4	7.9	8.1
Dissolved oxygen (mg O_2/l)	9.2	5.2	5.4	9.7	4.6	7.6
Nitrate (mg NO_3-N/l)	1.6	1.1	1.2	2.5	3.3	3.9
Nitrite (mg NO_2-N/l)	0.01	0.05	0.21	0.02	0.05	0.11
Total ammonia (mg NH_4-N/l)	0.02	0.17	0.12	0.06	0.77	0.37
Unionized ammonia (µg NH_3-N/l)	0.84	7.58	5.20	1.38	16.9	8.12
Phosphate (mg PO_4-P/l)	<0.01	0.23	0.22	0.05	0.42	0.39

Table 2. Relative contributions (%) of macroinvertebrate families at each sampling site for 1986 and 2006 surveys. *Contribuciones relativas (%) de las familias de macroinvertebrados en cada sitio de muestreo durante las campañas de 1986 y 2006.*

Macroinvertebrate families	S-1 (1986)	S-2 (1986)	S-3 (1986)	S-1 (2006)	S-2 (2006)	S-3 (2006)
Planariidae	2.86	0.00	0.00	3.14	0.00	6.45
Lumbricidae	0.00	0.00	0.00	0.00	0.22	0.00
Lumbriculidae	0.78	0.00	0.00	0.00	2.19	0.05
Tubificidae	7.86	29.9	2.77	1.45	46.8	1.02
Erpobdellidae	0.59	0.65	4.97	0.00	6.74	0.42
Glossiphoniidae	0.07	1.04	0.78	0.18	0.00	0.18
Planorbidae	0.00	0.72	0.86	0.00	0.57	1.16
Lymnaeidae	0.00	0.00	0.00	0.00	0.45	0.08
Physidae	0.00	0.00	0.00	0.00	6.67	0.00
Ancylidae	3.91	0.00	0.57	0.86	0.00	1.28
Hydrobiidae	12.5	0.00	0.00	25.4	0.35	0.67
Sphaeridae	0.00	1.60	1.24	0.47	2.32	7.23
Unionidae	0.00	0.00	0.00	0.00	0.00	0.10
Gammaridae	27.7	0.00	0.00	23.9	0.00	0.00
Baetidae	1.12	0.00	12.7	5.40	0.00	6.63
Heptageniidae	1.33	0.00	0.00	0.65	0.00	0.05
Leptophlebiidae	0.28	0.00	0.00	0.00	0.00	0.08
Ephemerellidae	3.26	0.00	0.00	0.00	0.00	0.00
Ephemeridae	2.23	0.00	0.00	0.00	0.00	0.00
Perlidae	0.35	0.00	0.00	0.75	0.00	0.00
Perlodidae	0.56	0.00	0.00	0.18	0.00	0.60
Leuctridae	1.26	0.00	0.00	0.00	0.00	0.00
Nemouridae	0.07	0.00	0.14	0.00	0.00	0.00
Calopterygidae	0.00	0.00	0.00	2.21	0.00	0.00
Cordulegasteridae	0.00	0.00	0.00	0.10	0.00	0.00
Sialidae	0.00	0.13	0.00	0.00	0.22	0.00
Elmidae	17.6	0.00	0.00	16.3	0.00	3.70
Helodidae	0.00	0.00	0.00	0.00	0.00	0.03
Gyrinidae	0.00	0.00	0.00	0.96	0.00	0.00
Haliplidae	0.00	0.00	1.39	0.00	0.00	0.00
Hydropsychidae	0.49	0.00	1.19	0.26	0.00	4.85
Glossosomatidae	1.89	0.00	0.00	1.71	0.00	0.00
Lepidostomatidae	0.63	0.00	0.00	0.00	0.00	0.00
Sericostomatidae	1.54	0.00	0.00	5.72	0.00	0.73
Polycentropodidae	0.14	0.00	0.00	0.00	0.00	0.18
Rhyacophilidae	0.35	0.00	0.00	0.00	0.00	0.08
Hydroptilidae	1.82	0.00	3.06	0.00	0.00	0.39
Chironomidae	6.49	70.3	32.8	6.57	32.9	48.6

Table 2. (cont.)

