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Title	Evaluation of candidate biological indicators of hydrological and morphological degradation
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Dissemination Level

PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Summary

Work Package 3 of REFORM focuses on the impacts of hydromorphological changes on river and floodplain ecosystems. The main research objectives of deliverable 3.3 are:

- To establish empirical relationships between the biota, flow dynamics, substrate complexity/habitat heterogeneity and sediment dynamics.
- To select and develop candidate indicators for WFD quality elements that quantify impacts of hydrological and morphological degradation in rivers.
- To develop novel biological indicators capable of diagnosing hydromorphological stressors in a multiple stressor environment.
- To advise on the design of monitoring programs to detect hydrological and morphological degradation.
- To address the importance to quantify uncertainty in biology based hydromorphological assessment and discuss the implications of uncertainty for monitoring and assessment.

The empirical relationships between biota, flow dynamics, substrate complexity and sediment dynamics are investigated in chapter 1. The performance of existing indicators/metrics to assess hydromorphological degradation has been determined in a case study of the Regge catchment in the Netherlands and a study of Danish streams (Chapter 2). Novel designs for developing ‘new’ diagnostic metrics to solve issues related to multiple stressors including hydromorphological stress are presented in Chapter 3. Subsequently chapter 4 describes how monitoring programs can be designed to assess hydromorphological degradation using biological indicators. Finally, chapter 5 adresses the importance to quantify uncertainty with its implications for monitoring when assessing the ecological status of river systems.

Conclusions and recommendations:

Empirical relationships

Invertebrates – The experiment described in chapter 1.1 showed that the effects of patchiness and flow on lowland stream caddisflies are species-specific. The wide range of effects found in the experiment indicates that small-scale heterogeneity in the form of substrate patchiness is an important driver of the community patterns in streams and that change in substrate composition or spatial arrangement of patches due to natural or anthropogenic disturbances could have considerable effects on macroinvertebrate populations.

The experimental addition of fine sediment (chapter 1.2) to the substrate affected oxygen penetration, nutrient concentrations (ammonium, nitrate, nitrite and SRP), and both benthic and hyporheic invertebrate assemblage structure. Different flow rates also affected these attributes. Nevertheless, the hypothesis that higher flows would ameliorate any effects of added fines, although generally supported for oxygen penetration, was not supported for benthic or hyporheic invertebrates (no interaction between flow type and sediment treatment). However, the rates of flow used in the mesocosms did not appear to reach the threshold required to remove fines or alter sediment dynamics.

Periphyton – Experimental results (chapter 1.3) support the assumption that fine sediment has the potential to confound investigations of other stressors on periphyton in river environments, including phosphorous concentration (Jones et al. 2014). Under reduced phosphorous conditions the chlorophyll-*a* and ash-free dry weight concentrations

were lower with sediment treatment. Whereas, under control conditions, the sediment treatment showed increased chlorophyll-*a* and ash-free dry weight concentrations. This is an interesting result, and clearly needs further study, as it has potential implications for management and other studies involving phosphorous reduction in river systems.

Performance of existing indicators

Macroinvertebrates – River zonation preferences (upstream vs downstream species) have the potential to indicate hydrological and/or morphological stress (chapter 2.1). Although overlap between samples of different quality classes is apparent, locations of good hydrological or morphological quality are dominated by upstream or rhithral species, whereas downstream or potamal species are more abundant in locations of poor hydrological or morphological quality.
Macrophytes - The Danish Stream Plant Index (DVPI) declined with increased frequency of weed cutting (chapter 2.2). A significant relationship was found also between DVPI and stream morphology (channel sinuosity and profile) in small streams. Such a relationship was not detected in middle-sized and large streams most likely reflecting that weed cutting often correlates with the degree of channelization, thereby making it difficult to distinguish these two types of impacts in these stream types.

Novel metrics

Macroinvertebrates - AMOEBE provides an appealing visual tool for water managers to diagnose the cause of stream degradation, including morphological and hydrological degradation (chapter 3.1). Because AMOEBE is a diagnostic tool it makes it easier to decide on appropriate restoration measures when a stream fails to meet the ecological quality objectives. Apart from AMOEBE, macroinvertebrate traits, that are indicative of low and high fine sediment conditions, have been identified (chapter 3.4). This indicates the potential to develop a biological trait-based index for fine sediment stress. It further validates the incorporation of the metric percentage of silt dwelling species in the AMOEBE, and also offers potential for improvement of AMOEBE in the future.
Fish - For fish the overall shape of size spectra was identified as a potential novel metric for assessing impacts of hydromorphological pressures and restoration projects.
Diatoms - Results suggest, in line with deliverable 3.1, that the TDI scoring system, developed to assess the degree of eutrophication, is robust to hydromorphological stress (percentage cover of fine sediment). Furthermore, no relationship between motility (proposed metric of sediment stress based on phytobenthos) and sediment stress could be detected. However, the strong influence of fine sediment on diatom community composition indicates the potential for a robust metric relating diatoms to fine sediment.

Designs for monitoring

The results from chapter 1, 2 and 3 suggest it is possible to develop indices for fish, macroinvertebrates and diatoms that can indicate (hydrolo)morphological degradation. Results described in deliverable 2.2 clearly demonstrate the importance of both aquatic and riparian vegetation as a key physical control of river form and dynamics and a crucial component of river restoration. This, in combination with the diagnostic tool AMOEBE descibed in chapter 2, is a first step in the development of stressor specific bioassessment methods.

Uncertainty

Based on current literature it is very difficult to gain insight on the extent to which different sources of variation (i.e., natural spatial, natural temporal, and within-site variation) contribute to overall variation (Vlek, 2014). Also, information on variability varies depending on the metric, ecological status and stream type studied and the monitoring techniques applied. It is, therefore, unlikely that data on variation/uncertainty can be applied universally. So, instead of providing quantified information on variation/uncertainty, chapter 5 provides guidelines to develop a biological assessment system that is both affordable and provides managers with meaningful results.

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Table of Contents

1 Empirical relationships between biota, flow dynamics, substrate complexity and sediment dynamics.

1.1 *Relationship between substrate patchiness, flow regime and emergence success and adult fitness of four lowland stream Trichoptera*

1.1.1 Introduction

Caddisflies are often used as one of the main indicator groups when assessing the ecological status of streams, amongst others, to gain information on the flow regime (Rosenberg & Resh, 1993). Fluctuations in current velocity resulting from human impacts on the stream and its watershed, especially extended periods of low flow and peak discharges, could affect the species composition and densities of caddisfly communities (Cobb et al., 1992; Boon, 1993; Suren & Jowett, 2006). Flow could affect invertebrates directly, by interfering with respiration and feeding, or by initiating drift. Indirect effects of flow fluctuations are often related to changes in substrate composition and heterogeneity of the stream bed, for example, as a result of the deposition of fine sediments during low flows or patches of coarse organic material being washed away during spates (Wood & Armitage, 1997; Dewson et al., 2007; Death, 2008). It is well known that many invertebrate species prefer specific resource types within the mosaic of substrates and structures which make up the stream bed (Schröder et al., 2013). If these resources disappear after hydrological extremes, so will its associated species. More commonly, such disturbances only lead to the redistribution of resources, changing the spatial configuration of patches within a reach. The effects of these more subtle, small-scale changes in habitat heterogeneity on stream invertebrates are not fully understood. In the light of the lack of effect on invertebrates of numerous restoration projects aiming at the restoration of instream habitats through creating large-scale substrate and flow heterogeneity (e.g. Palmer et al., 2010; Haase et al., 2013), it is important not to overlook the influence of mechanisms acting on the scale of the organisms.

Direct tests of the importance of the spatial arrangement and configuration of resource patches for stream invertebrates are scarce, but clear effects of microscale patchiness on densities, emergence success and larval biomass have been reported (Palmer, 1995; Palmer et al., 2000; Silver et al., 2000; Lancaster & Downes 2014). Resource acquisition efficiency might explain the observed effects of patchy environments (Palmer, 1995), besides the predation or dislodgment risk involved with inter-patch movements. In caddisflies resource deficits experienced during larval development cannot be fully overcome by adult feeding, which is often minimal or even does not occur at all (Boggs, 2009; Jannot, 2009). As a result, poor habitat conditions resulting from hydrologic disturbances during the larval stage could eventually lead to negative consequences on adult fitness correlates, such as reduced body size, longevity and fecundity (Beveridge & Lancaster, 2007; Jannot, 2009). Also, strategic allocation decisions might be observed when environmental or biotic conditions are suboptimal; the allocation of resources among parts of the body during the last stages of larval development can occur in a manner consistent with the minimization of the effects on adult fitness (Stevens et al., 2000). For example, resources can be allocated to the abdomen at the expense of the thorax to preserve reproduction capacity (Stevens et al., 1999).

Aim of this study was to experimentally test 1.) if the emergence success of caddisflies was affected by the arrangement of resource patches and the flow regime of the larval microhabitat, and 2.) if there was an effect of patch configuration and flow on adult fitness correlates. In indoor artificial recirculating channels a fixed amount of terrestrial detritus, in the form of patches of autumn-shed leaves, was offered to the larvae of four detritivorous Trichoptera species in three spatial arrangements (aggregated to dispersed) under three flow regimes (slow to fast current, constant or fluctuating flow regime). The number of emerging caddisflies was counted, and for each adult fitness correlates in the form of total mass, mass of the abdomen en forewing length were measured. The hypothesis is that both the spatial configuration of resource patches and the flow regime influence the efficiency of resource acquisition in the caddisfly larvae during the experiment, which in turn affects the emergence success as well as fitness correlates in the adults. It is expected that the direction and magnitude of the response will vary between species as a result of morphological, physiological and behavioural differences among species, for example, traits regarding mobility, body size, habitat use and food processing rate.

1.1.2 Methods

Stream mesocosm setup

Stream bottoms consisting of sand (layer of 5 cm thick, grain size 0-2 mm) and patches of leaves were created in 93x13.5x19 (LXWXH) compartments in indoor stream mesocosms, filled with 10 cm deep aerated water. Leaf patches consisting of abscised oak (*Quercus robur*) leaves, offered in three different spatial arrangements, differing in the number of patches, individual patch size, interspersion and perimeter length (Figure 1.1.1, Table 1.1.1). Total patch size and biomass of leaves was kept constant in all compartments; the experiment started with 12 g of leaves per compartment, divided equally over the patches. To keep patch configuration intact during the experiment and especially to prevent mixing of the substrates PVC slots were placed between the patches of sand and leaves (Figure 1.1.2). Each compartment was covered with a perspex plate to prevent emerged caddisflies from escaping. To facilitate collection of adults, traps were installed above each compartment (Figure 1.1.3).

To prevent downstream transport of the leaves by the current patches were covered by a 1x2.2 cm mesh. Three different flow regimes were used: constant 5 cm s^{-1}, constant 25 cm s^{-1}, and a fluctuating flow regime with 5 cm s^{-1} and 25 cm s^{-1} alternating every 3.5 days. The mesocosm water was constantly recirculated from a 600 L reservoir and consisted of preconditioned water (preconditioning time was 2 weeks, water consisted of a mixture of 550 L of tap water and 50 L of water from a nearby stream). Light regime followed natural conditions. Water temperature was kept constant at 12°C (range 11.9-12.2), air temperature at 16°C.

Table 1.1.1. Characteristics of the patch configurations used in the experiment.

Patchiness measures	Configuration		
	I	II	III
Number of patches	1	3	6
Individual patch area (cm^2)	607.5	202.5	101.25
Total perimeter (cm)	13.5	67.5	157.5
Perimeter to area ratio	0.022	0.133	0.281
Downstream distance between patches (cm)	0	15	0

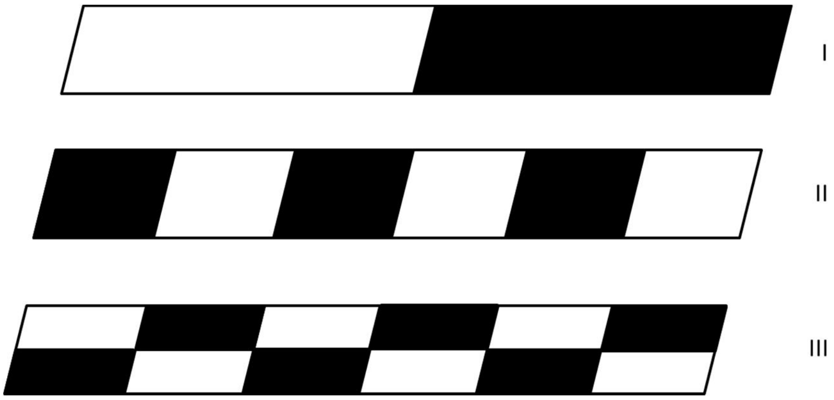


Figure 1.1.1. Spatial arrangements of leaf patches (black) and sand (white) used in the experiment.

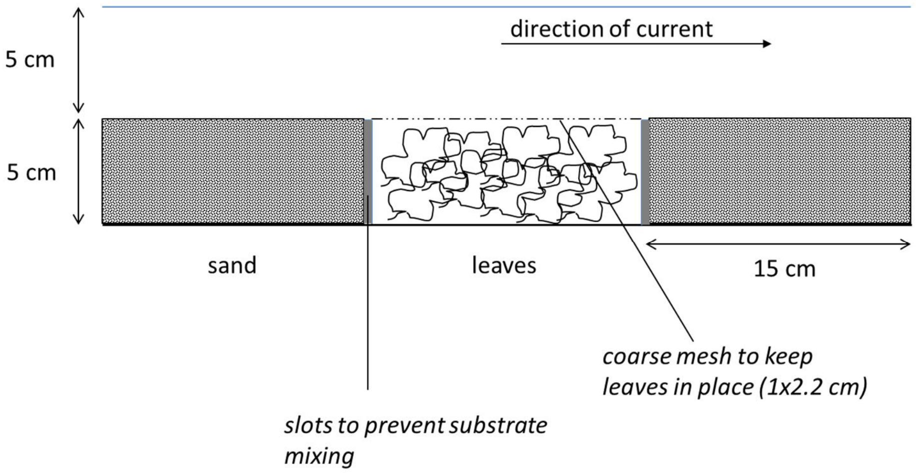


Figure 1.1.2. Layout of the bottom substrate of the experimental compartments.

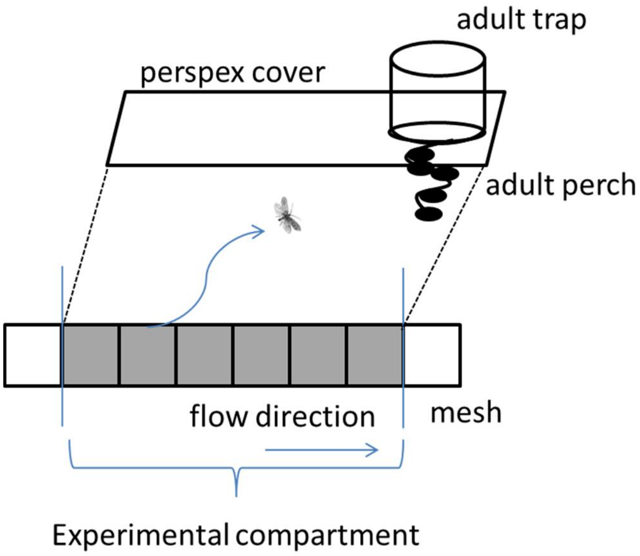


Figure 1.1.3. Layout of the experimental compartments. Each compartment was closed at the up- and downstream end by slots with 1-mm-mesh- covered openings to retain sufficient water flow but to prevent animals from escaping the experimental compartment. The top of the compartment is covered by a Perspex plate to prevent the escape of emerged caddisflies. To facilitate collection of adults, traps are installed above each compartment. An artificial plant which just touches the water surface is used to attract adults by facilitating perching.

Experimental design

In total 6 stream mesocosms were available, each consisting of 4 channels. Each channel was divided into 2 experimental compartments. Since flow cannot be varied within one channel of a stream without influencing the current velocity in the other three channels, flow treatment was not randomly assigned to the channels but is randomly assigned to the streams (2 stream mesocosms with constant slow flow, 2 with constant fast flow and 2 with fluctuating flow). With 3 types of patch arrangement, this resulted in 9 treatment combinations, which were replicated 5 times (45 experimental units).

Test species

Species characteristic of northwestern European low-gradient, lowland streams with a streambed dominated by sand and patches of detritus were used as test species: *Sericostoma personatum* (Sericostomatidae), *Lepidostoma basale* (Lepidostomatidae), *Micropterna sequax* (Limnephillidae) and *Potamophylax rotundipennis* (Limnephildae). All are members of the shredder functional feeding group. Larvae were hand-collected in the field in the first half of March. Only late instars were used in the experiment, generally latest instar larvae (*L. basale*, *M. sequax* and *P. rotundipennis* have 5 instars, *S. personatum* approximately 7 instars) (Table 1.1.2). Ten larvae of each species were released in each of the experimental compartments at the start of the experiment.

To ensure that food did not become a limiting factor, every two weeks 4 g of additional leaves was added to the compartments to compensate for consumed organic material, again equally divided over the compartments. Furthermore, additional food in the form of 1 cube (4 g) of frozen *Tubifex* oligochaetes (Dutch Select Food Tubifex; crude protein 6,2%, crude fat 2,6%) was supplied every two weeks in each compartment to prevent cannibalism or predation in species which supplement their diet with (dead) invertebrates (Wissinger et al. 2004). These extra food sources are added because we wanted to ensure that all potential components of the caddisflies diet were available in the experiment.

Table 1.1.2. Overview of species' overall development time and emergence success. Size of the larvae (n = 28) at the start of the experiment is expressed as head width (HW), together with its corresponding instar (between brackets; Wallace et al. 2003, Wagner 1990).

Species	Initial larval HW and instar		Adult emergence			Development time	
	Mean (mm)	Min-max (mm)	Tot. (%)	m (#)	f (#)	Mean (d)	Min-max (d)
L. basale	1.010 (V)	0.625 (IV)-1.148 (V)	57.1	133	123	105.5	68-158
S. personatum	1.793(VII)	1.122 (V)-2.122 (VII)	64.0	155	133	76.4	47-177
M. sequax	1.737 (V)	1.221 (IV)-2.130 (V)	79.8	155	196	154.4	48-203
P. rotundipennis	1.834 (V)	1.714 (V)-2.078 (V)	92.7	238	179	171.2	137-200

Data collection and statistical analysis

After emergence, adults were collected and frozen at -18°C until further processing. I counted the number of emerging individuals (emergence success) and the number of males and females (sex-ratio) and measured the length of the left forewing, the total adult dry biomass, and the dry biomass of the abdomen. Forewing length was measured using a microscope with an eyepiece micrometer to the nearest 0.1 mm. To determine dry mass, the caddisflies were dry to a constant weight at 60°C (48 h) and weighed on a microbalance to the nearest 0.0001 g. All normally distributed data was analyzed with two-way ANOVA's with both flow regime and patch configuration as fixed factors, followed by Tukey's post hoc procedures. Non-normal data was analysed using Kruskal-Wallis non-parametric ANOVA's, with Bonferroni-corrected Mann-Whitney U-tests to examine differences between treatment pairs. Statistical tests were conducted in IBM SPSS Statistics version 19.0 (IBM Corp, Armonk, NY, USA).

1.1.3 Results

Treatment effects on adult emergence

There were differences in overall (all treatments combined) emergence success between species; the number of successfully emerged individuals was highest in *P. rotundipennis* and lowest in *L. basale* (Table 1.1.2). Mean emergence of *S. personatum* differed between treatments for current velocity and patch configuration (two-way ANOVA: flow regime $F_{2,45} = 7.02$, $P = 0.003$; patchiness $F_{2,45} = 19.18$, $P < 0.001$; no significant interaction $F_{4,45} = 1.36$, $P = 0.266$). More individuals emerged in the constantly low current velocity treatment in comparison to the constantly high and variable treatments and emergence success was higher in patch configuration III in comparison to I, with configuration II taking an intermediate position (Fig. 1.4ab). Emergence of *L. basale* differed between treatments for patch configuration (two-way ANOVA: patchiness $F_{2,45} = 3.98$, $P = 0.027$, flow regime $F_{2,45} = 0.07$, $P = 0.931$, no significant interaction $F_{4,45} = 0.39$, $P = 0.817$), with a higher emergence in patch configuration III in comparison to configuration II, with configuration I taking an intermediate position (Fig. 1.4cd). Emergence in *M. sequax* (two-way ANOVA: flow regime $F_{2,44} = 5.13$, $P = 0.011$; patch configuration $F_{2,44} = 6.90$, $P = 0.003$; no significant interaction $F_{4,44} = 0.76$, $P = 0.560$) was lower in the constantly high current velocity treatment in comparison to the emergence in the other two flow regimes, whilst the effect of patch configuration was comparable to that of *S. personatum* (Fig. 1.4ef). In *P. rotundipennis* no differences in emergence between treatments were detected (Kruskal Wallis tests: flow regime $X^2{}_{2,45} = 3.55$, $P = 0.059$; patch configuration $X^2{}_{2,45} = 2.89$, $P = 0.084$) (Fig. 1.4gh).

Treatment effects on fitness emerged adults

In both sexes of *S. personatum* mean FW-length of the emerged individuals differed between patch configurations, but not between current velocity treatments (two-way ANOVA's, females: patch configuration $F_{2,133} = 3.97$, $P = 0.021$; flow regime $F_{2,133} = 0.09$, $P = 0.918$; interaction not significant $F_{4,133} = 1.28$, $P = 0.281$, males: patch configuration $F_{2,155} = 4.51$, $P = 0.013$; flow regime $F_{2,155} = 0.241$, $P = 0.786$; interaction not significant $F_{4,155} = 1.22$, $P = 0.305$). Both males and females in patch configuration III had longer forewings in comparison to I, with configuration II taking an intermediate position (Figure 1.1.5). No differences were detected between treatments for total dry mass and abdomen dry mass.

Total dry mass, abdomen dry mass and FW-length did not differ between treatments in L. basale and *P. rotundipennis* females. In *P. rotundipennis* males FW-length differed between patch configurations (two-way ANOVA, patch configuration $F_{2,238} = 5.57$, $P = 0.004$; flow regime $F_{2,238} = 0.89$, $P = 0.413$; interaction not significant $F_{4,238} = 1.48$, $P = 0.210$). Forewings were significantly larger in patch configuration III in comparison to configuration II, with configuration I taking an intermediate position (Figure 1.1.6). The pattern found for total mass (two-way ANOVA, patch configuration $F_{2,238} = 5.21$, $P = 0.006$; flow regime $F_{2,238} = 1.23$, $P = 0.295$; interaction not significant $F_{4,238} = 1.77$, $P = 0.136$) and abdomen mass (two-way ANOVA, patch configuration $F_{2,238} = 3.77$, $P = 0.024$; flow regime $F_{2,238} = 0.95$, $P = 0.389$; interaction not significant $F_{4,238} = 1.90$, $P = 0.111$) was comparable to that of FW-length (Figure.1. 1.6).

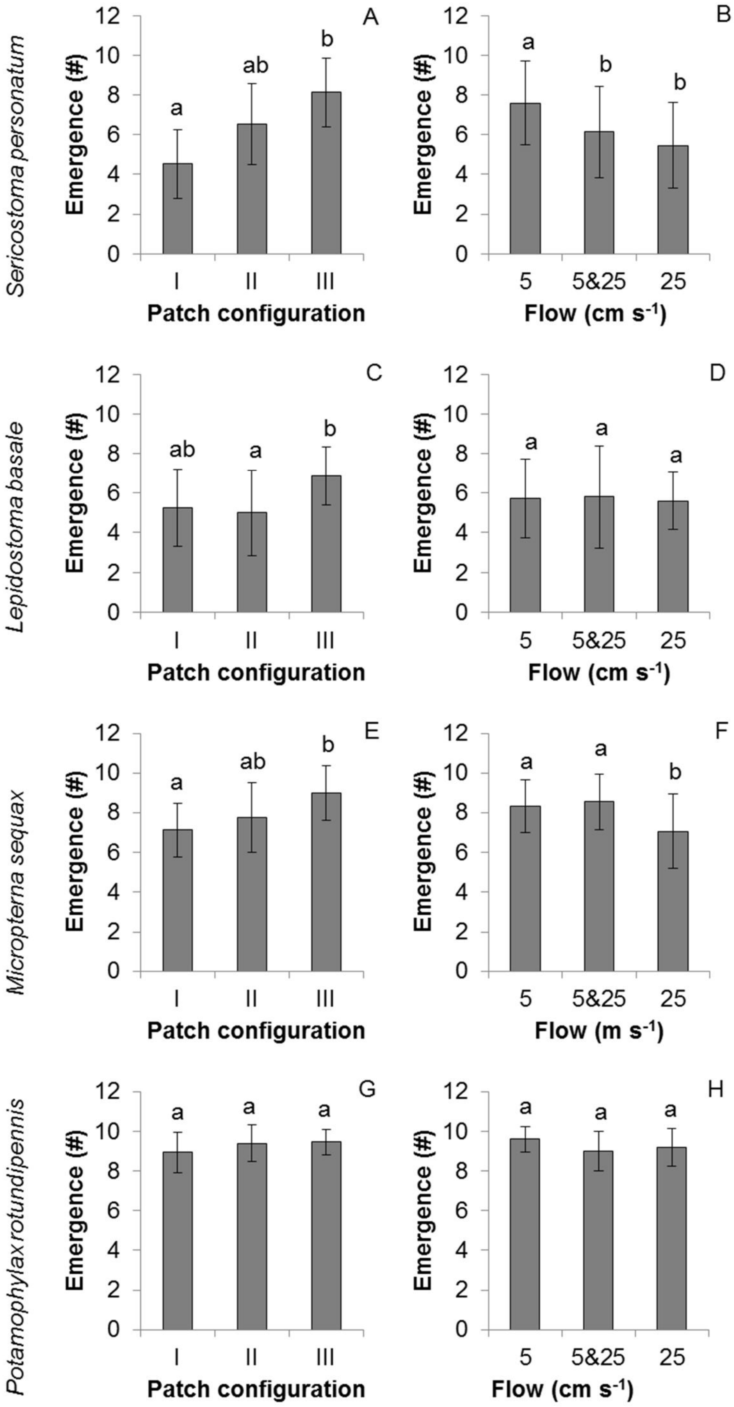


Figure 1.1.4. Comparison of the mean emergence success for the four caddisfly species in treatments with different spatial arrangements of patches (configuration I to III, Fig. 1.1) and different flow regimes (constant low flow of 5 cm s^{-1}, constant high flow of 25 cm s^{-1} and fluctuating flow alternating every 3.5 days between 5 cm s^{-1} and 25 cm s^{-1}). Bars with different letters are significantly different.

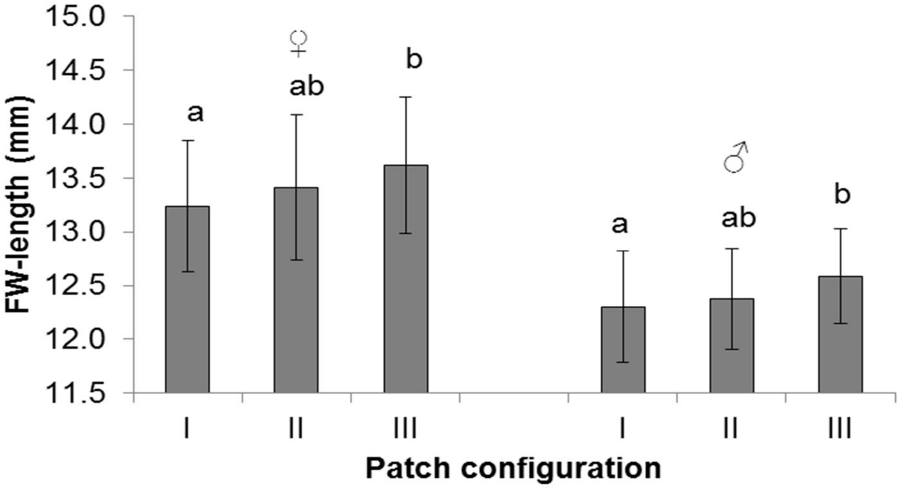


Figure 1.1.5. Mean forewing length (±1SD) of emerged female and male *Sericostoma personatum* in treatments with different spatial arrangement of patches (configuration I to III, Fig. 1.1). Bars with different letters are significantly different within female and male groups.

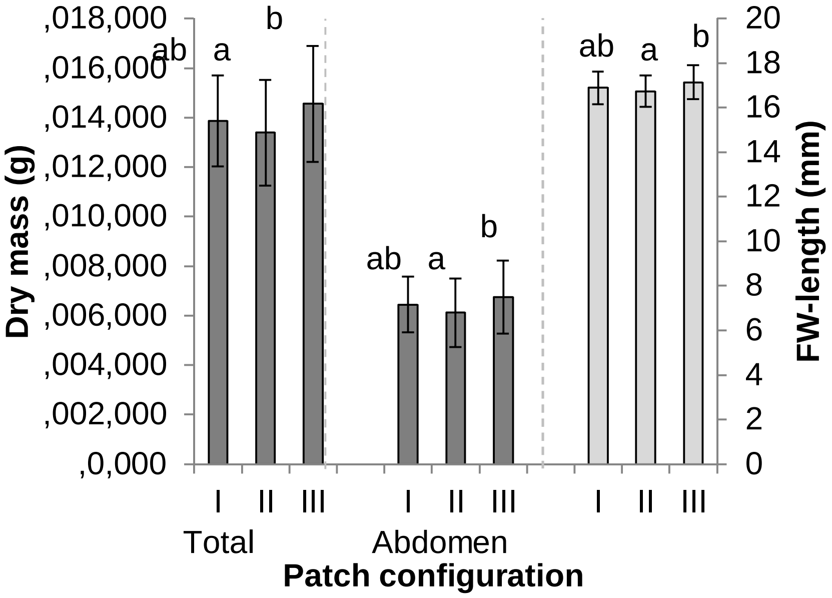


Figure 1.1.6. Mean (±1SD) total dry mass, abdomen dry mass and forewing length of emerged male *Potamophylax rotundipennis* in treatments with different spatial arrangement of patches (configuration I to III, Figure 1.1.1). Bars with different letters are significantly different within parameter groups.

In *M. sequax* females there was an effect on FW-length of both the flow regime and the patch configuration (Kruskal Wallis tests: flow regime $X^2_{2,196} = 7.83$, $P = 0.020$; patch configuration $X^2_{2,196} = 10.172$, $P = 0.006$). Forewings were larger in individuals which emerged from the constant high current velocity treatment in comparison to the fluctuating flow regime treatment, and the constant low current velocity treatment took an intermediate position (Figure 1.1.7A). Forewings were longer in patch configuration I in comparison to configuration II, whilst the forewings of the females which emerged from configuration III were of intermediate length (Figure 1.1.7B). In *M. sequax* males forewing length differed between flow regime treatments but not between patch configuration treatments (two-way ANOVA, flow regime $F_{2,155} = 4.05$, $P = 0.019$; patch configuration $F_{2,155}$ = 2.89, P = 0.059; no significant interaction $F_{4,155}$ = 0.61, P = 0.659). The effect of flow regime on the forewing length of the males was comparable to that recorded for the females (Figure 1.1.7A). In females total mean body mass and abdomen mass differed only between the patch configurations (Kruskal Wallis tests: total mass $X^2_{2,196}$ = 8.54, P = 0.014; abdomen $X^2_{2,196}$ = 10.08, P = 0.006) and not for the different flow regimes, with the heaviest individuals and abdomens recorded in treatments with patch configuration I (Figure 1.1.7C,E). For total male mass (two-way ANOVA: flow regime $F_{2,155}$ = 4.08, P = 0.019; patch configuration $F_{2,155}$ = 4.36, P = 0.014; no significant interaction $F_{4,155}$ = 0.21, P = 0.934) and male abdomen mass (two-way ANOVA: flow regime $F_{2,155}$ = 3.78, P = 0.025; patch configuration $F_{2,155}$ = 4.24, P = 0.016; no significant interaction $F_{4,155}$ = 0.24, P = 0.917) both the treatments' flow regime and the patch configuration affected the mean mass of the individuals, which was lowest for variable flow conditions and patch configuration II (Figure1. 1.7D,F).

1.1.4 Discussion

Effects of patch configuration on emergence success

The effect of the spatial arrangement of patches and flow regime on adult emergence success was species specific, but in three out of four species the configuration with many small patches, a larger total perimeter as well as a higher perimeter to area ratio (configuration III) resulted in a higher emergence success. This finding is in line with the laboratory experiment of Silver et al. (2000), who found that the survival of the chironomid *Chironomus riparius* was greater in subdivided leaf patches in comparison to large aggregations of leaves. Given that there was no interaction with the flow regime to which the larvae were exposed and the patch configuration it indicates that the observed patterns were the result of active habitat selection and not of passive accumulation due to flow effects in the constant high and fluctuating flow regimes.

Potential explanations for the enhanced emergence success in a patchy environment in comparison to aggregated resources could be found in the life histories of the species. The increase in *S. personatum* emergence success with patchiness could be well explained by the behaviour of the larvae: it feeds on coarse organic material during the night and lives burrowed in sandy sediments during at daytime (Wagner 1990). Since the increase in number of patches results in an increase in sand-leaves perimeter length, it is likely that this facilitates the efficiency of resource acquisition in this species. Interestingly, the same increase with patchiness was also observed in the mean wing length of the emerged individuals, both in males and females. Feminella & Resh (1990) showed that larvae living under high densities had smaller wings in comparison to the wing length recorded at lower densities, which could be an explanation for the pattern observed here. If *S. personatum* larvae aggregate along the leaf-sand perimeter, total available habitat increases with increasing patchiness, leading to lower larval densities in the subdivided spatial arrangements. In insects, adult body size and/or mass often decreases in response to suboptimal conditions during larval development (Svensson 1975, Feminella & Resh 1990, Boggs & Freeman 2005). In *S. personatum* I observed only an effect on wing length, and not on mass of the adults, which might be an indication of allocation of resources to preserve adult weight at the expense of wing development (Stevens et al. 2000).

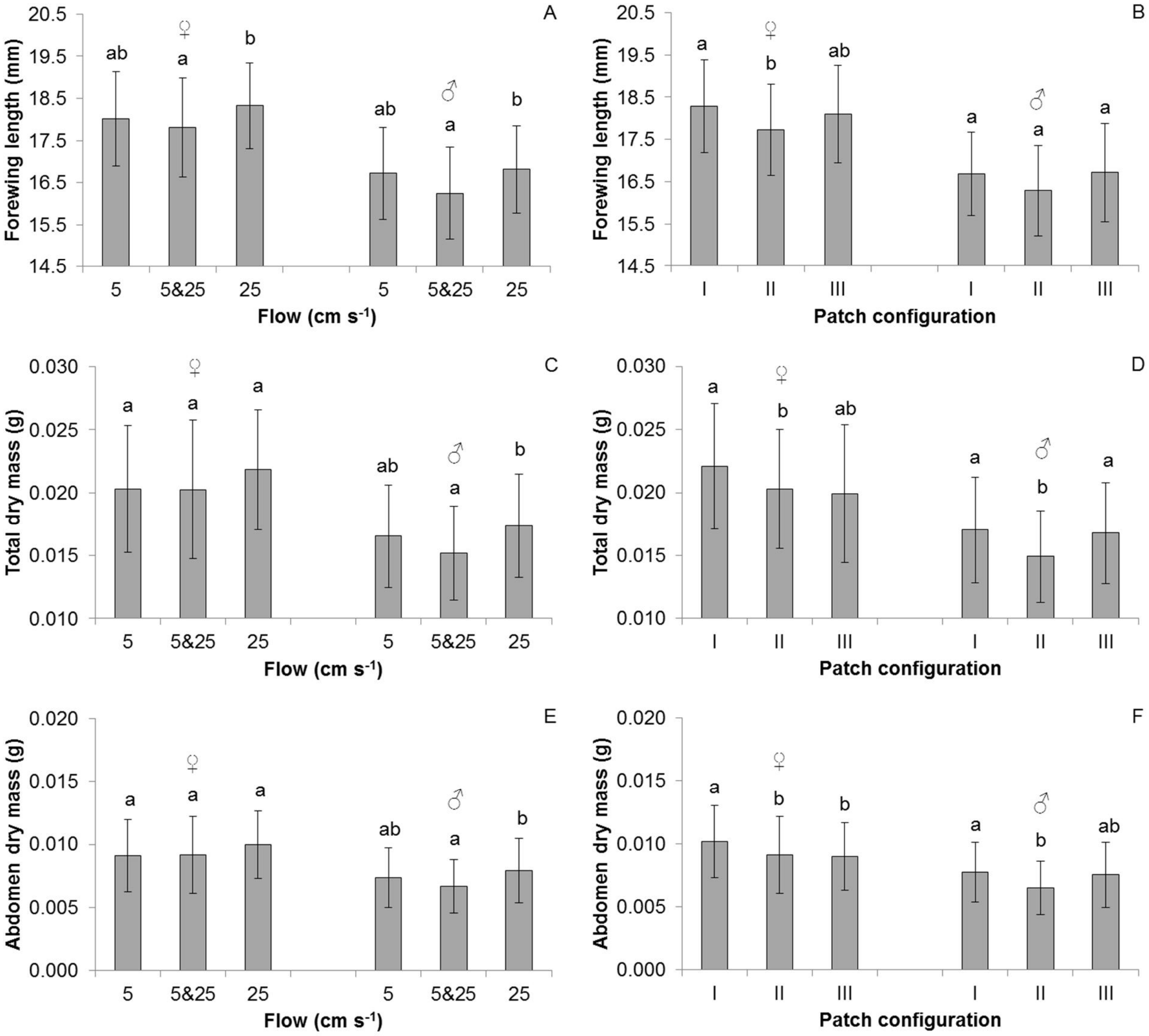


Figure 1.1.7. Mean (±1SD) forewing (FW) length, total dry mass and abdomen dry mass of emerged female and male *Micropterna sequax* in treatments with different flow regimes (constant low flow of 5 cm s^{-1}, constant high flow of 25 cm s^{-1} and fluctuating flow alternating every 3.5 days between 5 cm s^{-1} and 25 cm s^{-1}) and different spatial arrangement of patches (configuration I to III, Figure 1.1.1). Bars with different letters are significantly different within female and male groups.

M. sequax displayed a similar pattern in emergence success as in the former species. Its microhabitat preference in lowland streams comprises leaf packs in combination with mineral substrates, although the causal reason underlying this observation is not clear (Tolkamp, 1982; Verdonschot et al. 2012). One of the possibilities could be the demand for specific case building material (Otto & Svensson, 1980), in the case of the species large sand grains. Since this species is relatively immobile (Verdonschot et al., 2012), I speculate that a in a patchy environmental with much edge-habitat a combination of food in the form of leaf patches and case building material within a short distance to each other could be beneficial for survival. Nonetheless, when looking at the fitness correlates, emerged females from the fully aggregated patches had the largest wings and highest biomass, indicating that resource acquisition efficiency was higher in these large leaf patches. As in emergence success, smallest wings and lowest biomass were associated with the completely isolated smaller leaf patches of configuration II. In males, effects on fitness correlates were comparable, except that there was no effect on wing length.

The pattern in emergence success found in *L. basale* does not match the increase in perimeter length or perimeter to patch area ratio as in *S. personatum* and *M. sequax,* with the highest emergence success in configuration III and the lowest in configuration II. In contrast to the other three species, *L. basale* is closely associated with organic patches as well as with wood; mineral substrates are avoided. Furthermore, a mark-recapture study showed that the species was very mobile, actively searching for suitable food patches (Hoffmann 2000). Possibly, the individuals reared in the spatial patch arrangement with fragmented patches (configuration II) had a lower emergence success because their foraging movements were impeded by the patches of sand, resulting in mortality in the smaller isolated patches. On the other hand, no effect on fitness correlates were detected, something which could be expected when conditions were less optimal. Furthermore, if resource availability was the mechanism behind the observed pattern, it does not explain why emergence was higher in configuration III in comparison to the fully aggregated patches in configuration I.

Emergence in *P. rotundipennis* was not affected by patch configuration. This species was very successful in all treatments, which is in line its ubiquitous microhabitat distribution under natural conditions (Urbanič et al., 2005). Nonetheless, fitness correlates in emerged males of this species did show a consistent response to patch configuration: wing length, total biomass and abdomen biomass were highest in the most subdivided patches and lowest in spatial configuration II. As in the emergence success of *L. basale*, the smaller fragmented isolated patches had a negative effect on the adult fitness. Suprisingly, this pattern was not found in the females. An explanation for this difference between sexes could be that the females are larger and heavier than males, which could be an advantage when nutrient reserves are used for further development, e.g. during the pupal stage (Nylin & Gotthard, 1998).

Effects of flow regime on emergence success and fitness correlates

Each of the species studied in the experiment has a preference for slowly flowing streams and the lentic zones in faster flowing streams, based on which it could be expected that a high current velocity might have a considerable impact on the larvae. This was not the case, high flows affected the emergence success in two species only: *S. personatum* and *M. sequax*. The difference in the observed response among species is likely the result of behavioural or morphological differences among species, which determine how well the species can cope with fast flow. Interestingly, Verdonschot et al. (2014) showed that *M. sequax* larvae were able to withstand considerable hydraulic forces during spates. This research showed that this ability was costly on the long term because high flows resulted in a lower emergence.

I expected that a lower emergence success would also be visible in the fitness correlates, since caddisfly larvae developing under poor or suboptimal environmental conditions are often smaller and have a lower weight (Eggert & Wallace, 2003; Miller et al. 2012). Surprisingly, no effect on the fitness correlates in *S. personatum* could be detected under the flow conditions characterized by a lower emergence. The contrast between survival and fitness was even larger in *M. sequax*, were the highest male biomass and in both sexes the longest forewings were found under constant fast flow conditions. In the latter species especially the fluctuating current velocity treatment had a negative impact on the fitness correlates. Although drift is rare in *M. sequax* (Verdonschot et al., 2014), increased time spend in the drift could have been an explanation for the lower biomass and smaller wing size under alternating high and low flow conditions, when individuals are frequently dislodged by the suddenly increased current from their resource patches, lowering their resource acquisition efficiency. Since I did not observe the behaviour of the larvae, the cause of the observed differences among treatments remains speculative. The smaller wings but no decrease in biomass in the females in contrast to the uniform response in the male fitness correlates might again be an indication of a strategic allocation decision as a response to suboptimal flow conditions, as seen in *S. personatum*, in which biomass was preserved at cost of investments in wing development (Stevens et al., 1999; 2000).

Role of biotic interactions

Biotic interactions could be another explanation for the differences observed among patch configurations, in the form of inter- or intraspecific competition for resources or through predation. Because the same species combinations were used in all treatments I was not able to test if the results were influenced by competition or predation. Given that inter- and intraspecific aggregation is common in invertebrate species inhabiting stream leaf patches (Murphy et al., 1998) and sufficient food was provided, including animal dietary supplements to prevent predation and cannibalism (Wissinger et al., 1996), strong biotic interactions — resulting in increased mortality — are not likely to be expected here. This assumption is further supported by the studies of Wagner (2005) and Schmera et al. (2007), who found that the spatial distribution of caddisfly larvae belonging to the shedder guild is largely structured by the environmental characteristics of the stream microhabitat instead of by competition. Nevertheless, within other guilds, such as periphyton grazers, there is evidence of competition for resources (Feminella & Resh, 1990; Wagner, 2005). Thus, more subtle effects on, for example, biomass or wing length, cannot be ruled out. To test if biotic interactions could explain part of the patterns I found in this experiment, follow-up experiments in which the species are tested in isolation and/or in different densities are needed.

