

## Habitat-specific benthic metabolism in a Mediterranean-type intermittent stream

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**Abstract** – A modified flow-through chamber method was used to measure gross primary production (GPP), net primary production (NPP), community respiration (CR) and associated environmental variables in an intermittent Mediterranean-type stream in Southern Portugal. Three common types of in stream habitats were targeted: cobble (C), cobble covered with filamentous algae (C + A) and leaf litter (LL). NPP, GPP and CR differed significantly among all three habitats. GPP increased with chlorophyll *a* and, less strongly, with photosynthetic active radiation and, therefore, was highest in C + A habitat. The highest CR was in LL and its variation was best determined by ash-free dry mass (AFDM) of plant litter. Higher respiration in LL was related to heterotrophic activity and, to a lesser extent, to autotrophic respiration associated with periphyton. We observed a decrease of production efficiency of primary producers with AFDM in C + A and C habitats. Our results demonstrate that each habitat type should be considered as a discrete metabolic entity and that particular sets of environmental factors are responsible for habitat specific metabolic responses. Scaling up measurements from discrete habitat patches to the entire reach or stream should not be done by extrapolating the results of a single habitat type and will require quantification of habitat coverage, at the appropriate scale.

**Key words:** benthic metabolism / Quarteira River Basin / light and dark incubations / benthic habitats

### Introduction

Streams are heterogeneous environments, with fluctuating channel structure shaped by geological and hydrological processes across broad spatial and temporal scales (Poff and Ward, 1989; Elosegi *et al.*, 2010). These dynamics produce patchy distributions of autotrophic and heterotrophic organisms, influencing resource use and metabolic processes (Southwood, 1977; Pringle *et al.*, 1988; Warnars *et al.*, 2007). A rich body of literature has been built around the influence of habitat characteristics and habitat heterogeneity on organization of community structure (Rabeni and Minshall, 1977; Townsend and Hildrew, 1994; Palmer and Poff, 1997; Wallace *et al.*, 1997; Beisel *et al.*, 1998, 2000; Armitage and Cannan, 2000; Brown, 2003). We know that habitat heterogeneity contributes directly and indirectly to biodiversity and

ecosystem functions such as primary production, decomposition and nutrient cycling (Wallace *et al.*, 1997; Lepori *et al.*, 2005; Gessner *et al.*, 2010). However, relatively few studies directly investigated how habitat patchiness modulates ecosystem-level processes (Pringle *et al.*, 1988; Gustafson, 1998). This is of increasing concern, as the rising global threat of habitat loss and fragmentation impels us to better understanding how habitat-specific dynamics influence overall ecosystem processes. Benthic community metabolism is a conspicuous biological process and it integrates how whole communities are influenced by environmental variables across spatial and temporal scales (Fellows *et al.*, 2006).

Few studies dedicated to benthic metabolism have demonstrated important differences in gross primary production (GPP), net primary production (NPP) and community respiration (CR), among different stream habitats (Rier and King, 1996; Clapcott and Barmuta, 2010). González Pinzón *et al.* (2014) demonstrated large

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spatial variation in metabolism within a stream, related to presence of different geomorphic units, bed materials and type of transient storage. Additionally, [Fellows \*et al.\* \(2006\)](#) demonstrated that separating experimental sites according to habitat type, improved the ability to explain variation in GPP and CR along an agricultural land-use disturbance gradient. Considerable differences in GPP and CR have also been reported along gradients of biofilm structural complexity ([Sabater and Romani, 1996](#)) and community composition ([Busch and Fisher, 1981](#); [Velasco \*et al.\*, 2003](#)). These studies suggest that factors driving ecosystem metabolism are habitat-specific and different habitats act as separate metabolic components. Considering the functional role of individual habitats separately is, therefore, paramount to understanding how spatial heterogeneity and habitat patchiness influence ecosystem processes. This will greatly enhance our ability to predict how shifts relative habitat proportions can affect scaling estimates of stream metabolism.

The natural complexity of streams presents many challenges in measuring metabolism and numerous approaches have been developed to address these ([Odum, 1956](#); [Marzolf \*et al.\*, 1994](#); [Bott \*et al.\*, 1997](#)). By far, chamber-based measurements offer the most straightforward way to investigate the influence of separate river units on the metabolism ([Fuss and Smock, 1996](#); [Rier and King, 1996](#); [Whitledge and Rabeni, 2000](#); [Fellows \*et al.\*, 2006](#); [Clapcott and Barmuta, 2010](#)). One of the main advantages of metabolic chambers is the capacity to separate specific habitats and isolate factors affecting metabolism within those habitats ([Bott \*et al.\*, 1978](#)).

However, metabolic chambers can alter environmental conditions in ways that influence ecosystem metabolism ([Dodds, 1991](#); [Bott \*et al.\*, 1997](#)). For example, metabolic chambers have been shown to induce nutrient limitations and alter temperature, dissolved oxygen and water velocity conditions during the incubation. It is therefore important to improve the design of metabolic chambers, in order to obtain reliable data and facilitate direct comparison between studies.

Most chamber-based measurements focus on a single substrate unit, usually cobbles or gravel. This approach often fails to account for spatial changes in habitat structure, which can result in localized autotrophy in some habitats and heterotrophy in others ([Whitledge and Rabeni, 2000](#)). This oversimplification can lead to erroneous conclusions when extrapolating results from the habitat to the reach scale, especially in streams subjected to frequent changes in habitat structure, such as Mediterranean-type intermittent streams.

