# Impacts of watercress farming on stream ecosystem functioning and community structure

Shaun Cotter

School of Biological and Chemical Sciences
Queen Mary, University of London

Submitted for the degree of
Doctor of Philosophy
of the University of London
September 2012

## Abstract.

Despite the increased prominence of ecological measurement in fresh waters within recent national regulatory and legislative instruments, their assessment is still almost exclusively based on taxonomic structure. Integrated metrics of structure and function, though widely advocated, to date have not been incorporated into these bioassessment programmes.

We sought to address this, by assessing community structure (macroinvertebrate assemblage composition) and ecosystem functioning (decomposition, primary production, and herbivory rates), in a series of replicated field experiments, at watercress farms on the headwaters of chalk streams, in southern England. The outfalls from watercress farms are typically of the highest chemical quality, however surveys have revealed long-term (30 years) impacts on key macroinvertebrate taxa, in particular the freshwater shrimp *Gammarus pulex (*L.), yet the ecosystem-level consequences remain unknown.

Initial studies were at Europe's largest watercress farm at St Mary Bourne, Hampshire, during the bioremediation of its complex wastewaters and changes to farm management practices. These widened to include larger scale spatiotemporal studies at other watercress farms. Detrimental ecological impacts at the start of the study were detected by the structural and functioning measures, but they did not respond to bioremediation. However, an increase in *G. pulex* abundance was detected, providing evidence of recovery in response to altered practices, which may be attributable to the cessation of chlorine use. The detrimental impacts were unique to the St Mary Bourne watercress farm and were not consistent across the other watercress farms in the study. Our results demonstrate the importance of integrated metrics of both ecosystem structure and functioning, to derive a more comprehensive view of aquatic ecosystems and highlights the difficulties associated with extrapolating from laboratory studies in response to stressors.

## Acknowledgements.

I am deeply indebted to a number of people and organisations for their support and guidance over the last few years, from the projects inception one afternoon in Winchester to the completion of this PhD.

I am extremely grateful to my first supervisor Dr. Guy Woodward for his unerring patience, enthusiasm (knowledge on fire) and assistance throughout the life of this project, without which this would not have been possible. Thank you also to Prof. Alan Hildrew my second supervisor and also to Guy for their comments, advice and discussions on the direction and scope of the work undertaken to underpin and present this thesis.

I am very grateful to Graham Roberts of the Hampshire and Isle of Wight Wildlife Trust who put me in touch with Vitacress salads and their Technical Director Dr. Steve Rothwell, which allowed the initial research idea to become reality and for the project to begin. I am thankful to Vitacress who also allowed me unrestricted access to their watercress farms and for their financial help in the early stages of the project. I would also like to thank Shirley Medgett of the Environment Agency (EA) for access to her invaluable local knowledge, professional expertise, EA laboratory services and for financial assistance over the fieldwork stages of this project.

I have had a number of friends assist and accompany me on my early morning sojourns for the collection of samples and the deployment of sampling arrays, I send a special thank you to Chris Dyer, Sue Philbey and Dave and Fergus Crawley. I also

thank Charmaine Grieger for her advice and assistance with Photoshop and the proof reading of an early draft of this thesis.

And saving the best to last, I am extremely grateful to my parents Dr. John Leon and Marian Cotter for always providing the right environment, for their belief and for being there…

**Table of Contents.**

**List of tables.**

## List of figures.

**List of plates.**

## Chapter 1.

# General Introduction.

Water is abundant on Earth, covering 70% of its surface, but only 2.5% of this is in the form of fresh water. 68.7% of this is bound in the form of ice and snow, 29.9% as groundwater and yet only 0.26% is concentrated in lakes, reservoirs and river systems (Gleick 1996). Running waters are unique amongst aquatic ecosystems being linear and unidirectional in flow, contributing to shape the morphology of river channels, their spatial and temporal variability and biotic adaptations to these dynamic conditions (Giller & Malmqvist 1998). In addition to being relatively isolated within a predominantly terrestrial landscape, the susceptibility of these properties to environmental change, are exacerbated by anthropogenic activity within their catchments (Malmquvist & Rundle 2002). Rivers and streams provide a multitude of ecosystem goods (drinking water, farming) and services (recreation, fishing) of value to humans, and encouraging human settlement which often reaches high population densities along river corridors with associated high levels of resource exploitation.

Globally, fresh water ecosystems are under intense and increasing pressure from anthropogenic stressors (Townsend, Hildrew & Francis, 1983; Griffith, Perry & Perry, 1993; Ventura & Harper, 1996; Dangles & Guerold, 1999, 2001; Pascoal, Cassio & Marvanova 2005; Ricciardi, & Macisaac, 2011). In the UK, Europe and the United States extensive and comprehensive protective environmental legislation have been introduced, in attempts to mitigate or even remediate these threats. In the USA the 'American Clean Water Act' (ACWA) (EPA 2002) and across Europe, the Water Framework Directive (WFD) (European Commission 2003) both explicitly focus on the ecological status and integrity of waters, through the assessment of community structure and ecosystem functioning. Functional indicators measure the services or functions provided (the processes), while structural indicators measure what lives in an ecosystem (the pattern) (Matthews *et. al.*, 1982; Minshall 1988; Young, Townsend & Matthaei 2004). Biomonitoring uses the biota itself to identify and monitor changes in an ecosystem, which can be assessed using a variety of community structure and ecosystem functioning measures. Structural measures are the far more commonly used of these two types of approach (Norris & Thomas, 1999), being relatively quick and simple to apply and having had a longer history of implementation. As a consequence structural ‘‘assemblage’’ basal assessments have been embedded in many national monitoring schemes since the 20th Century (Matthews *et al*., 1982). Structural methods are usually based on species diversity e.g. indices of biological integrity (IBI’s), or the presence / absence of indicator species (Herricks & Cairns, 1982; Armitage *et al*., 1983; Wright *et al*., 1984; Hawkes, 1997; Davy-Bowker *et al*., 2005; Statzner & Beche 2010). Measurements of ecosystem functioning are far less commonly used, despite being specifically referred to in the WFD and ACWA and being advocated by many scientists (Matthews *et al*., 1982; Bunn and Davies 2000; Gessner & Chauvet 2002; Carlisle & Clements 2005; Young, Matthaei & Townsend 2008; Hopkins, Marcarelli & Bechtold 2011; Haldyz *et al*., 2011a & b; Woodward *et al*., 2012).

Acidification (Dangles *et al.*, 2004) and eutrophication (Gulis, Ferreira & Graca 2006; Lecerf *et al.*, 2006; Lecerf & Chauvet 2008) have been the primary foci of much of the functional research in running waters. However, these represent only two of a host of other stressors, which include siltation, climate change, habitat destruction, species removal and addition (see Malmqvist & Rundle, 2002), and the use of pesticides (Rasmussen 2012) and other agrochemicals, which can also combine to have complex synergistic effects (Giller *et al*., 2004). Clearly, there is an urgent requirement for research into the impacts of these stressors on both ecosystem processes and community structure, especially given the current heavy bias in the favour of the latter. In particular, there is a need to develop integrated structural-functional approaches to improve our understanding and ability to predict the impacts of anthropogenic stressors (Woodward 2009) and their potential to provide an early indication of degradation or recovery (Palmer & Febria 2012). This thesis sought to examine this within the context of headwater chalk streams and the impact watercress farming has upon them, by examining their basal energy sources and the communities that mediate them.

The relationship between aquatic and terrestrial ecosystems is particularly close for headwater streams as narrow stream widths and overhanging riparian canopies can maximize the impact of the surrounding terrestrial ecosystem (Vannote *et al.*, 1980). Riparian vegetation influences the way energy enters a freshwater system either directly, through an input of material such as leaf-litter and woody debris derived from streamside trees (Cummins *et al.*, 1989; Young, Matthaei & Townsend 2008) or indirectly, through the shading effects of the stream channel, limiting the solar energy available for primary production via the photosynthesis of green plants and algae (Hill, Ryon & Schilling 1995). The primary sources of energy underpinning the food web in chalk streams are therefore a combination of allochthonous terrestrial leaf litter (Hieber & Gessner 2002; Pascoal & Cássio 2004) and autochthonous algal and macrophyte production (Bernhardt & Likens 2004), and stressors that disrupt either trophic pathway can alter overall ecosystem functioning. In headwater streams, particularly those in wooded areas, allochthonous inputs are often significantly higher than autochthonous inputs from in-stream algal primary production, so the relative importance of the two pathways, and their vulnerability to stressors, varies among systems (Naiman *et al.*, 1987; Giller & Malmqvist 1998). Allochthonous detritus can account for as much as

99% of the basis of secondary production within the entire stream food web (Woodward, Speirs & Hildrew 2005) and consequently is often considered the main energy source within small, low order streams (Wallace *et al.,* 1997). Algal primary production is considered the main autochthonous pathway in most streams, as algae is more palatable to macroinvertebrates than live macrophyte consumption (Gregory 1983). The decomposition of leaf-litter (Hieber & Gessner 2002; Pascoal & Cássio 2004) and epilithic algal primary production (Bernhardt & Likens 2004) are therefore critical processes in chalk streams and both energy inputs need to be considered simultaneously, to provide an overall picture of ecosystem functioning. Like other headwater streams, chalk headwater streams also receive most of their energy from allochthonous sources (Westlake *et al.,* 1972; Dawson 1976; Hynes 1983) but are less dependent on this subsidy than are non-chalk streams (Berrie 1976). This is because chalk streams in their natural state, i.e. upland moorland streams, are typically open canopy, shallow, clear and contain little suspended sediment (Sear, Armitage & Dawson 1999; Heywood & Walling 2003; EA 2004). This, together with their high nutrient status drives photosynthesis (Berrie 1976; Davies-Colley *et al.,* 1992) and as a result chalk streams in a natural state can exhibit very high primary (and secondary) production (Dawson 1976, Mullholland *et al*., 2001; Fellows *et al.,* 2006).

### ***Allochthonous pathways.***

The pathways of allochthonous energy flux rely upon the supply of detrital material from sources outside the stream. The most important component of these allochthonous inputs to streams is leaf litter from the surrounding vegetation (Webster *et al.,* 1999), providing both a habitat and a food source for macroinvertebrate detritivores and microbial decomposers (Harmon *et al.,* 1986; Romero *et al.,* 1994). Leaf litter entering a stream can become ensnared in riffles and shallow areas, where it is rapidly colonised, broken down and assimilated as secondary production by a combination of aquatic hyphomycetes, bacteria and stream macroinvertebrates (McArthur *et al*., 1988; Hieber & Gessner 2002). In temperate streams, biotic factors account for the largest proportion of overall leaf mass loss, typically dominated by macroinvertebrate shredders, followed by aquatic fungi and bacteria, with the remainder attributable to abiotic factors such as fragmentation and leaching (Hieber & Gessner 2002; Graça & Canhoto 2006; Hladyz *et al.*, 2011a, b). The breakdown rates of leaf litter in streams can be affected by environmental factors such as temperature (Liski *et al*., 2003), physical abrasion (Heard *et al*., 1999), pH (Griffith & Perry 1993) and plant chemical and physical defences (Graça & Canhoto 2006; Hladyz *et al.*, 2011a, b). The incorporation of leaf material into macroinvertebrate secondary production proceeds at a faster rate in nitrogen rich and soft leaves (e.g. alder), when compared with nitrogen poor, chemically protected, recalcitrant leaves (e.g. oak), (Canhoto & Graça, 1995; González & Graça 2003). Leaves of these more resistant species, such as oak, can take a longer time to be fully colonized and degraded by microorganisms and in some instances can often be found in streams all year round, even in temperate systems with highly seasonal inputs (Graça & Canhoto 2006). Oak litter is therefore an ideal choice for studies taking place during any season, and for this reason were used as a key component of the standardised bioassays employed in this study.

The dominant microbial consumers of detritus in stream food webs are aquatic hyphomycete fungi, in terms of both their standing biomass and production (Hieber & Gessner 2002; Pascoal & Cassio 2004; Abelho, Cressa & Graca 2005). In addition to direct leaf breakdown (Gessner & Chauvet 1994) they also enhance the palatability of leaves for shredder consumption (Suberkropp 1992; Graça 1992). The stream macroinvertebrates that consume terrestrial derived coarse detritus are commonly characterised under the functional feeding group (FFG) ‘shredders’ (e.g. *Gammarus pulex L*); defined as macroinvertebrates possessing mouthparts formed for tearing coarse particulate organic matter (after Cummins & Klug 1979; see review by Wallace & Webster 1996). Once in the water, leaves are rapidly exposed to large numbers of aquatic hyphomycetes spores (Bärlocher & Graça 2002), and within a few days germinate and grow into the leaf substrates (Webster & Benfield 1986; Canhoto & Graça 1999), where they produce leaf degrading enzymes (Canhoto, Barlocher & Graca 2002). Most stream macroinvertebrates, except Tipulidae larvae (Graça & Bärlocher 1998), do not possess the enzymes necessary to breakdown cellulose and lignin, the structural plant cell wall compounds of leaf litter. Fungi and bacteria however, produce enzymes that can digest these to release simpler compounds. These can be assimilated directly by shredders (Suberkropp 1992), many of which feed selectively upon patches on leaves that have been colonised by fungi, as these are generally more nutrient enriched than the leaf itself (Arsuffi & Suberkropp 1985). This microbial conditioning is important for shredders (Suberkropp 1992; Graça 2001; De Lange *et al.,* 2005), which benefit from both the fungal action on leaves and also by feeding directly on the fungi.

### ***The relative importance of decomposers and detritivores.***

Detritus in the form of senesced leaves is the most obvious and the most frequently studied input of allochthonous particulate organic matter to streams (Wallace *et al.,* 1999; Lecerf *et al.,* 2006). The interaction between leaves, decomposers and detritivores has been a central research area for stream ecologists for decades (Petersen & Cummins 1974; Webster & Benfield 1986; Gessner & Chauvet 1994; Wallace *et al.,* 1999; Lecerf *et al.,* 2006; Hladyz *et al.,* 2011a, b). Methods to determine the relative importance of bacteria, fungi, and invertebrates, include measurements of the concentrations of polysaccharides, total nitrogen, protein, tannin, lignin, ergosterol (an indicator of fungal biomass in the leaves) and the use of different mesh apertures in litter bags to selectively exclude different consumer groups (Suberkropp, Godshalk & Klug 1976; Boulton & Boon 1991; Young, Matthaei & Townsend 2008). The most common measures used to assess this detrital pathway, being relatively cost-effective, simple and easy to implement (Young, Matthaei & Townsend 2008) are leaf litter decomposition rates (Boulton & Boon 1991); in particular the relative importance of microbial and leaf shredding macroinvertebrate activity (Gessner & Chauvet 2002). One of the most widely used methods to assess the relative importance of shredders and microbes on leaf litter breakdown is via the simultaneous use of coarse (~10mm) and fine-mesh (~0.5mm) leaf bags, respectively. Coarse mesh allows access to large shredders, such as the freshwater shrimp *G. pulex,* one of the key consumers investigated in this study, and can be used as a proxy measure for shredder activity; whereas fine mesh excludes large macroinvertebrates and acts as a proxy measure for microbial processes (e.g. Boulton & Boon 1991; Hladyz *et al.,* 2011a, b). The biomonitoring of the streams presented in this thesis employed these methods to quantify leaf breakdown rates and to apportion the contribution of shredder breakdown, microbial breakdown and physical fragmentation, to the decomposition process.

#### ***Autochthonous pathways.***

This basal energetic pathway relies upon the supply of energy from sources within the stream. Biofilms are an important autochthonous source of carbon in streams (Augspurger *et al.,* 2008). In freshwater ecosystems, biofilms are complex matrices that can develop on any submerged or introduced surface, consisting of periphytic algae, bacteria, fungi, protozoa and their secretory products such as extracellular polymeric substances (EPS) and organic and inorganic non-living materials (Lock *et al.*, 1984; Finlay & Esteban 1998; Newman & McIntosh 1989; Burkholder 1996; Sekar *et al.*, 2002; Marxsen 2006). Biofilms contribute to the organic material in stream water through leached exudates, senescent material and sloughed dead, and live cells (Lock *et al.* 1984; Rounick & Winterbourn 1986), which can surpass that of catchment inputs (Minshall 1978). Biofilms are low in biomass, but high in turnover rate and thus can form the base of food webs (Lock *et al.* 1984; Rounick & Winterbourn 1986; Stevenson 1996). Biofilm organism assemblages characteristically have short life cycles, allowing a rapid response to changing conditions and can be the first to respond to and recover from stress (Lowe & Pan 1996). In low light environments biofilms are predominantly heterotrophic and dominated by bacteria (Blenkinsopp & Lock 1994), but in the presence of light, biofilms are dominated by photosynthetic organisms (autotrophs) the algae (Lock *et al.*, 1984), particularly Chlorophyta (green algae), Bacillariophyta (diatoms) and Cyanobacteria ("blue-green algae") (Peterson 1996). The algae component of these biofilms are fed upon by macroinvertebrate herbivores, and generalist omnivores, by grazing and scraping algae from submerged substrates and are often referred to as "grazers" (after Cummins & Klug 1979).

Aquatic macrophytes contribute to both the detrital, as dead macrophytes, and primary production pathways, through attached algae and its direct consumption through herbivory (Newman 1991). Few riverine macroinvertebrates feed on living macrophytes (Newman 1991; Pinder 1992) and herbivory by shredders upon this resource is low, as macrophytes contain a high content of refractory compounds together with a low nutrient content and the presence of chemical deterrents (Cummins

& Klug 1979; Mann 1988; Newman 1991). Macrophytes have been documented as the dominant energetic resource for some stream consumers in the Amazon (Forsberg *et al.*, 1993; Leite *et al.*, 2002), but this is not the case in temperate climate streams.

Algal primary production is an important complementary ecosystem process in many streams, providing a year round resource and can be assimilated up to three times more efficiently by macroinvertebrates than plant detritus (Benke & Wallace 1980). Even in some forested shaded streams, algae can contribute to almost 50% of macroinvertebrate secondary production (McCutchan & Lewis 2002). Algal primary production can directly influence the grazer community and vice versa and primary consumers can thus affect the supply rate of the resource (Richardson 1993), which is not the case with the donor-controlled detritus-detritivore interactions within the food web. The latter should, in theory, tend to counteract the destabilising effects of faster algal pathways by slowing the rate of energy transfer through the web, so the balance of the two is important for determining the stability of the system as a whole (Rooney *et al.*, 2006), and both pathways were examined in our study.

Riverine algae biomass can be regulated by interactions among the supply of resources (nutrients and light), grazing, differences in substrate or flow conditions, the frequency of disturbances associated with the hydrological regime and the presence of toxins (Biggs *et al*., 1998; Barbour *et al.*, 1999; Hillebrand, 2008). A positive relationship between benthic algal biomass and nutrient concentrations of total nitrogen (N) and total phosphorous (P) in the water column (see review Dodds 2006), have been found in field manipulations (Stevenson *et al.*, 2006; Rier & Stevenson 2006) and stream surveys (Biggs 2000). Grazing by herbivores can lead to a reduction of algal biomass and to changes in its composition and/or morphology (Feminella & Hawkins 1995; Hillebrand 2008; Holomuzki, Feminella & Power 2010). Different taxa and morphological forms of algae vary in the extent to which grazer taxa are able to consume and assimilate them (Dudley, Cooper & Hemphill 1986; Hill, Weber & Stewart 1992; Steinman, Mulholland & Hill 1992). Stressors could alter the proportions of algae that are palatable to grazing macroinvertebrates, and in response they may switch partly or totally between algal and detrital food resources (Chessman *et al.*, 2009). Algal accrual and the relative importance of bottom-up drivers of ecosystem functioning and food web dynamics (e.g., nutrients, light) versus top down effects (e.g., grazing by invertebrates), are measures commonly used to assess algal primary production and herbivory (after Lamberti & Resh 1983). The concentration of chlorophyll *a* is positively correlated with primary productivity (Wetzel 2001), and is often used as a surrogate measure for algal biomass stocks and growth rates (Kalff 2002; Gregor & Marsalek 2004), because chlorophyll is the major pigment used for photosynthesis in algal cells (Barbour *et al.*, 1999). Chlorophyll *a* measures were selected in this study because the most likely algae species encountered would be from the Chlorophyta and Bacillariophyta divisions, which predominantly use chlorophyll *a* to capture light for photosynthesis (Peterson 1996). However this does differ by species which can be dependent upon substrata, light, nutrients, disturbance regimes, flow regulation and grazing (Burns & Ryder 2001).

