

Periphyton control on stream invertebrate diversity: is periphyton architecture more important than biomass?

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Abstract. There is little consensus on the form of the periphyton biomass–macroinvertebrate diversity relationship in streams. One factor that these relationships do not account for is the growth form of primary producers. We (1) examined the periphyton biomass–macroinvertebrate diversity relationship in 24 streams of Cantabria, Spain, in July 2007, and (2) determined whether this relationship was underpinned, and better explained, by specific responses to the growth form of the periphyton community. We hypothesised that macroinvertebrate diversity would be a log-linear function of periphyton biomass and would respond differently to two coarse divisions of the periphytic community; i.e. positively to %cover of non-filamentous algae and negatively to %cover of streaming filamentous algae. There was no relationship between benthic periphyton biomass and macroinvertebrate diversity in these streams but, as predicted, this relationship was underpinned by responses to the growth form of periphyton community. Generally, macroinvertebrate diversity responded positively to %cover of non-filaments and negatively to %cover of streaming filaments, although results were variable. These findings suggest that periphyton biomass–macroinvertebrate diversity relationships in streams can be underpinned by interactions with specific growth forms of periphyton. We suggest that further research is required to develop robust thresholds of %cover of filamentous algae cover that would benefit managers wishing to minimise negative effects of eutrophication on stream communities.

Additional keywords: algae, biomonitoring, Cantabria, changepoint, diatom, filamentous, macroinvertebrate, rapid assessment, river management, Spain, thresholds.

Received 16 October 2013, accepted 10 December 2013, published online 16 June 2014

Introduction

Although the relationship between productivity and diversity is a central theme in ecological research (Abrams 1995; Mittelbach *et al.* 2001), we are far from reaching a consensus on the form of the relationship either empirically or theoretically for both producers and consumers (e.g. Mittelbach *et al.* 2001; Adler *et al.* 2011). Differences in the observed patterns may be a result of several factors, including the spatial scale of observation (Chase and Leibold 2002; Tonkin and Death 2013), disturbance (Huston 1994), history of community assembly (Fukami and Morin 2003) and differences between ecosystems and organisms studied (Mittelbach *et al.* 2001).

Compared with lentic systems and indeed most other environments, few studies have specifically investigated whether higher productivity or, in fact, standing crop of algal biomass leads to greater diversity in lotic systems. The few to look specifically at this periphyton standing crop–invertebrate diversity relationship in streams have found both unimodal (Death

and Zimmermann 2005) and log-linear (Death 2002; Tonkin *et al.* 2013) increases in diversity with productivity. Primary producers are principally periphytic algae in streams, which vary greatly in their growth form and include prostrate, stalked and filamentous forms (Hoagland *et al.* 1982; Steinman and McIntire 1986), all of which respond differently to environmental conditions and grazing. However, typically, biomonitoring involves assessing periphyton biomass using either chlorophyll *a* and/or ash-free dry weight (AFDW) and although these two measures are often highly correlated, they do not always respond in the same way to environmental conditions (Biggs and Hickey 1994; Feminella and Hawkins 1995). Detailed assessment of periphyton community has been less widely used as an index for biomonitoring environmental conditions (Pan *et al.* 1996; Hill *et al.* 2000). Typically, the focus of these assessments of biotic integrity has been diatoms (Kelly and Whitton 1995; Pan *et al.* 1996), although Whitton and Kelly (1995) advocated the use of the full community of plants including bryophytes.

Not only do various growth forms of periphytic algae respond differently to environmental conditions, but they can provide diverse habitat and resources for higher trophic levels (Dudley *et al.* 1986). Different periphyton growth forms can also fulfil different functional roles in benthic communities (Steinman *et al.* 1992). When levels of periphyton reach greater densities and epilithic films such as diatoms are replaced by macroalgae such as filamentous green algae, interactions between grazers and periphyton can shift from simple plant–herbivore interactions to more complex relationships. As well as providing food for a few specialist taxa, macroalgae can both provide and remove habitat and compete for space with invertebrates. Dudley *et al.* (1986) classed invertebrates into those negatively affected by macroalgae because of competition for space, positively affected because of habitat provision, and positively affected by food provision. This can be reflected in the typical shift from pollution (nutrient)-sensitive taxa associated with thin periphytic films, to pollution-tolerant taxa and filamentous-algae growth forms often associated with nutrient eutrophication (Suren *et al.* 2003).

We set out to (1) test the response of stream invertebrate-diversity metrics, often used in biomonitoring, to periphyton biomass (assessed as chlorophyll *a*) and (2), because biomass measurements do not account for variation in the growth form of primary producers, to examine whether this link can be better explained by underlying responses to different growth forms of periphyton categorised coarsely into two major groups (i.e. all non-filamentous films and mats and streaming filamentous

green algae). We also use a common stream-specific metric, %EPT (Ephemeroptera, Plecoptera and Trichoptera), to assess whether this metric is more sensitive to environmental gradients in streams than are simple invertebrate diversity measures. Percentage EPT is commonly used in stream bioassessment because, as a result of their sensitivity, EPT taxa often respond in predictable manners to changes in environmental conditions (Lenat 1988). As a result of previous work in streams (Death 2002; Tonkin and Death 2012; Tonkin *et al.* 2013), we hypothesise that invertebrate diversity, including richness and rarefied richness, will increase logarithmically with increasing periphyton biomass, but we predict that this relationship will be underpinned by particular responses to different growth forms of periphyton. Specifically, because of the view that diatoms, which make up a large part of non-filamentous periphytic film and mat growth forms, are considered favourable food and habitat to many stream invertebrates and filamentous algae can be poor habitat for many (but not all, e.g. Dudley *et al.* 1986; Power 1990) invertebrates (Suren and Riis 2010), invertebrate diversity will respond positively to %cover of non-filamentous and negatively to %cover of streaming filamentous algae.

Materials and methods

Study sites

Twenty-four streams were sampled in the Cantabria region, Northern Spain, on one occasion in July 2007 (Fig. 1, Appendix 1).



Fig. 1. Map showing the location of 24 streams in Cantabria, Spain, sampled in July 2007. Latitudes and longitudes are given in Appendix 1.

Cantabria is a mountainous and coastal region, with both characteristics playing an important role in determining climate and river morphology. Near the northern coast, valleys are below 400 m asl, whereas the Cordillera Cantábrica, a west to east running mountain range, reaches more than 2600 m asl in the south-west of the region. These steep inland valleys run northward and contain short 'flashy' rivers with high erosive power. Cantabria has a humid oceanic temperate climate, with an average annual temperature of 14°C and an average annual precipitation of ~1200 mm. Rainfall is regularly distributed throughout the year, being heaviest in winter and spring. Storms occur in any season, and snow is common from late autumn to early spring on the mountain ranges (for a more detailed description see Barquín *et al.* 2012). Land use surrounding sampling sites varied from Atlantic deciduous forest consisting predominantly of oak (*Quercus* spp.) and European beech (*Fagus* spp.) to pasture and small urban settlements.