Conclusions

The experiment showed that the effects of patchiness and flow on lowland stream caddisflies were species-specific. Nonetheless, in three out of four species emergence success was highest in the most patchy environments, characterized by the highest perimeter length and perimeter to area ratio. Patch size was another important aspect, small isolated patches resulted in lower emergence success in *L. basale* and negative effects on fitness correlates in *P. rotundipennis* and *M. sequax*. Fitness correlates generally did not match the pattern found for emergence success and differed between males and females in some species. Indications for strategic allocation decisions, in which biomass was preserved at cost of investments in wing development were found in *S. personatum* and *M. sequax* females only. The wide range of effects found in the experiment indicates that small-scale heterogeneity in the form of substrate patchiness is an important driver of the community patterns in streams and that changes in substrate composition or spatial arrangement of patches due to natural or anthropogenic disturbances could have considerable effects on macroinvertebrate populations.

1.2 *Relationship between flow, fine silt and macroinvertebrate community composition*

1.2.1 Introduction

The hyporheic zone of a river is the interface between aquifer and surface water and is an important ecotone, acting as habitat for a range of organisms (Malcolm et al., 2002; Malcolm et al., 2008; Stubbington et al., 2009) and as a zone of biogeochemical cycling of nutrients and contaminants (Mulholland et al., 2008; Pinay et al., 2009). The zone is both temporally and spatially active with its boundaries changing according to its sediment structure, and the nature of internal and external hydrological exchange (Boulton & Stanley, 1995; Robertson & Wood, 2010). Due to widespread anthropogenic influences, alteration of hydrological exchange in stream beds through processes such as increased fine sediment delivery from altered land-use, or changes to hydrology through river regulation, lead to modified hyporheic processes. Alterations to hydrology and sediment delivery may occur independently or concurrently affecting both nutrient dynamics and hyporheic and surface dwelling invertebrate assemblages (Marmonier et al., 2012; Krause et al., 2011).

Increased sediment load, particularly in the form of fines, and/or increased deposition of fine
sediment due to changes in flow can lead to siltation, or colmation, of bed sediments. As a result of low flows, the settling of particles on the sediment surface can cause external colmation or during high flows (flooding) the interstices of bed sediments can become clogged by silt depositing directly below the armor layer, causing internal colmation (Olsen et al., 2010). Colmation may form a seal that disconnects surface water from hyporheic water (Brunke, 1999). Colmated sediments are characterized by reduced porosity and hydraulic conductivity as well as by a consolidated texture, which has significant implications for the efficiency of hyporheic biogeochemical cycling and habitat conditions (Nogaro et al., 2010). Weakened exchange between the surface and hyporheic zone can starve the interstitial environment of oxygen and promote reducing conditions, thus altering the nutrient dynamics of the river ecosystem (Brunke & Gonser, 1997), either directly by limiting oxygen (Baker, Dahm & Valett, 2000) or indirectly by altering hyporheic bacterial communities (Findlay & Sobczak, 2000). Nutrient dynamics may be affected as anoxic conditions within the bed sediment can reduce nitrate concentrations due to denitrification (House & Denison, 1998; House et al., 2001) and the presence of fine particles can sequester soluble forms of phosphorus in the through-flowing water (Vervier et al., 2009). Invertebrates occupying interstitial spaces are likely to play a role in maintaining or modifying porosity through their feeding and burrowing activities (Danielopol, 1989). Boulton et al. (1997) suggested that once conditions exceed those which can be tolerated by hyporheic organisms the interstitial spaces can rapidly fill with fine sediment, creating anoxic zones that may lead to decreased abundances and diversity of the hyporheos. Although there is much research examining the role of colmation on hyporheic oxygen concentrations (e.g. Meyer et al., 2008) there is relatively little work aimed at determining what changes occur in nutrient dynamics and the invertebrate community.

The exchange of water in the hyporheic zone is complex, but generally depends on the rate of flow of the surface waters (Fellows et al., 2001; Hancock & Boulton, 2005). During low river flows, limited surface water enters the groundwater/surface water ecotone and the main contribution to flow comes from deeper groundwater or lateral aquifers. Conversely, at high river flows or during spates, surface river water predominantly downwells into the hyporheic zone, altering subsurface water chemistry and residence time (Stanley & Boulton, 1995). The potential for river regulation to affect hyporheic zones stems mainly from weakening fluxes between the sediment and river due to interstitial sedimentation and decreased hydraulic exchange (Hancock, 2002). Hydrological influences may be direct and capable of physically transporting organisms (e.g., Marmonier & Châtelliers, 1991) or indirect, whereby both physical and chemical aspects of the habitat of hyporheic invertebrates are altered (Dole-Olivier, 2011). There are strong associations between the magnitude and direction of surface-hyporheic hydrologic exchange and hyporheos assemblage structure (Schmid-Araya, 2000; Swan & Palmer, 2000; Olsen & Townsend, 2003). These patterns are evident in both large and small rivers indicating that hydrologic exchange is a potent and ubiquitous determinant of hyporheic assemblage structure.

Hydrologic factors are known to have a major influence upon the distribution of biota and the nature of ecological processes occurring at the surface of streams (Growns & Davies, 1994; Armitage & Cannan, 2000; Brooks et al., 2005). However, much less is known about how these factors affect the ecology of the hyporheos. Changes in the hyporheos have been recorded in response to spates, particularly when the bed structure has been disturbed (e.g. Marmonier & Dole, 1986; Boulton et al.,1992; Dole-Olivier & Marmonier, 1992). Although some workers have suggested that low flows are likely to have a limited influence on both hyporheic nutrients and invertebrates (Boulton & Harvey, 2003; Boulton et al., 2004; Hancock & Boulton, 2005), the importance of flow in determining the exchange of water in the hyporheic zone would suggest otherwise. Furthermore, as flow is an important parameter controlling the colmation of sediments, through its influence on depositional and erosional processes, there is considerable potential for any effect of flow on the hyporheic environment to be altered by fine sediment.

The relationship between flow and colmation of sediments is not straightforward. Whilst peak flows can be associated with the delivery of large quantities of fine sediment from the catchment (Gibson, 2002), in areas of the river bed experiencing high flow, frequently occurring flood events tend to winnow out fine sediments allowing an exchange of oxygen-rich surface water with the pore water of hyporheic interstices (Gibson, 2002; Mürle et al., 2003). In simple terms, the behaviour of fine sediment within rivers is influenced by hydrologic conditions, the load of fine sediments entering rivers is largely dependent on landscape erosion within the catchment (Boulton et al., 1997), with both factors interacting to govern the rate of colmation. As drivers such as forestry and agricultural intensification tend to be associated with changes to both flow and fine sediment load (hydromorphological changes to increase drainage and increased tillage) there is considerable advantage to understanding their separate and combined influence on hyporheic conditions. Despite the potential for flow and fine sediments to interact to govern physical and chemical hyporheic conditions, we lack experimental investigations of the combined effects of fine sediments and flow on hyporheos.

We present an experimental investigation where we artificially manipulated stream bed fines
and flow rates and examined their individual and joint effects on hyporheic nutrients and invertebrate communities. We hypothesised that differences in discharge and fine sediment bed loads would individually alter hyporheic physicochemical properties and

invertebrates assemblages. In addition, we tested a third hypothesis that there would be an interaction between hydrology and sedimentation whereby higher flow rates would ameliorate the effects of increased levels of fines.

1.2.2 Methods

Experimental design

The experiment was carried out between May and August 2012 in twelve open air, flow-through flume mesocosms located at the Freshwater Biological Association's River Laboratory (50°40'49"N, 2°11'05"W) in Dorset, U.K. Four blocks of mesocosms were sited adjacent to and fed from the Mill Stream, a side channel of the River Frome [mean annual discharge = 6.38 $m^3 s^{-1}$, baseflow index = 0.84 (Marsh and Hannaford 2008); median nutrient concentrations, ammonium = 0.04 (range 0.013 to 0.182) mg L^{-1}, nitrite = 0.07 (0.023 to 0.250) mg L^{-1}, nitrate = 26.85 (15.80 to 37.37) mg L^{-1}, soluble reactive phosphorus = 50 (30 to 190) µg L^{-1} (Wessex Water, unpublished data)]. Each block consisted of three aluminium linear flumes (0.33 m width, 12.4 m length and 0.30 m depth) positioned at approximately 140° to the riverbank and aligned side by side in an east-west direction.

Mesocosms were filled to a depth of 20 cm with sediment sourced from a local quarry to replicate the sediment-size distribution of the Mill Stream (volumetric proportions of particle sizes, 85% 11–25 mm, 5% 2–11 mm, 5% 0.35–2 mm, 5% 0-0.35 mm (Armitage, 1995; Ledger et al., 2008)). To mimic internal colmation, river fines (<2 mm size fraction, obtained from dredged river bed material) were added to sediment placed in the downstream 4 m stretch of each mesocosm. In these sections total fines represented 10% of sediment weight. We chose 10% as ecological impairment potentially occurs when fines reach this level (Kemp et al., 2011). Therefore, each mesocosm contained 2 bed sediment types; clean sediment in the upstream most 4 m section and experimentally colmated (EC) sediment in the downstream most 4 m section.

River water was delivered at the head of each block through an upstream inflow pipe (110 mm diameter) into a reservoir, approximately 2 m long, 1 m wide and 0.35 m deep. From the reservoir, water flowed by gravity over a low weir into the upper end of each channel; flow rates in the individual mesocosms were controlled by adjusting the height of the weir.

Two flow rates were initially established in the twelve mesocosms. The higher flow rate (mean 5.3 x 10-3 $m^3 s^{-1}$ range 4.7 x 10^{-3} to 6.1 x 10^{-3}) was chosen to create turbulent flow (Reynolds number >2000) and the lower flow rate (3.2 x 10-4 $m^3 s^{-1}$ range 2.7 x 10^{-3} to 3.9 x 10^{-3}) was chosen to create a transitional flow between turbulent and laminar flows (Reynolds number, 500 < 2000). The higher and lower flow rates were assigned to each mesocosm alternately along the east-west direction. Flow was started in each mesocosm in May 2012. After 40 days, flow treatments in six out of twelve of the mesocosms were reversed; three of the higher flow flumes were altered to the low flow rate and three of the slower flow mesocosms were increased to the high flow rate (see Table 1.2.1). The experiment was concluded after sampling invertebrates and physico-chemistry at day 70. Prior to loading sediment into the mesocosms three replicate five litre sediment samples were randomly taken from clean and EC bed sediments before loading into the flumes. Bed sediment types assessed by freeze coring. A 12 mm diameter copper pipe 40 cm in length with a pointed end was pushed into the bed sediment till it touched the bottom of the mesocosm. Liquid nitrogen was poured into the tube to freeze the surrounding water and sediment particles. The resulting core was divided into equal lengths, defrosted and the weight of fines and organic content measured.

Table1.2.1. Flow Scenarios.

Label	FF	FS	SS	SF
Flow scenario	70 days x fast flow (no change)	40 days x fast flow; 30 days x slow flow	70 days x slow flow (no change)	30 days x slow flow; 40 days x fast flow
No. of channels	3	3	3	3

Sampling protocol

Electrical conductivity, pH and temperature were measured with a hand-held electronic meter in each of the experimental sections at the start of the experiment, then at four day intervals, at the end of day 40 when the flows in some channels were changed, then at 8 day intervals and at the termination of the experiment on day 70. Benthic invertebrates were sampled using 25 x 25 cm surber sampler, with the substrate disturbed to a depth of approximately 3 cm, at day 30 and day 70. Six randomly placed samples were taken from of each 4m sediment-type section in each mesocosm.

Before the start of the experiment four clusters of standpipes were inserted into the sediment of each mesocosm, in order to sample bed chemistry and hyporheic invertebrates. Each cluster comprised three standpipes inserted to depths of 5, 11 and 18 cm, with two clusters positioned within the top 4 m section and two within the bottom 4 m section of each mesocosm. The standpipes were made from 12 mm diameter PVC pipe with four 5 mm diameter holes located on the vertical sides of the pipe 10 mm from the sealed bottom. A foam plug sealed the holes between sampling occasions. Hyporheic invertebrates and water samples were collected from the standpipes 30 and 70 days after the commencement of flow within the channels. At sampling, the foam plug was pulled from the base of the standpipe drawing water from the zone immediately adjacent to the 5 mm holes into the standpipe. Water for nutrient analyses was collected by drawing 20 ml of standpipe water through a 0.45 µm filter using a syringe fitted with a flexible hose. Water samples were frozen immediately for subsequent analysis. Soluble reactive phosphorus (SRP) concentrations were determined using the colorimetric methods of Murphy & Riley (1962). Ammonium, NH^{4+}-N concentrations were determined colorimetrically using an indophenol blue method (Leeks et al., 1997). Nitrate and nitrite concentrations were determined by Ion Chromatography (Dionex DX500). Hyporheic invertebrates were sampled by drawing 500 ml of water from the standpipe, using a separate syringe fitted with a flexible hose. The water was then passed through a 250 µm sieve and the animals preserved in 4% formaldahyde. Invertebrates were identified to Family level, with the exception that chironomid larvae were taken to subfamily, and mites, nematodes and ostracods to Order. Depth of oxygen penetration into the bed sediment was assessed using 6 mm diameter untreated softwood dowels, as described by Marmonier et al. (2004). Dowels were inserted vertically to the bottom of the mesocosms. Four such dowels were inserted at even spacing along each of the upper and lower 4m sections of each mesocosm. Dowels were inserted at the start of the experiment and removed following water quality and invertebrate sampling on day 30. The depth of oxygen penetration was assessed by measuring the length of each dowel that remained unstained below the bed surface. Fresh dowels were inserted on day 40, when flows were altered in six of the mesocosms, and removed and measured on day 70.

Data analysis

Differences in the bed sediment particle size between the two sediment types at the start of the experiment were tested using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). PERMANOVA can be used to perform a main factors type analysis of variance (ANOVA), based on a matrix of similarities between sites. Significant relationships between the main factors (particle size and sediment type) and their interaction were tested using 9999 randomisations, based on Euclidean distances between samples. Similarly, differences in the bed sediments characteristics between sediment type and flow scenario at the send of the experiment were also tested with PERMANOVA. Differences in depth of oxygen penetration into the mesocosm bed between sampling occasions, flow scenarios and sediment types were tested using PERMANOVA, using Euclidean distances between samples.

We tested differences in physicochemical variables, and benthic and hyporheic invertebrate assemblage structure between sampling occasions, flow scenarios, depths and bed sediments using PERMANOVA. Position of samples in the mesocosms was incorporated as a covariate to account for any potential longitudinal effects. The main factors of interest to test our hypotheses were sediment type and flow scenario and the interaction between the two factors to test for any ameliorative effect of increased flow on experimental colmation. To compare among flow scenarios, we used planned contrasts to compare physicochemical variables and invertebrate assemblage of the fast-flow scenarios with the slow-flow scenarios at time 1 (FF and FS with SS and SF) and time 2 (FF and SF with SS and FS). In addition, planned contrast were used to compare within the fast-flow and slow-flow scenarios on both sampling occasions, i.e. FF with FS and SS with SF at time 1 and, FF with SF and SS with FS at time 2.

Nutrient data and invertebrate abundance data were modified to using logarithmic and square root transformations, respectively, to minimise potential effects of skewed distributions. Invertebrate abundance data was range standardised prior to analysis. Euclidean distance was used as input for the PERMANOVA analysis of physicochemical data and Bray-Curtis distance was used to form similarity matrices for the invertebrate data. Significant relationships between the main factors and interactions were tested using 9999 randomisations. Patterns of differences in invertebrate assemblages identified by PERMANOVA were presented diagrammatically using non-metric multidimensional scaling (NMDS) (Clarke, 1993). Similarity percentages (SIMPER) (Clarke, 1993) were used to identify nutrients or invertebrate species contributing to differences in main factors identified by PERMANOVA.

1.2.3 Results

Sediments

At the start of the experiment, there was a significant difference in the percentage (by weight) between particle size categories and bed sediment type (pseudo-F = 8.5 (p = 0.007) and 314 (p = 0.0001) respectively; Figure 1.2.1). The average total percentage of particles smaller than 2 mm was greater (9.8 % w/w) in EC sediment than clean sediment (6.2 % w/w). The average organic content of EC sediment was also greater (4.8% w/w) when compared with clean sediment (0.0 w/w).

At the end of the experiment, the distribution of fines at different depths from the freeze cores corroborated that significant differences still existed between the two sediment types (interaction between depth and sediment type (pseudo-F = 13.2, p = 0.0001, Figure 1.2.2). Percentage fines increased with depth but at a greater rate in the EC sediment than the clean sediment. There was no significant difference in the distribution of fines between flow scenarios. The distribution of percentage organic matter at different depths was significantly different between the two sediment types (interaction between depth and sediment type (pseudo-F = 3.4, p = 0.027, Figure 1.2.2). The percentage organic content decreased with depth for both sediment types but the rate of decline was greater in the EC sediment. There was no significant difference in the percentage organic content between flow scenarios or any interaction between flow scenarios and sediment types.

Surface water

There were significant differences in electrical conductivity, temperature and pH between the two sampling occasions (Table 1.2.2). Temperature and pH were also significantly different between sediment types. Average temperature in the experimental colmated substrate (15.9°C) was slightly greater than the clean sediment (15.7°C). Average pH was also slightly greater in the experimental colmated substrate (8.5) compared with the clean sediment (8.4). There was no significant difference in water quality between the different flow scenarios.

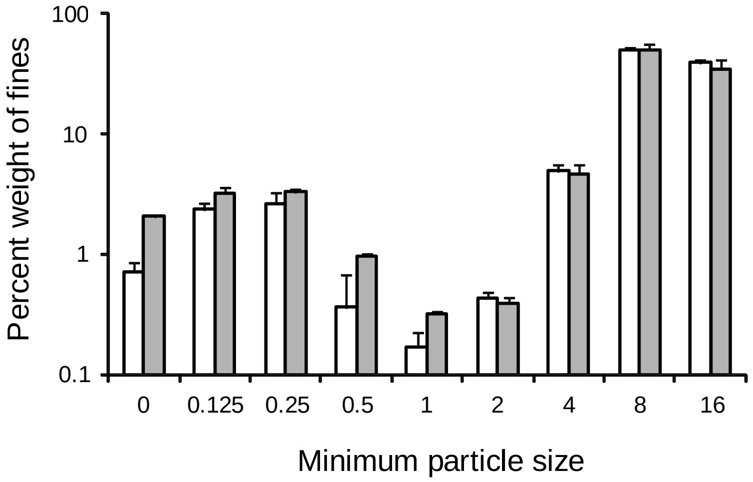


Figure 1.2.1. Mean percentage weight (± 1 S.E.) of bed sediment particles among sizes classes for sediment loaded into mesocosms at Day 0. White columns indicate clean sediment and grey columns sediment with fines added.

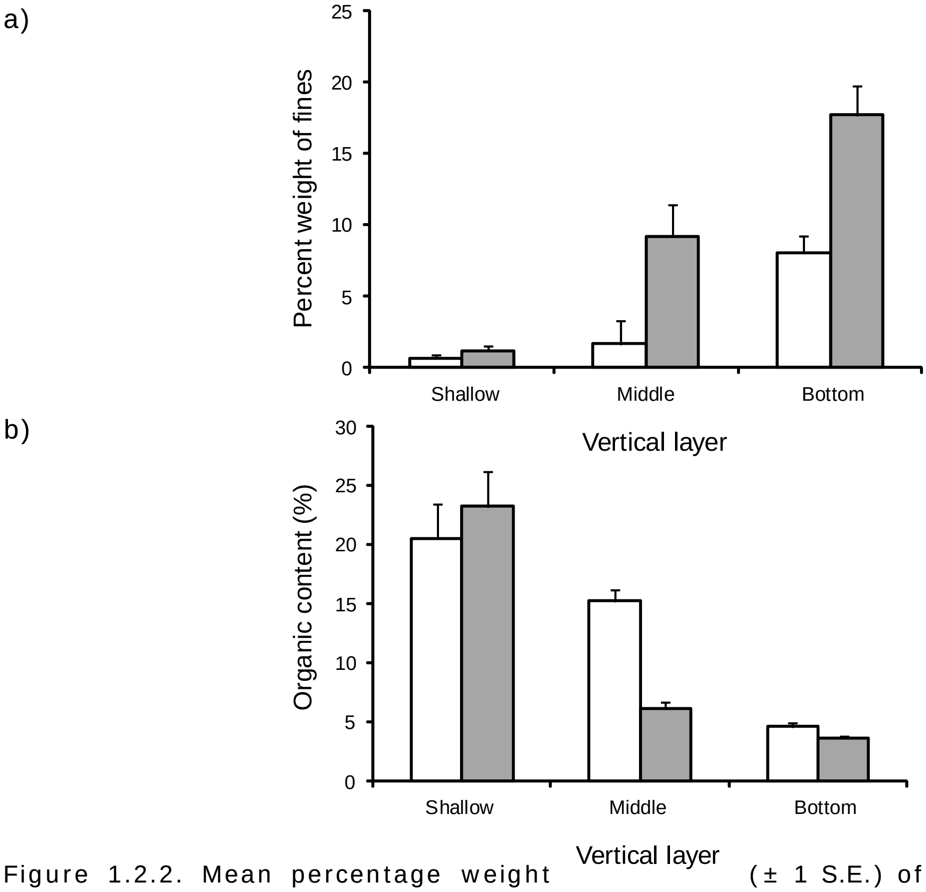


Figure 1.2.2. Mean percentage weight (± 1 S.E.) of a) fines (particles < 2mm) and b) percentage organic content in the shallow (0-x cm), middle (x – y cm) and bottom (y – z cm) layers of bed sediment in mesocosms at Day 70. White columns indicate clean sediment and grey columns experimentally colmated sediment.

Table 1.2.2. Effect of experimental treatments on physico-chemical variables and invertebrate richness, density and assemblage structure. Pseudo-F values and probability levels given for each source of variance in PERMANOVA models.

Source of variation	Degrees of freedom	Electrical conductivity	pH	Temperature	Richness	Density	Assemblage structure
Sediment type (ST)	1	ns	42***	5**	15.6***	16.4***	3.9***
Sampling occasion (SO)	13	1139***	54***	216***	14.1***	ns	23.5***
Flow scenario (FS)	3	ns	ns	ns	7.3***	ns	5.0***
ST x SO	13	ns	ns	ns	ns	4.2*	ns
ST x FS	3	ns	ns	ns	3.4*	ns	ns
SO x FS	39	ns	ns	ns	7.4***	ns	4.1***
SO x ST x FS	39	ns	ns	ns	ns	ns	ns

Table 1.2.3. Effect of experimental treatments on oxygen penetration, nutrient concentrations and invertebrate structure and assembly. Pseudo-F values, probability levels and percentage variation given for each source of variance in PERMANOVA models. Significant sources of variation are shown where relevant.

Source of Variance	d.f	Oxygen penetration		Ammonium		Nitrite		Nitrate		Soluble Reactive Phosphorus (SRP)		Assemblage Structure	
		Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%
Position (Po) (Covariate)	1	NA		ns		ns		ns		ns		4.8***	4
Sediment type (ST)	1	81.7***	15	9.6**	17	ns		16.9***	23	ns		3.5***	5
Flow scenario (F)	3	5.1**	2	6.8***	5	ns		11.4***	6	5.8***	9	5.0***	4
Depth (D)	2	NA		3.3*	1	ns		ns		3.3*	2	4.0***	2
Sampling occasion (SO)	1	74.7***	14	11.4***	4	ns		10.6***	3	8.2**	5	42.3***	18
ST x F	3	3.3*	2	3.4*	4	ns		5.1**	5	ns		1.5*	1
ST x D	2	NA		5.9**	6	ns		4.3*	<1	5.5**	9	ns	
ST x SO	1	96.0***	36	ns		ns		8.3**	4	ns		3.8***	2
F x D	6	NA		ns		ns		ns		ns		1.3*	1
F x SO	3	10.6***	7	6.6***	9	ns		14.2***	15	ns		4.2***	6
SO x D	2	NA		ns		ns		ns		4.0*	1	ns	
SO x ST x F	3	4.5**	5	2.8*	4	ns		5.0**	9	ns		ns	

NA – not applicable, ns – not significant, *** – $p < 0.001$, ** – $p < 0.01$, * – $p < 0.05$.

Table 1.2.4. Pseudo-F values and probability levels for planned contrasts in PERMANOVA models of the effect of experimental treatements on bed chemistry and hyporheic invertebrates.

Source of Variance	d.f.	Oxygen penetration	Ammonium	Nitrite	Nitrate	Soluble Reactive Phosphorus (SRP)	Assemblage Structure
		Pseudo F	Pseudo F	Pseudo F	Pseudo F	Pseudo F	Pseudo F
Time 1							
(FF,FS) vs (SS,SF)	1	41.4***	5.4*	ns	3.5*	ns	11.2***
(SS) v (SF)	1	ns	ns	ns	ns	ns	ns
(FF) v (FS)	1	ns	ns	ns	ns	ns	ns
Time 2							
(FF,SF) vs (SS,FS)	1	11.5***	24.5***	ns	38.1***	5.3*	10.7***
(SS) v (FS)	1	ns	ns	ns	4.7*	ns	ns
(FF) v (SF)	1	ns	ns	ns	3.5*	10.7***	2.2*

*** – $p < 0.001$, * – $p < 0.05$.

Oxygen

Oxygen penetration was significantly different between all main factors including sediment type, flow scenarios and sampling occasions, explaining 15%, 2% and 14% of the variation, respectively (Table 1.2.2). However, there were significant pair-wise interactions between all three main factors (Table 1.2.2). Average oxygen penetration was generally greater for clean sediments than EC sediments (Figure 1.2.3).Between days 30 and 70 average oxygen penetration declined in all clean sediments irrespective of flow. Oxygen penetration remained the same in EC sediments when flows were not reversed between days 30 and 70. However, where flows were reversed from fast to slow, oxygen penetration was significantly reduced and when flows were reversed from slow to fast oxygen penetration significantly increased (Figure 1.2.3, Table 1.2.2).

The interaction between sediment type and flow scenario can be explained because for all scenarios, except the FF scenario, the average oxygenation penetration was greater for the clean sediment compared with the EC sediment (Figure 1.2.3). For the FF scenario the average oxygen penetration was similar between sediment types (Figure 1.2.3). At the end of 30 days the average oxygen penetration was lower in the EC sediment compared with the clean sediment, but by day 70 oxygen penetration was similar (~ 100 mm) in both sediment types, giving a significant interaction between sediment type and time. The flow scenario by time interaction is explained by a decrease in average oxygen penetration from day 30 to 70 for all flow scenarios, except the SF scenario where the average penetration was similar between the two times. However, the fast flow scenarios had significantly greater oxygen penetration compared with the slow flow scenarios on both sampling occasions and there was no significant difference within the fast or slow flow scenarios at either time (Table 1.2.3).

Nutrients

Median concentrations of nutrients 5 cm below the bed in the mesocosms during the experiment were 0.06 mg L^{-1} (range 0.003 to 4.375 mg L^{-1}) for ammonium, 0.02 mg L^{-1} (below detection to 1.00 mg L^{-1}) for nitrite, 20.88 mg L^{-1} (below detection to 28.25 mg L^{-1}) for nitrate and 23.0 µg L^{-1} (1.0 to 129 µg L^{-1}) for SRP. These values are similar to the concentrations observed in the River Frome (see above). The main factors of interest, sediment type and flow scenario significantly influenced the concentration of ammonium and nitrate and only flow scenario influenced SRP and explained between 5% and 17% of the total variation (Table 1.2.3). However, the main factors also significantly interacted with depth and sampling occasion. None of the main factors influenced nitrate (Table 1.2.2). The interaction between flow scenario and sediment type was significant for ammonium and nitrate but explained 5% or less of the total variation.

The change in the concentration of ammonium with depth was significantly influenced by sediment type (Table 1.2.3). Pair-wise comparisons indicated that ammonium concentrations in the surface layer were not significantly different between sediment types, but were significantly higher in the middle and deep sections of the EC sediment (Figure 1.2.4).

The concentration of ammonium differed significantly between flow scenarios and was influenced by sampling occasion (Table 1.2.3). The planned significant effects of depth, flow scenario and sampling occasion on SRP concentration and significant interactions between some of these factors (Table 1.2.2).

Pair-wise tests indicated that on the first sampling occasion the mean concentration of SRP on the bottom of the flumes was significantly lower than either the middle or surface layers. However, on day 70 there was no significant difference between the different depths. Pair-wise tests indicated that the mean concentration of SRP was significantly lower in the SF flow scenario compared with the other scenarios, potentially explaining the significant main factor flow scenario.

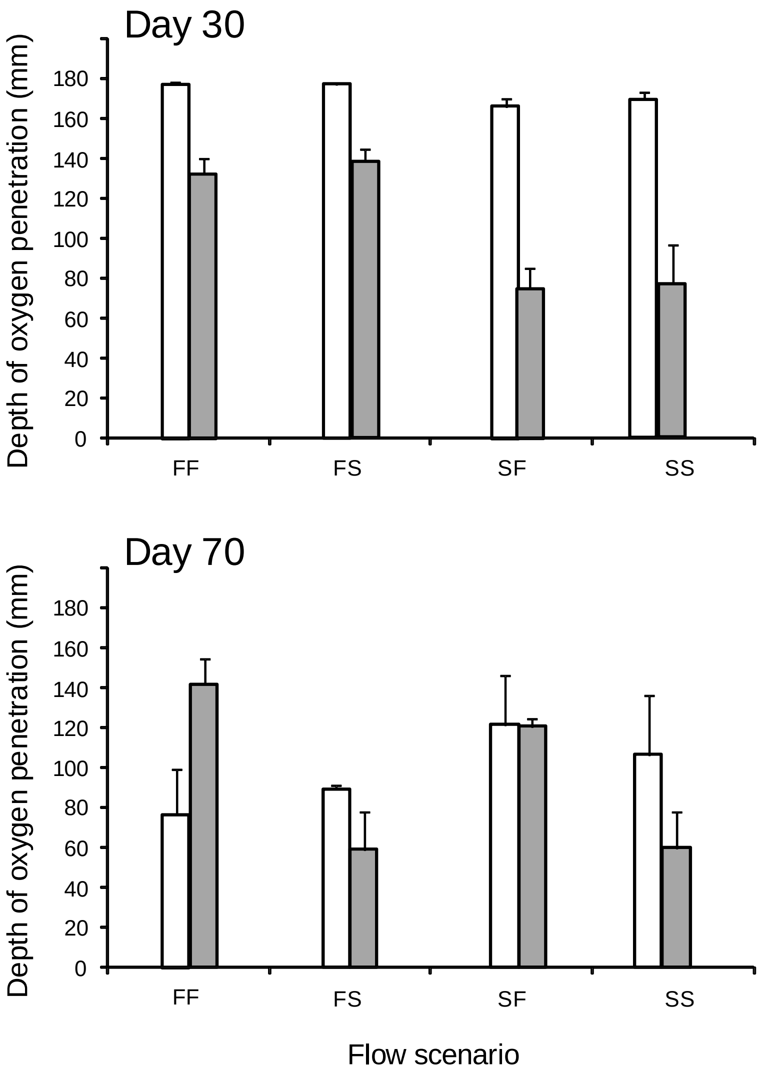


Figure 1.2.3. Mean depth of oxygen penetration (± 1 S.E.) at day 30 and day 70 among flow scenarios, times and sediment types. White columns indicate clean sediment and grey columns experimentally colmated sediment. Note on day 30 the first letter of the flow scenario code is applicable (FF = FS; SS = SF) whereas on day 70 the second letter is applicable (FF = SF; SS = FS).

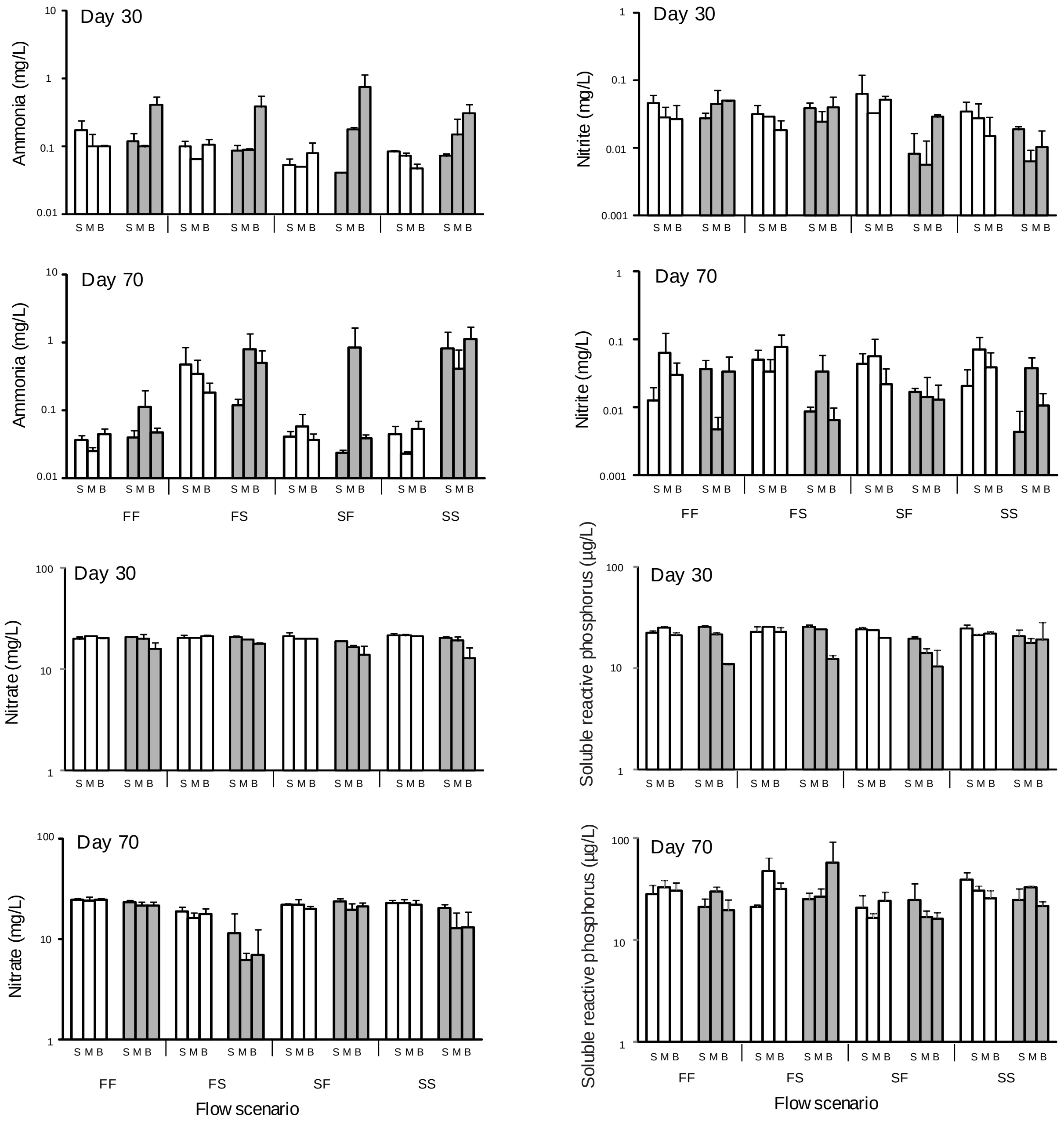


Figure 1.2.4. Mean (± 1 S.E.) concentration of four nutrients at Day 30 and Day 70 in surface (S), mid (M) and bottom (B) depth layers in clean sediment (white columns) and experimentally colmated sediment (gray columns) for four flow scenarios. Note on day 30 the first letter of the flow scenario code is applicable (FF = FS; SS = SF) whereas on day 70 the second letter is applicable (FF = SF; SS = FS).

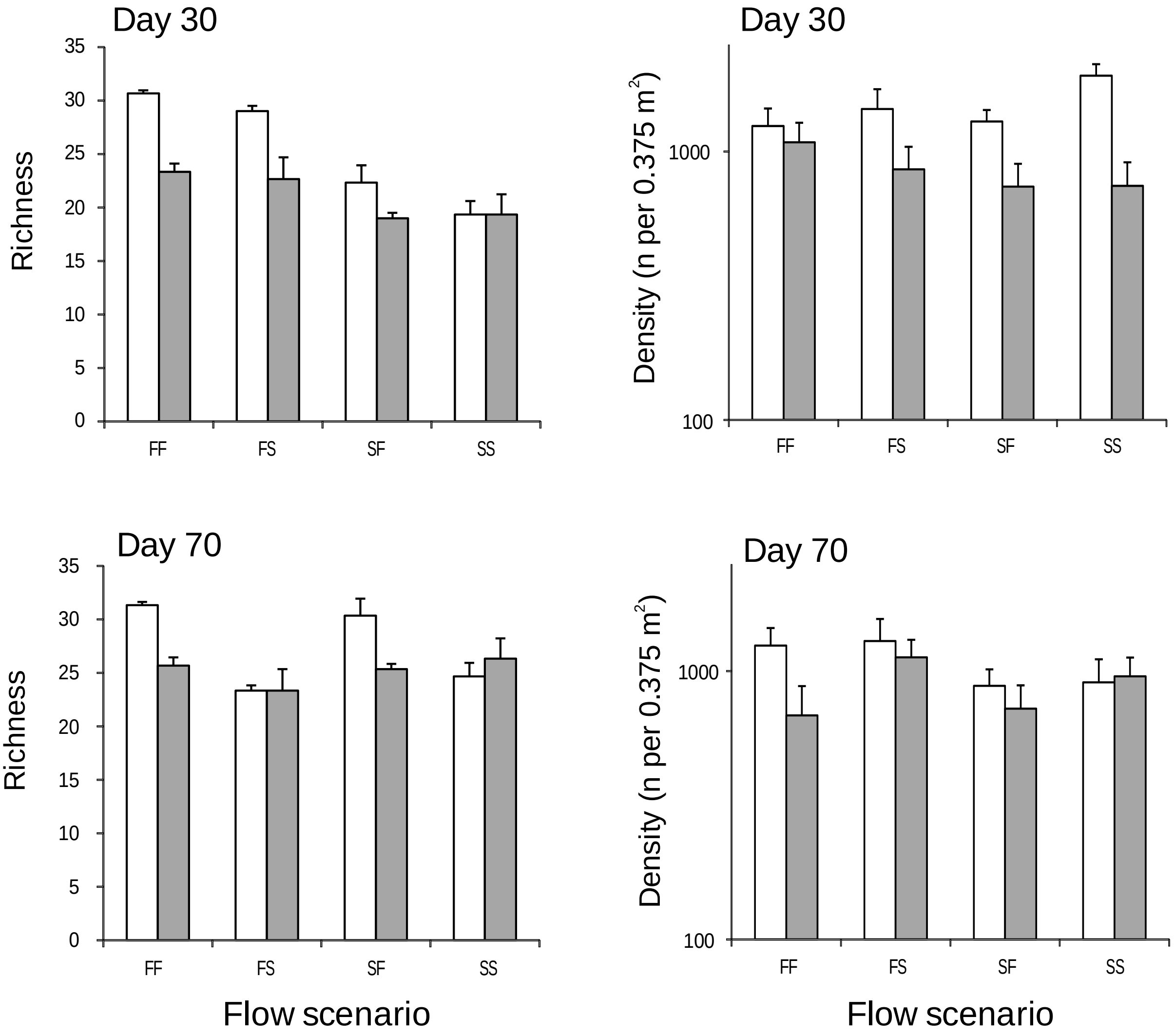


Figure 1.2.5. Mean benthic invertebrate richness and density (± 1 S.E.) at Day 30 and 70 in four flow scenarios. White columns indicate clean sediment and grey columns experimentally colmated sediment. Note on day 30 the first letter of the flow scenario code is applicable (FF = FS; SS = SF) whereas on day 70 the second letter is applicable (FF = SF; SS = FS).

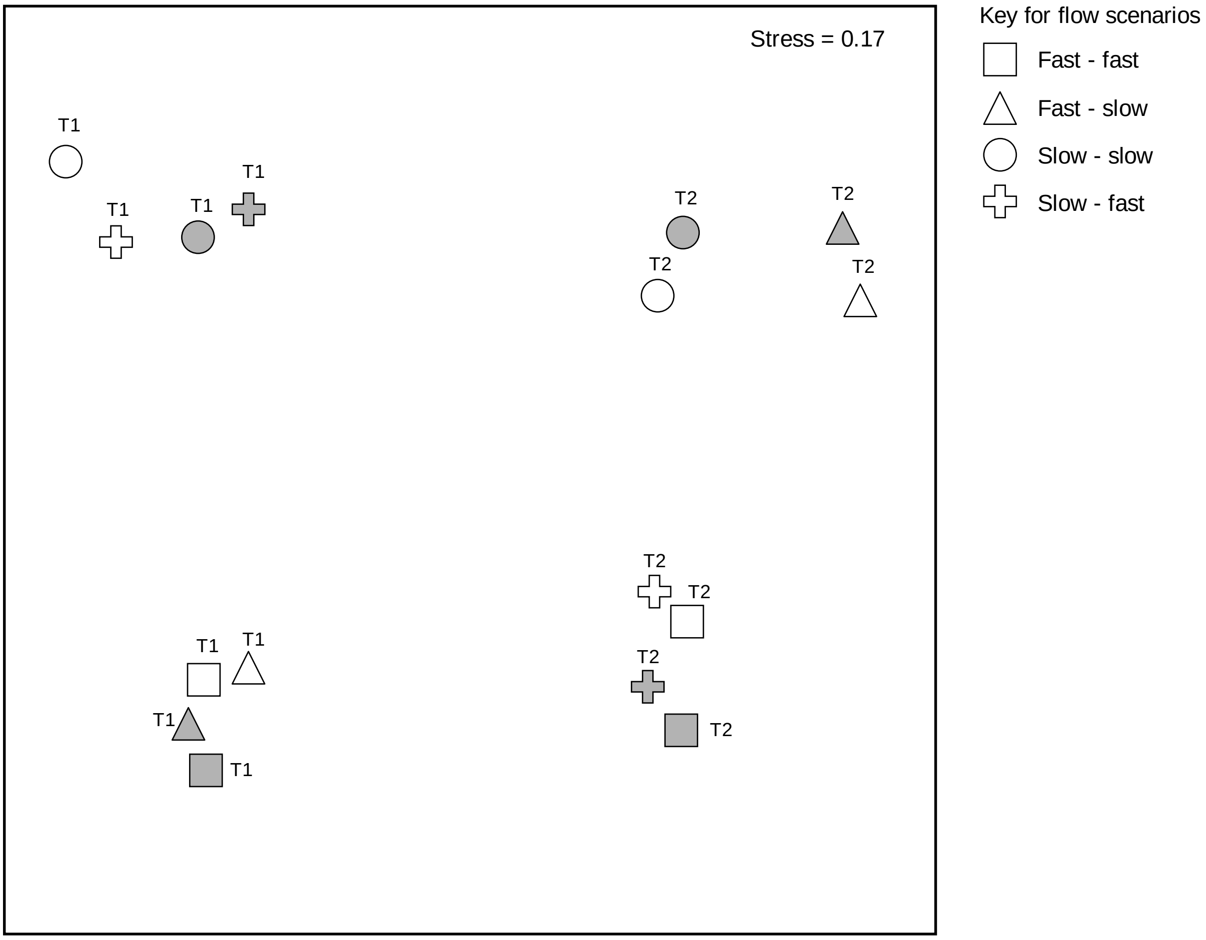


Figure 1.2.6. Ordination of centroids of benthic invertebrate assemblages from different flow scenarios at day 30 (T1) and day 70 (T2). White symbols indicate clean sediment and grey symbols experimentally colmated sediment. Note on day 30 the first part of the flow scenario code is applicable (FF = FS; SS = SF) whereas on day 70 the second part is applicable (FF = SF; SS = FS).