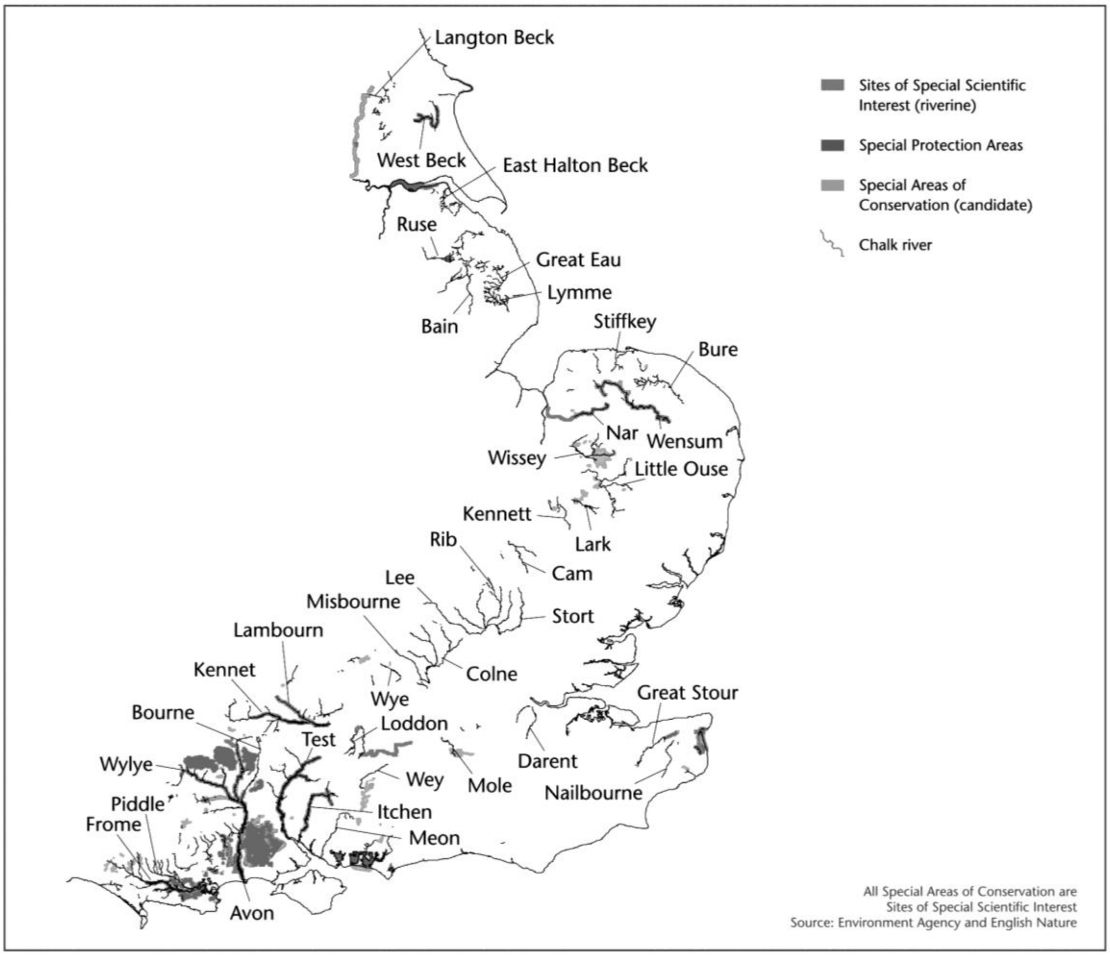
We focused specifically on the response of the functional measures primary production and herbivory in this study, through the accrual of algae and its consumption by grazing macroinvertebrates, by the selective exclusion of consumers in field manipulations, after Hladyz *et al.,* (2011a, b).

### ***Ecosystem functioning measures***.

In this study we sought to conduct the simultaneous examination of both main energy pathways as they enter the ecosystem, rather than the traditional ecosystem analysis approach of considering a single (usually structural) response variable. This was achieved using primary production, herbivory and leaf litter breakdown (both microbial and macroinvertebrate activity), to quantify consumer-resource interactions and to test ecological questions and hypotheses related to ecosystem and community responses. Most ecosystem functioning studies to date have concentrated on either the detrital or primary production pathways in isolation e.g. Dangles *et al.*,2004; Lecerf & Chauvet 2008; Riipinen, Davy-Bowker & Dobson 2008; Gücker, Boëchat & Giani 2009; Riipinen *et al.*, 2010, and very few have examined both simultaneously (but see Magbanua *et al*., 2010; Hladyz *et al*., 2011a, b). A recent land use study demonstrated the efficacy of an integrated structural and functioning approach their potential to assist in the identification of stressors and support their inclusion in future bioassessments (Hladyz *et al*., 2011a, b). Our study represents a relatively unexplored novel integrated approach and to our knowledge is the first to apply these in a replicated fashion, across multiple chalk stream ecosystems.

### ***Chalk streams and watercress farming.***

Chalk streams are found only in England, Belgium, France, the Netherlands and New Zealand (van Lanen & Dijksma 1999; Environment Agency 2004), with the English sites representing by far the greater number of this resource type in Europe (HCC 2000; Holmes 2006) (Fig 1.1).



All Special Areas of Conservation are Sites of Special Scientific Interest
Source: Environment Agency and English Nature

(For colour map see (p4) http://adlib.everysite.co.uk/resources/000/057/248/Summary_chalk_rivers.pdf)

Figure 1.1. Map showing the location of the UK’s chalk rivers and streams and the rivers used in the thesis. The rivers studied are located to the bottom left of the map. The Frome and Piddle are to the left of the Avon and the Test, Itchen and Meon are to the right.

These ecosystems are among the most productive (Westlake *et al.*, 1972) and species-rich of all temperate fresh waters (Wright 1992; Woodward *et al.,* 2008), but about one third of England's chalk streams are presently classified as "poor" or "very poor" in terms of their channel habitat quality, reflecting the impacts of multiple stressors, including those associated with organic pollution (e.g. oxygen depletion and suspended solids contamination), over-abstraction (e.g. low flows) which serve to concentrate pollutants, intensive agriculture (e.g. nitrate and phosphate enrichment), fish farming and watercress farming (EA 2004). Chalk streams are especially susceptible to meteorological drought, as up to 90% of their annual discharge is derived from groundwater in the underlying chalk aquifer, and their flow is strongly related to rainfall in the preceding months (Casey, Smith, & Clarke 1990; Berrie 1992). The fragile ecosystems of these streams are threatened further by drought, caused by river diversions, groundwater abstraction and their adverse effect on groundwater recharge (Wright & Berrie 1987; Castella *et al.,* 1995; Agnew *et al.,* 2000). The threat is further exacerbated because maximum periods of groundwater extraction from the chalk aquifer coincides with natural periods of low flow (Agnew *et al.,* 2000) and because these aquifers are the most important groundwater source in south east England, accounting for 72% of the water public supply (EA 2008). As a result, chalk streams have been identified as a key habitat under the EC Habitats Directive and UK Biodiversity Action Plan (BAP) and listed as a priority for protection (HMSO 1995; HCC 1998; Acreman *et al.,* 2000; Jarvie *et al.,* 2006).

The chalk streams of Southern England are fed by groundwater aquifers to produce clear waters of good chemical quality, relatively stable temperatures and hydrology within their shallow channels, which protect macrophytes from frost and promote growth during the winter months (Berrie 1992; Sear, Armitage & Dawson

1999; EA 2004). In addition, because the majority of chalk stream discharge can be derived from groundwater, violent floods are rare. Chalk streams are regarded as stable biological habitats with a ratio of < 6:1 maximum to minimum discharge, in contrast to mountain streams which can have ratios > 40:1 (Casey, Smith, & Clarke 1990). These features strongly buffer chalk streams against changes in temperature and flows (Ibbotson *et al.,* 1994), features that also favour watercress farming (Casey 1981; Stevens 1983; Casey & Smith 1994).

Watercress has been produced in the UK since Roman times e.g. River Mimram, Hertfordshire. However, the first British large scale commercial watercress farm opened in 1808 (Glenny 1897) and currently 39 of the 161 UK chalk rivers and streams (EA 2004) have such farms on their headwaters (Cox 2006) (Fig 1.1.). The headwaters of most chalk streams in Dorset and Hampshire are now occupied by watercress beds or trout farms, and natural undisturbed and unmanaged water sources are almost non-existent (Casey, Smith, & Clarke 1990).

The watercress industry has boomed in recent years, with UK annual sales in 2010 in excess of £55m, having grown by £18m since 2006 (Nielsen UK market data). This surge in production has been driven partly by the perceived status of watercress as an anticarcinogen (Gill *et al.,* 2007; Wu, Zhou & Xu 2009; Wu *et al.,* 2010), due to the high levels of phenylethylisothiocyanate (PEITC) that it contains, and its designation as a “superfood”. Watercress is also high in antioxidants, Vitamin C, calcium, iron and folate, which are again often marketed as important constituents of health foods (Hedges & Lister 2005).

### ***The problem at St Mary Bourne and other watercress farms and its remediation.***

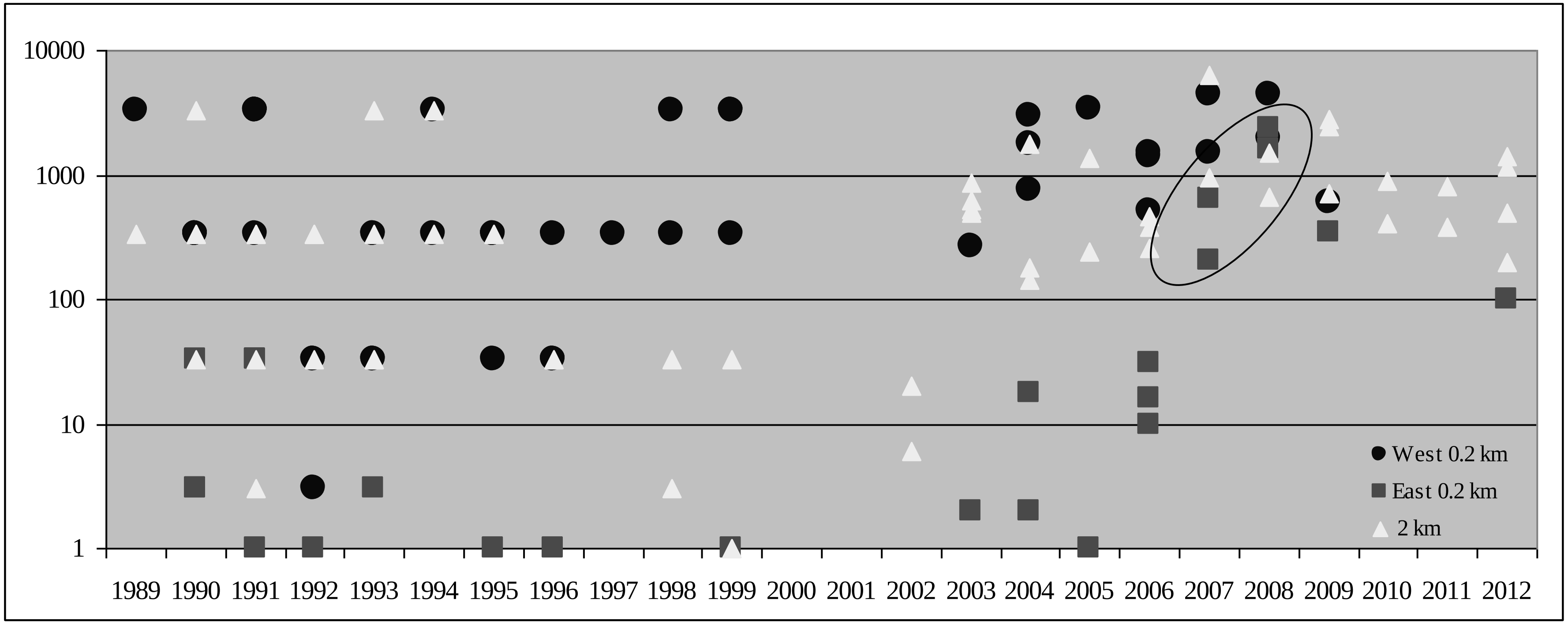
The Bourne Rivulet, the focal site of this study, is a first order tributary of the River Test (EA 2004), famed as the birthplace of fly-fishing (EA 2006). Located at the head of the Bourne rivulet is the Vitacress Salads Ltd (VSL) watercress farm (the largest in Europe) and an associated factory. The factory washes and packs ready for sale watercress sourced from SMB, other VSL UK & EU farms and other salad leaves from Europe, Africa and America. The overseas salad leaves are flown to Amesbury, Wiltshire and transported by road to SMB.

The outfalls from this and other watercress farms are typically of the highest chemical quality (River Ecosystem level 1 [RE1]), as defined by UK Environment Agency (EA) standards (Medgett 1998; Martin 2007; EA 2010). However, EA surveys have revealed localised but a long-term decline in the abundance of key macroinvertebrate taxa, in particular the freshwater shrimp *G. pulex* (Fig 1.2 EA data pp. 29). This phenomenon has been reported both at the SMB site (Soulsby 1975; Medgett 1998; McSwan 2005; White & Medgett 2006) and at other watercress farms (Roddie, Edwards & Crane 1992; EA 1992, 1995, 1997-1998, 2004a).

*G. pulex* is common in most running waters across Europe, from headwaters to medium sized rivers (Moenickes *et al*., 2011). The species is considered characteristic of chalk streams (EA 2004) where it is often observed in high abundance (Berrie 1992). *G. pulex* can be the dominant macroinvertebrate in UK chalk streams (Joyce & Wotton 2008), in terms of numbers and population biomass, (MacNeil, Dick & Elwood 1999; Harrison & Harris 2002), attaining densities of 1,000–10,000 individuals / m$^2$ (Pardo & Armitage 1997; Armitage & Cannan 2000). *G. pulex* is also a key processor of detrital inputs to the food web (Woodward *et al*., 2008) as well as being important prey for bullheads *(Cottus gobio L.)* (Mills & Mann 1990) and salmonid fishes (Maltby 1994).

*G. pulex* is also widely used as an indicator of good water quality (Maltby *et al.*, 2002), as it is sensitive to a number environmental contaminants (Forrow & Maltby 2000; Bloor, Banks & Krivtsov 2005; see review Kunz, Kienle & Gerhardt 2010) and used in monitoring their impact e.g. eutrophication (Whitehurst 1991), pesticide bioassays (Matthiessen *et al.*, 1995) and acidification (Guerold *et al.*, 2000). Thus *G. pulex* is an important species for studies of watercress farming impacts on chalk streams. The absence of this provider of key “ecosystem goods and services” is therefore of both ecological and economic concern, since chalk streams and rivers are of high amenity value (Neal & Whitehead 2002) and support valuable and productive sport fisheries (Mann, Blackburn & Beaumont 1989).

.



Source Environment Agency.

Fig 1.2. St Mary Bourne long-term *G. pulex* numbers. Depicting the long-term decline in *G. pulex* numbers downstream of the SMB watercress farm. Data from standard Environment Agency methodology - three minute un-replicated kick samples. Samples taken: i) west Bourne Rivulet 0.2 kms below the farm where no effluent from the watercress farm is released, ii) the west Bourne Rivulet 0.2 kms below the farm where effluent from the watercress farm are released, iii) 2 kms downstream of the watercress farm after both the east and west Bourne Rivulet converge (Fig 2.3 pp.60). The ringed area depicts an increase in *G. pulex* numbers, evidence of recovery from 2007 onwards, in the east Bourne rivulet. Lowland chalk stream (River Frome Dorset) studies have found densities of 1,000–10,000 individuals / m$^2$ (Pardo & Armitage 1997; Armitage & Cannan 2000).

In the 1980s, in response to the negative EA surveys, the shift to more intensive methods of watercress production, and the regulator-enforced need to reduce watercress farm impacts on key macroinvertebrate taxa including *G. pulex*, steps were taken by the National Rivers Authority (NRA) to license the discharges of all watercress farms, with specific limits on a number of parameters in the receiving water. These included: pH to be no less than 6 or greater that 9; free chlorine shall be absent; total zinc concentration should not exceed 75 µg/l; Malathion concentration (an organophosphorous insecticide) to not exceed 0.5 µg/l (pesticides are now no longer used at any watercress farms); the entire flow from a farm must pass through a settlement tank / lagoon or other treatment plant, where the final suspended solid effluent entering the carrier stream must not exceed 20 mg/l (Cox 2006). Specifically at SMB prior to the current study, a range of potential stressors had been curtailed in a series of unsuccessful attempts to mediate the impact of the watercress farm, in terms of restoring *G. pulex* populations to their historically far more abundant levels. For example, since 2004 the effluent from the factory salad washing and packing operation has passed over a parabolic screen to remove all leaf fragments >2mm in size (EA compliance standard >5mm), and a settling system to remove suspended silt and sand. This was to prevent the further accumulation of a fine red-coloured silt and sand that had accumulated in the receiving river (east Bourne rivulet), which was thought to be derived from the imported salad leaves. The east Bourne rivulet was dredged to remove these deposits in November 2005 at the request of the EA (Cox 2006). In addition: i) the use of zinc use as a fungicide ceased in 2002 (Cox 2006), ii) in an attempt to minimise the SMB farms impact on nutrient levels, the application of slow release fertiliser pellets was supplemented with liquid fertiliser sprayed directly into the incoming borehole water and iii) the watercress bed waste water effluents (i.e. during bed preparation), and the factory waste water effluents (i.e watercress sanitising wash) were all passed into a settlement tank. Despite all of these measures, and an exhaustive search for other pollutants, *G. pulex* populations had not recovered in the Bourne rivulet below the SMB watercress farm, prior to the current studies (White & Medgett 2006) (Fig. 1.2. pp. 29).

To sanitise the watercress and salad leaf at the SMB factory, sodium hypochlorite was used to chlorinate the secondary salad wash. Chlorine readily dissolves in water and in the presence of nitrogen can produce chloramines (Lind 1995), notably monochloramines (the most toxic chloramine) particularly when within a pH range of 7 - 8 (Palin 1950; White 1992), with a half-life ranging from 75 hours to 12.5 days, dependent upon temperature (Vikesland, Ozekin & Valentine 2001). Monochloramines, chlorine and ammonia, individually and synergistically, are toxic to aquatic life, as are their breakdown products (Fisher *et al*., 1999; Berenzen, Schultz, & Liess 2001; Beketov 2004). Chloramines can also persist in the presence of sodium metabisulphite (Bedner, MacCrehan & Helz 2004; MacCrehan, Bedner & Helz 2005), the dechlorinating agent used at SMB. It is therefore highly likely that monochloramine was produced in the settlement tank, formed in a reaction between chlorine from the dechlorination effluent and nitrogen from the watercress beds clearing effluent, although it was not specifically measured in this study. Monochloramine may be able to persist for days and even weeks after release into the carrier river at the outfall, where reduced *G. pulex* abundance and leaf litter breakdown rates began to be evident. In an ongoing search for potential pollutants, chlorine use in the factory was discontinued in July 2006. It was replaced by an additional spring water wash, amid industry concerns over carcinogenic chlorine disinfection by-products remaining on salad leaves, such as haloacetic acids and trihalomethanes (Hua & Reckhow 2007; Wei *et al.*, 2007). The initiative was also to improve the taste and nutrient content, and was in agreement with the UK grocery multiples e.g. Sainsbury, Tesco and Waitrose, major customers of Vitacress (S. Rothwell pers.comm.).

Further, it had been suggested (Fewings 1999) that the watercress itself could be responsible for the persistently low abundance of *G. pulex* immediately downstream of watercress farms, acting via the release into the stream during watercress harvesting and washing, of the allelochemical phenylethylisothiocyanate (PEITC) a mustard oil which gives watercress its characteristic peppery flavour. PEITC functions as an herbivory defence mechanism (Newman, Hanscom & Kerfoot, 1990, 1992, 1996) and is a breakdown product of the watercress glucosinolate / myrosinase chemical defence system (Newman, Hanscom & Kerfoot, 1992), and in laboratory experiments, at high concentrations is toxic to *G. pulex* (Worgan & Tyrell 2006). Thus PEITC could potentially be responsible for the deleterious effect on *G. pulex.* With this in mind, it has been suggested that watercress beds could act as mini-wetlands and bioremediate PEITC. Direct measurement of PEITC was not possible and beyond the scope of this study, because no reliable standard methodology has been established in an aqueous matrix, in part due to its high volatility. However, gas chromatography-mass spectroscopy (GC-MS) has been demonstrated as a method that may be able to provide a reliable assay in the future (Dixon 2010).

The horizontal sub-surface flow type of constructed wetland (Vyzamal 2005), widely used across Europe, has a number of features that resemble a watercress bed e.g. the presence of vegetation, flowing water, a filtration medium and an impermeable lining. Artificially constructed wetlands are widely used to treat agricultural effluents (see Kadlec *et al.*, 2000), and the industrial breakdown of isothiocyanates (mustard oils) found in other members of the *Brassicae* family (e.g. rapeseed oil), are routinely achieved by exposing flowing water to bacteria (Hardman, McEldowney & Waite 1993, Breen & Seymour 1994) and phytodegradation by rooted macrophytes, as occurs in natural wetlands. By re-routing the farm wastewaters through the watercress beds the action of bacteria in their root systems, phytodegredation, photolysis, temperature, oxidation and exposure to the environment, could thus be able to bioremediate the perceived effects of PEITC effluent release (S. Rothwell pers.comm.) (sensu Vymazal 2006).