Sites were selected from the following six river catchments: Río Besaya, Río Saja, Río Pas, Río Pisueña, Río Nansa and Río Ebro. To account for local variation in factors such as geology and land use, sites were selected in pairs *a priori* within each of the six catchments, so that one low- and one high-productivity site in close geographic proximity were sampled. Because these were selected before sampling, productivity estimates for the selection of *a priori* high- and low-productivity streams were based on one-off visual estimates of periphyton, which are detailed below. All sites were cobble-bottom streams. Altitude of the sites ranged from 163 to 1061 m asl and average channel width ranged from 1.9 to 30.7 m (Appendix 1). Riparian canopy cover ranged from 1% to 80% cover (Appendix 1).

Physicochemical variables

Several physical and chemical variables were measured once during the time of macroinvertebrate sampling. Depth and water velocity were recorded with a Marsh–McBirney flowmate current meter (Marsh–McBirney, Frederick, MD) at five equidistant points along the thalweg. Conductivity, temperature, dissolved oxygen concentration and percentage saturation, and pH were measured using a YSI 556 MPS meter (YSI Inc., Yellow Springs, Ohio, USA). A 250-mL unfiltered water sample was collected at each site and kept in the dark and on ice during transport, for later analysis of nitrate (NO_3^- : cadmium-reduction method), phosphate (PO_4^{3-} : molybdate method) and ammonia (NH_3 : salicylate method), calculated using a Beckman Coulter DU Series 700 UV/Vis Scanning Spectrophotometer (Beckman Coulter Inc., Brea, CA). Substrate size composition was assessed by sampling 100 stones using the Wolman walk methodology (Wolman 1954), and then converting these measurements to a substrate-size index following Jowett and Richardson (1990). Substrate heterogeneity was assessed using the Shannon diversity index, whereas bed stability was measured using the bottom component of the Pfankuch stability index (Pfankuch 1975). Finally, percentages of riparian vegetation and canopy cover were visually estimated over a ~50-m reach.

Biological collections

Periphyton biomass was measured by extracting chlorophyll *a* from five stones (mean area: 60 cm²) collected randomly from

riffles within the same ~50-m reach at each site. These were kept cool and in dark, before being frozen and taken back to the laboratory. Chlorophyll *a* and phaeophytin were extracted directly from the stones by using 90% acetone at 5°C for 24 h in the dark. Absorbances were read on a Beckman Coulter DU Series 700 UV/Vis Scanning Spectrophotometer and converted to pigment concentration following Steinman and Lamberti (1996). Stone surface area was estimated from axial dimensions following Graham *et al.* (1988) and then halved to correct for the proportion of the stone available for periphyton growth.

We calculated two other metrics to assess periphyton communities within riffles along the sampling reach, namely, %cover of all non-filamentous algae cover (i.e. periphytic films and mats) and %cover of streaming filamentous algae (i.e. clearly identifiable filamentous algae to the naked eye). These were visually assessed along three randomly located transects across the entire width of the stream bed within riffles along the sample reach, using modified rapid assessment protocols from the New Zealand Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000). We assessed coverage of the two periphyton categories on every stone directly beneath the transect line across the width of the streams. Where substrate was finer than ~10 mm, we did not examine the periphyton cover. Where coverage was uncertain between bare substrate and thin films, we also felt the rock surfaces with our hands to examine coverage. We grouped all algal forms into the two coarse groups of non-filaments (includes diatoms and all other crustose, prostrate and stalked algae growth forms, as well as non-streaming filamentous algae) and streaming filaments, with the remaining being classified as having no cover. Even though there is some potential for observer bias using this method, observations were made by the same person at all 24 sites. Recent research has highlighted that, given appropriate training, variability in estimates using visual assessment approaches may not be a major problem (Kilroy *et al.* 2013).

Five 0.1-m² 500- μm -mesh Surber samples were collected at random from riffles at each site and were preserved in 10% formalin in the field. In the laboratory, the samples were washed through 500- μm and 1-mm Endecott sieves before being identified and counted to the lowest possible taxonomic level. Invertebrates were mostly identified to morpho-species; however, where possible morpho-species were identified using available keys (e.g. Tachet *et al.* 2000).

The number of animals per 0.1 m² (density) was calculated for each individual sample and averaged per site, as was the number of taxa (richness). Rarefied richness ($ES[N]$) was calculated for 261 individuals, which was the lowest average number of animals at a site. Rarefaction accounts for the passive increase in the number of taxa collected with increasing number of individuals collected (Hurlbert 1971). This, in effect, standardises sites by predicting richness per a set number of animals rather than a set area. The final community metric used was the mean percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) animals per sample.

Statistical analysis

All analyses were performed using R version 2.15.2 (R Core Team 2013). First, to assess any clear linkages between

Table 1. Pearson's correlation coefficients for periphyton and macroinvertebrate community metrics against physicochemical variables collected from 24 streams of Cantabria, Spain, July 2007

DO, dissolved oxygen; OH cover, overhead cover; ES(261), rarefied taxonomic richness, calculated for 261 individuals; SI, size index; hetero., heterogeneity.
 *** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$ (significances are after correcting for false discovery rate)

