

# Metabolism of Basque streams measured with incubation chambers

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Received: 5/10/10

Accepted: 28/4/10

## ABSTRACT

### Metabolism of Basque streams measured with incubation chambers

The metabolism (gross primary production, GPP; respiration, R; and net metabolism, NM) of epilithon was measured by incubating randomly selected cobbles in recirculatory chambers in 21 Basque streams running to the Atlantic coast. The studied streams spanned a broad range of environmental conditions and ranged in ecological status from excellent to poor. The GPP ranged from negligible to  $35 \text{ g O}_2\text{m}^{-2}\text{d}^{-1}$ , and the R ranged from 1 to  $17 \text{ g O}_2\text{m}^{-2}\text{d}^{-1}$ . The NM showed that the epilithon was autotrophic in 15 sites and heterotrophic in the rest. None of the measured environmental variables was significantly correlated with R. Stepwise analyses showed that both the GPP and the NM were related to the epilithic chlorophyll *a* and phosphate concentrations. The metabolism values obtained were lower than those previously published from open-channel method, the number of autotrophic sites higher, and the controlling environmental variables different. The difference between open-channel and chamber methods was not related to the area covered with cobbles in the study sites. Although chamber measurements yield results in sites where open-channel methods do not work, they seem to overestimate the importance of autotrophy.

**Key words:** Stream, metabolism, epilithon, Spain.

## RESUMEN

### Metabolismo de los ríos vascos medido con cámaras de incubación

Se midió el metabolismo (producción primaria bruta, PPB; respiración, R; y metabolismo neto, MN) del epilíton en 21 ríos vascos que drenan a la costa atlántica, incubando cantos en cámaras recirculatorias. Los ríos estudiados comprendían un amplio abanico de condiciones ambientales y su estado ecológico iba desde excelente hasta pésimo. Los valores de PPB iban desde cercanos a 0 hasta  $35 \text{ g O}_2\text{m}^{-2}\text{d}^{-1}$ , los de R desde 1 hasta  $17 \text{ g O}_2\text{m}^{-2}\text{d}^{-1}$ . El MN indicaba que el epilíton de 15 ríos era autótrofo, el resto heterótrofo. Ninguna de las variables ambientales medidas estaba correlacionada de forma significativa con la R. Regresiones paso a paso indicaban que tanto la PPB como el MN estaban relacionados con la clorofila epilítica y con la concentración de fosfato. Los valores de metabolismo obtenidos fueron más bajos que los publicados previamente en base a métodos de cauce abierto, el número de tramos autótróficos era mayor, y las variables ambientales responsables diferentes. Las diferencias entre ambos métodos no eran proporcionales a la abundancia de cantos en el lecho. Aunque las medidas en cámara permiten obtener resultados en tramos en los que no funcionan los métodos en cauce abierto, aparentemente sobreestiman la importancia de la autotrofia.

**Palabras clave:** Río, metabolismo, epilíton, España.

## INTRODUCTION

The stream metabolism is an integrative measurement of ecosystem functioning, and thus, can po-

tentially be used as a functional indicator of the ecological status of stream ecosystems (Young *et al.*, 2006; Fellows *et al.*, 2006). Its main components, gross primary production (GPP) and res-

piration (R), are affected by factors such as nutrients, turbidity, canopy cover and temperature (Acuña *et al.*, 2004; Young *et al.* 2004; Izagirre *et al.* 2008), and therefore, they respond to many human activities occurring in the basin.

The stream metabolism can be measured at the scale of individual substrata (typically cobbles) in closed chambers (Bott *et al.* 1996; Bunn & Davies, 2000; Uehlinger *et al.* 2002) or at a reach scale by measuring diel changes in oxygen concentration on open channels (Marzolf *et al.* 1994; Mulholland *et al.*, 2001; Bott *et al.*, 2006). The open-channel approach critically depends on estimating the diffusion of oxygen between water and air, and the methods developed for that purpose often yield contrasting results (Generaux & Hemond, 1992; Young & Huryn, 1999; Aristegi *et al.*, 2009). Nevertheless, these methods can be adapted to use data from continuous monitoring of water quality, and thus, they offer the possibility to monitor the ecosystem functioning almost in real time (Izagirre *et al.*, 2007). The main drawback of the open-channel approach is that it cannot be used in situations where the oxygen saturation shows no diel changes, such as in steep mountain brooks, in highly shaded streams, or during floods. In addition, the chamber and open channel methods measure different parameters. The chamber method measures the metabolism of an enclosed community (e.g., epilithon), whereas the open-channel methods measure the ecosystem metabolism of an area that can be several km long (Reichert *et al.* 2009). Therefore, although the driving factors might not vary, the chamber and open-channel estimations are expected to differ. However, it is possible to obtain a better agreement between chamber and reach-scale estimations in areas dominated by the type of substrate analysed in chambers.

