

Accepted Manuscript

This is the peer reviewed version of the following article:

Schneider, SC, Sample, JE, Moe, JS, Petrin, Z, Meissner, T, Hering, D. Unravelling the effect of flow regime on macroinvertebrates and benthic algae in regulated versus unregulated streams. *Ecohydrology*. 2018; 11:e1996, which has been published in final form at <https://doi.org/10.1002/eco.1996>

This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

It is recommended to use the published version for citation.

1 **Unravelling the effect of flow regime on macroinvertebrates and benthic algae in**
2 **regulated versus unregulated streams**

3
4
5
6 *Short title:* Effects of flow regime on macroinvertebrates and benthic algae
7
8
9

10
11 Susanne C. Schneider^{1*} susi.schneider@niva.no
12 James E. Sample^{1*} james.sample@niva.no
13 S. Jannicke Moe¹ jannicke.moe@niva.no
14 Zlatko Petrin² petrin.zlatko@gmail.com
15 Thomas Meissner³ thomas.meissner@uni-due.de
16 Daniel Hering³ daniel.hering@uni-due.de
17
18

19 ¹ Norwegian Institute for Water Research, Gaustadalléen 21, 0349, Oslo, Norway

20 ² Norwegian Institute for Nature Research, P.O. Box 5685 Sluppen, NO-7485 Trondheim,
21 Norway

22 ³ Department of Aquatic Ecology, Faculty of Biology, University of Duisburg-Essen, 45117
23 Essen, Germany

24
25 * Susanne C. Schneider and James E. Sample contributed equally to this study
26
27

28 **Supplementary information**

29
30 A more detailed description of the methodology, including fully documented code for the
31 statistical analysis, is available online [here](#).
32
33
34
35
36

37 **Keywords**

38 River; hydrology; periphyton; macrozoobenthos; flow variability; cyanobacteria; Indicators
39 of Hydrological Alteration;
40
41

42 **Abstract**

43

44 Variability in riverine flow regimes is important for aquatic biodiversity. However, across the
45 globe, management of water resources has altered natural flow dynamics. We explored
46 relationships between flow regime (calculated from three years' daily averaged discharge), and
47 water chemistry, benthic algae, as well as macroinvertebrate datasets from 64 sites across
48 Germany and Norway. To deal with multicollinearity while maintaining interpretability, we
49 performed principal component (PC) analyses for each dataset in each country, and selected
50 the metric with the highest absolute loading on each PC to represent that PC. We then used L1-
51 regularised (lasso) regression to link differences in water chemistry and hydrology to
52 differences in ecology, and compared this approach to the more popular best-subsets ordinary
53 least squares (OLS) regression.

54 The results obtained using lasso regression were broadly comparable to those produced by best-
55 subsets OLS, but the lasso approach "rejected" more models than the best-subsets approach.
56 When lasso identified a plausible model, it was the same or similar to the best model found by
57 best-subsets OLS. The lasso method was more "discerning", i.e. it identified a smaller number
58 of potentially interesting models, while best-subsets regression seemed to find "too many"
59 relationships. We identified two response variables that were potentially affected by regulation:
60 (i) river regulation may lead to higher cyanobacterial abundance, possibly via a less variable
61 flow regime; (ii) reduced flow variability may lead to a reduced proportion of grazers and
62 scrapers, possibly indicating a shift towards an increased importance of heterotrophic energy
63 sources in ecosystems with less variable flows.

64

65 **1. Introduction**

66

67 Natural variability in riverine flow regimes maintains ecological gradients, impacting aquatic
68 biodiversity (Poff et al., 2007; Richter et al., 1997; Schneider and Petrin, 2017). However,
69 exploitation of freshwater resources such as industrial abstractions, hydropower generation and
70 drinking water supply has significantly altered natural flow dynamics across the globe (Poff et
71 al., 2007). 37% of European rivers are affected by flow regulation (www.ecologic.eu/11663)
72 and, in Norway, where hydropower provides >95% of the total electricity generated, more than
73 two-thirds of river basins are affected by hydromorphological alteration (www.nve.no).
74 Understanding the effects of flow alteration on aquatic ecology is crucial for the sustainable
75 management of regulated river systems, but linking indicators of hydrological change to
76 ecology is not straightforward because (1) properly replicated and controlled experiments are
77 rarely possible in practice in fluvial ecosystems (Richter et al., 1997), (2) species assemblages
78 in experimental flumes often differ from the assemblages in adjacent watercourses, raising
79 questions on the relevance of flume experiments (Bækkeli et al., 2017), and (3) field data may
80 reflect the effects of correlated variables rather than the effects of the variables of interest.

81

82 The “natural flow paradigm” (Poff et al., 1997) provides a rationale for linking hydrological
83 indicators (HIs) to ecological responses, and a range of HIs have been proposed for assessing
84 the extent of ecohydrological change. Olden & Poff (2003) conducted a comparison of more
85 than 170 flow-derived metrics and concluded that the suite of indices commonly referred to as
86 the “Indicators of Hydrological Alteration” (IHA; Richter et al., 1996) typically provide an
87 adequate summary of the overall flow regime. The IHA methodology defines 33 statistics in
88 five broad classes, and the approach has been widely applied to characterise compensation
89 flows that mimic the natural regime. However, in the context of linking HIs to ecological
90 datasets, multicollinearity between the IHA variables usually necessitates dimensionality
91 reduction (Olden and Poff, 2003; Yang et al., 2008). A simpler approach is the “ecochange”
92 concept proposed by Vogel et al. (2007), in which hydrological alteration is estimated from the
93 difference between “before” and “after” flow duration curves. Gao et al. (2009) demonstrated
94 that this method provides an effective summary of the IHA output without multicollinearity
95 issues, but it is only applicable to study designs where flow data are available both before and
96 after some well-defined intervention (such as building a dam).

97

98 Although many studies focus on developing indicators of hydrological change, comparatively
99 few establish convincing links between these metrics and measures of ecological
100 health/resilience. In the presence of strong multicollinearity, common pre-processing
101 approaches for linking hydrological and ecological variables include using PCA or
102 (Non-)Metric (Multi-)Dimensional Scaling (NMDS) to identify relevant subsets of explanatory
103 variables from an initial broader suite, followed by regression analysis to identify potentially
104 interesting relationships: Monk et al. (2007) used PCA followed by stepwise linear regression
105 to investigate relationships between around 200 HIs and macroinvertebrate metrics at 83
106 locations in England and Wales; Yang et al. (2008) used a genetic programming approach to
107 perform variable selection and identify significant relationships between time series of fish
108 species diversity and the IHA parameters calculated for a catchment in Illinois, USA; and
109 Schneider and Petrin (2017) used NMDS and stepwise regression to link hydrology and water
110 chemistry variables to benthic algae and macroinvertebrate assemblages at 40 sites in Norway.

111

112 Here, we explored relationships between hydrology, water chemistry, benthic algae and
113 macroinvertebrate datasets collated for 64 sites across Germany and Norway. We use two
114 independent datasets collected in two countries to identify potentially causal relationships
115 between regulation/hydrology and aquatic biota. We used the approach of Richter et al. (1996)
116 to develop an initial set of HIs, which were then distilled to a more informative subset following
117 the recommendations of Olden and Poff (2003) and Gao et al. (2009). We expected (1)
118 ecological and (2) hydrochemical & hydrological differences between regulated and
119 unregulated streams, and we hypothesized (3) that the hydrochemical and hydrological
120 differences may plausibly explain the ecological differences.

121

122 When testing hypothesis 3, we avoided using stepwise or “best-subsets” regression techniques
123 – despite their popularity in the literature – because in cases where significance testing is of
124 interest these methods have been criticised for “overfitting” the data, leading to inflated
125 estimates of significance (Harrell, 2001). Instead, we present an alternative approach using L1-
126 regularised (lasso) regression with qualitative analysis of the “lasso path”, which we believe is
127 both more robust and more informative than iterative variable selection procedures. Results
128 obtained using “best-subsets” regression are included for comparison.

129

130 **2. Methodology**

131

132 **2.1 Site locations**

133

134 Data were collected from 64 sites (*Fig. 1*), half of which were classified as "regulated" i.e. they
135 had an anthropogenically modified flow regime. 24 sites (12 regulated) were in Western
136 Germany and the remaining 40 (20 regulated) in Southern Norway. The study sites were
137 selected to be located close to flow gauging stations, such that daily average flow
138 measurements were available. River regulation is a multifaceted term, and our dataset
139 comprises “minimum discharge” sites (those from which freshwater is abstracted and bypasses
140 the river), sites downstream from outlets of hydropower plants, and sites downstream from
141 dams and weirs.

142

143 The German sites were small and medium sized siliceous mountain streams in the state of
144 North Rhine-Westphalia. Elevations ranged from 50 to 350 m a.s.l. and the median drainage
145 area was 147 km² (range: 11 to 800 km²). Land cover comprised mostly deciduous forest
146 dominating the hillslopes with settlements and agricultural areas at lower elevations and along
147 valley floors. The geology was siliceous, with precipitation ranging from 800 to 1600 mm/yr.

148

149 The median drainage area of the Norwegian sites was 375 km² (range: 7 to 2335 km²).
150 Elevations ranged from 15 to 990 m a.s.l. and land cover from alpine mats to largely
151 coniferous forest, as well as settlements and agricultural areas along valley floors. The
152 geology was siliceous and average precipitation ranged from 600 to 3500 mm/yr.

153

154 **2.2 Ecological data**

155

156 Benthic algae and macroinvertebrate surveys were conducted at all 64 sites. The German data
157 originated from national monitoring surveys during late summers and autumns between 2006

158 and 2012. In Norway, fieldwork took place during September 2013, and samples were taken
159 as close as possible to the respective hydrological gauging stations. The distance between
160 sampling points and gauging stations was <5 km in Germany and <1 km in Norway, and the
161 differences in drainage area between sampling sites and gauging stations was < 10%.

162

163 Benthic algae

164

165 Samples of soft-bodied benthic algae (defined as algae, attached to the river bottom or in close
166 contact on or within patches of attached aquatic plants, including cyanobacteria but excluding
167 diatoms) were taken according to European standard procedures (EN 15708:2009) along an
168 approximately 10 m length of river bottom using an “aquascope” (a bucket with a transparent
169 bottom). Diatoms were not included due to the great differences in methodology for sample
170 preparation and enumeration between diatom and non-diatom benthic algae. In Germany, a 5-
171 level scale was used to record abundance of benthic algae at each site: 1, microscopically rare;
172 2, microscopically abundant; 3, maximum 5% cover; 4, 5% to 33% cover; 5, more than 33%
173 cover. In Norway, percentage cover of each form of macroscopically visible benthic algae was
174 recorded, and the abundance of each microscopic taxon was estimated in the laboratory as
175 “rare”, “common” or “abundant”. To enable comparative data analysis, we translated the cover
176 data recorded in Norway into the 5-level scale used in Germany. All samples were examined
177 under a microscope and all non-diatom algae identified to species, wherever possible. For some
178 genera of filamentous green algae, whose vegetative forms cannot be determined to species
179 level (e.g. *Spirogyra* Link or *Mougeotia* C. Agardh), categories based mainly on filament width
180 were used.