| Variable | No. of taxa (N) | Log(N) | ES(261) | %EPT animals | Chlorophyll a | %Non-filaments | %Streaming filaments | %Bryophytes |
|-------------------|---------------------|------------|---------|--------------|-----------------|----------------|----------------------|-------------|
| Pfankuch | -0.09 | 0.17 | -0.14 | -0.09 | -0.24 | 0.17 | -0.16 | -0.18 |
| Altitude | 0.27 | 0.20 | 0.16 | 0.10 | 0.00 | -0.07 | -0.01 | -0.09 |
| pH | -0.07 | 0.08 | -0.13 | -0.03 | 0.39 | -0.15 | 0.13 | 0.25 |
| Conductivity | -0.17 | 0.50 | -0.35 | -0.31 | 0.64* | -0.46 | 0.75*** | -0.19 |
| Temperature | 0.11 | -0.11 | 0.15 | -0.07 | -0.29 | 0.50 | -0.34 | -0.06 |
| DO | 0.27 | 0.18 | 0.20 | -0.04 | 0.43 | 0.04 | 0.22 | 0.02 |
| OH cover | 0.17 | -0.13 | 0.26 | 0.04 | -0.16 | -0.19 | 0.00 | -0.06 |
| Velocity | 0.18 | -0.29 | 0.30 | 0.31 | -0.57 | 0.44 | -0.27 | -0.31 |
| Depth | -0.26 | -0.31 | -0.12 | 0.05 | -0.07 | 0.21 | -0.05 | 0.13 |
| Width | 0.02 | -0.12 | 0.05 | -0.23 | -0.27 | 0.53 | -0.29 | -0.05 |
| Substrate SI | -0.06 | -0.44 | 0.15 | 0.05 | -0.38 | 0.07 | -0.29 | 0.04 |
| Substrate hetero. | -0.07 | 0.27 | -0.16 | -0.09 | 0.19 | -0.15 | 0.22 | 0.25 |
| Slope | 0.34 | -0.17 | 0.41 | -0.09 | -0.06 | -0.10 | -0.09 | 0.13 |
| Nitrate | -0.40 | 0.12 | -0.39 | -0.26 | 0.10 | -0.13 | 0.25 | 0.09 |
| Phosphate | -0.19 | 0.21 | -0.25 | -0.17 | 0.05 | -0.16 | 0.10 | -0.18 |
| Ammonia | -0.29 | 0.51 | -0.43 | -0.39 | 0.14 | -0.49 | 0.26 | -0.09 |

physicochemical variables and both periphyton and invertebrates, we correlated all invertebrate, periphyton and physicochemical variables using Pearson's correlation coefficient with the `rcorr()` function in the R package 'Hmisc'. We adjusted P -values for multiple comparisons by using the false discovery rate method (Benjamini and Hochberg 1995) with the `p.adjust()` function in the R 'stats' package.

We used linear regression to examine relationships among periphyton biomass, %cover of non-filaments, %cover of streaming filaments and invertebrate metrics, using the `lm()` function in the 'stats' package in R. Where required, we $\log(x+1)$ -transformed data to remove heteroscedasticity. Where both linear and quadratic regressions were run, we used Akaike's information criterion (AIC) to select the best-fitting curve. We also regressed the three dominant taxa against the three periphyton metrics. Where thresholds were apparent in the response of invertebrate metrics to %cover of streaming filamentous algae, we tested these using the `cpt.mean()` procedure in the R package 'changepoint' (Killick and Eckley 2011). We used Bayesian information criterion (BIC) and 'at most one change' (AMOC) to select the location of single change-points if present.

To visually assess the multivariate structure of the macroinvertebrate community, we performed a non-metric multidimensional scaling (NMDS) ordination using the `metaMDS()` function in the Vegan package (Oksanen *et al.* 2011). We used Bray–Curtis distances and limited the number of NMDS axes to two. To examine different influences of the three periphyton metrics (chlorophyll a , %cover of non-filaments, and %cover of streaming filaments), we fitted smooth-surface thinplate splines using the `ordisurf()` function in Vegan. This uses generalised additive models (GAMs) to overlay a smoothed response surface, which allows a more detailed interpretation than does a simple linear vector. More specifically, it enables non-linear effects of the three periphyton metrics on macroinvertebrate community structure to be examined visually.

Results

Physicochemical variables and periphyton

Periphyton biomass, assessed as mean chlorophyll a , ranged from 2.58 to 15.35 $\mu\text{g cm}^{-2}$, with a mean \pm s.e. of $5.8 \pm 0.7 \mu\text{g cm}^{-2}$. Chlorophyll a was positively correlated with %cover of streaming filamentous algae ($r = 0.63$, $P = 0.039$). Percentage cover of non-filaments ranged between 8.3% and 95.0%, with a mean of $48.3 \pm 5.6\%$ cover, and %cover of streaming filaments averaged $24.0 \pm 6.1\%$, ranging between 0.0% and 91.7%. Conductivity ranged from 68 to 402 $\mu\text{S cm}^{-1}$ (Appendix 1). Conductivity was positively correlated with chlorophyll a and %cover of streaming filamentous algae (Table 1).

Invertebrate community composition

Mean taxonomic richness ranged between 9.6 and 21.0 taxa per 0.1 m^2 with a mean \pm s.e. of 15.6 ± 0.6 taxa per 0.1 m^2 and the number of individuals averaged 928.2 ± 406.7 individuals per 0.1 m^2 , ranging between 261.4 and 10194.2 taxa per 0.1 m^2 . Percentage EPT ranged between 11.5% and 83.6%, with a mean of $56.8 \pm 3.5\%$.

Ephemeroptera was the most abundant family, making up between 12.6% and 75.2% of animals at each site, with an average (mean \pm s.e.) of $47.9\% \pm 3.2\%$ of the community composition, followed by Diptera ($25.9\% \pm 4.1\%$; range 3.1–83.2%), Coleoptera ($5.1\% \pm 0.7\%$), Trichoptera ($4.6\% \pm 0.8\%$) and Plecoptera ($3.6\% \pm 0.8\%$). These patterns were largely due to the dominance of three individual taxa. *Baetis* spp., on average, made up $41.4\% \pm 3.0\%$ of the community, followed by *Prosimulium* spp. ($17.8\% \pm 3.7\%$), and *Echinogammarus* spp. ($7.2\% \pm 2.1\%$).

In response to gradients of periphyton cover, % *Prosimulium* spp. was lowest at intermediate levels of %cover of non-filaments ($F_{2,21} = 7.39$, $P = 0.004$, $R^2 = 0.41$, $y = 55.45 - 2.09x + 0.02x^2$), whereas it was not related to %cover of streaming filaments ($F_{2,21} = 2.94$, $P = 0.075$, $R^2 = 0.22$,

$y = 19.48 - 0.79x + 0.01x^2$). Percentage *Baetis* spp. peaked at intermediate levels of %cover of non-filaments ($F_{2,21} = 6.25$, $P = 0.007$, $R^2 = 0.37$, $y = 11.88 + 1.61x - 0.02x^2$), whereas it was also not related to %cover of streaming filaments ($F_{2,21} = 2.53$, $P = 0.10$, $R^2 = 0.19$). Moreover, % *Echinogammarus* spp. did not respond to %cover of either non-filaments ($F_{1,22} = 1.49$, $P = 0.24$, $R^2 = 0.06$) or streaming filaments ($F_{1,22} = 1.98$, $P = 0.17$, $R^2 = 0.08$).

Ordination on $\log(x + 1)$ -transformed invertebrate data produced a reasonable fit, with a stress of 0.19 (Fig. 2). Overlaying GAM-fitted smooth surfaces for each of the three periphyton metrics indicated three different effects on the structure of multivariate invertebrate community (Fig. 2). Streaming filamentous algae loaded negatively on NMDS 1, whereas non-filaments cover exhibited a clear negative loading on NMDS 2 (Fig. 2). However, the influence of chlorophyll *a* on the structure of invertebrate community was more non-linear, with the strongest gradient on NMDS 1 but the lowest value situated central in ordination space.