Despite its importance, metabolism has been relatively little studied in Iberian streams, and most research has focused on its seasonal variations in few study areas (e.g., Guasch & Sabater, 1994; Mollá, 1996; Velasco *et al.*, 2003; Acuña *et al.*, 2004). Therefore, we are still far from getting an accurate picture of the spatial and temporal variations of metabolism and of the factors governing these variations in Iberian streams. The

Iberian Peninsula features a large variety of climatic and geomorphic zones, which can have profound implications for the structure and functioning of stream ecosystems (e.g., Sabater *et al.*, 2008). Recently, by applying the open-channel approach to data from multiple continuous-monitoring stations, Izagirre *et al.* (2008) studied the factors controlling the metabolism of Basque streams across a large range of environmental situations. Nevertheless, the absence of diel variations in oxygen concentration made it unfeasible to measure the whole stream metabolism of some areas.

Here, we present the results of epilithic metabolism measured with chambers in the same areas analysed in the Izagirre *et al.* (2008) study. In addition, we compare our results with those obtained with the open-channel method by Aristegi *et al.* (2009). The objectives of our study are 1) to get metabolism data of Basque streams at sites where the open-channel method could not be used, 2) to test the hypothesis that differences between epilithic and ecosystem metabolism are smaller in areas dominated by cobbles and boulders, and 3) to assess whether epilithic and whole-stream metabolism are controlled by the same environmental variables.

## METHODS

This study was conducted in July of 2005 in the provinces of Biscay and Guipuscoa (Basque Country, northern Spain). The climate is humid-oceanic, the average annual rainfall ranges from 1500 to 2500 mm, and the average monthly temperatures range from 8 °C to 19 °C. The area is hilly, with its lithology dominated by limestone, sandstone, and marl. Mountains of up to 1,500 masl are close to the sea, resulting in narrow, steep valleys, with streams that are short and steep. About 1.8 million people live in the industrialised areas of these provinces. Most rural areas are covered by intensively managed tree plantations of Monterey pine (*Pinus radiata* D. Don) and blue gum (*Eucalyptus globulus* Labill) harvested in short rotations with heavy machinery. Because of all these activities in such a small territory, most streams were severely polluted

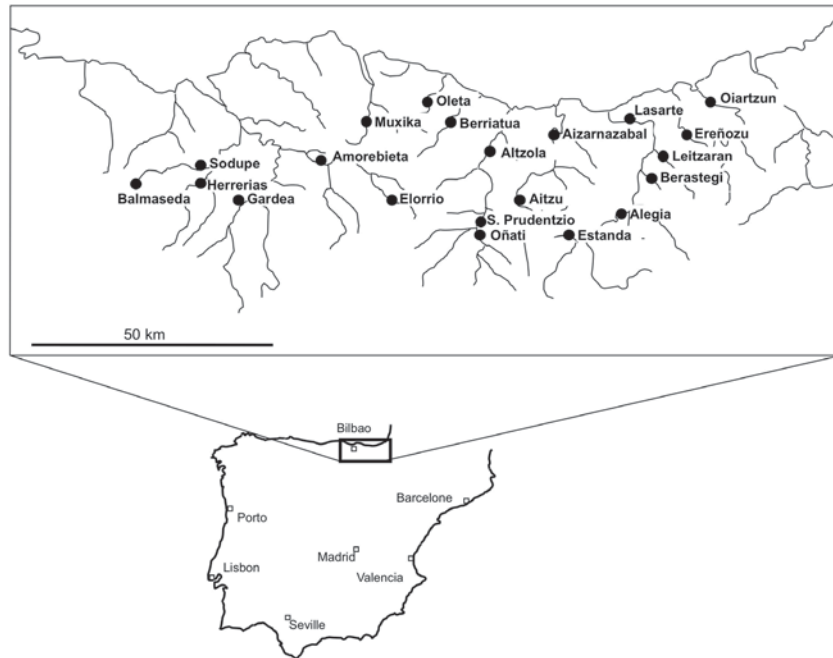


Figure 1. The study sites. *Área de estudio*.