To this end, we quantified ecosystem metabolism rates (GPP, NPP, CR), in the three most common substrates in Mediterranean streams: cobbles (C), cobbles covered with filamentous algae (C + A) and leaf litter (LL). We used closed metabolic chambers modified from [Wasiak \(2009\)](#) where we applied an improved flow-through approach to overcome common problems associated with closed chamber techniques. In addition, we quantified water temperature, photosynthetically active radiation – PAR,

chlorophyll *a*, ash-free dry mass – AFDM, volume rate of water flow passing through the chamber – VR and nutrients (nitrite, phosphate and ammonium), allowing us to assess which factors contributed most to variation in stream metabolism among habitats. We predicted that habitats rich in biofilm and algae (C and C + A) would be net autotrophic, while the metabolism of LL would be strongly heterotrophic.

## Methods

### Study site

We conducted our study in the Algibre, a first-order intermittent stream in the Quarteira River Basin, Southern Portugal (37°11'20" N, 8°5'33"W). The catchment area is ~324 km<sup>2</sup> with an elevation range from 14 to 515 m. The average monthly temperature varies from 8 to 29 °C and average annual rainfall is 625 mm. Catchment land use consists of arable land with shrub and herbaceous vegetation with occasional olive tree and citrus plantations. Catchment geology is predominantly limestone and non-calcareous clay ([Trindade \*et al.\*, 2013](#)). The studied reach was 400 m long and, on average, 8 m wide, with natural morphodynamics, uniform channel morphology and steady flow conditions. The average depth ranged from 20 to 50 cm in riffles and 50 to 100 cm in pools. At steady flow conditions (March–May) average discharge was 1.3 m<sup>3</sup>.s<sup>-1</sup> and it gradually decreased towards warmer months, being as low as 0.026 m<sup>3</sup>.s<sup>-1</sup> (July–August) just before the channel completely dries. Channel substrate was predominantly gravel and cobble that during the summer season was densely covered by filamentous algae, chiefly *Cladophora agg.* and *Vaucheria* sp(p.). Riparian vegetation was dominated by *Arundo donax*, herbaceous vegetation and carob and olive trees. It was moderately developed, with a mean width of 3 m and occasional spots of very sparse canopy cover.

Annual variability in stream discharge directly affects substrate characteristics, the development of algae and macrophytes and accumulation of organic debris ([Gasith and Resh, 1999](#)). Along the margins, depositional pool zones were filled with accumulated LL from adjacent riparian vegetation, underlain with clay. We focused on the three most common in stream habitats: cobble (C), cobble covered with filamentous algae (C + A) and leaf litter (LL) (ESM-1, online material). These habitats represent distinct biological units that emerge through interactions between hydrological and geomorphological processes. Water velocity in the study reach was, on average, 0.09 m.s<sup>-1</sup>, ranging from an average of 0.016 m.s<sup>-1</sup> in the LL deposition zones to 0.035 m.s<sup>-1</sup> in the cobble habitat and 0.178 m.s<sup>-1</sup> in cobble covered with algae.

### Experimental procedure

Metabolic measurements were done *in situ*, from April 11 to June 27, 2014, a period characterized by stable

flow conditions. Thirty-nine measurements of NPP, and CR were taken in haphazardly selected patches of each habitat type (C, 12; C + A, 15; LL, 12) within the same 400 m reach. NPP and CR were measured using enclosed acrylic metabolic chambers modified from Wasiak (2009), with several improvements to study habitat-specific metabolism (ESM-1 and ESM-2, online materials).

We buried a metal chamber base ~180 mm diameter in the stream sediments (two per habitat – randomly placed). A sample of intact substrate was removed from the river bottom and gently placed on the base, where it was left submerged in the stream for 3–5 days to allow macroinvertebrates recolonization (assuming that previous studies at perennial streams of O'Connor, 1991; Boyero, 2003; Oliveira *et al.*, 2014 will be similarly applicable to our system). In most benthic community studies, the bases were left for longer periods (Bott *et al.*, 1978). Unfortunately this was not possible in our study, due to the risk of theft or vandalism, which did occur, with several bases disappearing during these periods of recolonization. Chamber bases with LL were prepared by filling the base unit with naturally fallen leaves of *A. donax*. Litter material was composed of senescent leaves, consisting of entire and partially degraded specimens (~30–50% decomposed – personal observation) as well as remains of stems. During each 2 h incubation the base was sealed with an acrylic chamber (of 4.53 L volume), without disturbing the previously established community. The chambers were submerged to ensure they were free of air bubbles and to equilibrate the temperature in the chambers with the stream water (Bott *et al.*, 1978). Each chamber was connected to a submersible pump by the inflow and outflow ports, so that the water flow inside the chamber was continuously homogenized, to allow stable dissolved oxygen readings. The inflow tube was deep seated inside the chamber in a manner to not re-suspend the bottom sediment, but maximizing water homogenization. Submersible pumps were powered by a portable generator (ESM-2, online material).