Benthic Invertebrates

Overall 57 taxa were identified: the most common taxon was Gammaridae comprising 28% of the total density, followed by Tanytarsini chironomids (15%), Chironomini chironomids (13%), Cyclopoid copepods (10%), Ephemerellidae (6%), and Ephemeridae (5%). The remaining taxa each comprised less than five percent of the total density.

Benthic invertebrate richness, density and assemblage structure were all significantly influenced by the differences in sediment in the mesocosms (Table 1.2.2). Richness and assemblage structure were also influenced by sampling occasion and flow scenario. One of the terms of interest, the sediment/flow scenario interaction was not significant for density or assemblage structure suggesting that flow regimes did not greatly influence the effects of sediment type on benthic invertebrates. In addition, the sediment/sampling occasion interaction was not significant for richness or assemblage structure suggesting that any effects of sediment were constant through time.

Overall, the average richness was 26 taxa in clean sediments and 23 taxa in EC sediments and the average number of taxa increased from 23 at day 30 to 26 at day 70 (Figure 1.2.5). Clean sediments in the FF flow scenario had a significantly greater number of taxa than the EC sediments, but this did not occur for the other flow scenarios, potentially explaining the significant ST x FS interaction. Benthic invertebrate richness was generally greater in the fast-flow scenarios (i.e. FF and FS on day 30 and SF and FF on day 70), the increase in the SF from day 30 to day 70 and decrease in FS from day 70 to day 30, probably explains the significant FS x SO interaction.

Benthic invertebrate density was generally greater in the clean versus the EC sediment type in all flow scenarios and on both sampling occasions (Figure 1.2.5). Average density in clean sediments declined from 1474 to 1082 animals per 0.375 m^2 from day 30 to day 70. However, average density in the EC sediments increase slightly from 860 to 870 animals per 0.375 m^2, over the same time, potentially explaining the significant ST x SO interaction.

Flow scenario significantly interacted with sampling occasion for benthic invertebrate assemblage structure, indicating that the influence of the changed flow regime. On day 30, the benthic invertebrate assemblages in the fast (FF and FS) flow scenarios were not significantly different to each other but were significantly different to those from the slow (SS and SF) flow scenarios. Similarly on day 70, the fast (FF and SF) scenarios were not significantly different to each other but were significantly different to the slow (SS and FS) flow scenarios. In addition, on day 30 the benthic invertebrate assemblages in the slow flows (SS and SF) were not significantly different. However, on day 70 the slow flow scenarios were significantly different from each other but had a similar assemblage structure as indicated by their close proximity in ordination space (Figure 1.2.6). These results suggest that the invertebrate assemblages responded to the immediate effects of flow within the mesocosms; adjusting the flow from fast to slow or from slow to fast at day 40 resulted in the fauna becoming the same as the slow controls (SS) or fast controls (FF), respectively at day 70 (Figure1.2. 6).

Four benthic invertebrate taxa contributed up to 20% of the significant differences between fast and slow flow scenarios on day 30. Athericidae, Baetidae and Ephemerellidae were more abundant in the faster flowing mesocosms and Dytiscidae more abundant in the slower flow scenarios. Five taxa including, Hydropsychidae, Baetidae, Hydroptilidae, Polycentropodidae and Leuctridae, contributed up to 20% to the significant differences between the fast and slow flow scenarios on day 70. With the exception of Leuctridae, all these taxa were more abundant in the faster flow scenarios.
Seven taxa were identified as contributing up to 20% of the difference in invertebrate assemblages between sediment types. Leuctridae, Ephemeridae and Baetidae were generally more abundant in the EC sediment type and Dytiscidae, Hirudinea, Hydracarina and Caenidae were generally more abundant in the clean sediments.

Hyporheic Invertebrates

A total of 29 taxa were identified in the hyporheic samples. The most common taxon found was Cyclopoid copepods comprising 58% of the total abundance, followed by Chydoridae (15%), Gammaridae (7%), Ostracoda (4%), Tanytarsini chironomids (4%), Oribatidae (1%) and Ephemeridae (1%). The remaining 22 taxa each comprised less than one percent of the total abundance.
Each of the main factors significantly influenced hyporheic invertebrate assemblage structure (Table 1.2.3). Five of the six two-way interactions were significant, however, only the interaction between flow scenario and sampling occasion explained greater than 2% of the total variation. Pair-wise tests of hyporheic invertebrate assemblage structure indicated the bottom two sediment layers differed significantly from the surface layer but

not from each other. The interaction between depth and the other main factors was not significant or minor indicating that depth differences were generally maintained through time and between sediment types and flow scenarios. The taxa Oligochaeta, Ephemeridae and Tanytarsini were more abundant in the surface layer compared with the two deep layers. In contrast, four taxa, including Oribatidae, Ostracoda, Gammaridae and Cyclopoida were more abundant in the deep layers.

The differences in the hyporheic invertebrate assemblages between sediment types explained 5% of the total variation (Table 1.2.3). Seven taxa contributed 6% or more to the separation of assemblages between sediment types: six taxa including, Oligochaeta, Cyclopoida, Tanytarsini and Ephemeridae were more abundant in the clean sediment, whereas, Ostracoda, Oribatidae, and Gammaridae were more common in EC sediment.

On day 30, the hyporheic invertebrate assemblages in the fast (FF and FS) flow scenarios were not significantly different to each other but were significantly different to those from the slow (SS and SF) flow scenarios (Table 1.2.3). Similarly on day 70, the fast (FF and SF) scenarios were not significantly different to each other but were significantly different to the slow (SS and FS) flow scenarios, explaining the interaction between sampling occasion and flow scenario (Table 1.2.3). These results suggest that the hyporheic invertebrate assemblages responded to flow within the mesocosms; changing the flow from fast to slow or from slow to fast at day 40 resulted in the faunal assemblage switching to become the same as the slow controls (SS) or fast controls (FF), respectively at day 70 (Figure 1.2.7). The hyporheic invertebrate taxa that contributed more than 6% to the significant differences between flow scenarios on day 30 included, Oligochaeta, Gammaridae, Tanytarsini, Orthocladiinae, Ostracoda and Ephemeridae, which were all more abundant in the faster flowing mesocosms (Figure 1.2.7, Table 1.2.3). Three taxa more abundant in the fast flows (Oligochaeta, Ephemeridae, Oribatidae) and two taxa less abundant (Ostracoda and Sphaeridae), contributed more than 6% to significant differences between flow scenarios on day 70 (Figure 1.2.7).

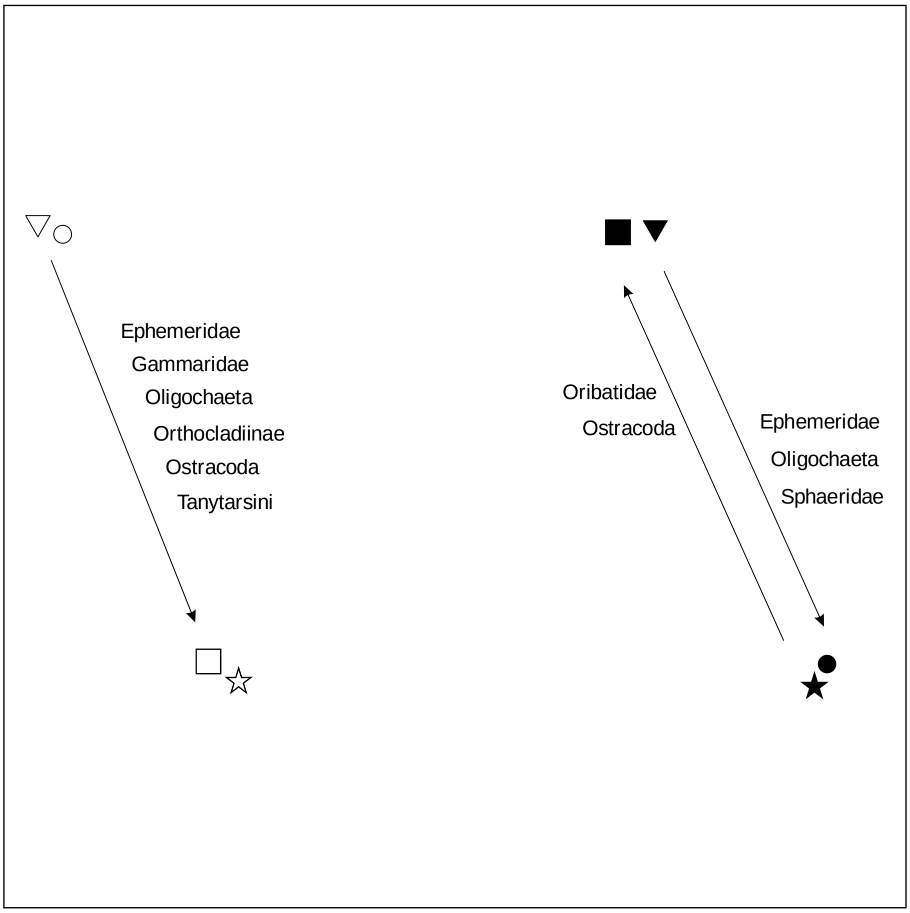


Figure 1.2.7. Ordination of centroids of hyporheic invertebrate samples from FF (stars), FS (squares), SS (triangles) and SF (circles) flow scenarios on day 30 (white symbols) and day 70 (black symbols). Arrows indicate direction of increased abundance of taxa.

1.2.4 Discussion

We have demonstrated that the addition of fines to stream sediment and the alteration of flow, affect hyporheic oxygen penetration, nutrients and invertebrates. Nevertheless, our hypothesis that higher flows would ameliorate any effects of added fines, although generally supported for oxygen penetration, was not supported for benthic or hyporheic invertebrates (no interaction between flow type and sediment treatment). We observed no major interaction effects between flow regime and sediment types either on sediment characteristics, nutrient concentrations or invertebrate assemblage structures. These results are in contrast with the assertion that increased flow can reduce the effects of sedimentation through winnowing (Gibson, 2002; Mürle et al., 2003). It has been reported that increased flow can reduce the effects of sedimentation on invertebrates (Mattaei et al., 2010) and it is possible that the differences between the turbulent and transitional flow regimes (and their alteration) in our mesocosms did not reach a requisite threshold to create enough shear stress to remove fines or alter sediment dynamics. Nevertheless, Boulton et al. (2004) observed only limited change in sediment dynamics within the hyporheic zone following an artificial spate that created a three-fold increase (at least) in discharge. Further manipulative experiments are required to determine the flow rates required to mitigate the impacts of excess fine sediments in stream beds. Such experiments would assist in the management of sedimentation of rivers. Gravel cleaning operations are often conducted as a management tool to restore the quality of gravel stream beds sufficient to make them suitable for salmonid spawning (Shackle et al., 1999; Merz et al., 2004). However, there has been a limited number of studies investigating the efficiency of gravel cleaning and river bed restoration (Sarriquet et al., 2007; Meyer et al., 2008). Without addressing the sources and retention of fine sediment, the indications are that such gravel cleaning approaches only provide a temporary improvement in the spawning success of salmonids (Pulg et al., 2013).

The experimental addition of fine sediment to the substrate affected oxygen penetration, nutrient concentrations (ammonium, nitrate, nitrite and SRP), and both benthic and hyporheic invertebrate assemblage structure. Although the substrate had been well mixed before adding it to the mesocosms, by the end of the experiment most of the fines, in both sediment types, had settled to the bottom of the mesocosms. There was also an increase in the amount of fines in the substrate, presumably as a consequence of deposition from the inflowing water; by the end of the experiment fine sediment, comprised 18% by weight in the experimentally colmated reaches. Oxygen penetration was lower in the EC sediment at day 30 but was similar in both sediment types at the end of the experiment. The concentration of ammonium was generally higher in the EC sediment compared with the clean sediment, the concentrations of nitrite similar and nitrate lower. Nitrite is produced as an intermediate in both nitrification and denitrification. The similarity of free nitrite in the sediment is, therefore, potentially a result of the complex balance between diffusive transport, aerobic ammonium oxidation, and anaerobic nitrate reduction. The addition of fines caused an increase in anoxic conditions (Figure 1.2.3) that altered the nutrient dynamics in favour of denitrification. These results are supported by Meyer et al (2005) who demonstrated that although nitrite accumulated from both aerobic ammonium oxidation and anaerobic nitrate reduction, the nitrate reduction rather than ammonium oxidation was the major direct source of nitrite in the anaerobic sediment layer. Another potential pathway of a lower concentration of ammonium in the clean substrate is conversion to dinitrogen gas via anammox. The clean substrate had a higher percentage organic content compared with the EC sediments and anammox activity has been positively correlated with concentration of the organic carbon content of estuarine sediments (Nicholls &Trimmer 2009). However, further research is required to determine the relative contribution of anammox and denitrification activity in freshwater sediments and how this may be modulated by organic carbon.

The mean concentration of SRP was generally lower in the EC sediment. Phosphorus dynamics in rivers are controlled by biotic (e.g. assimilation by both primary producers and decomposers) and abiotic factors (e.g. sorption to and release from suspended and benthic particle surfaces) (Reddy et al., 1999; Mainstone & Parr 2002). Abiotic control on P in streams and rivers is often driven by particle size (House & Denison, 2000), with small particles being particularly effective in sequestering soluble P (Klotz, 1988; He et al., 1995). Although there may be some biotic influence which caused the lower SRP concentrations in the fines-added sediment, it is highly likely that the increase in the percentage fines of the EC sediment resulted in an increase in the sorption potential, thereby reducing the SRP concentrations in the pore water. The median and range of nutrient concentrations within the mesocosms was comparable to those observed in the River Frome. The similarity of the nutrient concentrations suggests that the results observed within the mesocosms are likely to be transferable to real river ecosystems, as has been shown previously for the benthic invertebrate communities of these mesocosms (Harris et al., 2007).

Several studies have suggested an impact of fine sediment pollution on hyporheic invertebrates (Richards & Bacon, 1994; de Bovee et al., 1995; Boulton et al., 1997; Bo et al., 2007). However, the amount of fine sediment required to cause a detrimental effect is often not reported. We have demonstrated that an increase of 3.6% fines by weight (the difference between the fines in the clean and EC sediments) is enough to alter invertebrate assemblage structure. However, further experimentation is required to determine whether greater colmation will have greater impacts on the hyporheic invertebrates. In contrast to our findings, Pacioglu et al. (2012) observed that of 32 taxa in the hyporheic assemblage only abundances of Oligochaeta differed between colmated and clean sediments. Fine sediment pollution can affect invertebrates directly through altered water quality (Bretschko, 1994), loss of microhabitat (Rae, 1987), loss of access to food resources (Lenat et al., 1981) and the damage to respiratory systems of individuals (Lemly, 1982) or indirectly by changing trophic sources (Quinn et al., 1992). Just as the relationship between benthic invertebrates and fine sediments is poorly understood (Jones et al., 2011), the conditions under which excess fine sediment causes detriment to the hyporheos, and the mechanisms that cause such effects, require further study.

The difference between the benthic invertebrates in the experimentally colmated and clean sediments was primarily driven by the differences in abundances of seven taxa. The densities of four taxa including Caenidae, Dytiscidae, Hirudinea and Hydracarina were higher in clean sediment and three families Baetidae, Ephemeridae and Leuctridae were higher in the EC sediment, suggesting that colmation can have positive and negative impacts, depending on individual taxa. Mattaei et al. (2010) also demonstrated positive and negative impacts of sediment addition and suggested that extra sediment could provide additional habitat for some taxa. We set up our EC sediments to mimic changes to bed composition resulting from inputs of fine sediment to catchments where the average size of particles becomes smaller, interstices between larger particles become filled and where a where a surface drape of deposited sediment occurs (Kaufmann et al., 2009). Most invertebrate species have specific requirements of the substrate they occupy and tend to avoid patches that fail to meet their requirements (Culp et al., 1983; Peckarsky, 1991; Williams & Smith, 1996; Sarriquet et al., 2007). For example, blackfly larvae tether themselves by means of a posterior circlet of hooks onto strands of silk which they attach to comparatively clean substrate, and they avoid substrates covered by a surface drape of loose sediments (Bass, 1998). Several species of crawling mayfly larvae avoid finer, less stable substrates as they cannot grip them effectively (Ciborowski et al., 1977; Corkum et al., 1977). However, other species, for example, certain Chironomidae and Ephemeridae, select finer sediments into which they build tunnels. Jones et al. (2011) suggested that along with physical changes to bed sediments, colmation can also impact the chemical environment, particularly to decrease oxygen levels. However, we only noted small changes to water quality in our mesocosms and oxygen penetration in the bed was always greater than the sampling depth of 3 cm, suggesting that altered bed particle size structure was the main mechanism affecting invertebrates.

The responses of the invertebrates to the altered flows is consistent with Dewson et al (2007) who indicated that overall invertebrate abundance can increase or decrease in response to decreased flow, whereas invertebrate richness commonly decreases because habitat diversity decreases. The differences in assemblage structure between the fast and slow flows were primarily driven by eight taxa, six of which were more abundant in the fast flows. The increased abundance of three of the six taxa, including Baetidae, Ephemerellidae and Hydropsychidae is consistent with Extence et al. (1999) who indicated these taxa are primarily associated with moderate to fast flows. In contrast, Extence et al. (1999) also included Leuctridae in fast flowing taxa but this family was more abundant in our slow flow mesocosms. In addition, although Hydroptilidae and Polycentropodidae were more abundant in our fast flow mesocosms, Extence et al (1999) lists these taxa as primarily associated with slow flowing and standing waters. Only one taxon, Dytiscidae, was more abundant in the slow flow mesocosms and was listed by Extence et al. (1999) as associated with slow flowing waters. The contrasting results from the mesocosms and the classification of Extence et al. (1999) suggest that these taxa may have more flexible hydraulic requirements that previously thought.

We have demonstrated that benthic and hyporheic invertebrates responded to differences in the surface flow conditions and that a change in assemblage structure was evident after 30 days. Altering the surface flows resulted in a rapid change to both the benthic and hyporheic invertebrate assemblage structure. These results are supported by previous studies which demonstrate hyporheic invertebrate assemblages may respond to droughts and floods and that there can be differences in upwelling and downwelling parts of riffles (e.g. Dole-Olivier & Marmonier, 1992; Hancock, 2006; Stubbington et al., 2009; Olsen et al., 2010; Robertson & Wood 2010). However, this is the first study to establish that hyporheic invertebrate assemblages respond to differences in local near-bed hydraulics. The response of the hyporheos to localised changes in surface flows suggests that their hydrological relationships may be more sensitive than originally thought and, therefore, we advocate the incorporation of measures of near-bed hydraulics into future studies.

In conclusion, we have demonstrated an increase in the proportion of bed fines to 10% by weight decreases oxygen levels, increases ammonium concentrations, and alters invertebrate assemblage structure in the hyporheic zone. Different flow rates also affected these attributes. The rates of flow used in our mesocosms did not appear to reach the threshold required to remove fines or alter sediment dynamics. Furthermore, the response to flow appeared to be a direct effect of surface flows acting on the hyporheos, rather than any historic effects caused by past delivery or flow paths. Further research is required to evaluate if lower percentages of bed fines result in ecological impairment and to determine what flow rates are required to ameliorate sedimentation impacts.

1.3 *The response of periphyton growth rate to fine sediment additions and phosphorus concentration*

1.3.1 Introduction

Within Work Package 3 of the REFORM project we focus on using a variety of sources of information to disentangle the interactions between hydromorphology and other stresses. Specifically there is a description of work which is to test some empirical relationships and taxon/trait sensitivities in replicate experimental channels. Deliverable 3.1 focused on analysing large spatial datasets. In that deliverable and in chapter 1.3 of this deliverable it was found that it was not possible to develop an indicator metric for fine sediment impacts on diatoms. The underlying rationale was that that fine sediment, depositing on the benthic substrate, would favour motile species; those that could literally get out of the way and migrate to the top of the sediment where light was available. This rationale was based on the findings of previous studies. Such a metric would have allowed a distinction to be made between eutrophication impacts on benthic algae and fine sediment impacts. Robust metrics for eutrophication already exist for algae. In this chapter we explore the question further, experimentally testing the interaction between fine sediment and nutrient concentration on benthic algal production.

Across Europe it has become evident that rivers are commonly subject to multiple stresses, typically nutrient enrichment and at least one form of hydromorphological degradation (Schinegger et al. 2012). In catchments with significant amounts of arable farming, excess inputs of fine sediment from ploughed land are a common stressor. It is not surprising that fine sediment stress and nutrient enrichment co-occur as both are produced by intensive farming. Indeed, fine sediment is a primary source of particulate phosphorus. Reducing the amount of phosphorus (P) loading in rivers is a key step in limiting freshwater eutrophication issues, including excessive algal growth (Bowes et al. 2012). Restoration of rivers in order to prevent eutrophication is vital to meet the ‘good ecological status’ criteria demanded by the European Union’s Water Framework Directive (WFD). Consequently there has been work in many rivers to reduce P inputs from the catchment, to constrain primary productivity through P limitation. Many of these rivers have seen significant reductions in P concentrations due to the introduction of a number of schemes including the European Union’s Urban Waste Water Treatment Directive (EEC 1991), which led to phosphate removal at sewage treatment works (STW) (Foy, 2007; Kinniburgh & Barnett, 2010) and the Catchment Sensitive Farming Initiative, which has attempted to reduce diffuse, agricultural nutrient inputs to rivers.

WFD ecological status is assessed using metrics for a number of biological quality elements (BQEs), which cover algae, macrophytes, fish and invertebrates. Experiments investigating the ecological impact following reductions in P loading have seen mixed results in rivers; some have shown the intended improvements in river ecology (Kelly et al. 2009, Bowes et al. 2011), whilst others have failed to find evidence that the reduction in P had a significant positive impact on ecological status (Neal et al. 2010, Bowes et al. 2012). Further study is clearly needed to investigate the impact of a reduction in P loading on river ecology.

Periphyton (a mixture of algae, heterotrophic microbes and detritus, which is attached to substrata in aquatic environments) has been identified as a major driver of the ecologically damaging processes associated with eutrophication (Hilton et al. 2006, McCall et al. 2014). Therefore, factors which impact on algae in rivers are the subject of investigation in this report, and, in particular, methods for controlling periphyton growth rates.

The impact of fine sediment (<2mm) on river systems has also been investigated in recent research. River sediment is essential for ecological functioning and heterogeneity. However, increases in certain human activities, for example, agriculture, catchment urbanisation, mining and forestry practices, have led to increased mobilisation and more rapid delivery of fine sediments to waterbodies (Yamada and Nakamura 2002, Jones et al. 2014). High levels of sediment suspension and deposition in rivers can have major negative impacts on biological elements such as invertebrates, macrophytes and fish, and targets for sediment management are set based on their impact on fish (Yamada and Nakamura 2002, Jones et al. 2014). Much less is known about the impact of fine sediments on algae. However, a study by Yamada and Nakamura (2002) found that increases in fine sediment levels led to decreases in living periphyton levels. Other studies have focused on the impact of fine sediment on benthic diatoms, a particular group of algae, which form a major component of periphyton, and are assessed in the phytobenthos BQE (Dickman et al. 2005, Jones et al. 2014). Sediment is thought to negatively impact on periphyton directly in a number of ways, firstly, through shading by both deposited and suspended sediment, which reduces the amount of light the photosynthetic algae receive, leading to growth limitation. Burial may also result in shading and an altered physical and chemical environment, which can impact on nutrient availability. Sediment deposition can also lead to physical abrasion of the algae and create an unstable environment that is more easily eroded (Yamada and Nakamura 2002, Jones et al. 2014). Indirect effects may also occur due to the sediment impacting on other aspects of the river ecosystem, such as macrophytes and invertebrates. The impact of sediment on periphyton is an area in need of further study, particularly as the presence of fine sediment has the potential to confound investigations of other stressors in river environments, for example, P concentration (Jones et al. 2014).

In this study we aim to use river flume mesocosms to investigate the impacts of P reduction on periphyton growth, in a river that normally has P in excess (soluble reactive phosphorous (SRP) - 100 µg l^{-1} in preliminary investigations). The experiment also aims to expand on current knowledge available regarding the impact of fine sediment on periphyton growth. By considering these stressors simultaneously and investigating interactions in this way, a more realistic situation is replicated, that could be representative of a river management scenario.

1.3.2 Methods

Study site

The flume mesocosm experiments took place on the River Nadder in the village of Burcombe, UK (Latitude N51:04:45 , Longitude W1:54:06). The River Nadder is part of the Hampshire Avon catchment and flows east to join the main stem of the River Avon, at the city of Salisbury (Figure 1.3.1). The river was chosen to carry out nutrient limitation and sediment impact experiments as it has been subject to elevated nutrient levels and fine sediment for many years. It is also a safe site for fieldworkers to operate and the local river managers, residents and Environment Agency were supportive.

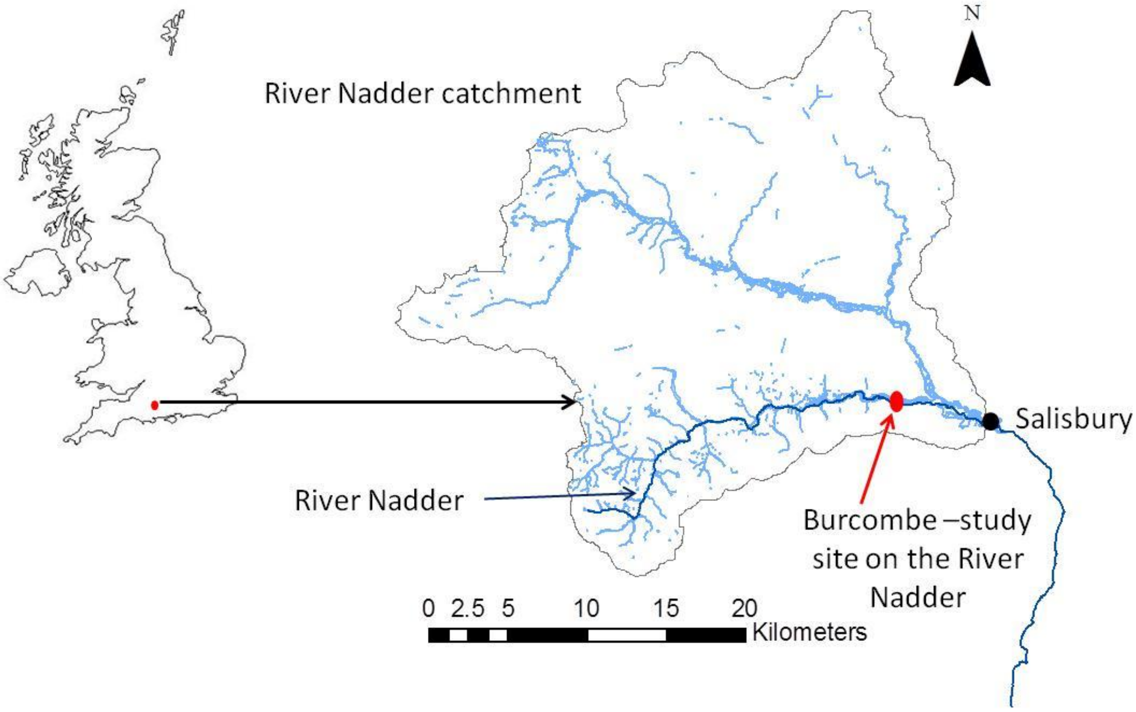


Figure 1.3.1. River Nadder catchment, Wiltshire, UK, with location of the flume experiment at Burcombe.

Flume mesocosm experiments

Eighteen flumes (six sets of three flumes) were installed along a 50 m stretch of relatively straight, uniform section of the river with a negligible amount of riparian shading. The maximum average river depth and width were ca. 0.9 m and 7 m respectively. Land use at the site was grassland, with rough pasture on one bank and housing on the other bank.

Flumes were grouped into sets of three, with each individual flume measuring approximately 0.26 m in width and 3 m in length. Figure 1.3.2 shows a diagrammatic representation of a set of three flumes. Each flume contained a sump (roughly 15 x 15 x 6 cm), approximately one third of the way along its length, to catch any large river debris and/or chemical precipitate (a possibility with the phosphorus reduction treatment that is described below). The flow rate of river water through the flumes was controlled by gates at the upstream end of each flume. At the start of the experiment the flow rates were adjusted until the water velocity was approximately 0.12 m s^{-1} in each flume (measured mid-channel just downstream of the sump).

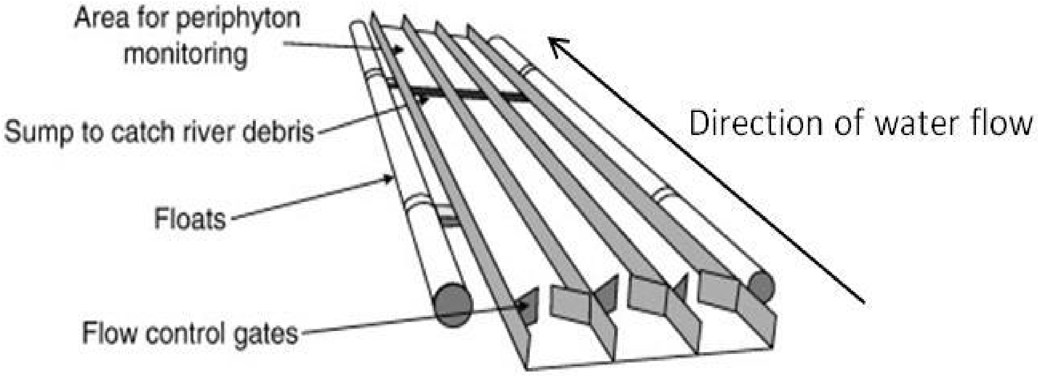


Figure 1.3.2. A set of three flumes with their associated floats.

The flumes were constructed from polyvinyl chloride (PVC) sheeting and secured within aluminium frames. The flumes were positioned with ample space between them to ensure that treatments from different flumes would not interact. Metal poles were driven into the river bed to fix the flumes in place, and floats were attached along the edges of each set of flumes to enable them to adapt to small fluctuations in the river depth. Consequently, the water level in the flumes was maintained at approximately three cm for the duration of the study. By maintaining the flumes in the upper portion of the water column (more than 0.5 m above the sediment) colonisation of the flumes by invertebrates, and any subsequent grazing on the accumulating biofilm, was minimised.

It should be noted that there were no statistically significant differences in velocity (mean ± s.e. 0.12 ± 0.003 ms^{-1}), depth (0.30 ± 0.005 m) or discharge ($11 \times 10^{-3} \pm 0.2 \times 10^{-3}$) between the flumes, and there was also no effect on these parameters resulting from the flumes being grouped into sets of three.

Differences in periphyton growth under varying experimental treatments were measured by providing an identical base substrate in each flume in the form of unglazed ceramic tiles (approximately 7 x 7 cm). Tiles were used as they were easy to remove for analysis without disturbing the ongoing experiment and they also acted as a good substrate for periphyton growth. Total periphyton biomass was estimated from these tiles by the quantification of chlorophyll-*a* concentration that is found in all green plants and constitutes approximately 1 to 2 % of the dry mass of planktonic algae (McCall *et al.* 2014).

Experimental treatments

Within stream flume mesocosm experiments were used in this study to investigate the impact of phosphorous limitation and fine sediment addition on periphyton accrual rates simultaneously.

In total, 16 flumes were used during the experiment and treatments were randomly assigned to each flume. Four flumes were kept as controls and had unmodified River Nadder water running through them. Four flumes had water with a reduced phosphorus content flowing through them, but no sediment added. Another four flumes had fine sediment applied to the lower section of the flumes and their ceramic tiles at the beginning of the experiment, but had unmodified water running through them. The final four flumes had both reduced phosphorus water flowing through them and sediment added to the tiles. The distribution of these treatments across the flumes can be seen in Figure 1.3.2.

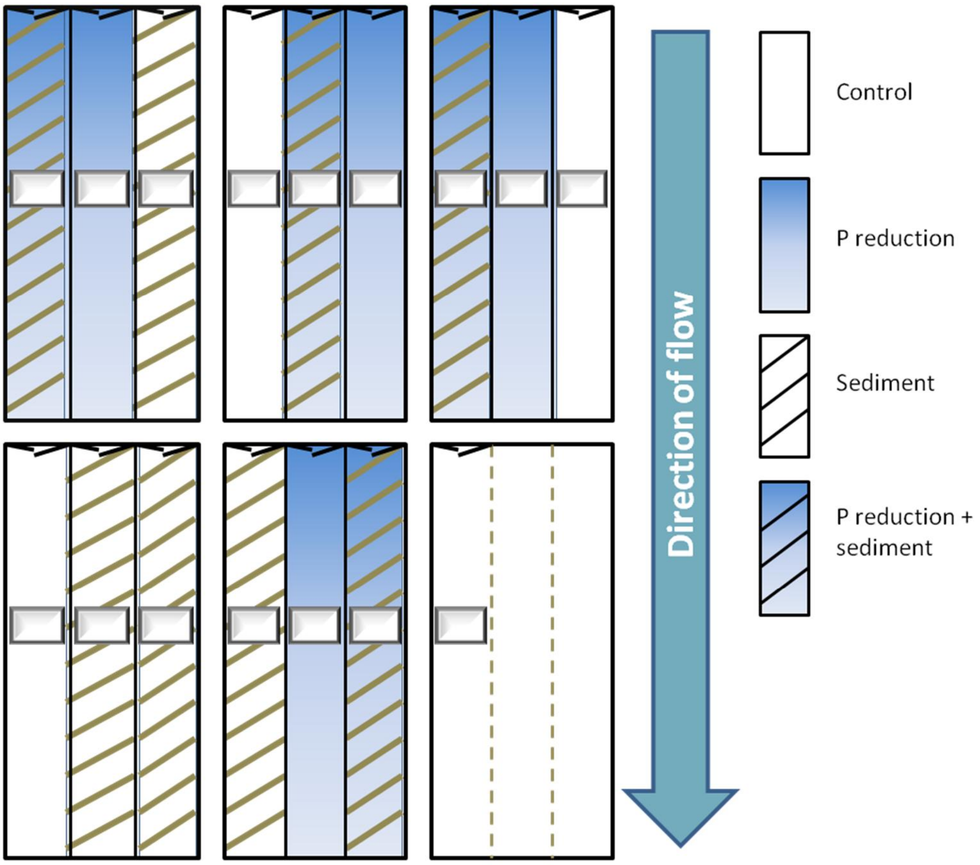


Figure 1.3.2. Diagram showing how the two treatments were assigned. Sumps are represented by the small rectangles and tiles were placed downstream of them.

Sampling and experimental procedure

Following installation of the flumes, but prior to the beginning of the experiment, it was necessary to alter the river water in some of the flumes to create the reduced phosphorus treatment. This was achieved using the P-stripping methodology developed by Bowes *et al.* (2007). The availability of phosphorus was lowered by introducing an iron chloride solution into each of the reduced P treatment flumes. The iron chloride solution consisted of 300 g of $FeCl_3$ dissolved in approximately 23 kg of distilled water. A peristaltic pump was used to continuously drip feed this solution into each of the relevant treatment flumes, immediately downstream of the flow control gates of the flumes. The iron reacts with the phosphate ions present in the incoming river water and rapidly forms an insoluble, non-bioavailable precipitate ($Fe_3(PO_4)_2$) (Reynolds and Davies, 2001). Initial investigations of the river had suggested that the ambient soluble reactive phosphorous (SRP) concentrations were *ca.* 100 µg l^{-1}. The phosphorus limiting concentration for the river was unknown, therefore, during the experiment the decision was made to try and lower the SRP of the reduced P treatment flumes to at least half the control SRP value (i.e. treatment of 50 µg l^{-1}). SRP is considered equivalent to bioavailable phosphorus.

SRP measurements were made twice a day from each flume using the following procedure. A syringe, rinsed in river water, was used to slowly collect 60mls of water flowing through the flume from the area directly above the sump. This water was then immediately filtered through a 0.45 µm cellulose nitrate membrane filter (WCN grade; Whatman Ltd., Maidstone, UK) and a 20 ml aliquot was taken and analysed within 30 minutes in the field for SRP concentration using a portable spectrophotometer (model DR2800; Hach Lange, Düsseldorf, Germany), and following the method of Murphy and Riley (1962), detailed below.

The filtered river samples were first inverted (to ensure they were thoroughly mixed) before the addition of 1ml of colour reagent (R1) and 1 ml of reducing agent (R2) by pipette. Batch quantities (500 mls) were made of each reagent in the laboratory prior to analysis in the field. When not in use they were stored in sealed containers in the fridge. Reagents were also added to four phosphate standards with concentrations of 0, 25, 50 and 100 µg P l^{-1}. Once the reagents had been added the samples were mixed via inversion and left to stand for 12 minutes. During this time the phosphate in the water reacts with acid-molybdate (R1) to form molybdo-phosphoric acid, a complex, which is then reduced to form a blue complex when it mixes with ascorbic acid (R2). The intensity of the blue colour was then measured spectrophotometrically. Each sample was transferred to a rinsed cuvette and the absorbance measured at a wavelength of 880 nm. A graph with linear regression was plotted of each standard concentration against the absorbance at 880 nm in order to quantify the SRP concentrations of the experimental samples.

Measurements of SRP were used to ensure the P concentrations in the reduced P treatment groups had been sufficiently lowered and were reasonably stable prior to the beginning of the experiment. Once suitably low P concentrations were achieved, the periphyton accumulation portion of the experiment was started. This involved placing 16 tiles in an evenly spaced distribution in each flume in the area downstream of the sump. Care was taken to avoid placing tiles too close to the end of the flume in case of disturbances in the water flow, and to avoid filamentous algae from one tile growing over the one adjacent one downstream. The sediment treatment was then applied to each of the relevant flumes.

Mixed sediment was taken from the bed of the river downstream of the experimental site prior to the beginning of the experiment. This sediment was then autoclaved to eliminate any microbial communities already living in the sediment that might have influenced subsequent periphyton growth and thus confound our results. Two Duran bottles containing roughly 1400 mls of autoclaved mixed sediment each were used per flume. River water was used to create a slurry of fine sediment that was then gently applied to the lower flume and tiles, taking care to avoid deposition of larger sediment material. The flow in the flumes was temporarily suspended by closing the flow gates during sediment addition to allow the sediment to settle. Each Duran bottle was mixed approximately five times to maximise the amount of fine sediment used. Application resulted in a complete coating of the tiles with fine sediment (approximately 3-4 mm thick) that is visible in Figure 1.3.3. Autoclaved fine sediment was retained for chlorophyll a analysis, for comparison.

During the experimental period, water samples were taken for SRP analysis twice a day. This allowed accurate monitoring of the P concentrations of the river and treatment water in the flumes so that appropriate alterations to the treatments could be made quickly when necessary.

Figure 1.3.3. Photograph showing two flumes at the beginning of the experiment, one of which has been treated with sediment.

The experiment began on the 30th of May 2014(day 1). On the 3rd June (day 5) three tiles were carefully removed from each flume to determine periphyton biomass by chlorophyll-*a* analysis. Tiles were placed into clearly labelled zip-lock bags and frozen at -20°C before being transported back to the laboratory for analysis. By day 5 a clear layer of periphyton had developed on some of the tiles, although it was not yet sloughing.

Sample analysis

Chlorophyll-*a* (Chl-*a*) analysis was performed, as in McCall *et al. (*2014), to estimate the periphyton biomass on the tiles for Chl-*a* analysis were defrosted in a dark room before being scrubbed with a toothbrush into a plastic tray. Deionised water was then used to thoroughly rinse the tiles, toothbrush and storage bags to ensure all of the material had been collected. The tray was agitated to evenly distribute the material, before the solution was poured into a 500 ml Duran bottle (Fischer Scientific, Loughborough, UK) and made up to 300 ml with deionised water. This suspension was then homogenised for ten minutes using a magnetic stirrer, before a 40 ml aliquot was taken and filtered through a 0.45 µm membrane glass fibre filter paper (GF/C grade; Whatman Ltd., Maidstone, UK), under vacuum. Each filter paper was placed in an individual vial with 20 ml of 90% (v/v) acetone overnight under refrigerator conditions (4°C), for chlorophyll-*a* extraction. The filter papers were then removed and the samples gently mixed and allowed to come to room temperature. Approximately three ml of each sample was transferred to a cuvette for analysis using a spectrophotometer (model DU520; Beckman Coulter, California, USA). The absorbance of each sample was measured at wavelengths of 630, 645, 665 and 750 nm (APHA, 2005), and the Chl-*a* concentration (µg cm^{-2}) of each sample was determined using equation 2.1 (APHA, 2005). Between each sample, the pipette and cuvette were rinsed thoroughly using 90 % acetone to prevent cross-contamination. Total tile area for each sample was also calculated to allow the total quantity of Chl-*a* on each tile to be back-calculated.

For each flume a second aliquot was taken from the same 300 ml homogenised periphyton suspension for dry mass and ash free dry mass analysis (AFDM). AFDM is a useful metric for measuring biomass as it is the mass of all organic material (algae, bacteria, small fauna and detritus) in the sample in the absence of silt, sediments and other inorganic components (APHA, 2005).The AFDM analysis followed the methods of McCall *et al.* (2014), in short a 40 ml sub-sample was filtered through ashed (500°C for 2 hr), pre-weighed GF/C grade glass microfibre filter papers (Whatman, Maidstone, UK). The filter paper was dried overnight at 105°C to constant mass, and then re-weighed once it had been cooled. The dry mass (DM) (mg cm^{-2}) was then calculated according to equation 2.2 (APHA, 2005). As with Chl-*a* concentration, the mass per tile was calculated by dividing the mass by the tile area within the sub-sample. Each filter paper was then placed into a muffle furnace (model AAF 1100; Carbolite Ltd., Hope, UK) and incinerated at 500°C for two hours to volatilise all organic material. The filter papers were then cooled and reweighed to determine the ashed mass (mg cm^{-2}) (equation 2.3 APHA, 2005). The AFDM was them calculated by subtracting ashed mass from dry mass, and used alongside the Chl-*a* results to calculate the Autotrophic Index (AI). The AI is simply the ratio of AFDM to Chl-*a* concentration and is used to determine the trophic nature of the periphyton community (APHA, 2005). Higher AI values tend to indicate heterotrophic dominated communities. However, the index can be affected by non-viable organic matter (detritus) (APHA, 2005), which increases the AFDM value, and so caution must be used when interpreting AI values.