by the mid-20<sup>th</sup> century. However, over the last 20 years, the water quality has been improving steadily (Arluziaga, 2002). Today, some streams are still severely degraded, whereas others have recovered enough to maintain salmon populations (Galera & Antón, 2001). Apart from the chemical pollution, many Basque streams were canalised and have degraded margins and floodplains (Basque Government 2003).

Twenty-one stream areas (Fig. 1) with contrasting environmental conditions were studied. The catchment areas ranged from 2 981 to 78 677 ha, the average discharge ranged from 30 to 4 800 l/s, the channel width ranged from 9 to 46 m, the canopy cover ranged from 0 to 70 %, and the ecological quality ranged from excellent to very poor (Table 1). These are the same areas where Izagirre *et al.* (2008) measured the open-channel metabolism continuously for more than one year and where Aristegi *et al.* (2009) compared methods for measuring the re-aeration rate.

The epilithic metabolism was measured in the central hours of the day by means of six perspex chambers (20 × 30 × 15 cm) where water was

kept in motion by water pumps (Maxi Jet 750) for two hours. A randomly selected cobble was enclosed in each chamber, the chambers were submerged in water, and three of them were covered with black opaque plastic. The oxygen was measured with an oxymeter WTW Oxi315i/SET at the onset of the experiment, after an hour and after two hours through a hole in the lid of each chamber that was otherwise kept closed with a screwed cap. After the incubation, we measured the volume (using a bucket and a beaker) and the area (by covering the cobble with aluminium foil, which was later weighed in the laboratory) of the cobble. The oxygen data from dark chambers were used to calculate the R, the oxygen balance in light chambers to calculate the net metabolism (NM), and the sum of both to calculate the GPP of epilithon (all in  $\text{g O}_2\text{m}^{-2}\text{h}^{-1}$ ). To allow for comparisons, these data were converted to daily metabolism. The daily R ( $\text{g O}_2\text{m}^{-2}\text{d}^{-1}$ ) was calculated by multiplying the hourly respiration by 24; the daily GPP was calculated by multiplying the hourly NM by the number of daylight hours and adding R multiplied by daylight hours;

**Table 1.** Environmental characteristics of the studied areas (Izagirre *et al.*, 2008). The biotic index was calculated by the Basque Government (2003) with the invertebrate based Iberian Biological Monitoring Working Party (IBMWP) method (Alba-Tecedor & Sanchez-Ortega 1988). *Características ambientales de los tramos estudiados (Izagirre et al., 2008). El índice biótico fue calculado por el Gobierno Vasco (2003) mediante el método Iberian Biological Monitoring Working Party (IBMWP, Alba-Tecedor & Sanchez-Ortega 1988), basado en invertebrados.*

Site	Drainage area (ha)	Q (m <sup>3</sup> s <sup>-1</sup> )	Width (m)	Depth (m)	Water velocity (m s <sup>-1</sup> )	Canopy cover (%)
Aitzu	5 973	0.10	12.9	0.27	0.33	20.3
Aizarnazabal	26 896	1.61	22.7	1.34	0.30	51.4
Alegia	32 974	0.92	36.2	1.34	0.26	14.9
Altzola	46 254	1.28	31.1	0.51	0.24	12.3
Amorebieta	23 718	0.93	23.3	0.45	0.23	0.0
Balmaseda	19 576	1.33	23.9	0.23	1.54	20.9
Berastegi	2 866	0.36	12.2	0.49	0.55	14.5
Berriatua	9 281	0.17	18.7	0.24	0.30	31.7
Elorrio	2 936	0.03	8.8	0.39	0.27	39.2
Ereñozu	21 550	1.71	15.8	0.77	—	18.2
Estanda	5 469	0.11	24.0	0.30	0.26	21.9
Gardea	32 974	0.13	19.0	0.13	0.12	0.0
Herrerias	25 436	0.07	26.6	0.18	0.34	3.4
Lasarte	78 677	4.79	46.4	0.39	0.66	1.1
Leitzaran	11 449	0.77	22.2	0.46	0.16	19.6
Muxika	3 141	0.09	9.5	0.42	0.21	69.8
Oiartzun	5 613	0.28	22.0	0.57	0.25	34.5
Oleta	6 742	0.14	14.4	0.23	0.32	48.1
Oñati	9 929	0.26	14.5	0.38	0.29	35.1
S. Prudentzio	12 180	0.32	14.0	0.46	0.24	24.0
Sodupe	27 680	0.71	30.0	2.14	0.57	0.0