181

182 Prior to analysis, the taxonomic levels were harmonized between German and Norwegian
183 datasets. From the harmonised dataset, we calculated overall taxon richness, as well as richness
184 of the most abundant algal groups, i.e. red algae, green algae, and cyanobacteria (Table 1). We
185 approximated total benthic algal abundance at each site by summarising the cubed 5-level
186 values for each taxon. This was done because the 5-level scale used for abundance estimation
187 of benthic algae in Germany is non-linear. Using cubed 5-level values for total abundance is a
188 method commonly used for submerged macrophytes (Melzer, 1999) and is regarded as the
189 “best possible” approximation for comparing abundances among algal groups and among sites.
190 Other response variables were calculated (e.g. cover of *Phormidium* sp., cover of cyanobacteria
191 with heterocysts, eutrophication indices used for ecological status assessment, etc.), but
192 omitted from further analysis since they either showed little variation, co-varied with other
193 response variables, or were inapplicable in one of the countries.

194

195 Macroinvertebrates

196

197 In Germany, the multi-habitat sampling procedure was applied. Benthic invertebrates were
198 collected from a total of 20 sample units from representative substrates (i.e. those covering
199 more than 5% of the sample reach). Each sampling unit had a size of 25 x 25 cm (resulting in
200 1.25 m² of stream bottom being sampled), and was sampled by means of kick sampling. At
201 each site in Norway, macroinvertebrates were collected in ten replicates using a Surber net
202 (sampling area 0.1 m²; mesh size 500 µm). The substrate mainly consisted of gravel, pebbles,

203 cobbles or small boulders, that were agitated to a depth of ~10 cm for one minute during
204 sampling. All samples were immediately preserved in 70% ethanol for later analysis.

205

206 In the laboratory, samples were sorted using a 500 µm sieve and the macroinvertebrates
207 classified to the lowest possible taxonomic level, usually species. In Germany,
208 macroinvertebrate identification was performed to the level of the Operational Taxalist
209 (<http://www.fliessgewaesserbewertung.de/en/download/bestimmung/>), which means most
210 taxa were identified to species, but with genus level for certain Plecoptera and Trichoptera,
211 family level for Oligochaeta, and from genus to subfamily level for Diptera. In Norway, some
212 dipteran taxa and microcaddisflies (Hydroptilidae) could only be identified to genus. In
213 addition, bryozoans, nematodes, oligochaetes, water mites, cladocerans, ostracods, non-biting
214 midges and blackflies were not identified any further. Prior to data analysis, taxonomic levels
215 were harmonized between the German and Norwegian datasets.

216

217 We calculated common bioassessment indices and abundance ratios of functional feeding
218 groups (FFG) of macroinvertebrates using ASTERICS (www.fliessgewaesserbewertung.de/;
219 Table 1). FFGs are used to characterize ecosystem attributes such as the relative importance of
220 autotrophic and heterotrophic organic carbon as the basis of the food web (Doledec et al.,
221 2015).

222

223 2.3. Water chemistry

224

225 In Germany, water samples were taken within one month of biological sampling, while in
226 Norway, water samples were taken together with the biological samples. At four German sites,
227 there was a considerable time gap between the dates of macroinvertebrate surveys and the
228 collection of hydrochemical samples. The corresponding sites were therefore removed from
229 the macroinvertebrate dataset, leaving 64 sites for benthic algae and 60 for macroinvertebrates.
230 Water chemistry was analysed at accredited laboratories using the following national standard
231 procedures (Norway/Germany): total organic carbon (TOC; NS/DIN EN 1484), Total
232 phosphorus (TP; NS/DIN EN ISO 15681-2), and Total nitrogen (TN; NS 4743/ DIN 38409
233 H28). In addition, pH and conductivity were measured in both countries using handheld
234 instruments.

235

236 2.4. Hydrological indices

237

238 Near-complete time series of average daily flow were available for all locations for a period of
239 three years prior to sampling. Beyond three years, some of the discharge records had substantial
240 data gaps, so the three-year period prior to sampling was chosen to represent medium-term
241 hydrological conditions at each watercourse. Three years seem an appropriate time frame for
242 our analysis because macroinvertebrates and benthic algae may rapidly recolonize a stream site
243 after an extreme event (Power et al., 2013). Consequently, older records of river flow are
244 increasingly unlikely to have persistent effects on present day macroinvertebrate and benthic
245 algal composition.

246

247 Small data gaps in each series, up to a maximum of 7 days in length, were filled using linear
248 interpolation (because we had no indications of extreme events during these short periods), and

249 a suite of 62 HIs were calculated from the daily flow values at each location (Table 2). Selection
250 of HIs was initially based on the 33 IHA metrics defined by Richter et al. (1996), but modified
251 to produce a single statistic for each variable for the entire three-year period. For example,
252 rather than using the Julian date of each annual maximum to represent the timing of extreme
253 events (as per Richter et al.), we calculated the number of days prior to sampling to the
254 maximum flow in the series (and also to the last point when flows exceeded the 95th percentile).

255
256 Exploratory analysis of the modified set of IHA metrics revealed that some variables exhibited
257 very little variation in our dataset. In addition, previous analysis of the Norwegian data found
258 that important predictors included metrics representing flow *ranges*, as well as indices of
259 monthly maxima and minima (Schneider and Petrin, 2017). This finding was broadly consistent
260 with the work of Olden and Poff (2003), who noted that the IHA methodology often fails to
261 adequately quantify the magnitude of extreme flow conditions. We therefore expanded the set
262 of HIs in our analysis to include monthly minimum and maximum discharges, together with a
263 number of overall indices of flow variability, such as the coefficient of variation (CV), the
264 interquartile range (IQR) and the 90-percentile-range (Table 2).

265

266

267 2.5. Analysis procedure

268

269 The workflow for the analysis is summarised below and described in detail in Appendices A1-
270 A4. Data processing was performed using Python 2.7 (Python Software Foundation, 2016) and
271 all code is available in the Supplementary Information.

272

- 273 1. Define hydrological regimes and stratify the dataset
- 274 2. Use dimensionality reduction to ameliorate collinearity among variables, separately for
275 each dataset (benthic algae, macroinvertebrates, water chemistry, hydrology) and each
276 country (Norway, Germany); identify subsets of variables that broadly represent overall
277 variability in each dataset
- 278 3. Test hypotheses 1 and 2 by comparing the metrics selected in step 2 at regulated versus
279 unregulated sites
- 280 4. Use regression techniques on the variables showing substantial differences in step 3 to
281 quantify relationships between hydrology, water chemistry and biota (hypothesis 3),
282 and interpret their ecological significance

283

284 Germany and Norway have different climates and hydrological regimes (Appendix A1).
285 German sites are characterised by high autumn and winter flows generally declining throughout
286 the summer. In contrast, Norwegian sites are heavily influenced by snow accumulation and
287 melting processes, typified by low flows during the winter and peak discharges during May
288 and June. A number of previous studies (Monk et al., 2007; Olden and Poff, 2003) found that
289 the most representative hydrological metrics vary according to stream type. For this reason, we
290 began by performing separate analyses on the German and Norwegian datasets before
291 analysing the pooled data (to create an analysis with greater statistical power). We regard the
292 occurrence of similar relationships in the Norwegian, German and pooled datasets as
293 particularly interesting, as they increase the weight of evidence.

294

295 Dimensionality reduction

296

297 The set of calculated metrics comprised two binary categorical variables (Germany/Norway;
298 regulated/unregulated) and almost 100 continuous variables (62 HIs, 23 macroinvertebrate
299 metrics, 8 benthic algae metrics and 5 water chemistry variables), many of which are similar
300 in nature (e.g. maxima, minima, and percentiles of flow). Exploratory data analysis using
301 pairwise correlation matrices revealed substantial multicollinearity, which was reduced using
302 the PCA algorithm in Scikit-Learn (v0.18.1; Pedregosa et al., 2011) to identify a smaller set of
303 near-orthogonal variables capable of explaining most of the variance. Olden and Poff (2003)
304 suggested PCA as a pragmatic method for variable selection in the context of eco-hydrological
305 data analyses. One disadvantage, however, is that linear combinations of the input variables
306 can become difficult to interpret in a meaningful way. We therefore followed the approach of
307 Gao et al. (2009) by choosing the metric with the highest absolute loading on each principal
308 component (PC) to represent that PC. In cases where several metrics had approximately the
309 same maximum loading, all candidate variables were carried forward for further analysis (such
310 variables are marked in brackets in Table 3). This method has the advantage of retaining
311 meaningful variables and facilitating interpretation, while also reducing multicollinearity.
312 Note, however, that collinearity is not eliminated completely, because the selected variables
313 for each PC are no longer orthogonal.

314

315 We use the Kaiser-Guttman criterion (KGC; Gao et al., 2009) to decide how many PCs to keep.
316 The KGC recommends keeping all components with eigenvalues greater than 1, which in the
317 analysis presented here typically selects between 3 and 10 PCs, explaining around 80 – 90%
318 of the overall variance. Variables were first standardized by subtracting the mean and dividing
319 by the standard deviation, and separate PCAs were applied to each of the four datasets (benthic
320 algae, macroinvertebrates, water chemistry, hydrology) in each country. Further details are
321 provided in Appendix A2.

322

323 Tests for differences

324

325 The metrics selected by PCA were tested to explore statistical differences between regulated
326 and unregulated sites using a robust Bayesian approach (Kruschke, 2012) implemented using
327 the PyMC3 package (v3.0; Salvatier et al., 2016). A detailed explanation is provided in
328 Appendix A3. Within a Bayesian framework, probabilities are interpreted as “degrees of
329 belief”, so our approach permits statements such as (for example), “*given our data, there is*
330 *85% probability that regulated sites in Norway have a higher macroinvertebrate abundance*
331 *than unregulated sites*”.

332

333 Regularised multiple linear regression

334

335 The 64 sites comprising our dataset encompassed a range of regulation and also a variety of
336 natural flow regimes, so there may be no clear-cut distinction between regimes at “regulated”
337 and “unregulated” sites. As well as analysing differences between regulated and unregulated
338 sites, we therefore also analysed the dataset as a continuous spectrum of hydrological regimes,
339 rather than making a binary classification, in order to better understand the relationships
340 between variables.

341

342 The regression analysis considered the same set of response and explanatory variables for both
343 countries, as this makes it possible to directly compare models between countries, and to
344 estimate combined models based on the “pooled” data from both countries. The set of response
345 and explanatory variables included all those showing substantial differences between regulated
346 and unregulated sites in either country (i.e. the *union* of the variables for Norway and Germany
347 shown in Table 4).

348

349 Despite dimensionality reduction using PCA, the number of combinations of response and
350 explanatory variables under consideration was still large. Previous studies (e.g. Monk et al.,
351 2007; Schneider and Petrin, 2017) applied stepwise or best-subsets regression in this situation,
352 but these techniques are problematic when significance testing is of interest (Harrell, 2001).
353 We therefore used the more robust approach of lasso regression (Hastie et al., 2009), using
354 standardized data to identify the most important relationships between ecological (response)
355 and explanatory variables. A detailed explanation of this method is given in Appendix A4. The
356 lasso can easily be used to assess which predictors are important in the model, but estimating
357 the strength of the relationships (i.e. the model coefficients) is more difficult. One pragmatic
358 solution is to use the lasso to identify the best model (or a small number of candidate models),
359 and then use OLS regression with the unstandardized data to estimate the coefficients directly
360 in the original data units (Hastie et al., 2009). This is the approach adopted here.