Macroinvertebrate families	S-1 (1986)	S-2 (1986)	S-3 (1986)	S-1 (2006)	S-2 (2006)	S-3 (2006)
Psychodidae	0.00	0.00	0.00	0.00	0.00	0.31
Simuliidae	1.61	0.00	36.3	3.43	0.00	14.3
Athericidae	0.74	0.00	0.00	0.36	0.00	0.23
Empididae	0.00	0.00	0.43	0.00	0.00	0.34
Ceratopogonidae	0.00	0.00	0.62	0.00	0.57	0.00
Stratiomyidae	0.00	0.00	0.00	0.00	0.00	0.05
Anthomyidae	0.00	0.00	0.00	0.00	0.00	0.03

found only during the 2006 sampling survey (also at S-1). *Lumbricidae*, *Lymnaeidae*, *Physidae*, *Unionidae*, *Helodidae*, *Psychodidae*, and *Stratiomyidae* were found during the 2006 sampling survey (downstream from the trout farm outlet) but not during the 1986 sampling survey.

Biological parameter values at each sampling site are presented in Table 3. Total density and total biomass increased downstream from the trout farm effluent. Significant ($P < 0.01$; t-test) increases in these biological parameters were more evident at S-2 during the 1986 sampling survey, and at S-3 during the 2006 sampling survey (Table 3). On the contrary, the number of families decreased downstream from the trout farm effluent, especially at S-2. However, during the 2006 sampling survey, the number of families at S-3 was higher than at S-1.

Values of biotic indices at each sampling site are also presented in Table 3. During both sampling surveys, the water quality in ecological terms appeared to be degraded by the trout farm effluent (from good quality to poor quality), values of all biotic indices being markedly reduced at S-2. However, a spatial recovery of the water quality was evident with the distance to the fish farm, with values of total scores (BMWP, BMWP′, t-BMWQ) being higher at S-3 than at S-1 during the 2006 sampling survey. In contrast, values of average scores (ASPT, ASPT′, a-BMWQ) were higher at S-1 than at S-3 during both sampling surveys.

Pearson correlation coefficients for chemical parameters (dissolved oxygen, nitrate, nitrite, ammonia, phosphate) and biotic indices (BMWP, ASTP, BMWP′, ASPT′, t-BMWQ,

Table 3. Values of biological parameters and biotic indices at each sampling site for 1986 and 2006 surveys. *Valores de los parámetros biológicos e índices bióticos en cada sitio de muestreo durante las campañas de 1986 y 2006.*

Biotic indices	S-1 (1986)	S-2 (1986)	S-3 (1986)	S-1 (2006)	S-2 (2006)	S-3 (2006)
Number of families	28	7	15	21	12	29
Total density (individuals/m^2)	2864	6468	4186	3849	3143	14181
Total biomass (grammes/m^2)	4.87	10.2	6.68	3.26	3.95	8.15
BMWP	168	19	53	106	25	112
ASTP	6.7	2.7	4.1	5.6	2.8	5.3
BMWP′	180	19	60	125	29	146
ASTP′	6.7	2.7	4.0	6.0	2.9	5.2
t-BMWQ	286	34	108	204	47	227
a-BMWQ	10.6	4.9	7.2	9.7	4.7	8.4