Data analysis

Relationships between the sediment and reduced P treatments and Chl-*a* concentration and AFDM of the resulting periphyton were quantified using two-way analysis of variance (ANOVA) tests. Statistical analyses of Chl-*a* and AFDM data were based on the mean value of three tiles. All statistical analyses were carried out in Minitab® 16.0 Statistical Software. Results of the statistical tests were assessed against the standard *p* value of 0.05.

1.3.3 Results

Levels of nutrients in treated channels

The control P flumes had average SRP concentrations between 106 and 112 µg P L^{-1} over the course of the five day experiment, with an overall average of 109.78 µg L^{-1}. The average SRP concentrations in the reduced P treatment flumes were between 35 and 52 µg L^{-1}. This is means that the reduced P treatment flumes contained water with SRP concentration reduction of between 52 and 68%. Figure 1.3.4 shows how the SRP concentrations fluctuated during the experimental period for each of the flumes.

A two-way ANOVA was used to test if the P treatment and/or sediment treatment had made a significant difference to the average SRP concentration of each flume. Phosphorus treatment was found to significantly affect average SRP concentration (P <

0.0001), however sediment treatment did not significantly affect average SRP concentration (P = 0.192) (Table 1.3.1).

Table 1.3.1 Two way ANOVA results to test if SRP was significantly different between treatments.

Source	DF	SS	MS	F	P
Phosphorus	1	17401.4	17401.4	1123.71	0.0001
Sediment	1	29.6	29.6	1.91	0.192
Interaction	1	43.0	43.0	2.78	0.122
Error	12	185.8	15.5		
Total	1517659.8				

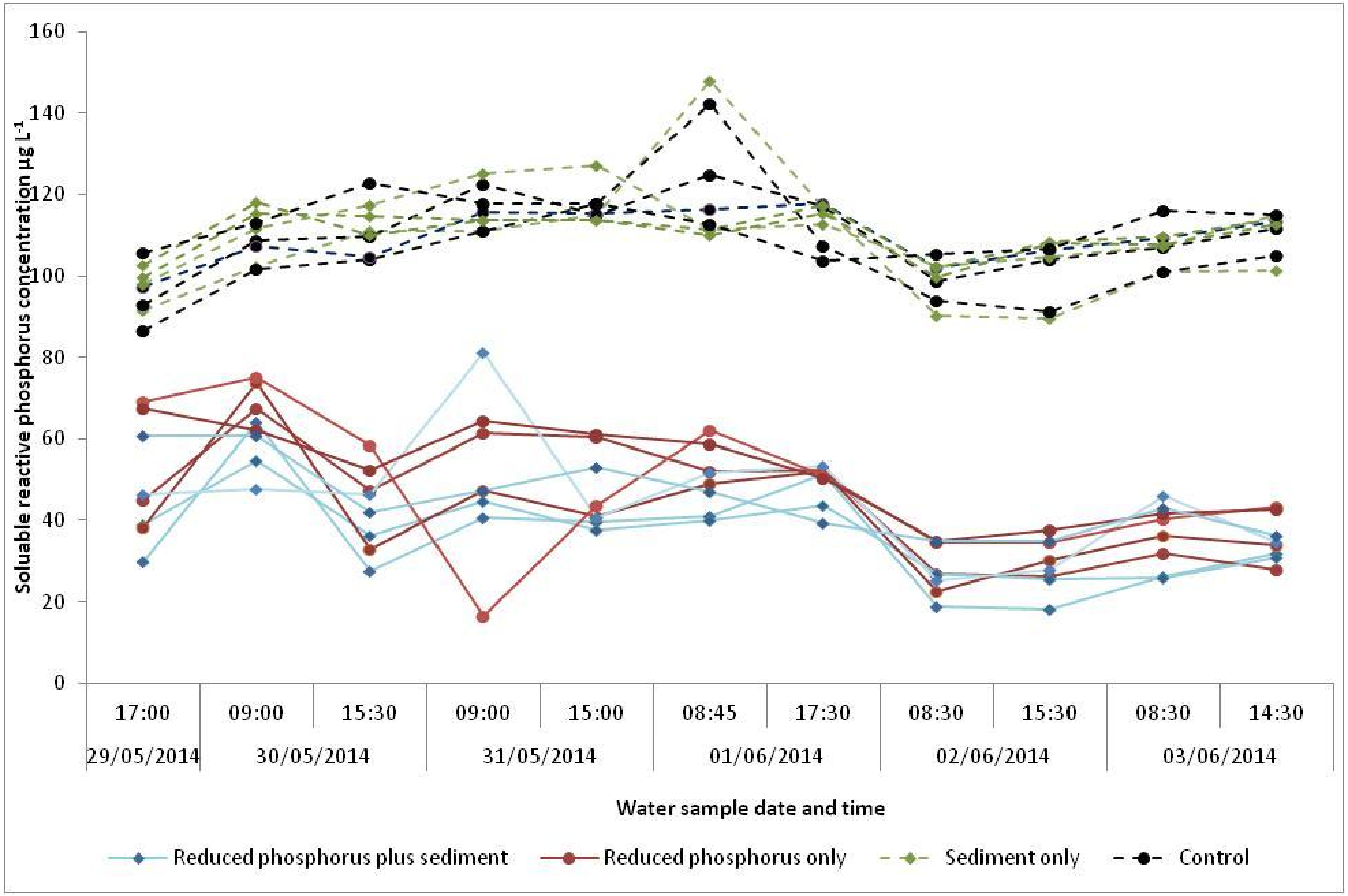


Figure 1.3.4. Soluble reactive phosphorus levels in the four treatments across the duration of the experiment.

Periphyton Chl-a response to treatment

A two-way ANOVA revealed that the Chl-*a* concentration of tile periphyton was significantly different between the control (background) and reduced SRP treatments, Table 1.3.2. The reduced P treatment tiles had lower Chl-*a* concentrations than the control flume tiles on average, but there was some overlap in the data range, which can be seen in Figure 1.3.5.

The sediment treatment was not found to significantly affect the Chl-*a* concentration after five days of experiment, (

Table 1.3.2 However, a significant interaction was found between the sediment and reduced P treatments. In the reduced P flumes which also had sediment, the Chl-*a* concentrations were lower on average than the concentrations in the flumes with reduced P only. Conversely, in the control flumes which had sediment added, the Chl-*a* concentrations were higher than (mean 6.91 µg cm^{-2}) in the control flumes without sediment (mean 5.31 µg cm^{-2}).

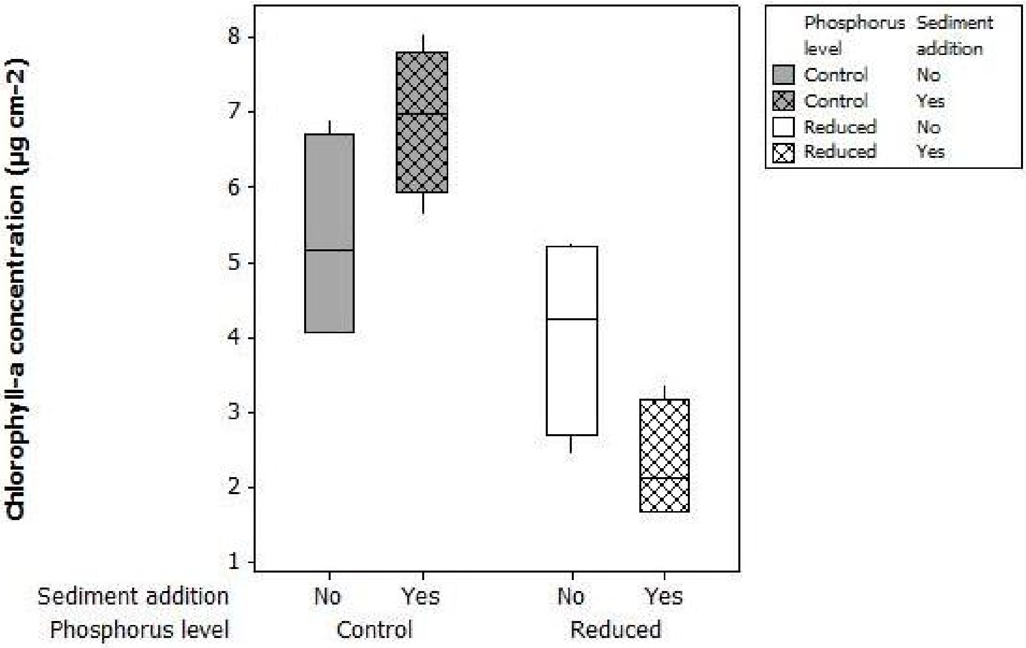


Figure 1.3.5. A boxplot of chlorophyll a values on day 5 of the experiment.

Table 1.3.2 Two Way ANOVA of chlorophyll a concentration by treatment.

Source	DF	SS	MS	F	P
P	1	34.2749	34.2749	25.01	0.000
Sediment	1	0.0184	0.0184	0.01	0.910
Interaction	1	11.0632	11.0632	8.07	0.015
Error	12	16.4431	1.3703		
Total	15	61.7996			

Table 1.3.3 Summary table showing the average (n=4) SRP, Chl-*a* concentration, AFDM and AI for each of the treatment groups. Chl-a, AFDM and AI scores are the averages of three tiles from each flume that are then used to create an average per treatment.

Treatment	Average SRP (µg L^{-1})	Average Chl-*a* concentration (µg cm^{-2})	Average AFDM (mg cm^{-2})	Average AI score
Reduced P plus sediment	39.69	2.32	9.80	3.80
Reduced P only	44.58	4.05	11.16	2.79
Sediment only	109.37	6.91	33.26	4.73
Control	110.93	5.31	16.16	2.96

Periphyton Ash Free Dry Mass- response to treatment

The control P with sediment treatment has a noticeably higher AFDM (33.26 mg cm^{-2}) compared to any of the other treatment groups (Figure 1.3.6). A two-way ANOVA found P treatment significantly affected AFDM, with lower AFDM levels found in the reduced P treatments (Table1.3.4). Sediment did not significantly affect the AFDM levels, however, the interaction term was found to be significant. This suggests that sediment may have an impact in combination with P levels. As with the Chl-*a* results, in the reduced P flumes the AFDM appears lower in those flumes with sediment, and in the control P flumes the AFDM is higher in those flumes with sediment.

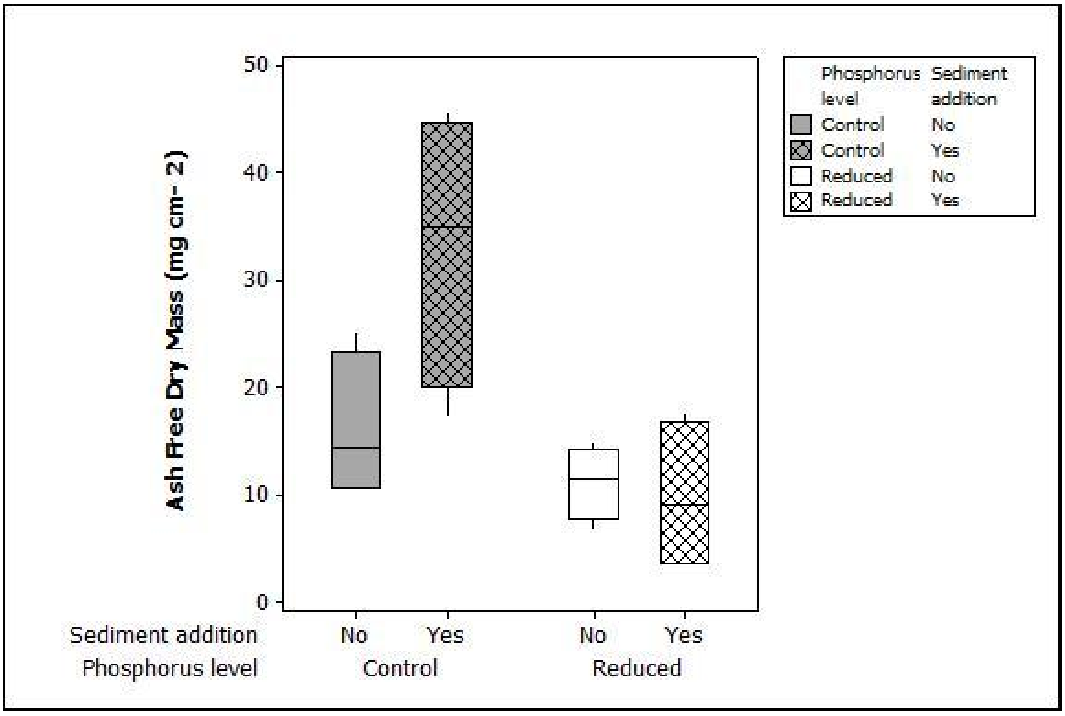


Figure 1.3.6 Periphyton response as ash free dry weight to the treatments.

Table 1.3.4. Two way ANOVA analysis with ash free dry weight as the response variable.

Source	DF	SS	MS	F	P
Phosphorus level	1	810.41	810.411	11.56	0.005
Sediment addition	1	247.56	247.565	3.53	0.085
Interaction	1	340.62	340.625	4.86	0.048
Error	12	841.05	70.087		
Total	15	2239.65			

1.3.4 Discussion

Phosphorus concentrations in this experiment were successfully manipulated to create treatment flumes with SRP concentrations of at least half that of the control flumes. Past experiments have shown that similar reductions in P loading can lead to improvements in river ecology (Kelly et al. 2009, Bowes et al. 2011). The findings from this investigation lend further support to this theory, as reducing the P concentrations was found to significantly reduce periphyton growth rate (as measured by Chl-a concentration and AFDM). Periphyton growing on tiles in the reduced P treatment flumes, was found to have a significantly lower Chl-a concentration that strongly suggests a restriction in algal growth due to P limitation. Furthermore, the AFDM was also significantly lower in reduce P flume periphyton implying a lowered organic content. Reducing the amount of P loading in rivers is seen as a key step in limiting freshwater eutrophication issues, including excessive algal growth (Bowes et al. 2012), in order to meet the ‘good ecological status’ criteria demanded by the European Union’s Water Framework Directive. This experiment provides support for the idea that P reduction can be used to limit algal growth in river systems, however, the P-limitation concentration is likely to be highly variable between different river systems (Bowes et al. 2012, McCall et al. 2014).

The impact of sediment on periphyton growth was much more muted than the response to SRP reduction. Fine sediment is known to have a detrimental impact on a number of biotic elements, including fish, macrophytes and invertebrates (Yamada and Nakamura 2002, Jones et al. 2014), however, much less is known about their impact on algae in river systems. A study by Yamada and Nakamura (2002) found that increases in fine sediment levels led to decreases in Chl-a and increases in Autotrophic index values, that suggested a decrease in living periphyton levels. However, in this experiment sediment treatment did not significantly impact on either Chl-a concentrations or AFDM, suggesting that algal growth was unaffected.

Both of these factors can have detrimental impacts on algal growth; however, during our experiment these processes may have been of relatively minor importance. Our sediment application, in place from the start of the experiment, formed a substrate rather than acting as a shade cover. However, all the channels were subject to some fine sediment deposition from the background load in the river but it produced a barely visible covering.

It is worth noting that some studies assume sediment deposition will select for motile species, however, this assumes that the sediment deposition is at such a rate that sessile species cannot grow over any deposit (Dickman et al. 2005, Jones et al. 2014). Evaluation of changes in community structure in groups such as the diatoms can be highly valuable in assessing the ecological status of a river, and should be considered in future experiments.

Although the sediment treatment did not significantly impact on either Chl-a concentration of AFDM on its own, significant interactions with the P treatment were reported for both analyses. This supports the suggestion that fine sediment has the potential to confound investigations of other stressors on periphyton in river environments, including P concentration (Jones et al. 2014). In this experiment the sediment appears to have different effects depending on the SRP concentration. Under reduced P conditions the sediment treatment tiles, have lower Chl-a and AFDM concentrations, whereas under control conditions the sediment treated tiles have increased Chl-a and AFDM concentrations. This is an interesting result, and clearly needs further study, as it has potential implications for management and other studies involving P reduction in river systems.

The use of within-river flume mesocosms for an experiment such as this provides the advantage of being able to study the impact of numerous stressors on periphyton simultaneously at a single location. Nutrient concentrations within the flumes can be controlled, maintained and measured simply and accurately without disturbing the experiment or causing any ecological damage to the river. Although this study only looked at two P treatments it is possible to produce a wide range of nutrient concentrations and study their impacts on periphyton. A gradient of concentrations has the potential to identify threshold concentrations, such as the P-limiting concentration that are vital for effective catchment management and nutrient target setting (McCall et al. 2014). The flumes also allow other factors that affect algal growth, for example, light, sediment, and flow to be largely controlled, or manipulated, as required. The flumes are portable and have minimal power requirements, which make them more flexible than other artificial stream facilities, and allows for their deployment at sites of particular scientific or environmental interest.

Freshwater eutrophication issues are widespread both in the UK and EU, and identification of factors which negatively impact on biological elements can help target management strategies in these systems to improve their ecological status. River systems are highly variable and so wide-ranging studies are needed to inform management decisions. However, at a local scale this study would suggest that in the River Nadder P reduction could be used to control periphyton growth via P limitation. This also suggests that the recent introduction of schemes designed to reduce P loading can be effective at reducing periphyton growth in rivers with high background SRP concentrations. There is clearly a need to consider not only how factors such as nutrient concentrations are impacting on biological elements, but also how they are interacting with other factors such as sediment, particularly, in light of increased human activities that are leading to more rapid delivery of large quantities of sediment to water bodies. Other abiotic variables known to affect periphyton growth, including flow regime, light intensity and food-web interactions also need to be studied. A better understanding how these factors interact and affect the biological elements of river ecosystems, should allow more effective management in the future.

2 Performance of existing indicators to diagnose hydomorphological stressors.

2.1 *A case study of the Regge and Dinkel catchment, The Netherlands.*

2.1.1 Introduction

In The Netherlands, regional water district managers conduct routine surveys of the flora and fauna inhabiting drainage ditch networks. Assessment of the ecological quality of these sampling locations is mainly based on national assessment and Water Framework Directive criteria. We refer to good ecological quality as a state of ecological integrity, in which all appropriate (a)biotic elements are present in the ecosystem and processes occur at appropriate rates (Angermeier and Karr, 1994), reflecting the conditions under which human influence is limited. Although sites with a good ecological quality as well as heavily degraded systems are easily discerned, both in terms of vegetation and macroinvertebrate assemblage composition, recognition of the patterns of loss of ecological integrity proved to be difficult.

To assess the ecological quality of a water body, a combination of components reflecting the structure and functioning of the ecosystem should be used (Karr and Chu, 1999; Barbour et al., 1999). By combining parameters providing information on different ecosystem features into a multimetric index, the ecological quality of a water body can be derived. In case of drainage ditches, both macroinvertebrates and macrophytes can potentially be used to develop such an index. In this study, we chose to focus on macroinvertebrates. We investigated if it was possible to identify a hydrologic and morphologic stressor gradient and assess the ecological quality along these gradients based on the ecology of the macroinvertebrates present. Aim of this study was to 1.) Identify one stressor gradient based on hydrologic stress and another stressor gradient based on morphologic stress, and analyse if hydrologic and morphologic stressors can be analysed separately. 2.) Identify indicators based on macroinvertebrate ecology traits that can reflect hydrologic and morphologic stressors. A large dataset collected by regional water district managers was used to evaluate the response of macroinvertebrate assemblages to hydrologic or morphologic stressor gradients. Based on their discriminatory efficiency with increasing hydrologic or morphologic stressors, we selected metrics that could respond to hydrologic and/or morphologic stressor gradients and studied whether these metrics could be useful as indicator tools for such stressors.

2.1.2 Methods

Data collection

In the Netherlands, streams are monitored on a regular base. Both abiotic and biotic parameters are monitored. The stream sampling of macroinvertebrates follows a standardized sampling procedure: a representative section of the stream (approximately 50 m) is selected. For this section the proportion of surface area covered by the habitat types present (e.g. sand, gravel, silt, emergent and submerged vegetation) is estimated. Subsequently, the different habitats are sampled in proportion to the surface area covered by each of the major habitat types using a pond net (mesh-size 500 µm, width 30 cm), up to a total length of 5 m. Samples are transported to the laboratory, where they are sieved using 1.0 mm and 500 µm sieves. Macroinvertebrates are sorted alive and identified to the lowest taxonomical level practical.

The data used for this study were collected from the Regge and Dinkel catchment between 1981 and 2009. In total, the macroinvertebrate dataset contained 69 sites, including spring areas, upper, middle and down stream areas, which had been sampled regularly. In the data analysis of this study, we only included sites at which macroinvertebrates were assessed more than once and environmental data were available, containing information on the parameters pH, temperature, nitrate, ammonium, total nitrogen, orthophosphate, total phosphate, sulfate, dissolved oxygen, biochemical oxygen demand, chlorofyl-a, turbidity, depth, water transparency, and conductivity.

The macroinvertebrate datasets were combined into a single database. Inconsistencies in data formats and scientific nomenclature were resolved. The resulting taxonlist showed considerable taxonomic overlap, mainly as a result of difficulties identifying early instar specimens. Since it was unknown if a specimen identified to, for example, genus level in one sample was actually an early instar of a species recorded in another sample a taxonomic adjustment procedure was necessary to avoid multiplication of the same information during analysis (Nijboer & Verdonschot, 2000; Schmidt-Kloiber & Nijboer, 2004; Vlek et al., 2004). When specimens were identified to species (lowest taxonomic level), apart from a few exceptions, which were only identified to genus or family (higher taxonomic levels), the higher taxonomic levels were omitted and the lowest taxonomic level was kept. When specimens identified to genus or family level were abundant compared to the specimens identified to species level (frequency of occurrence >20% of all the species belonging to this genus or family), the lower taxonomic level(s) were aggregated to the higher taxonomic level.

Hydrology stressor gradient

Data on the hydrologic status of Regge and Dinkel sites were needed to identify a hydrologic stressor gradient corresponding to the sites included in the macroinvertebrate and environmental dataset. We used two sources of information on the hydrologic status of the Regge and Dinkel catchment: 1) Hydrologic scores of Regge and Dinkel water bodies as reported by the water manager (Knol et al. 2009), which were determined using the Dutch Water Framework Directive index (WFDi) method (STOWA 2012), and 2) Hydrologic study of Twente (Heydeman & Van ‘t Oever, 1975). The hydrology status scores were expressed in hydrology quality classes, ranging from bad (score 1) to high (score 5). The scores for hydrology were connected to the macroinvertebrate dataset.

Samples from locations not included in WFDi quality descriptions, were assessed for hydrology status using the qualification by Van der Hoek et al. (2003). Of samples that were included in both quality determination methods, scores were compared. We found that quality scores given by the two methods resulted in a comparable quality class.

Morphology stressor gradient

The morphologic status of the sites included in the dataset was based on 1) the morphologic survey study of the Regge and Dinkel catchment performed by van der Hoek et al. (2003) and 2) on morphologic scores of Regge and Dinkel water bodies on morphologic status as reported by the water manager (Knol et al. 2009), which were determined using the Dutch Water Framework Directive index (WFDi) method (STOWA 2012). The morphology status scores were expressed in hydrology quality classes, ranging from bad (score 1) to high (score 5). The scores for morphology were connected to the macroinvertebrate dataset. Samples from locations not included in WFDi quality descriptions, were assessed for morphology status using the qualification by Van der Hoek et al. (2003). Of samples that were included in both quality determination methods, scores were compared. We found that quality scores given by the two methods resulted in a comparable quality class.

Hydrology and morphology gradient analysis

The hydrology gradient and morphology gradient did not have comparable class distributions (Figure 2.1.1). From the data with an insufficient quality score (classes 1 and 2), the morphology gradient contained more samples from locations with a bad morphology, whereas the hydrology gradient contained more data from locations with a poor quality score. There were more samples from moderate morphology locations than from moderate hydrology locations. Both gradients contained equal numbers of samples from good quality locations (class 4 and 5). The samples that scored class 5 for morphology were all located in spring areas. The hydrology and morphology gradients are not fully independent of each other as most of the locations that score insufficient for morphology also score insufficient for hydrology, whereas locations with good morphology also have a good hydrology (matrix in table 2.1.1). Morphology and hydrology interfere in the Regge and Dinkel catchment.

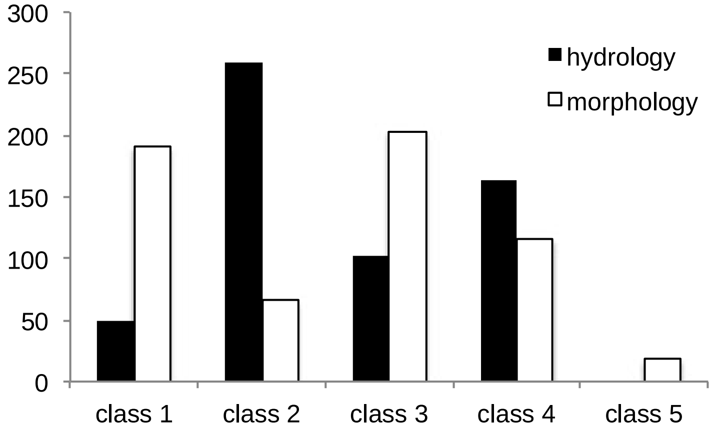


Figure 2.1.1. Class distribution of the morphology and hydrology gradients.

Regge and Dinkel macroinvertebrate dataset contained more data with a combined insufficient score for morphology and hydrology (n=213, red cells in table 2.1.1) than data with a combined good score for morphology and hydrology (n=97, green cells in table 2.1.1). The yellow cells in table 2.1.1 refer to locations that score well on one of the two gradients, but insufficient on the other. These samples (n=103, of which 67 morphologically stressed and 36 hydrologically stressed) are interesting when studying the separate effects of morphology and hydrology on macroinvertebrates.

Table2.1.1. Morphology versus hydrology class distribution across the macroinvertebrate dataset.

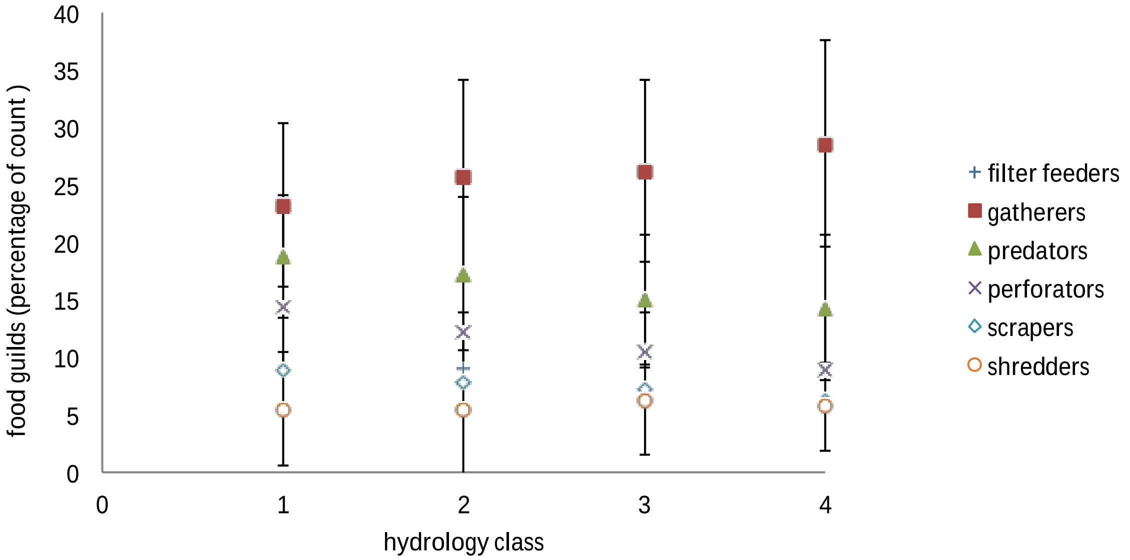
morfology	hydrology					
	n/a	class 1	class 2	class 3	class 4	class 5
n/a	0	0	3	0	0	0
class 1	0	49	95	46	0	0
class 2	0	0	66	0	0	0
class 3	21	0	73	42	67	0
class 4	0	0	22	14	79	0
class 5	0	0	0	0	18	0

Metric selection

Metrics derived from the autoecological information collected for the AQEM assessment program (Hering et al., 2004), environment and habitat preferences of Dutch macroinvertebrates (Verberk et al., 2012), and river zonation preferences (Hering et al. 2014, AQEM online dataset) were calculated from the macroinvertebrate data. The selected metrics were based on functional characteristics and habitat preference, comprising functional feeding group, locomotion characteristics, river zonation preference and microhabitat (substrate) preference. A tolerance/sensitivity metric based on taxa and individuals indicating the tolerance for saproby was also selected. All metrics within the functional and habitat preference category and tolerance/sensitivity category were based on fuzzy-coded data (10 points distributed per taxon over the relevant classes).

2.1.3 Results and discussion

Metrics based on food guilds showed comparable responses to morphology stress and hydrology stress. For both gradients, no significant differences were observed for macroinvertebrate food guild distributions (Figure 2.1.2).



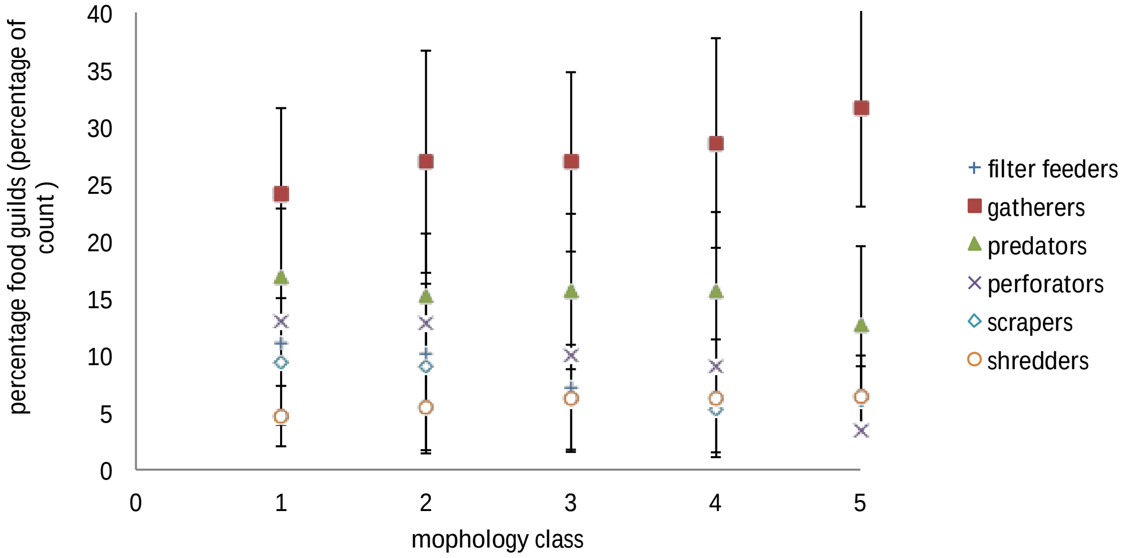


Figure 2.1.2: food guild distributions across the hydrology and morphology stressor gradient in mean±SD. Class 1: most stress, class 4,5: least stressed locations.

Neither did metrics based on locomotion charateristics show any significant differences across the stressor gradients (Figure 2.1.3).

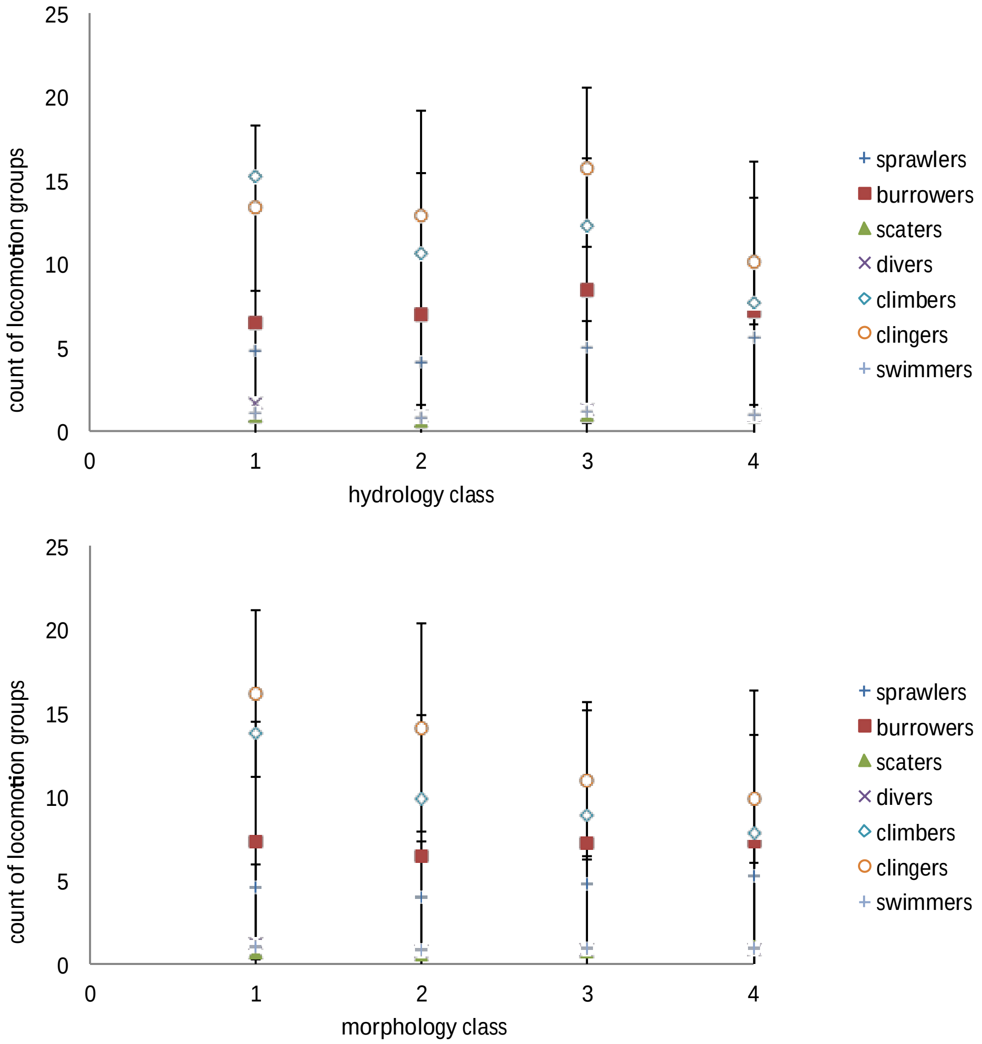


Figure 2.1.3. Distributions of locomotion characteristics across the hydrology and morphology stressor gradient in mean ±SD. Class 1: most stress, class 4,5: least stressed locations.

Metrics based on rheophilic species, relative or absolute numbers, did also not differ along the stressor gradients (Figure 2.1.4). Although a decrease in the relative amount of rheophilic species was observed for the lesser-stressed samples, the absolute number of rheophilic species did not confirm this pattern. Probably, there are more species present at the lesser-stressed sites, resulting in a higher biodiversity. Hydrologic stress as well as morphologic stress could result in a less diverse habitat, with a more uniformed streambed containing less varying adjacent habitat.

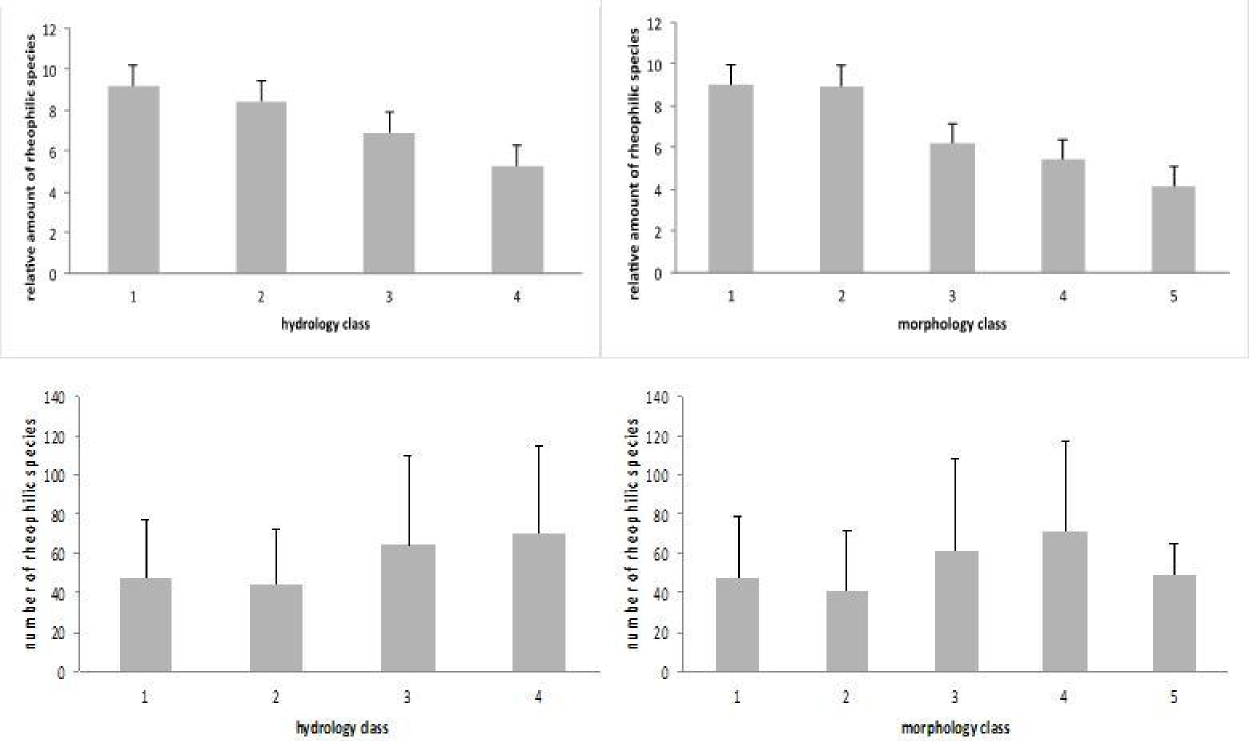
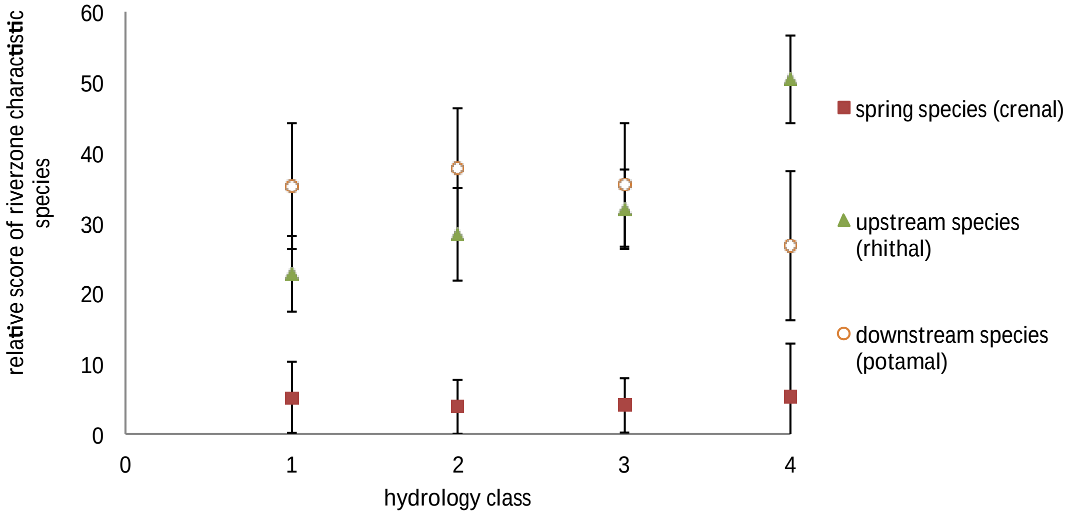


Figure 2.1.4. Relative abundances of rheophilic species, and absolute abundance (number of rheophilic species) across the hydrology and morphology stressor gradient in mean±SD. Class 1: most stress, class 4,5: least stressed locations.

However, metrics based on characteristic river zone macroinvertebrates did show a pattern in the response of macroinvertebrates to hydrologic and morphologic stress (Figure 2.1.5). Locations that are not under hydrologic stress are dominated by upstream species, whereas downstream species are more abundant in locations that are under hydrologic stress. The same pattern is found for the morphology stressor gradient.



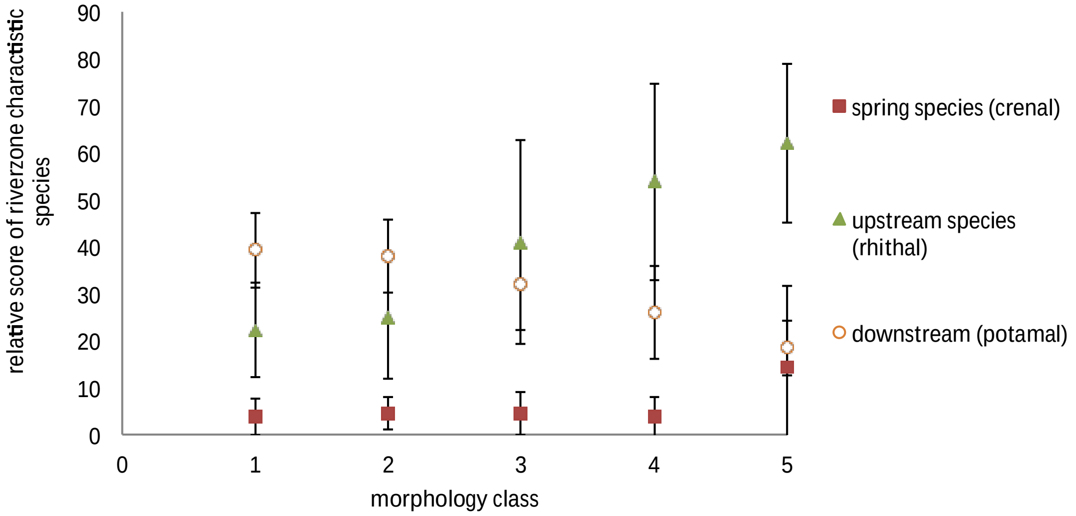


Figure 2.1.5. Distributions of riverzone characteristic species across the hydrology and morphology stressor gradient in mean±SD. Class 1: most stress, class 4,5: least stressed locations.

To explore how this metric responds to hydrologic and morphologic stress, we compared the best locations in the dataset (green cells in table 2.1.1) to the worst locations in the dataset (red cells in table 2.1.1) (Figure 2.1.6). The best sites contained significantly more upstream species than downstream species, but the stressed sites were dominated by downstream species.