NPP was measured as changes in dissolved oxygen inside each chamber, by using an oxygen sensor (YSI, Professional Plus), encased in an external flow cell (YSI, model 3059), which was sequentially connected to each of the chambers. When one measurement was finished, the flow cell was disconnected and connected to the next chamber. The tube connectors were always submerged during these operations, in order to avoid any air bubbles entering the line. This design allowed simultaneous incubation of several chambers, using only one oxygen probe. When incubations in light were finished, the water inside the chamber was completely exchanged. This was achieved by disconnecting the tubes from the circulation system and allowing the fresh water from the stream to enter the line. Considering that the flow rate of water passing through the chamber was, on average,  $1.66 \times 10^{-5} \text{ m}^3 \cdot \text{s}^{-1}$  the volume of the system was totally exchanged in 5 min. Including this time interval we allowed approximately 20 min to half an hour time of acclimation before starting to measure community

respiration. CR measures were done after covering the chamber with a black plastic wrapper to inhibit the light. Light and dark incubations lasted 2 h (DO concentration recorded at 12 min intervals). Incubations were initially paired with “blank” chambers, filled only with stream water (no substrate added to the bases), to assess the metabolism of the water column. *T*-tests ( $P > 0.05$ ) showed that differences between corrected and uncorrected metabolic rates were negligible, so benthic metabolism rates were ultimately not corrected for water column metabolism. NPP and CR ( $\text{mg DO m}^{-2} \cdot \text{h}^{-1}$ ) were calculated as follows:

$$(\text{NPP, CR}) = (\Delta\text{O}_2) \times V \times (S^{-1})$$

where  $\Delta\text{O}_2$  is the change of oxygen concentration between beginning and end of the experiment per unit volume and time ( $\text{mg DO L}^{-1} \cdot \text{h}^{-1}$ ),  $V$  the remaining volume (l) of the chamber (subtracted by the volume of substrate),  $S$  the stone surface area ( $\text{m}^2$ ) and in case of chamber with LL is the area occupied by LL, which was approximately the area of the base.

GPP was estimated as the sum of rates in light and dark incubations ( $\text{GPP} = \text{NPP} + \text{CR}$ ; Bott, 2006). Production-to-respiration ratio (P/R) describes the balance of metabolic processes during 24 h period and was calculated as GPP converted to daily metabolism divided by the CR during 24 h period following the equation:  $\text{GPP}/\text{CR}_{24}$  (Bott, 2006).

Pre- and post-incubation water samples were collected to assess nutrient depletion within chambers during incubations [nitrite ( $\text{NO}_2^- \text{-N}$ ), ammonium ( $\text{NH}_4^+ \text{-N}$ ) and phosphate ( $\text{PO}_4\text{-P}$ )]. Nutrient analyses were done on a MERCK Spectroquant Nova 60, using Spectroquant® Test kits for  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and  $\text{PO}_4^{2-}$ . To validate oxygen readings taken by the DO probe, we simultaneously collected water samples for oxygen analysis, using the spectrophotometric Winkler method (Labasque *et al.*, 2004).

Simultaneously with oxygen measurements, temperature inside the chamber was monitored (using the YSI probe model) as well as PAR in  $\mu\text{mol quanta m}^{-2} \cdot \text{s}^{-1}$  (LI-250A Light Meter). The light sensor was placed in the water in the proximity of chambers to ensure that the amount of light that reach the sensor was the same that reach the chambers. Current velocity [ $\text{m} \cdot \text{s}^{-1}$ ] in each habitat type used for the experiment was measured using a two-dimensional acoustic-Doppler velocimeter (FlowTracker Handheld ADV, Sontek YSI Inc.). After the experiment finished, the content of each chamber was taken to the laboratory and processed for determination of periphyton biomass and macroinvertebrate identification.

We scrubbed periphyton from stones and leaves into a known volume of water using a toothbrush (Biggs and Kilroy, 2000). In case of LL, each piece of leaf material was gently placed in the tray and superficial biofilm layer was scrubbed from both sites. The resultant slurry was thoroughly homogenized, subsampled and filtered on glass fibre filters (GF/C, 47 mm Whatman) for determinations

of chlorophyll *a* (Chl *a* mg.m<sup>-2</sup>) and AFDM (mg.m<sup>-2</sup>). AFDM of LL was measured separately including all the leaves biomass. Chl *a* was extracted in 90% boiling ethanol and kept in the freezer for 24 h. The absorbance was read on spectrophotometer (Thermospectronic GENESYS 10UV). AFDM filters were dried at 60 °C to constant weight and AFDM represents the weight difference before and after 4 h at 450 °C (Biggs and Kilroy, 2000).

For AFDM analyses in the chambers containing LL, all the biomass was collected, placed on the tray, dried and ashed as described above. For the chambers containing cobbles and cobbles covered with algae, Chl *a* and AFDM were calculated per stone surface area assuming that metabolically active area of stones is 60% (Biggs and Close, 1989).

Macroinvertebrates from each chamber were sorted and identified to family level (except Ostracoda and Oligochaeta). In order to verify whether the macroinvertebrate assemblages inside the chambers reflected the macroinvertebrate assemblages collected using kick sampling method (with hand net of 0.5 mm mesh and 25 cm width), we compared data from the chambers and from routine sampling done previously on the same type of habitats (Sroczyńska *et al.*, 2014).