Density and diversity patterns

The only aspect of the invertebrate community to respond to chlorophyll *a* was the number of individuals, which increased monotonically with increasing biomass; however, although significant, the explained variance was low (Fig. 3, Table 2). Taxonomic richness, rarefied richness and %EPT animals were not related to chlorophyll *a* (Fig. 3, Table 2).

Density and diversity measures exhibited opposing responses to the two growth forms of periphyton measured. Taxonomic richness and rarefied richness increased log-linearly with increasing substrate cover of non-filaments, but the number of individuals was not related to %cover of non-filaments (Fig. 3, Table 2). The percentage of EPT animals appeared more sensitive to higher percentage cover of non-filamentous algae, and peaked strongly at intermediate levels and declined at higher levels of %cover of non-filamentous algae (Fig. 3, Table 2). However, this was largely dependent on one site which had 95% cover. Removing this site strengthened the fit and altered the relationship to a quadratic increase ($F_{2,20} = 9.77$, $P = 0.001$, $R^2 = 0.49$, $y = 24.36 + 1.36x - 0.01x^2$).

Taxonomic richness was not linearly related to %cover of streaming filamentous algae; however, density of individuals exhibited a quadratic increase with increasing %cover (Fig. 3, Table 2). Both rarefied richness and %EPT animals responded negatively to streaming filamentous algae, exhibiting a curvilinear decline with an increasing cover of streaming filamentous algae (Fig. 3, Table 2). However, removing the site with 92% cover of streaming filamentous algae removed any relationship with %EPT ($F_{2,20} = 0.41$, $P = 0.67$, $R^2 = 0.04$). Change-point analysis indicated that taxonomic richness exhibited a threshold response to increasing streaming filaments at 40% cover, with a drop in mean richness from 16.27 taxa below and including 40% cover to 13.60 taxa above 40% cover. Rarefied richness exhibited a similar threshold response, with a drop from 15.32 to 11.73 taxa above 40% cover of streaming filaments. Change-point analysis did not return a significant threshold response of %EPT animals to the cover of streaming filamentous algae, despite %EPT being considerably lower at the last data point of 92% cover.

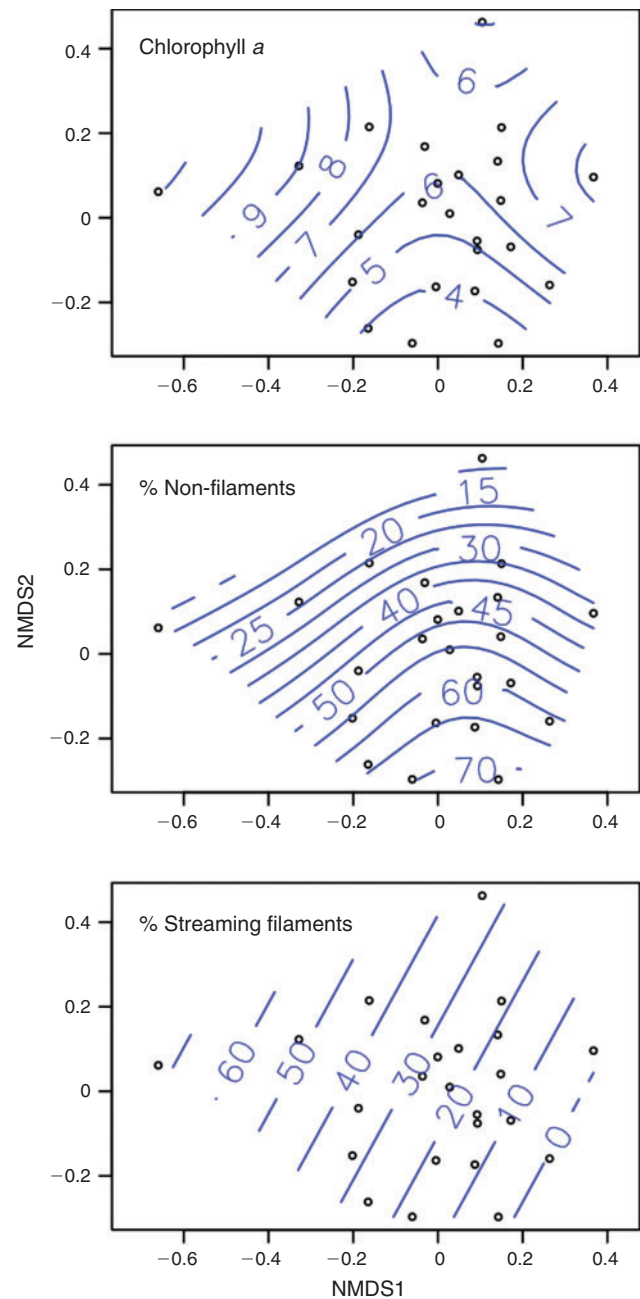


Fig. 2. Non-metric multidimensional scaling (NMDS) ordination on $\log(x + 1)$ -transformed invertebrate-community data collected from 24 streams in Cantabria, Spain, July 2007. Individual plots display overlaid smooth-surface thin-plate splines using generalised additive models (GAMs) for the three periphyton metrics. Numbers on the splines represent the values of the periphyton metric. 2D stress = 0.19.

Discussion

There was no relationship between periphyton biomass and invertebrate diversity in the present study. Recent studies in stream communities have found log-linear increases in diversity with periphyton biomass (e.g. Death 2002; Tonkin and Death 2012; Tonkin *et al.* 2013), which, along with the belief that stream-wide competitive exclusion does not often materialise at