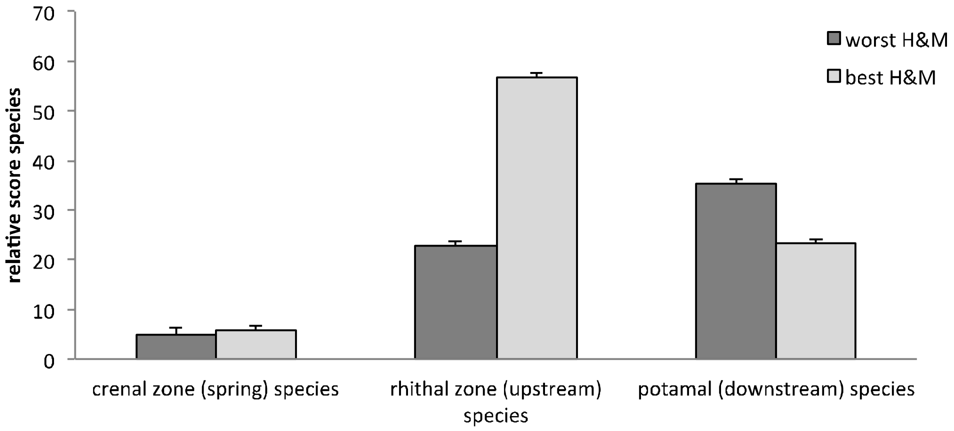


Figure 2.1.6. Comparing the best sites to the worst sites in terms of both hydrologic and morphologic stress, using mean relative abundances of river zone characteristic species±SD.

In the Regge and Dinkel catchment, however, the least stressed sites are located upstream, whereas both morphologic and hydrologic stress increase downstream. This might be a bias in the method, resulting of using catchment data in which the stressor gradient is geographically distributed with the flow direction. In the Dutch multistressed catchments, stress caused by disturbances in morphology and/or hydrology are difficult to separate. We have made an attempt to do so, by comparing the samples that are either hydrologically stressed and morphologically intact with sites that are morphologically altered and hydrologically functioning (the yellow cells in table 1). Both types of stressors still contained significantly more upstream characteristic species than downstream species (Figure 2.1.7).

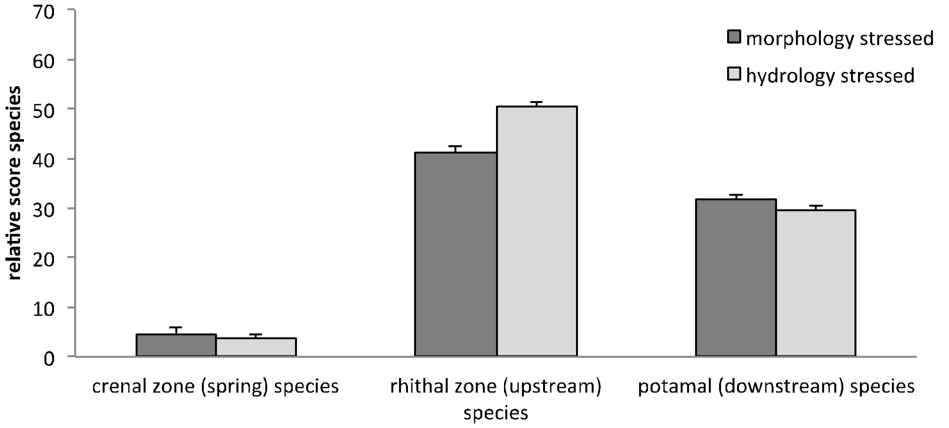


Figure 2.1.7. Comparing sites under either hydrologic or morphologic stress, using mean relative abundances of river zone characteristic species ±SD.

The hydrologically stressed samples contained the highest amount of upstream species. This indicates that when sites are either stressed in hydrology or morphology, they still are in relatively good condition compared to sites that suffer from both hydrologic and morphologic stress. However, seen the geographic bias in our catchment area, we should need to test this hypothesis using data from a catchment in which the morphologic and hydrologic stressor gradients are not following the geographical upstream to downstream pattern.

2.2 *Danish Stream Plant Index and its ability to capture impacts in Danish streams*

2.2.1 Introduction

Macrophytes are found in all types of Danish streams, from the smallest brooks to the major rivers such as the River Gudenå and the River Skjern. Their widespread occurrence reflect that habitat conditions are suitable for plant growth i.e. the streams are generally small and shallow (>95% of the stream reaches are less than 10 m wide and 1 m deep), they have low slopes (<0.005 m m-1), low water velocities (<0.8 m s-1) and sediments dominated by clay, silt and sand suitable for root attachment. In addition, light penetration is generally high because most streams are located in open areas without shading from trees and bushes, primarily due to agricultural land use in adjacent areas. Additionally, the supply of nutrients (nitrogen and phosphorus) is typically ample via both the water and the stream bottom from where they can be taken up.

In the streams three main groups of plants are found: (1) submerged plants, (2) amphibian plants and (3) terrestrial plants that can be found growing in water. All submerged plants species are adapted to life under water, and they are only rarely observed above water. Most of them are rooted in the stream bottom, but some flow freely around. The amphibian plants, which comprise several species, can live both on land and in water. In many cases the water forms grow out of the water during the growing season. Flowering and seed setting typically occur only for the terrestrial plant forms. The third group of plants in streams grows mainly on land but may also occur under the water. Some species are, however, rarely observed under water, while other species do it relatively often. This implies a gradual transition from the terrestrial plants that thrive in water to the amphibian plants and the submerged plants. In terms of number, most species belong to the group of amphibian plants and terrestrial plants, but the true aquatic plants and the amphibian plants are those having the largest coverage in the streams.

In addition to contributing significantly to biological diversity (biodiversity), stream plants have important functions. Via their growth they can change the water flow and in this way create channels with especially fast flow or standing water where fine-grained mineral or organic particles can be deposited. Thereby the plants provide physical structures, both vertically and horizontally in the stream channel and habitats for other organisms such as small animals (macroinvertebrates) and fish. In addition, the macroinvertebrates graze on microorganisms (bacteria, hyphomycetes and epiphytic algae) that are growing on the plants, and some species directly eat the leaves of certain aquatic plants. The plants also contribute to the turnover of nutrients. Together with their attached microalgae, they remove significant amount of nutrients transported through the streams.

Species composition in streams are controlled by a number of factors both natural environmental conditions and anthropogenic impacts. Stream size, light (penetration, attenuation) and alkalinity are factors exerting a strong influence on species composition. Certain species are typically found in small streams such as brooks, while other species virtually only occur in large streams, reflecting variations in the range of habitats and their suitability for different species. Light is an absolutely necessary prerequisite for plant photosynthesis. If the light penetration is low, only mosses survive.

Light penetration is inhibited by shading of riparian vegetation and, for instance, by high concentrations of suspended matter in the stream water (periodically found in certain streams in clayey soils). Stream water alkalinity is a very important factor as well, because CO_2 and HCO_3 are important carbon sources for plant photosynthesis. Alkalinity in Danish streams varies from below 0.05 (in the northern part of Western Jutland) to almost 10 meq/L (on Zealand and Lolland-Falster). A number of plant species are particularly frequently in streams with low-alkaline water, as is the case in the northern part of Western Jutland. These species include, for instance, *Myriophyllum alterniflorum*, *Potamogeton natans* and *Glyceria maxima* (Riis et al., 2000), while the coverage of other species is largely limited to the low-alkaline streams in Western Jutland (*Oenantha fluviatilis*, *Luronium natans*, *Callitriche hamulata*, *Juncus bulbosus*), see, for instance, Moeslund et al. 1990).

Stream plants are - and have for several decades been - influenced by a number of different human-induced impacts. Thus, the natural course of most Danish rivers has to some extent been changed through straightening of sinuous natural curves, excavation of the bottom and construction of dams for water mill operation and meadow irrigation. Drainage has typically affected stream catchments and has partly led to faster runoff and partly to local (large parts of Jutland) leaching of acid, iron-rich water, causing precipitation of ochre. In addition, the plants have routinely been removed by cutting or excavation, a still ongoing practice. The objective is to improve drainage of the cultivated or urban areas adjacent to the streams. Finally, farming and the discharge of wastewater from towns and rural settlements have resulted in increased input of nitrogen and phosphorus. Thus, the stream water content of dissolved phosphorus varies between 0.002 to locally almost 5 mg/L, while the content of dissolved nitrogen can vary from 0.005 to more than 30 mg/L (Wiberg-Larsen et al.
2012).

Stream plants as environmental indicators

Stream plants have a relatively short history as environmental indicators. This may seem surprising because they like other groups of organisms such as microalgae, macroinvertebrates and fish are affected by various anthropogenic impacts. We generally consider increased nutrient concentrations, changes in hydrological and morphological conditions as well as weed cutting and excavation in streams as the most important impacts affecting plant communities in Danish streams (Baattrup-Pedersen et al. 2004).

As part of the implementation of the EU's Water Framework Directive, a Danish Stream Plant Index has been developed (see Baattrup-Pedersen & Larsen 2013; Baattrup-Pedersen, Larsen & Riis, 2013; Søndergaard et al. 2013). The index, DVPI, is determined using a prediction model that directly classifies a stream to an environmental category with an accompanying EQR value from the list of existing plant species and their coverage.

DVPI was intercalibrated in 2011 (Birk et al. 2011). The intercalibration exercise included countries from the Central-Baltic GIG (Geographical Intercalibration Group), which in addition to Denmark comprises the Netherlands, Belgium, Germany, Poland, Estonia, Latvia, Lithuania and parts of Sweden, the UK and France. This work has, among other things, included a harmonisation of the individual countries' methods for ecological classification and has, in the case of Denmark, resulted in an adaptation of the originally proposed methods and of the thresholds between environmental categories (Søndergaard et al.

Within the Central-Baltic GIG, a total of three streams types are defined (Anonymous 2013), of which R-C1 ("Sandy lowland brooks") and R-C4 (“Medium-sized lowland streams") correspond to Danish type 2 and 3 streams, i.e. streams with catchment areas greater than 10 km^2 (see Table 2.2.1; Baattrup-Pedersen et al. 2004). The Danish type 2 and 3 streams have a bottom width of 2-10 m and >10 m and a catchment area of 10-100 km2 and >100 km^2, respectively. The Danish type 1 streams, width<2 m are, thus, not part of the Central-Baltic stream types.

Table 2.2.1. Characteristics of the three stream types included in the intercalibration of plant indices of the Central-Baltic Intercalibration Group (CB-GIG); see table 3 in Anonymous (2013).

Common IC type	Type characteristics
Sandy lowland brooks (R-C1)	Catchment area:10 - 100 km^2 Altitude: <200 m Geology: siliceous Channel substrate: sand Alkalinity: >1 meq/l
Siliceous mountain brooks (R-C3)	Catchment area: 10 - 100 km^2 Altitude: 200 - 800 m Geology: siliceous Channel substrate: boulders, cobble and gravel Alkalinity: <0.4 meq/l
Medium-sized lowland streams (R-C4)	Catchment area: 100 - 1,000 km^2 Altitude: <200 m Geology: mixed Channel substrate: gravel and sand Alkalinity: >2 meq/l

Purpose of the present study

It was a prerequisite for the DVPI intercalibration that the index was sensitive to changes in the streams’ degree of eutrophication, similar to the other EU countries (Birk & Willby 2010, Birk & Willby 2011). As part of the intercalibration process it was demonstrated that DVPI responds negatively to (1) higher concentrations of dissolved phosphorus in Danish streams and to (2) increased productivity in the streams expressed as a weighted average of Ellenberg N based on the plants’ indicator values for nutrients and the frequency of the species (Ellenberg et al. 1991; Birk et al. 2011).

Here we analyse if DVPI is sensitive to physical disturbances (weed cutting) and stream morphological modifications in addition to eutrophication. For the purpose of REFORM we have concentrated on type 2 and 3 streams and only initially included type 1 streams that are not part of the Central-Baltic stream types (width <2m).

2.2.2 Methods and results

Selection of data sets and environmental parameters

Plant data

A data set of plant lists collected via NOVANA in the period 2012-2013 was used as basis. The reason for the choice of this particular period is that some significant changes were made in 2012 to the existing method for collection of field data. In this way, the registration of plants in large, deep streams has become more optimal by use of boat (whereby the whole cross section of a stream can be investigated compared to the situation before where registration could only be made in a relatively narrow, shallow riparian zone). In addition, it has been attempted to make the description of shading of the investigated sections more objective and reproducible. The procedure for collection of data is described in Wiberg-Larsen & Baattrup-Pedersen (2013).

In total, 310 plant lists were available from 115 type 1 streams and 163 type 2 and 3 streams. Note that these plan lists were not part of the plant lists used to develop DVPI (Baattrup-Pedersen et al. 2013).

Physico-chemical environmental variables

Several data were used to characterize physic-chemical conditions of the streams:

- Disturbances caused by stream maintenance: This is described by the share of cross sections with weed cutting, the number of years in which cutting has been performed and the cutting intensity calculated by means of simple multiplication of the two factors (Box 1). These data were provided by the Danish municipalities in connection with the project.

Box 1. Stream maintenance

Share of stream cross sections where weed cutting has been performed: Weed cutting is performed within a limit of 0-100% of the cross section. The percentages have been translated into a frequency scale of 0-5 via division by 20. For private streams (typically small) where weed cutting is supposedly performed but the method is unknown, the share is set to 100%. Weed cutting is typically performed in a stream channel, which means that the location of the plants relative to that of the stream channel decides the size of the impact. Some plants are found along the stream bank where cutting is typically not performed, whereas the plants in the middle of the stream are particularly vulnerable. It is not possible to take this into account. In some cases weed cutting is performed at different widths depending on whether the cutting takes place once or twice. If this is the case, an average of the cut extent has been used.

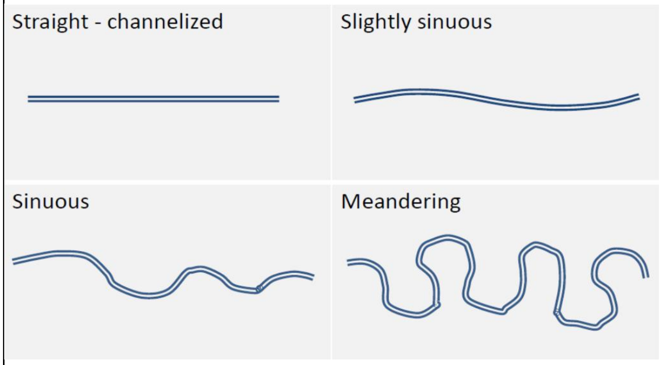
Weed cutting frequency: A scale of 0, 1, 2, 3 etc. is used, corresponding to the number of annual weed cutting events. In some cases 0-1, 1-2 or 2-3 cutting events are recorded, i.e. the number of events varies from one year to the next. If this is the case, intermediate values are given (0.5 - 1.5 - 2.5 - etc.). For private, supposedly maintained, streams, the frequency is set to 1.

- Physical conditions in the streams: These are described in the Danish Physical Index (Wiberg-Larsen 2013), converted to EQR values (Ecological Quality Ratio, scale 0-1) based on the formula: DFI+12/ (12 +54) and sub-elements of this that describe the degree of naturalness/regulation of the stream channel. The first element is stream sinuosity, which can have the values 0-3 (see Box 2). The second element is the stream profile, which can also have the values 0-3 (see Box 2). Finally, width and slope were used as physical parameters. All data were collected via NOVANA.

Box 2. Physical conditions: Sinuosity and cross-sectional profile

Sinuosity:

0	Straight-channelised	(SI < 1.05)
1	Weakly sinuous	(1.05 < SI < 1.25)
2	Sinuous	(1.25 < SI < 1.50)
3	Meandering	(SI > 1.50)



The assessment is made either visually in the field using the figure or up-to-date aerial photos (e.g., Google Earth). In the latter case, the index values (SI: Sinuousity Index) calculated as the actual length of the section divided by the length of the straight line between the start and the end of the section.

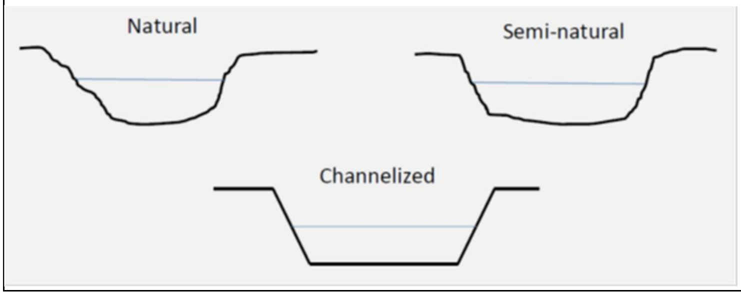
Cross-sectional profile:

0 The cross section is clearly rectangular and channelised - no discernible variation in water flow and substrate across the stream.

1 Semi-natural (deep) - overall rectangular cross section with clear signs of previous channelisation. Banks are typically long and sloping and covered with different types of grasses. A secondary path of flow has been created within the excavated channel with variations in flow, depth and substrate conditions. The stream is far (more than 1 m) below ground level and does not have good hydrological contact with the riparian zone.

2 Semi-natural (not deep) - signs of previous channelisation. The stream is not markedly excavated below ground level. There is no hydrological contact with the surrounding areas.

3 Natural - no clear signs of channelisation, natural contact between stream and riparian area.



Water chemical conditions: The content of plant nutrients is described by the concentrations of dissolved phosphorus (PO_4-P) and dissolved nitrogen (NH_3+NH_4-N), and the availability of carbon is described via total alkalinity. For each station a mean value is calculated based on all the available measurements from NOVANA in the period 2004-2012.

- Water chemical conditions: The content of plant nutrients is described by the concentrations of dissolved phosphorus (PO4-P) and dissolved nitrogen (N3+NH4-N), and the availability of carbon is described via total alkalinity. For each station a mean value is calculated based on all the available measurements from NOVANA in the period 2004-2012.
- Land use in the riparian zone: Farming in the riparian zone - here defined to 50 m along the stream on both sides of the 100 m long study reach - will, together with slope, influence the physical conditions of the stream and the extent of maintenance, and there will also be a nutrient supply. Similarly, paved areas have an influence on the physical conditions of the stream and on the maintenance. To illustrate this, the total contribution of "cultural impact" in the riparian zone (agriculture + paved areas) has been calculated. AIS data were applied in the calculations.
- Shading by high herbs, trees and bushes along the streams significantly impacts stream plant communities. The shading depends on the stream’s profile and width, the density and height of herbs, trees and bushes along the stream, the distance of the shading elements to the stream and the orientation of the stream relative to the corners of the world (and the sun path). In the analyses performed here, a shading index was calculated based on the density of trees/bushes in, respectively, a 0-2 and a 2-5 (10) m riparian zone along each bank as well as the average height of these trees/bushes within the two zones (Wiberg-Larsen & Baattrup-Pedersen 2013). The index does not take into consideration other elements of relevance to the shading of the stream vegetation, including shading from the banks which especially depends on the excavation depth of the stream.

Calculations & statistics

$DVPI_{EQR}$ values were calculated using a prediction model. This model was developed on the basis of more than 1,200 plant lists showing occurrence frequencies for the species present (Baattrup-Pedersen & Larsen 2013; Baattrup-Pedersen, Larsen & Riis, 2013; Søndergaard et al. 2013). From a given species composition of plants, the model calculates the probability for a stream to belong to a certain ecological state category. This probability is used to calculate EQR (EQR = Ecological Quality Ratio), which can assume a value on a scale from 0 to 1. The calculations were done in the software program S-Plus. $DVPI_{EQR}$ was only calculated for plant species with a total coverage of >2%, which is specified as a criterion for the use of DVPI (Baattrup-Pedersen & Larsen 2013).

The relationships between the applied environmental variables were investigated using Principal Components Analysis (PCA). Initially, an analysis was conducted of all variables (N = 18) followed by an analysis of a reduced number of variables (N = 9) after exclusion of a number of strongly intercorrelated variables that in reality reflect the same impact. The analyses were performed in PC-ORD 6.0.

The correlation between $DVPI_{EQR}$ and selected environmental variables was examined by multiple regression. We chose this approach because Danish streams are affected by a number of simultaneously acting stressors, which means that it is relevant to include several factors at the same time in the analyses

2.2.3 Results and discussion

Interactions between environmental variables

In an initial PCA (results not shown graphically) of all the included environmental variables (N= 18), the first 3 axes explained 66.7% of the variation; axis 1 (PCA1) alone explained 43.9%. All weed cutting parameters (number of cuttings, share of cut cross-sectional area and cutting intensity - for the periods 2012-2013 and 2007-2011, respectively) were strongly positively correlated with PCA1 (r>0.80), while stream physical state (sinuosity, cross-sectional profile and Physical Index) was strongly negatively correlated with this axis (r>0.57). The physical condition of the streams was also strongly negatively correlated with PCA2 (r>0.61), while the cultural impact within the 50 m wide riparian zone was strongly positively correlated with this axis (r=0.58). Several of the variables were inter-correlated and to exclude the correlation between parameters expressing the same thing, a PCA was undertaken using only one variable to characterise the weed cutting (number of weed cuttings in the years 2012-2013) and physical state (cross-sectional profile). In this PCA with 9 variables, the first 3 axes explained 56.3% of the variation, PCA1 accounting for 25.9%, PCA2 for 18.5% and PCA3 for 11.9%. However, only PCA1 and PCA 2 were significant axes. The result shown in Figure 2.2.1 is based on both small (type 1), middle-sized (type 2) and large (type 3) streams.

The physical state of the streams (r=-0.76) and shading from tree vegetation (r=-0.54) were strongly negatively correlated with PCA1, while alkalinity (r=0.59) and the proportion of cultural impact within the 50 m wide riparian zone (r=0.64) were strongly positively correlated with this axis. PCA1 thus represents the physical conditions of the streams: At one end of the axis are the least physically impacted streams, which are also those most shaded by riparian trees/forest and with a simultaneously small degree of cultural impact in the riparian zone; at the opposite end of the axis are the most regulated streams, which are completely open to natural light with minimal shading by trees and with a simultaneously high degree of cultural impact. Alkalinity is probably positively correlated with the cultural impacts as the alkalinity is highest in eastern Denmark where the lime content of the soil is highest, while the opposite is the case in Western Jutland.

Weed cutting (r=0.51) and width (r=0.56) were strongly positively correlated with PCA2, while slope (r=-0.73) and dissolved-N (r=-0.50) were strongly negatively correlated with this axis. PCA2 can be interpreted as an axis which primarily represents physical disturbance combined with stream size.
Thus, there is a natural strongly negative correlation between stream size and slope - the small streams typically have a high slope, while the large rivers always have a relatively low slope. And while weed cutting (or other type of maintenance) is nearly always performed in the large-sized streams, a relatively high number of small streams are not maintained, particularly streams with high slope.

The result from the PCA reveals that the plant composition in streams and thereby $DVPI_{EQR}$ is affected by many simultaneously acting factors and that the importance of the individual factors varies from stream site to stream site. For this reason, a low $DVPI_{EQR}$ value could reflect a high level of eutrophication in some streams, whereas in others a low $DVPI_{EQR}$ value could reflect a high degree of disturbance. A low $DVPI_{EQR}$ value could also reflect that there is a simultaneously high eutrophication level and high degree of disturbance.

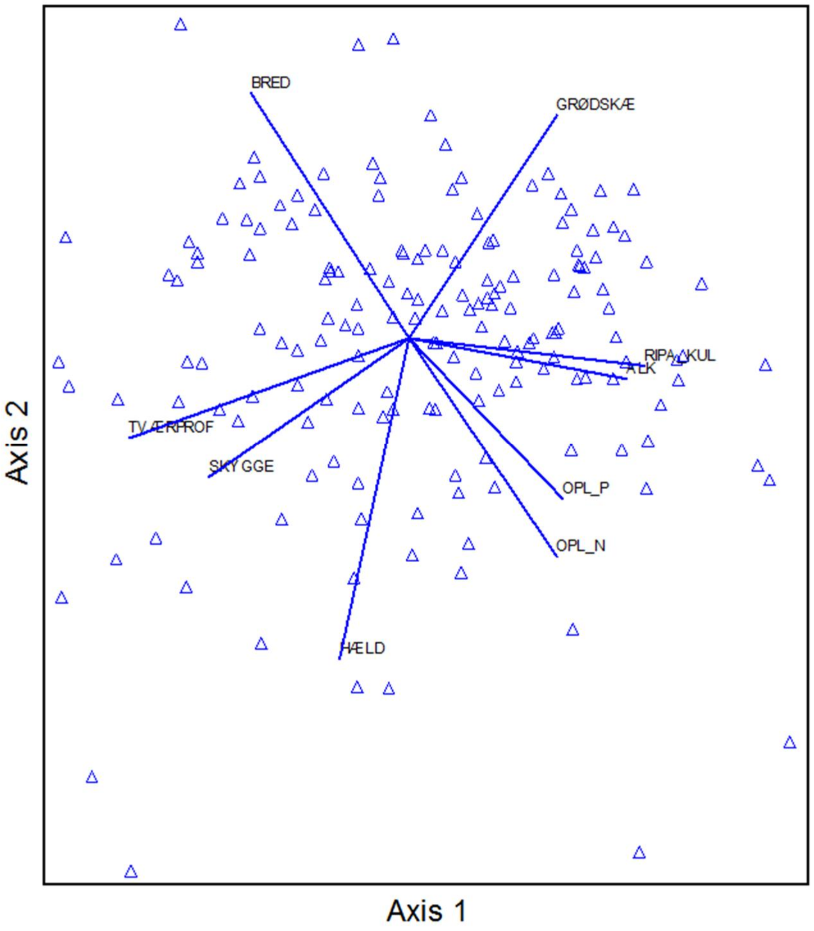


Figure 2.2.1. Principal Components Analysis (PCA) of environmental variables measured at the 186 stream stations included in the test of the Danish Stream Plant Index (DVPIEQR). Only the first two axes (PCA1 and PCA2) are shown. Legend: BRED = stream width, HÆLD = stream slope, TRÆRPROF = cross-sectional profile, SKYGGE = shading from trees and bushes, RIPA_KUL = extent of agricultural cultivation and urban impact within a 50 m riparian zone, GRØDSKÆ = number of annual weed cuttings, ALK = total alkalinity, OPL_N = dissolved nitrogen in the water phase; OPL_P = dissolved phosphorus in the water phase.

DVPI and environmental variables

The occurrence of more simultaneously acting stressors in the streams makes it appropriate to include several impact variables in the analyses of how $DVPI_{EQR}$ changes as a function of impacts. We decided to include the physical state of the stream (assessed on the basis of the cross-sectional profile), stream maintenance (assessed from weed cutting frequency because this parameter is simple and unambigious) and dissolved phosphorus as an indicator of eutrophication level. Table 2.2.2 shows median, mean value as well as minimum and maximum values of the various impact variables in type 2 and 3 streams.

Table 2.2.2. Concentrations of dissolved phosphorus, cross-sectional profile and weed cutting frequency in the group of medium-sized and large (type 2-3) streams. The values are given as median, medium, minimum and maximum values and 95% quantiles. The statistical values for weed cutting frequency are calculated on the basis of the average number of annual cuttings in the period 2012-2013.

	Median	Min.-max.	95% fractile
Type 2-3 (n=121)			
Dissolved-P (mg L^{-1})	0.050	0.005-1.160	0.105
Cross-sectional profile	1.75	0-3	3
Weed cutting frequency ($year^{-1}$)	1.27	0-4	3

We found that $DVPI_{EQR}$ changed significantly as a function of changes in the amount of dissolved phosphorus and weed cutting frequency in type 2 and 3 streams. $DVPI_{EQR}$ decreased with increased phosphorus concentrations and increased weed cutting frequency. For the small type 1 streams we found that $DVPI_{EQR}$ responded to stream morphology as well. We found an increase in $DVPI_{EQR}$ the more natural the cross-sectional profile is (data not shown). The results for the middle-sized and large streams (type 2 and 3) are summarised in Table 2.2.3 where the parameter estimates show the importance of the individual factors for $DVPI_{EQR}$, and the sign indicates whether the impact is positive or negative. Similar multiple regression analyses were performed in which the environmental variable cross-sectional profile was replaced by the variable sinuosity. The results were, however, virtually the same and are thus not shown here.

Table 2.2.3. Results of a multiple regression analysis between the Danish Stream Plant Index (DVPI) EQR values and three selected impact variables at 175 NOVANA stream stations. Dissolved phosphorus was logarithm transformed in the model. Weed cutting frequency is given as the average number of annual cuttings in the period 2012-2013. The parameter estimate for the individual variables in the overall model as well as t value and P value are given. * indicates that the given variable contributes significantly to the overall model.

	Parameter estimate	t values	*P*
Dissolved phosphorus	-0.0367	-2.36	0.0201*
Cross-sectional profile	0.0014	0.10	0.9212
Weed cutting frequency	-0.0717	-4.91	<0.0001*

Overall, the model explained 22% of the variation in data for type 2 and 3 streams (F=12.22; p<0.0001), implying also that a large part of the variation in $DVPI_{EQR}$ cannot be explained by the included variables. This is not surprising since several factors besides the ones included (phosphrorus, cross-sectional profile, weed cutting frequency) affect plant communities in Danish streams such as alkalinity and slope, as mentioned previously. These factors are not correlated with the factors included in the analysis (see PCA). Also, even though many variables were included in the initial PCA analysis, only about 45% of the variation in data can be explained via the first two significant axes. This means that there are a number of factors - in addition to the most essential impact variables - that may influence the plant communities. Additionally, we find it likely that in addition to the current level of impact also the historic level of impact influence $DVPI_{EQR}$. Pollution from point sources was considerably more significant historically, weed cutting was also more frequent and dredging occurred more frequently (Wiberg-Larsen et al. 2012; Iversen & Ovesen 1997). These former impacts may still be of importance for community composition today and, consequently for $DVPI_{EQR}$. Thus, many species may have disappeared from stream sites to which re-colonization cannot easily occur despite lower levels of impact. Also, it is possible that species may still be in decline in sites that continue to be impacted or where the impact has been intensified, and the frequency of the present species may therefore not be closely linked to the current impact level (Lindborg & Eriksson, 2004; Cavalli et al. 2014). This applies mainly to species that are locally frequent but with dispersed regional occurrence (see discussion in Cavalli et al. 2014).

2.2.4 Conclusions

We found that $DVPI_{EQR}$ changed as a function of changes in the types of impacts that we consider to be significant for the plant communities in Danish streams. Thus, $DVPI_{EQR}$ declined with increasing concentrations of dissolved phosphorus in the streams and increased frequency of weed cutting. A significant relationship was found also between $DVPI_{EQR}$ and stream morphology in small streams, with the highest index values found in streams with a natural profile and natural sinuosity. The reason for not finding such a relationship in middle-sized and large streams may reflect that weed cutting often correlates with degree of channelization thereby making it difficult to distinguish these two types of impacts in these stream types.

3 Designs to develop novel metrics or other indicator tools.

3.1 *Hypothesis design: a diagnostic tool for bioassessment based on macroinvertebrates*

Apart from the general asessment of ecological status, water authorities would benefit from a system that enables them to identify the cause of an observed change in ecological quality or the reason surface water fails to meet the ecological quality objectives. Especially in countries were different streassors exert their influence on ecological quality a diagnostic system is essential. Unfortunataly, most of the bioassessment systems in use today cannot serve as diagnostic tools. Altough several studies have used a multimetric approach to develop an assessment system , they use this approach to 'better' indicate general degradation rather than to assess the separate effects of individual stressors.

In the Netherlands a diagnostic tool has been developed to distinguish between eutrophication/organic pollution, morphological stress and hydrological stress. As a result of studies into the value of different indicators for assessment of ecological quality we have developed a multimetric index for streams called AMOEBE (Verdonschot & Verdonschot 2010, Verdonschot et al. 2012). The ecological preferences of macroinvertebrate species (Verberk et al. 2012) have been ascribed to four key components of the stream ecosystem, being: stream velocity, structures, substances and species (5-S model; Verdonschot 1995). Each key component is represented by one or two metrics that have been known to indicate the ecologial quality of streams and to represent the most important gradient from pristine to degraded streams.The seven metrics that together form theAMOEBE are:

1. Current preference index (Stream velocity): index that indicates the preference of species for certain current velocities and varies from species that only occur in standing waters to species that occur only in waters chracterised by high current velocities. A low percentage of rheofilic species indicates very low current velocity or stagnation.
2. Number of Trichoptera genera (Species): metrics that uses Trichoptera, because Trichoptera are a good indicator of ecological quality of aquatic eccosystems for both streams and standing waters .
3. Number of Ephemeroptera and Plecoptera genera (Species): metric that uses the presence of many Ephemeroptera and Plecoptera because they are characteristic for streams of very good ecological quality.
4. Percentage of aquatic plant dwelling species (Structures): in comparison to streams, swamps, ditches, side-arms disconnected from the river and other standing waters characterised by high plant biomass accommadate a large share of macroinvertebrates associated with aquatic plants. This metric uses species that are depending on aquatic plants for their survival , e.g., for reproduction, emergence, attachment, food source. Mass production of plant biomass is considered to be negative for streams of high ecological status and often results from a combination of lack of shading, altered hydrologic conditions and elevated nutrient concentrations.

5. Percentage of silt dwelling species (Structures): siltation, resulting for exapmple from stagnation, leads to an increase in species living in or feeding on silt. The metric is based on this principle.
6. Saprobic index (Substances): index that incorporates the sensitivity of species for organic pollution, including the low oxygen availability to goes with it and subsequent release of toxic substances.
7. Salinity index (Substances): metric that incorporates the sensitivity of species for high ion concentrations, including chloride. High or varying ion concentrations indicate disturbance, such as diverting water of a different origin to an area in case of drought or run-off of nutrient rich water from adjacent farmland. Many species are not resistent to this are.

To calulate metric/index values the species lists of the sample locations and their log 2(x+1)-transformed abundances are linked to the indicative values of the different species described by Verberk et al. (2012). The indicative values are devided over different classes according to the so-called ‘fuzzy coding’ technique: depending on the ecological preference of a species 10 points are divided over the relevant classes. For example a species with a preference for fast flowing water, but that also occurs in places with moderate current velocity, scores: high current velocity = 8 points, moderate current velocity = 2 points, low current velocity = 0 points etc. Since the abundance of species is also important for assessment purposes (this is a measure of success of the species in the system) the scores are corrected for abundance. For each sample the weighted score of all species is summed and the percentage per class is calculated. In the case of the current preference, salinity and saproby the percentages have been converted into an index. The weighed percentage of plant and slit dwelling individuals is calculated.

The next step is that the metric/index values are scaled linearily from 0 to 1 based on values for Dutch streams and ditches of high ecological quality (reference situation) (Nijboer et al. 2003, Verdonschot & Nijboer 2004). This step makes the different metrics comparable. Each metric discerns between five ecological quality classes: 0-0.2 = high, 0.2-0.4 = good, 0.4-0.6 = moderate, 0.6-0.8 = poor, 0.8-1.0 = bad. The results are represented in an AMOEBE-diagram (Figure 3.1.1). In the diagram the metrics indicate which key components are under stress, visualised by the shape of a buldge (hence, the name AMOEBA) in the diagram (Figure 3.1.2).

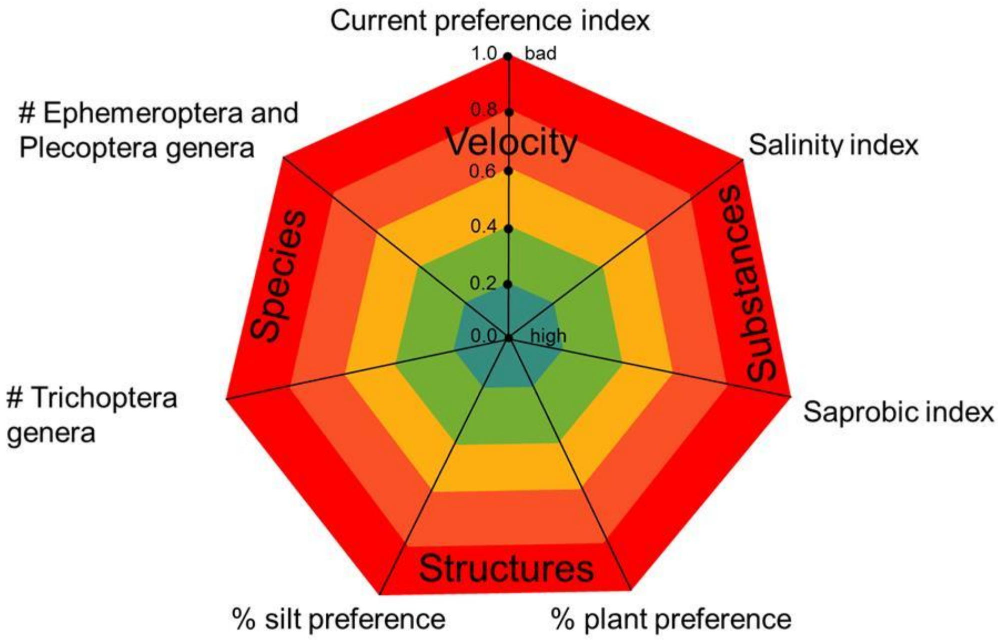


Figure 3.1.1. The AMOEBE for streams integrates the key components of the stream ecosystem based on the ecological preferences of macroinvertebrate species.

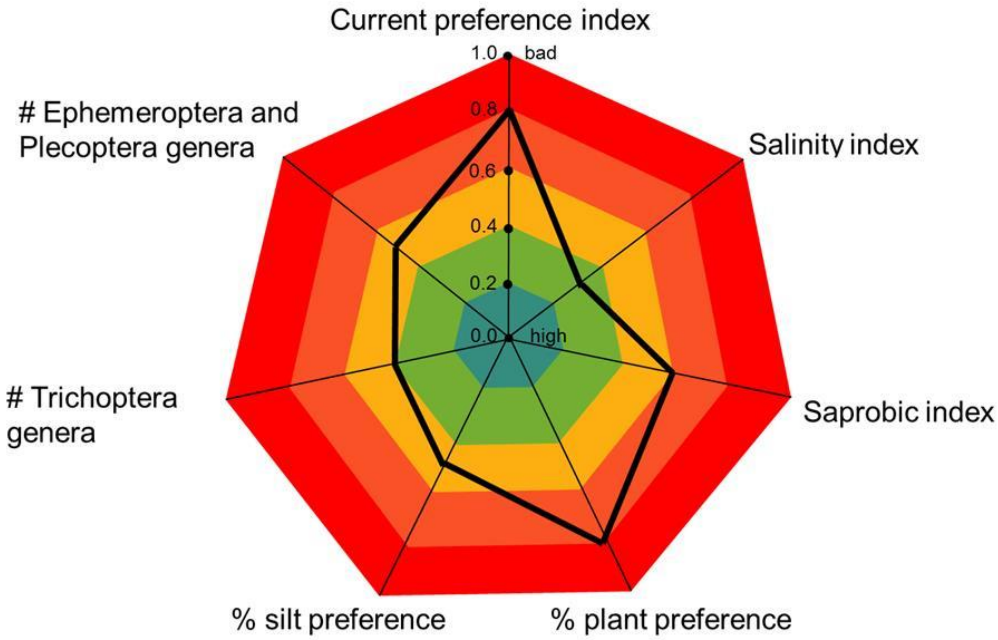


Figure 3.3.2. Example of an AMOEBE-diagram based on a macroinvertebrate sample from an arbitrary stream, the 'bulges' indicate the severity of the different sources of anthropogenic stress.

The AMOEBE provides an appealing visual tool for water managers to diagnose the cause of stream degradation, including morphological and hydrological degradation. This way the AMOEBE is making it easier to decide on appropriate restoration measures when a stream fails to meet the ecological quality objectives. However, before the AMOEBE can be widely applied for water management purposes it has to be validated with data from both Dutch streams and other streamtypes in Europe.

3.2 *Fish community size spectra as a new potential metric for assessing impacts of HYMO pressures*

3.2.1 Introduction

[literally] "…aquatic ecologists have developed size spectra [frequency distributions of individual length classes for a multiple species community] to compare the density of organisms belonging to different body size classes, regardless of their taxonomic affiliation (e.g. Sheldon *et al*. 1972, Sprules and Munawar 1986, Ahrens and Peters 1991). Size spectra show that, over a large range of body sizes, the total density of organisms in each size class decreases roughly log-linearly with increasing body size. Small organisms (of all taxa combined) are much more abundant in nature than large organisms. Size spectra are valuable in comparing large-scale patterns among communities and, because they include information both on the size of organisms and on community biomass, they are intimately tied to ecosystem function and dynamics. Most community size spectra have been measured in marine or freshwater plankton (Ahrens & Peters 1991, San Martín *et al*. 2006). The approach has rarely been used for benthic or terrestrial communities, where the substrate makes sampling and sorting of organisms much more difficult (but see Strayer 1991, Stork and Blackburn 1993). Since the total density of organisms in a body size class, density-body size relationships and size spectra are closely linked to each other. However, their inter-conversion would seem to require knowledge of the density of all species in the community, and in practice such information is rarely available, because the range of species that can be enumerated simultaneously is limited by sampling methodology and taxonomic expertise." (Cyr *et al*. 1997)

Authors (e.g. Cyr et al. 1997) have theorised that differences in the size spectra of different communities might be due to: (1) differences in numbers of species in different environments (e.g. aquatic vs. terrestrial); (2) differences in the proportion of ectotherms (generally more abundant than endotherms of similar size (Peters and Wassenberg 1983, Damuth 1987), and considering that the smallest endotherms are about 2 g in size, larger than many ectotherms) and endotherms in different environments; (3) differences in the total amount of energy transferred from primary producers to consumers, due to both different rates of herbivory (Cyr and Pace 1993) and food qualities, in different environments; and (4) different access to a third dimension, over which primary production is often distributed, of organisms living in different environments (e.g. aquatic vs. terrestrial). But also thermodynamic reasons have been used to explain the differences of size spectra of communities; being the slope of the log-linear relationship between density and ataxonomic size-class positively correlated to the relative energy fluxes passing through the system (or with different production/biomass ratios) (Lurie and Wagensberg 1984, Margalef 1991).

Accounting that the degree of rheophily amongst fishes in a community might be considered as a continuous gradient to describe the variation of physical-chemical and energetic conditions of riverine habitats along the river continuum from rhithral to potamal habitats; one can expect that there might be some kind of relation among the variation of the size spectra of fish communities and the extent of rheophily. The Fish Region Index (FRI, Wolter *et al*. 2013) is a descriptor of this rheophilic gradient. Since early succession stages of ecosystem patches are characterized by higher production rates with lower biomass than mature stages, P/B ratio is usually higher in the former than in the latter habitats. According to the river continuum concept, upper reaches of the rivers are more prone to be affected by frequent occasional catastrophic events, and subsequently less likely to reach mature succession stages. This might lead to an inverse relation among P/B (and therefore different slopes of the size spectra) and the FRI. In addition, it is well known that size spectra show steeper slopes in communities affected by seasonally pulsed disturbances (which are typically found in the upper reaches of rivers) (Jul-Larsen *et al*. 2003). According to this, one may expect to find a theoretically continuous variation of the slope of the size spectra along the gradient of FRI values, like shown in Figure 3.2.1.