## Data analysis

We compared ecosystem metabolism among habitats with analysis of variance (ANOVA) when assumptions of normality (Shapiro–Wilk test for small sample size) and homogeneity of variance (Leven’s test) were met. When the homogeneity of variance assumption was not met, we applied the non-parametric Kruskal–Wallis Rank Sum Test using the “kruskalmc” function in the R package “pgirmess” (R Development Core Team, 2012; Giraudoux, 2013). We performed Tukey’s *post hoc* analyses and generated 95% confidence intervals using the “TukeyHSD” function in the R package “multcomp” (Hothorn *et al.*, 2008; R Development Core Team, 2012). Univariate analyses and graphs were done using R package (R Development Core Team, 2012). When ANOVA demonstrated significant differences between habitats, correlation analysis were done, treating each habitat separately. Spearman product moment correlation was used to assess the relationship between explanatory and response variables as well as correlation between DO measurements and Winkler spectrophotometry oxygen determination.

Multivariate analyses were performed on normalized (Euclidean distances) metabolic responses (NPP, GPP and CR) and explanatory variables (Chl *a*, AFDM, VR, PAR, temp, nitrite, phosphate and ammonium), with habitat as a fixed factor. PERMANOVA (permutational multivariate analysis of variance) was used to test for significant differences in metabolism between the habitats. Distance-based linear models (DistLM) were used to examine the relationship between response variables and explanatory

variables. Firstly, the significance of the relationship was assessed for individual environmental variables with marginal tests (999 permutations). Significant variables ( $P < 0.01$ ) were subsequently included in model selection using the BEST procedure, which tests all possible combinations between explanatory variables and the response matrix. This procedure helps eliminate the effect of covariance between the variables, as it considers all the response variables together. Distance-based redundancy analysis (dbRDA) was used for the ordination and visualization of the best overall DistLM solution, according to the Akaike information criterion (AIC). All multivariate analyses were done using the PRIMER 6 statistical package with the PERMANOVA + add-on (Clarke and Warwick, 2001).

## Results

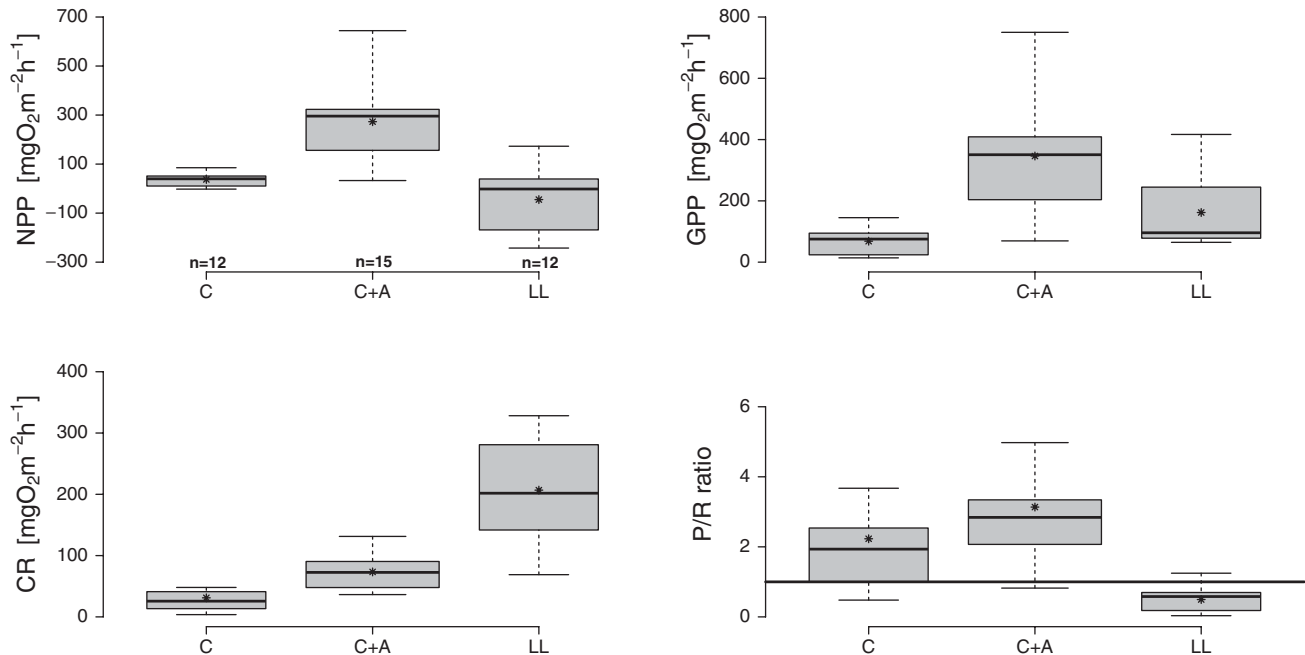
### Benthic metabolism

The highest significant average GPP was recorded for the habitat C + A and the lowest for the habitat C (Fig. 1, Table 1). Average oxygen consumption (CR) during dark phase was 3-fold and 7-fold higher in habitat LL, relative to C + A and C, respectively (Fig. 1). The variability in oxygen consumption during dark phase in C and C + A was lower than during light hours, while LL habitat demonstrated high variation in both respiration rate and oxygen production. All three habitats show significant differences in terms of GPP, NPP and CR (Table 2). For NPP and GPP variables, Tukey multi pairwise comparisons showed significant differences between C + A – C ( $P < 0.001$ ) and C + A – LL ( $P < 0.001$ ), but not for LL – C ( $P = 0.32$  and  $P = 0.25$  respectively). Multiple comparisons after Kruskal–Wallis for CR showed significant differences ( $P < 0.05$ ) among all the groups.