and the daily NM was calculated as the difference between the daily GPP and the daily R.

The photosynthetically active radiation (PAR) reaching the water surface was measured during the incubations every 15 min with a sensor LI-COR LI-240SA. After the incubations, the epilithon was sampled from the incubated cobbles. All the epilithic material was scraped from an area of 18.85 cm<sup>2</sup> per stone using scalpels, toothbrushes and Pasteur pipettes. The samples were stored in plastic containers, enclosed in an ice box, and carried to the laboratory where they were frozen (−20 °C). Later, the samples were thawed and homogenised with a domestic blender. One subsample was used to determine the ash-free dry mass (AFDM) by drying it at 105 °C for 24 h and ashing at 500 °C for 4 h. Another subsample was used to measure the epilithic chlorophyll *a* content spectrophoto-

metrically after extraction in hot ethanol (Sartory & Grobbelaar, 1984).

In addition, water samples for chemical analyses were also collected from the stream. All these samples were taken to the laboratory in an ice box and frozen until the analyses (−20 °C). Later, the samples were thawed and filtered (Whatman GF/C), and their nutrient were analysed. Nitrate, nitrite, ammonium and silicate in water were measured with TRAACS 800 auto analyser. Phosphate was measured by the stannous chloride method (APHA, 1992) on a Shimadzu UV-1603 spectrophotometer.

The water temperature, pH (pH meter Hanna HI 9025), oxygen concentration (oxymeter WTW OXI 96), and conductivity (conductimeter WTW LF 90) were measured in the stream at the onset of the incubations. The characteristics of the area (width, depth, water velocity, substrate and riparian cover) were obtained from 10 cross-

transects evenly spaced along an area five times longer than channel width (Izagirre *et al.* 2008).

To discern the environmental variables controlling the epilithon, Pearson correlations were performed with epilithic biomass and chlorophyll (log-transformed for normality) as dependent variables and environmental variables as independent ones. To test the hypothesis that differences between epilithic and ecosystem metabolism are smaller in areas dominated by similar rock material, where communities other than epilithic are rare, we computed the standardised difference between chamber and open-channel methods, comparing our results to those simultaneously measured by the open-channel method by Aristegi *et al.* (2009). The standardised difference was calculated as the absolute value of the metabolism (either R, GPP

or NM) calculated by the open method minus the metabolism calculated in chambers divided by the metabolism calculated by the open method. Furthermore, we performed linear regressions with cover of rock material (cobbles, cobbles + boulders, cobbles + boulders + rocks) as the independent variables and the standardised difference in R, GPP, and NM estimations as the dependent ones. Finally, to determine the environmental variables controlling the metabolism, we performed stepwise regressions with epilithic metabolism (R, GPP and NP) as dependent variables and 12 independent variables: benthic AFDM, chlorophyll *a*, incident PAR, nitrate, nitrite, ammonium, phosphate and silicate concentrations, discharge, width, depth, and riparian cover. All the statistical analyses were performed with Statview 5.0.1 for Macintosh.