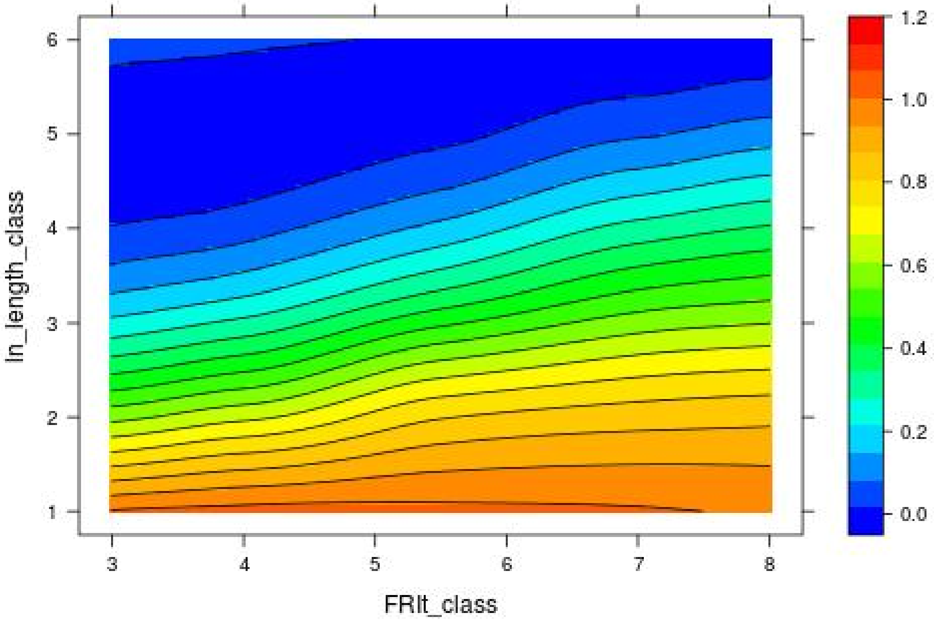


Figure 3.2.1. Theoretical variation of size spectra slopes along the rheophily gradient measured by the Fish Region Index (FRI). For a site with a give value of the Fish Region Index (X axis) a relative abundance (Z axis, colour scheme 0 to 1 proportion) is expected for every ln(length[mm]) class (Y axis).

Community size spectra have also been shown to assess the degree of impact caused by some sort of degradation (Jung and Houde 2005) (namely fishing pressure). Impacted or fished communities show steeper slopes than non-impacted (or non-fished) ones. So it could be expected that, if the relationship between the FRI and natural size spectra slopes could be modelled, then the difference between the expected (modelled) and the observed size spectra slopes could become an indicator the degree of deviation of an impacted fish community from its reference conditions.

The first challenge in the development and modelling of size spectra metrics is to model the expected size spectra slopes along the gradient of fish communities of the river continuum. According to the methods to measure the expected FRI (FRI_e, approximately equal to Huet zones) and the observed FRI for a given fish community (FRI_t) exposed in D1.3 (Wolter *et al*. 2013), impacted communities can be identified where the difference between the observed and expected FRI values (FRI_t-FRI_e) are high. Therefore, a calibration dataset of sites for which FRI_t-FRI_e is less than a given value, can be used to describe the expected variation of the size spectra slopes along the FRI_t gradient of the sites that are approximately reference sites at high ecological status. Deviations of size spectra from this reference frame in impacted fish communities might indicate the degree of the impact on the fish community in terms of its size spectrum.

3.2.2 Sensitiveness to multi-stressor conditions: limiting factors hypothesis

To test the sensitiveness of the community size-spectra to multiple anthropogenic stresses, their response to several conditions must be analysed: presence of single water quality stressors; presence of single HYMO stressors; presence of concurrent water quality and HYMO stressors. However, the data availability for each type of conditions are limited, and certainly insufficient to conduct proper parametric tests. In this situation, one proposal is to test the limiting factors hypothesis. This hypothesis states that given a combination of stressors, the most important one is acting as limiting stressor. Once this limiting stressor is removed, the community will shift until the effect of the next most important stressor becomes limiting. Therefore, this analysis tests which stressor induces a higher difference between the expected (Figure 3.2.4 and 3.2.5) and the observed fish community size-spectra. Sorting the different stressors according to their effect, will identify which one will be the limiting factor when present in combination of other stressors.

The question to be addressed is, therefore: are size spectra sensitive to HYMO alterations? To address this question, the fish community data from the degraded sites (see above paragraphs in this Methods section) will be analysed. Every site was characterized by its HYMO impacts, and checked whether the deviation of their size spectra can be explained by the presence/absence of a given HYMO impact. This was done by testing for significant differences in the shapes and slopes of size spectra between degraded and calibration sites.

The results may identify which HYMO impacts induce noticeable and predictable responses on the community size spectra, and subsequently in the functional structure of fish communities.
To use a different dataset of that used to build the reference size spectra variation, the rejected (due to differences between FRI_e and FRI_t) dataset was used.

3.2.3 Methods

Data from the EFI+ Spanish dataset (EFI+ Consortium, Spanish Team 2009) were used to build this reference frame of variation of size spectra along the FRI gradient. Previously, sites for which FRI_t-FRI_e was less than |1| were selected, thus rejecting sites with significantly altered fish communities.

A total of 1,534 sampling sites were initially considered, and after removing the site with FRI_t-FRI_e >|1|, 1,080 sites remained (including 132,780 individual fish total length data). FRI_t and FRI_e ranged from 3.75 to 7.39 and 3.5 to 7, respectively. And total fish lengths in the EFI+ Spanish dataset ranged from 10 to 1,039mm.

With this reference dataset, the relative abundance (number of individuals/total individuals in the site) of every ln(total length [mm]) 0.2 class was calculated for every site. Then the average value of ln(relative abundances) of every ln(total length) class was calculated for every FRIt 0.5 class. A smoothened surface was obtained from the representation of the distribution of average ln(relative abundances) data in a 2D plot where X axis represents the FRI_t gradient and Y axis is the ln(total length) range.

The outcome of these procedures was used as the reference variation of size spectra to which the observed size spectra of impacted sites would be compared. Size spectra were compared by means of the following procedure: (1) For every 0.5 FRI_e class and every 0.2 ln(length) class the difference between the observed and the reference relative abundances were calculated; (2) the sum of absolute values of the differences for all the ln(length) classes in the impacted site was considered as a measure of the alteration of the shape of its size spectrum [Δ*size spectrum (obs-ref)*]; (3) the slope of the size spectrum of every impacted site was determined by means of a generalized linear model, the difference between the expected slope for the corresponding FRI class to which the site belongs and the observed slope was calculated and considered as a measure of the alteration of the trend of its size spectrum [Δ*size spectrum slope (obs-ref)*] (Figure 3.2.2).

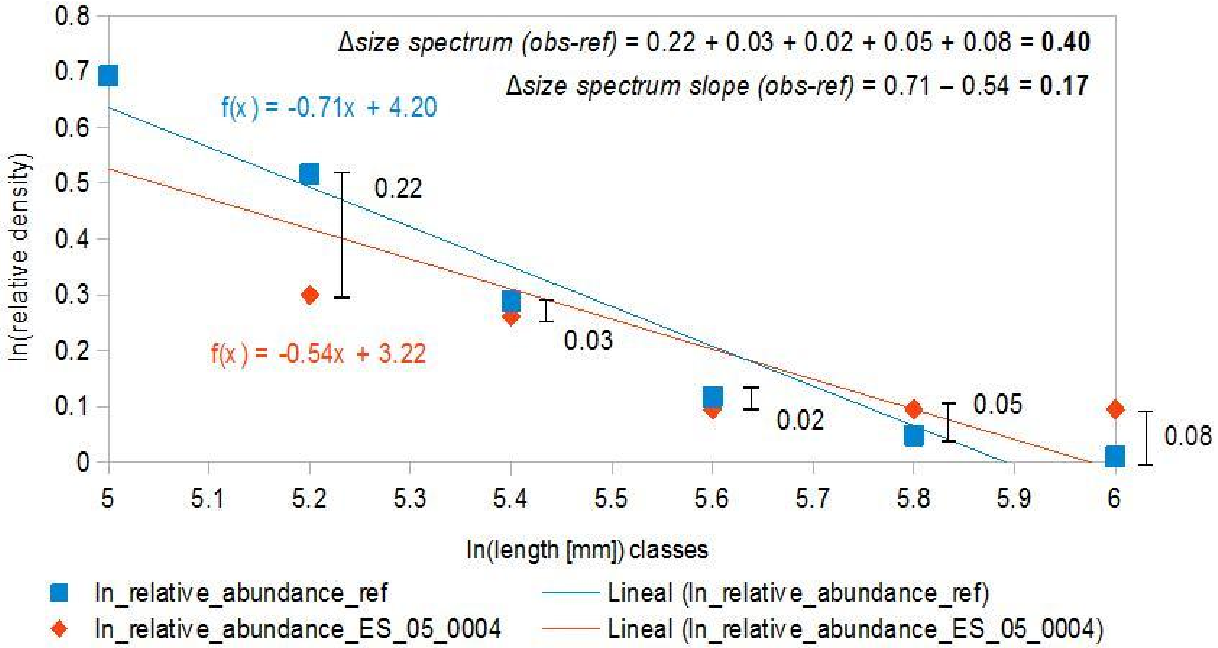


Figure 3.2.2. Example (with real site coded ES_035_0590 from the impacted dataset) of the methodological procedure to calculate both variables of size spectra alteration: **Δ***size spectrum (obs-ref)* and **Δ***size spectrum slope (obs-ref)*.

The alteration of the overall shape of the size spectrum, Δ*size spectrum (obs-ref)*, describes the total amount of change induced by a pressure on the fish community size structure, regardless the sign of the change. This is to say that it does not indicate whether there are more or less small fish or larger fish. That effect is described by the change in the slope of the size spectrum, Δ*size spectrum slope (obs-ref)*. For example, a positive value of Δ*size spectrum slope (obs-ref)* will indicate that the pressure induces an increase in the steepness of the size spectrum. This is to say that there are less large fish in the impacted conditions than what was expected in unimpaired conditions.

Pairwise comparison t-tests (Bonferroni correction) were conducted to test for differences in the values of Δ*size spectrum (obs-ref)* and Δ*size spectrum slope (obs-ref)* between sites with different degrees of each pressure category (listed in Table 3.2.1).

All analyses were done in R statistical environment.

Table 3.2.1. Pressures considered in the comparison t-test of the **Δ***size spectrum (obs-ref)* and *size spectrum slope* values of different sites; and their description.

Pressure	Description
Acidification	No= all the sites, since there are not acidification problems in the sites provided.
Barriers_catchment_down	Yes= There is at least 1 barrier in the catchment, downstream from the site. No= There are no barriers in the catchment, downstream from the site.
Barriers_river_segment_down	Yes= There is at least 1 barrier within the river segment, downstream from the site. No= There are no barriers within the river segment, downstream from the site.
Barriers_river_segment_up	Yes= There is at least 1 barrier within the river segment, upstream from the site. No= There are no barriers within the river segment, upstream from the site.
Channelisation	Straightened= the stream is forced to flow in a straight line. Intermediate= the natural sinuosity of the stream is reduced. No= there is no reduction of the natural sinuosity.
Cross_sec	Technical crossec/U-profile= there is a technical profile covering all the width of the stream. Intermediate= there is an alteration of the cross-section in one or both banks, but not a U-profile. No= there is not alteration of the cross-section.
Embankment	Cont.no permeab.= there is a continous embankment made of concrete or similar. Cont.permeable= there is a continous embankment made of permeable material. Local= there is only few local embankments (like a bridge). No= there is no embankment.
Eutrophication	Extreme= the site is affected by an intense eutrophication. Intermediate= the site is affected by a moderate eutrophication. Low= there is only a slight eutrophication. No= there is no eutrophication.
Floodplain_fishing_occasion	No= there is <10% of the former floodplain remaining. Small= 10-40% remaining. Medium= 40-70% remaining. Large= >70% remaining.
Hydro_mod	Yes= sites with a dam upstream, within the river segment. No= the rest of the sites.
Hydropeaking	Yes= there is an hydroelectric dam within the river segment, upstream of the site. No= The rest of the sites.
Impoundment	No= there is no flow velocity reduction due to impoundment. Weak= Some flow velocity reduction due to impoundment. Strong= Site is inside an artificial impoundment.
Instream_habitat	High= there is an important alteration of the presence and distribution of instream habitats (rapids, runs, glides and pools). Intermediate= there is a moderate alteration of the instream habitat. No= instream habitat is not altered.
Organic_pollution	Strong= the site is affected by an intense organic pollution. Weak= the site is affected by a moderate organic pollution. No= there is no or very slight organic pollution.
Organic_siltation	Yes= the site is significantly affected by organic siltation. No= there is no or very slight organic siltation.
Reservoir_flushing	Yes= sites with a dam upstream, within the river segment. No= the rest of the sites.
Riparian_vegetation	No= 75-100% riparian vegetation remaining. Slight= 50-75% remaining. Intermediate= 25-50% remaining. High= <25% remaining.
Sedimentation	High= very important input of fine sediment into the stream. Medium= moderate input of fine sediment. Weak= slight input of fine sediment. No= there is no artificial input of fine sediment into the stream.
Temperature_impact	Summer decrease= sites with a dam upstream, within the river segment, because all the big dams have a bottom outlet. No= the rest of the sites.
Toxic_substances	High concentration= sites downstream a "point with dangerous substances", at a distance of 0-1km from it. Intermediate= sites downstream a "point with dangerous substances" at a distance of 1-10km from it. No= the rest of the sites.
Velocity_increase	Yes= there is a significant pressure (channelisation or floodprotection) which changes the water velocity. No= there is not a significant pressure likely to change the water velocity.
Water_abstraction	Strong= the site is affected by an intense water abstraction. Weak= the site is affected by a moderate water abstraction. No= there is no or very slight water abstraction.
Water_quality_index	5=Tox.HC+Pollut.Weak; Pollut.Strong. 4=Tox.HC+Poll.No; Tox.Inter+Poll.Weak; Poll.Weak+Eutr.Extr or Siltat. 3=Tox.Inter+Poll.No+Eutr.Low; Tox.No+Poll.Weak or Low. 2=Tox.Int.+Poll.No+Eutr.No; Tox.No+Poll.No+Eutr.Low or Int. 1= Tox.No+Poll.No+Eutr.No.
Water_use	The main human activity for which water is used at the site: No, Drinking water, Fish ponds, Hydropower, Industrial water, Irrigation, Other.

3.2.4 Results

The 2D plot of the distribution of average ln(relative abundances) by ln(total length) classes shows that the variation of the slopes of size spectra is not regular along the FRI_t gradient (Figures 3.2.3 and 3.2.4).

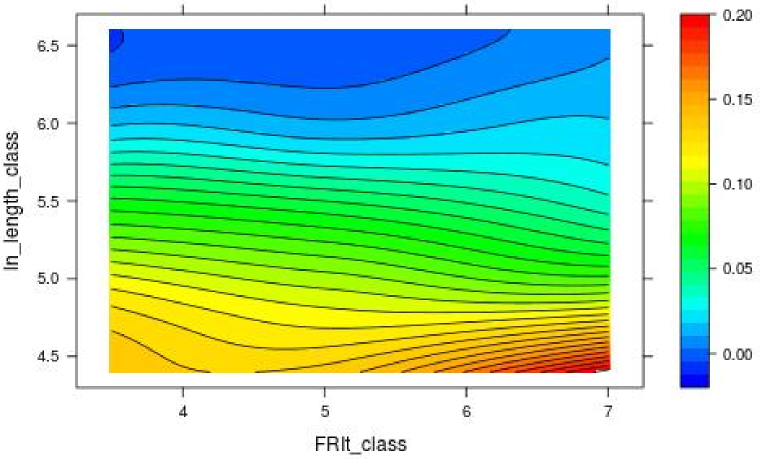


Figure 3.2.3. Distribution of average ln(relative abundance) of every 0.2 ln(total length) class and 0.5 FRIt class, smoothened by the whole range of length classes.

When looking at the smoothed surface of the whole length range (Figure 3.2.3), the size spectra show lower slopes for the smaller length classes in low(FRI_t <4) FRI_t sites (i.e. upper reaches), whereas at lower reaches (FRI_t 6 to 7) the slope is steeper at smaller length classes yet less steep in large length classes. In intermediate reaches (FRI_t ~ 4.5 to 5.5) the slopes of the size spectra are quite constant along the length class gradient.

If the plots are divided in to 0.6 ln(total length) classes, much more detailed can be observed (Figure 3.2.4). In these plots a less linear profile of the size spectra is observed. There is an elevation of the surface around ln(length) ~ 5 (total length ~ 150mm), especially for FRI_t values less than 6 (upper and middle reaches). Although bimodality in size spectra has been related to the presence of different trophic strategists (zooplanktivorous and piscivorous) within the fish community (Jung and Houde 2005), these effects might also be due to other biological reasons (e.g. immigration or emigration of some length classes from other habitats; or self-thinning based density-dependent mortality only acting when cohorts reach a given size; up to that size the abundance dynamics are highly variable among years), or to methodological reasons (e.g. the catch ability of lower length classes my differ from upper to lower reaches). There are no data with which to test the influence of these two reasons and hence we assume them not to be influencing. Therefore, to avoid spurious conclusions, the length classes to be included in the size spectra were restricted to those showing a monotonic size spectra. As shown in Figure 3.2.4, a monotonic trend is present in ln(length) classes larger than 5 (total length~150mm). To make the data comparable among FRI_t classes, ln(relative abundance) average data for every FRI_t class were standardized by making them relative to the abundance of ln(length) class 5

$\ln(\text{relative abundance})^{std}_{i,j} = \ln(\text{relative abundance})_{i,j} / \ln(\text{relative abundance})_{5,j}$

(e.g. standardised ln(relative abundance) of length class *i* for FRI_t class *j* is calculated as ln(relative abundance) of length class *i* divided by ln(relative abundance) of length class 5 for FRI_t class *j*). After this standardization, it was assumed that data remain ecologically meaningful since [literally] "variations in the intercept with the vertical axis indicate that average overall biomass in the system is a function of the availability of nutrients to the system, rather than a function of the structure of the particular biological community" (Boudreau and Dickie, 1992).

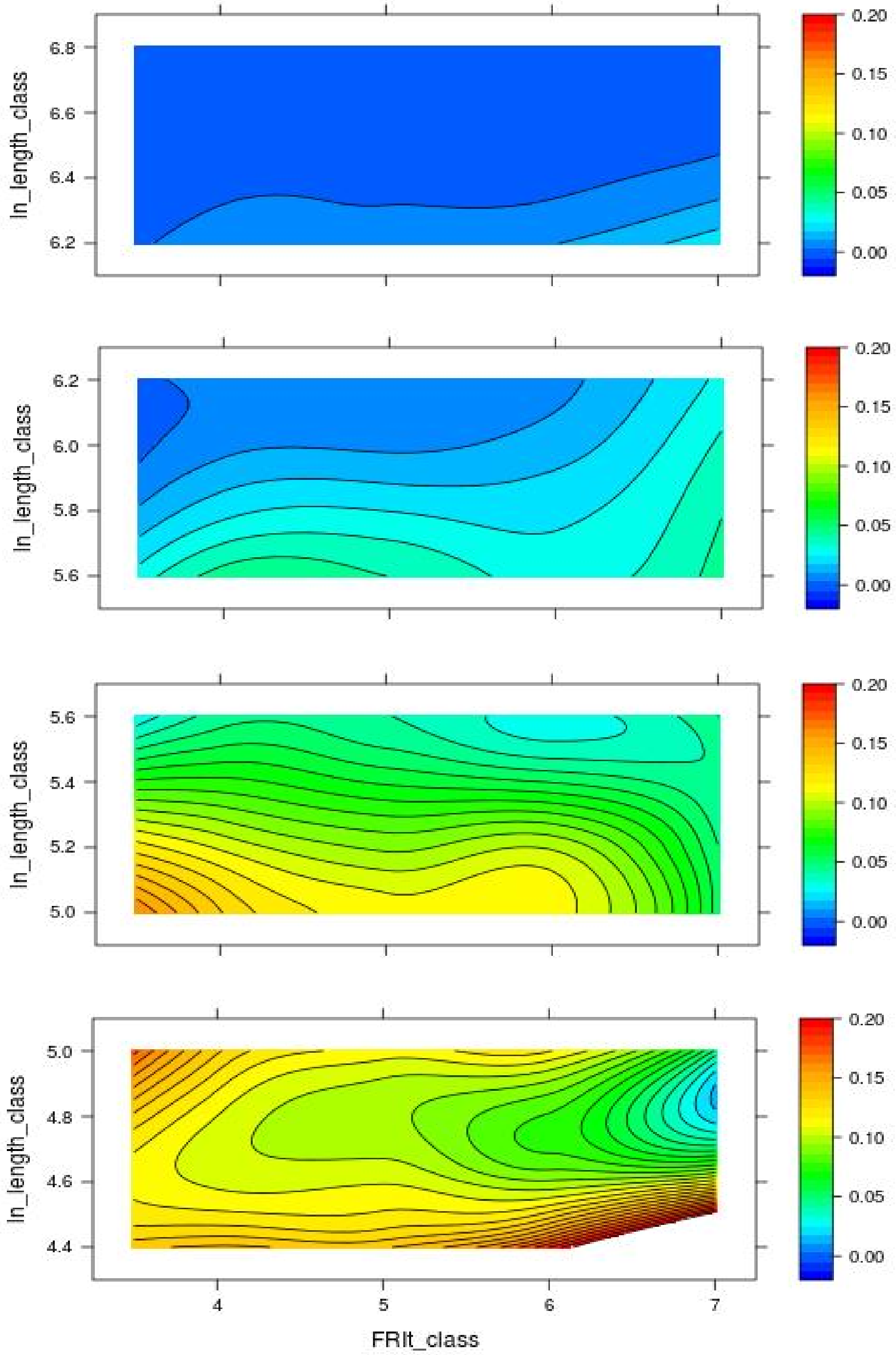


Figure 3.2.4. Distribution of average ln(relative abundance) of every 0.2 ln(total length) class and 0.5 FRI$_t$ class, smoothened by intervals of four 0.2 ln(total length) classes.

This new plot (Figure 3.2.5) shows a steeper slope of the size spectra for the lowest values of FRIt (headwaters) and smoother slope at the largest values of FRIt (lowest reaches), with a plateau in FRIt ranging from 4 to 6.

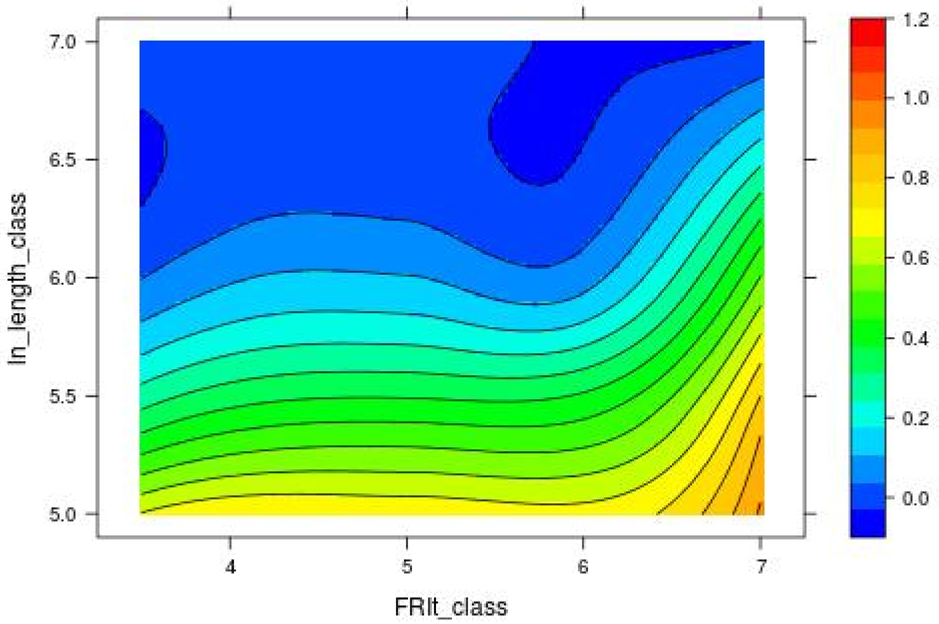


Figure 3.2.5. Distribution of standardised (relative to the value of class 5.0 of every FRI_t class) average ln(relative abundance) of every 0.2 ln(total length) class and 0.5 FRI_t class, smoothened by the range of ln(total length) classes from 5 to 7.

This last plot might be considered as the reference frame of variation of size spectra along the FRI gradient, and it is rather consistent with the theoretical frame deduced in Figure 3.2.1. The ratio at which the slope of the size spectra decreases along the FRI_t gradient is 10%, approximately (Figure 3.2.6).

The expected size spectrum slope for a given site has been considered according to the regression line of Figure 3.2.6. This is to say that a site whose expected FRI (FRI_e) is 5, the expected slope of its size spectrum would be:
FRI_e^5 = -0.1012*5+1.0411=0.5351.

Assuming that there would be a certain amount of natural variability around the reference size spectra, natural variance in the calibration dataset has to be allowed. Therefore, a comparison t-test was conducted between the values of Δ*size spectrum (obs-ref)* and *Δsize spectrum slope (obs-ref)* of the calibration (unimpaired) dataset and the impacted sites. To determine if a site/community has a significantly altered size spectra, observed difference in both variables has to be greater than that observed in unimpaired sites. Figure 3.2.7 shows the results of this comparison tests. It can be seen that that observed difference was significantly ($p<0.05$) greater in the impacted sites than in unimpaired sites for Δ*size spectrum (obs-ref)*, but not for *Δsize spectrum slope (obs-ref)*. Therefore, the results regarding this latter variable cannot be conclusive.

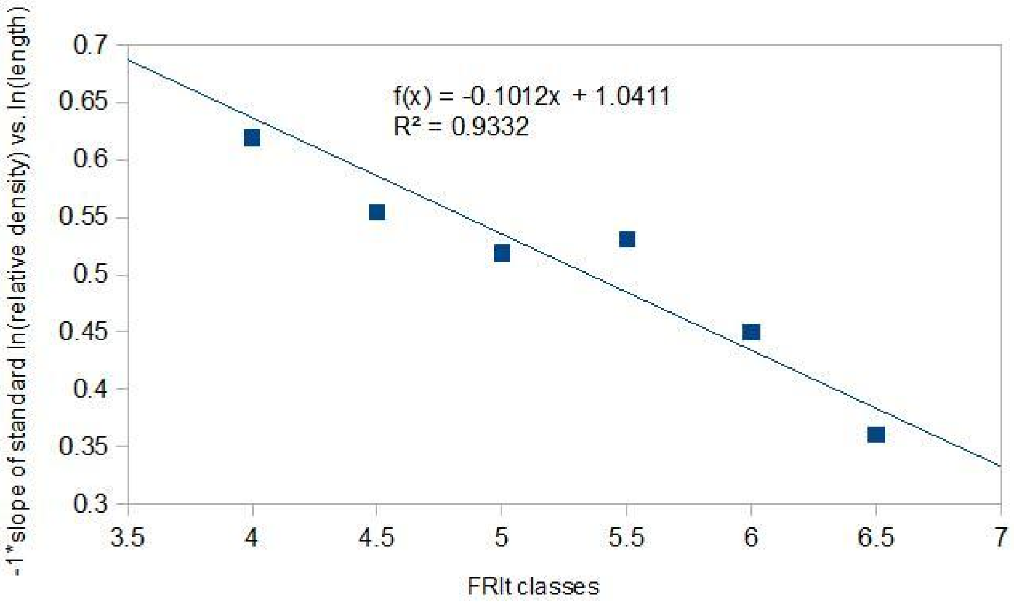


Figure 3.2.6. Value of the negative slope of the size spectra (ln(length) classes from 5 to 7) for every 0.5 FRIt class.

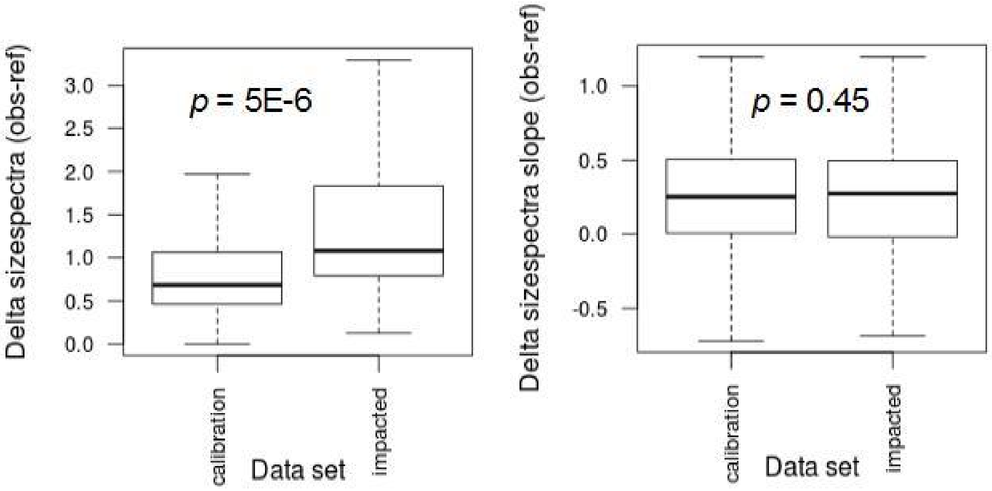


Figure 3.2.7. Results of the t-test comparison (Bonferroni correction) between values of **Δ***size spectrum (obs-ref)* and *Δsize spectrum slope (obs-ref)* of unimpaired (calibration) and impacted sites.

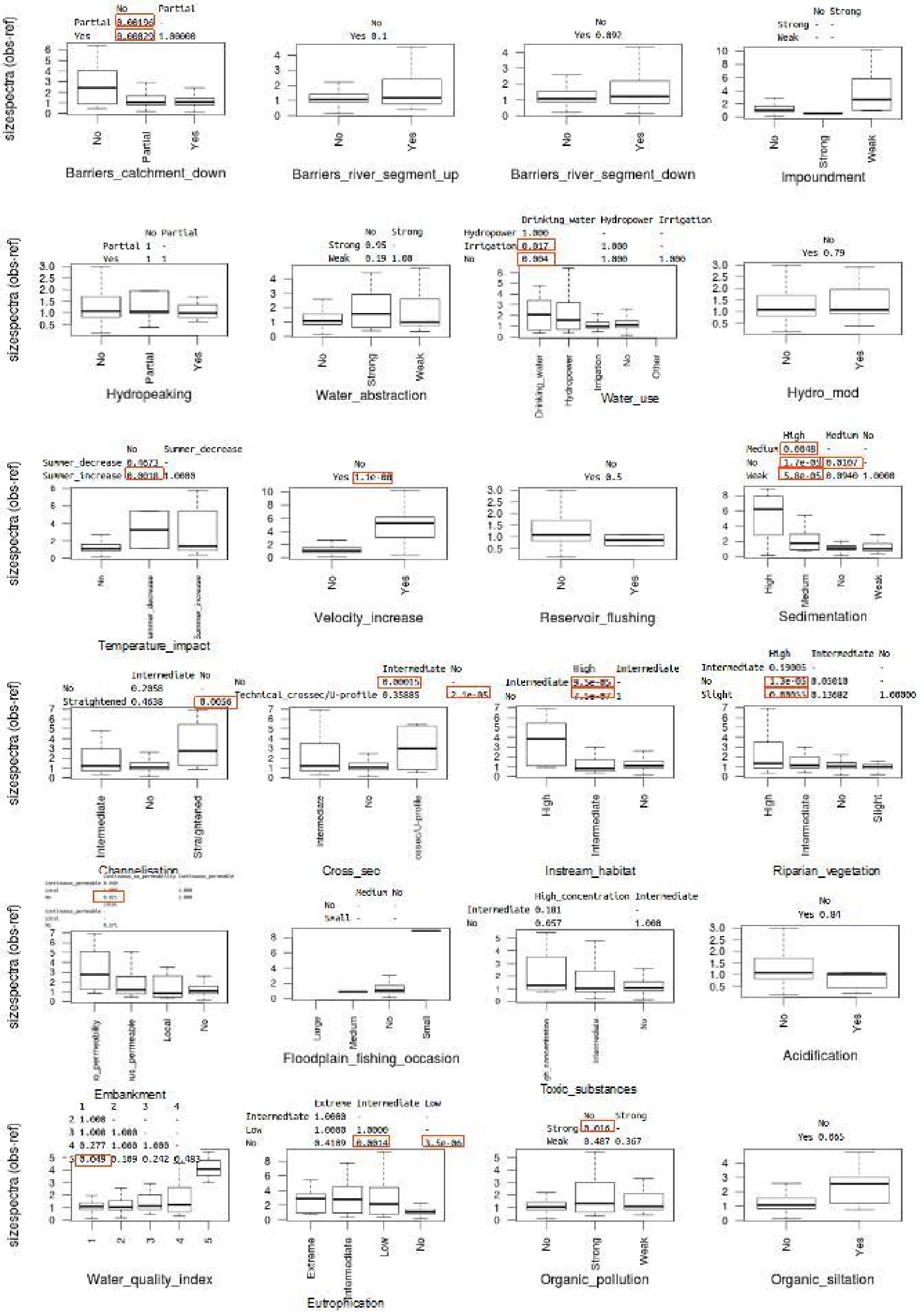


Figure 3.2.8. Results of the t-test comparison (Bonferroni correction) between values of **Δ***size spectrum (obs-ref)* of sites grouped by their degree of different HYMO and non-HYMO pressures. Matrices show the significance (p-value) of the pairwise comparisons; p-values<0.05 are highlighted.

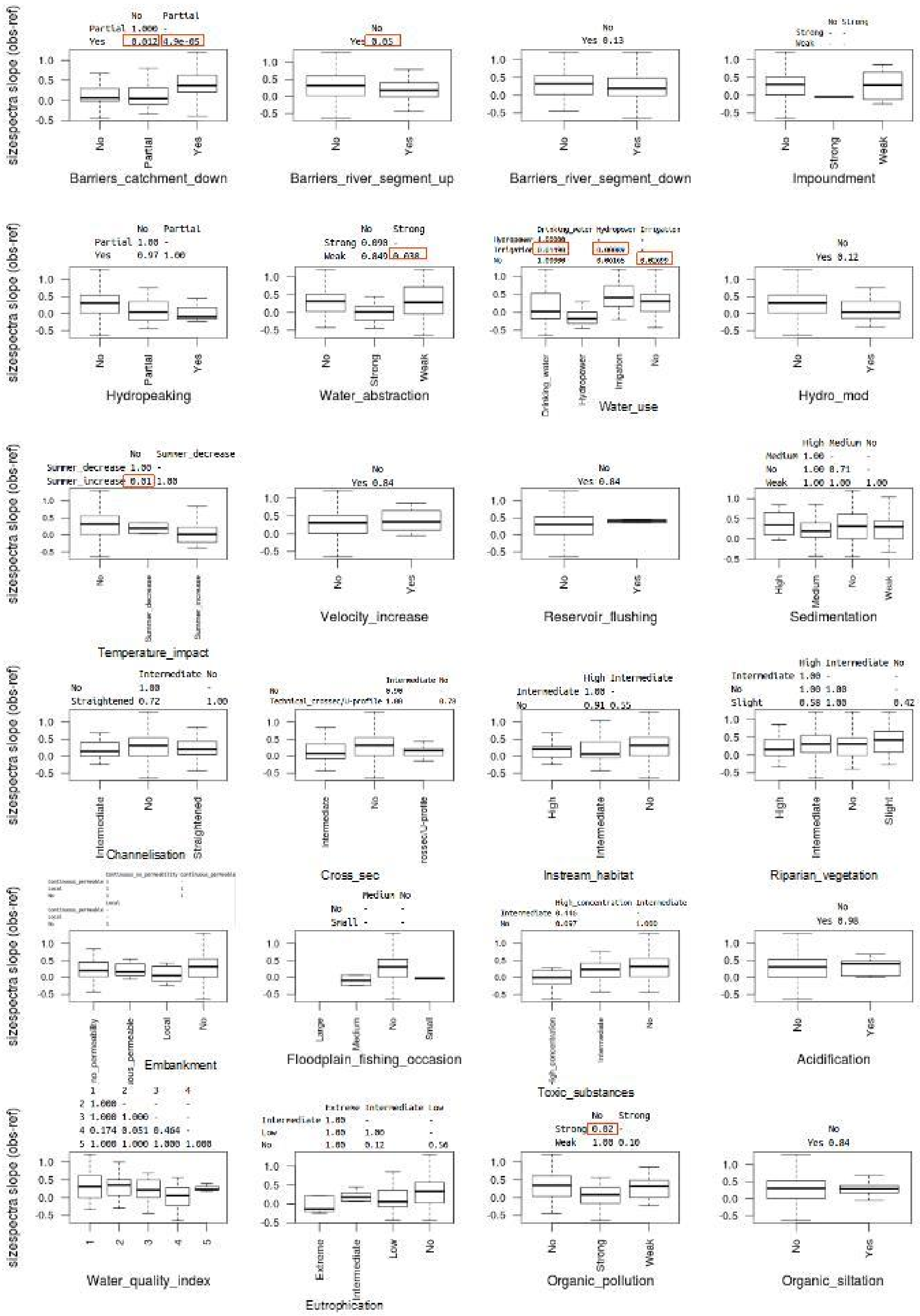


Figure 3.2.9. Results of the t-test comparison (Bonferroni correction) between values of *Δsize spectrum slope (obs-ref)* of sites grouped by their degree of different HYMO and non-HYMO pressures. Matrices show the significance (p-value) of the pairwise comparisons; p-values<0.05 are highlighted.

Δsize spectrum (obs-ref) quantitatively describes how different is the actual shape of the size spectrum of a given fish community from the expected in the absence of sensitive alterations in its specific composition. Here it is assumed that this 'reference' situation would be found when the observed FRI is not very different from the expected FRI (see Deliverable 1.3 for the details). Similarly, *Δsize spectrum slope (obs-ref)* accounts for the differences in the steepness of the slope of these size spectra.

One might expect that when fish population/community dynamics are sensitive to a given pressure the actual size spectra might differ from its reference, both in shape and in the slope. And, furthermore, the higher the difference between observed and expected, the greater the sensitivity to that pressure. This approach is the basis for the concept of indicators of integrity. So it may be deduced that when *Δsize spectrum (obs-ref)* shows higher values in sites where the degree of a given pressure is higher, then *Δ size spectrum (obs-ref)* can be useful to estimate the impact of that pressure on the fish community size structure. On the other hand, *Δ size spectrum slope (obs-ref)* may provide information on what changes in the fish community are induced by that given pressure.

So both variables, *Δsize spectrum (obs-ref)* and *Δsize spectrum slope (obs-ref)* provide complementary information on how hydromorphological impacts may affect the community dynamics.

According to the results (Figure 3.2.8), *Δsize spectrum (obs-ref)* is significantly ($p<0.05$) higher in sites affected by an artificial increase of summer water temperature, *Temperature_impact*, (2.97) than in unaffected sites (1.55). Paradoxically, the impacted sites show no alteration of the slope of their size spectra, and significantly different ($p<0.05$) from the non-impacted sites (mean value of *Δsize spectrum slope (obs-ref)* =-0.01 and 0.35, respectively, in impacted and unimpacted sites) (Figure 3.2.9). This means that thermal alteration (summer increase) induces changes in the shape, but not in the steepness, of the fish community size structure. Since there is no clear change in the slope of the size structure, there is not a consistent pattern in the change of the shape. Therefore it is not easy to be translated into what it means (e.g. more or less small fish, fewer larger fish, more mortality, reduced growth or longevity). This lack of consistency in the change of shape among sites can evidence that the effects of the thermal alteration might be dependent on the local (presumably thermal) conditions of the site.

Strong *Organic_pollution* is not a HYMO pressure, but it induces significantly higher values of *Δsize spectrum (obs-ref)* in communities subject to this pressure than those communities at sites without this pressure. Similarly to *Temperature_impact*, sites not subject to strong organic pollution show low differences in slope compared to what it should be expected (mean value of *Δsize spectrum slope (obs-ref)* =0.09 and 0.37, respectively, in impacted and unimpacted sites). Organic pollution induces changes in the overall shape of the size spectra, but not in its slope.

In sites where there is a significant pressure (channelisation or flood protection) which changes the water velocity, *Velocity_increase*, *Δ size spectrum (obs-ref)* shows significantly higher values (4.97) than in unimpacted sites (1.56). However, there are no significant changes in the slope, meaning that this pressure induces changes in the shape but not in the steepness of the community size structure. Similar effects are found in sites with very high input of fine sediment into the stream (*Sedimentation*); where the stream is forced to flow in a straight line (*Channelisation*); where there is a technical profile covering all the width of the stream (*Cross_sec*), where there is an important alteration of the presence and distribution of instream habitats (rapids, runs, glides and pools) (*Instream_habitat*); sites with less than 25% riparian vegetation remaining (*Riparian vegetation*); where there is a continuous embankment made of concrete or similar (*Embankment*); and sites with the highest value of the water quality alteration index (*Water_quality_index*).

3.2.5 Discussion

Some results have already been obtained (D 3.1) from the observation of the presence/absence of species in HYMO impacted sites, thus leading to conceptual models. However, the presence/absence of species is such a coarse variable that it lacks the power to describe a mechanistic understanding of community change, along with indicators of the degree of impact.

Since individual size and growth is one of the most biologically sensitive variables of the biota, community size spectra seems a reasonable way to synthetically look at the functional structure of a fish assemblage. Size spectra have been profusely used in marine ecology to assess the status of fisheries, along with the integrity of plankton based trophic chains (since it is much easier to systematise the plankton community).

The analysis presented here indicate that size spectra show a regular pattern of variation along a synthetic index of rheophily (Fish Region Index) in a large dataset of fish communities in Spain. This response has found to be consistent with the currently known theoretical behaviour of size spectra in other types of animal communities.

Many HYMO pressures (but also non-HYMO pressures) generate a clear response in the fish community size structures, particularly inducing changes in the overall shape of the size spectra, rather than altering its steepness. Steeper slopes are predicted for impacted than for unimpaired sites. This effect has been explained as the response of a biotic community to impacts, which generally consist of its retraction to earlier succesional stages due to both intended or unintended changes in habitat. Early stages of ecosystems show generally higher production rates and lower biomasses than mature stages. Human activities can drive changes in ecosystems intendedly to increase this production rates at the cost of a reduction in the biomass. This leads to faster life strategies where organisms show both higher recruitment rates and higher mortality rates. The response of this retraction on the size structure of biotic communities will therefore produce steeper slopes of the size-spectra. However, contrarily to what could be expected from the theoretics on the response of size-spectra to impacts, no significant differences have been detected among size-spectra slopes of impacted and unimpaired sites, Δsize spectrum slope (obs-ref). This might be the result of two counteracting types of hydromorphological impacts, namely; (1) those driving mature fish communities in lower reaches (higher FRI values) to earlier succesional stages, and thus causing steeper slopes as expected (e.g. … [impacts that smoothen the slope]); and (2) those stabilizing the natural disturbances regimes driving pioneer fish communities with steep size-spectra slopes inhabiting upper reaches (lower FRI values) towards more mature communities showing slower life strategies (lower recruitment and lower mortality rates) (e.g. ... [impacts that steepen the slope]).