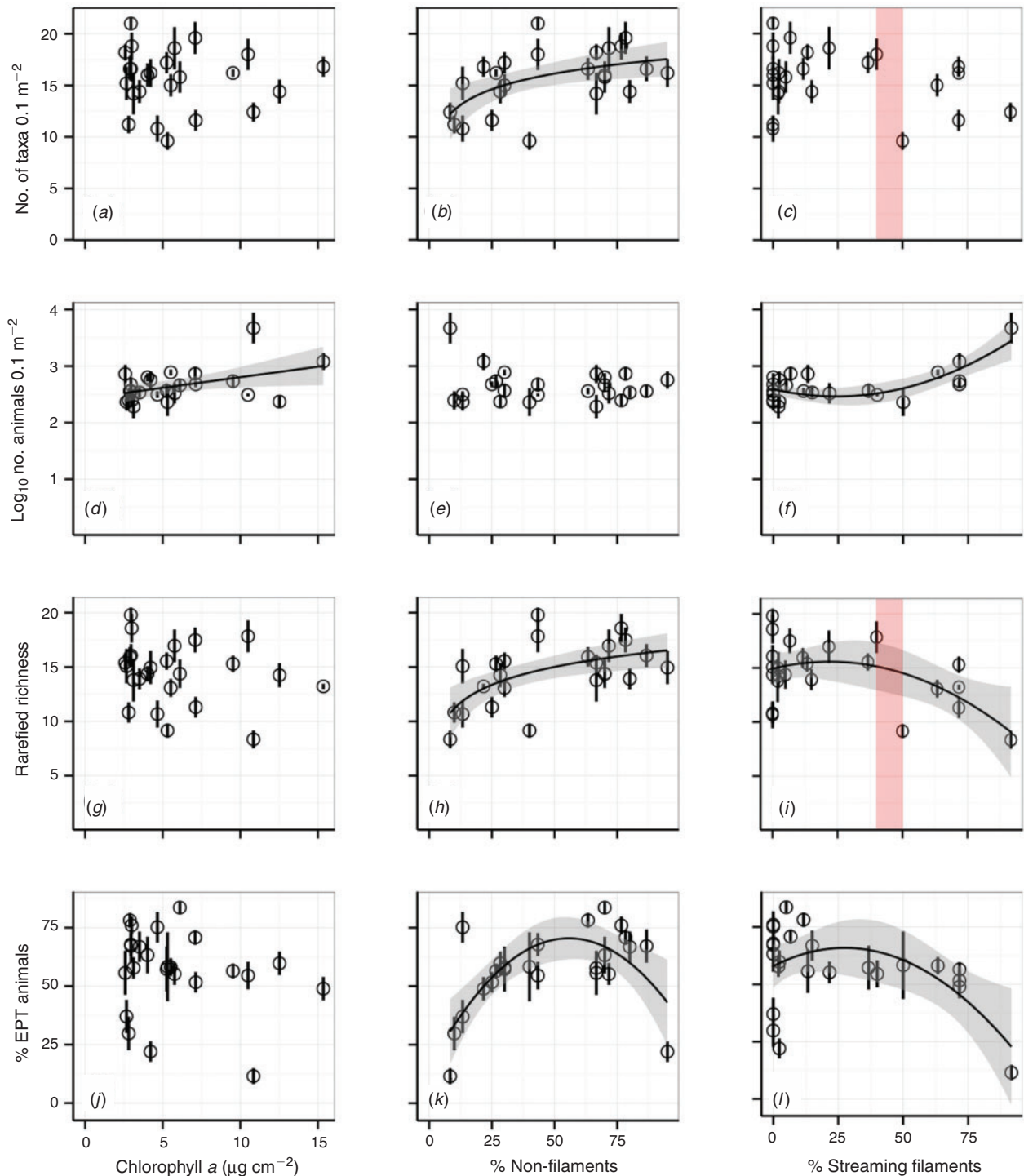


Fig. 3. Mean (± 1 s.e.) (a–c) taxonomic richness, (d–f) number of animals, (g–i) rarefied richness (ES(261)), and (j–l) %EPT animals as a function of (a, d, g, j) chlorophyll *a*, (b, e, h, k) %cover of non-filamentous algae, and (c, f, i, l) %cover of streaming filamentous algae in 24 streams of Cantabria, Spain, July 2007. Grey area represents 95% confidence interval of the regression line. Vertical shaded area (light grey), between two dotted lines, on *c* and *i* represent the significant threshold point identified using changepoint analysis. Regression equations are given in Table 2.

high periphyton biomass in streams, led us to predict that this log-linear trend would occur in these Spanish streams. However, no clear link was evident between periphyton biomass and any of the metrics used.

Relationships with periphyton growth form

Although invertebrate communities did not respond clearly to changes in periphyton biomass, the growth form of the periphyton community was important in determining diversity

Table 2. Results for regression analysis for taxonomic richness, number of animals, rarefied richness and %EPT animals against periphyton metrics for 24 streams in Cantabria, Spain, July 2007

Degrees of freedom for linear and log-linear models are 1,22 and for quadratic models 2,21. AIC = Akaike's information criterion for the selection of the best model among linear, log-linear and quadratic curves. Lowest values represent the best model

| Parameter | F (AIC) | P | R ² | Equation |
|---|---------------|--------|----------------|---------------------------------|
| Chlorophyll <i>a</i> (µg cm ⁻²) | | | | |
| No. of taxa | 0.05 | 0.83 | 0.002 | Non-significant |
| Log(no. of animals) | 5.31 | 0.03 | 0.194 | $y = 2.43 + 0.037x$ |
| ES(261) | 0.91 | 0.35 | 0.040 | Non-significant |
| %EPT animals | 1.14 | 0.30 | 0.049 | Non-significant |
| Films and mats cover (%) | | | | |
| No. of taxa | 9.62 (42.8) | 0.01 | 0.304 | $y = 7.55 + 2.2 \ln(x)$ |
| Quadratic | 4.91 (43.1) | 0.02 | 0.319 | $y = 10.7 + 0.19x - 0.002x^2$ |
| Log(no. of animals) | 0.62 | 0.44 | 0.027 | Non-significant |
| ES(261) | 12.77 (42.3) | 0.002 | 0.367 | $y = 5.73 + 2.37 \ln(x)$ |
| Quadratic | 6.38 (42.8) | 0.007 | 0.378 | $y = 9.08 + 0.21x - 0.0016x^2$ |
| %EPT animals | 3.70 | 0.07 | 0.144 | Non-significant |
| Quadratic | 9.36 | 0.001 | 0.471 | $y = 15.35 + 1.98x - 0.02x^2$ |
| Filamentous algae cover (%) | | | | |
| No. of taxa | 1.59 | 0.22 | 0.067 | Non-significant |
| Log(no. of animals) | 12.30 (4.18) | 0.002 | 0.359 | $y = 2.51 + 0.006x$ |
| Quadratic | 13.71 (-3.21) | 0.0002 | 0.566 | $y = 2.59 - 0.011x + 0.0002x^2$ |
| ES(261) | 5.54 (118.1) | 0.028 | 0.201 | $y = 15.45 - 0.043x$ |
| Quadratic | 4.22 (117.3) | 0.03 | 0.287 | $y = 14.93 + 0.059x - 0.001x^2$ |
| %EPT animals | 3.67 | 0.07 | 0.143 | Non-significant |
| Quadratic | 4.19 | 0.03 | 0.285 | $y = 57.93 + 0.58x - 0.01x^2$ |

patterns. There has been extensive research on the effects of grazers on algal communities, and this top-down control has been the central focus of periphyton–invertebrate community relationships (Hillebrand 2009). However, of note is the fact that the majority of this research has focused solely on the control of periphyton biomass and not on different growth forms. Our results have demonstrated that focusing simply on biomass is potentially masking important underlying relationships. Other than grazer-specific responses (e.g. Gresens and Lowe 1994; Maasri *et al.* 2008), the bottom-up effects of algal assemblages on invertebrate communities has received little direct attention in streams (but see Dudley *et al.* 1986; Koksvik and Reinertsen 2008). Nonetheless, it is clear that the growth form of periphyton has strong influences on the structure of stream benthic communities (Dudley *et al.* 1986; Koksvik and Reinertsen 2008), and grazing communities can in fact grow at different rates depending on the dominant algal growth form (Feminella and Resh 1991).