**Table 2.** Characteristics of the stream water and photosynthetically active radiation (PAR) during the metabolism incubations. b.d.l. = below detection level. The PAR was measured every 15 minutes, and the rest of the variables were measured once, at the onset of incubation. *Características del agua y radiación fotosintéticamente activa (PAR) durante las incubaciones de metabolismo. b.d.l. = por debajo del nivel de detección. El PAR se midió cada 15 minutos, el resto de las variables una vez, al inicio de la incubación.*

Site	Temperature (°C)	pH	Conductivity ( $\mu\text{S cm}^{-1}$ )	Oxygen (%)	Nitrate ( $\mu\text{g N/l}$ )	Nitrite ( $\mu\text{g N/l}$ )	Ammonium ( $\mu\text{g N/l}$ )	Phosphate ( $\mu\text{g P/l}$ )	Silicate ( $\mu\text{g Si/l}$ )	PAR ( $\mu\text{E m}^{-2}\text{s}^{-1}$ )
Aitzu	22.5	8.15	—	125.8	b.d.l.	75	816	969	2308	1134
Aizarnazabal	17.8	7.90	479	99.7	151	23	78	24	444	628
Alegia	22.1	7.24	591	76.8	871	78	72	107	1239	214
Altzola	21.5	7.59	753	86.4	116	32	393	237	4243	1763
Amorebieta	22.1	7.79	587	101.0	1022	37	82	149	846	1197
Balmaseda	17.9	8.04	620	136.0	1319	72	76	95	1756	1758
Berastegi	19.1	7.48	1039	86.5	1607	110	89	122	980	996
Berriatua	19.5	7.75	410	94.1	1816	393	1795	134	2649	52
Elorrio	19.1	7.94	548	82.5	36	16	99	212	689	706
Ereñozu	18.9	7.85	134	99.9	1268	124	77	85	347	1699
Estanda	18.0	7.92	764	75.8	1661	29	140	72	1014	366
Gardea	18.9	8.45	4964	107.5	101	23	68	99	1343	1218
Herrerías	29.2	8.71	355	167.2	1997	205	99	343	433	787
Lasarte	19.5	7.13	546	96.7	1907	172	125	24	330	717
Leitzaran	18.1	7.50	216	97.3	2663	245	131	401	471	743
Muxika	18.5	7.78	417	89.4	2021	38	197	51	1682	282
Oiartzun	16.4	7.79	259	98.8	15	15	79	807	1876	864
Oleta	19.0	7.36	359	91.4	974	91	196	95	1562	1382
Oñati	25.1	8.24	314	208.0	951	51	96	74	541	1037
S. Prudentzio	21.1	—	—	11.7	2021	38	197	51	1682	1366
Sodupe	22.8	—	—	96.6	419	29	49	96	430	1405

## RESULTS

There were large differences in environmental characteristics, reflecting differences in catchments (Table 2). The water temperature ranged from 16.4 to 29.2 °C; the lowest value occurred at small streams with good riparian forests, while the highest, at larger streams with more stagnant waters. The pH was circumneutral in all the areas. The conductivity ranged from 134  $\mu\text{S}/\text{cm}$  in Ereñozu, a clear water stream with siliceous catchment, to 4 964 in Gardea, which is an area affected by an upstream salty diapir. The oxygen saturation ranged from 12 % in the extremely polluted San Prudentzio to 208 % in Oñati and reflected the balance between primary production and respiration. The concentration of nutrients changed in 1-2 orders of magnitude. The nitrate ranged from non detectable to over 2 600  $\mu\text{g N}/\text{l}$ , while the nitrite levels almost reached 400  $\mu\text{g N}/\text{l}$  and the ammonium, almost 1 800  $\mu\text{g N}/\text{l}$ . Overall, the inorganic nitrogen showed different re-

dox states from waters dominated by reduced forms (e.g., Aitzu, located below a water treatment plant) to waters dominated by oxidised forms. The concentration of phosphate ranged from 24 to over 800  $\mu\text{g P}/\text{l}$ , and the one of silicate, from 300 to over 4 000  $\mu\text{g Si}/\text{l}$ . The photosynthetically active radiation ranged from 52 to over 1 700  $\mu\text{E m}^{-2}\text{s}^{-1}$  showing the effects of both weather and riparian cover.