VSL were very keen to implement the re-routing (July 05). However, to have a baseline dataset (June 05) prior to re-routing, we negotiated a delay of 35 days in which to plan, design, and obtain our biomonitoring data, initially to detect the effects of watercress farming and subsequently the effects of the re-routing. This precluded the running of leaf litter breakdown trials to determine the optimum immersion period for chalk streams, i.e. tannins to be leached and for hyphomycetes to colonise and condition the oak leaves necessary prior to macroinvertebrate feeding. However, the EU Rivfunction project researching into the effects of litter decomposition in response to eutrophication and the modification of riparian vegetation, at 60-paired sites in ten Eco-regions throughout Europe; used leaf bags with 5g of leaf litter immersed for between 20 – 70 days (Rivfunction 2013). Specifically, Hladyz *et al.*, (2011a, b) experimentally determined a T50 for oak leaves: i.e. when 50% of the initial leaf mass (5g) had been lost from a set of leaf bags placed in a repeatedly sampled headwater reference stream, at 35 days. We were also concerned that 35 days might be too long an immersion time, because we had observed large numbers of *G. pulex* in the east Bourne rivulet at SMB (side site), which might consume and empty the leaf bags, thus preventing the calculation of process rates. We therefore made regular inspections during our initial experiments, without disturbing the bags, to ensure that leaf litter was still present during their period of immersion. With regard to *G. pulex* recolonisation within and downstream of the SMB watercress farm, *G. pulex* are very robust and rapid upstream colonisers of newly wetted winterbourne reaches, generally limited to two kilometres upstream from the perennial head (House & Punchard 2010) which in this case is the SMB watercress farm. *G. pulex* may also be present further upstream of SMB where typically they are able to recolonise from small spring pools and refugia, which may not have completely dried out in the winterbourne section. *G. pulex* are also present within the SMB watercress beds (WC site) and in the main body of the east Bourne rivulet (side site) flowing through SMB, all of which are upstream or within the affected region of the Bourne rivulet. Given that *G. pulex* are highly mobile and rapid recolonisers in stream assemblages (Graca *et al.*, 2010) and that PEITC is highly volatile and has a short residence time of 30 - 120 minutes in freshwaters (Environ 2008), we reasoned that a time frame of between 30 -35 days should be more than sufficient to see a measurable biological recovery in terms of *G. pulex* abundance and their consumption of leaf litter. We concluded that a 31 day immersion period was the best choice taking into consideration our experimental, logistical and time constraints.

It was proposed (S. Rothwell pers.comm.) that if the watercress beds were shown to be able to successfully bioremediate the farm effluent i.e. the return of *G. pulex*, then a long-term solution in the form of a permanent artificial wetland may be constructed below the farm. A base line dataset was determined in June 2005 and the VSL farm’s effluent was re-routed back through the watercress beds from July 2005 onwards, the land immediately below the farm was purchased and plans for the wetland (Appendix 1 pp. 227) were drawn up for the project with this in mind (S. Rothwell pers.comm.). Ultimately a permanent wetland was not required and therefore was not constructed.

### ***Aims and structure of the thesis.***

This study sought to assess through a series of complementary case studies, how integrated measures of ecosystem processes and community structure, as advocated by the EU WFD, could offer greater insights into the impacts of anthropogenic stressors, than offered by the use of structural or functional measures in isolation. We investigated the effects that watercress farms have upon the headwater chalk streams that they utilise, to test ecological questions and hypotheses related to their impact, on both ecosystem processes and the structure of their macroinvertebrate community assemblages. The SMB watercress farm known to have a downstream invertebrate impact, in particular the absence of the freshwater shrimp *G. pulex*, the subject of much debate and conjecture, upon which this thesis seeks to add some experimental rigour and more formal analysis. Having determined the extent of the decline in ecosystem functioning and community structure across the SMB watercress farm (Chapter 4), we investigated the response of ecosystem processes and community structure to a series of changes to farm management practices, specifically designed to remediate the perceived negative ecological impacts of watercress farming on the Bourne Rivulet (Chapter 4 and 5). We also sought to determine whether this pattern of impairment was general and consistent across other chalk streams with watercress farms on their headwaters, in

Hampshire and Dorset and over time (Chapter 6). By using a combination of existing techniques in an integrated novel way, we were able provide new insights into ecosystem impairment that cannot be achieved using current bioassessment techniques in isolation.

Specifically our objectives were as follows: (the structure of the thesis and the data chapters are summarised in Fig.1.3 & Table. 1.1).

1) Obtain a baseline dataset i.e. pre re-routing.

2) Is there a response to re-routing?

3) What is the situation at other watercress farms?

4) What is the effect of season at SMB and other farms?

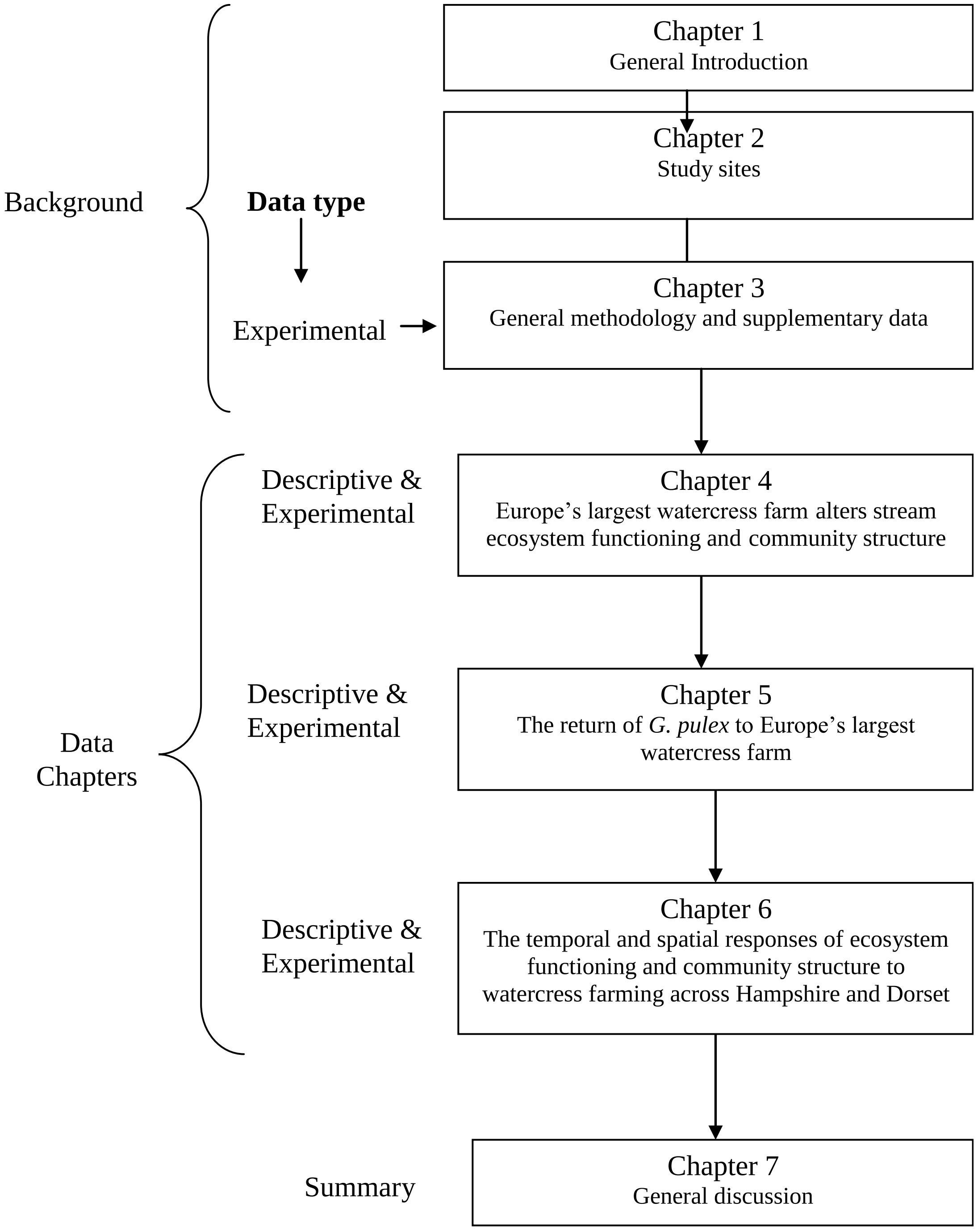


Figure 1.3. Structure of the Thesis.

Table 1.1. Structure of the Data Chapters.

| **Chapter** | **Date** | **Sample site** | **Scale of study** |
|---|---|---|---|
| 4 | June 2005<br>July 2005 | 5 sites | Temporal: Inter-annual<br>Spatial: 5 sites, 1 river |
| 5 | June 2005<br>June 2006<br>June 2007 | 3 sites | Temporal: Inter-annual<br>Spatial: 3 sites, 1 river |
| 6 | Autumn 2006<br>Winter 2006/7<br>Spring 2007<br>Summer 2007 | 3 sites | Temporal: Inter-seasonal<br>Spatial: 3 sites, 3 river |
| 6 | Summer 2007 | 3 sites | Temporal: Summer 2007<br>Spatial: 3 sites, 6 river |

## References.

Abelho, M., Cressa, C. & Graca, M., A., S., (2005) Microbial biomass, respiration and decomposition of *Hura crepitans* L. (Euphobiacea) leaves in a tropical stream. *Biotropica*, **37**, 397-402.

Acreman, M., C., Adams, B., Birchall, P. & Connorton, B., (2000) Does Groundwater Abstraction Cause Degradation of Rivers and Wetlands? *Journal of the Chartered Institution of Water and Environmental Management*, **14**, 200-206.

Agnew, C.T., Clifford, N.J. & Haylett, S., (2000) Identifying and alleviating low flows in regulated rivers: the case of the Rivers Bulbourne and Gade, Hertfordshire, UK. *Regulated Rivers: Research & Management*. **16**, 245–266.

Armitage, P. D. & Cannan, C. E., (2000) Annual changes in summer patterns of mesohabitat distribution and associated macroinvertebrate assemblages. *Hydrological Processes*, **14**, 3161–3179.

Armitage P.D., Moss D., Wright J.F. & Furse M.T. (1983) The performance of a new biological water-quality score system based on macroinvertebrates over a wide-range of unpolluted running-water sites. *Water Research*, **17**, 333–347.

Arsuffi, T., L. & Suberkropp, K., (1985) Selective feeding by caddisfly (Trichoptera) detritivores on leaves with fungal colonized patches. *Oikos*, **45**, 50-58.

Augspurger, C., Gleixner, G., Kramer, C., Küsel, K., (2008) Tracking carbon flow in a 2-week-old and 6-week-old stream biofilm food web. *Limnology and Oceanography* **53**:, 642−650.

Barbour, M., T., Gerritsen, J., Snyder, B., D. & Stribling, J., B., (1999) Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. 2nd edn. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Barlocher, F. & Graca, M., A., S., (2002) Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. *Freshwater Biology*, **47**, 1123-1135.

Bedner, M., MacCrehan, W., A. & Helz, G., R. (2004) Making chlorine greener: Investigation of alternatives to sulfite for dechlorination. *Water Research*, **38**, 2505-2514.

Beketov, M., A., (2004) Different sensitivity of mayflies (Insecta, Ephemeroptera) to ammonia, nitrite and nitrate: linkage between experimental and observational data. *Hydrobiologia*, **528,** 209–216.

Benke, A., C. & Wallace, J., B., (1980) Trophic basis of production among net-spinning caddisflies in a Southern Appalachian stream, *Ecology*, **61**, 108 -118.

Berenzen, N., Schultz, R. & Liess, M., (2001) Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. *Water Research*, **35,** 3478–3482.

Berrie, A., D., (1976) *Detritus, micro-organisms and animals in fresh water.* In: J. M. Anderson & A. Macfadyen (eds), The Role of Terrestrial and Aquatic Organisms in Decomposition Processes, 17th Symposium of British Ecological Society. Blackwell, Oxford: 323-338.

Berrie, A., D., (1992) The chalk-stream environment. *Hydrobiologia*, **248**, 3-9.

Bernhardt, E., S. & Likens, G., E. (2004) Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology*, **49**, 14-27.

Biggs, B., J., F., (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society*, **19**, 17-31.

Biggs, B., J., F., Stevenson, R., J. and Lowe, R., L., (1998) A habitat matrix conceptual model for stream periphyton. *Archiv fur Hydrobiologie*, **143**, 21–56.

Blenkinsopp, S. A. & Lock M. A., (1994) The impact of storm-flow on river biofilm architecture. *Journal of Phycology*, **30**, 807–818.

Bloor, M., C., Banks, C., J. & Krivtsov, V., (2005) Acute and sublethal toxicity tests to monitor the impact of leachate on an aquatic environment. *Environment International*, **31**, 269-273.

Boulton, A., J. & Boon, P., I., (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over a new leaf? *Australian Journal of Marine and Freshwater Research*, **42**, 1–43.

Breen, P., F. & Seymour, B., S., (1994) The Combination of a Flood-Retarding Basin and a Wetland to Manage the Impact of Urban Runoff. *Water Science Technology*, **29**, 103-109.

Bunn, S., E. & Davies, P., M., (2000) Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia*, **422/423**, 61–70.

Burkholder, J. M., (1996) Interactions of benthic algae with their substrata. In: Stevenson, R.J., Bothwell, M.L., Lowe, R.L. (Eds.), Algal Ecology of Freshwater Benthic Ecosystems, Aquatic Ecology Series. Academic Press, Boston, pp. 253–298.

Burns, A., & Ryder, D. S., (2001) Potential for biofilms as biological indicators in Australian riverine systems. *Ecological Management and Restoration*, **2 (1)**, 53–63.

Canhoto, C. & Graca, M., A., S. (1995) Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis*. *Freshwater Biology*, **34**, 209-214.

Canhoto, C. & Graca, M., A., S., (1999) Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. , *Microbial Ecology*, **37**, 163-172.

Canhoto, C., Barlocher, F. & Graca, M., A., S., (2002) The effects of *Eucalyptus globulus* oils on fungal enzymatic activity. *Archives of Hydrobiology*, **154**, 121-132.

Carlisle DM, Clements WH. (2005) Leaf litter breakdown, microbial respiration and shredder production in metalpolluted streams. *Freshwater Biology*, **50**, 380– 90.

Casey, H., (1981). Discharge and chemical-changes in a chalk stream headwater affected by the outflow of a commercial watercress bed. *Environmental Pollution, Series B,* 2, 5, pp. 373–385.

Casey, H. & Smith, S., M., (1994) The effects of watercress growing on chalk headwater streams in Dorset and Hampshire. *Environmental Pollution*, **85,** 217–228.

Casey, H., Smith, S., M. & Clarke, R., T. (1990). Trends and seasonality in the nitrate concentrations and loads of the Bere stream (Dorset) for the period 1966-1986. *Chemistry and Ecology*, **4**, 85-94.

Chessman, B., C., Westhorpe, D., P., Mitrovic, S., M., & Hardwick, L., (2009) Trophic linkages between periphyton and grazing macroinvertebrates in rivers with different levels of catchment development. *Hydrobiologia*, **625**, 135-150.

Castella, E., Bickerton, M., Armitage, P.D. & Petts, G.E., (1995) The effects of water abstractions on invertebrate communities in UK streams. *Hydrobiologia*, **308**, 167–182.

Cox, J., Associates (2006) *Watercress growing and its environmental impacts on chalk rivers in England.* Report by Jonathan Cox Associates. English Nature Contract No. EIT-05-020 p 56.

Cummins, K., W. & Klug, M., J., (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, **10**, 147-172.

Cummins, K., W., Wilzbach, M., A., Gates, D., M., Perry, J., B., and Taliaferro, W., B. (1989) Shredders and riparian vegetation: leaf litter that falls into streams influences communities of stream invertebrates. *BioScience*, **39**, 24.30.

Dangles, O. & Guerold, F. (1999) Impact of headwater stream acidification on the trophic structure of macroinvertebrate communities. *International Review of Hydrobiology*, **84**, 287–297.

Dangles, O. & Guerold, F. (2001) Influence of shredders in mediating breakdownrates of beech leaves in circumneutral and acidic forest streams. *Archiv f¨ur Hydrobiologie* **151**, 649–666

Dangles, O., Gessner, M., O., Guérold, F. & Chauvet, E., (2004) Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. *Journal of Applied Ecology***,** **41**, 365-378.

Davies-Colley, R., J., Hickey, C., W., Quinn, J., M., & Ryan, P., .A. (1992) 'Effects of clay discharges on streams: 1. Optical properties and epilithon', *Hydrobiologia*, **248**, 215–234.

Davy-Bowker, J., Murphy, J., F., Rutt, G., R., Steel, J., E., C., & Furse, M., T., (2005). The development and testing of a macroinvertebrate biotic index for detecting the impact of acidity on streams. *Archives of Hydrobiology***,** **163**, 383–403.

Dawson, F., H. (1976) Organic contribution of stream edge forest litter fall to the chalk stream ecosystem. *Oikos*, **27**, 13-18.

De Lange, H., J., M. Lurling, M., B., Van den Borne, E., T., & Peeters, H., M. (2005) Attraction of the amphipod *Gammarus pulex* to water-borne cues of food. *Hydrobiologia*, **544**, 19–25.

Dixon, M. J., (2010) *The sustainable use of water to mitigate the impacts of watercress farms on chalk streams in Southern England*, Ph.D. thesis, Department of Civil Engineering and the Environment, University of Southampton. http://eprints.soton.ac.uk/195397/1.hasCoversheetVersion/DIXON_MJ_Corrected_PhD_Thesis.pdf Retrieved 27.01.2013.

Dodds, W., K., (2006) Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography*, **51**, 671–680.

Dudley, T., L., Cooper. S., D. & Hemphill. N., (1986) Effects of macroalgae on a stream invertebrate community. *Journal of the North American Benthological Society* **5**, 93–106.

Environ (2008) *Literature review of the behaviour of mustard oils in aquatic ecosystems.* Report for Vitacress Salads Limited by Environ UK. Ltd.

Environmental Agency (1992) *Invertebrate data Dorset / Wiltshire 1992*

Environmental Agency (1995) *Invertebrate data Dorset / Wiltshire 1995*

Environmental Agency (1997-1998) *Invertebrate data Dorset / Wiltshire 1997-1998*

Environmental Agency (2004a) *Invertebrate data Dorset / Wiltshire 2004*

Environment Agency (2004) *The State of England's Chalk Rivers*.

Environment Agency (2006) The Test and Itchen Catchment Abstraction Management Strategy (CAMS) available at Retrieved 31.08.2012 http://publications.environment-agency.gov.uk/PDF/GESO0306BKMB-E-E.pdf

Environment Agency (2008) *Draft River Basin Management Plan, South East River Basin District December 2008.*

Environment Agency (2010). River Ecosystem (RE) Classification System. Retrieved 20.03.2010 http://www.environment-agency.gov.uk/research/planning/40297.aspx Retrieved 20.03.2010. http://www.environmentagency.gov.uk/static/documents/Research/river_e1.doc Retrieved 28.03.2012. http://www.ashford.gov.uk/pdf/Appendix%203.2%20-%20River%20Ecosystem%20Classification%20System.pdf

European Commission (2003) *The new EU Water Framework Directive*. Available from: Retrieved 28.08.2012. http://ec.europa.eu/environment/water/water-framework/index_en.html.

EPA (2002) Environmental Protection Agency. American clean water act (EPA ACWA). Available from: Retrieved 28.08.2012. http://epw.senate.gov/water.pdf

Fellows, C., S., Clapcott, J., E., Udy, J., W., Bunn, S., E., Harch, B., D., Smith, M., J. & Davies, P., M. (2006) Benthic metabolism as an indicator of stream ecosystem health, *Hydrobiologia*, **572**, 71-87.

Feminella, J., W. & Hawkins, C., P., (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of North American Benthological Society,* **14**, 465–509.

Fewings, S., (1999) *Survey of Operational Practice on Watercress Farms in Hampshire*. Collaborative Project of the Environment Agency, Horticultural Development Council and Watercress Growers Association.

Finlay, B. J., & Esteban, G. F., (1998) Freshwater protozoa: biodiversity and ecological function. *Biodiversity and Conservation,* **7**, 1163-1186.

Fisher, D. J., Burton, D. T., Yonkos, L. T., Turley, S. D. & Ziegler. G. P. (1999) The relative acute toxicity of continuous and intermittent exposures of chlorine and bromine to aquatic organisms in the presence and absence of ammonia. *Water Research*, **33**, 760-768.

Forrow, D., M. & Maltby, L., (2000) Toward a mechanistic understanding of contaminant-induced changes in detritus processing in streams: direct and indirect effects on detritivore feeding. *Environmental Toxicology and Chemistry*, **19**, 2100 – 2106.

Forsberg, B. R., Araujo-Lima, C., A., Martinelli, L., A., Victoria, R., L. & Bonassi, J., A., (1993) Autotrophic carbon sources for fish of the Central Amazon. *Ecology,* **74,** 643-652.

Gessner, M., O. & Chauvet, E., (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology,* **75**, 1807-1817.

Gessner, M., O. & Chauvet, E., (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications,* **12**, 498–510.

Gill, C., I., R., Haldar, S., Boyd, L., A., Bennett, R., Whiteford, J., Butler, B., Pearson, J., R., Bradbury, I. & Rowland, I., R. (2007) Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy volunteers. *American Journal of Clinical Nutrition,* **85,** 504-510.

Giller, P., S., Hillebrand, H., Berninger, U., G., Gessner, M., O., Hawkins, S., Inchausti, P. *et al.*, (2004) Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos,* **104**, 423–436.

Giller, P., S. & Malmqvist, B., (1998) *The Biology of Streams and Rivers*. Oxford University Press, Oxford.

Gleick, P. H., (1996) Water resources. In Encyclopedia of Climate and Weather, ed. by S. H. Schneider, Oxford University Press, New York, vol. 2, pp.817-823.

Glenny**,** W., W., (1897) Watercress, its history and cultivation. *Journal of the Royal Agricultural Society of England,* **8**, 607–622.

Gonzalez, J., M. & Graca, M., A., S. (2003) Conversion of leaf litter to secondary production by the shredder caddisfly *Sericostoma vittatum. Freshwater Biology,* **48**, 1578-1592.

Graca, M., A., S., (1992) Starvation and food selection by stream detritivores. *Ciencia Biologica. Ecology and Systematics,* **12**, 27-35.