In the present study, although variation was evident in the shape of relationships, general patterns suggest that there were opposing influences of the two main growth forms detected. Namely, %cover of non-filamentous algae exerted a positive response and %cover of streaming filamentous algae a negative response on invertebrate diversity. Differences were mainly due to changes in the densities of the three dominant taxa, namely, the blackfly larvae, *Prosimulium* spp., the mayfly, *Baetis* spp., and the amphipod, *Echinogammarus* spp. Moreover, the response to the dominant growth forms appeared to be highly species specific depending on feeding habits, such as favouring more palatable epilithic films or drift-feeding on filamentous algal cells.

Percentage of non-filaments was the best predictor of diversity, with both taxonomic and rarefied richness increasing logarithmically as cover increased. This mirrors the hypothesis we set of a log-linear curve for the relationship between periphyton biomass and diversity that several recent studies have found in benthic communities (e.g. Death 2002; Tonkin and Death 2012). Although diatoms are just one of the groups of algae that comprise our 'non-filaments' classification, they are likely to make up a large component of these films and mats. Diatoms are the most important food source for a high proportion of benthic invertebrates, because grazers tend to be able to assimilate diatoms better than other algal taxonomic classes (Lamberti *et al.* 1989).

The percentage of EPT animals can respond to shifts in periphyton biomass (Tonkin *et al.* 2009); however, we found no such relationship in the present study. Percentage EPT did respond to the growth form of periphyton, declining at the highest levels of %cover of both filaments and non-filaments; however, these trends were influenced by individual sites at the end of the spectrum of cover. The decline at higher levels of cover of non-filaments may be due to the fact that more palatable forms of periphytic films, such as diatoms in particular, are replaced by other mat-forming taxa when growth becomes more prolific. *Baetis* often use diatoms as a food source and their prevalence can vary greatly with types of algae depending on the stage in their lifecycle (Dudley *et al.* 1986). Mayflies generally tend to favour grazing diatoms (Jacoby 1987) and due to the large proportion of grazers, EPT taxa often respond negatively to filamentous algae (Quinn and Hickey 1990; Suren 2005). Consequently, we expected a strong decline in %EPT with an increasing cover of streaming filamentous algae; however, it

remained relatively high up to three-quarters of bed cover of streaming filamentous algae, and although there was a strong decline in %EPT beyond this point, this was affected by one site with 92% cover of streaming filaments. One potential explanation for this is that some filamentous algae can host other palatable epiphytic algae such as diatoms, which in turn provides a food source for grazers. Recent research has shown that epiphytic diatoms can lead to reduced shear stress on photosynthesising filamentous algae (Hansen *et al.* 2014), which could in fact alter the hydrodynamic environment for grazers.

Filamentous algae may also provide a greater retention of organic detritus that in turn would support more taxa from other functional feeding groups such as shredders. However, the shredder and/or predator *Echinogammarus* spp. did not respond to either growth form of periphyton. Barquin and Death (2004) found *Echinogammarus* to be dominant in spring-fed streams in Cantabria and suggested that this may be a consequence of increased biotic interactions associated with environmental stability, thus causing suppression of other invertebrates. We found no evidence to suggest lower diversity at sites with greater densities of *Echinogammarus*.

The number of animals increased rapidly with increasing %cover of streaming filaments, largely as a result of *Prosimulium* spp. becoming dominant. The response of *Prosimulium* spp. to increased cover of filamentous algae is variable due to their life histories (Townsend 1981; Dudley *et al.* 1986; Morin and Peters 1988); black flies (Simuliidae) are often associated with bare substrates, whereas smaller individuals are often found in high densities attached to filamentous algae (Dudley *et al.* 1986). Black-fly larvae are filter feeders, capturing their food from drifting organic seston and so do not directly browse on periphyton, but can be found in high densities in enriched rivers, feeding on drifting algal cells (Peterson *et al.* 1985) as do other filter-feeding invertebrates (Benke and Wallace 1980; Wallace and Merritt 1980).

The number of taxa inhabiting substrates did not change with an increasing cover of streaming filaments. However, because of marked increases in *Prosimulium* spp., rarefied richness declined strongly at intermediate levels. When levels of periphyton reach greater densities and epilithic diatoms are replaced by macroalgae such as filamentous green algae, more complex relationships tend to develop, involving habitat provision and exclusion as well as direct food interactions (Dudley *et al.* 1986). Dense layers of filamentous algae can lead to the displacement of sensitive taxa by those that can tolerate large diurnal fluctuations in dissolved oxygen. For instance, chironomids are often associated with macroalgae, whereas EPT taxa are not (Power 1990; Koksvik and Reinertsen 2008).

Although grouping into two broad categories is a simplification of the underlying composition of periphyton, benthic invertebrates often have specialist feeding traits for either filamentous or non-filamentous growth forms. For example, stream herbivores typically reject filamentous algae in favour of other algae when selecting food sources (Gregory 1983; Steinman *et al.* 1992), possibly because they often have high cellulose content and thick walls (Lamberti and Moore 1984). The dense levels of streaming filamentous algae found in the present study are potentially a result of such selective grazing, which has been shown to alter plant communities in many

ecosystems by reducing palatable algae, leaving non-palatable algae behind (e.g. Feminella and Resh 1991; Bråthen *et al.* 2007). Grazers can alter periphyton community structure within and between micro- and macro-algal growth forms in lotic systems (McAuliffe 1984; Dudley *et al.* 1986; Feminella and Resh 1991), and the suppression of palatable forms can lead to communities consisting of resistant prostrate blue-green algae (Hart 1985; Power *et al.* 1988). In fact, selectivity is often so strong that grazing insects may remove, without ingesting, unfavourable forms so as to allow favourable forms to remain for grazing (Hart 1985). Therefore, caution is needed when inferring top-down or bottom-up control in streams because it is likely to change through time with the arrival of specialist grazers (Lamberti *et al.* 1989) or with physical disturbance resetting the process (Fisher *et al.* 1982).