361

362 Finally, for purposes of comparison, we also applied “best-subsets” regression to identify the
363 best OLS model out of all possible combinations of explanatory variables (judged according to
364 the Bayesian Information Criterion; BIC). This method is computationally intensive and prone
365 to “overfitting”, but it is nevertheless widely used and therefore offers an interesting contrast
366 to the lasso.

367

368 All regression analyses were carried out separately for each country. In addition, datasets were
369 “pooled” and additional analyses of the combined Norwegian and German data carried out.
370 Individually, the ecological, chemical, and hydrological gradients in each country may be
371 small, whereas in the combined dataset they were larger. The combined analyses therefore
372 made it easier to constrain regression relationships, albeit with the caveat that additional
373 complications were introduced by combining measurements from two different hydrological
374 regimes, and by introducing a possible “country effect”. However, if similar relationships
375 occurred in the Norwegian, German and combined dataset, that strengthened the significance
376 of the findings.

377

378 **3. Results**

379

380 **3.1. Differences between regulated and unregulated sites**

381

382 Table 3 summarizes the variables with the strongest gradients in each dataset and each country.
383 See Appendix A2 and the [online code repository](#) for further details of the PCA procedure.

384

385 Each of the variables listed in Table 3 was tested for differences between regulated and
386 unregulated sites using a Bayesian approach (Table 4; see Appendix A3 and the [online code](#)

387 [repository](#) for further details). Table 4 only includes metrics with a posterior probability of a
388 difference between regulated and unregulated sites assessed to be worthy of further
389 investigation: cases where posterior probabilities of differences were small ($< 75\%$), or the
390 model diagnostics indicated problems with the assumptions (based on the posterior predictive
391 check – see Appendix A3) were disregarded.

392

393 Consistent differences between regulated and unregulated sites in both countries included (1)
394 the higher abundance of cyanobacteria, (2) the slightly higher German saprobic index, (3) the
395 higher mean flow, (4) the lower coefficient of variation in flow regime and (5) the slightly
396 higher total nitrogen concentration at regulated than unregulated sites.

397

398 The observed differences in biota between regulated and unregulated sites (Table 4) may reflect
399 coincidence, river regulation, or a causal relationship with a co-variate. Our data cannot
400 differentiate among these. To gain a more detailed understanding of the relationships between
401 response and explanatory variables, we used lasso regression.

402

403 3.2. Regression analysis

404

405 We summarised the results of the OLS analysis obtained for the best lasso model and compared
406 them with results from a “best-subsets” approach, separately for the Norwegian, German and
407 combined datasets (Table 5; see Appendix A4 and the [online code repository](#) for further
408 details). The same sets of response and explanatory variables were used in both countries (i.e.
409 all variables in Table 3) to facilitate model comparison.

410

411 The positive relationship between the coefficient of variation of flows (CV) and the
412 proportion of grazers and scrapers consistently occurred in the Norwegian, German and
413 combined datasets. In both, Germany and Norway, a 10% decrease in flow variability was
414 associated with a 1.3% to 2% decrease in the proportion of grazers and scrapers (Fig. 2,
415 Table 5). A similar result was also achieved with OLS “best-subset” regression, although in
416 the Norwegian dataset the best model was achieved using December maximum flow and the
417 number of flow reversals per year, instead of CV (Table 5).

418

419 Using the best-subset approach, three more relationships consistently occurred in the German,
420 Norwegian and combined datasets: the German saprobic index, Shannon-Wiener diversity, and
421 the proportion of swimmers and divers were significantly related to flow variables (Table 5).
422 However, for the German saprobic index, explanatory variables differed between Norway and
423 Germany (Table 5), rendering the relationship unreliable. For the proportion of swimmers and
424 divers, the number of high pulses consistently occurred in all three datasets, but with different
425 signs (positive in Germany and negative in Norway), also rendering the relationship unreliable.
426 However, a high October maximum flow was consistently associated with a higher
427 macroinvertebrate diversity in the German, Norwegian and combined datasets (Table 5).

428

429 All other relationships were either unexplained in one country (no model performed
430 significantly better than the null model), weakly explained, or inconsistent between countries.
431 Since we did not want to overinterpret our data, we only show the results (Table 5), but do not
432 discuss them further, so that other researchers may compare our results with their own data.

433

434 **4. Discussion**

435

436 We used two independent datasets that were collected in two countries to identify potentially
437 interesting relationships between hydrological regulation and aquatic biota, supposing that the
438 occurrence of similar relationships in Germany and Norway reduces the likelihood that the
439 relationships occurred by chance. We found systematic differences in water chemistry and
440 hydrology between regulated and unregulated streams, regardless of differences in general flow
441 patterns between Norway and Germany (Appendix A1). In both countries, regulated rivers
442 were characterised by a lower coefficient of variation in flow (Table 4), consistent with the
443 expectation that regulated rivers have a more stable flow regime (Poff et al., 2007). Also,
444 regulated rivers were characterized by higher TN concentrations. The reason for this, however,
445 remains unclear, given that river regulation may have ambivalent effects on water chemistry.
446 Hydropower plants usually withdraw hypolimnetic water, where nutrient concentrations are
447 typically higher than at the surface (Kunz et al., 2013), leading to higher nutrient concentrations
448 downstream from the outlets. On the other hand, reservoirs may act as nutrient traps, reducing
449 nutrient concentrations downstream (Kunz et al., 2011). As regulated rivers also had a higher
450 mean flow (Table 4), we suspect that the enhanced TN concentrations may simply reflect river
451 size. We lack data on land use in the catchments, but a larger proportion of agricultural and
452 urban land use further downstream is likely to be associated with higher nitrogen levels in
453 aquatic ecosystems (Schindler, 2006). The higher mean flow at regulated sites is simply related
454 to the fact that many large rivers are regulated today (Poff et al., 2007), so rivers with an
455 unregulated flow regime will mostly be found upstream, where mean flow is low.

456

457 Only two biological variables consistently differed between regulated and unregulated sites in
458 both Germany and Norway. The abundance of cyanobacteria and the German saprobic index
459 were higher at regulated sites (Table 4). However, the increase in the German saprobic index
460 was very small, and likely is only indirectly related to regulation through the generally larger
461 size of the regulated rivers: macroinvertebrate assemblages will often shift towards species
462 tolerating higher organic pollution levels along the river continuum (Rosenberg and Resh,
463 1993). An effect of stream flow on the abundance of cyanobacteria has been shown before
464 (Schneider, 2015) suggesting that the reduced flow variation in regulated rivers (Table 4) may
465 indeed lead to an increase in cyanobacterial abundance. This may be due to reduced scouring
466 in regulated rivers (prolonged periods of high discharge may decrease *Phormidium* cover,
467 probably due to substrate movement; Schneider, 2015), or indirectly related to higher fine
468 sediment deposition in regulated rivers (*Phormidium* is able to trap sediment (Aristi et al.,
469 2017), and use phosphorus released from the entrapped sediment (Wood et al., 2015)).

470

471 When relating the observed differences in ecology to differences in hydrology and water
472 chemistry, we found that the results obtained using lasso regression were broadly comparable
473 to those produced by the more commonly used best-subsets OLS. However, the lasso approach
474 seems more conservative, presumably because the cross-validation procedure employed by
475 lasso provides a more rigorous test for actual predictive power. When lasso does suggest a
476 plausible model, it is usually the same or similar to the best model found by best-subsets OLS.
477 In the context of significance testing, the lasso is statistically more robust and, in addition,
478 consideration of the lasso path (Appendix A4) provides valuable additional insights concerning

479 the relationships between variables and trade-offs between model complexity and goodness-
480 of-fit. We believe the combined methodology presented here offers a pragmatic approach to
481 variable selection and significance testing that will also perform well using larger datasets
482 (unlike best-subsets or stepwise approaches).

483

484 The key pattern in our models was that the proportion of grazers and scrapers increased with a
485 more variable flow regime (Table 5). These results agree with results from flume experiments
486 (Ceola et al. 2013) suggesting that a stochastic flow regime increases grazing rates compared
487 to stable flow conditions, probably due to a larger number of microhabitats with reduced shear
488 stress and hence better foraging conditions for grazers than under constant flow conditions.
489 Doledec et al. (2015) found an increased proportion of grazers following an increase in the
490 daily minimum flow in regulated rivers. Grazers and scrapers feed on periphyton, and a higher
491 proportion of grazers and scrapers therefore suggests a shift towards a more autotrophic basis
492 of the food web (Doledec et al., 2015). Although the relationship between the coefficient of
493 variation in flow and the proportion of grazers and scrapers was not very strong (Table 5), it
494 nevertheless indicated that reduced flow variability may coincide with a shift from an
495 autotrophic towards a more heterotrophic basis of the food web. A reduced proportion of
496 grazers and scrapers in rivers with a less variable flow regime may also contribute to the
497 increased cover of cyanobacteria observed in regulated rivers (because they are less grazed
498 upon).

499

500 The best-subset approach also indicated that high October maximum flows were associated
501 with higher macroinvertebrate diversity (Table 5). However, Poff and Zimmermann (2010)
502 reported that both increasing and decreasing macroinvertebrate diversity may occur in response
503 to elevated flows. Consequently, this relationship should not be over-interpreted; since it only
504 occurred in the best-subset approach and was not picked up by the lasso, it perhaps illustrates
505 the argument that best-subset regression is prone to finding “too many” significant
506 relationships.

507

508 In conclusion, we identified two biological response variables that were potentially affected by
509 regulation/flow regime: (i) river regulation may lead to higher cyanobacterial abundance,
510 possibly via a less variable flow regime, and (ii) reduced flow variability may lead to a reduced
511 proportion of grazers and scrapers, possibly indicating a shift towards an increased importance
512 of heterotrophic energy sources in the ecosystem. We cannot exclude that other response
513 variables also were affected by regulation/flow regime, but these were not picked up in our
514 analyses (e.g. because the gradient in our data was too short). The high number of potentially
515 interesting variables, combined with strong multicollinearity, complicates the interpretation of
516 our results. Nevertheless, we believe the changes in community composition indicated by our
517 analyses are strong enough to warrant further investigation.

518

519

520 **Acknowledgements**

521

522 The study was funded by the Research Council of Norway (ECOREG, 221398/E40) and by
523 the EU 7th Framework Programme, Theme 6 (Environment including Climate Change)

524 (MARS, contract no.: 603378; <http://www.mars-project.eu>). The authors declare no conflict of
525 interest.

526

527 **References**

528

529 Aristi, I., Clapcott, J. E., Acuña, V., Elozegi, A., Mills, H., Wood, S. A., & Young, R. G.
530 (2017). Forestry affects the abundance of Phormidium-dominated biofilms and the
531 functioning of a New Zealand river ecosystem. *Marine and Freshwater Research*, *68*,
532 1741-1751.

533 Bækkelie, K. A. E., Schneider, S. C., Hagman, C. H. C., & Petrin, Z. (2017). Effects of flow
534 events and nutrient addition on stream periphyton and macroinvertebrates: an
535 experimental study using flumes. *Knowledge and Management of Aquatic Ecosystems*,
536 *418*, article number 47. doi:10.1051/kmae/2017041.

537 Ceola, S., Hoedl, I., Adlboller, M., Singer, G., Bertuzzo, E., Mari, L., Botter, G., Waringer,
538 J., Battin, T. J., & Rinaldo, A. (2013). Hydrologic Variability Affects Invertebrate Grazing
539 on Phototrophic Biofilms in Stream Microcosms. *PLOS ONE*, *8*, Article Number: e60629.