Graca, M., A., S., (2001) The role of invertebrates on leaf litter decomposition in streams – A review. *International Review of Hydrobiology,* **86**, 383-393.

Graca, M., A., S. & Barlocher, F., (1998) Proteolytic gut enzymes in *Tipula caloptera* – interaction with phenolics. *Aquatic Insects,* **21**, 11-18.

Graça, M., A., S. & Canhoto, C. (2006) Leaf litter processing in low order streams. *Limnetica,* **25**, 1–10.

Graca, M., A., S., Cressa, C., Gessner, M., O., Feio, M., J., Callies, K., A. & Barrios, C., (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology,* **46**, 947–957.

Gregor, J., & Marsalek, B. (2004) Freshwater phytoplankton quantification by chlorophyll a: A comparative study of in vitro, in vivo and in situ methods. *Water Research,* **38**, 517–522.

Gregory, S., V. (1983) Plant-herbivore interactions in stream systems. pp 157–189 i*n* Barnes, J., R, Minshall, G., W. (eds) Stream Ecology: Application and Testing of General Ecological Theory. New York: Plenum Press.

Griffith, M., B., Perry, S., A. & Perry, W., B., (1994) Secondary production of macroinvertebrate shredders in headwater streams with different baseflow alkalinity. *Journal of the North American Benthological Society,* **13**, 345–356.

Gucker, B., Boechat, I. G., & Giani A. (2009) Impacts of agricultural land use on ecosystem structure and whole-stream metabolism of tropical Cerrado streams. *Freshwater Biology,* **54**, 2069 - 2085.

Guerold, F., Boudot, J., P., Jacquemin, G., Vein, D., Merlet, D. & Rouiller, J., (2000) Macroinvertebrate community loss as a result of headwater stream acidification in the Vosges mountains (N-E France). *Biodiversity and Conservation,* **9**, 767–783.

Gulis V., Ferreira V. & Graca A., S. (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. *Freshwater Biology,* **51**, 1655–1669.

Hardman**,** D., J., McEldowney, S. & Waite, S. (1993) Pollution: Ecology and Biotreatment, Longman Scientific and Technical, London, England.

Harrison, S., S., C. & Harris, I., T., (2002) The effects of bank side management on chalk stream invertebrate communities. *Freshwater Biology,* **47**, 2233-2245.

Harmon, M., E., Franklin, J., F., Swanson, F., J., Sollins, P., Gregory, S., V., Lattin, J., D., Anderson, N., H., Cline, S., P., Aumen, N., G., Sedell, J., R., Lienkaemper, G., W., Cromack, Jr., K. and Cummins, K., W. (1986) Ecology of coarse woody debris in temperate ecosystems. In *Advances in Ecological Research,* Vol. 15, pp. 133-302.

Hawkes, H., A., (1997) Origin and development of the Biological Monitoring Working Party (BMWP) score system. *Water Research,* **32(3)**, 964-968.

H.C.C., (1998) Biodiversity Action Plan for Hampshire: Volume One, Hampshire Biodiversity Partnership.

H.C.C., (2000) Biodiversity Action Plan for Hampshire: Volume Two, Hampshire Biodiversity Partnership. Chalk streams. Retrieved 28.08.2012 from http://www.hampshirebiodiversity.org.uk/pdf/PublishedPlans/ChalkStreamsHAPjjDTP2.pdf

Heard, S., B., Schultz, G., A., Ogden, C., B. & Griesel, T., C. (1999) Mechanical abrasion and organic matter processing in an Iowa stream. *Hydrobiologia*, **400**, 179–186.

Hedges, L., J., & Lister, C., E. (2005) Crop & Food Research Confidential Report No. 1473. Nutritional attributes of salad vegetables. Vegfed New Zealand Institute for Crop & Food Research Limited. Private Bag 4704, Christchurch, New Zealand.

Herricks, E., E. & Cairns, J., J., (1982) Biological monitoring. Part III – receiving system methodology based on community structure. *Water Research*, **16**, 141–153.

Heywood, M., J., T., & Walling, D., E., (2003) Suspended sediment fluxes in chalk streams in the Hampshire Avon catchment, UK In 'The Interactions between Sediments andWater. Proceedings of the 9th International Symposium on the Interactions between Sediments and Water'. (Ed. B. Kronvang.) pp. 111–117. (Kluwer Academic Press: London.)

Hieber, M. & Gessner, M., O., (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026-1038.

Hill, W., R., Ryon, M., G. & Schilling, E., M. (1995) Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology,* **76**, 1297-1309.

Hill, W., R., Weber, S., C. & Stewart, A., J., (1992) Food limitation of two lotic grazers: quantity, quality, and size-specificity. *Journal of the North American Benthological Society,* **11**, 420–432.

Hillebrand, H., (2008) Grazing regulates the spatial variability of periphyton biomass. *Ecology*, **89**, 165–173.

Hladyz, S., Abjornsson, K., Giller, P., S., and Woodward, G., (2011a) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology* **48**, 443–452.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M., O., Giller, P., S., Gulis, V., Hutton, S., A., Lacoursie`re, J., O., *et al.*, (2011b) Stream ecosystem functioning in an agricultural landscape: The importance of terrestrial–aquatic linkages. *Advances in Ecological Research*, **44**, 211–276.

H.M.S.O., (1995) Biodiversity: *The UK Steering Group Report***,** Volume 1 and 2.

Holmes, N., T., H., (2006) The importance of long-term data sets in science and river management. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **16**, 329 – 333.

Holomuzki, J., R., Feminella, J., W. & Power, M., E., (2010) Biotic interactions in freshwater benthic habitats. *Journal of North American Benthological Society, **29**,* 220–244.

Hopkins, J., Marcarelli, A. & Bechtold, H., (2011) Ecosystem Structure and Function are Complementary Measures of Water Quality in a Polluted, Spring-Influenced River. *Water, Air, & Soil Pollution,* **214,** 409-421.

House, A. & Punchard, N., (2010) A method to assess the effect of public water supply abstraction on flow duration and winterbourne invertebrate communities.Wessex Water Operations Centre, Bath, UK. British Hydrological Society. Third International Symposium, Managing Consequences of a Changing Global

Environment, Newcastle 2010. Retrieved 19.02.2013. http://www.hydrology.org.uk/assets/2010%20papers/066House&Punchard.pdf

Hua, G. & Reckhow, D., A., (2007) Comparison of disinfection by products formation from chlorine and alternative disinfectants. *Water Research*, **41**, 1667–1678.

Hynes, H., B., N. (1983) Groundwater and stream ecology. *Hydrobiologia,* **100**, 93-99.

Ibbotson, A., Armitage, P. D., Beaumont, W., Ladle, M. & Welton, S. (1994) Spatial and temporal distribution of fish in a small lowland stream. *Fisheries Management and Ecology,* **1**, 143-156.

Jarvie, H., P., Neal, C., Juergens, M., D., Sutton, E., J., Neal, M., Wickham, H., D., *et al.,* (2006) Within-river nutrient processing in Chalk streams: The Pang and Lambourn, *The UK. Journal of Hydrology,* **330**, 101–25.

Joyce, P. & Wotton, R., S., (2008) Shredder fecal pellets as stores of allochthonous organic matter in streams. *Journal of the North American Benthological Society,* **27**, 521–528.

Kadlec, R., H., Knight, R., L., Vymazal, J., Brix, H., Cooper, P. & Haberl, R., (2000) Constructed wetlands for pollution control - processes, performance, design and operation. IWA Scientific and Technical Report No. 8. IWA Publishing, London, UK.

Kalff, J., (2002) *Limnology: inland water ecosystems*. Prentic Hall, Upper Saddle River, NJ, U.S.A.

Kunz. P., Y., Kienle, C. & Gerhardt, A., (2010) *Gammarus* spp. in aquatic ecotoxiolocy and water quality assessments: towards integrating multilevel tests. *Reviews of Environmental Contamination & Toxicology,* **205**, 1–76.

Lamberti, G., A. & Resh, V., H., (1983) Stream pe-riphyton and insect herbivores: an experimental study of grazing by a caddisfly population. *Ecology,* **64**, 1124-1135.

Lecerf, A. & Chauvet, E., (2008) Diversity and functions of leaf-associated fungi in human-altered streams. *Freshwater Biology,* **53**, 1658-1672.

Lecerf, A., Usseglio-Polatera, P., Charcosset, J., Y., Lambrigot, D., Bracht, B. & Chauvet, E. (2006) Assessment of functional integrity of eutrophic streams using litter breakdown and benthic invertebrates. *Archiv fur Hydrobiologie*, **165**, 105–126.

Leite, R., G., Araujo-Lima, C., Victoria, R., L. & Martinelli, L., A., (2002) Stable isotope analysis of energy sources for larvae of eight fish species from the Amazon floodplain. *Ecology of Freshwater Fish,* **11**, 56–63.

Lind, C., (1995) Wastewater Dechlorination Options. *Public Works*. 126 (September): 69-70.

Liski, J., Korotkov, A., V., Prins, C., F., L., Karjalainen, T., Victor, D., G., & Kauppi, P., E., (2003) Increased carbon sink in temperate and boreal forests. *Climatic Change,* **61**, 89– 99.

Lock, M. A., Wallace, R. R., Costerton, J. W., Ventullo, R. M., Charlton, S. E., (1984) River epilithon: towards a structural-functional model. *Oikos,* **42**, 1022.

Lowe, R. L. & Pan, Y., (1996) Benthic algal communities as biological monitors. In: Algal Ecology: Freshwater Benthic Ecosystems (eds R. J. Stevenson, M. L. Bothwell and R. L. Lowe) pp. 705–739. Academic Press, San Diego.

MacCrehan, W., A., Bedner, M. & Helz, G., R. (2005) Making chlorine greener: Performance of alternative dechlorination agents in wastewater. *Chemosphere,* 60, 381-388.

MacNeil, C., Dick, J., T., A., & Elwood, R., W., (1999) The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). *Biological Reviews*, **74**, 375–395.

Magbanua, F., S., Townsend, C., R., Blackwell, G., L., Phillips, N. & Matthaei, C., D., (2010) Responses of stream macroinvertebrates and ecosystem function to conventional, integrated and organic farming. *Journal of Applied Ecology,* **47,** 1014-1025.

Maltby, L., (1994) Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: *Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organisation* (Editors: Sutcliffe, D.W.). Freshwater Biological Association, Ambleside, UK.

Maltby, L., Clayton, S., A., Wood, R., M. & McLoughlin, N., (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. *Environmental Toxicology and Chemistry,* **21**, 361–368.

Malmqvist, B. & Rundle, S., (2002) Threats to running water ecosystems of the world. *Environmental Conservation* **29**, 134–153.

Martin, D., (2007) Environmental Agency Report: *A review of water quality in the upper reaches of the Bourne Rivulet between November 2004 and December 2006.*

Marxsen, J., (2006) Bacterial production in the carbon flow of a central European stream, the Breitenbach. *Freshwater Biology*, **51**, 1838-1861.

Mann, K., H., (1988) Production and the use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *American Society of Limnology and Oceanography*, **33**, 910--930.

Mann, R., H., K., Blackburn, J., H. & Beaumont, W., R., C., (1989) The ecology of brown trout *Salmo trutta* in the English chalk streams. *Freshwater Biology*, **21**, 57-70.

Matthews, R., A., Buikema, A., L., Cairns, J. & Rodgers, J., H., (1982) Biological monitoring. Part IIA. Receiving system functional methods, relationships and indexes. *Water Research*, **16**, 129–139.

Matthiessen, P., Sheahan, D., Harrison, R., Kirby, M., Rycroft, R., Turnbull, A., Volkner, C. & Williams, R., (1995) Use of a Gammarus pulex bioassay to measure the effects of transient carbofuran run-off from farmland. *Ecotoxicology and Environmental Safety*, **30**, 111–119.

McArthur, J., V., Barnes, J., R., Hansen, B., J. & Leff, L., G. (1988) Seasonal dynamics of leaf litter breakdown in a Utah alpine stream. *Journal of the North American. Benthological Society,* **7,** 44–50.

McCutchan, J., H., Jr., Lewis, W., M., Jr. (2002). Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography* **47**, 742–52.

McSwann, E., (2005) Environmental Agency Report: Invertebrate data for the Bourne rivulet 1989 - 2004.

Medgett, S., (1998) Environmental Agency Report: *The impact of St. Mary Bourne Cress Farm on the Bourne Rivulet.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Mills, C., A. & Mann, R., H., K., (1990) The bullhead Cottus gobio, a versatile and successful fish. 51st Annual Report Freshwater Biology Association pp.76-88.

Minshall, G. W., (1978) Autotrophy in stream ecosystems. *BioScience,* **28**, 767–771.

Minshall, G., W., (1988). Stream ecosystem theory: a global perspective. *Journal of the North American Benthological Society,* **7**, 263–288.

Moenickes, S., Schneider, A., Mühle, L., Rohe, L., Richter, O. & Suhling, F., (2011) From population-level effects to individual response: modelling temperature dependence in Gammarus pulex**.** *The Journal of Experimental Biology,* **214**, 3678-3687.

Mulholland, P., J., Fellows, C., S., Tank, J., L. *et al.*, (2001) Interbiome comparison of factors controlling stream metabolism. *Freshwater Biology,* **46**, 1503–1517.

Naiman, R., J., Melillo, J., M., Lock, M., A., Ford, T., E. & Reice, S., R. (1987) Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum, *Ecology,* **68**, 1139-1156.

Neal, C., & Whitehead, P., G., (2002) Nutrient studies within the Thames Basin: biological and sediment-water interactions and the effects of point source reductions on water quality functioning. *Science of the Total Environment*, **282/283**, 3-7.

Newman, M.C., & McIntosh, A.W., (1989) Appropriateness of aufwuchs as a monitor of bioaccumulation. *Environmental Pollution*, 60, 83–100.

Newman, R., M., (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. *Journal of the North American Benthological Society,* **10**, 89–114.

Newman, R., Hanscom, Z. and Kerfoot, C., W., (1992) The watercress glucosinate-myrosinase system: a feeding deterrent to caddisflies, snails and amphipods. *Oecologia,* **92**, 1-7.

Newman, R., N., Kerfoot, W., C. & Hanscom, Z., (1990) Potential chemical defence in a spring stream macrophyte. *Journal of Chemical Ecology,* **16**, 245-259. 94

Newman, R., N., Kerfoot, W., C. & Hanscom, Z., (1996) Watercress allelochemcial defends high nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology,* **77,** 2312-2323.

Norris, R., H. & Thomas, M., C., (1999) What is river health? *Freshwater Biology,* **41**, 197–209.

Palin, A. (1950) A Study of the Chloro Derivatives of Ammonia *Water and Water Engineering*. **54**, 248-258.

Palmer, M., A. & Febria, C., M. (2012) The Heartbeat of Ecosystems, *Science.* **336**, 1393 -1394.

Pardo, I. & Armitage, P. D., (1997) Species assemblages as descriptors of mesohabitats. *Hydrobiologia*, **344**, 111–128

Pascoal, C. & Ca´ssio, F. (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Applied and Environmental Microbiology, 70, 5266–5273.

Pascoal, C., Cassio F. & Marvanova, L., (2005) Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. *Archiv fur Hydrobiologie,* **162**, 481–496.

Petersen, R., C., and Cummins, K., W., (1974) Leaf processing in a woodland stream. *Freshwater Biology,* **4**, 345-368.

Peterson C. G. (1996) Response of benthic algal communities to natural physical disturbance. In Algal Ecology: Freshwater Benthic Ecosystems (eds R. J. Stevenson, M. L. Bothwell & R. L. Lowe) pp. 375–403. Academic Press, San Diego.

Pinder, L., C., V., (1992) *Biology of epiphytic Chironomidae (Diptera: Nematocera) in chalk streams, Hydrobiologia,* **248**, 39-51

Rasmussen, J., J, Wiberg-Larsen, P., Baattrup-Pedersen, A., Friberg, N., Kronvang, B. (2012) Physical structure of stream microhabitats influences the response of stream macroinvertebrate communities to pesticide stress. *Environmental Pollution*, **164**, 142–9.

Ricciardi, A. & Macisaac, H., J. (2011) Chapter 16 - Impacts of biological invasions on freshwater ecosystems. *Fifty years of invasion ecology: the legacy of Charles Elton*, 1st Edition, Blackwell Publishing Ltd, p. 211-224.

Richardson, J., S., (1993) Limits to productivity in streams: evidence from studies of macroinvertebrates, *Canadian Special Publication of Fisheries and Aquatic Sciences*, **118**, 9 -15.

Rier, S., T. & Stevenson, R., J., (2006) Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia*, **561**, 131-147.

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P. & Dobson, M. (2010) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology*, **55**, 346-359.

Riipinen, M., P., Davy-Bowker, J. & Dobson, M., (2008) Comparison of structural and functional stream assessment methods to detect changes in riparian vegetation and pH. *Freshwater Biology*, **54**, 2127–2138.

Rivfunction (2013) accessed 14.02.2013. http://www.ecolab.ups-tlse.fr/rivfunction/ see results / papers.

Roddie, B., Edwards, T. & Crane, M., (1992) Potential impact of watercress discharges on the freshwater amphipod Gammarus pulex L. *Bulletin of Environmental Contamination and Toxico*logy, **48**, 63-69.

Rooney, N., McCann, K., Gellner, G. & Moore, J., C., (2006) Structural asymmetry and the stability of diverse food webs. *Nature*, **442**, 265–269.

Romero, J., Perez, M., Mateo, M., A. & Sala, E., (1994) The below ground organs of the Mediterranean seagrass Posidonia oceanica as a biogeochemical sink. *Aquatic Botany*, 4**7**, 13–19.

Rounick, J. S. & Winterbourn M. J., (1986) The formation, structure and utilisation of stone surface organic layers in two New Zealand streams. *Freshwater Biology*, **13**, 57–72.

Sear, D., A., Armitage, P., D., & Dawson, F., D., H. (1999) Groundwater dominated rivers. *Hydrological Processes*, **11,** 255-276.

Sekar, R., Nair, K.V.K., Rao, V. N. R., Venugopalan, V. P., (2002) Nutrient dynamics and successional changes in a lentic freshwater biofilm. *Freshwater Biology*, **47**, 1893–1907.

Soulsby, P., G., (1975) Biological report; Summary report on cress farm investigations. Southern Water.

Statzner, B. & Bêche L., A., (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology*, **55,** 80-119.

Steinman, A., D., Mulholland, P., J. & Hill, W., R., (1992) Functional responses associated with growth form in stream algae. *Journal of the North American Benthological Society*, **11**, 229–243.

Stevens, C., P., (1983) *Watercress.* ADAS/MAFF Reference Book 136. Grower Books, London, England. p.53.

Stevenson, R. J., (1996) An introduction to algae in aquatic ecosystems. In. Algal Ecology: Freshwater Benthic Ecosystems (eds R. J. Stevenson, M. L. Bothwell and R. L. Lowe) pp. 3–30. Academic Press, San Diego.

Stevenson, R., J., Rier, S., T., Riseng, C., M., Schultz, R., E. & Wiley, M., J., (2006) Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. *Hydrobiologia,* **561**, 149-165.

Suberkropp, K., (1992) Interactions with invertebrates. In: *The Ecology of Aquatic Hyphomycetes.* Felix Bärlocher (ed): 118-134. Ecological Studies 94, New York, Berlin: Springer-Verlag.

Suberkropp, K., Godshalk, G., L. & Klug, M., J., (1976) Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology,* **57**, 720–727.

Townsend, C., R., Hildrew, A., G. & Francis, J., (1983) Community structure in some southern English streams: the influence of physicochemical factors. *Freshwater Biology,* **13**, 521–544.

van Lanen, H., A., J., & Dijksma, R., (1999) Water flow and nitrate transport to a groundwater-fed stream in the Belgian- Dutch chalk region. *Hydrological Processes,* **13**, 295–307.

Vannote, R., L., Minshall, G., W., Cummins, K., W., Sedell, J., R. & Cushing C., E., (1980) The River Continuum Concept. Canadian Journal of Fisheries and *Aquatic Sciences,* **37**, 130–137.

Ventura, M. & Harper, D., (1996) The impacts of acid precipitation mediated by geology and forestry upon upland stream invertebrate communities. *Archives of. Hydrobiology*. **138**, 161–173.

Vikesland, P., J., Ozekin, K. & Valentine, R., L., (2001) Monochloramine decay in model and distribution system waters. Water Research. **35**, 1766–1776.

Vymazal, J., (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological Engineering,* **25**, (5), 478–90.

Vymazal, J., (2006) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment,* **380**, 48-65.

Wallace, J., B., and Webster, J., R., (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology,* **41**, 115-139.

Wallace, J., B., Eggert, S., L., Meyer, J., .L. & Webster, J., R., (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102-104.

Wallace, J., B., Eggert, S., L., Meyer, J., L. and Webster, J., R., (1999) Effects of resource limitation on a detrital-based ecosystem, *Ecological Monographs,* **69**, 409–442.

Webster, J., R. and Benfield, E., F., (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics,* **17**, 567-594.

Webster, J., R., Benfield, E., F., Ehrmann, T., P., Schaeffer, M., A., Tank, J., L., Hutchens, J., J. and D'Angelo, D., J. (1999) What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology,* **41**, 687-705.

Wei, K., Zhou, H., Zhou, T. & Gong, J., (2007) Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: impact on fresh agricultural produce quality. *Ozone: Science & Engineering,* **29**, 113–120.