From a practical point of view, a measure of the change of this overall shape of size spectra [e.g. Δ*size spectrum (obs-ref)*)] might therefore be useful as a new potential metric for assessing impacts of HYMO pressures and restoration projects.

3.3 *The potential of a diatom trait based indicator to assess altered sediment dynamics*

3.3.1 Introduction

Excessive mobilisation and delivery of fine sediments to water bodies has detrimental impacts on those biotic elements used for waterbody status classification. Typically changes in the diatom assemblage (as either phytobenthos or phytoplankton) are used to assess the extent of stress from eutrophication (nutrient pollution as dissolved inorganic phosphorus or to a lesser extent dissolved inorganic nitrogen (e.g. Kelly & Whitton, 1995; Kelly et al., 2001)) acidification (Battarbee et al., 1999) or salinisation (van Dam et al., 1994). As increased delivery of fine sediment has the potential to impact diatom assemblages in many ways, it is not surprising that indices based on benthic diatom assemblage structure have been proposed. These comprise simply of the relative abundance of motile species (e.g. Bahls, 1993). This measure is based on the fact that many raphid species are capable of migrating through deposited sediment to avoid its negative impacts. However, the use of such an index has yet to be fully tested (Jones et al. 2014). Kelly et al. (2007) note that the gradient of TDI ecological quality ratio, indicating a shift from nutrient sensitive to nutrient tolerant taxa, is also correlated with a shift to motile taxa; whilst this may indicate a functional change in the assemblage, such change confounds interpretation when motility responds to nutrient availability rather than sediment load (and vice versa). Porter et al. (2008) reported that the abundance of taxa with continuously high dissolved oxygen requirements decreased significantly with increases in nutrient and suspended sediment concentrations, confounding interpretation of indices developed for the detection of organic pollution (Lowe, 1974; van Dam et al., 1994). Similarly, impacts of suspended sediment that do not entail burial (e.g. scouring) are not reflected in indices of motility and their effects may be interpreted as evidence of other stressors. Such issues are not exclusive to diatoms, but arise whenever biological quality elements are interpreted as change across a single gradient of pressure (Jones et al., 2010).

3.3.2 Methods

Data were compiled from surveys undertaken on behalf of the Welsh Government to assess the effectiveness of agri-environment schemes in Wales (Agri-environment Monitoring and Services Contract Lot 3 183/2007/08 and Axis II monitoring). Sites were scattered across Wales, covering a wide range of physico-chemical conditions. Representative samples of attached benthic algae were collected for the estimation of the relative abundance of diatom taxa following the DARES methodology. 5 replicate stones (or macrophytes where suitable stones were lacking) were randomly selected from the benthos and attached algae removed from the surface with a toothbrush and rinsed with algal free water into clean nalgene bottles. Samples were preserved with Lugol's iodine for identification. On return to the laboratory, samples were digested with hydrogen peroxide and mounted on microscope slides. The slides were examined under x 1000 magnification, with 300 diatom valves from random fields of view in each sample being identified to species level following Kelly & Yallop (2012). Data were summarised for each sample as a list of taxa present and their relative abundance (as percentage of valves counted). All taxa that were found in less than 3 % of samples were excluded from analyses. Data on the trait of interest (i.e. mobility) were acquired from Jones et al. (2014) and on nutrient affinity (TDI score) from Kelly & Yallop (2012).

The physical characteristics of each river reach from which diatom samples were collected was assessed either in the field or from maps, together with visual assessments of substrate composition as percentage cover of boulders/cobbles (>64 mm), pebbles/gravel (64 mm – 2 mm), sand (2 mm – <62.5 µm) and silt/clay (<62.5 µm). Conductivity and pH were determined in the field with dip probes. Nutrient concentrations were determined by standard analystical techniques on water samples collected at the time of sampling or modelled using frameworks capable of estimating pollutant loading from land use within each of the selected catchments (Anthony et al., 2012).

Data were analysed using partial ordination, which involved a two step process. The initial step in the analysis was to establish the relationship between diatom community composition and a number of candidate environmental variables characterising river condition and type. Here CCA was used to establish which environmental variables had a significant influence on the variation in diatom data and how much variation could be described by the variables used. The environmental variables offered to the analysis included physical (e.g. distance from source, altitude, slope, cross-sectional area) and chemical (nutrient concentrations, pH, alkalinity) and the percentage cover of fine sedimant (sand, silt and clay). Variables were selected from this suite sequentially for inclusion in the model after testing the significance of their influence using Monte Carlo simulation tests.

The next step in the analysis was to remove the influence of the environmental variables describing river type, leaving only the relationship between fine sediment and diatom taxa. This was done by including those variables describing river type which had a significant influence, as covariables. The variation in diatom taxa remaining is that which is explained by the amount of deposited fine sediment. In simple terms this analytical process is equivalent to establishing: “When all other things are equal, what is the response of diatoms to fine sediment?” The output of the analysis is a single ranking of sensitivity of taxa to fine sediment irrespective of river type. The distribution of the traits of interest, mobility and nutrient affinity, across this axis of fine sediment was then established.

3.3.3 Results

The unconstrained CCA indicated that alkalinity, percentage fine sediment cover, orthophsophate concentration and river slope at the site were best at describing the variation in the diatom taxa (Figure 3.3.1a). This does not imply that these are the drivers of change in the community, simply that they are the best statistically at describing the observed variation in the community (shown in Figure 3.3.1b). The resulting model with just these four variables could account for 9.2% of the variation in diatom taxa (see Table 3.3.1). Hence, alkalinity, percentage fine sediment cover, orthophsophate concentration and river slope at the site were used as covariables in the partial ordination, leaving only the influence of percentage fine sediment cover.

Table 3.3.1. Summary statistics for CCA relating diatom taxa to selected physical, geographical and chemical environmental variables.

Axes	1	2	3	4	Total inertia
Eigenvalues :	0.288	0.106	0.095	0.067	6.061
Species-environment correlations :	0.700	0.658	0.600	0.567	
Cumulative percentage variance					
of species data :	4.7	6.5	8.1	9.2	
of species-environment relation:	51.7	70.9	88.0	100.0	
Sum of all eigenvalues					6.061
Sum of all canonical eigenvalues					0.556

The first axis of the partial CCA (herafter pCCA) was correlated with percentage fine sediment cover; this axis explained 4.7% of the variation in the diatom taxa (Table 3.3.2). The distribution of the taxa along the 1st axis, an increasing gradient of percentage cover of fine sediment (including a line in the opposite direction from the intercept, which represents the average condition in the data set), was used to rank the diatom taxa from most to least sensitive to fine sediment (Figure 3.3.2). The contour gradients of mean percentage cover of fine sediment relate to the distribution of the diatom taxa (Figure 3.3.2 inset) in the same way (but note the scale difference). The taxa most strongly correlated with a low percentage cover of fine sediment were *Brachysira* (other species), *Frustulia krammeri*, *Nitzschia tubicola*, *Diadesmis contenta*, *Nitzschia gracilis*, and *Surirella crumena*, whilst those most strongly associated with a high cover of fine sediment were *Cocconeis* (other species), *Luticola mutica*, *Navicula* [small species], *Navicula capitatoradiata* and *Gyrosigma acuminatum*.

Table 3.3.2. Summary statistics for pCCA relating diatom taxa to percentage fine sediment cover.

Axes	1	2	3	4	Total inertia
Eigenvalues :	0.270	0.702	0.446	0.381	6.061
Species-environment correlations :	0.698	0.000	0.000	0.000	
Cumulative percentage variance					
of species data :	4.7	16.8	24.6	31.2	
of species-environment relation:	100.0	0.0	0.0	0.0	
Sum of all eigenvalues					5.774
Sum of all canonical eigenvalues					0.270

Despite there being a strong influence of percentage cover of fine sediment on diatom community composition, motility appeared to be distributed across the whole of the gradient of fine sediment. Whilst the taxa associated with a high percentage cover of fine sediment were mostly motile, so were the taxa associated with a low percentage cover of fine sediment. Both motile and non-motile taxa were found throughout the gradient of percentage fine sediment cover. Nutrient affinity appeared to have some realtionship with the gradient of percentage fine sediment cover, with higher scoring (higher affinity to nutrients) taxa tending to have an association with a high percentage cover of fine sediment. However, nutrient affinity was scattered across the gradient of percentage cover of fine sediment. It is not surprising that the association between the trait of nutrient affinity and the gradient of percentage cover of fine sediment was not strong, as orthophosphate was one of the covariables used in the pCCA.

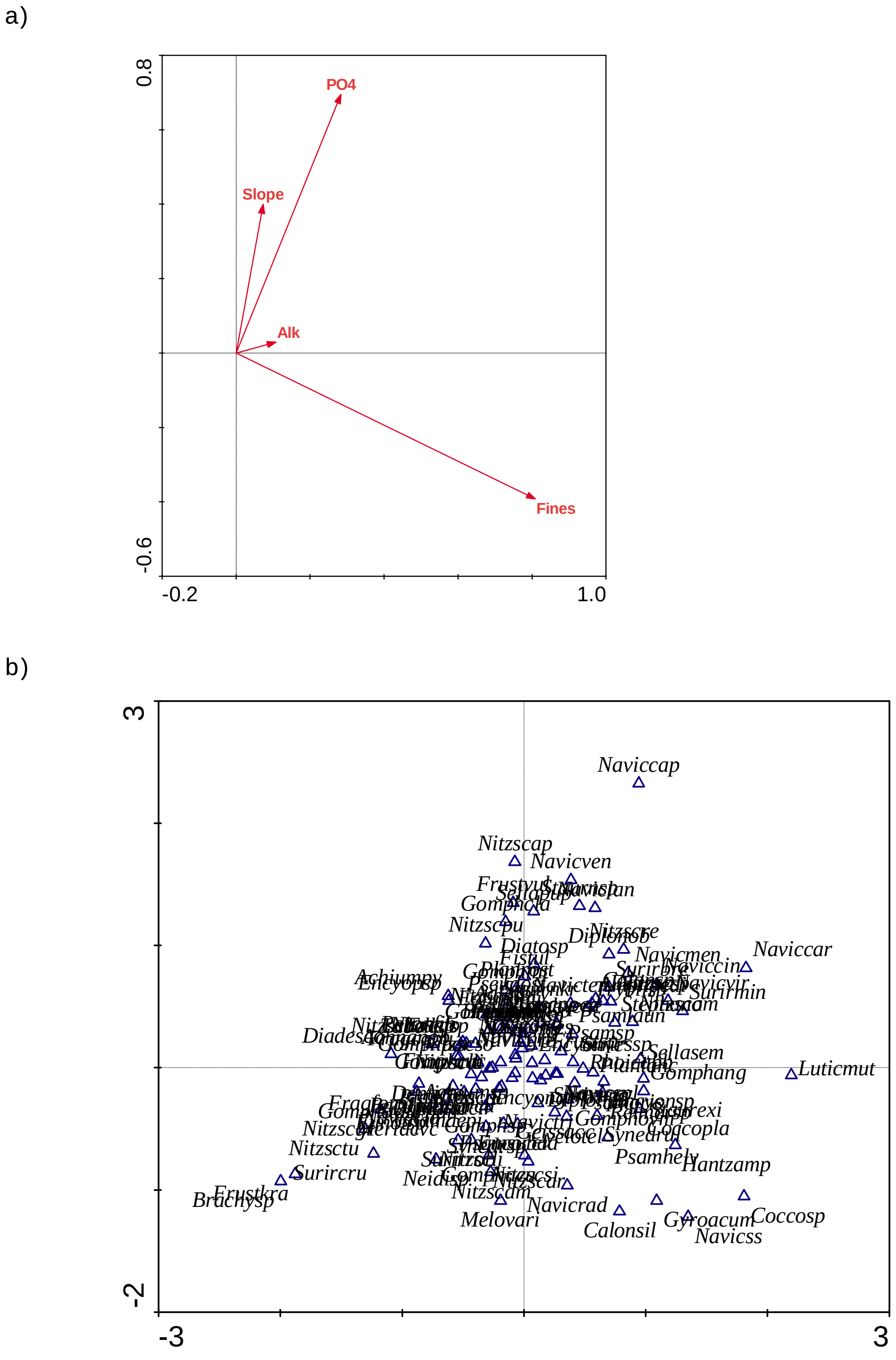


Figure 3.3.1. Ordination plot from unconstrained CCA showing a) relationships amongst the environmental variables and b) distribuition of diatom taxa. Alk = alkalinity, Fines = percentage fine sediment cover (sand, silt & clay), PO4 = orthophsophate concentration, and Slope = river slope at site.

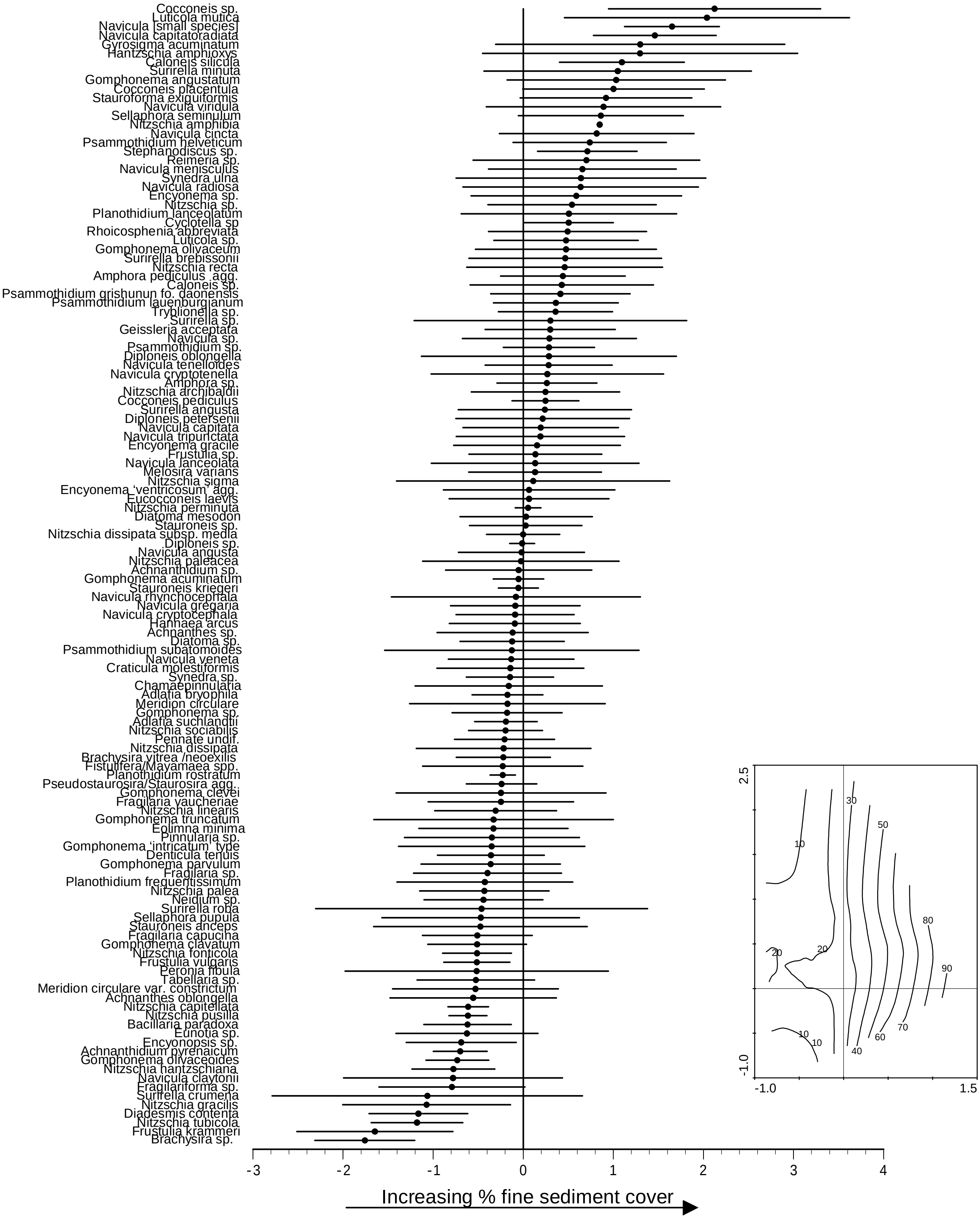


Figure 3.3.2. Optimum (point) and amplitude (line) of diatom taxa along the first canonical axis of pCCA, correlated with increasing % fine sediment cover. Taxa are ranked from least sensitive to most sensitive to fine sediment (top to bottom). Inset shows contour gradients of percentage fine sediment cover through pCCA ordination space.

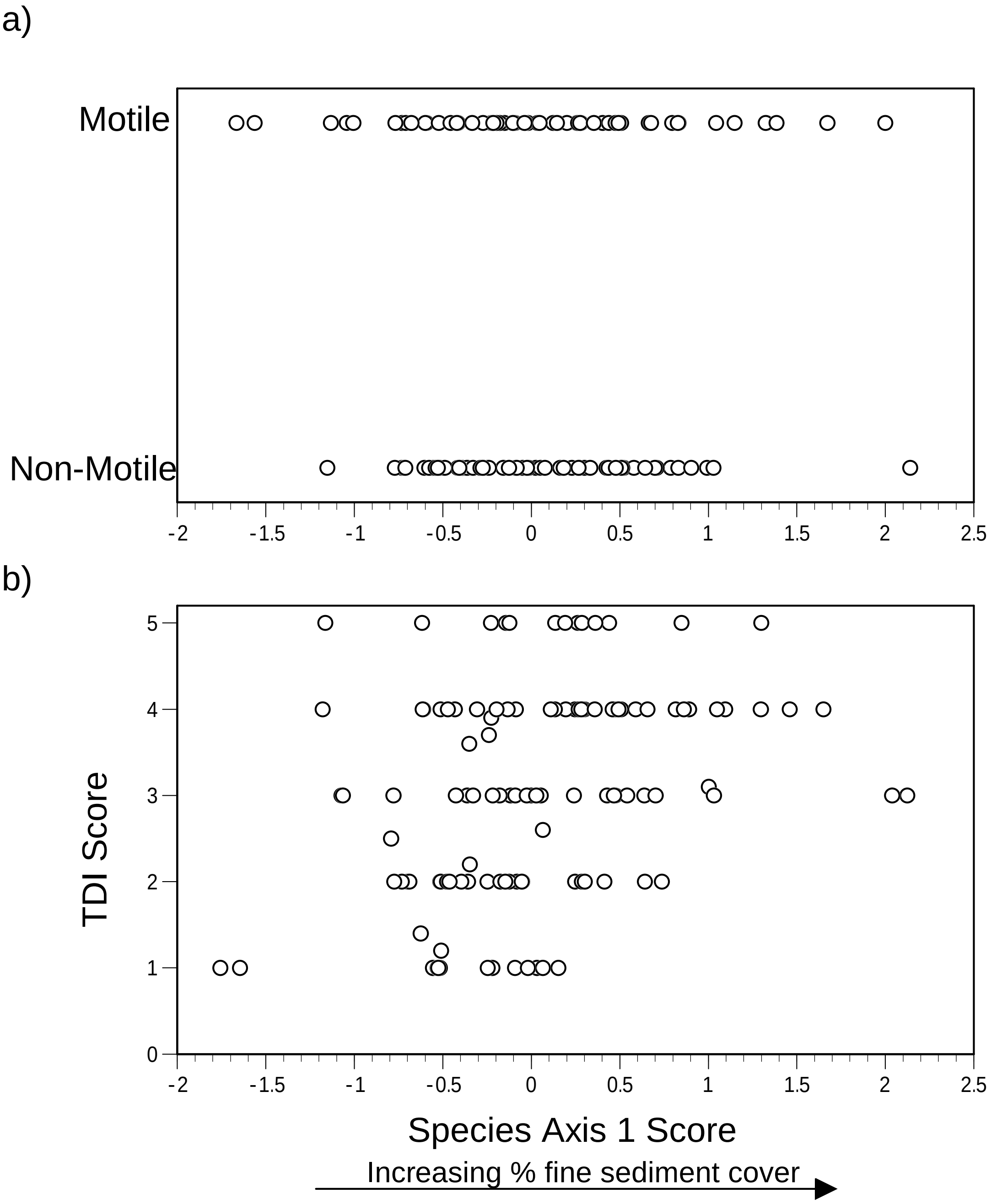


Figure 3.3.3. Distribution of two diatom traits, a) motility and b) nutrient affinity (as TDI score) along the first canonical axis of pCCA, correlated with increasing % fine sediment cover. The optima of taxa, and their corresponding trait characteristic, are plotted by their pCCA axis 1 scores.

3.3.4 Discussion

As excessive mobilisation and delivery of fine sediments to water bodies are likely to result in increased rates of siltation (deposition of fine sediment), and hence burial of benthic diatoms, it has been suggested that the relative abundance of motile taxa could be used as an index of fine sediment pressure (e.g. Bahls, 1993). Many raphid taxa are capable of motility, and this trait could provide an advantage if motile species are capable of migrating through deposited sediment to avoid the negative impacts of burial. Indeed, many species that live in fine grained sediments undergo migrations to and from the sediment-water interface (Harper, 1976; Hay et al., 1993). However, burial is not the only consequence of pressure from fine sediment for diatoms. Diatoms may suffer from damage to frustules as the result of abrasion or may benefit where they can exploit nutrients released from deposited fine sediments. Hence, diatoms may exploit a range of strategies to cope with the pressure of increased fine sediment (such as rapid growth, small size or damage resistant frustules). Correspondingly, motility may provide taxa with an advantage under a variety of other environmental conditions. Here we have collated data to explore the relationship between fine sediment and two traits of diatoms, motility and nutrient affinity.

Percentage cover of fine sediment had a strong influence on diatom community composition, and it was possible to rank the taxa according to their affinity to this gradient. Despite this, motility did not show a strong association with axis 1 of the partial CCA, describing the gradient of precentage cover of fine sediment. Although the taxa associted with a high percentage cover of fine sediment were mainly motile, so were taxa associated with the opposite extreme and motile taxa were found throughout the gradient. It appears that motility is a trait characteristic of taxa associated with a wide range of fine sediment conditions and cannot be reliably attributed to any part of the gradient of sediment pressure. Hence, it is not recommended that % motile is used as an index of fine sediment. This is not surprising as motility provides advantages under a range of conditions; examples include avoiding competition for light from other taxa, avoiding photo-damage or migration towards patches of high nutrient availability. Furthermore, a number of strategies may provide advantages to diatoms under high sediment conditions. A more thorough compilation of diatom morphological and (if possible) physiological traits would be an advantage to understanding how diatoms respond to environmental variation.

Although diatom community composition responded to the percentage cover of fine sediment, nutrient affinity appeared to be only weakly associated with axis 1 of the pCCA. Whilst this is not surprising as orthophosphate was one of the covariables in the analysis, it does suggest that the TDI scoring system is robust to variation in precentage cover of fine sediment. Here we have tried to separate the gradient of nutrient availability from that of percentage cover of fine sediment. Nevertheless, sites with fine grained bed sediments will inherently tend to have higher nutrient availability at the sediment water interface due to the higher geochemical activity of small particles, and nutrient re-release from anoxic silts and muds. This association between bed composition and nutrient availability has lead to concerns that fine sediments could confound diatom indices of nutrient availability: our results suggest this is not the case.

Despite the lack of a relationship between motility and percentage cover of fine sediment, the strong influence of fine sediment on diatom community composition indicates the potential for a robust metric relating diatoms to fine sediment. Here, with we have refrained from constructing an index as we had limited data on which to base the scoring of taxa. Taxa could be scored following a fuller analysis, encompassing a wider range of taxa and sites, provided careful selection of sites not considered to be affected by any other stresses other than sediment inputs, and to provide an even distribution of sites across a wide gradient of pressure from fine sediment. An uneven distribution of sites across the pollution gradient causes bias in the results: If stressed sites are over-represented there is an increased probability of taxa being described as having a restricted distribution simply because by chance they are more likely to occur at this end of the gradient. Although this screening procedure here reduced the total amount of data used in the analyses, it increased the likelihood of a statistically significant (and real) result being found. Inclusion of data that failed to pass the criteria would introduce confounding variables, reduce the likelihood of a statistically significant result and increases the probability of committing a type I error (accepting a difference that is not real).

3.4 *The potential of macroinvertebrate trait based indicators to assess altered sediment dynamics*

3.4.1 Introduction

While there is a reasonably good understanding of the response of river macroinvertebrate community structure to fine sediment stress (Wood & Armitage, 1997; Jones et al., 2012; Relyea et al., 2012; Extence et al., 2013), there has been less focus on how macroinvertebrate biological trait characteristics are affected by excessive fine sediment deposition. It is likely that the response of the macroinvertebrate community to such stress will be mediated through their intrinsic biological traits, with some taxa being more susceptible to the chemical changes associated with organically rich fine sediment, and others being more susceptible to the physical impacts of mineral fines (Culp et al., 1986). Focussing on the biological trait response could provide the basis for a widely applicable diagnostic index to fine sediment impacts as opposed to the composition-based indices that can be limited to the original development biogeographic region.

Firstly, as part of REFORM Deliverable 3.1 we compiled macroinvertebrate trait data for an existing spatially extensive dataset. This dataset originated from a UK Government-funded project seeking to extend the evidence base on ecological impacts of fine sediment on freshwaters. The dataset was interrogated, as part of REFORM Deliverable 3.1, from a biological trait perspective, with the aim of identifying suites of traits that were associated with fine sediment stress, and conversely those associated with low stress conditions. While the analyses did identify potential indicator traits e.g. ovoviviparity, the laying of isolated cemented eggs, active aerial disperal, and crawling, there was no robust statisitical test of these associations.

We are now aiming to revisit these data to extend and hopefully improve the trait analysis by use of the Fourth-corner method (Legendre et al., 1997; Dray & Legendre, 2008; ter Braak et al., 2012) and a new approach that combines the complimentary outputs of RLQ and Fourth-corner analyses to allow better interpretation of the observed patterns focusing on those confirmed to be statistically significant (Dray et al., 2014).

Secondly, we will also revisit the macroinvertebrate community data from the flow-fine silt manipulative experiments detailed in Section 1.2. We will analyse these data from a biological trait perspective using the same suite of approaches as applied to the spatially extensive correlative dataset with the objective to experimentally confirm that those traits identified in the latter analysis to be associated with high/low stress conditions are actually responding to the fine sediment stress gradient.

3.4.2 Methods

Spatially extensive dataset

In total, 205 stream sites across England and Wales were sampled between spring 2010 and autumn 2011 (Figure 3.4.1). Each site was on an independent watercourse, was sampled once with a sample of the macroinvertebrate community and deposited fine sediment being collected. These sites were selected from a larger pool of sites each of which was confirmed to be:

- not impacted by sewage inputs
- not to have large urban areas in the catchment
- not to have upstream reservoirs or lakes

The delivery of fine sediment from the catchment to each river sites was modeled using PSYCHIC, a process-based model of suspended sediment mobilisation in land run-off and subsequent delivery to watercourses (Davison et al., 2008). Based on these data we only considered sites with predominantly (>75%) agricultural fine sediment sources.

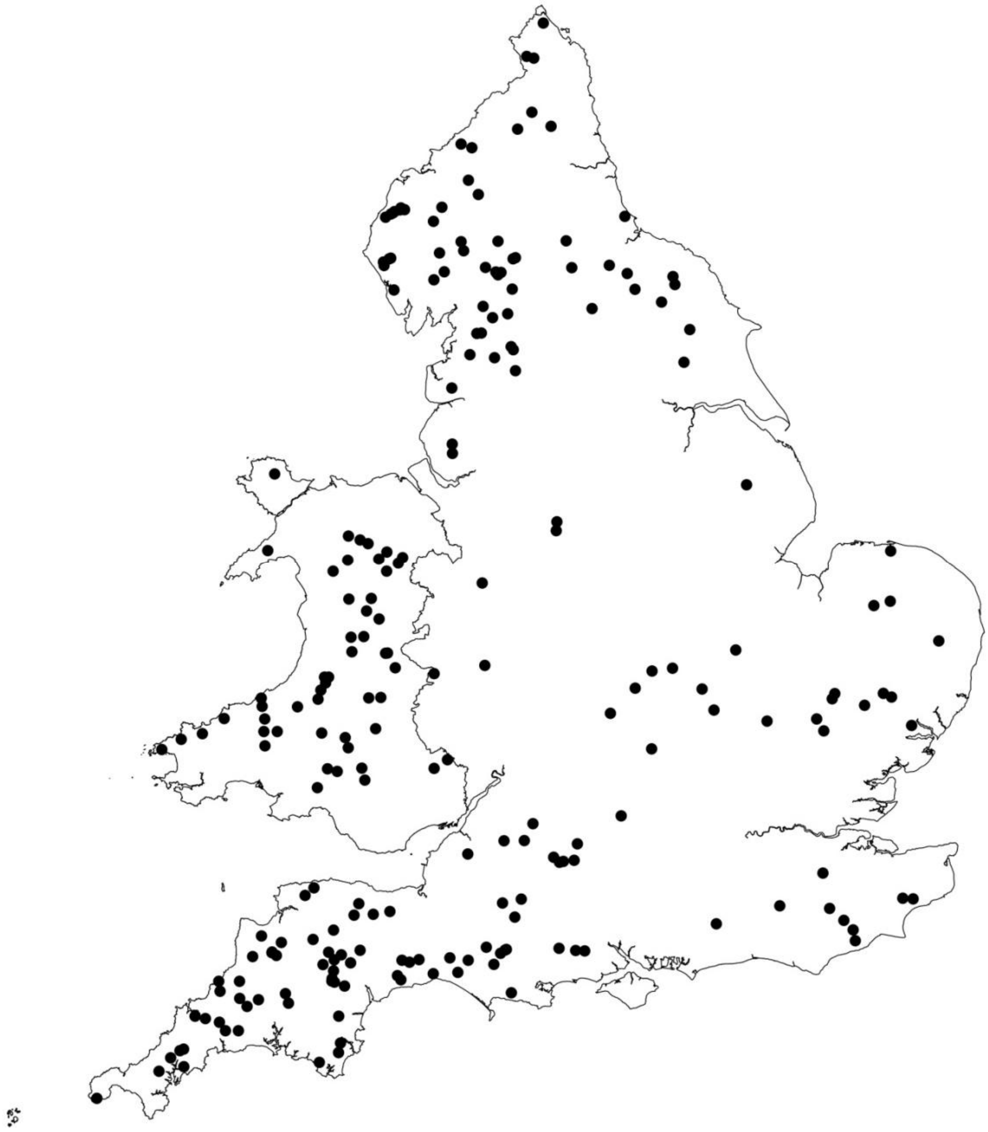


Figure 3.4.1. Location of sampled stream sites across England and Wales.

To ensure that the sampled macroinvertebrate communities came from as wide a range of natural river types as possible, within the limits set by the other site selection criteria, each site was allocated to one of four approximate stream types based on four map-based physical variables; their catchment geology, distance from source (km), altitude (m asl) and slope (m km^{-1}). The boundary values for this guideline stream typology were loosely based on those associated with the seven RIVPACS IV super end groups (Davy-Bowker et al., 2008). The fundamental aim was to ensure as equal a sampling effort as possible across the fine sediment stressor gradient for each broad stream type, thus ensuring that a representative sample of streams was included in the study where fine sediment pressure was the main driver of differences in species occurrence.

Macroinvertebrates were sampled at each site using the RIVPACS method; a standard three-minute kick/sweep and one minute search sample with a pond net (1 mm mesh-size) (Furse et al., 1981; Murray-Bligh et al., 1997). A field measurement of pH and conductivity was also taken (Hanna Instruments Combo HI98129). Associated RIVPACS environmental variables were recorded either at the site (stream width and depth, velocity, substrate composition), or from map-based data (discharge category, altitude, distance from source and slope). Macroinvertebrate community samples returned to the laboratory for subsequent identification and quantification to the lowest practicable taxonomic level.

Fine sediment deposits on the stream bed were quantified immediately upstream of the macroinvertebrate sampling area using the disturbance technique described in Lambert and Walling (1988) and refined by Collins and Walling (2007). Here a steel cylinder (height 75 cm, diameter 48.5 cm) was inserted into an undisturbed section of the stream bed and the water column vigorously agitated for one minute, without touching the stream bed, to raise any fine sediment deposited on the surface of the stream bed. A pair of water samples was then collected quickly from within the cylinder. The stream bed was then disturbed to a depth of approximately 10 cm, and the water and bed vigorously agitated for one minute to raise any sub-surface fine sediment in addition to the re-suspended surface deposits. A second pair of water samples was then collected from within the cylinder. Four such sets of water samples (surface, and combined surface and subsurface) were collected from each site, two from erosional patches and two from depositional patches. The samples were then refrigerated and returned to the laboratory within 5 days, where they were processed for dry mass and organic content (i.e. volatile solids following combustion at 550°C). The particle size distribution of material <1mm diameter was also measured using a Malvern Mastersizer 2000. Reach-averaged values for surface and total (combined surface and subsurface) deposited fine sediment were subsequently derived.

In summary, for each site, there was an estimate of the quantity of fine sediment being delivered from the catchment (kg ha^{-1} yr^{-1}), derived from the PSYCHIC model, as well as actual measurements of deposited fine sediment mass and composition and the in-stream biological community.

Table 3.4.1. Environmental variables recorded at each of the 205 sites in the spatially extensive dataset.

Variable type	Environmental variable	Abbreviation
Natural environmental variable	Discharge Category (long-term historical average discharge; 1-10) (1= <0.31, 2= 0.31-0.62, 3= 0.62-1.25, 4= 1.25-2.5, 5= 2.5-5.0, 6= 5-10, 7= 10-20, 8= 20-40, 9= 40-80, 10= 80-160 m^3s^{-1})	DISCHCAT
	Distance from source (km) (log-transformed)	DISTSOU
	Altitude of site (masl) (log-transformed)	ALTITUDE
	Slope of site ($m.km^{-1}$) (log-transformed)	SLOPE
	Catchment area (km^2) (log-transformed)	CatcArea
	Stream width (m) (log-transformed)	WIDTH
	Stream depth (cm) (log-transformed)	DEPTH
Measured fine sediment variables	Geomean total sediment mass (log-transformed)	SedMass
	Geomean Depositional area sediment mass (log-transformed)	DpSedMas
	Geomean Erosional area sediment mass (log-transformed)	ErSedMas
	Geomean total organic mass (log-transformed)	VsMass
	Geomean Depositional area organic mass (log-	DpVsMas

Variable type	Environmental variable	Abbreviation
	transformed)	
	Geomean Erosional area organic mass (log-transformed)	ErVsMass
	Mean % organic (log-transformed)	PctOrg
	Mean Depositional area % organic (log-transformed)	DpPctOrg
	Mean Erosional area % organic (log-transformed)	ErPctOrg
	% by volume of particles in sand size category	PctSa
	% by volume of particles in silt size category	PctSi
	% by volume of particles in clay size category	PctCl
Modelled fine sediment inputs	PSYCHIC estimate of local bank erosion fine sediment load to site from catchment (kg/ha/yr) (log x+1-transformed)	LBESedLd
	PSYCHIC estimate of agricultural fine sediment load to site from catchment (kg/ha/yr) (log x+1-transformed)	AgSedLd

Manipulative experiment dataset

Details of the experimental manipulation of stream bed fines and flow rates to examine their individual and joint effects on benthic macroinvertebrate communities are given in Section 1.2. In summary, 12 replicate flumes (arranged in four blocks of three) containing 20cm depth of local alluvial gravels, were each assigned to one of two initial flow treatments; slow or fast flowing. The downstream half of each flume was also assigned to one of two fine sediment treatments; no fine sediment applied or fine sediment applied to achieve 10% of sediment weight. After 40 days, flow treatments in six out of twelve of the flumes were reversed; three of the higher flow flumes were altered to the low flow rate and three of the slower flow flumes were increased to the high flow rate (see Table 3.4.1). Therefore the experiment consistent of 24 experimental areas each assigned to one of eight treatments (Table 3.4.2). Benthic macroinvertebrates were sampled in all experimental areas prior to the flow alterations. The experiment was concluded after 70 days when the benthic macroinvertebrate community was sampled again in each experimental area. Macroinvertebrates were sampled using 25 x 25 cm Surber sampler, with the substrate disturbed to a depth of approximately 3 cm, at day 30 and day 70. Six randomly placed Surber samples were taken from of each 4m sediment-type section in each flume. Specimens were identified to family-level with the exception of Chironomidae larvae which were taken to subfamily, mites, nematodes and Ostracoda to Order, and Oligochaeta to Class.

Table 3.4.2. Assignment of treatment to experimental areas within flumes.

Flume	Experimental Area	Initial Flow Treatment	Day 40 Flow Treatment	Sediment treatment
A	1	F	F	No sediment added
A	13	F	F	Sediment added
B	2	S	S	No sediment added
B	14	S	S	Sediment added
C	3	F	S	No sediment added
C	15	F	S	Sediment added
D	4	S	F	No sediment added
D	16	S	F	Sediment added
E	5	F	F	No sediment added
E	17	F	F	Sediment added
F	6	S	S	No sediment added
F	18	S	S	Sediment added
G	7	F	S	No sediment added
G	19	F	S	Sediment added
H	8	S	F	No sediment added
H	20	S	F	Sediment added
I	9	F	F	No sediment added
I	21	F	F	Sediment added
J	10	S	S	No sediment added
J	22	S	S	Sediment added
K	11	F	S	No sediment added
K	23	F	S	Sediment added
L	12	S	F	No sediment added
L	24	S	F	Sediment added

Compilation of biological trait data

Three existing freshwater macroinvertebrate species trait resources were used to gather available biological trait information.

- www.freshwaterecology.info
- French Genus Trait Database (Tachet et al., 2000)
- Hyperheos trait data (Descloux et al., 2014)

The first was originally compiled as part of the EU FP5-funded AQEM project and supported and further developed by subsequent EU-funded projects; STAR (FP5), Euro-limpacs (FP6), BIOFRESH and REFRESH (both FP7). The second dataset was compiled by French biologists for those taxa found in French waters; many of which are also found in the UK. The third resource was compiled by P. Marmonier and made available through Descloux et al. (2014). The French data was the primary source of information and was supplemented with information from the other two resources for those taxa or traits that were not included in the French database.

Each biological trait was described by several trait-classes. The trait characteristics of each taxon were scored by assigning a value to each trait-class reflecting the affinity of the taxon to the trait-class. Scores ranged from 0 to 5 indicating no to high affinity respectively (Chevenet et al., 1994). The way traits were scored differed between the two databases, as did the range of trait-classes for some traits. For those traits that did match (life cycle duration, resistance form, reproduction, dispersal, respiration) we identified the taxa in freshwaterecology.info that were not already included in the French data. These data were then appended to the French data to create the final trait dataset. As the two datasets use a different scoring method, we also had to convert the freshwaterecology.info 1-10 method to the French 1-5 method by simply converting 5s to 3s and 10s to 5s, leaving 1s as 1s.

The spatially extensive dataset contained information on 10 biological traits for 192 distinct taxa and the experimental dataset contained information on 7 biological traits for 52 distinct taxa (Table 3.4.3).

Table 3.4.3. Biological traits used in the analyses of the spatially extensive dataset, with their associated trait classes. Traits analysed in experimental dataset are also indicated.

TRAIT	TRAIT-CLASS	Abbreviation	Used in experimental dataset
Maximal potential size	⩽ .25 cm	MaxS_25cm	x
	> .25-.5 cm	MaxS_5cm	x
	> .5-1 cm	MaxS_1cm	x
	> 1-2 cm	MaxS_2cm	x
	> 2-4 cm	MaxS_4cm	x
	> 4-8 cm	MaxS_8cm	x
	> 8 cm	MaxSm8cm	x
Life cycle duration	⩽ 1 year	Lcyc_m1	
	> 1 year	Lcyc_l1	
Potential number of cycles per year	< 1	Pcyc_lt1	x
	1	Pcyc_1	x
	> 1	Pcyc_gt1	x
Aquatic stages	egg	AqSt_eg	
	larva	AqSt_la	
	nymph	AqSt_ny	
	adult	AqSt_ad	
Reproduction	ovoviviparity	Repr_ovo	x
	isolated eggs, free	Repr_ief	x
	isolated eggs, cemented	Repr_iec	x
	clutches, cemented or fixed	Repr_ccf	x
	clutches, free	Repr_cfr	x
	clutches, in vegetation	Repr_cvg	x
	clutches, terrestrial	Repr_ctr	x
	asexual reproduction	Repr_asr	x
	parthenogenesis	Repr_par	x
Dispersal	aquatic passive	Disp_aqp	
	aquatic active	Disp_aqa	
	aerial passive	Disp_aep	
	aerial active	Disp_aea	
Resistance forms	eggs, statoblasts	Rest_egg	x
	cocoons	Rest_coc	x

TRAIT	TRAIT-CLASS	Abbreviation	Used in experimental dataset
	housings against desiccation	Rest_hou	x
	diapause or dormancy	Rest_dia	x
	none	Rest_non	x
Respiration	tegument	Resp_teg	x
	gill	Resp_gil	x
	plastron	Resp_pla	x
	spiracle	Resp_spi	x
Locomotion and substrate relation	flier	Loco_fli	x
	surface swimmer	Loco_ssw	x
	full water swimmer	Loco_swi	x
	crawler	Loco_crw	x
	burrower	Loco_bur	x
	interstitial	Loco_int	x
	temporarily attached	Loco_tpa	x
	permanently attached	Loco_pat	x
Food	microorganisms	Food_mio	x
	detritus (< 1mm)	Food_det	x
	dead plant (>= 1mm)	Food_dep	x
	living microphytes	Food_mip	x
	living macrophytes	Food_map	x
	dead animal (>= 1mm)	Food_dea	x
	living microinvertebrates	Food_mii	x
	living macroinvertebrates	Food_mai	x
	vertebrates	Food_vrt	x

Data Analysis

Spatially-extensive dataset

Previously the spatially extensive dataset had been analysed using redundancy analysis and RLQ methods (See REFORM Deliverable 3.1). Here our objective was to advance this work by applying Fourth-corner analysis and then a novel RLQ-Fourth-corner combined analysis to statistically test the significance of apparent associations between traits and environmental variables.

RLQ ordination assigns scores to species, samples, traits, and environmental variables along orthogonal axes allowing the plotting of graphical summaries of the main structures. It does not however allow for any robust statistical test of the significance of any evident trait environmental variable relationships. On the other hand, the Fourth-corner approach measures and tests the correlation between each trait/trait-class and each environmental variable but does not take into account any potential covariance between traits or between environmental variables (Dray & Legendre, 2008; Dray et al., 2014). The significance of correlations were tested using the combined results of 4999 permutations of sites and 4999 permutations of species, with *P*-values adjusted for multiple comparisons using the false discovery rate method (Benjamini & Hochberg, 1995).

By applying the Fourth-corner approach directly to the outputs from RLQ as opposed to the original R (environmental variables), L (community data) & Q (trait data) tables, we can test the correlations between each trait or trait-class and each RLQ axis and the correlations between each environmental variable and each RLQ axis. This combined approach is used to maximise the complementarity between these two methods and improve our interpretation of trait–environment relationships.