540 Doledec, S., Forcellini, M., Olivier, J. M., & Roset, N. (2015). Effects of large river
541 restoration on currently used bioindicators and alternative metrics. *Freshwater Biology*,
542 *60*, 1221-1236.

543 Gao, Y., Vogel, R. M., Kroll, C. N., Poff, N. L., & Olden, J. D. (2009). Development of
544 representative indicators of hydrologic alteration. *Journal of Hydrology*, *374*, 136–147.
545 doi:10.1016/j.jhydrol.2009.06.009.

546 Harrell, F.E., 2001. Regression Modelling Strategies with applications to linear models,
547 logistic regression and survival analysis, Springer Series in Statistics. Springer
548 International Publishing, Cham. doi:10.1007/978-3-319-19425-7

549 Hastie, T., Tibshirani, R., Friedman, J., 2009. The Elements of Statistical Learning, Springer
550 Series in Statistics. Springer New York, New York, NY. doi:10.1007/978-0-387-84858-7

551 Kruschke, J.K. (2012). Bayesian Estimation Supersedes the t Test. *Journal of Experimental*
552 *Psychology*, *142*, 573–603. doi:10.1037/a0029146.

553 Kunz, M., Wueest, A., Wehrli, B., Landert, J., & Senn, D. B. (2011). Impact of a large
554 tropical reservoir on riverine transport of sediment, carbon, and nutrients to downstream
555 wetlands. *Water Resources Research*, *47*, Article Number: W12531.

556 Kunz, M., Senn, D. B., Wehrli, B., Mwelwa, E. M., & Wueest, A. (2013). Optimizing turbine
557 withdrawal from a tropical reservoir for improved water quality in downstream wetlands.
558 *Water Resources Research*, *49*, 5570-5584.

559 Melzer, A. (1999). Aquatic macrophytes as tools for lake management. *Hydrobiologia*, *395*,
560 181-190.

561 Monk, W. A., Wood, P. J., Hannah, D. M., & Wilson, D. A. (2007). Selection of river flow
562 indices for the assessment of hydroecological change. *River Research and Applications*,
563 *23*, 113–122. doi:10.1002/rra.964.

564 Olden, J. D., & Poff, N. L. (2003). Redundancy and the choice of hydrologic indices for
565 characterizing streamflow regimes. *River Research and Applications*, *19*, 101–121.
566 doi:10.1002/rra.700.

567 Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M.,
568 Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D.,
569 Brucher, M., Perrot, M., Duchesnay, É., 2011. Scikit-learn: Machine Learning in Python.
570 *J. Mach. Learn. Res.* *12*, 2825–2830.

571 Poff, N. L., & Zimmermann, J. K. H. (2010). Ecological responses to altered flow regimes: a
572 literature review to inform the science and management of environmental flows.
573 *Freshwater Biology*, *55*, 194-205.

574 Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegard, K. L., Richter, B. D., Sparks,
575 R. E., & Stromberg, J. C. (1997). The Natural Flow Regime. *Bioscience*, 47, 769–784.
576 doi:10.2307/1313099.

577 Poff, N. L., Olden, J. D., Merritt, D. M., & Pepin, D. M. (2007). Homogenization of regional
578 river dynamics by dams and global biodiversity implications. *Proceedings of the National
579 Academy of Sciences U. S. A.* 104, 5732–5737. doi:10.1073/pnas.0609812104.

580 Power, M. E., Holomuzki, J. R., & Lowe, R.L. (2013). Food webs in Mediterranean rivers.
581 *Hydrobiologia*, 719, 119-136.

582 Python Software Foundation, 2016. Python Language Reference, version 2.7.

583 Richter, B. D., Baumgartner, J. V., Powell, J., & Braun, D. P. (1996). A Method for
584 Assessing Hydrologic Alteration within Ecosystems. *Conservation Biology*, 10, 1163–
585 1174.

586 Richter, B., Baumgartner, J., Wigington, R., & Braun, D. (1997). How much water does a
587 river need? *Freshwater Biology*, 37, 231–249.

588 Rosenberg, D. M., & Resh, V. H. (eds.)(1993). *Freshwater Biomonitoring and Benthic
589 Macroinvertebrates*. Springer, NY.

590 Salvatier, J., Wiecki, T. V., Fonnesbeck, C., 2016. Probabilistic programming in Python
591 using PyMC3. *PeerJ Comput. Sci.* 2, e55. doi:10.7717/peerj-cs.55

592 Schindler, D. W. (2006). Recent advances in the understanding and management of
593 eutrophication. *Limnology and Oceanography*, 51, 356-363.

594 Schneider, S. C. (2015). Greener rivers in a changing climate?-Effects of climate and
595 hydrological regime on benthic algal assemblages in pristine streams. *Limnologica*, 55,
596 21–32.

597 Schneider, S. C., & Petrin, Z. (2017). Effects of flow regime on benthic algae and
598 macroinvertebrates - A comparison between regulated and unregulated rivers. *Science of
599 the Total Environment*, 579, 1059–1072. doi:10.1016/j.scitotenv.2016.11.060.

600 Vogel, R. M., Sieber, J., Archfield, S. A., Smith, M. P., Apse, C. D., & Huber-Lee, A. (2007).
601 Relations among storage, yield, and instream flow. *Water Resources Research*, 43, Article
602 Number W05403. doi:10.1029/2006WR005226

603 Wood, S. A., Depree, C., Brown, L., McAllister, T., & Hawes, I. (2015). Entrapped
604 Sediments as a Source of Phosphorus in Epilithic Cyanobacterial Proliferations in Low
605 Nutrient Rivers. *PLOS ONE*, 10, Article Number: e0141063.

606 Yang, Y.-C. E., Cai, X., & Herricks, E. E. (2008). Identification of hydrologic indicators
607 related to fish diversity and abundance: A data mining approach for fish community
608 analysis. *Water Resources Research*, 44, Article Number: W04412
609 doi:10.1029/2006WR005764.

610

611
 612
 613

Table 1: Ecological indices calculated at each site.

Benthic algae metrics	Macroinvertebrate metrics
<p>Species richness metrics</p> <ul style="list-style-type: none"> • Overall taxon richness • Red algae richness • Green algae richness • Cyanobacteria richness <p>Abundance metrics</p> <ul style="list-style-type: none"> • Overall abundance • Red algae abundance • Green algae abundance • Cyanobacteria abundance 	<p>Overall metrics</p> <ul style="list-style-type: none"> • Abundance (ind/m²) • Taxon richness • Number of Genera • Average score per Taxon • German Saprobic Index • Diversity (Shannon-Wiener-Index) • Life Index • Evenness • EPT-Taxa (%) <p>Feeding behaviour metrics</p> <ul style="list-style-type: none"> • Grazers and scrapers (%) • Miners (%) • Xylophagous Taxa (%) • Shredders (%) • Gatherers/Collectors (%) • Active filter feeders (%) • Passive filter feeders (%) • Predators (%) • Parasites (%) <p>Locomotion metrics</p> <ul style="list-style-type: none"> • Swimming/skating (%) • Swimming/diving (%) • Burrowing/boring (%) • Sprawling/walking (%) • (Semi-) sessile (%)

614

615
616
617
618

Table 2: 62 hydrological indicators (HI) calculated from daily flow data. IQR, interquartile range; *cv*, coefficient of variation; σ , standard deviation of flows; μ , mean flow.

Group	Variable(s)	Number of metrics	Description
Magnitude of overall water conditions	Mean discharge	1	The mean daily flow over the entire 3-year period
	Min, P05, P25, P50, P75, P95, max, range, IQR, 90-percentile-range	10	Flow percentiles. The range is calculated as (max-min); the IQR as (P75-P25); the 90-percentile-range as (P95-P05)
	Coefficient of variation of flows	1	A dimensionless measure of variability: $cv = \frac{\sigma}{\mu}$
Magnitude of monthly water conditions	Monthly minimum, mean and maximum discharge	36	The minimum, mean and maximum of all daily flow values in each month over the period of interest
Timing of extremes	Days to the last extreme event	4	The number of days from the sampling date to the minimum and maximum flows in the record. The numbers of days to the last point in the record where flows either exceeded the P95 value or fell below the P05 value
Magnitude, frequency and duration of extremes	Moving averages	6	The minimum and maximum of 7-, 30- and 90-day centred moving averages over the period of interest
	Average number of reversals per year	1	The average number of times per year where the flow record switches from rising to falling or vice-versa
	Number of high pulses	1	The average number of "events" each year where the flow is greater than P90
Rates of change	The average daily rise and fall rate	2	The average rate of change (m ³ /s/day) from all periods when flows are increasing and all periods when flows are decreasing. (Periods where flows are constant are not included)

619

620
 621
 622
 623

Table 3: Variables selected for further analysis using PCA. The selected variables represented the strongest gradients in each dataset and country. Metrics in brackets had loadings very close to the maximum, and were therefore also included – see text for details.

Variable type	Variable category	Germany	Norway
Response	Benthic algae	Overall PB richness	Overall PB richness
		Cyanobacteria abundance	Cyanobacteria abundance
		Green algae richness	Red algae abundance
			(Overall PB abundance)
	Macroinvertebrates	Number of taxa or genera	Overall abundance
		German saprobic index	German saprobic index
		Evenness	Shannon-Wiener diversity
		LIFE index	Shredders
		Passive filter feeders	Sprawlers and walkers
		Predators	Swimmers and divers
		Sprawlers and walkers	
		Burrowers and borers	
		(Active filter feeders)	
(Grazers and scrapers)			
Explanatory	Water chemistry	Conductivity	Conductivity
		TP	TP
		(TN)	TN
		(TOC)	(TOC)
	Hydrology	Mean	Mean
		Coefficient of variation	October maximum
		Number of high pulses	December maximum
		Days to p05	Days to p95
		Days to maximum	Days to maximum
		Days to minimum	Number of reversals
			Range
	(Coefficient of variation)		

624

625

626 **Table 4:** Metrics with a $\geq 75\%$ posterior probability of differences between regulated and unregulated
627 sites. Negative differences imply a metric is lower at regulated sites than at unregulated ones. Brackets
628 as in Table 3. HPD, Highest Posterior Density. Metrics marked in bold denote consistent differences
629 between regulated and unregulated sites in Germany and Norway.