Westlake, D., F., Casey, H., Dawson, H., Ladle, M., Mann, R., H., K. & Marker, A., F., H., (1972) The chalk stream ecosystem. *Symposium on Productivity Problems in Freshwater* (Kajak, Z. & Hillbricht-Ilkowska, A., eds.), pp., 615-635. Proceedings IBP-UNESCO.

Wetzel, R., G., (2001) *Limnology*. 3rd edn. Academic Press, San Diego, CA.

White, G.C. (1992) *Handbook Of Chlorination and Alternative Disinfectants.* Volume 3. Van Nostrand Reinhold Co., New York, NY.

White, S. & Medgett, S., (2006) Environmental Agency Report: *Bourne Rivulet Invertebrate Survey.*

Whitehurst , I., T., (1991) The Gammarus–Asellus Ratio as an index of organic pollution. *Water Research,* **25**, 333–340.

Woodward, G., Gessner, M., O., Giller, P., S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B., G., Tiegs, S., D., Cariss, H., Dobson, M., Elosegi, A. ,Ferreira, V., Graça, M., A.,,S., Fleituch, T., Lacoursiere, J., O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L., B., M. and Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science,* **336**, 1438-1440.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R., B., (2008) Trophic trickles and cascades in complex food web: impacts of keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshwater Biol*ogy, **54**, 2171–2187.

Woodward, G., Speirs, D., C. & Hildrew, A., G., (2005) Quantification and resolution of a complex, size-structured food web. *Advances in Ecological Research,* **36**, 85-135.

Worgan, A. & Tyrell, R., (2006) *Monitoring behavioural responses of Gammarus pulex to watercress oils.* Report for Vitacress Salads Ltd by the Centre of Ecology and Hydrology, (CEH), Dorset.

Wright, J., F., (1992) Spatial and temporal occurrence of invertebrates in a chalk stream, Berkshire, England. *Hydrobiologia,* **248**, 11–30.

Wright, J., F., Armitage, P., D., Furse, M., T. & Moss, D., (1984) The classification of sites on British rivers using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie,* **22**, 1939–1943.

Wright, J. F. & Berrie, A. D. (1987) Ecological effects of groundwater pumping and a natural drought on the upper reaches of a chalk stream. *Regulated Rivers: Research & Management,* **1**, 145–160.

Wu, X., Zhou, Q., H. & Xu, K., (2009) Are isothiocyanates potential anti-cancer drugs? *Acta Pharmacologica Sinica.* **30**, 501–512.

Wu, X., Zhu, Y., Yan, H., Liu, B., Li, Y., Zhou, Q., & Xu, K., (2010) Isothiocyanates induce oxidative stress and suppress the metastasis potential of human non-small cell lung cancer cells. *BioMed Central Cancer*, **10**, 269.

Young, R., Townsend, C. & Matthaei, C., (2004) *Functional indicators of river ecosystem health – an interim guide for use in New Zealand*. Prepared for Ministry for the Environment - Sustainable Management Fund Contract 2208. Retrieved 28.08.2012. from http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/functional-indicators-guide-final.pdf.

Young, R., G., Matthaei, C., D. & Townsend, C., R., (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society,* **27**, 605-625.

# Chapter 2.

# Study Sites.

An initial study was carried out at SMB (Bourne rivulet) to determine the extent of the potential impacts upon our measures of ecosystem functioning and the impacts on key macroinvertebrate taxa, in particular the freshwater shrimp *G. pulex* and the measures response to rerouting. Five biomonitoring sites (Up, Side, WC, Out, Down), representing five spatial treatments were used (Chapter 4). Subsequently the biomonitoring was simplified, and the five sites reduced to three sites (Up, Out, Down). These were used in the replicated BACI (Before, After, Control, Impact) experimental design in our further study at SMB (Chapter 5) and in our wider replicated Control, Impact (CI) seasonal and spatial studies at this and five other watercress farms (streams) (Chapter 6).

All six streams (Bourne Rivulet, Pillhill Brook, Candover Brook, River Meon, Bere stream, River Piddle) within the study were 1st order with watercress farms on their headwaters, owned and managed by Vitacress Salads Limited (VSL) (Fig 2.1 & 2.2). Watercress farms were chosen: similar in river catchment, position on their associated chalk river and riparian zone land use. There were no sewage treatment works or industrial facilities upstream of the chosen farms. All sampling sites at each farm were standardised as far as possible for physical variables both within and across sites, by using riffles that were 1-3m wide, ≥10cm deep, similar in flow and with a stony substratum. The watercress farms in these studies do not abstract water directly from their carrier streams but do so from artesian groundwater flow, which is returned to the stream and thus augments the natural flow regime of the stream. A site description with location (long. / lat.) and physico-chemical data for each sampling station on each chalk stream and watercress farm are summarised in the next sections and in the accompanying Tables 2.1 - 2.5. pp 65 - 71.

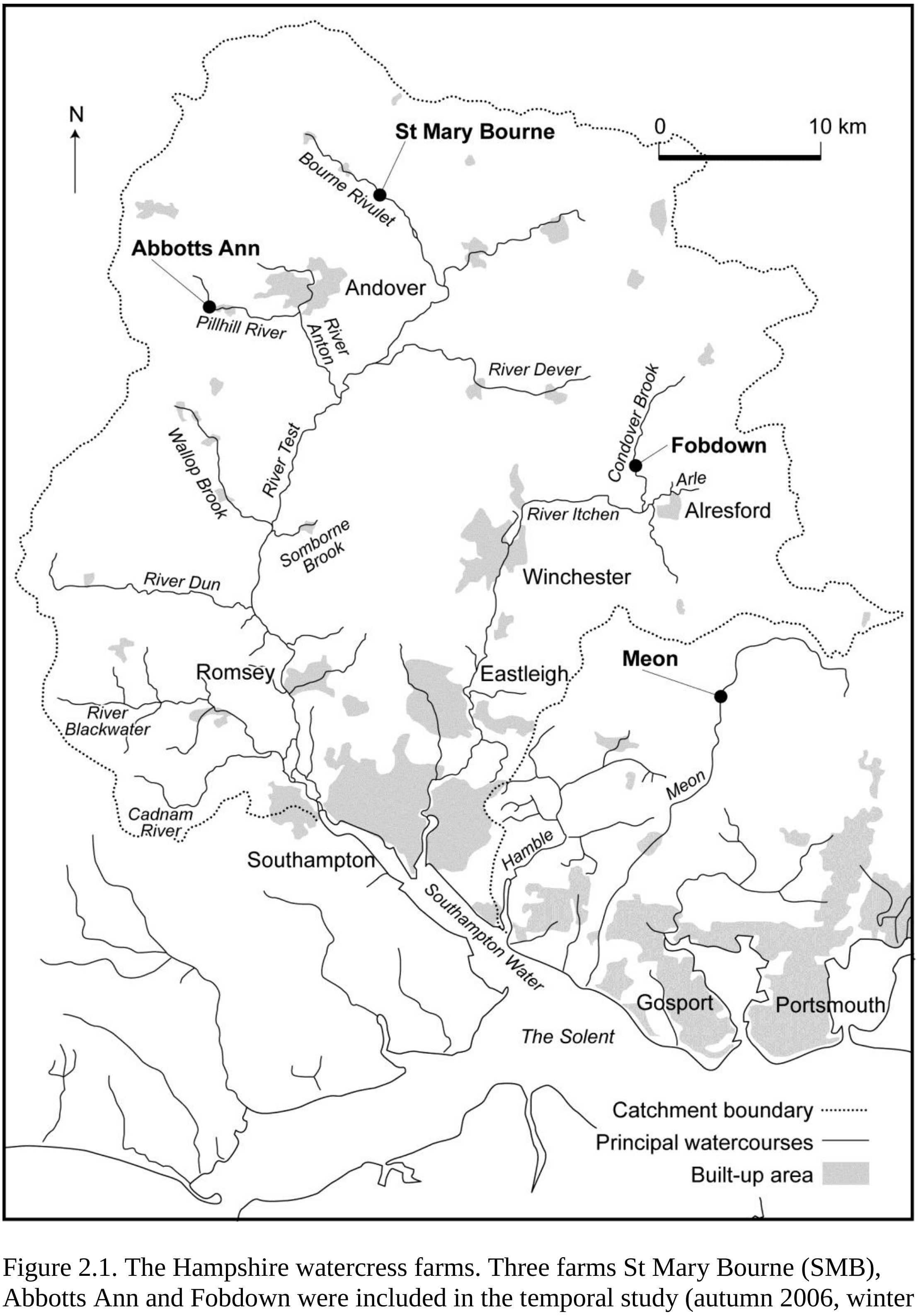


Figure 2.1. The Hampshire watercress farms. Three farms St Mary Bourne (SMB), Abbotts Ann and Fobdown were included in the temporal study (autumn 2006, winter 2006/7, spring 2007 and summer 2007). This was widened in the spatial study in summer 2007 to include Meon and the two Dorset watercress farm in Fig. 2.2. Five sites were chosen at SMB in the initial study in Chapter 4 (Up, Side, WC, Out, Down). This was simplified and reduced to three sites, in the further SMB study in Chapter 5 and the wider studies at St Mary Bourne and other farms in Chapter 6 (Up, Out, Down).

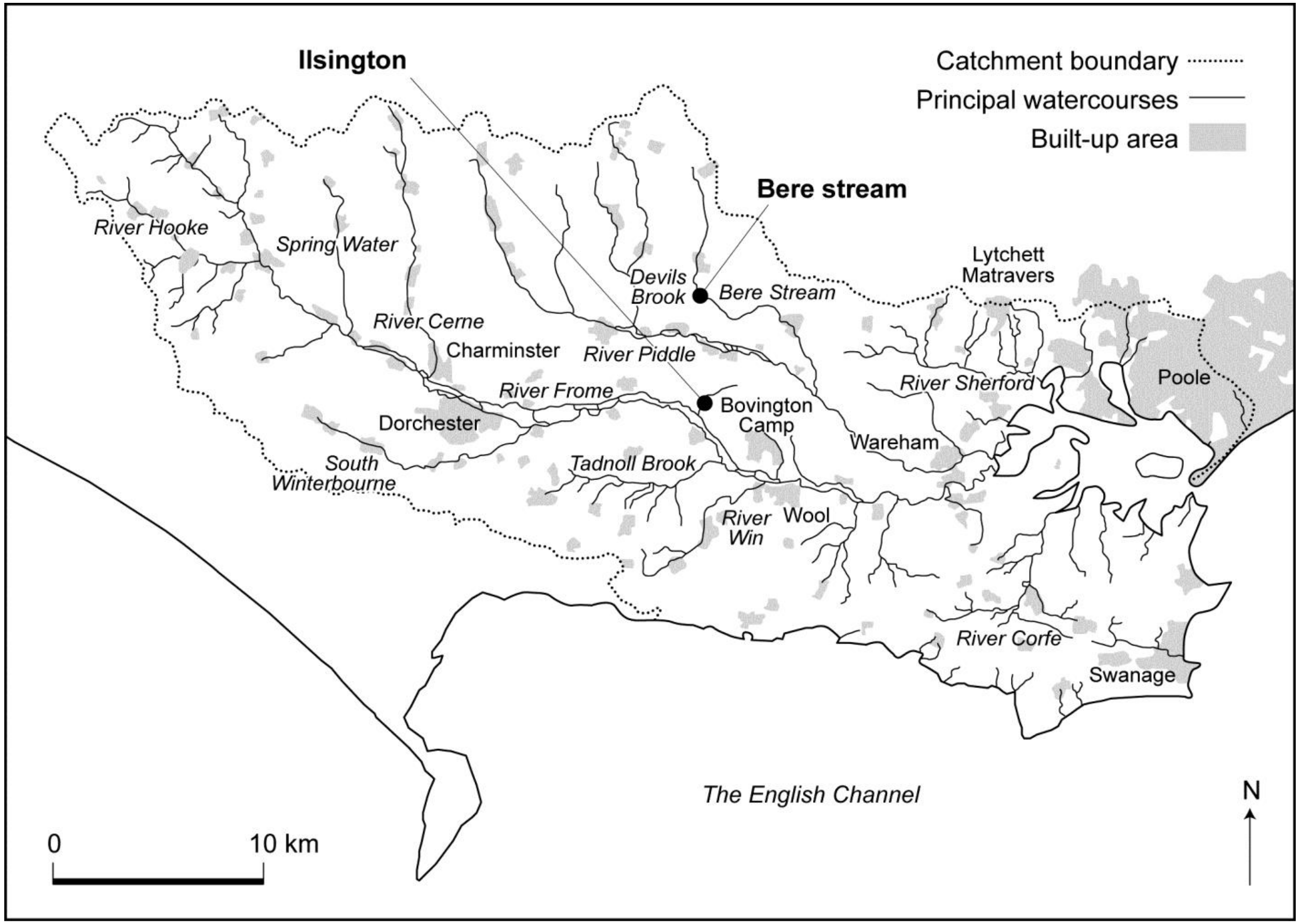


Figure 2.2. The Dorset watercress farms.These two farms Bere and Ilsington were used in the spatial study in June 2007 together with the four Hampshire watercress farms depicted in Fig. 2.1.

### *The Watercress farms*

The six watercress farms used in the study and their adjacent stream are described in turn in the following section. Watercress farms were chosen with as similar as possible catchments and position in relation to their associated chalk river, river basin and riparian zone land use, which were predominantly pasture, arable farming & deciduous woodland. The natural state of most lowland chalk streams in the UK is probably an ill-defined, braided channel running through alder (*Alnus glutinosa* L.*)* and willow *(Salix spp.* L.*)* woodland (Ladle & Westlake, 1995), although little of this native vegetation remains, following extensive clearance for agriculture over many centuries. The majority of streams now flow through catchments dominated by arable and pasture land use (Harrison & Harris 2002) with bankside vegetation ranging from grassland to bushes and trees which include oak. The headwaters of all the streams, naturally experience low flows in their 'winterbourne' stretches, upstream of the watercress farms sited on them and may partially or temporarily desicate and cease to flow, particularly in dry summers, because of water abstraction and the lack of rainfall to recharge the aquifer (Wood & Petts 1999). This causes the water table in the underlying chalk aquifer to fall, but returns once recharged from rainfall percolating through the permeable chalk geology, in the catchment area (Acreman & Dunbar 2010). Chalk streams are the most species-rich of all temperate fresh waters (Wright 1992; Woodward *et al.*, 2008) and have a characteristically diverse and abundant flora (Shamsudin & Sleigh 1994) and invertebrate and fish fauna (Mann, Blackburn & Beaumont 1989). This is a result of their characteristically stable patterns of temperature, discharge and shallow channels with beds composed of gravel and flints (Berrie 1992; Prenda, Armitage & Grayston 1997; Sear, Armitage & Dawson 1999).

The stream mid-channel area is often dominated by river water crowfoot (*Ranunculus penicillatus* L.) and water-starworts (*Callitriche* L.) particularly in spring and early summer, but as summer progresses wild watercress (*Rorippa nasturtium-aquaticum* L.*)* lesser water-parsnip (*Berula erecta L.*) and water forget-me-not (*Myosotis scorpioides* L.*)* encroach from the margins which also shades and lowers stream flows to provide cover and refugia (Westwood 2008; Acreman & Dunbar 2010). These streams support a great diversity of invertebrates e.g. caddisflies, mayflies, stoneflies, damselflies, dragonflies, the freshwater shrimp (*G. pulex*). Chalk streams are also notable for their important wild and farmed fisheries; the game species brown trout (*Salmo trutta* L.), Atlantic salmon (*Salmo salar* L.), grayling (*Thymallus thymallus* L.) and other fish species include the brook lamprey (*Lampetra planeri* L.) and bullhead (*Cottus gobio* L.). Important mammalian species include water vole (*Arvicola terrestris* L.) otter (*Lutra lutra* L.) and a wide variety of birds inhabit stream margins and their lower reaches (EA 2004). The presence of these rare and often protected species in conjunction with the unique features of chalk stream and rivers has contributed to the high degree of legislative protection that these fragile ecosystems have been afforded e.g. chalk streams are identified as a key habitat in the UK Biodiversity Action Plan (BAP) and are listed as a priority for protection (Cox 2006). Ten chalk rivers are designated as Sites of Special Scientific Interest (SSSIs) for their wildlife interest (including the Itchen and Test) and of these, four are Special Areas of Conservation (SACs) under the Habitats Directive (including the Test) (HCC 2000; EA 2004; EA 2006; Cox 2006) see Table. 2.1. pp. 65 for study river designations.

1). St Mary Bourne watercress farm lies on the Bourne Rivulet a tributary and headwater of the river Test (EA 2004), its source is eight km. north-west of the farm.

The watercress farm lies within a valley of arable and pasture land-use below the village of St Mary Bourne in Hampshire, within an area of outstanding natural beauty (AONB). This is the largest watercress farm in Europe with 6.9 hectares of watercress beds, and includes a washing and packing factory for watercress sourced from this and other watercress farms, and salad leaves from overseas. For further details, see reports on and references therein from Southern Water (Soulsby 1975), the EA (Medgett 1998; McSwan 2005; White & Medgett 2006), Natural England (Cox 2006) and Vitacress Salads Ltd (Marsden 2006).

2). Abbotts Ann watercress farm is sited on the Pillhill Brook a tributary of the River Anton and ultimately the River Test, whose source is four km. west of the site. The watercress farm lies in an area of water meadows within a valley of arable and pasture land-use. Two km. downstream is a trout fishery. For further background, see (EA 1997-8) and (Cox 2006) and references therein.

3). Fobdown watercress farm is situated on the Candover Brook, a tributary of the River Itchen whose source is four km. due north of the site; within a valley designated as a Special Area of Conservation (SAC). The Rivers Itchen (FD farm) and the Test (SMB & AA farms) are world renowned for their game fishing, provided by brown trout, both wild and stocked populations, grayling and to a lesser extent salmon and sea trout. For further background, see (Cox 2006).

4). Meon watercress farm lies on the River Meon, which rises four km. to the east of the site, within an area of outstanding natural beauty (AONB) and the South Downs National Park. For further background, see (Cox 2006).

5). Bere watercress farm lies on the Bere stream in Dorset; a tributary of the River Piddle, which rises six km. to the north of the site, within the Site of Special Scientific Interest (SSSI) designated section of the river. The River Piddle and the River Frome are in the same catchment area. For further background, see (EA 2004a; Cox 2006).

6). Ilsington watercress farm is situated on a tributary of the River Frome in Dorset, the most westerly Chalk stream in the UK (Howden & Burt 2008) and lies within the SSSI designated section of the river. The source rises two km. to the north east of the site. The River Frome and its tributaries have been the focus of much research over the last 50 years, mainly due to the presence of The Freshwater Biological Association River Laboratory at East Stoke, including watercress e.g. Crisp 1969; Crisp, Matthews & Westlake 1982; Casey & Smith 1994. For further background, see (EA 2004a; Cox 2006).

### ***Study sites.***

Five study sites were sampled in an initial survey of the SMB watercress farm in June 2005, based on their hydrological connectivity to the farm, and hence exposure to stressors and the experimental design requirements for our replicated BACI design. These sites represented five spatial treatments: “Up” is an upstream “reference” or control site in the channel that receives water directly from the aquifer. “Side” is situated in the western channel of the Bourne rivulet, and receives only water from watercress in block D, thus acting as a control for the management practices at the watercress farm at sites “Watercress beds (WC) ” and “Out” and to provide background levels for comparison. “WC” is directly below all the watercress beds receiving water from blocks C, B, E and R, which are rotations of cultivated watercress beds. Block D flows into the western arm of the Bourne Rivulet whereas blocks A, B, C, E and R flow into the outfall and the eastern arm of the Bourne Rivulet. The water from “WC” passes directly to the outfall site, “Out”, where all the waste waters (salad wash effluent, settlement tank effluent, clearing effluent, from the farm converge as they enter the stream. The “Down” site is 0.5 kilometres downstream of the “Outfall” site, chosen to detect evidence of potential recovery (Fig. 2.3 & 2.4) (Plates 2.1-2.5 pp. 72-75). The experimental design was simplified in Chapters 5 and 6, by omitting the “Side” and “WC”sites, which were replicate spatial controls in our initial intensive study, to leave three study sites “Up”, “Out” and “Down”. At SMB the “Up”, control site was in the carrier channel for the borehole water rather than upstream of the farm in the Bourne rivulet. This was because the Bourne rivulet is a winterbourne and the SMB watercress farm is its perennial head, and when we initially sampled in June 2005 the Bourne rivulet upstream of the farm had ceased to flow. This initial sampling was time critical because in 31 days time the re-routing would take place, we therefore had to choose the best upstream site we could find at that time. At all other watercress farms the upstream site was in the stream directly above the farm.