Autotrophy dominated in C + A and C habitats, with positive P/R ratios ( $> 1$ ), while LL was entirely heterotrophic ( $P/R < 1$ ; Fig. 1, Table 1). The highest mean production relatively to respiration was recorded for the C + A habitat (3.13) and the lowest for LL (0.49).

### Environmental variables

Chl *a* ranged from 1 to 33 mg.m<sup>-2</sup> on C habitat, 21 to 146 mg m<sup>-2</sup> on C + A habitat and 9–64 mg.m<sup>-2</sup> on LL (Table 3). The highest average Chl *a*, as well as the highest variability, was measured in habitat C + A and the lowest in habitat C. LL had twice the average Chl *a* than C habitat. AFDM ranged from 1 on C to 36 g.m<sup>-2</sup> on C + A, while values in LL habitat were two orders of magnitude higher than in the other habitats and showed a broad variation (900–1921 g.m<sup>-2</sup>). Temperature varied little between habitats, with average of 21–22 °C. Water flow passing through the chambers varied on a narrow interval ( $0.06 \pm 0.005 \text{ m}^3.\text{h}^{-1}$ ) and it did not differ among habitats.



**Fig. 1.** Boxplots with NPP, GPP, CR and P/R ratio for each habitat; where black asterisk is a mean; horizontal segment is a median; horizontal lines marking the box are first and third quartiles with “whiskers” that extends to minimum and maximum value. Horizontal line in P/R ratio boxplot marks the benchmark for autotrophy ( $> 1$ ) and heterotrophy ( $< 1$ ).

**Table 1.** Range for net primary production (NPP), gross primary production (GPP), community respiration, CR [ $\text{g O}_2 \text{ m}^2 \cdot \text{day}^{-1}$ ] and P/R ratio for each habitat (C, cobble; C + A, cobble covered by algae; LL, leaf litter).

| Habitat | NPP        | GPP       | CR         | P/R ratio |
|---------|------------|-----------|------------|-----------|
| C       | -0.03–1.22 | 0.17–1.93 | 0.086–1.94 | 0.48–7.72 |
| C + A   | 0.47–8.66  | 0.99–9.84 | 0.64–2.55  | 0.82–9.14 |
| LL      | -3.86–2.47 | 0.23–5.99 | 1.65–7.87  | 0.03–1.25 |

Nutrients varied little between measurements and paired *t*-test showed no significant differences in nitrites and phosphates between pre- and post- water samples for both light and dark incubations ( $P > 0.05$ ). Ammonium levels were mostly below the detection limits of our instrumentation ( $< 0.05 \text{ mg} \cdot \text{L}^{-1}$ ).

Correlation between the DO probe and Winkler spectrophotometric determination was very high ( $r = 0.86$ ,  $P < 0.001$ ), with consistently lower values obtained by Winkler spectrophotometry ( $3.2\text{--}18.0 \text{ mg O}_2 \cdot \text{L}^{-1}$ ) than measured by the oxygen probe ( $4.2\text{--}22.0 \text{ mg O}_2 \cdot \text{L}^{-1}$ ).

### Relationship between benthic metabolism and habitat characteristics

The best DistLM model provided by BEST procedure included three vector overlays: Chl *a*, AFDM and PAR, which significantly contributed to the ordination axes (Fig. 2). The model explained over 60.0 % of the total variability in data. From these three vectors, Chl *a* and AFDM were the strongest contributors to the dbrDA analysis, having higher explanatory potential in comparison with PAR. The variation in Chl *a* and PAR were

associated with the variation in NPP and GPP in C + A habitat, while AFDM was strongly associated with LL habitat and strongly correlated with CR.

PERMANOVA showed statistically significant differences in explanatory variables among all the habitats ( $F = 21.6$ ,  $P = 0.001$ , based on 999 permutations) and pairwise tests showed that LL habitat differed the most from the other two habitats, with higher dissimilarity between LL and C + A than LL and C. Due to differences existing between all three habitats, correlation analyses were performed separately for each habitat.