Country	Category	Metric	Mean difference	95% HPD interval	Probability of difference
Germany	benthic algae	Overall PB richness [-]	1.2	-1.5 to +3.8	83 %
		Cyanobacteria abundance [-]	22.7	+3 to +45	99 %
		Green algae richness [-]	0.5	-0.9 to +1.8	78 %
	macro-invertebrates	Number of taxa [-]	-4.4	-16 to +8	77 %
		Number of genera [-]	-3.6	-13 to +6	78 %
		German saprobic index [-]	0.05	-0.1 to +0.2	76 %
		Evenness [-]	-0.05	-0.14 to +0.04	89 %
		Sprawlers and walkers [%]	6.2	-3 to +15	92 %
		(Active filter feeders) [%]	-0.8	-2.6 to +1.1	83 %
		(Grazers and scrapers) [%]	-5.9	-18 to +6	85 %
	hydrology	Mean flow [m³.s⁻¹]	1.4	-2.4 to +5.6	77 %
		Coefficient of variation [-]	-0.33	-0.51 to -0.13	100 %
		Number of high pulses [-]	-2	-4 to -1	100 %
	water chemistry	Conductivity [$\mu\text{S.cm}^{-1}$]	66.9	-40.0 to +176.0	90 %
		(Total nitrogen) [mgN.l⁻¹]	0.5	-0.7 to +1.7	82 %
(Total organic carbon) [mgC.l ⁻¹]		-0.9	-2.7 to +0.9	87 %	
Norway	benthic algae	Cyanobacteria abundance [-]	37	-9 to +85	94 %
		(Overall PB abundance) [-]	61	-57 to +185	84 %
	macro-invertebrates	Overall MZB abundance [-]	305	-304 to +873	85 %
		German saprobic index [-]	0.04	-0.06 to +0.13	78 %
		Shannon-Wiener diversity [-]	0.17	-0.1 to +0.5	86 %
		Shredders [%]	-1.9	-4.7 to +0.9	92 %
		Swimmers and divers [%]	-9	-19 to 0	97 %
	hydrology	Mean flow [m³.s⁻¹]	5.7	-3.9 to +16.0	88 %
		October maximum flow [m ³ .s ⁻¹]	19.6	-18.3 to +58.6	84 %
		December maximum flow [m ³ .s ⁻¹]	14.2	+4.1 to +24.1	100 %
		Number of reversals per year [-]	25	+13 to +36	100 %
		Range of flows [m ³ .s ⁻¹]	51	-48 to +151	85 %
		(Coefficient of variation) [-]	-0.23	-0.43 to -0.02	98 %
water chemistry	Total nitrogen [mgN.l⁻¹]	0.08	+0.02 to +0.13	99 %	
	(Total Organic Carbon) [mgC.l ⁻¹]	1	0 to +2	98 %	

630

631

632

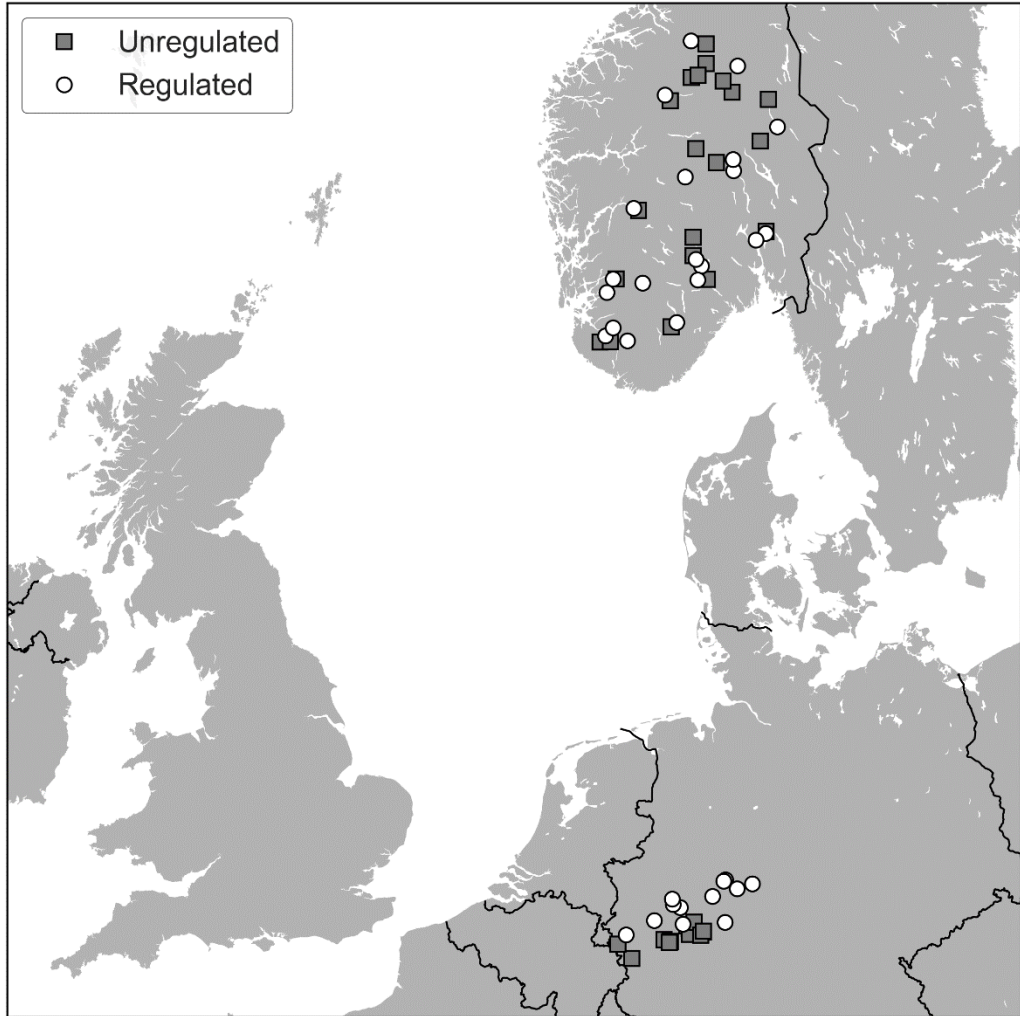
633

634
635
636
637

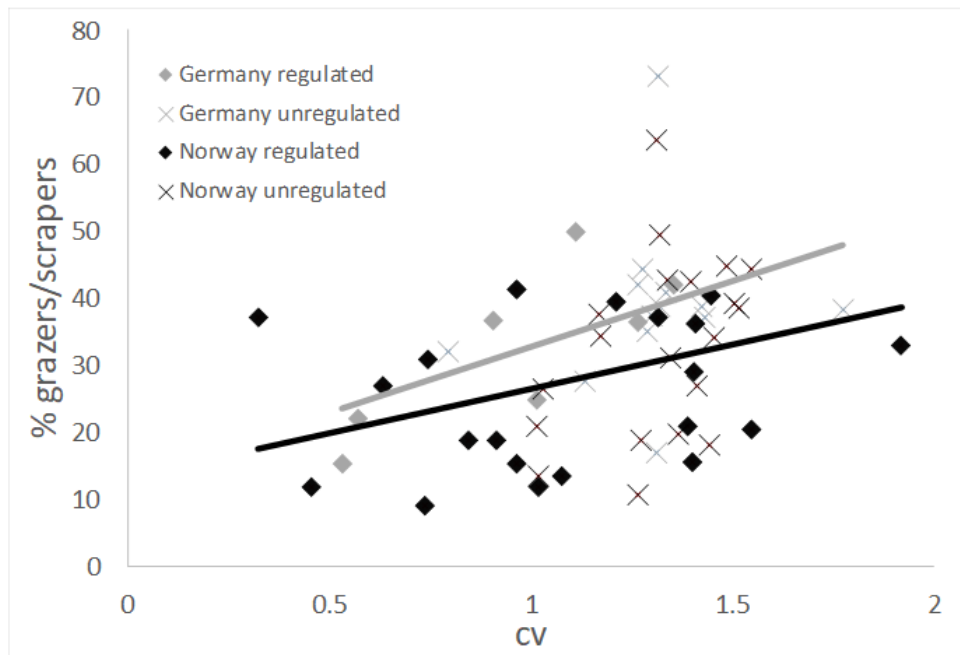
Table 5: Summary of fitted regression models. PB, Benthic algae (phytobenthos); MZB, macroinvertebrates (macrozoobenthos); F-prob, F-statistic for overall model significance; BIC, Bayesian Information Criterion. “country” is a binary variable (1 for Norway; 0 for Germany); colour code: red, there are obvious problems with the model (e.g. overfitting, residuals not normally distributed); yellow, the best identified model is no better than the null model; green, the model is worthy of further consideration. Bold letters: all variables in the model were significant at $p = 0.05$.