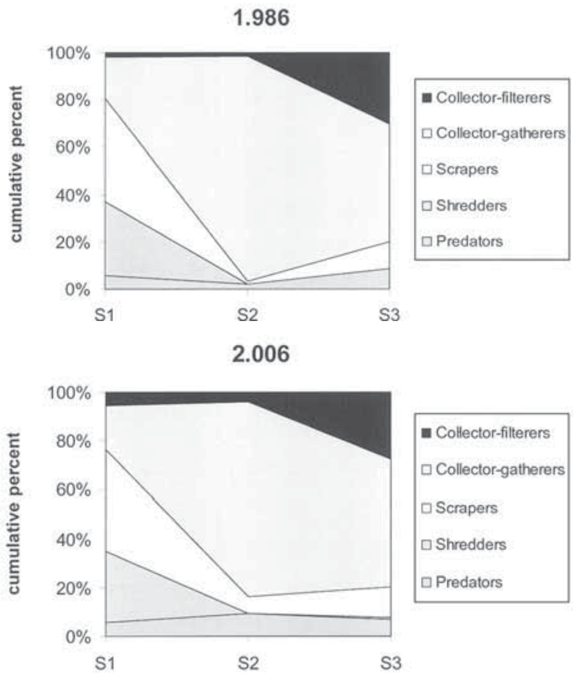

Figure 2. Relative abundances (%) of macroinvertebrate functional feeding groups at each sampling site for 1986 and 2006 surveys. *Abundancias relativas (%) de los grupos tróficos funcionales de la comunidad de macroinvertebrados bentónicos en cada sitio de muestreo durante las campañas de 1986 y 2006.*

a-BMWQ) are presented in Table 4. Positive correlations between all biotic indices were significant ($P < 0.01$). However, correlations of chemical parameters (dissolved oxygen, ammonia, phosphate) with average scores (ASPT, ASPT′, a-BMWQ) were higher than with total scores (BMWP, BMWP′, t-BMWQ).

Relative abundances of functional feeding groups at each sampling site are shown in figure 2. During both sampling surveys, the trophic structure of the macrobenthic community appeared to be altered by the trout farm outlet. Shredders and scrapers were the functional feeding groups most adversely affected (shredders, particularly). A spatial recovery of scrapers was evident as the distance from the fish farm increased, though the relative abundance of this trophic group never returned to values observed at the reference site. Conversely, collectors and predators increased their relative abundances downstream from the trout farm outlet, collector-gatherers becoming the dominant functional feeding group at S-2 and S-3.

Table 4. Pearson correlation (n = 6) matrix for chemical parameters (dissolved oxygen, nitrate, nitrite, ammonium, phosphate) and biotic indices (BMWP, ASTP, BMWP′, ASPT′, tBMWQ, aBMWQ). (a) = $P < 0.05$; (b) = $P < 0.01$. *Matriz de correlaciones de Pearson para parámetros químicos (oxígeno disuelto, nitrato, nitrito, amonio, fosfato) e índices bióticos (BMWP, ASTP, BMWP′, ASPT′, tBMWQ, aBMWQ). (a) = P < 0.05; (b) = P < 0.01.*

	Oxygen	Nitrate	Nitrite	Ammonia	Phosphate	BMWP	ASTP	BMWP′	ASTP′	tBMWQ	aBMWQ
Oxygen	1.0000										
Nitrate	0.1156	1.0000									
Nitrite	–0.4655	–0.1222	1.0000								
Ammonia	–0.5964	0.6570	0.0100	1.0000							
Phosphate	–0.7129	0.5860	0.3762	0.8599*	1.0000						
BMWP	0.8910[a]	0.1268	–0.3116	–0.5070	–0.5631	1.0000					
ASTP	0.9208[b]	0.0927	–0.2358	–0.5760	–0.6417	0.9857[b]	1.0000				
BMWP′	0.8916[a]	0.2372	–0.2644	–0.4573	–0.5045	0.9891[b]	0.9797[b]	1.0000			
ASTP′	0.9256[b]	0.1052	–0.2978	–0.5729	–0.6738	0.9720[b]	0.9937[b]	0.9653[b]	1.0000		
tBMWQ	0.8970[a]	0.2029	–0.2415	–0.4896	–0.5341	0.9906[b]	0.9882[b]	0.9986[b]	0.9744[b]	1.0000	
aBMWQ	0.9401[b]	0.0265	–0.2749	–0.6479	–0.7075	0.9549[b]	0.9901[b]	0.9469[b]	0.9930[b]	0.9610[b]	1.0000

DISCUSSION

In general, changes in the family composition of the macrobenthic community downstream from the trout farm outlet (especially at S-2) reflect a substitution of sensitive macroinvertebrate families for tolerant ones (Table 2): ephemeropterans, amphipods, plecopterans, trichopterans, coleopterans, and planarians decreased in abundance, whereas the abundance of tubificid worms, leeches, and dipterans (mainly chironomids) all increased. Reductions in dissolved oxygen concentrations and increases in inorganic nutrient concentrations (particularly the unionized ammonia) (Tables 1 and 4) would be the main causes for these changes in the community of benthic macroinvertebrates downstream from the trout farm. In addition, the siltation of organic matter, as a sludge deposit on the stream bottom at S-2, would be the primary environmental factor responsible for the marked alteration of the macrobenthic community just downstream the trout farm outlet.