Manipulative experiment dataset

The benthic macroinvertebrate data from the flow-fine sediment experiment were also analysed using the RLQ - Fourth-corner combined approach to identify correlations between trait-classes and treatments. In addition, treatment main and interaction effects on the prevalence of trait-classes were quantified using redundancy analysis on a matrix of the relative prevalence of each trait-class (within each of the 7 traits) in each of the 48 samples. This latter table was created by log-transformed abundance weighting the taxon scores for each trait-class for a given sample. The sums of weighted scores (one per trait-class) were then expressed as the relative abundance distribution (within a trait), giving the site trait profile (Archaimbault et al. 2010). In particular we wished to test whether the trait assemblage was significantly altered by an increase in benthic fine sediment and whether the extent of change was dependent on the preceding flow conditions.

RLQ and Fourth-corner analyses were undertaken using R 3.1.1 (R Core Team, 2014) with the additional ade4 package (Dray & Dufour, 2007). Redundancy analyses were carried out using Canoco 4.5 (ter Braak & Šmilauer, 2002).

3.4.3 Results

Spatially-extensive dataset

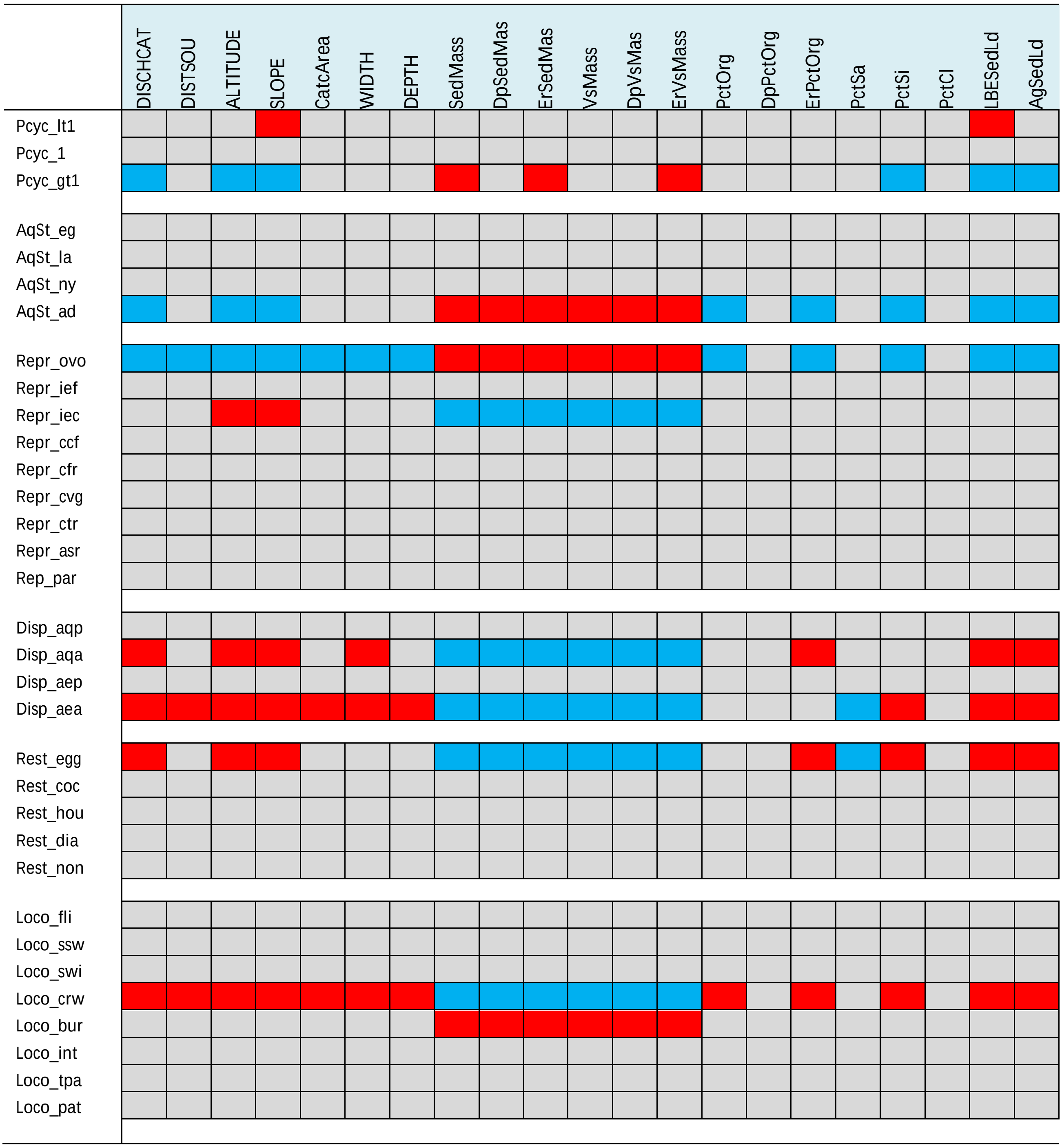
In total, 126 out of the 1134 pairwise correlations between trait-classes and environmental variables were found by Fourth-corner analysis to be statistically significant (11%), involving 10 of the 54 trait classes across 6 traits. Only % organic fines in depositional habitats (DpPctOrg) and % Clay (PctCl) were found to be uncorrelated with any trait-class (Table 3.4.4).

An increase in the mass of fine sediment in the stream bed was associated with a significant increase in the prevalence of adult aquatic stages, ovoviviparity, multiple life cycles within a year, and burrowing. An increase in mass of fine sediment in the stream bed was also associated with a significant decrease in the prevalence of active aerial and aquatic dispersal, the laying of isolated cemented eggs, egg aquatic stages, and crawling (Table 3.4.4). The traits maximum potential size, life cycle duration, mode of respiration and food were not related to any measure of benthic fine sediment stress.

The results of the combined RLQ-Fourth-corner analysis are presented in Table 3.4.5. They illustrate that there is a significant positive correlation between RLQ-axis1 and prevalence of >1 life cycle per year, adult aquatic stage, ovoviviparity and burrowing. RLQ-axis1 was also positively correlated with many measures of benthic fine sediment mass, and negatively correlated with organic content of fine sediment (PctOrg) and modelled delivery of fine sediment to the reach (Table 3.4.5). A significant negative relationship was found between RLQ-axis1 and ⩽1 life cycles per year, egg aquatic stage, isolated, cemented, eggs, aquatic dispersal, active aerial dispersal, egg resistance stage and crawling. The much less pronounced RLQ-axis2 was positively associated with gill respiration and negatively associated with laying eggs in terrestrial clutches.

RLQ-axis1 was also negatively correlated with variables describing river size e.g. discharge category, distance from source, catchment area, width and depth, but counter-intuitively also with altitude and slope, variables that decrease in magnitude as river size increases (Table 3.4.5). However, these latter two variables were distinguished from the other river-size variables by RLQ-axis2.

Table 3.4.4. Results of Fourth-corner analysis of spatially extensive dataset. Only traits with significant correlations to environmental variables are shown. Significant (*P*<0.05) positive associations are represented by red cells, and significant negative associations correspond to blue cells. Non-significant associations are in grey. *P*-values were adjusted for multiple comparisons using the false discovery rate procedure. Codes for environmental variables and traits are explained in Tables 3.4.1 and 3.4.3.



	DISCHCAT	DISTSOU	ALTITUDE	SLOPE	CatcArea	WIDTH	DEPTH	SedMass	DpSedMas	ErSedMas	VsMass	DpVsMas	ErVsMass	PctOrg	DpPctOrg	ErPctOrg	PctSa	PctSi	PctCl	LBESedLd	AgSedLd
Pcyc_lt1	⬜	⬜	⬜	🟥	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	⬜
Pcyc_1	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Pcyc_gt1	🟦	⬜	🟦	🟦	⬜	⬜	⬜	🟥	⬜	🟥	⬜	⬜	🟥	⬜	⬜	⬜	⬜	🟦	⬜	🟦	🟦
AqSt_eg	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
AqSt_la	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
AqSt_ny	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
AqSt_ad	🟦	⬜	🟦	🟦	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟦	⬜	🟦	⬜	🟦	⬜	🟦	🟦
Repr_ovo	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟥	🟥	🟥	🟥	🟥	🟥	🟦	⬜	🟦	⬜	🟦	⬜	🟦	🟦
Repr_ief	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_iec	⬜	⬜	🟥	🟥	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_ccf	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_cfr	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_cvg	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_ctr	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Repr_asr	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Rep_par	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Disp_aqp	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Disp_aqa	🟥	⬜	🟥	🟥	⬜	🟥	⬜	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	🟥	⬜	⬜	⬜	🟥	🟥
Disp_aep	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Disp_aea	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	⬜	🟦	🟥	⬜	🟥	🟥
Rest_egg	🟥	⬜	🟥	🟥	⬜	⬜	⬜	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	🟥	🟦	🟥	⬜	🟥	🟥
Rest_coc	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Rest_hou	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Rest_dia	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Rest_non	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_fli	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_ssw	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_swi	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_crw	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟥	⬜	🟥	⬜	🟥	⬜	🟥	🟥
Loco_bur	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_int	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_tpa	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Loco_pat	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜

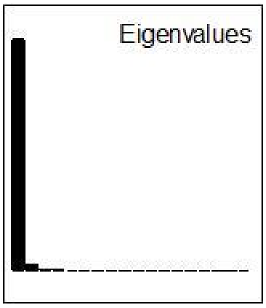
Table 3.4.5. Results of combined RLQ and Fourth-corner analysis of spatially extensive dataset. (a) Fourth-corner tests between the first two RLQ axes for environmental variables (RLQ-R1/R2) and traits. Only traits with significant correlations to RLQ axes are shown. (b) Fourth-corner tests between the first two RLQ axes for traits (RLQ-Q1/Q2) and environmental variables. Significant (*P*<0.05) positive and negative associations are represented by red and blue cells respectively. Non-significant associations are in grey. *P*-values were adjusted for multiple comparisons using the false discovery rate procedure. RLQ axes eigenvalues are also displayed.

(a)

	RLQ-R1	RLQ-R2
Pcyc_lt1	🟦	⬜
Pcyc_1	🟦	⬜
Pcyc_gt1	🟥	⬜
AqSt_eg	🟦	⬜
AqSt_la	⬜	⬜
AqSt_ny	⬜	⬜
AqSt_ad	🟥	⬜
Repr_ovo	🟥	⬜
Repr_ief	⬜	⬜
Repr_iec	🟦	⬜
Repr_ccf	⬜	⬜
Repr_cfr	⬜	⬜
Repr_cvg	⬜	⬜
Repr_ctr	⬜	🟦
Repr_asr	⬜	⬜
Rep_par	⬜	⬜
Disp_aqp	🟦	⬜
Disp_aqa	🟦	⬜
Disp_aep	⬜	⬜
Disp_aea	🟦	⬜
Rest_egg	🟦	⬜
Rest_coc	⬜	⬜
Rest_hou	⬜	⬜
Rest_dia	⬜	⬜
Rest_non	⬜	⬜
Resp_teg	⬜	⬜
Resp_gil	⬜	🟥
Resp_pla	⬜	⬜
Resp_spi	⬜	⬜
Loco_fli	⬜	⬜
Loco_ssw	⬜	⬜
Loco_swi	⬜	⬜
Loco_crw	🟦	⬜
Loco_bur	🟥	⬜
Loco_int	⬜	⬜
Loco_tpa	⬜	⬜
Loco_pat	⬜	⬜

(b)

	RLQ-Q1	**RLQ-Q2**
DISCHCAT	🟦	⬜
DISTSOU	🟦	🟥
ALTITUDE	🟦	⬜
SLOPE	🟦	🟦
CatcArea	🟦	🟥
WIDTH	🟦	⬜
DEPTH	🟦	🟥
SedMass	🟥	⬜
DpSedMas	🟥	⬜
ErSedMas	🟥	⬜
VsMass	🟥	⬜
DpVsMas	🟥	⬜
ErVsMass	🟥	⬜
PctOrg	🟦	🟦
DpPctOrg	⬜	🟦
ErPctOrg	🟦	🟦
PctSa	🟥	🟥
PctSi	🟦	⬜
PctCl	⬜	🟦
LBESedLd	🟦	⬜
AgSedLd	🟦	⬜



Manipulative experiment dataset

No significant associations were found between any of the trait-classes and the RLQ axes. Only three of the 16 treatment combinations were significantly associated with RLQ axes; RLQ-axis1 being positively associated with the first sampling of the slow-slow, no-sediment treatment and negatively associated with the second sampling of the fast-slow, sediment treatment. However the combined RLQ – Fourth-corner analysis could not consider interactions between treatments and as such may not have been the most appropriate analytical approach for the experimental dataset.

A better approach was to use a series of redundancy analyses (RDA), as set out in ter Braak & Šmilauer (2002), to test the significance of different treatment effects and their interaction. Having factored out the effect of time of sampling, there was a small (14% of variation) but significant flow effect and a small (3% of variation) but significant sediment effect on the macroinvertebrate trait assemblage but no interaction between the two treatments (Table 3.4.6, Figure 3.4.2). Over 40% of the variation in trait assesmblage was unaccounted for. The short length of the arrows and their proximity to the origin relative to the spread of trait scores indicates that the flow and sediment manipulations had small effects on macroinvertebrate trait assemblage. It is therefore not possible to confidently note the traits/trait-classes associated with high and low levels of benthic fine sediment. Nevertheless, trait-classes such as consumption of fine detritus, micro and macrophytes, temporary attacment and maxiumum size between 0.5-1 cm seemed to be associated with sediment addition areas (Figure 3.4.2). It is also clear from the relative positions of the four flow treatments that there was a logical pattern, with the initial slow-flowing flumes tending to the negative half of RDA axis 1 and the initial fast-flowing flumes tending to the positive half (Figure 3.4.2). Variation between flow treatments was greater than that between sediment treatment. The fine sediment manipulation had the most pronounced effect on the fast-fast flow treatment.

Table 3.4.6. Analysis of variance table of all trait-classes simultaneously obtained by redundancy analyses on a matrix of relative prevalence of each trait-class.

Source	df	Total SS	*F*	*P*
Time (co-variable)	1	0.364		
Flow	3	0.144	4.378	0.001
Sediment	1	0.033	3.042	0.004
Flow x Sediment	3	0.034	1.051	0.369
Residual	39	0.425		
Total	47	1.00		

Given that sampling occasion and flow were the main factors associated with variation in the trait assemblage a series of further RDAs were run, with sediment effects factored out, to explore in more detail the effect of the four different flow treatments. We found a significant interaction between sampling occasion and flow treatment, with the time effect being considerably more pronounced than the flow effect (Table 3.4.7, Figure 3.4.3). RDA axis1 was aligned with time, while RDA axis 2 was distinguished the slow-flow treatments from the fast-flowing ones. It was evident that the switching of flow after 40 days caused a change in the trajectory of temporal change such that the slow flow trait assemblage shifted to one resembling a consistently fast-flowing assemblage and the fast flow trait assemblage shifted to one resembling a consistently slow-flowing assemblage (Figure 3.4.3).

Larger sized, predatory, scavenging, crawling, ovoviviparous and asexually reproducing macroinvertebrates tended to be more prevalent in slow-flowing conditions. Smaller, burrowing, herbivorous, macroinvertebrates that laid isolated eggs or clutches of eggs tended to be associated with the faster flowing conditions.

Over the course of the experiment the prevalence of multiple life-cycles in a year decreased while semivoltinism increased. Respiration strategies shifted from predominantly via the tegument and spiracles to through gills (Figure 3.4.3). Having no resistance form was the dominant trait on the first sampling occasion but by day 70 resistance traits such as eggs, statoblasts and diapause were more prevalent.

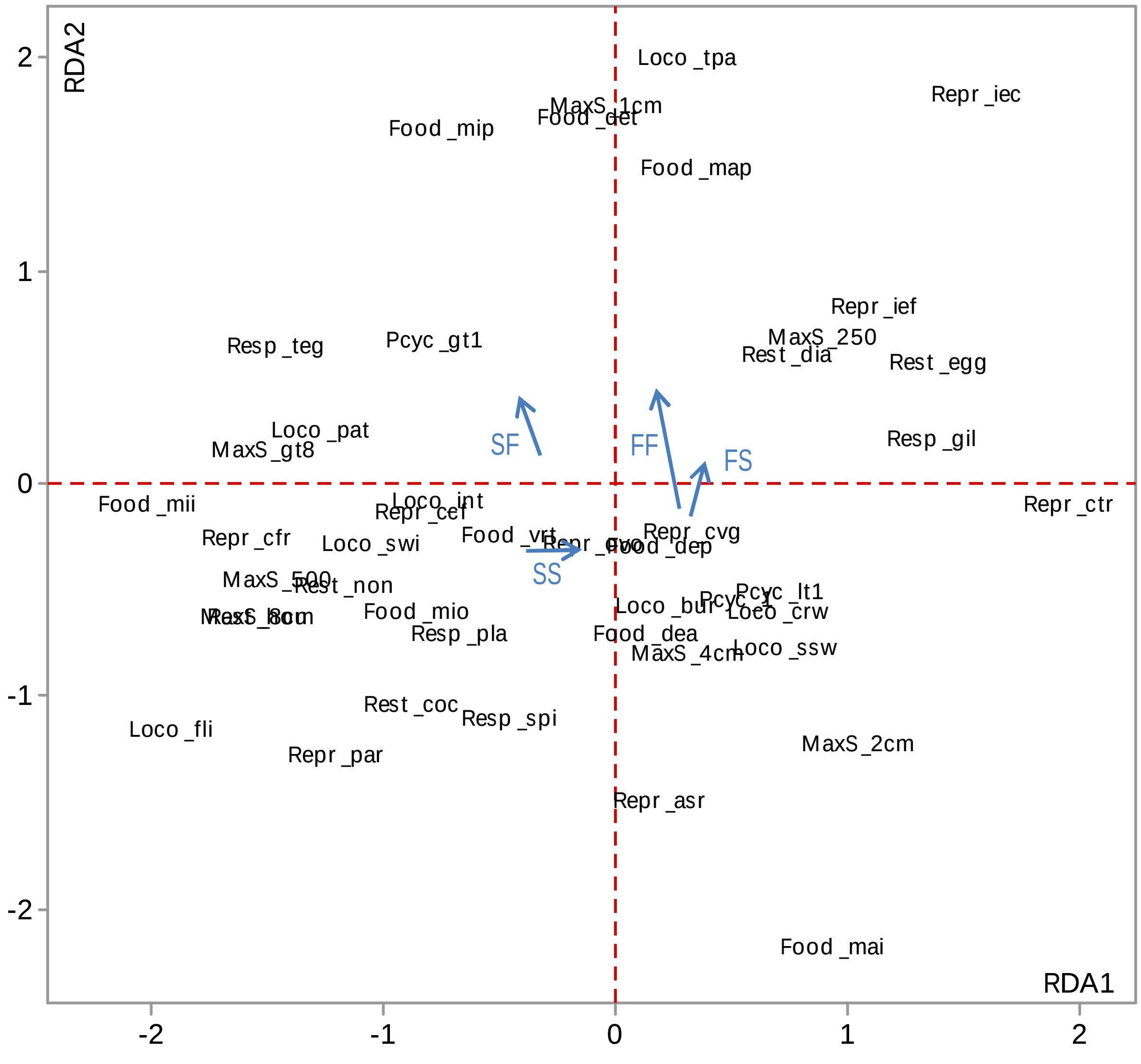


Figure 3.4.2. RDA ordination biplot illustrating the distribution of trait-class scores and treatment effects. Arrows indicate the effect of sediment addition (arrowhead) on the position of the centroid for a given flow treatment (FF = fast-fast, SS = slow-slow, FS = fast-slow, SF = slow-fast), having factored out the effect of time. Codes for trait-classes are explained in Table 3.4.3.

Table 3.4.7. Analysis of variance table of all trait-classes simultaneously obtained by redundancy analyses on a matrix of relative prevalence of each trait-class.

Source	df	Total SS	*F*	*P*
Sediment (co-variable)	1	0.0033		
Flow	3	0.144	4.378	0.001
Time	1	0.364	33.338	0.001
Flow x Time	3	0.064	2.095	0.003
Residual	39	0.395		
Total	47	1.00		

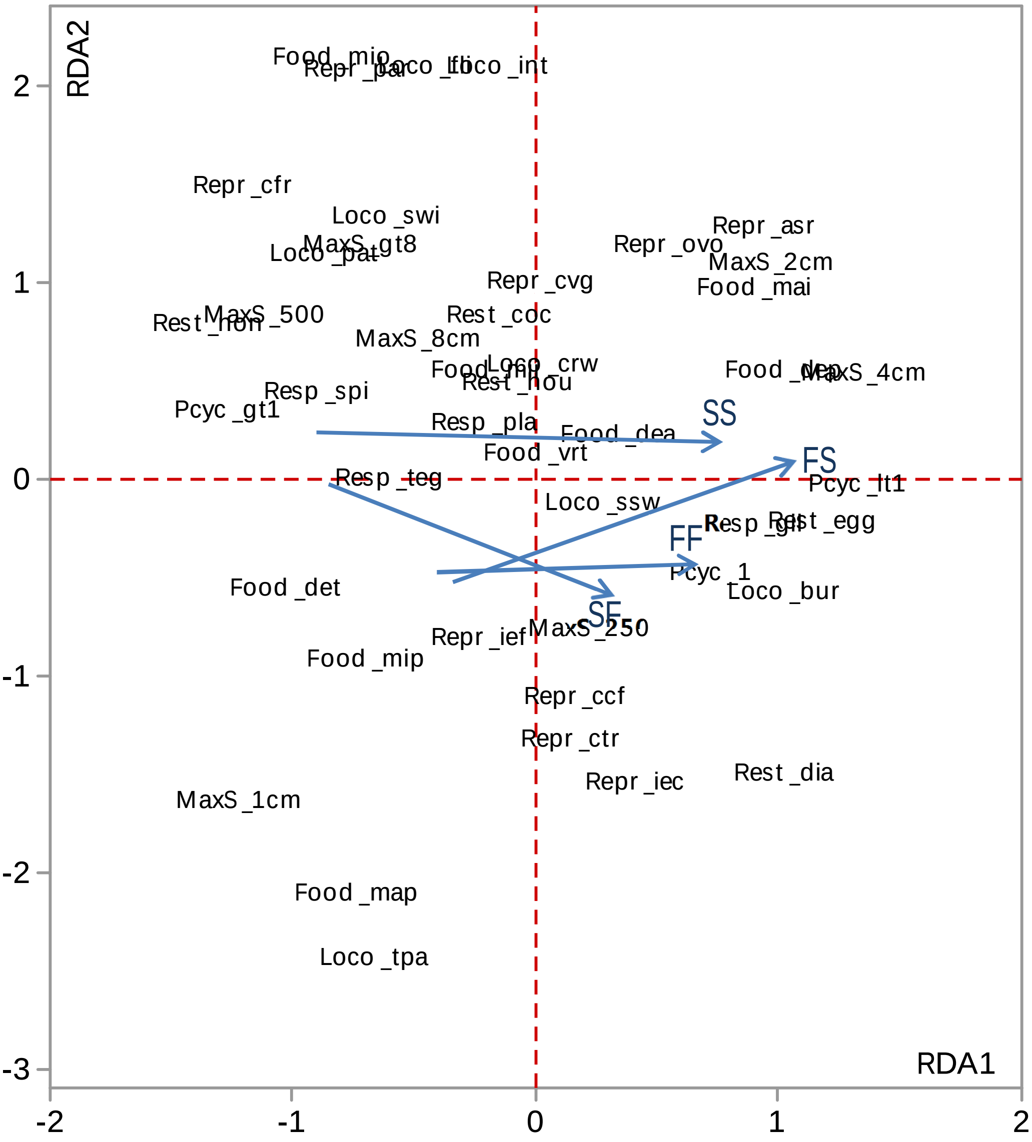


Figure 3.4.3. RDA ordination biplot illustrating the distribution of trait-class scores and treatment effects. Arrows indicate the effect of time (2^{nd} sampling occasion at arrowhead) on the position of the centroid for a given flow treatment (FF = fast-fast, SS = slow-slow, FS = fast-slow, SF = slow-fast), having factored out the effect of sediment manipulation. Codes for trait-classes are explained in Table 3.4.3.

3.4.4 Discussion

Spatially-extensive dataset

The additional analyses undertaken on the 205-sample fine sediment dataset have statistically reinforced the interpretations made in REFORM Deliverable 3.1, namely that the prevalence of ovoviviparity, prolonged adult aquatic stage, burrowing and multivoltinism significantly increased with an increasing mass of fine sediment in the stream bed. In contrast, the prevalence of univoltinism and semivoltinism, prolonged egg stage, the laying of isolated cemented eggs, aquatic and active aerial dispersal, egg and statoblast resistance forms and crawling decreased with an increasing mass of fine sediment in the stream bed.

Along RLQ axis 1 there was a stark negative association between measures of benthic fine sediment mass and measures of stream size such as distance from source, width and depth. This reflects the fact that there is a natural tendency for larger watercourses to have a greater quantity of benthic fine sediment as the hydromorphological character changes from turbulent eroding shallow headwaters to deeper, more laminar-flowing, depositing rivers. The RLQ – Fourth-corner analyses (Tables 3.4.4 and 3.4.5) did not seek to factor out this natural longitudinal gradient but this would be something worth exploring further if the approach allowed co-variables to be defined and a ‘partial RLQ’ to be carried out. Indeed an approach similar to this has been recently applied to a plant trait dataset where the impact of a grazing intensity gradient on the trait assemblage was being investigated across a range of different habitats and years, with the authors wishing to remove the effect of the latter two factors (Wesuls et al., 2012). However in this situation there was just a single categorical co-variable; further R code development work would be necessary to carry out the analysis we require.

Nonetheless, we have robustly identified those macroinvertebrate traits that are indicative of low and high fine sediment conditions. This knowledge will help lead to the development of a biological trait-based index of fine sediment stress.

Manipulative experiment dataset

We found a significant but small effect of fine sediment addition on the benthic macroinvertebrate trait assemblage in our experiment; this manipulation could only account for 3.3% of the overall trait assemblage variation. Trait data were compiled at family-level or higher as the macroinvertebrates were identified to this taxonomic level. This possibly limited the potential of the traits to discriminate between the two sediment treatments. Alternatively it may be that the addition of fines up to a point where they represented 10% of sediment weight may not have been sufficient to cause stress on the benthos. The 10% threshold had been chosen based on fish responses to fine sediment but perhaps a higher threshold was required for stream macroinvertebrates in the lowland setting of this experiment.

The dominant explanatory factor was time (sampling occasion) which implies that the macroinvertebrate community was still in a state of colonization flux as the experiment was running. The sediment treatment still had the potential to alter the colonisation trajectory, however we found no significant interaction between sediment and time, having factored out the flow effect. In contrast, the flow treatment did affect the colonisation trajectory, with the switch in flow conditions causing a subsequent drift in the trait assemblage from one associated with its initial flow state to one closely resembling that found in the alternatively consistent flow treatment.

Unfortunately we were unable to confirm, from the experimental results, the trait-fine sediment associations that we found in the correlative spatially extensive dataset. The taxonomic limitations of the experimental dataset precluded a direct comparison of results from both approaches. This work highlights the importance of identifying specimens to the lowest possible level, particularly when analyzing from a trait perspective. Many families contain genera and species with differing trait characteristics e.g. Hydrophilidae, Oligochaeta that are 'averaged away' when the data are compiled at less resolved taxonomic levels.

In conclusion, despite further experimental manipulation being needed to robustly confirm the causative link between the stressor and the trait response, we have successfully demonstrated the potential for macroinvertebrate biological trait data to be used to detect the ecological impact of excessive fine sediment stress on river communities.

4 Designs to monitor biological indicators of hydromorphological degradation.

The biological assessment systems used by member states to derive Ecological Quality Ratio's (EQRs) for the purpose of surveillance moniotoring are, in most cases, not stressor specific. Therefore, they cannot be used for diagnosic purpposes. In the case of operational monitoring the WFD states (regarding the selection of sites): "For bodies at risk from significant hydromorphological pressure, sufficient monitoring points within a selection of the bodies in order to assess the magnitude and impact of the hydromorphological pressures." Concerning investigative monitoring the WFD states: "Investigative monitoring shall be carried out where surveillance monitoring indicates that the objectives set out in Article 4 for a body of water are not likely to be achieved and operational monitoring has not already been established, in order to ascertain the causes of a water body or water bodies failing to achieve the environmental objectives,..." These phrases imply that in the case of operational and investigative monitoring it is imperative to know which biological indicators indicate hydrological and/or morphological degradation and which are the key abiotic factors driving biological degradation. Operational monitoring can take place after selection of the appropriate biotic and abiotic variables. However, the selection of appropriate abiotic and biotic variables is challenging.

For the purpose of operational and investigative monitoring member states "..shall monitor for those quality element which are indicative of the pressures to which the body or bodies are subject". Many authors have stressed the advantages of using macroinvertebrates compared to other groups for biological monitoring and assessment purposes (e.g. Hellawell 1986; Metcalfe 1989). These advantages have made macroinvertebrates the most commonly applied group of organisms for assessing water quality (Bailey et al., 2001; Carter & Resh, 2001; Hering et al., 2006) and, therefore, macroinvertebrates are the main focus of this chapter.

The majority of existing bioassessment systems based on macroinvertebrates were developed to detect organic pollution (Friberg et al., 2006, Friberg, 2014). Friberg et al. (2009) stressed the importance of developing biological indices related to hydromorphological degradation. Their view is based on the extensive literature on linkages between the in-stream physical environment and benthic macroinvertebrates. However, Friberg et al. (2009) found only relatively weak relationships between various measures of hydromorphological stress and commonly used macroinvertebrate assessment tools. For instance, Friberg et al. (2009) found a maximum r2 value of just 0.21 between RHS (River Habitat Survey) indices and a number of macroinvertebrate indices in streams along a hydromorphological degradation gradient. RHS is used in the UK to categorise the physical features of rivers over 500 m reaches (Raven et al., 1998). Results from the same study suggested a more detailed assessment of hydromorphological features (e.g. sediment characteristics at the smaller scales at which macroinvertebrates are sampled) provided a far better fit with macroinvertebrate responses than RHS indices. In combination with the many studies mentioned by Friberg et al. (2009) that report sediment conditions as one of the primary drivers of macroinvertebrate community composition, this indicates the necessity to link the abiotic hydromorphological classification of river reaches to sediment dynamics.

Lorenz et al. (2004) developed an index to detect the impact of hydromorphological degradation, the German Fauna Index. Although this index shows a strong correlation with hydromorphological degradation (R2=0.67), it is almost as sensitive to organic pollution (R2=0.55). This suggests that organic pollution and hydromorphological degradation are not the variables driving community composition, but that both stressors (at least in part) are influencing the same variable (i.e., the oxygen concentration), which is driving macroinvertebrate community composition. It is imperative that we unravel the primary drivers of macroinvertebrate community composition (e.g., oxygen concentration, shear stress, biotic interaction) at the appropriate temporal and spatial scales. The only way to do this is to combine observational data and data from experimental work. The development of bioassessment systems has been based primarily on observational data. However, observational data are only suited for detecting general patterns and correlations (data on primary drivers at the appropriate temporal and spatial scale are usually lacking). Experimental datasets are required to confirm whether correlations derived from observational data actually represent cause-effect relationships.

Although Friberg et al. (2009) and Lorenz et al. (2004) implied to have studied hydromorphological degradation, the environmental variables considered in their studies mainly relate to the morphology of streams, not the hydrology. Apart from the Lotic-invertebrate Index for Flow Evaluation (LIFE) developed by Extence et al. (1999), no attempt has been made (to our knowledge) to assess hydrologic degradation using biological indicators. Although the LIFE method is very valuable in the sense that it provides the opportunity to relate several hundreds of flow variables to the macroinvertebrate community, it also has two important disadvantages. First, the LIFE method is designed to reflect the faunal responses to ‘flow conditions’ and their change over time, not for the sole purpose of biological assessment. This means that the interpretation of LIFE scores is not straightforward, limiting its applicability in biological assessment. Second, Extence et al. (1999) stated that “a link exists between poor habitat quality and depressed LIFE scores”, which means that the LIFE methodology, although developed to study changes in flow, also responds to at least one other stressor, namely habitat quality.

To develop a stressor-specific biological assessment system remains a major challenge, especially since the variation in macroinvertebrate community composition remains, for a large part, unexplained by the abiotic variables considered in aquatic monitoring programs. This is due, in part, to stochastic mechanisms related to differential colonization/extinction dynamics after disturbance (Chase, 2010; Verdonschot, 2012). Merovich & Petty (2010) suggested that metacommunity dynamics might prevent a strong correspondence between macroinvertebrate community composition and the water quality template in the Monongahela River basin of West Virginia, despite the localized effects of water chemistry. However, the fact remains that we might not be measuring the appropriate abiotic variables at the appropriate temporal and/or spatial scale. Diagnosing specific stressors will remain difficult until we have completely unraveled ecosystem functioning, including the effects of species interactions (e.g., competition, predation), invertebrate dispersal, and assemblage dynamics on a watershed scale. For example, many studies have indicated that (biological) restoration might fail without the presence of near-natural and undisturbed sites within range of the dispersal capacity of source populations (e.g., Brooks et al., 2002; Muotka et al., 2002; Parkyn et al., 2003). The results from chapter 1, 2 and 3 suggest it is possible to develop indices for fish, macroinvertebrates and diatoms that can indicate (hydrolo)morphological degradation. Results described in deliverable 2.2 clearly demonstrate the importance of both aquatic and riparian vegetation as a key physical control of river form and dynamics and a crucial component of river restoration. This, in combination with the diagnostic tool AMOEBE descibed in chapter 2, is a first step in the development of stressor specific bioassessment methods.

5 Uncertainty in biological assessment of hydromorphological degradation.

Since the introduction of the WFD, water authorities have been obliged to monitor changes in ecological quality on larger spatial scales as opposed to site scale and to indicate the level of confidence and precision of the results provided by the monitoring programs in their river basin management plans (European Commission, 2000). To meet these requirements, the statistical power of the monitoring programs should be analyzed. The statistical properties associated with freshwater monitoring programs are often unknown. Power analysis (assessing the ability of a program to accurately detect change) could help avoid unnecessary expenditures for monitoring programs that cannot provide meaningful results or that lead to overspending. The statistical power of monitoring programs depends, in part, on the variability of biological assessment results.

Before the extent of hydromorphological degradation at a site can be assessed, samples from the macroinvertebrate community present at the site will have to be collected and processed. The collection and processing of macroinvertebrate samples consists of a sequence of steps (Figure. 5.1.1). Each step in this sampling and sample processing chain represents choices that have to be made, such as “Do we sample all habitats?” and “Do we identify to genus or species level?” Depending on the choice, the actual composition and condition of the macroinvertebrate community may be misinterpreted (Diamond et al., 1996). The choice will influence the final result, the taxa list, including the number of individuals per taxon. Because assessment of the extent of hydromorphological degradation of a site is based on this taxa list, results can vary based on the choices made during sampling and sample processing.

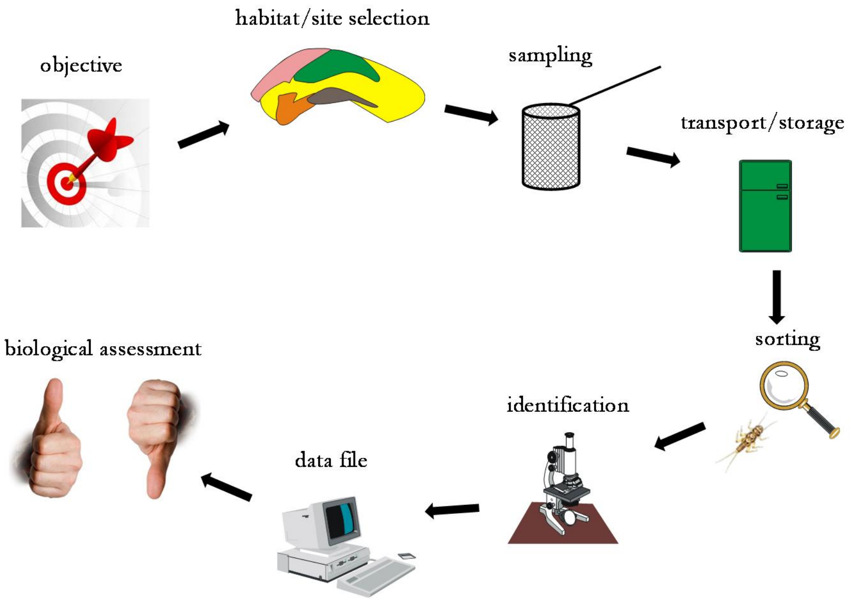


Figure 5.1.1. Sampling and sample processing chain: overview of the different steps that have to be taken before a biological assessment system can be applied (modified after Nijboer, 2006).

Biological monitoring usually has two purposes: (1) to estimate variables of interest at a site and (2) to make comparisons among sites or time intervals. Variables of interest in biological monitoring are primarily metric values (e.g., the number of taxa, Average Score Per Taxon, Saprobic Index) and ecological quality classes resulting from biological assessment systems. Metric values and ecological quality classes are calculated based on the macroinvertebrate community composition. Various methods have been developed to collect macroinvertebrates from streams and to process macroinvertebrate samples. These sampling and sample processing methods can vary in terms of sampled area, mesh size of sampling gear, sampled habitats, intensity of sorting, and taxonomic resolution of identification, among other parameters. The methodology that is applied influences the accuracy and variability of bioassessment results (expressed as metric values and/or ecological quality classes) (e.g., Barbour & Gerritsen, 1996; Diamond et al., 1996; Haase et al., 2004). Also, each method can be selective for certain species or groups of species that vary in their exposure and sensitivity to anthropogenic stress (Barton & Metcalfe-Smith, 1992).

Accuracy and variability are both important aspects of bioassessment. Variability refers to the extent to which data points in a statistical distribution or data set diverge from the average or mean value. Accuracy refers to the closeness of a measurement to its true value (Norris et al., 1992). Therefore, differences in accuracy between methods may result in different bioassessment results. Variability is important when making comparisons because the validity of conclusions depends on data variability (Norris et al., 1992); higher variability increases the probability of incorrect bioassessment results. An increase in accuracy or a reduction in variability is not always possible because the associated costs are often high. However, when assessing ecological quality for biological monitoring purposes, catching all organisms or taxa present at a site is not necessary (Barbour & Gerritsen, 1996). The standardization of sampling is required, though, for valid comparisons among sites and points in time (Courtemanch, 1996; Vinson & Hawkins, 1996). Thus, the question to focus on is which steps of sampling and sample processing need to be standardized. When two methods are equally variable and provide comparable bioassessment results, standardization is not necessary. Extensive evidence indicates that at least two steps in the sampling and sample processing chain require standardization when metrics based on taxa richness are considered: the sampled area and the effort spent sorting samples. For example, several studies have shown that the number of taxa collected from a sample increases asymptotically with an increase in sampled area and/or sorting effort (e.g., May, 1975; Verdonschot, 1990; Colwell & Coddington, 1995; Vinson & Hawkins, 1996).

In addition to accuracy and variability, cost plays an important role in decision-making related to method standardization. The cost of collecting and processing macroinvertebrate samples is high and can depend strongly on the sampling technique used (e.g., Barbour & Gerritsen, 1996; Metzeling et al., 2003; Vlek et al., 2006). Higher variability and lower accuracy increases the risk of incorrect assessment results. In the case that ecological quality at a site is incorrectly assessed as less than good, water managers will unnecessarily take costly restoration measures to reach a good ecological quality by 2015 (European Commission, 2000). From this point of view, the consequences of poor decision-making due to low accuracy and/or high variability potentially outweighs the savings associated with a less time consuming sampling and sample processing method (Doberstein, 2000).

Changes in the extent of hydromorphological degradation at a site can be the result of restoration measures or anthropogenic disturbance. However, such changes can be masked by several sources of variation, i.e., natural spatial variation, natural temporal variation and within-site variation. Whithin-site variation is defined here as the inability of a given sampling and sample processing technique to capture all organisms present at a site at a certain moment in time. Whitin-site variation will vary depending on the sample and sample processing method applied.

To determine whether change is the result of anthropogenic disturbance requires the determination of natural variability (Johnson, 1998; Leunda et al., 2009; Resh & Rosenberg, 1989). Based on the current literature it is very difficult to gain insight on the extent to which the different sources of variation contribute to overall variation. For example, most studies that considered seasonal variation looked at a combination of seasonal and within-site variation. Thus, what is noted as seasonal variation could in fact be within-site variation. Only a few cases used variance partition to separate the different sources of variability. Carlisle & Clements (1999) showed that the proportion of variance explained by different sources (site, season, year, and interaction terms) varies extensively depending on the metric. However, their ‘site term’ in the variance partition included both natural variation and variation due to different ecological quality at the sites. Carlisle & Clements (1999) did show relatively high statistical power for richness measures (total number of taxa, number of Ephemeroptera, Plecoptera, and Trichoptera taxa, and number of Ephemeroptera taxa) compared to metrics based on (relative) abundance.

To develop an assessment system, it is not crucial to know the different sources of within-site variability. As long as overall variation due to natural temporal, natural spatial, and within-site variability is known, we can determine metric performance. For all of the different sources of within-site variation, they are only important when within-site variability is large compared to natural temporal, natural spatial variation and the range in metric values among sites of varying quality (Clarke et al., 2006). When within-site variation appears to be relatively large, the different sources of within-site variability should be determined. For example, if within-site variability appears to be primarily the result of subsampling variance, the variability can be reduced by sorting more individuals from the sample or sorting a larger fraction of the sample. Sorting and identification errors during sample processing are common (Haase et al., 2006; Stribling et al., 2008; Haase et al., 2010). This automatically implies that the implementation of quality control mechanisms in macroinvertebrate monitoring and training personnel involved with processing macroinvertebrate samples will reduce within-site variation. In cases where natural temporal variation is high, there are several options to deal with it. First, in the case of seasonal variation, it is possible to standardize sampling to a single season or to combine samples from different seasons. Second, increasing sample size is an option as, in many cases, the variation described as temporal in the literature is in fact the inability to collect all taxa present at a site. The third option is to develop metrics that are less responsive to variables driving temporal variation in the composition of macroinvertebrate communities.

When developing an assessment system, variability is not the only important issue that needs to be considered. The cost associated with sampling and sample processing and the sensitivity of the assessment system to anthropogenic stress are also important. To weigh these different aspects, information is required regarding the variability of different metrics on different temporal and spatial scales. In an ideal world, the selection of metrics for the development of an assessment system would be based on labor-intensive sampling and sample processing methods to create an optimal situation to study the ability of a metric to separate ‘signal’ (responsiveness to stress) from ‘noise’ (variation). Replicate samples should be collected to gain insight into within-site variability, and samples from pristine sites should be collected to establish natural temporal and spatial variability. After a metric is proven to be capable of separating signal from noise, it can be determined whether less time-consuming sampling and sample processing methods may be adequate to achieve similar accuracy and variability.

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