The epilithic biomass ranged (Table 3) from 7-8 g AFDM/m<sup>2</sup> (Aizarnazabal, Leitzaran) to 146 g AFDM/m<sup>2</sup> (Oleta). The chlorophyll *a* content ranged from 14.8 mg/m<sup>2</sup> (Aizarnazabal) to 847.9 mg/m<sup>2</sup> (Altzola). The epilithic communities were dominated by filamentous green algae, while diatoms and red algae were less abundant. In San Prudentzio, the cobbles were covered by thick *Sphaerotilus* mats. Pearson correlation between epilithic variables and environmental variables showed that the AFDM was highly correlated with chlorophyll ( $r = 0.706$ ,  $p < 0.01$ ) and significantly correlated with PAR ( $r = 0.464$ ,

**Table 3.** Biomass, chlorophyll *a*, and metabolism of the epilithon in the incubated cobbles. NM = net metabolism; R = respiration; GPP = gross primary production. Average and standard deviation shown. There was no SD for GPP. *Biomasa y clorofila a*, y *metabolismo del epilithon en los cantos incubados*. NM = *metabolismo neto*; R = *respiración*; GPP = *producción primaria bruta*. Se proporcionan los datos medios y la desviación típica. No hay desviación típica para los datos de producción primaria bruta.

Site	AFDM (g m <sup>-2</sup> ) (n = 6)	Chl a (mg m <sup>-2</sup> ) (n = 6)	NM (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> ) (n = 3)	R (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> ) (n = 3)	GPP (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )
Aitzu	48.1 ± 244.3	110.9 ± 110.9	16.1 ± 3.1	11.8 ± 8.7	27.8
Aizarnazabal	7.2 ± 213.8	14.8 ± 14.8	-0.8 ± 0.4	2.7 ± 0.5	1.9
Alegia	28.9 ± 21.9	139.4 ± 66.6	1.1 ± 0.4	17.2 ± 21.9	18.3
Altzola	88.2 ± 45.2	847.9 ± 86.0	29.0 ± 4.8	6.3 ± 2.3	35.3
Amorebieta	41.8 ± 11.3	246.0 ± 123.2	7.3 ± 3.5	3.7 ± 3.1	10.9
Balmaseda	111.0 ± 1015.9	289.2 ± 289.2	-1.8 ± 19.7	10.2 ± 3.5	8.5
Berastegi	48.4 ± 46.0	125.0 ± 104.5	2.6 ± 0.4	3.0 ± 2.2	5.6
Berriatua	15.1 ± 5.1	48.2 ± 20.8	0.2 ± 0.4	3.9 ± 1.2	4.0
Elorrio	8.8 ± 4.8	62.4 ± 64.6	1.2 ± 0.3	10.0 ± 7.7	11.2
Ereñozu	8.9 ± 3.5	59.2 ± 36.2	2.1 ± 1.8	1.3 ± 0.8	3.3
Estanda	46.7 ± 47.1	271.0 ± 293.4	-3.3 ± 1.5	12.6 ± 7.9	9.3
Gardea	34.4 ± 18.5	238.4 ± 220.2	5.8 ± 16.8	3.7 ± 0.4	9.5
Herrerias	29.8 ± 7.2	81.4 ± 29.7	-0.3 ± 15.1	9.9 ± 3.2	9.7
Lasarte	16.3 ± 9.6	288.9 ± 327.5	-3.3 ± 14.0	8.8 ± 0.7	5.5
Leitzaran	7.9 ± 54.7	46.9 ± 46.9	3.1 ± 1.3	5.5 ± 5.5	8.6
Muxika	33.5 ± 28.9	37.1 ± 10.3	0.7 ± 0.2	1.1 ± 0.2	1.8
Oiartzun	13.6 ± 4.6	73.4 ± 8.8	3.5 ± 1.3	10.7 ± 16.5	14.2
Oleta	146.0 ± 111.8	277.4 ± 254.5	9.8 ± 25.0	5.6 ± 7.4	15.4
Oñati	39.6 ± 14.9	96.2 ± 46.7	4.2 ± 0.0	6.7 ± 0.0	10.9
S.Prudentzio	40.6 ± 48.2	39.4 ± 35.9	-8.7 ± 4.9	8.0 ± 1.7	0.0
Sodupe	91.9 ± 26.0	288.2 ± 173.0	11.7 ± 1.1	4.3 ± 0.8	16.0

$p < 0.05$ ). In addition, chlorophyll *a* showed a highly significant correlation ( $p < 0.01$ ) with cover ( $r = 0.566$ ) and significant correlations ( $p < 0.05$ ) with PAR and channel width ( $r = 0.494$  and  $0.458$ , respectively).