Dataset	Variable type	Response	Best lasso model OLS results			Best subsets OLS results				
			Model	Adj. R ²	F-prob	BIC	Model	Adj. R ²	F-prob	BIC
Germany only	PB	Overall PB richness	-0.005*cond+0.069*max10-0.61*n_hi_pulse_yr-0.03*revs_per_yr-0.25*toc+17	0.43	0.008	116.5	-0.0075*cond+0.17*max10-0.4*mean-0.59*n_hi_pulse_yr+14	0.51	0.001	111.3
		Cyanobacteria abundance	Null model	-	-	-	+65*cv+4.7*max10+3.4*max12-23*mean-1.1*range+2.9*revs_per_yr-4.1*toc-3.6e+02	0.60	0.002	232.1
		Green algae richness (Overall PB abundance)	-0.0044*cond-0.37*n_hi_pulse_yr-0.031*revs_per_yr+11	0.53	0.000	75.2	-0.0044*cond-0.37*n_hi_pulse_yr-0.031*revs_per_yr+11	0.53	0.000	75.2
	MZB	Overall MZB abundance	-21*n_hi_pulse_yr-14*toc+3.6e+02	0.24	0.021	285.0	-20*n_hi_pulse_yr+3.1e+02	0.18	0.022	284.9
		Number of taxa	Null model	-	-	-	9.3e+02*cv+74*max10-9.4*range-28*revs_per_yr+4.9e+03	0.52	0.004	309.0
		German saprobic index	Null model	-	-	-	-28*cv+0.87*max10-0.69*revs_per_yr+1.5e+02	0.38	0.012	157.9
		Evenness	Null model	-	-	-	-0.01*max10+0.039*mean+0.036*tn+1.6	0.37	0.016	-23.4
		Shannon-Weiner diversity	Null model	-	-	-	Null model	-	-	-
		Sprawlers and walkers	Null model	-	-	-	+0.018*max10-0.013*revs_per_yr+4	0.26	0.030	19.6
		Shredders	Null model	-	-	-	Null model	-	-	-
		Swimmers and divers (Active filter feeders)	+2*n_hi_pulse_yr+0.32*revs_per_yr-1.2*tn-0.99*toc-27	0.38	0.022	140.7	+2*n_hi_pulse_yr+0.29*revs_per_yr-32	0.36	0.000	138.1
		(Grazers and scrapers)	Null model	-	-	-	Null model	-	-	-
		20*cv+13	0.18	0.038	157.7	20*cv+13	0.18	0.038	157.7	
Norway only	PB	Overall PB richness	Null model	-	-	-	Null model	-	-	-
		Cyanobacteria abundance	-0.69*cond-55*cv+0.51*max12+4.9*n_hi_pulse_yr-0.067*range+2.9e+02*tn+1.3e+02	0.25	0.015	476.4	-86*cv+8.4*n_hi_pulse_yr+1.7e+02	0.20	0.006	468.6
		Green algae richness (Overall PB abundance)	Null model	-	-	-	Null model	-	-	-
	MZB	Overall MZB abundance	-1.6e+03*cv+3.2e+03	0.12	0.017	690.7	-1.6e+03*cv+3.2e+03	0.12	0.017	690.7
		Number of taxa	+42*tn+19	0.16	0.007	291.4	+0.13*cond-9.3*cv+1.8*toc+31	0.26	0.003	291.2
		German saprobic index	-0.014*n_hi_pulse_yr+1.7	0.10	0.024	-37.1	-0.014*n_hi_pulse_yr+1.7	0.10	0.024	-37.1
		Evenness	+0.0011*max10+0.00025*max12+0.0051*n_hi_pulse_yr-0.00038*range+0.4*tn-0.0024*toc+0.46	0.33	0.003	-49.9	+0.0011*max10-0.00042*range+0.41*tn+0.51	0.35	0.000	-59.0
		Shannon-Weiner diversity	+1.9*tn+0.063*toc+1.4	0.27	0.001	49.9	+0.0034*max10-0.0014*range+2.5*tn+1.5	0.44	0.000	42.3
		Sprawlers and walkers	Null model	-	-	-	Null model	-	-	-
		Shredders	Null model	-	-	-	Null model	-	-	-
		Swimmers and divers (Active filter feeders)	+10*cv-0.041*max10-0.08*max12-0.81*n_hi_pulse_yr+0.033*range-24*tn-1.9*toc+21	0.43	0.000	326.5	+15*cv-1.3*n_hi_pulse_yr-3.7*toc+20	0.41	0.000	318.2
		(Grazers and scrapers)	-4.4*cv-0.0068*range+11	0.21	0.005	215.6	-6.7*cv-0.078*mean+14	0.26	0.002	213.2
	13.2*cv + 13.2	0.09	0.034	318.5	-0.16*max12-0.17*revs_per_yr+53	0.16	0.015	317.9		
All data	PB	Overall PB richness	-0.013*cond+11*country+11	0.61	0.000	422.8	+16*country+6.5	0.61	0.000	420.4
		Cyanobacteria abundance	+1.2e+02*country-63*cv+0.49*max12+6.3*n_hi_pulse_yr+37	0.50	0.000	731.7	+1.1e+02*country-71*cv+6.9*n_hi_pulse_yr+55	0.49	0.000	730.0
		Green algae richness (Overall PB abundance)	-0.0099*cond+7.6*country+6.3	0.63	0.000	369.6	+11*country+2.7	0.63	0.000	367.6
	MZB	Overall MZB abundance	-1.4e+03*cv+11*mean-14*revs_per_yr-1.4e+02*tn+4.6e+03	0.18	0.005	1023.0	-1.6e+03*cv-15*revs_per_yr-1.7e+02*tn+5.1e+03	0.18	0.003	1020.0
		Number of taxa	-13*country+1.1*toc+37	0.29	0.000	464.0	-14*country+42	0.28	0.000	462.1
		German saprobic index	+0.00029*cond-0.044*country-0.014*n_hi_pulse_yr+0.027*tn+1.7	0.45	0.000	-51.8	+0.00066*cond-0.014*n_hi_pulse_yr+1.7	0.45	0.000	-58.7
		Evenness	country+0.001*max10+0.00032*max12+0.0063*n_hi_pulse_yr-0.00034*range-0.00045*revs_per_yr+0.014*tn	0.44	0.000	-88.7	-0.13*country+0.0011*max10-0.00038*range+0.015*toc+0.68	0.44	0.000	-97.1
		Shannon-Weiner diversity	-0.54*country+0.0039*max12-0.00078*range+0.082*toc+2.3	0.49	0.000	78.5	-0.67*country+0.0034*max10-0.0012*range+0.084*toc+2.4	0.52	0.000	74.3
		Sprawlers and walkers	Null model	-	-	-	Null model	-	-	-
		Shredders	+0.011*cond-3*country+7.7	0.21	0.000	389.6	+0.018*cond+4.7	0.21	0.000	386.2
		Swimmers and divers (Active filter feeders)	+0.021*cond+6.8*cv-0.048*max10-0.061*max12-0.75*n_hi_pulse_yr+0.037*range-2.5*toc+20	0.37	0.000	473.4	+0.02*cond-0.31*mean-0.97*n_hi_pulse_yr+0.046*range-2.7*toc+30	0.36	0.000	467.8
		(Grazers and scrapers)	+2.7*country-3*cv+0.14*n_hi_pulse_yr-0.0048*range+4.7	0.20	0.002	318.1	+3.4*country-4*cv+0.035*max12-0.085*mean+6.3	0.25	0.001	314.7
	-7.6*country+15*cv+19	0.18	0.002	475.8	-7.6*country+15*cv+19	0.18	0.002	475.8		

638
639
640
641



643
644 **Fig. 1:** Map of sampling locations.



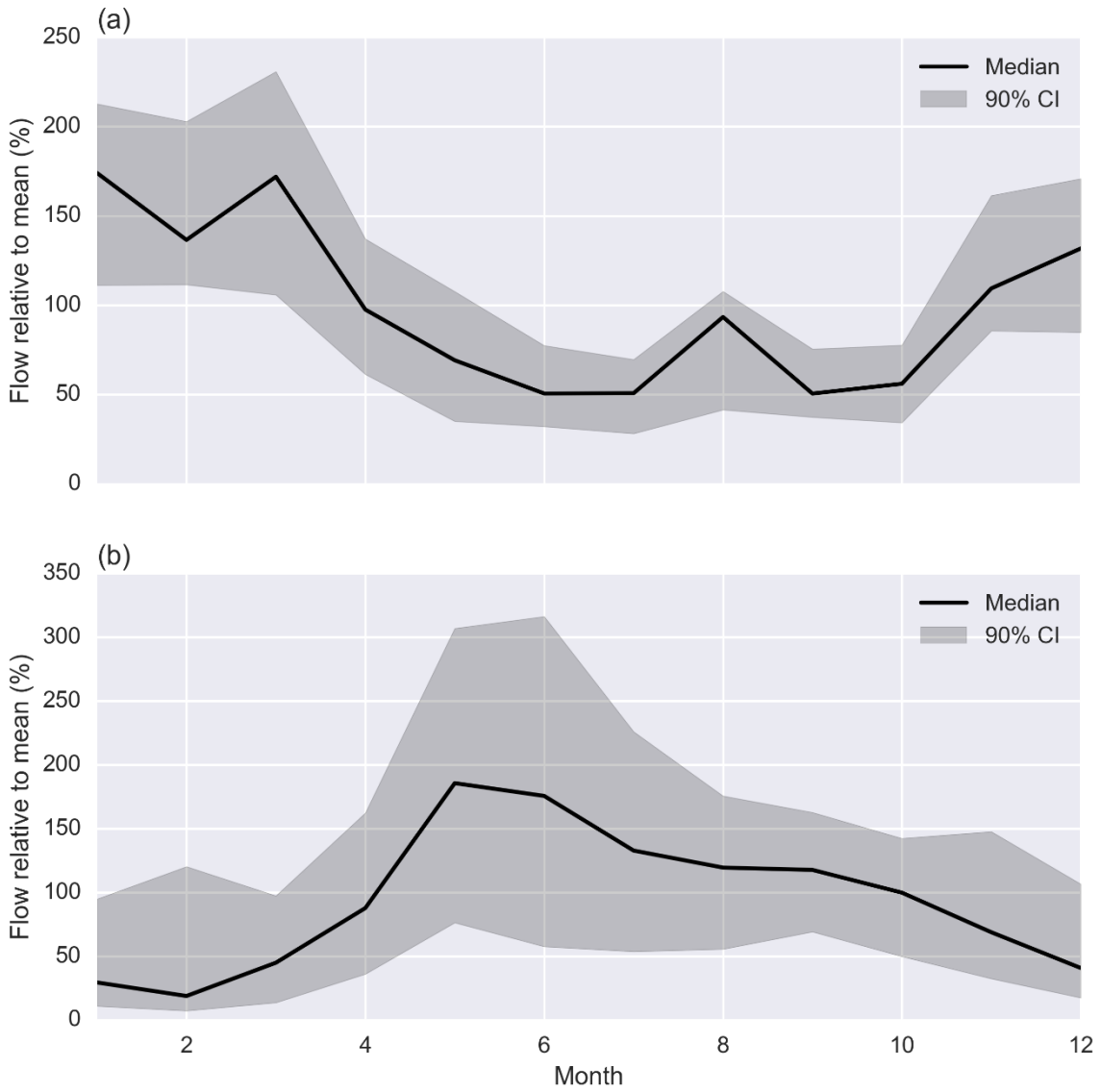
645
646 **Fig. 2.** Proportion of grazers and scrapers in relation to the coefficient of variation (CV) in the flow regime;
647 regression lines are drawn from the combined regulated and unregulated sites in each country
648
649

650 **Appendices**

651

652 **Appendix A1: Hydrological regimes**

653



654

655

656

657

Fig. A1: Monthly flows relative to the mean in (a) Germany (n=24) and (b) Norway (n=40). CI, confidence interval.

658 **Appendix A2: PCA**

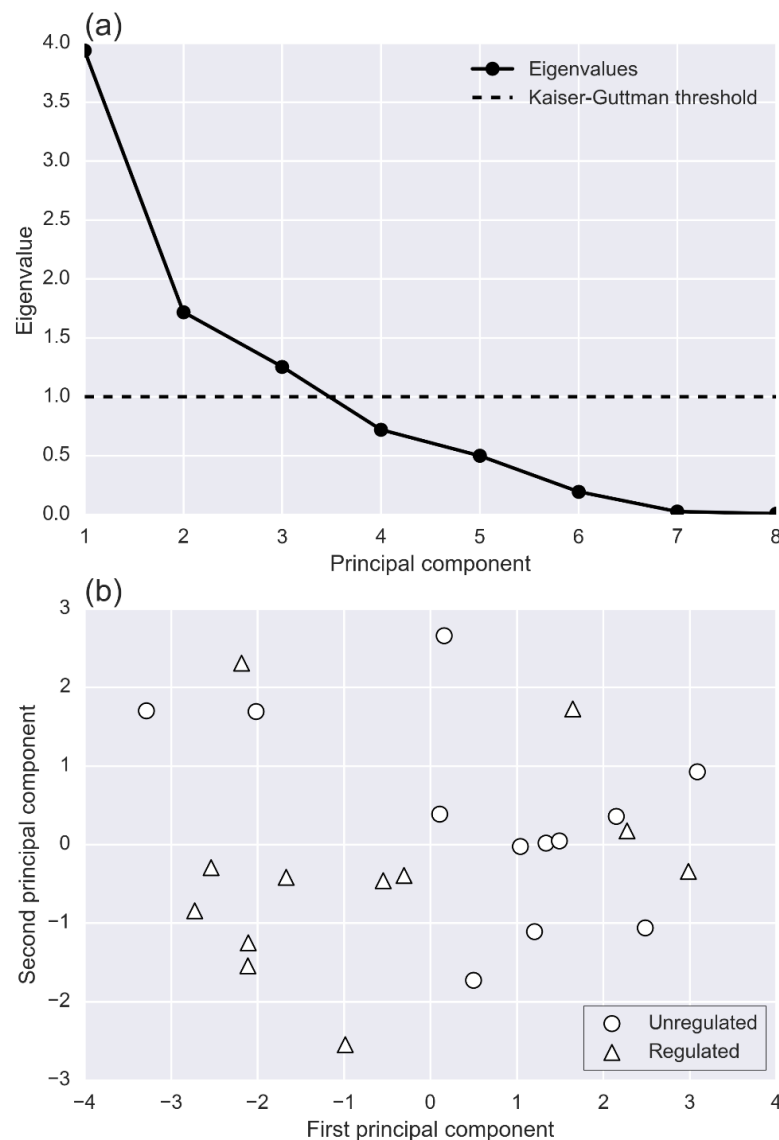
659

660 *Fig. A2* shows the results of the PCA for benthic algae in Germany. The KGC implies keeping the first three PCs,
661 which together explain 83% of the total variance. The PC loadings (see [online code repository](#)) indicate that PC1
662 is dominated by an inverse correlation with overall benthic algae richness and abundance, so sites with high scores
663 on PC1 tend to have lower richness and abundance. PC2 is dominated by an inverse relationship with green algae
664 richness and abundance, and PC3 is dominated by a similar relationship for cyanobacteria abundance.