Leeches, tubificid worms, and chironomids have been found to be characteristic macroinvertebrates in organic sludge deposits (Mantle, 1982; Hellawell, 1986; Rosenberg & Resh, 1993; Camargo, 1994; Oscoz *et al.*, 1999; Kirkagac *et al.*, 2004). Furthermore, in such cases, increases in the abundance of the whole macrobenthic community were mainly related to the increase of those taxonomic groups (Mantle, 1982; Hellawell, 1986; Rosenberg & Resh, 1993; Camargo, 1994; Oscoz *et al.*, 1999; Kirkagac *et al.*, 2004). On the other hand, many field studies have already shown that reductions in dissolved oxygen concentrations and/or increases in inorganic nutrient concentrations may be responsible for significant changes in the species composition of macrobenthic communities, with sensitive taxa being replaced by tolerant taxa (Mantle, 1982; Armitage *et al.*, 1983; Hellawell, 1986; Domezain *et al.*, 1987; Alba-Tercedor & Sánchez-Ortega, 1988; Rosenberg & Resh, 1993; Camargo, 1993, 1994; Oscoz *et al.*, 1999; Kirkagac *et al.*, 2004; Camargo *et al.*, 2005; Ortiz *et al.*, 2005). Moreover, the unionized ammonia (NH_3) in fresh waters is much more toxic to aquatic animals than other nutrients (such as nitrite and nitrate ions, ionized ammonia or ammonium ion, phosphate ion), and our study estimated that NH_3 levels downstream from the trout-farming outlet (Table 1), were sometimes higher than the recommended water quality criteria for long-term exposures (see Camargo & Alonso, 2006).

Changes in the family composition of the macrobenthic community are also responsible for changes in the values of biotic indices (Table 3): in both sampling surveys, values of biotic indices were markedly reduced at S-2, with a clearly spatial recovery at S-3 (especially during the 2006 survey). However, while values of total scores (BMWP, BMWP′, BMWQ) were higher at S-3 than at S-1 for the 2006 sampling survey, values of average scores (ASPT, ASPT′, a-BMWQ) were lower at S-3 than at S-1 during both sampling surveys (Table 3). Furthermore, correlations of chemical parameters with average scores were generally higher than with total scores (Table 4), suggesting a better indicator value of freshwater quality (in ecological terms) for average scores than for total scores. This fact has already been pointed out in previous studies (Armitage *et al.*, 1983; Camargo, 1993, 1994; Camargo *et al.*, 2004; Morais *et al.*, 2004; Alonso, 2005).

The marked alteration in the macrobenthic trophic structure downstream from the trout farm (Fig. 2) was mainly due to a decrease in the abundance of shredders (e.g., *Gammaridae*, *Sericostomatidae*, *Lepidostomatidae*) and scrapers (e.g., *Ancylidae*, *Hydrobiidae*, *Heptageniidae*, *Elmidae*, *Glossosomatidae*), and an increase in the abundance of collector-gatherers (e.g., *Tubificidae*, *Chironomidae*) and collector-filterers (e.g., *Sphaeridae*, *Hydropsychidae*, *Simuliidae*). Regarding the River Continuum Concept (Vannote *et al.*, 1980), this marked alteration would indicate that the trout farm effluent causes "potamological" effects on the trophic structure of the macrobenthic community. However, those effects appear to be more drastic in the 1986 survey than in the 2006 survey (Fig. 2), probably because of a higher annual trout production twenty years ago. Similar changes in the trophic structure of macrobenthic communities have already been found in other rivers and streams with organic pollution and nutrient enrichment (Camargo, 1994; Camargo *et al.*, 2005; de la Puente & Camargo, 2006).

It is concluded that, during the last twenty years, the wastewater depuration system of the trout farm has been clearly insufficient to prevent marked alterations in the recipient river. As a consequence, the trout farm must significantly improve its wastewater depuration system in order to recover the ecological characteristics of the upper Tajuña River.

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