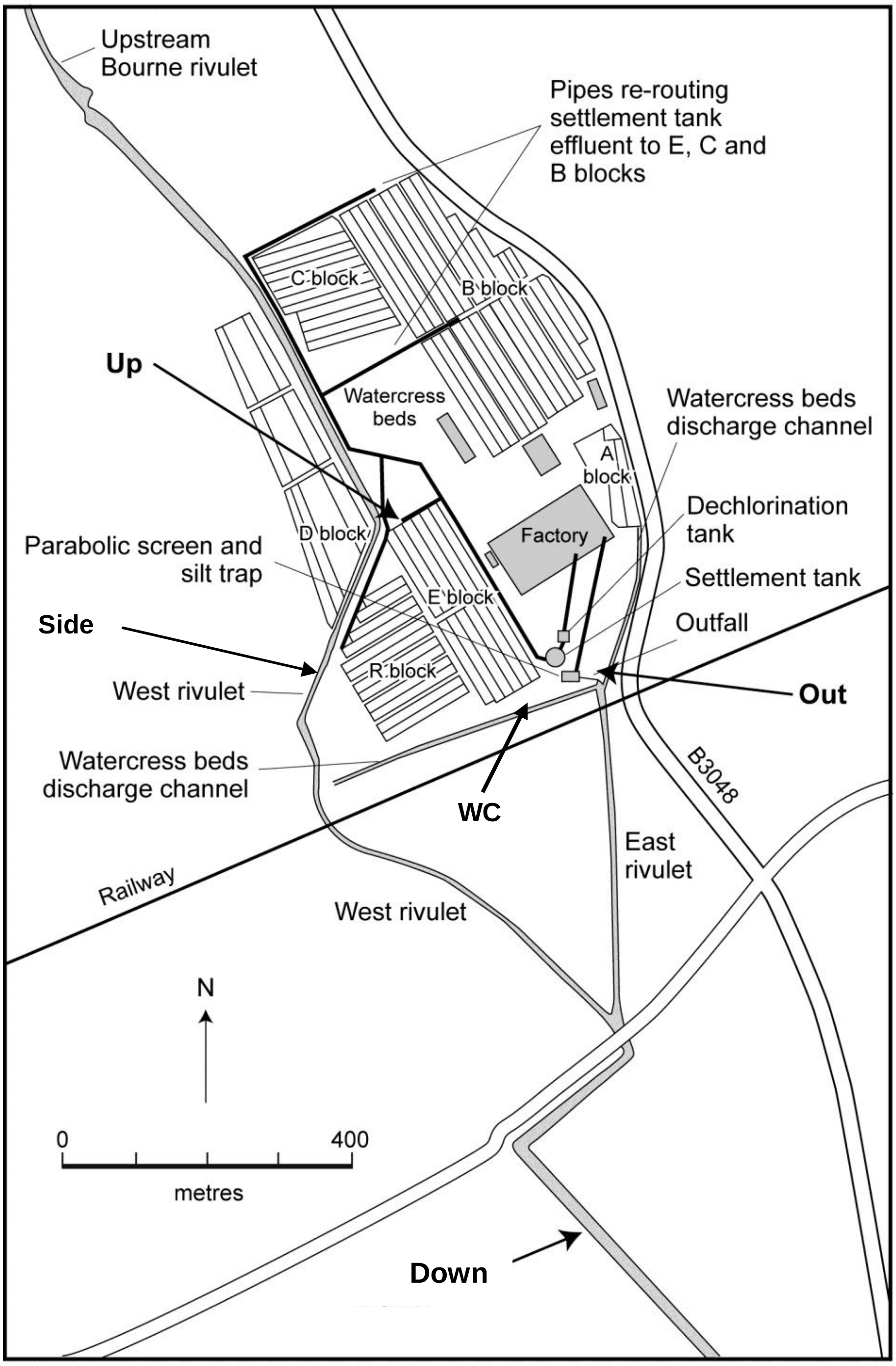


Figure 2.3. St Mary Bourne study site, showing sample sites, watercress beds, factory and layout of the farm: Watercress bed E discharges to the outfall and East Rivulet. Blocks D and R discharge to West Rivulet. Sample sites “Up” (= “Upstream” reference site), “Side” (= “Side channel” site acting as a control for the management practices at the watercress farm at sites “WC” and “Out’’), “WC” (= “Watercress beds” site), “Out” (= “Outfall” heavily impacted site), “Down”, (= Downstream site 0.5 km below the “Outfall”, used to detect evidence of potential longitudinal recovery). The “settlement tank effluent” was re-routed through the watercress beds on 28th June 2005. The use of chlorine in the secondary wash ceased on July 8th 2006. N.B. Up, Side, WC, Out, Down sites used in Chapter 4; Up, Out, Down sites used in Chapter 5.

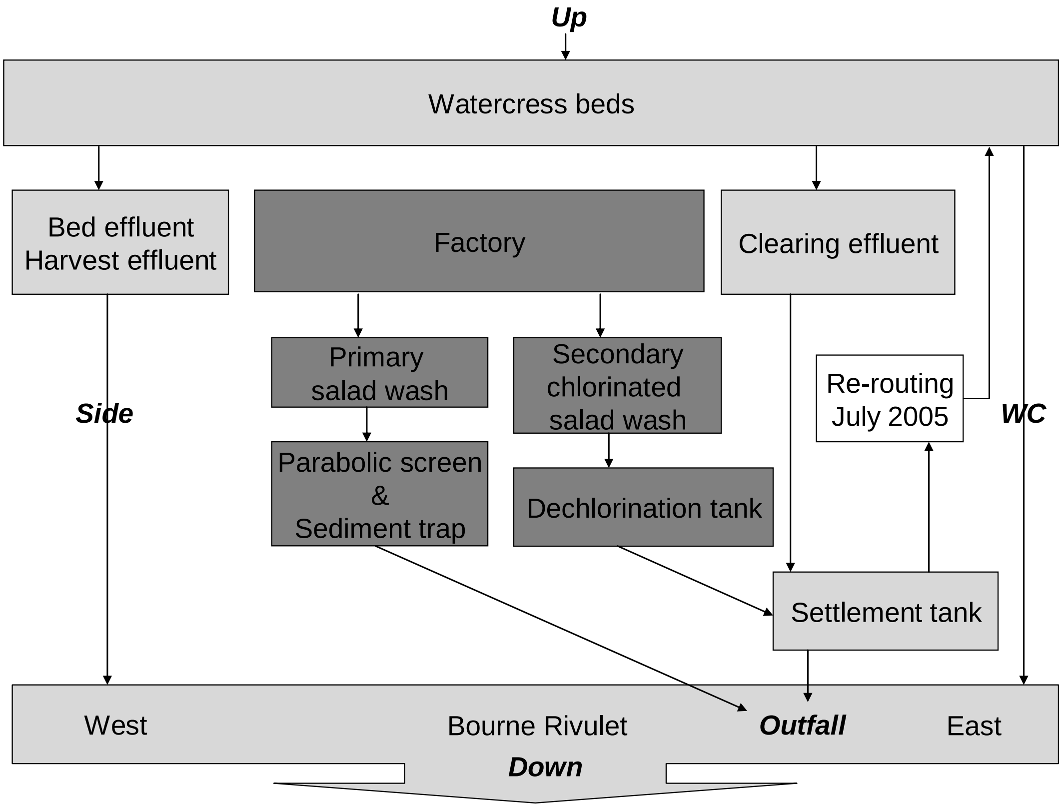


Figure 2.4.a). Flow diagram of effluent waters from processes and treatments at the SMB watercress farm, sample sites indicated in bold italics. Sample sites “Up” (= “Upstream” reference site), “Side” (= “Side channel” site acting as a control for the management practices at the watercress farm at sites “WC” and “Out’’), “WC” (= “Watercress beds” site), “Outfall” (= “Outfall” heavily impacted site), “Down” (= “Downstream” sites 0.5 km below the “Outfall”, used to detect evidence of potential longitudinal recovery). The “settlement tank effluent” was re-routed through the watercress beds on 28th June 2005. The effluent mixes freely with bed waters to pass through discharge channels below the beds, prior to release into the outfall “Out” and on to the East rivulet (see Fig 2.3 & Fig. 4.2.b). The use of chlorine in the secondary wash ceased on July 8th 2006. N.B. Sites: Up, Side, WC, Out, Down used in Chapter 4; Up, Out, Down used in Chapter 5.

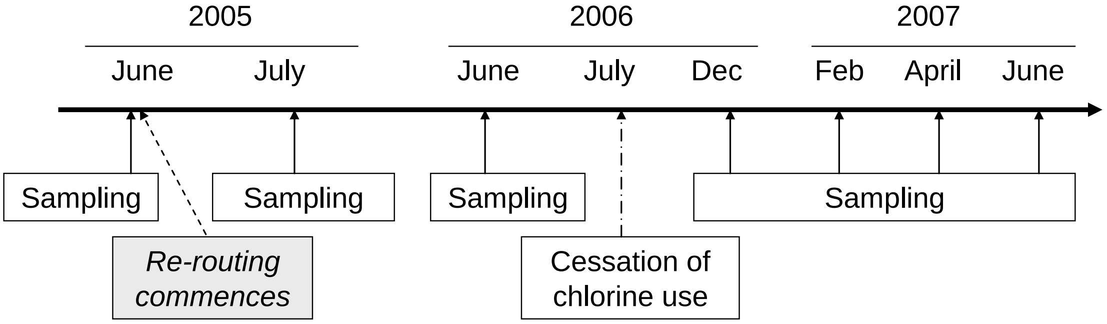


Figure 2.4.b). Timeline at SMB denoting sampling occasions. Dec (2006); Feb, April & June (2007) = Autumn, Winter, Spring and Summer respectively.

### ***St Mary Bourne watercress farm, bed layout and operational detail.***

The upstream section of the Bourne rivulet bisects the VSL watercress farm to form the western section of the rivulet. The Bourne rivulet is not connected to and does not supply water to the VSL farm. The farm receives water from artesian groundwater boreholes, which is returned to the carrier stream, thus augmenting the natural flow regime of the stream via the eastern section of the Bourne rivulet. Water is continuously abstracted from 25 – 30 pumped boreholes from the underlying chalk aquifer to supply all 6.9 hectares of watercress beds. Watercress harvesting can cause suspension of the bed sediment, which is reduced by lowering borehole water inputs. Wastewaters produced when no farming activities are conducted upon the watercress beds ("bed effluent") and during harvesting ("harvest effluent"), flow into discharge channels situated below the watercress beds. Block E is a rotation of cultivated watercress beds and discharges into the outfall and on to the east rivulet, whereas D and R blocks discharge directly into the west rivulet. After harvesting, the beds are prepared for either re-growth or replanting. This generates large volumes of sediment and this "clearing effluent", from all the watercress beds (i.e. Blocks A, B, C, E, D and R) is diverted to a settlement tank, which facilitates sediment deposition, before the waters are released into the outfall and on directly to the Bourne rivulet (Fig 2.3 & 2.4.).

A factory operates at the VSL site, to wash and pack ready for sale, watercress and other salad leaves sourced from the UK, Portugal, Spain, USA and Kenya. The watercress and salad leaves undergo a primary water wash in borehole water to remove dirt and foreign material. This "salad wash effluent" is discharged to a parabolic screen which removes leaf particles (>2mm), and a sediment trap (Plate 2.6 & 2.7; pp. 74 & pp. 75) which removes suspended solids (>5mg/l) prior to being released at the outfall into the East rivulet. The salad leaves pass through a secondary sanitising chlorinated salad wash, prior to being packed ready for sale. The chlorinated wash effluent is neutralised with sodium metabisuphite in a dechlorination tank and this “dechlorination effluent” is then released to the settlement tank (Fig 2.3 & 2.4.). Up to 30 metric tonnes of salad is washed per day, producing up to 2,500 $m^3$ of salad wash effluent using up to 40,000 gallons of borehole water an hour (Cox 2006). Approximately 40% of the washed salad is watercress or rocket, both of which release PEITC. In the autumn and winter, the settlement tank, 1,100 $m^3$ in capacity, is emptied directly into the farm outfall and on into the east rivulet once a week, in the spring and summer large volumes of “clearing effluent” requires the tank to be discharged daily. VSL farm waste waters are routed back into the carrier river rather than the sewage system as it is a condition of the watercress farms non consumptive licence that at least 95% of the water taken from the aquifer via the boreholes is returned to the carrier river (Cox 2006). This augments the natural flow and thus SMB watercress farm has become the perennial head of the Bourne rivulet.

### ***Temporal study, Chapter 6.***

Three 1st order chalk streams with watercress farms were selected for this study. The Bourne Rivulet a tributary of the River Test (St Mary Bourne watercress farm), the Pillhill Brook a tributary of River Anton and ultimately the River Test (Abbotts Ann watercress farm), and the Candover Brook a tributary of the River Itchen (Fobdown watercress farm) (EA 2004) (Fig 2.1).

### ***Spatial study, Chapter 6.***

Six 1st order chalk streams with watercress farms were selected for this study, which included those in the temporal study. Four of the streams were in Hampshire, the

Bourne Rivulet a tributary of the River Test (St Mary Bourne watercress farm); the Pillhill Brook a tributary of River Anton and ultimately the River Test (Abbotts Ann watercress farm); the Candover Brook a tributary of the River Itchen (Fobdown watercress farm) and the River Meon (Warnford watercress farm) (EA 2004) (Fig 2.1). The two remaining streams were in Dorset, the Bere stream a tributary of the River Piddle (Holly Bush watercress farm) and a tributary of the River Frome (Ilsington watercress farm) at Ilsington near Tincleton (EA 2004) (Fig 2.2).

Table 2.1. Physical characteristics of the study streams. All farms owned and managed by Vitacress salads Ltd.

| Watercress Farm | Stream | Tributary River | Underlying Geology* | Catchment Land use | Legislative conservation protection* | Altitude (m) | Farm size (Ha) | Farm management practices | Riparian land-use | Chapter |
|---|---|---|---|---|---|---|---|---|---|---|
| St Mary Bourne | Bourne Rivulet | Test | Chalk | ** | SSSI<br>AONB | 75 | 6.9 | C | ** | 4, 5, 6 |
| Fobdown | Candover Brook | Itchen | Chalk | ** | SAC<br>SSSI<br>AONB | 65 | 3.2 | C | ** | 6 |
| Abbotts Anne | Pillhill Brook | (1) Anton<br>(2) Test | Chalk | ** | SSSI<br>AONB | 75 | 2.4 | C | ** | 6 |
| Meon (Warnford) | - | Meon | Chalk | ** | SINC<br>AONB | 75 | 1.2 | C | ** | 6 |
| Ilsington | - | Frome | Chalk | ** | SAC<br>SSSI | 35 | 0.8 | C | ** | 6 |
| Bere (Holly Bush) | Bere Stream | Piddle | Chalk | ** | SSSI | 45 | 0.8 | C | ** | 6 |

Key: SSSI = Site of Special Scientific Interest, AONB = Area of Outstanding Natural Beauty, SAC = Special Area for Conservation, SINC = Site of Importance for Nature Conservation, C = conventional, = Pasture, arable farming & deciduous woodland,

Table 2.2. St Mary Bourne Studies (Chapter 4 & 5), July 2005 post re- routing only. Location and physico-chemical data for sample sites. Values are single sample measurements except temperature, width and depth, which are average values. See Chapter 3 for details - General Methods - Physical and chemical analyses of stream water. Figures within brackets = Environmental Quality Standard (EQS).

| Site | Width (m) | Depth (m) | °C | Phosphate mg /L. ( 0.1) | Nitrite mg/L. ( 0.01) | Nitrate mg/L (17.5) | Ammonia mg/L. (0.015) | BOD ATU mg/L. | Organic Carbon (Filtered) mg/L. |
|---|---|---|---|---|---|---|---|---|---|
| Up (upstream "control" ) | 0.3 | 0.2 | 10.5 | 0.023 | 0.0040 | 6.4 | 0.03 | 1 | 0.43 |
| Side (parallel "control" ) | 2.0 | 0.5 | 15.5 | 0.082 | 0.0722 | 6.2 | 0.143 | 1 | 1.08 |
| WC (parallel "control" ) | 1.2 | 0.2 | 15.4 | 0.099 | 0.0732 | 5.7 | 0.134 | 1.36 | 1.14 |
| Out (impacted outfall) | 1.7 | 0.6 | 15.4 | 0.093 | 0.0514 | 5.3 | 0.066 | 1.84 | 1.01 |
| Down (0.5km downstream "recovery") | 2.2 | 0.3 | 14.3 | 0.087 | 0.0338 | 5.1 | 0.030 | 1 | 0.84 |

Table 2.3. St Mary Bourne Studies (Chapter 5), Sept 2006 (post re- routing). Physico-chemical data for sample sites. Values are single sample measurements except temperature, width and depth, which are average values. See Chapter 3 for details - General Methods - Physical and chemical analyses of stream water. Figures within brackets = Environmental Quality Standard (EQS).

| Site | Width (m) | Depth (m) | °C | Phosphate mg /L. ( 0.1) | Nitrite mg/L. ( 0.01) | Nitrate mg/L (17.5) | Ammonia mg/L. (0.015) | BOD ATU mg/L. | Organic Carbon (Filtered) mg/L. |
|---|---|---|---|---|---|---|---|---|---|
| Up (upstream "control" ) | 0.3 | 0.2 | 10.5 | 0.02 | 0.004 | 5.79 | 0.03 | 1 | 0.26 |
| Out (impacted outfall) | 1.7 | 0.6 | 14.4 | 0.289 | 0.0725 | 6.9 | 0.171 | 4 | 1.26 |
| Down (0.5km downstream "recovery") | 2.2 | 0.3 | 14.3 | 0.0663 | 0.0525 | 6.29 | 0.104 | 1 | 1.03 |

Table 2.4.Temporal and Spatial Study (Chapter 6): Physico-chemical data for sample sites. Values are single sample measurements except temperature, width and depth, which are average values. See Chapter 3 for details - General Methods - Physical and chemical analyses of stream water. Key: S05 = Summer 2005, A06 = Autumn 2006, W06/7 = Winter 2006/7, Sp07 = Spring 2007, S07 = Summer 2007. Figures within brackets = Environmental Quality Standard (EQS).

| Site | Latitude | Longitude | Width (m) | Depth (m) | | | | | Organic Carbon (Filtered) mg/L. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S05 | A06 | W06/7 | Sp07 | S07 | S05 | A06 | W06/7 | Sp07 | S07 |
| St Mary Bourne Up | 51˚ 23’ 98’’ N | 01˚ 38’ 73’’ W | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.43 | 0.48 | 0.44 | 0.55 | 0.54 |
| St Mary Bourne Out | 51˚ 23’ 84’’ N | 01˚ 38’ 52’’ W | 1.7 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 1.01 | 0.88 | 0.61 | 1.27 | 1.14 |
| St Mary Bourne Down | 51˚ 23’ 47’’ N | 01˚ 38’ 60’’ W | 2.2 | 0.3 | 0.3 | 0.4 | 0.3 | 0.2 | 0.84 | 0.69 | 0.74 | 0.96 | 0.93 |
| Fobdown Up | 51˚ 09’ 82’’ N | 01˚ 18’ 78’’ W | 1.6 | - | 0.3 | 0.4 | 0.3 | 0.3 | - | 1.2 | 0.85 | 1.83 | 1.19 |
| Fobdown Out | 51˚ 09’ 62’’ N | 01˚ 18’ 96’’ W | 1.0 | - | 0.4 | 0.4 | 0.4 | 0.4 | - | 1.13 | 0.77 | 1.08 | 1.24 |
| Fobdown Down | 51˚ 10’ 24’’ N | 01˚ 18’ 87’’ W | 2.4 | - | 0.3 | 0.4 | 0.3 | 0.2 | - | 1.14 | 0.74 | 0.98 | 1.16 |
| Abbotts Ann Up | 51˚ 19’ 86’’ N | 01˚ 53’ 99’’ W | 1.4 | - | 0.5 | 0.6 | 0.5 | 0.4 | - | 1.64 | 0.93 | 1.14 | 1.32 |
| Abbotts Ann Out | 51˚ 19’ 27’’ N | 01˚ 53’ 32’’ W | 1.1 | - | 0.5 | 0.5 | 0.5 | 0.5 | - | 1.11 | 0.85 | 1.37 | 1.1 |
| Abbotts Ann Down | 51˚ 19’ 21’’ N | 01˚ 53’ 64’’ W | 2.8 | - | 0.2 | 0.3 | 0.2 | 0.2 | - | 1.17 | - | 1.35 | 1.19 |

Table 2.4. (continued).

| Site | Phosphate mg/L. ( 0.1) | | | | | Nitrate mg/L. (17.5) | | | | | Ammonia mg/L. (0.015) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S05 | A06 | W06/7 | Sp07 | S07 | S05 | A06 | W06/7 | Sp07 | S07 | S05 | A06 | W06/7 | Sp07 | S07 |
| St Mary Bourne Up | 0.023 | 0.022 | 0.025 | 0.022 | 0.022 | 6.4 | 5.9 | 6.7 | 6.4 | 6.6 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| St Mary Bourne Out | 0.093 | 0.035 | 0.071 | 0.158 | 0.230 | 5.3 | 5.2 | 6.3 | 9.3 | 4.4 | 0.066 | 0.03 | 0.03 | 0.037 | 0.071 |
| St Mary Bourne Down | 0.087 | 0.075 | 0.033 | 0.072 | 0.118 | 5.1 | 5.6 | 6.5 | 6.2 | 5.3 | 0.030 | 0.03 | 0.03 | 0.03 | 0.036 |
| Fobdown Up | - | 0.020 | 0.020 | 0.020 | 0.020 | - | 6.2 | 7.5 | 6.5 | 5.7 | - | 0.03 | 0.03 | 0.031 | 0.03 |
| Fobdown Out | - | 0.021 | 0.021 | 0.020 | 0.054 | - | 6.1 | 7.3 | 6.3 | 5.6 | - | 0.03 | 0.03 | 0.03 | 0.03 |
| Fobdown Down | - | 0.024 | 0.044 | 0.038 | 0.063 | - | 5.8 | 7.2 | 6.3 | 5.5 | - | 0.03 | 0.03 | 0.03 | 0.03 |
| Abbotts Ann Up | - | 0.026 | 0.032 | 0.040 | 0.027 | - | 9.1 | 9.5 | 8.4 | 8.4 | - | 0.03 | 0.03 | 0.03 | 0.03 |
| Abbotts Ann Out | - | 0.033 | 0.035 | 0.134 | 0.153 | - | 8.6 | 8.5 | 10.5 | 9.2 | - | 0.032 | 0.03 | 0.616 | 0.006 |
| Abbotts Ann Down | - | - | 0.044 | 0.140 | 0.134 | - | 8.6 | 7.2 | 10.9 | 8.9 | - | 0.03 | 0.5 | 0.56 | 0.053 |