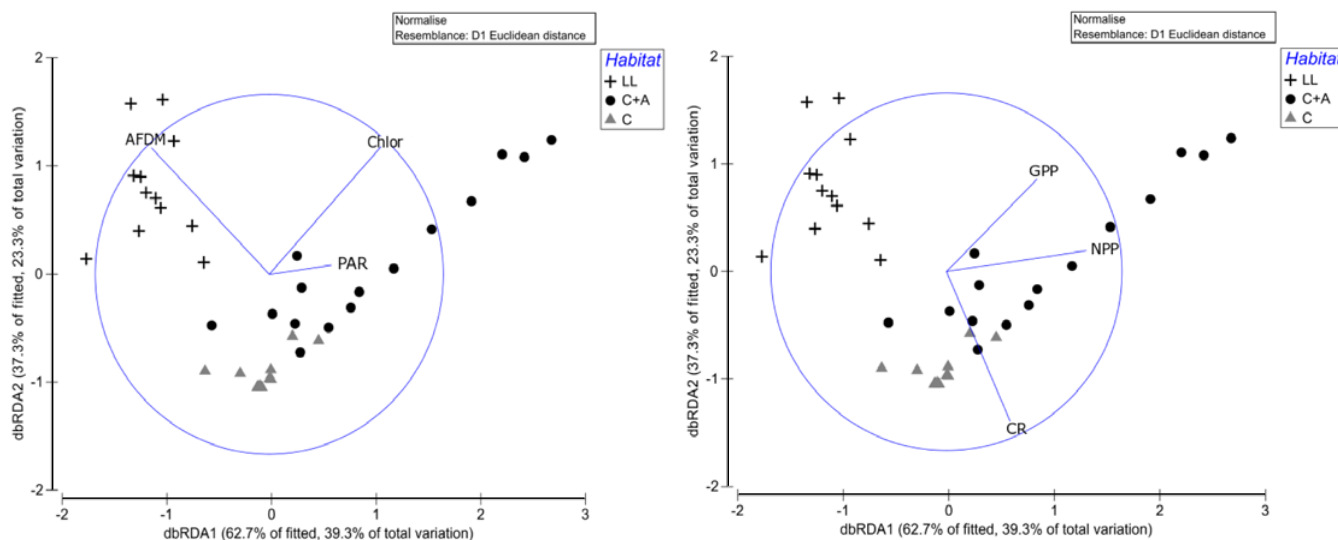
Both C and C + A habitats displayed decrease of production efficiency (expressed as GPP/Chl *a* ratio) with increasing standing stock (AFDM, Table 4). Chlorophyll *a* concentration was positively correlated with oxygen consumption (CR) in C + A and LL habitats (Table 4), although not in C habitat. High correlation existed between primary production and oxygen consumption in C and C + A habitats. By contrast it was not the case in LL habitat, where no correlation existed between GPP and CR. GPP, NPP and production efficiency (GPP/Chl *a* ratio) significantly increased with PAR in the C + A habitat. In C habitat, temperature was positively correlated with GPP, NPP and CR, regardless of PAR,

**Table 2.** Analysis of variance (ANOVA) for net primary production (NPP), gross primary production (GPP) and community respiration (CR) for the differences between habitats.

| Statistical test    | df | ANOVA   |         |                         | ANOVA   |         |                         | Kruskal–Wallis Rank Sum Test                             |                         |
|---------------------|----|---|---------|-------------------------|---|---------|-------------------------|--|-------------------------|
|                     |    | GPP (mg O <sub>2</sub> m <sup>-1</sup> .h <sup>-1</sup> ) |         |                         | NPP (mg O <sub>2</sub> m <sup>-1</sup> .h <sup>-1</sup> ) |         |                         | CR (mg O <sub>2</sub> m <sup>-1</sup> .h <sup>-1</sup> ) |                         |
| Source of variation |    | Mean Sq.  | F-ratio | Pr (> F)                | Mean Sq.  | F-ratio | Pr (> F)                | χ <sup>2</sup>   | P                       |
| Habitat             | 2  | 27 2841.00  | 13.71   | 3.75 × 10 <sup>-3</sup> | 373 635   | 19.96   | 1.47 × 10 <sup>-6</sup> | 27.01  | 1.37 × 10 <sup>-6</sup> |
| Residual            | 36 | 199.03  |         |                         | 18 719  |         |                         |  |                         |

**Table 3.** Mean ± SE for environmental variables (chlorophyll a, AFDM, photosynthetic active radiation, temperature, PO<sub>4</sub><sup>-</sup>-P, NO<sub>2</sub>-N, NH<sub>4</sub><sup>+</sup>-N) and GPP/Chl a measured during incubation experiments.

| Measured variables                                    | Habitat type  |               |               |
|---|---------------|---------------|---------------|
|   | C             | C + A         | LL            |
| Chlorophyll a (mg.m <sup>-2</sup> )                   | 9.95 ± 3.09   | 76.13 ± 10.45 | 22.55 ± 4.47  |
| AFDM (g.m <sup>-2</sup> )                             | 5.02 ± 1.15   | 17.00 ± 2.10  | 1382 ± 94.99  |
| PAR (μE m <sup>-2</sup> .s <sup>-1</sup> )            | 1799 ± 110    | 1495 ± 181    | 1554 ± 186    |
| Temperature (°C)                                      | 21.51 ± 0.55  | 22.57 ± 0.37  | 21.68 ± 0.46  |
| PO <sub>4</sub> -P (mg L <sup>-1</sup> )              | 0.046 ± 0.003 | 0.048 ± 0.005 | 0.041 ± 0.003 |
| NO <sub>2</sub> -N (mg.L <sup>-1</sup> )              | 0.042 ± 0.009 | 0.035 ± 0.006 | 0.036 ± 0.007 |
| NH <sub>4</sub> <sup>+</sup> -N (mg.L <sup>-1</sup> ) | 0.30 ± 0.27   | 0.31 ± 0.21   | 0.33 ± 0.26   |
| GPP/Chl a ratio                                       | 13.70 ± 2.97  | 5.51 ± 1.03   | 8.15 ± 1.34   |



**Fig. 2.** Diagram of distance-based redundancy analysis (dbRDA) for the best distance-based linear models (DistLM) solution. Vector overlays represent significant variables included in the model, selected with the BEST procedure based on Akaike’s information criterion. Left panel represents explanatory variables and right panel the response variables. Lengths of vectors indicate the relative influence of each variable for the ordination.

which was not significantly correlated with any of these variables.