The epilithic metabolism showed large differences between sites. The respiration varied from 1 to 17 g O<sub>2</sub>m<sup>-2</sup>d<sup>-1</sup>, and the gross primary production ranged from 0 to 35 g O<sub>2</sub>m<sup>-2</sup>d<sup>-1</sup>. As a result, the epilithon's net metabolism ranged from -8.7 to 29 g O<sub>2</sub>m<sup>-2</sup>d<sup>-1</sup>, and it was autotrophic in 15 out of 21 areas during the measurements. Stepwise regressions between chamber metabolism (GPP, R and NP) and environmental variables showed no significant relationship for the epilithic respiration. In contrast, both GPP and NP were directly related to chlorophyll and phosphate, as described by the following models:

$$\text{GPP} = 0.0322\text{Chla} + 0.014\text{Phosp} - 1.487$$

$$(r^2 = 0.673, p < 0.0001)$$

$$\text{NP} = 0.0344\text{Chla} + 0.020\text{Phosp} + 0.722$$

$$(r^2 = 0.890, p < 0.0001)$$

Contrary to our expectations, the standardised differences in metabolism between chamber and open-channel calculations were not related to the proportion of stream bed covered by cobbles (linear regression,  $p \gg 0.05$ ). We only got a marginally significant relationship between the cover of particles larger than gravel (pebbles, cobbles, boulders and rocks) and the standardised differences in GPP ( $r^2 = 0.196$ ;  $p = 0.099$ ) and NM ( $r^2 = 0.250$ ;  $p = 0.058$ ). Nevertheless, in both cases the relationship was positive, i.e., the standardised difference between chamber and open-channel estimations increased with the area covered with hard sediments.

## DISCUSSION

Our work presents, to our knowledge, the first dataset of chamber metabolism in multiple Iberian streams. Although restricted to a single ecoregion, the 21 studied sites included areas

in all the major basins in Guipuscoa and Biscay, and they covered most environmental situations found in Basque Atlantic streams, with the exception of small headwater reaches. Basque Atlantic streams typically show little seasonality (Sabater *et al.*, 2008) but large interannual variability derived from changing rainfall patterns (Izagirre & Elosegi, 2005; Elosegi *et al.*, 2006). Therefore, caution is necessary when comparing the present data with other results, even if they were obtained at the same streams. For instance, the epilithic biomass value of our study was much lower than the data already published for the same areas (Sabater *et al.*, 2000; Izagirre *et al.*, 2008). However, those data coincided with dry and hot summers, which are situations that promote the development of large algal mats (Mosisch & Bunn, 1997), whereas our data corresponded to a rainy year, where both light availability and discharge would limit algal growth (Biggs, 2000).

Not only can the epilithic biomass be highly variable from year to year, but also the factors governing it can change in relation to environmental conditions, as has been described in the Agüera stream (Biscay). Elósegui & Pozo (1998) reported that the spatial distribution of epilithic biomass mainly depended on light and nutrients, and that the temporal variations were controlled by floods. Similarly, Izagirre & Elosegi (2005) showed that in open canopy areas, the seasonal variations in epilithic biomass were controlled by the stage of the river, whereas in closed canopy areas, the seasonality of irradiance was an important factor. In normal years, the epilithic biomass could be adequately modelled based on the river discharge, whereas in dry years, the number of environmental variables affecting the biomass is higher (Boulêtreau *et al.*, 2008). In the present study, cover and irradiance seemed to be the main environmental variables controlling the spatial differences in epilithic biomass, whereas the role of nutrients seems to be negligible. The influence of light on epilithic biomass has been shown both observationally (Sabater *et al.*, 2008) and experimentally (von Schiller *et al.*, 2007), whereas the relationship between nutrients and epilithon is complex because algae are both dependent

and controllers of nutrient concentrations as well as because the internal recycling of nutrients in biofilms is important (Mulholland, 1996).