Table 2.4. (continued).

| Site | °C | | | | | Nitrite mg/L. ( 0.01) | | | | | BOD ATU mg/L. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S05 | A06 | W06/7 | Sp07 | S07 | S05 | A06 | W06/7 | Sp07 | S07 | S05 | A06 | W06/7 | Sp07 | S07 |
| St Mary Bourne Up | 10.5 | 10.6 | 10.3 | 10.4 | 10.6 | 0.0040 | 0.004 | 0.004 | 0.004 | 0.004 | 1 | 1 | 1 | 1 | 1 |
| St Mary Bourne Out | 15.4 | 9.4 | 8.6 | 10.8 | 13.5 | 0.0514 | 0.0198 | 0.0068 | 0.0798 | 0.0315 | 1.84 | 1 | 1 | 1.8 | 2.2 |
| St Mary Bourne Down | 14.3 | 9.2 | 8.7 | 10.8 | 13.5 | 0.0338 | 0.0171 | 0.0046 | 0.0231 | 0.0188 | 1 | 1 | 1 | 1.8 | 1 |
| Fobdown Up | - | 8.9 | 8.1 | 12.1 | 15.5 | - | 0.0118 | 0.0079 | 0.0187 | 0.0105 | - | 1 | 1 | 2.8 | 1 |
| Fobdown Out | - | 9.4 | 9.1 | 11.5 | 13.5 | - | 0.0109 | 0.0071 | 0.0156 | 0.0111 | - | 1 | 1 | 1.4 | 1 |
| Fobdown Down | - | 9.1 | 8.4 | 12.0 | 14.3 | - | 0.011 | 0.0065 | 0.0159 | 0.0123 | - | 1 | 1 | 1.5 | 1 |
| Abbotts Ann Up | - | 9.4 | 8.8 | 11.0 | 13.5 | - | 0.0119 | 0.0041 | 0.0331 | 0.0158 | - | 1 | 1 | 1.4 | 1 |
| Abbotts Ann Out | - | 9.4 | 9.0 | 11.6 | 14.5 | - | 0.0135 | 0.008 | 0.0434 | 0.0352 | - | 1 | 1 | 2.4 | 1 |
| Abbotts Ann Down | - | 9.5 | 9.2 | 11.6 | 14.0 | - | 0.0154 | 0.1 | 0.049 | 0.0308 | - | 1 | 1 | 2.1 | 1 |

Table 2.5. Spatial Study (Chapter 6), summer 2007. Location & physico-chemical data for sample sites. Values are single sample measurements except temperature, width and depth, which are average values. See Chapter 3 for details - General Methods - Physical and chemical analyses of stream water. Figures within brackets = Environmental Quality Standard (EQS).

| Site | Latitude | Longitude | Width (m) | Depth (m) | °C | Phosphate mg/L. ( 0.1) | Organic Carbon (Filtered) mg/L. | Nitrate mg/L. (17.5) | Ammonia mg/l. (0.015) | BOD ATU mg/L. | Nitrite mg/L. ( 0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Meon Up | 51˚ 00’ 63’’ N | 01˚ 11’ 01’’ W | 1.2 | 0.4 | 11.9 | 0.087 | 1.43 | 5.1 | 0.03 | 1 | 0.0074 |
| Meon Out | 51˚ 00’ 59’’ N | 01˚ 11’ 03’’ W | 1.8 | 0.3 | 12.0 | 0.081 | 1.22 | 5.9 | 0.03 | 1 | 0.0101 |
| Meon Down | 51˚ 00’ 42’’ N | 01˚ 10’ 98’’ W | 2.5 | 0.3 | 12.0 | 0.074 | 1.26 | 5.8 | 0.03 | 1 | 0.0095 |
| Ilsington Up | 50˚ 72’ 58’’ N | 02˚ 34’ 74’’ W | 1.4 | 0.3 | 11.9 | 0.02 | 0.7 | 6.4 | 0.047 | 1 | 0.0134 |
| Ilsington Out | 50˚ 72’ 39’’ N | 02˚ 34’ 70’’ W | 1.0 | 0.3 | 14.0 | 0.02 | 0.77 | 5.8 | 0.044 | 1 | 0.0324 |
| Ilsington Down | 50˚ 72’ 27’’ N | 02˚ 34’ 41’’ W | 1.1 | 1.0 | 13.1 | 0.02 | 0.91 | 6.0 | 0.062 | 1 | 0.03 |
| Bere Up | 50˚ 76’ 20’’ N | 02˚ 23’ 25’’ W | 1.5 | 0.5 | 12.3 | 0.02 | 1.13 | 9.8 | 0.03 | 1 | 0.0141 |
| Bere Out | 50˚ 75’ 94’’ N | 02˚ 22’ 94’’ W | 1.6 | 1.0 | 13.1 | 0.104 | 1.23 | 9.3 | 0.201 | 1 | 0.0548 |
| Bere Down | 50˚ 75’ 66’’ N | 02˚ 22’ 75’’ W | 1.8 | 0.5 | 13.4 | 0.036 | 1.15 | 9.7 | 0.168 | 1 | 0.0735 |

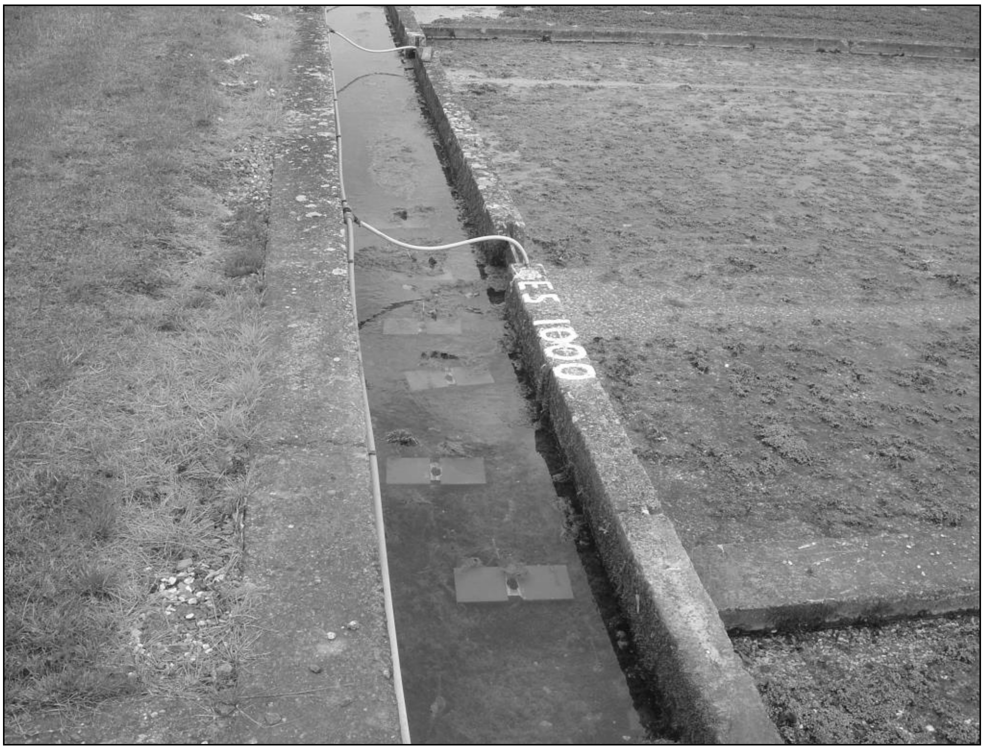


Plate 2.1. “Up” = “Upstream” reference site.

Plate 2.2. “Side” = “Side channel” site acting as a control for the management practices at the watercress farm at sites “WC” and “Out’’.

Plate 2.3. “WC” = “Watercress beds” site.

Plate 2.4. “Out” = “Outfall” heavily impacted site. Settlement tank in background.

Plate 2.5. “Down” = Downstream site 0.5 km below the “Outfall”, used to detect evidence of potential downstream recovery.

Plate 2.6. Parabolic screen.

Plate 2.7. Sediment trap.

**References.**

Acreman, M., & Dunbar, M., (2010) Ecologically acceptable flows in Chalk rivers. Retrieved 14.02.2013 from http://nora.nerc.ac.uk/10308/1/Ecological_flow_methods_for_chalk_rivers_final_report_19_April_10.pdf

Berrie, A., D., (1992) The chalk-stream environment. *Hydrobiologia,* **248**, 3-9.

Casey, H. and Smith, S., M., (1994) The effects of watercress growing on chalk headwater streams in Dorset and Hampshire. *Environmental Pollution,* **85**, 217–228.

Cox, J., Associates (2006) *Watercress growing and its environmental impacts on chalk rivers in England.* Report by Jonathan Cox Associates. English Nature Contract No. EIT-05-020 p 56. Retrieved. 28.08.2012.http://publications.naturalengland.org.uk/publication/40010

Crisp, D., T., (1969) Input and output of minerals for a small watercress bed fed by Chalk water. *Journal of Applied Ecology,* **7**, 117–40.

Crisp, D., T., Matthews, A., M. & Westlake, D., F., (1982) The temperatures of nine flowing waters in southern England. *Hydrobiologia*, **89**, 193–204.

Downes, J., D., Barmuta, L., A., Fairweather, P., G., Faith, D., P., Keough, M., J., Lake, P., S., Mapstone, B., D. and Quinn, G., P., (2002) Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. University Press, Cambridge

Environment Agency (2004) *The State of England's Chalk Rivers*.

Environmental Agency (1997-1998) *Invertebrate data Dorset / Wiltshire 1997-1998.*

Environmental Agency (2004a) *Invertebrate data Dorset / Wiltshire 2004.*

Environment Agency (2006) The Test and Itchen Catchment Abstraction Management Strategy (CAMS) available at Retrieved 31.08.2012 http://publications.environment-agency.gov.uk/PDF/GESO0306BKMB-E-E.pdf

Howden, N., J., K. & Burt, T., P., (2008) Temporal and spatial analysis of nitrate concentrations from the Frome and Piddle catchments in Dorset (UK) for water years 1978 to 2007: evidence for nitrate breakthrough? *Science of the Total Environment,* **407**, 507–526.

H.C.C. (2000) Biodiversity Action Plan for Hampshire: Volume Two, Hampshire Biodiversity Partnership. Chalk streams. Retrieved 28.08.2012. from http://www.hampshirebiodiversity.org.uk/pdf/PublishedPlans/ChalkStreamsHAPjjDTP2.pdf

Harrison, S., S., C. & Harris, I., T., (2002) The effects of bankside management on chalk stream invertebrate communities. *Freshwater Biology*, **47**, 2233–2245.

Ladle, M. & Westlake, D., F., (1995) River and Stream Ecosystems of Great Britain. In: River and Stream Ecosystems (Eds C.E. Cushing, K.W. Cummins & G.W.Minshall), pp. 343–388. Elsevier, Harrison New York.

Mann, R., H., K., Blackburn, J., H. & Beaumont, W., R., C., (1989) The ecology of brown trout *Salmo trutta* in the English chalk streams. *Freshwater Biology*, **21**, 57-70.

Marsden, C., D., (2006) *Combining chemistry, bioassay and biotic data to investigate the invertebrate decline in the Bourne Rivulet*. Report for Vitacress Salads Ltd by the Centre for Ecology and Hydrology (CEH), Dorset.

McSwann, E., (2005) Environmental Agency Report: Invertebrate data for the Bourne rivulet 1989 - 2004.

Medgett, S., (1998) Environmental Agency Report: *The impact of St. Mary Bourne Cress Farm on the Bourne Rivulet.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Prenda, J., Armitage, P., D. & Grayston, A., (1997) Habitat use by the fish assemblages of two chalk streams. *Journal of Fish Biology*, **51**, 64–79.

Sear, D., A., Armitage, P., D. & Dawson, F., D., H., (1999) Groundwater dominated rivers. *Hydrological Processes*, **11,** 255-276.

Shamsudin, L. & Sleigh, M., A., (1994) Seasonal changes in composition and biomass of epilithic algal floras of a chalk stream and a soft water stream with estimates of production. *Hydrobiologia*, **273,** 131-146.

Soulsby, P., G., (1975) Biological report; Summary report on cress farm investigations. Southern Water.

Underwood, A., J., (1994) On beyond baci – sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3–15.

Westwood, C., (2008) Ecological functioning of chalk stream headwaters.

White, S. & Medgett, S., (2006) Environmental Agency Report: *Bourne Rivulet Invertebrate Survey.*

Wood, P., J. & Petts, G., E., (1999) The influence of drought on chalk stream macroinvertebrates. *Hydrological Processes*, **13**, 387–399.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R., B., (2008) Trophic trickles and cascades in complex food web: impacts of keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Wright, J., F., (1992) Spatial and temporal occurrence of invertebrates in a chalk stream, Berkshire, England. *Hydrobiologia*, **248**, 11–30.

# Chapter 3.

# General Methods.

This study utilises ecosystem structure and functioning methods to examine the effects of watercress farming on chalk streams. Our measures of structure examine abundance both in the benthos and leaf litter bags, with a specific focus on shredding macroinvertebrates in particular *G. pulex*, the normally dominant shredder in chalk streams. A general description of these methods is contained in this Chapter. It is beyond the scope of this thesis to compare various index methods of deriving species diversity e.g. indices of biological integrity (IBI's) such as BMWP, ASPT and RIVPACS with one another or directly with our ecosystem structure and functioning measures.

## Physical and chemical analyses of stream water.

### *Temperature.*

TGP- 0050 data loggers (Gemini Data Loggers (UK) Ltd) recorded water temperatures at each site at fifteen-minute intervals during the experimental period (28 or 31d litter-bag and algal colonisation tile immersion), enabling temperature-corrected comparisons to be made by expressing ecosystem processes (e.g. decomposition and algal production) as rates per degree-day (after Minshall *et al.*, 1983).

### *Water Chemistry.*

A visit was made during each study period (28 or 31d) to each site to collect water samples, which were taken using standard EA protocols (Environment Agency 2008). On the same day, at each stream, a 1 L PET bottle of stream water was collected at each site within one hour of each other, and submitted to the EA Winchester for collection and processing by the Central EA laboratory. The following parameters were recorded: temperature, nitrate, phosphate, nitrite, ammonia, BOD and Organic Carbon (Environment Agency 2008). Dissolved Oxygen % Saturation (DO) and pH for each site were also taken and recorded in the field using an YSI 556 Multiprobe sonde.

## **Biological sampling.**

### ***Functional measures: Leaf litter decomposition rates.***

Detrital processing was measured as leaf-litter decomposition rates. Oak leaves (*Quercus robur L.*) were representative of leaf litter across our sites and were collected immediately after abscission during December 2004 and air-dried to constant mass. Sufficient leaves were collected for use throughout our studies and oak leaves were the only leaf species used. Oak leaves are physically robust, slow in decomposing and less susceptible to mechanical fragmentation compared to other leaves (Gessner & Chauvet 1994; Gulis, Ferreira, & Graca 2006). Oak leaves are found all year round in natural leaf packs within temperate streams (Graça & Canhoto 2006) and consequently were ideally suited for use in our studies.

A sample of litter (5g ± 0.10g) was then placed into litter-bags constructed of either 10mm aperture plastic mesh (henceforth referred to as “coarse bags”) to measure total decomposition (physical + microbial + macroinvertebrate) or 0.5mm aperture nylon mesh (“fine bags”) to measure microbial-only decomposition (after Riipinen *et al.*, 2009; Hladyz *et al.*, 2008, 2011a, b). The coarse bags were sewn and the fine bags were stapled closed into a tetrahedral shape, which holds the bags open rather than flat allowing stream water to circulate mimicking natural leaf packs, enclosing a suitably sized label of waterproof paper detailing site and replicate number (after Hladyz *et al.*, 2010). A single coarse bag and a fine bag were secured together with nylon cable ties to form a pair of bags. Prior to transport to the study site all the paired leaf bags were sprayed wet with distilled water to assist in the prevention of the break up of the leaves due to handling and transport to the study sites. At each of the study sites, labelled pairs of both 10mm and 0.5 mm mesh bags were secured to a house brick with two terracotta tiles glued to the top (henceforth referred to as “arrays”), with nylon ratchet ties (Fig. 3.1 & 3.2).

The arrays were anchored to the riverbed with a metal rebar driven into the substrate with a sledgehammer, cut level just below the water surface (to prevent snagging of floating material), and made secure with foam padding between the surfaces of the brick and rebar. Six replicate arrays (Fig. 3.1 & 3.2) were deployed at each of the sites, to provide statistical resolution, and left in the stream for 28 or 31d. 31 days were used in the field experiments at SMB during June / July 2005 and June 2006 (Chapter 4 and 5), this was revised to 28 days in the spatial and temporal study in late 2006 and 2007 (Chapter 6).

The general pattern of leaching from immersed whole leaves is a rapid loss over the first 24 hours, followed by a more gradual decline for an extended period. Depending on variables such as water temperature, turbulence, and leaf species, up to 25% of the initial dry weight of leaves may be lost to leaching in the first 24 hours. Leaves are colonized by a variety of aquatic microbes within a few days of deposition in freshwater. Fungi, principally hyphomycetes, in general dominate early colonization of tree leaves, gradually giving way to bacteria as decay advances. Mechanical and macroinvertebrate fragmentation follows microbial colonization. In streams, microbially softened leaves may be fragmented by current and abrasives or by high flows. A second mechanism of fragmentation is macroinvertebrate mediated. Leaf-shredding macroinvertebrates, or shredders, preferentially colonize and feed on microbially conditioned leaves and may contribute significantly to leaf breakdown in streams (see review Webster & Benfield 1986). Shredding macroinvertebrate activity was visually evident in our collected leaf bags, as a characteristic web like pattern of leaf veins, left behind after the material between the veins had been consumed (Plate. 3.1.).

Benthic macroinvertebrate assemblages were sampled at the same time as the collection of the algal tiles and litter bags, and from the same riffles within which the experimental arrays were located. Six replicate Surber samples (0.0625m² quadrat; 250µm mesh aperture) were collected at each site.

Following the collection of samples in plastic bags, they were stored in a cool box on ice at the field site and transferred in <1hr to a mobile freezer and then laboratory frozen at –20ºC. In the laboratory, after thawing, the leaf bags were washed in tap water into a fine mesh (500µm aperture) sieve where the leaves were rinsed, and separated from the invertebrates, gravel, sand and detritus, before being placed into an ovenproof aluminium foil dish. These were individually labelled and oven dried at 105º C to constant mass, and weighed. Leaf bag colonising invertebrates were picked out from the detritus, aided by a white sorting tray, and stored in vials of 70% industrial methylated spirits (IMS) for sorting, identification and counting. The remaining debris was washed in a semi fine sieve and any additional leaf material added to the appropriate foil container.

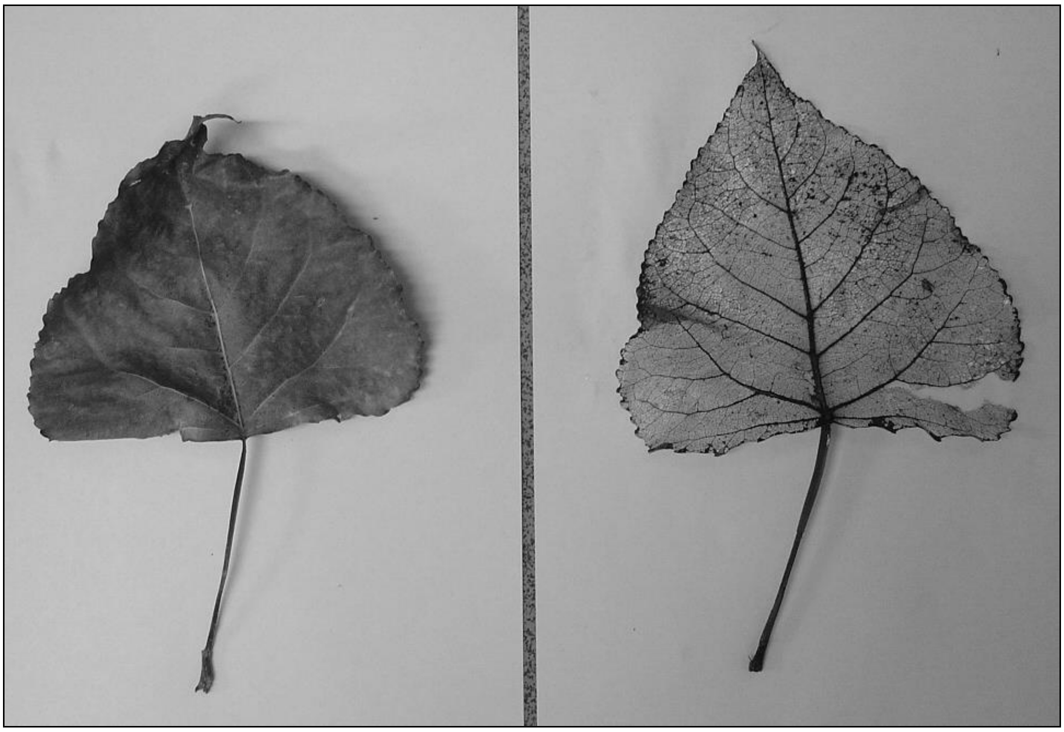


Plate 3.1. Macroinvertebrate evidence of feeding on leaves. Leaf before (left) and after (right) immersion in stream depicting the characteristic web like pattern of leaf veins, indicative of macroinvertebrate feeding activity (right).