665

666 *Fig. A2b* suggests it may be possible to use PC1 to distinguish between regulated and unregulated locations: a
667 straight line drawn at approximately $PC1 = 0$ broadly divides the data into “regulated” and “unregulated”
668 subsets, with only a small number of misclassification errors. The implication is that unregulated sites in Germany
669 tend to have lower overall benthic algae richness and abundance than regulated sites – an observation that is
670 tested more rigorously in subsequent phases of the analysis.

671



672

673

674

675

Fig. A2: PCA results for German benthic algae data. (a) Eigenvalues for each PC. (b) Projection of the data onto the first 2 PC axes

676 **Appendix A3: Bayesian test for differences**

677

678 For testing for differences between regulated and unregulated sites, we adopted a robust Bayesian approach
 679 (Kruschke, 2012), in which the variables of interest are assumed to be t-distributed, rather than Normally
 680 distributed (as in a standard t-test). Compared to a Normal distribution, the t-distribution has an additional
 681 degrees-of-freedom parameter, ν . As $\nu \rightarrow \infty$, the t-distribution becomes a Normal distribution, while values of ν
 682 close to 0 give the distribution heavier tails. These heavy tails mean the t-distribution penalises extreme values
 683 less severely than a Normal distribution, making the test more robust to outliers.

684

685 In each test we have two groups of data, from the regulated and unregulated sites. Following Kruschke (2012),
 686 we assume that each group is drawn from a t-distribution with the same number of degrees-of-freedom, and the
 687 aim is to estimate whether the other distribution parameters are different.

688

$$689 \quad \text{reg}_i \sim T(\nu, \mu_1, \sigma_1^2) \tag{1}$$

690

$$691 \quad \text{unreg}_i \sim T(\nu, \mu_2, \sigma_2^2) \tag{2}$$

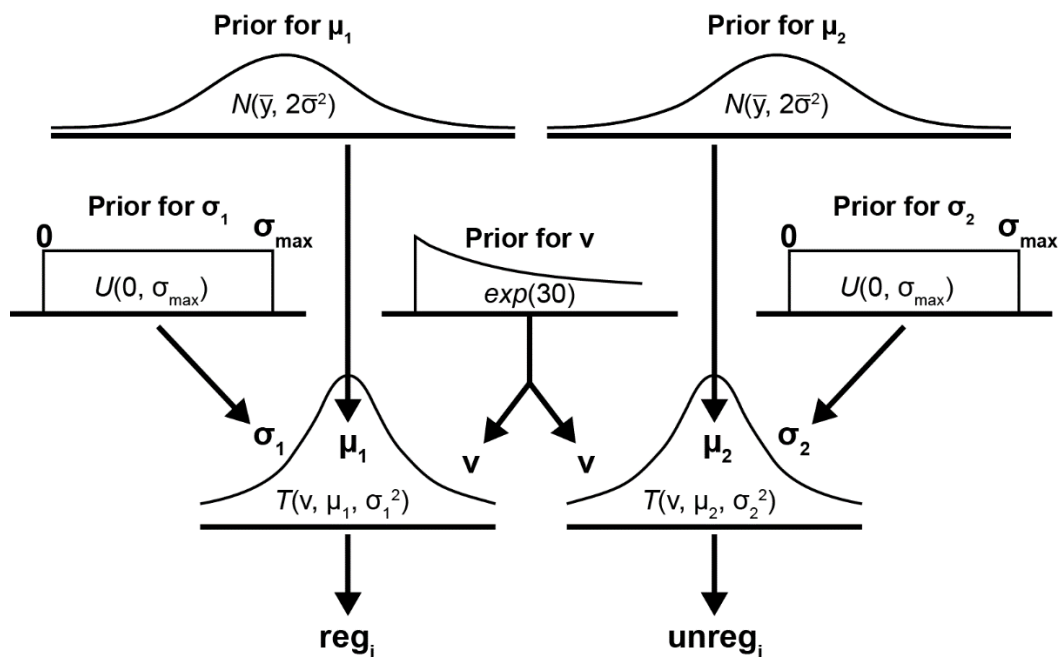
692

693 Where reg_i and unreg_i are the observed values for regulated and unregulated sites, respectively; ν is the number
 694 of degrees-of-freedom (assumed the same for both groups); μ_1 and μ_2 are the (possibly different) means for each
 695 group; and σ_1 and σ_2 are the (possibly different) standard deviations.

696

697 We set broad, uninformative priors on these quantities: the priors for the μ_j are Gaussian with the means equal
 698 to the overall mean of the pooled data, \bar{y} , and a variance that is twice the variance of the pooled data, $2\bar{\sigma}^2$; the
 699 priors for the σ_j are assumed to be Uniform on the interval between 0 and σ_{\max} , where σ_{\max} is large relative to
 700 the variance in the pooled data; and the prior for ν is an exponential distribution with mean 30, chosen because
 701 it allocates credibility evenly over the range between "nearly normal" and "heavy tailed" (Kruschke, 2012). This
 702 arrangement is illustrated in Fig. A3_1.

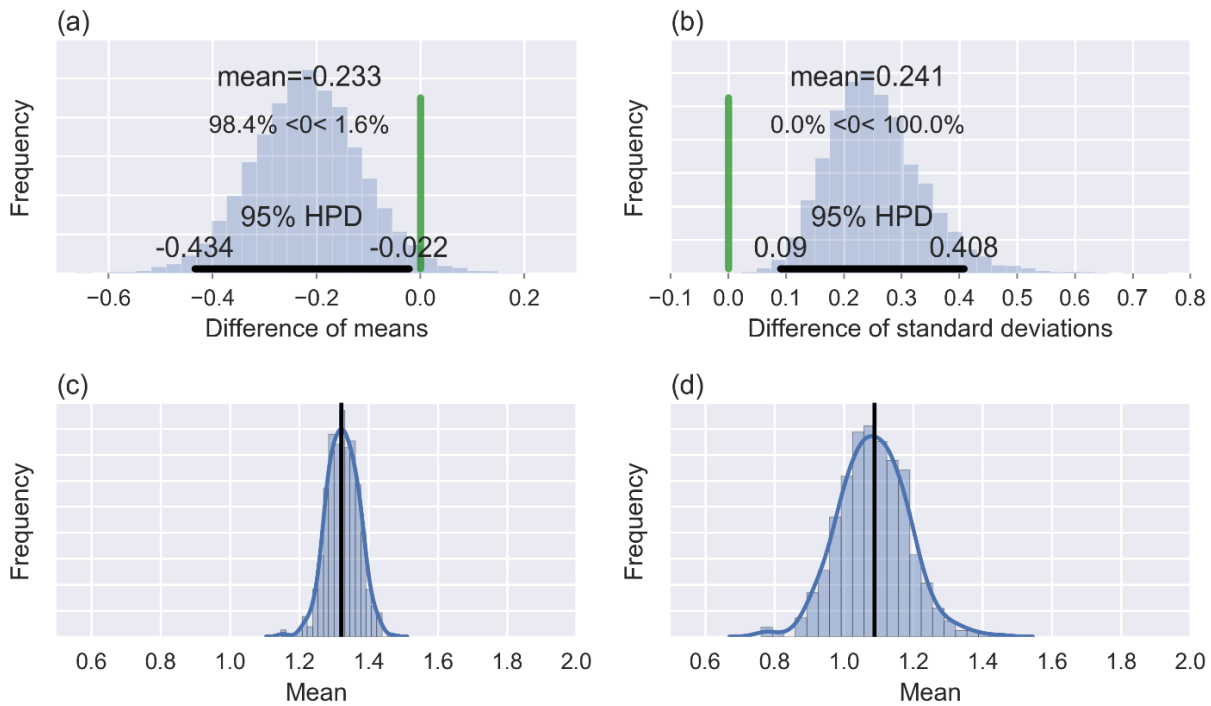
703



704

705 **Fig. A3_1:** Hierarchical diagram illustrating a robust Bayesian test for differences between two groups. After
 706 Kruschke (2012)

707 The posterior distribution $P(\mu_1, \mu_2, \sigma_1, \sigma_2, v|D)$, where D are the observed data, was explored via Markov chain
 708 Monte Carlo (MCMC) sampling using the PyMC3 package (Salvatier et al., 2016) for the Python programming
 709 language (Python Software Foundation, 2016). The goodness-of-fit of the model was assessed using “posterior
 710 predictive checks” (Gelman et al., 2004) and, when the fit was deemed acceptable, marginal posterior
 711 distributions were constructed for the differences between means, $(\mu_1 - \mu_2)$, and standard deviations, $(\sigma_1 -$
 712 $\sigma_2)$. The Highest Posterior Density (HPD) intervals and the proportions of each distribution greater than or less
 713 than zero were then used to estimate the (Bayesian) probability that differences between regulated and
 714 unregulated sites were statistically significant.
 715



716 **Fig. A3_2:** Differences in the coefficient of variation of flows between regulated and unregulated sites in
 717 Norway. (a) Posterior distribution for the difference between group means, $(\mu_1 - \mu_2)$. (b) Posterior for the
 718 difference between group standard deviations, $(\sigma_1 - \sigma_2)$. (c) PPC for unregulated sites. (d) PPC for regulated
 719 sites.
 720

721
 722 *Fig. A3_2* shows an example of the output for differences in the coefficient of variation of flows between regulated
 723 and unregulated sites in Norway. *Fig. A3_2a* shows the posterior distribution for the difference between group
 724 means, $(\mu_1 - \mu_2)$, while *Fig. A3_2b* shows the difference between group standard deviations, $(\sigma_1 - \sigma_2)$. The
 725 mean difference in the coefficient of variation between the two groups is -0.23 (*Fig. A3_2a*), implying that flows
 726 at regulated sites are, on average, 23% less dispersed than under natural flow regimes. The 95% HPD extends
 727 from -2% to -43%, and more than 98% of the posterior distribution is less than zero. There is therefore high
 728 probability that, given the data and the prior assumptions, flows at regulated sites are less variable than at
 729 unregulated sites. Similarly, there is strong evidence that regulated sites exhibit a greater range of coefficients of
 730 variation than unregulated ones (*Fig. A3_2b*).
 731

732 The lower row of plots on *Fig. A3_2* shows the Posterior Predictive Checks (PPCs) for unregulated (*Fig. A3_2c*) and
 733 regulated (*Fig. A3_2d*) sites. Black vertical lines show the means of the observed data in each group, while the
 734 smoothed histograms show distributions for *synthetic* means, generated by simulating from the fitted model.
 735 Substantial differences between simulated and observed values indicate a poorly fitting model and should be
 736 investigated further, but in this example the fit seems adequate. Additional details regarding model checking are
 737 provided in the [online code repository](#).

738 Appendix A4: L1-regularised (“lasso”) regression

739

740 The commonly used stepwise and best-subsets regression approaches have a number of well-documented
741 limitations in the context of variable selection and significance testing (Harrell, 2001). A more robust approach is
742 to use *regularized* regression, such as *ridge* or *lasso*, both of which accept a degree of bias in the model predictions
743 in return for parameter estimates that have lower variance and are therefore more stable (Hastie et al., 2009).