**Discussion**

Results were consistent with our predictions, in that all three habitats showed positive gross primary production, but with significantly higher net oxygen production in cobble and cobble covered with algae. Half of the LL habitat patches exhibited negative NPP, indicating that oxygen was consumed more rapidly than it was produced. The LL habitat was an important site for high microbial

activity, however, it constituted less than 10% of overall benthic substrate and thus its contribution at the reach-scale is probably limited. Accordingly, during stabilized flow conditions, benthic metabolism was dominated by net autotrophy, in cobble and cobble covered by algae habitats, with localized heterotrophy in LL.

Physical substrate heterogeneity and biological characteristics of substrata were demonstrated to influence both GPP and CR ratio (Guash *et al.*, 1995; Sabater and Romani, 1996; Sabater *et al.*, 1998; Cardinale *et al.*, 2002; Clapcott and Barmuta, 2010). Similar range of GPP and CR values, measured using open-channel and metabolic chamber methods were obtained in other Mediterranean

**Table 4.** Spearman's rank correlation for the response and independent variables.

| Habitat | Variable  | NPP    | GPP     | CR      | Chl a    | AFDM     | PAR     | Temp   |
|---------|-----------|--------|---------|---------|----------|----------|---------|--------|
| C       | NPP       |        | 0.82*** | 0.31    | 0.73***  | 0.55     | −0.36   | 0.62** |
|         | GPP       |        |         | 0.76*** | 0.60**   | 0.52     | −0.24   | 0.73** |
|         | CR        |        |         |         | 0.45     | 0.48     | −0.20   | 0.66** |
|         | GPP/Chl a | −0.41  | −0.09   | −0.06   | −0.81*** | −0.62**  | 0.39    | −0.31  |
| C + A   | NPP       |        | 0.95*** | 0.44    | 0.31     | −0.38    | 0.78*** | 0.10   |
|         | GPP       |        |         | 0.65*** | 0.51**   | 0.28     | 0.73*** | 0.16   |
|         | CR        |        |         |         | 0.75***  | 0.16     | 0.37    | 0.28   |
|         | GPP/Chl a | 0.73*  | 0.60**  | 0.08    | 0.35     | −0.70*** | 0.65*** | −0.16  |
| LL      | NPP       |        | 0.89*** | −0.38   | 0.13     | 0.10     | 0.21    | −0.08  |
|         | GPP       |        |         | −0.14   | 0.22     | 0.16     | 0.06    | 0.15   |
|         | CR        |        |         |         | 0.59**   | 0.29     | −0.53   | 0.48   |
|         | GPP/Chl a | 0.68** | 0.76*** | 0.31    | −0.36    | 0.09     | 0.43    | 0.03   |

Significant codes are as: < 0.01 \*\*\*, < 0.05 \*\*.

ecosystems (Molla *et al.*, 1994, 1996, Suárez and Vidal-Abarca, 2000; Aristegi *et al.*, 2010) and in desert streams (Busch and Fisher, 1981; Grimm and Fisher, 1984; Mulholland *et al.*, 2001). Molla *et al.* (1994) obtained similar values of GPP (3.24 g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup>), but higher average values of respiration (2.9 g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup>) for periphyton communities, using dial oxygen curve method. Our estimates of GPP and CR are most similar to the results obtained by Suárez and Vidal-Abarca (2000) for whole periphyton communities by using diurnal oxygen change method (range 0.24–10.7 for GPP and 0.26–7.29 g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup> for CR). Aristegi *et al.* (2010) reported wide ranges of GPP (0–35.3 g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup>) and CR (1.1–17.2 g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup>) using recirculatory chambers. However, their study encompassed streams with large variability in environmental conditions, whereas the small ranges of GPP and CR in our study were measured in only one reach. Similar values were also reported for a desert stream by Mulholland *et al.* (2001) (3.0 for GPP and 8.3 for CR g O<sub>2</sub> m<sup>2</sup>.day<sup>−1</sup>), using two-station diurnal oxygen change method. Accordingly, our results with metabolic chambers on C and C + A habitats are consistent with previous studies in intermittent streams in regions of Mediterranean and semiarid climate.

### Primary production

Previous studies found that algae production increases with the Chl *a* standing crop (Morin *et al.*, 1999; Bernot *et al.*, 2010). Similarly, we found that the most productive cobble covered with algae had the highest Chl *a* concentration compared to other habitats. A positive effect of algae biomass on GPP has been reported across different regions, as measured by open channel methods (Bott *et al.*, 1985; Morin *et al.*, 1999; Bernot *et al.*, 2010). However, high periphyton biomass does not necessarily translate into higher production efficiency (as seen from the results of GPP/Chl-*a* ratio) and also reported by Velasco *et al.* (2003). Decrease of GPP/Chl *a* ratio with increasing standing stock of periphyton is common for stream ecosystems (Guash *et al.*, 1995; Morin *et al.*, 1999; Velasco *et al.*,

2003). This pattern can be related to different composition of the algal assemblages and biofilm thickness in cobble and cobble covered by algae habitats. Shifts in algae community along the gradient of biofilm development are well documented (Hudon and Bourget, 1983; Sabater and Romani, 1996). In our study, the biofilm in C + A habitat was dominated by green filamentous algae, while the biofilm in the cobble habitat was scarce and nearly invisible. Phytoplankton photosynthetic rates are known to decrease with the increasing cell wall thickness (Enríquez *et al.*, 1996) and therefore, thicker walls of filamentous algae in C + A habitat were potentially responsible for the negative trend between GPP/Chl *a* and periphyton biomass. Another contributory mechanism that could explain this pattern is self-shading, which is a common process associated with periphyton of well-developed and complex biofilm structures (Guash *et al.*, 1995).