Management implications

Like many relationships in nature, linkages between periphyton growth form and invertebrate community metrics in the present study were non-linear. It is crucial to understand these non-linear relationships for the setting of ecological thresholds of anthropogenic impact (Hilderbrand *et al.* 2010) and assessing recovery from stressors (Clements *et al.* 2010) in lotic systems. Given we only spot-measured nutrients in the present study, we cannot directly attribute nutrients as the main underlying stressor here. However, long-term increases in nutrients can lead to the proliferations of filamentous algae similar to those found here, which in turn exert a threshold response of macro-invertebrate diversity (Wang *et al.* 2007; Evans-White *et al.* 2009). Of course, these relationships are also underpinned by the presence of riparian canopy cover, with proliferations of filamentous algae only able to occur with sufficient light levels (Bunn *et al.* 1999). Evans-White *et al.* (2009) suggested that the reduction in diversity at enriched sites may be a result of food-resource quality exceeding the level at which many taxa have evolved, and thus leading to a dominance of fast-growing primary consumers.

We suggest that the cover of streaming filamentous algae could be a useful threshold indicator in streams. In the present study, 40% cover of streaming filamentous algae led to a decline in diversity (richness and rarefied richness), whereas %EPT did not respond with a threshold response. A lack of sites above 75% cover may have limited this response because the final point at 92% cover had considerably lower %EPT. Welch *et al.* (1988) found levels of cover of filamentous algae greater than 20% were considered nuisance levels, whereas Biggs (2000) recommended a maximum of 30% cover as a management objective for aesthetics, recreation and trout habitat. As with any study, the timing of sampling is critical, especially for assessing nuisance growth of filamentous algae because these are vulnerable to flood events. Ideally, this should occur throughout the year and at least during summer low-flow periods, as was the case in the present study, but not following a high-flow event. We are unaware of any high-flow events leading up to the sampling performed in the present study.

The fact that invertebrate diversity responded more strongly to the growth form of periphyton than chlorophyll *a* implies that this could be a useful addition to typical biomass measures for managers interested in preserving biodiversity. Many have used

components of the periphyton community as an index for biomonitoring environmental conditions (Pan *et al.* 1996; Hill *et al.* 2000); however, most use diatoms exclusively and require large investments in time and money to complete. We have found that periphyton community composition does not necessarily need to be measured to a low taxonomic level, and rapid protocols have been developed for this assessment (e.g. Biggs and Kilroy 2000). Although losing information on species-specific responses, these rapid assessment methods overcome the high spatial variability associated with methods such as measuring chlorophyll *a* from stones. Often the distinction between invertebrate communities with shifts in relative abundance of films and mats to filamentous algae-dominated is obvious, such as the shift from insect- to non-insect-dominated communities (e.g. Suren *et al.* 2003). Nonetheless, more research is required to test the robustness of rapid assessment protocols such as recent work by Kilroy *et al.* (2013) that assessed consistency of visual estimates of periphyton standing crop in streams.

Conclusions

Our research found no relationship between chlorophyll *a*, a commonly used measure of periphyton biomass, and macroinvertebrate diversity in these streams. As hypothesised, this relationship was underpinned by contrasting responses of invertebrate communities to the growth form of the periphyton community. Generally, diversity responded positively to increasing %cover of non-filamentous algae and negatively to increasing %cover of streaming filamentous algae. This underlying response to the growth form of periphyton community provides some explanation for the lack of a clear relationship between productivity and diversity in lotic systems, as well as providing a possible mechanism for lowering diversity at greater nutrient concentrations. Further research is needed to explore the development of robust thresholds of filamentous cover by managers wishing to minimise negative effects of eutrophication on stream biota.

Acknowledgements

We are grateful to Jessica Costall for help with fieldwork. This manuscript was improved by comments from Angus McIntosh, Ian Henderson, Christopher Robinson and two anonymous reviewers. A Massey University Doctoral Scholarship supported JDT during the study. JB was supported by a Ramon y Cajal grant (Ref: RYC-2011-08313) of the Spanish Ministry of Economy and Competitive Affairs.

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Appendix 1. Selected mean physicochemical variables and site characteristics recorded at 24 streams in the Cantabrian Mountains, Spain, in July 2007
 Alt., altitude; Pflank. bottom, the bottom component of the Pflankuch stability index; Cond., conductivity; Temp., temperature