Our metabolism results are in the same order of magnitude of previously published works on Iberian streams from chamber incubations (e.g., Mollá *et al.*, 1996; Velasco *et al.*, 2003). Our results are also similar to those obtained from open-channel methods in the summer of 2003 in the same areas (Izagirre *et al.*, 2008), and therefore, they are useful to fill the gaps in that study, which occurred because the diel changes were too small at some sites or because of the malfunction of the oxygen electrode. Interestingly, according to the chamber data, the sites where the open-channel method failed were not because of the intensity of the metabolism, but the failure was related to the characteristics of the channel (turbulence, variable water quality).

By contrast, although the values of epilithic GPP and R seem to be similar to the ecosystem metabolism calculated by the open-channel method, they suggest an entirely different trophic balance. All ecosystems except one stream were heterotrophic during the summer of 2003, whereas the epilithic communities were autotrophic in 15 out of 21 sites in the summer of 2005. The chambers can limit the availability of nutrients and, thus, affect the primary production estimates, especially at oligotrophic sites (Bott *et al.* 1997). Conversely, the underestimation of respiration likely results from the exclusion of macrofauna (Marzolf *et al.*, 1994), and especially of hyporheos, which contributes to up to 70 % of the ER (Naegeli & Uehlinger, 1997; Fellows *et al.*, 2001). Furthermore, the metabolic activity differs markedly between different stream compartments such as macrophytes, algal mats and fine sediments (Velasco *et al.*, 2003) making it difficult to get a representative sampling.

When comparing our results with those obtained simultaneously by the open-channel method (Aristegi *et al.*, 2009), the epilithic GPP values were below the whole ecosystem GPP in 82 % of the occasions, and the epilithic R values in 91 % of the occasions. As a result, 91 % of the NM estimations in chambers were higher, more autotrophic than the open-channel estima-

tions. As noted by Odum (1956), the diel oxygen curve gives accurate information on the autotrophic or heterotrophic characteristics of each area, even when the calculated GPP or R values are inaccurate. Thus, the metabolic chambers are less reliable than the open methods to measure stream trophic status. In our case, it seems that the exclusion of hyporheos was the main factor behind the overestimation of the NM. Indeed, many Basque streams still receive fairly large amounts of labile organic matter that results in a high biological oxygen demand in the sediments (Basque Government, 2003), which suggest that a large fraction of the ecosystem respiration is linked to the hyporheos. The differences between methods affected not only the results but also the environmental factors controlling the metabolism. When using the open channel method, the whole-ecosystem GPP was related to turbidity and catchment activities, not to the epilithic biomass (Izagirre *et al.*, 2008), whereas the production in the chambers was related to the epilithic biomass and phosphate.

Thus, chamber and open-channel methods offer different pictures of stream metabolism, both in terms of spatial cover –some cm<sup>2</sup> in the case of chambers up to several km<sup>2</sup> in open channel– and of the components involved. Stream ecosystems are spatially complex entities where substantial differences between patches are common (Elosegi *et al.* 2010). Therefore, to adequately reflect whole-stream metabolism, the chamber measurements should include a sufficient number of replicates of metabolically important components (Bott, 1996), which is rarely carried out. Instead, most chamber studies focus on which are considered the main drivers of ecosystem metabolism, such as the cobbles in our case. Indeed, because other producers, such as plankton or macrophytes, are rare in Basque streams, one might hypothesise a good agreement between GPP in chambers and in entire areas, but that was not the case in the present study. Furthermore, there was either no statistically significant relationship between the covered area of the studied substrate type (cobbles) and the standardised differences between chamber and open-channel measurements, or the trends run in op-



posite directions, i.e., larger differences where stones (pebbles to rocks) were more abundant. All these findings suggest that the results from chamber incubations are highly limited in spatial scope and that extrapolating them, even in streams dominated by a single type of substrate, is highly risky.

## ACKNOWLEDGMENTS

This research was funded by the Spanish Department of Science and Technology, the University of the Basque Country, and the European Regional Development Fund through projects 9/UPV00118.310-14476/2002 and BOS2003-04466. Oihana Izagirre took part in this work thanks to a pre-doctoral grant by the Basque Government.

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