744

745 Ridge regression has advantages when there are lots of collinear predictors, whereas the lasso incorporates
746 “feature selection” and can be useful when the aim is to produce a parsimonious model, which may be easier to
747 interpret. Since multicollinearity had already been addressed to some extent using PCA, lasso regression was
748 chosen to identify the most important relationships between ecological (response) and explanatory variables.

749

750 The “loss function” minimised by lasso regression is:

751

$$752 \min_{\theta} \left[\frac{1}{2n} \| X\theta - y \|_2^2 + \alpha \| \theta \|_1 \right] \quad (3)$$

753

754 Where θ is the vector of model parameters; n is the number of samples; X is the design matrix; y is the vector of
755 observations; and α is a parameter controlling the amount of regularisation. The first term in this expression is
756 proportional to the usual loss function for Ordinary Least Squares (OLS) regression, whereas the second is
757 proportional to the L1-norm of the parameter vector. Large values of α therefore impose a heavy penalty on large
758 parameter values, producing “sparse” models where most parameters are set to zero. In contrast, $\alpha = 0$
759 corresponds to the OLS solution.

760

761 Lasso regression must be performed on *standardized* predictors, as otherwise the regularization penalty is applied
762 unevenly across the variables. Regularization also means the lasso coefficient estimates are not *consistent* (i.e.
763 they do not necessarily converge as the sample size grows) and they are biased towards zero. The lasso can
764 therefore be easily used to assess which predictors are important in the model, but estimating the strength of the
765 relationships (i.e. the model coefficients) is more difficult. One pragmatic solution is to use the lasso to identify
766 the best model (or a small number of candidate models), and then use OLS regression with the unstandardized
767 data to estimate the coefficients directly in the original data units (Hastie et al., 2009).

768

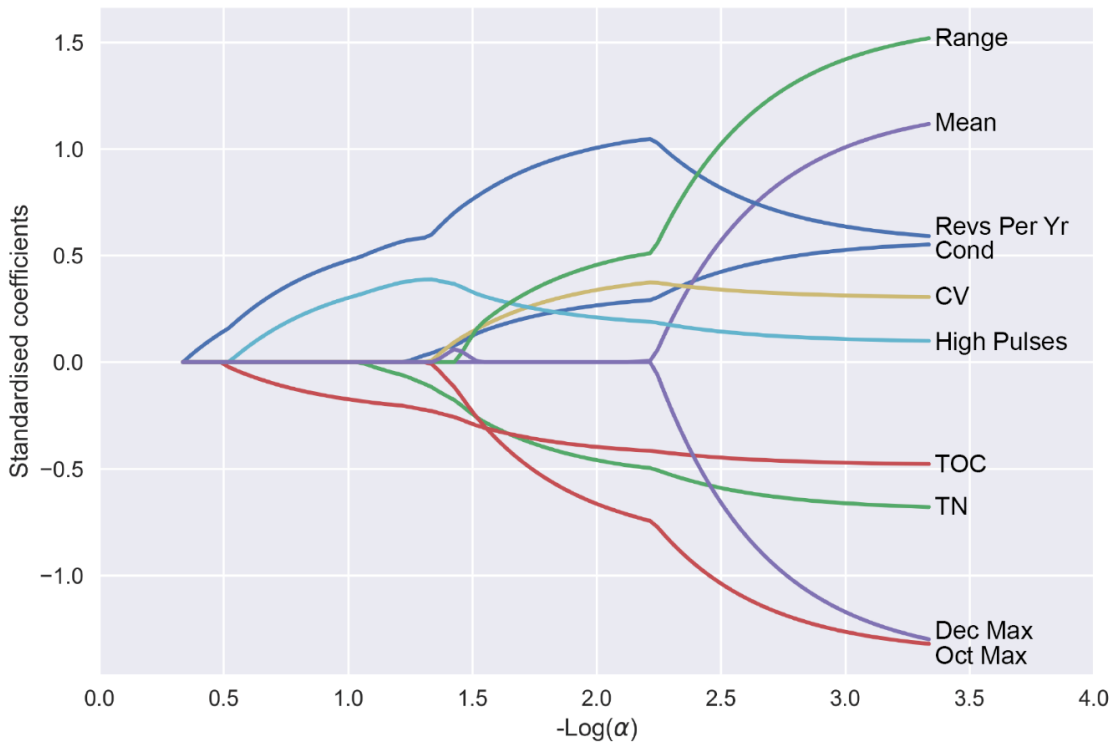
769 In the approach adopted here, the best fitting lasso model was chosen as the one with the lowest mean squared
770 test error under k-fold cross-validation across a range of values for α . In addition, plots of the “lasso path” (Fig. 6)
771 were assessed in order to better understand relationships between variables – in particular to identify and remove
772 strong collinearity between predictors. The analysis was performed using scikit-learn (Pedregosa et al., 2011).

773

774 For small to medium sized datasets, the choice of k in k-fold cross-validation can substantially affect the validation
775 curve. For each model, a range of k-values (usually 3, 5, 7 and, sometimes, 9) was explored to see whether the
776 location of the test-error minimum was robust. Models where the minimum was strongly dependent on k were
777 categorised as unreliable (highlighted in red on Table 5).

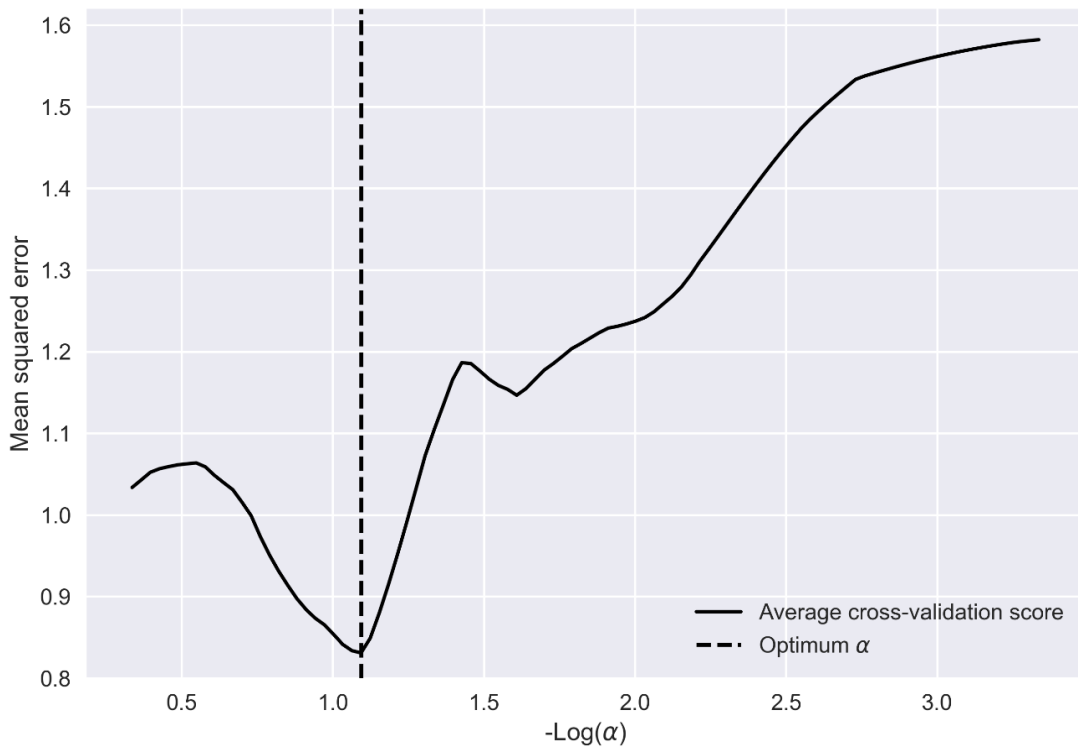
778

779 Fig. A4_1 shows the lasso path for a model where the response variable is “proportion of macroinvertebrate
780 swimmers and divers (%) in Germany”. The plot illustrates how the standardised model coefficients of the best
781 model (selected through cross-validation) vary with the penalty weight, α (equation 3). Small values of $-\text{Log}(\alpha)$
782 correspond to strong regularisation, such that all model coefficients are forced to zero (the “null” model). Larger
783 values of $-\text{Log}(\alpha)$ mean less regularisation, so models towards the right-hand margin of the plot approximate
784 the OLS solution.



786
787
788
789
790
791
792

Fig. A4_1: Lasso path for “proportion of macroinvertebrate swimmers and divers (%) in Germany”. Range, range of flows (maximum – minimum); Mean, average long-term flow; Revs Per Yr, average number of flow reversals per year; Cond, conductivity; CV, coefficient of variation; High Pulses, Number of high pulses (>90th percentile) per year; TOC, total organic carbon; TN, total nitrogen; Dec Max, December maximum flow; Oct Max, October maximum flow.



793
794

Fig. A4_2: Average cross-validation score as a function of the regularisation parameter, α .

795 As the amount of regularisation is reduced (reading from left to right along the x-axis), parameters that come into
796 the model first can be interpreted as having a stronger relationship to the response. In addition, the shape of the
797 lasso path for each variable provides information regarding any remaining collinearity between predictors. The
798 plot can be interpreted as follows:

- 800 • The first variable to enter the model is “Revs Per Yr”. The coefficient value is positive and increases rapidly,
801 implying a strong positive correlation between the proportion of swimmers and divers and the average
802 number of flow reversals per year.
- 803 • The next variable to enter is “TOC”. This relationship is negative and the magnitude increases steadily
804 throughout the path, implying that high TOC concentrations are associated with fewer swimmers and
805 divers.
- 806 • The third variable to enter the model is the number of high flow pulses. The coefficient is positive and
807 increases steadily until around $-\text{Log}(\alpha) = 1.3$, at which point both “Oct Max” and “CV” enter the model.
808 These two new variables are collinear with “High Pulses” and “Revs Per Yr”, as shown by the distinct kinks
809 in the paths for these variables: the trace for “Revs Per Yr” appears to be increasing to offset the negative
810 influence of “Oct Max”, while the coefficient for “High Pulses” slowly decreases, implying that once “CV”
811 and “Oct Max” are included in the model, the importance of “High Pulses” is reduced. This provides
812 evidence that, by around $-\text{Log}(\alpha) = 1.3$, the model is already beginning to “overfit” the data. By the
813 time the variables “Mean” and “Dec Max” enter the model, at around $-\text{Log}(\alpha) = 2.2$, there is very clear
814 evidence of overfitting, with obvious collinearity between “Mean”, “Oct Max”, “Dec Max”, “Range” and
815 “Revs Per Yr”.
816
817
818

819 Consideration of the lasso path provides useful qualitative insights into relationships between explanatory
820 variables and the response, and also between the explanatory variables themselves. For the example shown in
821 *Fig. A4_1*, a relatively parsimonious model with little obvious collinearity can be constructed for values of
822 $-\text{Log}(\alpha)$ less than around 1.3. The best overall lasso model can be identified quantitatively by calculating the
823 mean squared test error for a range of values of the regularisation parameter, α (*Fig. A4_2*). The minimum mean
824 squared cross-validation error occurs at $-\text{Log}(\alpha) \approx 1.1$, which is in agreement with the qualitative assessment
825 of the lasso path. By comparison to *Fig. A4_1*, it is clear that this model includes four explanatory variables with
826 non-zero coefficients: “Revs Per Yr”, “TOC”, “High Pulses” and “TN”. According to the lasso approach, these
827 variables are “significantly” associated with the response. Further details of this model, including plots of residuals
828 and fitted versus observed values, can be found in the [online code repository](#).

829
830
831