| Site Name | Catchment | Longitude | Latitude | Alt. (m) | Width (m) | Depth (cm) | Velocity (ms ⁻¹) | Substrate size index | Pflank. bottom | pH | Cond. (µS cm ⁻¹) | Temp. (°C) | DO (mg L ⁻¹) | DO (%) | OH cover (%) | Substrate hetero. | Slope (m 100 m ⁻¹) | Nitrate (mg L ⁻¹) | Phosphate (mg L ⁻¹) | Ammonia (mg L ⁻¹) |
|--|-----------|-----------|-----------|----------|-----------|------------|------------------------------|----------------------|----------------|------|------------------------------|------------|--------------------------|--------|--------------|-------------------|--------------------------------|-------------------------------|---------------------------------|-------------------------------|
| 1 Río Besaya @ Helguera | Besaya | -4.032760 | 43.158628 | 214 | 10.34 | 27.0 | 0.49 | 123.07 | 23 | 9.30 | 371 | 15.49 | 11.37 | 114.1 | 20 | 2.07 | 2.93 | 1.50 | 0.39 | 0.04 |
| 2 Río Ereña @ Helguera | Besaya | -4.030378 | 43.159622 | 222 | 7.60 | 23.0 | 0.36 | 141.54 | 29 | 9.03 | 166 | 15.91 | 10.00 | 101.3 | 60 | 2.01 | 3.53 | 0.40 | 1.16 | 0.05 |
| 3 Río Bisueña @ Barcena Pie de Concha | Besaya | -4.068260 | 43.121292 | 309 | 4.31 | 26.0 | 0.56 | 131.88 | 39 | 8.70 | 269 | 14.70 | 10.12 | 100.5 | 20 | 1.94 | 6.73 | 0.90 | 0.20 | 0.03 |
| 4 Río Torina @ Barcena Pie de Concha | Besaya | -4.054722 | 43.126793 | 299 | 7.30 | 22.8 | 0.20 | 142.69 | 27 | 9.19 | 124 | 14.96 | 10.18 | 100.8 | 20 | 2.15 | 5.64 | 1.00 | 0.24 | 0.03 |
| 5 Bco. de Santiurde @ Santirude de Reimosa | Besaya | -4.078611 | 43.063231 | 634 | 4.45 | 15.2 | 0.48 | 127.15 | 44 | 8.99 | 221 | 12.73 | 10.66 | 100.9 | 20 | 1.98 | 3.46 | 1.30 | 0.26 | 0.16 |
| 6 Ayo. Rumadero @ Pesquera | Besaya | -4.074784 | 43.083177 | 574 | 2.10 | 12.6 | 0.19 | 132.02 | 41 | 8.84 | 329 | 13.77 | 9.80 | 94.5 | 40 | 2.18 | 5.08 | 1.80 | 0.57 | 0.26 |
| 7 Río Leon @ San Martín de Quevedo | Besaya | -4.038779 | 43.139996 | 264 | 5.13 | 18.0 | 0.35 | 142.14 | 43 | 8.76 | 193 | 16.00 | 9.68 | 98.2 | 80 | 2.10 | 5.42 | 0.50 | 0.47 | 0.15 |
| 8 Río de los Llares @ Pedro | Besaya | -4.067773 | 43.192911 | 185 | 6.13 | 16.4 | 0.23 | 152.16 | 26 | 8.65 | 205 | 16.55 | 9.20 | 94.3 | 10 | 1.80 | 4.8 | 0.80 | 0.68 | 0.02 |
| 9 Río Argoza @ Barcena Mayor | Saja | -4.232753 | 43.156963 | 422 | 17.50 | 30.2 | 0.63 | 178.53 | 27 | 9.02 | 127 | 15.20 | 10.36 | 103.2 | 10 | 1.81 | 4.67 | 0.60 | 0.28 | 0.02 |
| 10 Río Saja @ Renedo | Saja | -4.304550 | 43.194076 | 293 | 30.70 | 23.4 | 0.56 | 145.22 | 42 | 8.97 | 130 | 17.79 | 10.25 | 108.1 | 1 | 1.89 | 7.00 | 1.00 | 0.33 | 0.03 |
| 11 Río de la Magdalena @ San Andrés | Pas | -3.897027 | 43.112736 | 412 | 9.63 | 23.2 | 0.31 | 166.81 | 30 | 9.04 | 194 | 12.77 | 9.50 | 88.5 | 50 | 1.95 | 5.13 | 0.90 | 0.38 | 0.03 |
| 12 Ayo. Salceira @ San Miguel de Luena | Pas | -3.899921 | 43.096378 | 347 | 2.67 | 13.4 | 0.46 | 158.08 | 19 | 8.85 | 216 | 12.94 | 10.70 | 101.5 | 60 | 2.01 | 10.36 | 1.10 | 0.31 | 0.10 |
| 13 Río Viña @ Viña | Pas | -3.804674 | 43.155633 | 326 | 6.97 | 19.4 | 0.47 | 139.45 | 24 | 8.66 | 81 | 18.63 | 9.69 | 103.6 | 40 | 1.94 | 3.48 | 0.50 | 0.14 | 0.03 |
| 14 Río Pas @ Vega de Pas | Pas | -3.759107 | 43.164859 | 366 | 9.50 | 20.6 | 0.54 | 192.32 | 30 | 8.77 | 130 | 18.36 | 9.16 | 97.4 | 5 | 1.64 | 2.55 | 0.70 | 0.66 | 0.04 |
| 15 Río Llerana @ Coterrillo | Pisuena | -3.794589 | 43.263832 | 207 | 5.37 | 18.4 | 0.38 | 137.48 | 32 | 8.44 | 256 | 15.38 | 9.56 | 95.5 | 30 | 2.14 | 1.23 | 2.50 | 0.50 | 0.05 |
| 16 Río Pisuena @ Barcena de Carriedo | Pisuena | -3.823485 | 43.241998 | 181 | 7.33 | 17.2 | 0.39 | 102.32 | 45 | 8.48 | 283 | 16.82 | 9.97 | 102.5 | 10 | 1.97 | 5.81 | 1.10 | 0.19 | 0.03 |
| 17 Río Nansa @ Puenteansa | Nansa | -4.406832 | 43.257111 | 168 | 9.47 | 20.8 | 0.36 | 121.21 | 41 | 8.75 | 303 | 15.41 | 10.00 | 100.2 | 10 | 1.92 | 4.44 | 0.40 | 0.19 | 0.04 |
| 18 Río Quivierda @ Puenteansa | Nansa | -4.406179 | 43.257101 | 163 | 6.15 | 16.8 | 0.33 | 110.22 | 33 | 8.59 | 280 | 14.02 | 10.67 | 103.7 | 50 | 2.14 | 2.95 | 0.20 | 0.56 | 0.03 |
| 19 Río Tanea @ Quintanilla | Nansa | -4.476253 | 43.254480 | 238 | 6.40 | 17.0 | 0.35 | 112.50 | 44 | 8.78 | 144 | 16.42 | 9.82 | 100.4 | 10 | 2.03 | 3.68 | 0.40 | 0.43 | 0.05 |
| 20 Ayo. de Traveseras @ Quintanilla | Nansa | -4.473238 | 43.256427 | 239 | 4.00 | 13.6 | 0.22 | 196.25 | 24 | 8.76 | 247 | 15.13 | 10.08 | 100.2 | 70 | 1.79 | 10.00 | 0.60 | 0.60 | 0.02 |
| 21 Bco. Palomba @ Paracuelles | Ebro | -4.211634 | 43.019856 | 895 | 1.93 | 14.4 | 0.24 | 61.12 | 20 | 9.24 | 402 | 12.99 | 11.90 | 113.2 | 5 | 1.95 | 1.79 | 0.20 | 0.51 | 0.05 |
| 22 Río Hjar @ Espinilla | Ebro | -4.226527 | 43.019571 | 937 | 13.00 | 13.4 | 0.45 | 119.91 | 43 | 9.15 | 121 | 17.17 | 10.30 | 96.7 | 2 | 2.04 | 2.11 | 0.50 | 0.18 | 0.04 |
| 23 Río de Soto @ Soto | Ebro | -4.222249 | 43.035916 | 960 | 5.00 | 14.6 | 0.52 | 131.04 | 34 | 9.06 | 68 | 13.01 | 10.12 | 96.2 | 70 | 2.18 | 7.31 | 0.06 | 0.19 | 0.04 |
| 24 Río Guares @ Abiada | Ebro | -4.289066 | 43.016011 | 1061 | 2.27 | 15.2 | 0.47 | 173.46 | 26 | 8.63 | 83 | 11.55 | 10.44 | 96.1 | 80 | 1.88 | 14.21 | 0.40 | 0.23 | 0.04 |