PAR was found to be positively correlated with GPP among different reaches, in open channel measurements (Bott *et al.*, 1985; Mulholland *et al.*, 2001; Acuna *et al.*, 2004; Bernot *et al.*, 2010) as well as incubation chambers, considering only epilithic assemblages (Velasco *et al.*, 2003) and whole communities (Rosenfeld and Roff, 1991). By contrast, in our study PAR explained very little variation in metabolic parameters and had distinct influence on primary production for different habitats (Table 4). Lack of correlation between PAR and cobble is corroborated by the low levels of chlorophyll *a* on this habitat. Interestingly, temperature independently of PAR was positively correlated with GPP on cobble, but not on cobble covered with algae. This additionally confirms that biofilm structure has important role in regulating GPP response in this temporary stream.

Primary production in LL was greater than in cobble. These differences appear to be driven by distinct substrate characteristics of C and LL. Organic conditioning of litter enhances algae GPP by allowing the use of the underlying substratum as a nutrient source (Sabater *et al.*, 1998). Additionally, the oligotrophic nature of our study stream could exacerbate the nutrient limitation effect on algal colonization of cobbles. It is important to mention, however, that GPP in LL habitat could have been

overestimated, as the total area of leaves and remaining litter material probably exceeded the area of the chamber base.

### Community respiration

The highest respiration was in the LL habitat. LL and woody substrata host greater amount of heterotrophic organisms, such as bacteria, fungi and macroinvertebrates, which utilize the underlying substratum to acquire nutrients and eventually contribute to organic matter decomposition (Graça *et al.*, 2001; Romani and Sabater, 2001; Gulis and Suberkropp, 2003). In our study, AFDM (detrital standing crop) was responsible for variation in CR and differentiated the heterotrophic LL habitat, with large amounts of AFDM, from other two habitats, with smaller values of AFDM (RDA analyses). When we examined only the LL habitat, however, litter biomass did not contribute to the variation in community respiration. This lack of correlation is presumably because CR in LL is fuelled by a combination of heterotrophic utilization of the allochthonous organic matter itself, but also some contribution of microbial and algal respiration associated with periphyton. Leaves used for the experiment were senescent and the long period in the water allowed microorganisms to be associated with leaf mesophyll as well as create a layer of biofilm on their surface. Additionally, the main groups of macroinvertebrates were collector-gatherers and scrapers, with only few shredders, which confirm the trophic potential of the biofilm. However, the short acclimation time of base units in the stream did not allow proper colonization of macroinvertebrates, in comparison with benthic community encountered in this habitat during conventional sampling with hand net. Base units had more mobile and drifting taxa and less burrowing organisms. Accordingly, some important burrowing shredders, such as Diptera family, may have been under represented.

In contrast, the cobble habitat had the lowest CR from all the habitats studied and, although the average Chl *a* concentration in LL was only twice higher than in C habitat, CR in LL was almost 7-fold higher than in cobble. Therefore, higher respiration in LL is likely a result of heterotrophic activity and microbial respiration associated with biofilm layer, rather than autotrophic respiration. The organic nature of leaves promotes settlement of fungi and bacteria, which obtain nutrients *via* LL degradation. In contrast, the major part of respiration in cobble and cobble covered by algae habitats came from autotrophic respiration, which is also reflected in high correlation between GPP and CR in these habitats.

### Conclusions

Most metabolism studies with benthic chambers focus only on the dominant habitat type in a river reach (Whitledge and Rabeni, 2000; Rees *et al.*, 2005; Aristegi *et al.*, 2010). Our study clearly emphasizes that

extrapolating from a single habitat to the entire reach or stream will result in significant under or over estimation of the metabolic rates, depending on the proportional dominance of habitat types. For example, benthic habitat mapping of the reach used in our study, done in previous years, indicated striking differences in habitat coverage between winter (5% of algae cover) and summer (50% of algae cover, Wasiak *et al.*, 2013). Considering that, during summer, at least half of the substrate in intermittent Mediterranean streams is covered by filamentous algae, using only the cobble substrate for incubations would underestimate GPP by 67%. Accordingly, scaling up measurements from discrete habitats to the entire reach or stream requires quantification of habitat coverage. This can be achieved by the use of various techniques such as GIS-based analysis, based on visual benthic habitat mapping, for small reaches, or side scan sonar imagery to map larger areas of streams to whole catchments.

Employment of flow cell into chamber design successfully overcame two main drawbacks related to chamber metabolism measurements: bubble formation and inadequate water circulation within chambers (Bott *et al.*, 1997; Dodds and Brock, 1998; Uzarski *et al.*, 2001). Furthermore, measurements taken by DO oxygen probe are linearly related to the oxygen concentrations measured using the Winkler method. This validates the use of *in situ* DO probes to monitor oxygen concentration during the incubation experiments and the accurateness of the metabolic rates obtained by this improved chamber design.

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