Plate 3. 2. The effect of herbivory on algal accrual tiles. Algal accrual tiles after in stream immersion, demonstrating higher algae accumulation on tiles with Vaseline ‘fence’ (left) (ungrazed tile), which exclude crawling grazing macroinvertebrates, compared to those without (right) (grazed tile); indicative of an increase in the macroinvertebrate grazing pressure of herbivory.

Figure 3.1. Experimental set-up of the standardised field arrays used at each sampling site to assess ecosystem functioning, through the use of proxy measures for detrital processing, primary production and herbivory (see insert i). Main picture shows six replicated arrays deployed at a sampling site in a stream.

Insert i) the ecosystem functioning experimental array: a coarse-mesh leaf-litter bag (10mm) to allow access to shredding macroinvertebrates (right) and a fine-mesh leaf-litter bag (0.5 mm) to exclude shredding macroinvertebrates (left), secured with nylon ratchet ties to a house brick, with two terracotta algal colonisation tiles glued to the top. Detrital processing was measured by proxy as leaf-litter decomposition rates. Assessed by enclosing 5g ± 0.10g of oak leaves in both the coarse and fine leaf-litter bags: total decomposition (physical + microbial + macroinvertebrate) and microbial-only decomposition respectively. Primary production and herbivory (grazing activity) was measured as the rate of algal colonisation of the terracotta tiles by proxy as chlorophyll *a*. Assessed by excluding grazing macroinvertebrates from one of the paired tiles, by coating Vaseline petroleum jelly on the underside and leading edge, avoiding the upper surface (right), while the other tile was left Vaseline free (left) which allowed grazing macroinvertebrates free access.

Insert ii) six replicated arrays prior to deployment in a stream, one array of the six with a temperature logger attached (foreground).

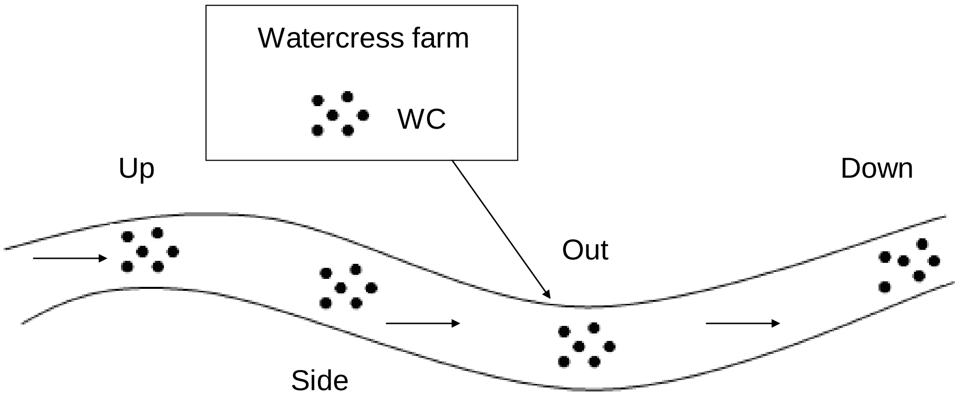


Figure 3.2a Experimental design used in Chapter 4.

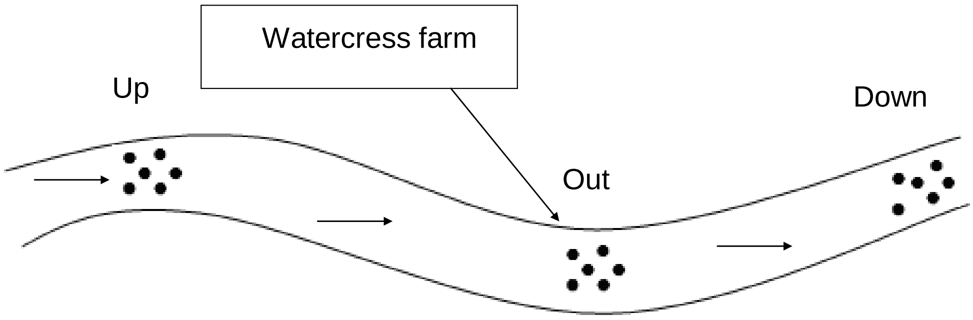


Figure 3.2b Experimental design used in Chapters 5 and 6.

Figure 3.2a & 3.2b Schematic diagrams of the experimental design used in a series of spatiotemporal studies, to assess the response of the ecosystem functioning measures (see insert i), to watercress farming and altered farm management practices. The arrows denote the flow of water. The study watercress farms do not abstract water directly from the stream but do so from artesian groundwater flow, which is returned to the carrier stream and thus augments the natural flow regime of the stream. Sample sites denoted as Up, Side, WC, Out and Down with the six sample replicates.

Decomposition rates were calculated for each litter-bag using the exponential decay coefficient, “*k*”, a standard metric used in decomposition studies (Petersen and Cummins 1974).

**Equation 3.1:** $-k = \ln (DW\ remaining / DW\ initial) / time$

Where DW initial (g) is the initial dry mass in the litter-bag, DW remaining (g) is the dry mass remaining after exposure in the stream. Time was expressed as degree-days over °C, by calculating mass loss per degree-day ($k_{dd}$), to correct for potential temperature effects across streams and sampling dates (after Minshall *et al.*, 1983; Woodward *et al*., 2012). Minor corrections to initial masses were made for losses due to handling, transportation, leaching and moisture content (after Riipinen *et al*., 2009; Hladyz *et al*., 2008, 2010).

***Conversion factors and leaching.***

The weight loss effects of leaching, oven drying and transportation were found by experiment and applied as corrections. A factor was obtained for leaching by using a water tank and running tap water arrangement, which provides a steady flow of chlorinated tap water to exclude biological activity. A set of six fine mesh replicate leaf bags were immersed in the tank for forty-eight hours, as the majority of losses due to leaching in leaves immersed in freshwater will have occurred within a 24 - 48 hour period (Nykvist, 1963; Petersen & Cummins, 1974; Suberkropp & Klug, 1976; Webster & Benfield, 1986).

A further set of six coarse leaf bags were subjected to the same procedures as the deployed leaf bags and taken to the site and back on the first day of array deployment to correct for the weight loss effects of handling and transportation. Loss effects of handling and transport, accounted for 4.3% of the weight loss and the effect of leaching and oven drying accounted for 14.4% of the loss; these values were used as a correction factor in the leaf breakdown calculations throughout the thesis (after Riipinen *et al*., 2009; Hladyz *et al*., 2008, 2010).

### ***Functional measures: Algal production and herbivory.***

Algal production and herbivory were assessed using two unglazed terracotta algal colonisation tiles glued to a three holed house brick's upper surface with Evostick 528 Trade Contact Adhesive, "arrays" (after Lamberti & Resh 1983; 1985; Lamberti & Feminella 1996). The effect of grazing macroinvertebrates was assessed by excluding their presence with the use of Vaseline petroleum jelly 'fences' (ungrazed tile), spread liberally on the underside and leading edge of one of the paired tiles avoiding the upper surface, which excludes crawling grazing macroinvertebrates, while the other tile was left Vaseline free (grazed tile) (after McAuliffe 1984; Hladyz *et al.,* 2011a, b) (Fig. 3.1 & 3.2). The exclusion of crawling grazing macroinvertebrates was evident in our study as higher algae accumulation on tiles with the Vaseline 'fence' (ungrazed tile) compared to those without (grazed tile), indicative of the effect of herbivory (Plate 3. 2). Each site and replicate was inspected every two days and cleared of any accumulated debris, such as floating vegetation.

Algae that had accumulated on the tiles was scrubbed off with a toothbrush and washed into a small dark bottle (after Ledger & Hildrew 1998; 2000), and quickly transferred to a mobile freezer to minimise chlorophyll breakdown, prior to storage in the laboratory at –80ºC prior to processing. In the laboratory, algal samples were thawed overnight in a dark cold room (at 4°C), and thoroughly shaken. For each sample, a 20 ml aliquot of algal suspension was filtered through a Whatman GF/C glass fibre filter placed in a Büchner funnel by applying a vacuum. The filter was then rapidly placed into 10 ml of ice-cold 90 % acetone, mashed with a glass rod, and placed on ice covered with a dark plastic bag to prevent chlorophyll degradation, which becomes highly sensitive to UV light during this extraction process. Samples were kept on ice in a dark cold room overnight, centrifuged at 4,000 rpm and the chlorophyll *a* content was then measured spectrophotometrically (APHA. 1995, Aminot & Rey 2000). Spectrophotometry was selected as the best method due to the availability of materials and equipment. Appropriate methods were chosen to maximise the extraction of chlorophyll *a* e.g. maceration of cells and acetone use (Schagerl & Künzl 2007). Absorption of the extraction was measured at 664 (chlorophyll *a*) and 750 nm (turbidity). Chlorophyll *a* concentration was calculated using the following equation (after Lorenzen 1967).

Equation 3.2: $Chl\ (\mu g / cm^2) = A * K * (A_{664} - A_{750}) * V / S * l$

Where $A$ is the absorption coefficient of chlorophyll *a* (= 11). $K$ is a factor to equate the reduction in absorbancy to initial chlorophyll *a* concentration (1.7:0.7, or 2.43). $A664$ and $A750$ is the absorption of the solution at 664 and at 750 nm, respectively. $V$ is the volume of acetone used for extraction (ml). $S$ is the tile surface area ($cm^2$) scrubbed during sampling (taking into account that only a fraction of each algal sample taken was being used in this analysis). $l$ is the path length of the light in the cuvette (1 cm).

***Structural measures: macroinvertebrates abundances in leaf litter bags and benthos.***

Macroinvertebrates from the benthic samples and those colonising the exposed litter-bags, sampled on the same occasion, were sorted and identified to species level where possible. Individuals were identified, using dissection and a binocular light microscope (at 8x - 80x magnification) where applicable, and a combination of published identification keys (see Appendix 2) and classified into functional feeding groups (FFG's) using (Tachet *et al.,* 2002).

***Data analysis and transformations.***

Ecosystem process rates (leaf litter decomposition and chlorophyll *a* accrual) and structural community attributes (benthic and leaf-litter bag macroinvertebrate abundance) were analysed as response variables using Linear Mixed-Effects Models (LMEM). Data were $\log_{10} x$ (or $\log_{10} x + 1$) transformed to meet the assumptions of homogeneity of variance and normality, where appropriate. LMEM was used to analyse both survey and experimental data and not ANOVA, as our (1) experiments consisted of both fixed and random effects and (2) to account for temporal non-independence involving repeated measurements from the same spatial location (i.e. six riffle blocks within each site measured on several occasions. Details of the spatial and temporal components tested in each data chapter are shown in Table. 3.1. Riffles (i.e. blocks) were fitted as a random effect in the models, and to include an assessment of in-stream variation, site and time (as month / season / year where appropriate) were fitted as fixed effects. For repeated measures models, site was used as the between-group factor, whereas time was the within-group factor: thus the variance it accounted for was partitioned from the model. For breakdown rate data, mesh was also included in the model as an additional between-group factor (fixed), for assessing differences between the two mesh types (Coarse and Fine). For algal colonisation tile data the additional between-group factor Herbivory (fixed) was incorporated for assessing top-down effects of grazers (i.e. ungrazed vs. grazed tile surfaces). Our experimental designs were unbalanced due to loss of a few replicates during exposure in the field, and to correct for this we used restricted maximum likelihood (REML) estimation to model error terms (after Hladyz *et al*., 2011a, b). When significant effects were identified, *post hoc* analyses were undertaken using Bonferroni pairwise comparisons. Analyses were conducted using SPSS Version 17.0 (SPSS, Inc., Chicago, IL, USA).

Ordination was used to explore the community level effects of watercress farming. Initial detrended correspondence analysis (DCA) of the macroinvertebrate community data revealed a gradient length <3 S.D., so linear models were used for subsequent ordinations of community composition in the litter bags and benthos (ter Braak & Prentice 1988). Unconstrained ordination - principal components analysis (PCA) and constrained ordination - redundancy analysis (RDA) were then performed in CANOCO version 4.5, to identify correlations between environmental variables, ecosystem process rates and community composition among sites and over time (ter Braak & Prentice, 1988).  RDA was used to explicitly test for site differences, where site and month were coded as dummy variables and tested with Monte Carlo permutations (999) and forward selection.

Table 3.1. Details of the temporal and spatial components tested in each Chapter; the number and position of the sampling sites on each stream, the number of streams and the number of sampling occasions. The following were sampled and determined at each of the replicated (six) sampling sites (Fig. 3.1 & 3.2), Functional measures: *Primary production*: Algal accrual - grazers excluded, Algal accrual – grazers; *Leaf litter decomposition*: Microbial decomposition *k*F; Macroinvertebrate & Microbial decomposition *k*C; Community measures: Benthic macroinvertebrate abundances, Leaf litter bag macroinvertebrate densities.

| **Chapter** | **Description** | **Spatial** | | **Temporal** |
|---|---|---|---|---|
| | | **Sampling sites** | **Streams** | **Sampling occasions** |
| 4 | SMB June & July 2005 | 5<br>(Up, Side, WC, Out, Down.) | 1<br>(SMB) | 2<br>(June & July 2005) |
| 5 | SMB June 2005, 2006 & 2007. | 3<br>(Up, Out, Down.) | 1<br>(SMB) | 3<br>(June 2005, 2006 & 2007) |
| 6 | Temporal study. 3 farms sampled on 4 occasions (seasonally). | 3<br>(Up, Out, Down.) | 3<br>(inc. SMB) | 4<br>(Autumn, Winter, Summer & Spring 2006 / 07) |
| 6 | Spatial study. 6 farms sampled on 1 occasion only (June 2007). | 3<br>(Up, Out, Down.) | 6<br>(inc. SMB) | 1<br>(June 2007) |

.

**References.**

Aminot, A. and Rey, F., (2000) Standard procedure for determination of chlorophyll *a* by spectroscopic methods. ICES, Denmark.

APHA., (1995) Standard methods. 19th Edition. American Public Health Association, Washington, DC.

Environment Agency (2008) Index of SCA Blue Book Methods. Index of Methods for the Examination of Waters and Associated Materials 1976 – 2011. Retrieved 28.08.2012. http://www.environment-agency.gov.uk/static/documents/Research/SCA_Blue_Book_236.pdf

Gessner, M., O. & Chauvet, E., (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807-1817.

Graça, M., A., S. & Canhoto, C., (2006) Leaf litter processing in low order streams. *Limnetica*, **25**, 1–10.

Gulis, V., Ferreira, V. & Graca, M., A., S., (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshwater Biology, **51**, 1655–1669.

Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J. & Woodward, G. (2008) Resource quality and stoichiometric constraints in a stream food web. *Freshwater Biology*, **54**, 957–970.

Hladyz, S., Tiegs, S., D., Gessner, M., O., Giller, P., S., Risnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. and Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology,* **55**, 1916-1929.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M., O., Giller, P., S., Gulis, V., Hutton, S., A., Lacoursie`re, J., O., *et al.*, (2011a) Stream ecosystem functioning in an agricultural landscape: The importance of terrestrial–aquatic linkages. *Advances in Ecological Research*, **44**, 211–276.

Hladyz, S., Abjornsson, K., Giller, P., S., and Woodward, G., (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology,* **48**, 443–452.

Lamberti, G., A. & Feminella, J., W., (1996) Plant–herbivore interactions. Methods in Stream Ecology (eds F.R. Hauer&G.A. Lamberti), pp. 409–430. Academic Press, NewYork.

Lamberti, G., A. & Resh, V., H., (1983) Stream periphyton and insect herbivores: an 6 experimental study of grazing by a caddisfly population. *Ecology,* **64**, 1124–1135.

Lamberti, G., A. & Resh, V., H., (1985) Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macroinvertebrates. *Freshwater Biology,* **15**, 21-30.

Ledger, M., E. & Hildrew, A., G., (1998) Temporal and spatial variation in the epilithic biofilm of an acid stream. *Freshwater Biology,* **40**, 655-670

Ledger, M., E. and Hildrew, A., G., (2000) Resource depression by a trophic generalist in an acid stream. *Oikos,* **90**, 271_278.

Lorenzen, C., J., (1967) Determination of chlorophyll and pheo-pigments: spectrophotometric equations**.** *Limnology and Oceanography,* **12**, 343-346

McAuliffe, J., R., (1984) Resource depression by a stream herbivore: effects on distribution and abundances of other grazers. *Oikos* **42**, 327-333.

Minshall, G., W., Petersen, R., C., Cummins, K., W., Bott, T., L., Sedell, J., R., Cushing, C., E. & Vannote, R., (1983) Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs,* **53**, 1-25.

Nykvist, N., (1963) Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. *Studia Forestalia Suecica*, **3**, 1-29

Petersen, R., C. and Cummins, K., W., (1974) Leaf pack processing in a woodland stream. *Freshwater Biology,* **4**, 343-368.

Peterson C. G. (1996) Response of benthic algal communities to natural physical disturbance. In Algal Ecology: Freshwater Benthic Ecosystems (eds R. J. Stevenson, M. L. Bothwell & R. L. Lowe) pp. 375–403. Academic Press, San Diego.

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P., & Dobson, M. (2009) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology*, Published Online: Aug 24 2009.

Schagerl M., Künzl G., (2007) Chlorophyll *a* extraction from freshwater algae – a re-evaluation. *Biologia,* **62**, 270-275.

Suberkropp, K. and Klug, M., J., (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* , **57**, 707-719.

Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P., (2002) Invertebres d’Eau Douce, 2nd corrected impression. CNRS editions, Paris.

ter Braak, C., J., F. & Prentice, I., C., (1988) A theory of gradient analysis. *Advances in Ecological Research*, **18**, 271-313.

Webster, J., R. and Benfield, E., F., (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics,* **17**, 567-594.

Woodward G., M.O. Gessner, P. S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B.G. McKie,S. D. Tiegs, H. Cariss, M. Dobson, A. Elosegi, V. Ferreira, M. A.S. Graça, T. Fleituch, J. O. Lacoursiere, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B.-M. Vought, Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science*, **336**, 1438-1440.

# Chapter 4.

## *Watercress production alters stream ecosystem functioning and community structure.*

### Introduction.

Our principal aims were to assess the potential of multiple measures of ecosystem functioning, combined with community-based measures, as a new, integrated approach to biomonitoring. Specifically, we sought to investigate whether the simultaneous examination of primary production, herbivory and leaf litter decomposition by both microbial and macroinvertebrate activity, together with structural macroinvertebrate community measures, could be used to evaluate the impact of the VSL watercress farm on the ecology of the Bourne rivulet and to detect potential impacts and recovery following altered management practices. This was carried out both prior to (June 2005) and immediately after (July 2005) bioremediation i.e. re-routing the SMB watercress farm settlement tank waste waters through the watercress beds. The metrics were used in a large-scale field experiment with multiple spatial and temporal controls, in a BACI-style (Before, After, Control, Impact) study (see reviews in; Underwood 1994; Downes *et al.*, 2002), designed to detect both initial impacts and evidence of potential biological recovery.

Sampling prior to and after the re-routing enabled a comparison with which to test our hypothesis that re-routing the wastewaters from the SMB farm and factory back through the watercress beds, could bioremediate their effect and act as an artificial wetland. Our experimental hypotheses were:

1) The upstream “control” site would be unaltered by the presence of the farm and for this lack of impact to remain unaffected by re-routing. The outfall

site should show the strongest responses to the watercress farm, with evidence of at least partial recovery downstream.

2) Post manipulation one month later in July 2005, if the re-routing had been successful, the response of the outfall and downstream sites should resemble those of the upstream site.
3) If the re-routing has been successful then PEITC is likely to be the stressor. If not other potential stressors such as chlorine use and organic pollution may be implicated.

## Methods.

Refer to Chapter 2 & 3 – Sites & General Methods for a detailed description.

Five study sites were sampled in an initial survey of the SMB watercress farm in June 2005. These sites, representing five spatial treatments (Up, Side, WC, Out, Down), were selected based on their hydrological connectivity to the farm and hence exposure to stressors and the experimental design requirements for our replicated BACI design.

## Results.

This study took place in June and July of 2005. Spatial description, location (Fig. 2.1 & 2.3 pp. 53 & 60) physico-chemical data (Table 2.1-2.5 pp. 65-71) and site plates (Plates 2.1-2.7 pp. 72-75) are available in Chapter 2.

### *Physico-chemical parameters.*

Temperature ranged from 14.3 – 15.5°C at all sites, except water directly from the aquifer borehole at the Up site, which was a near constant 10.5°C. Water chemistry data was only available in July 2005 (post re-routing). EQS levels were not exceeded upstream except for ammonia. Nitrite and ammonia levels were also exceeded at all other sites, and phosphate levels were elevated at the outfall and downstream sites relative to the upstream site. BOD was high at the WC (1.36) and outfall sites (1.84).

***Functional measures: a) leaf litter decomposition rates.***

There was no significant difference between the LMEM decomposition rates per day and per degree-day analyses, suggesting that the differences were not temperature related. This pattern was found throughout our analyses in this thesis (Chapter 4, 5 & 6). Therefore only the degree-day data are included in the results and analyses. Per day results appear as tables in the Appendix for the appropriate Chapter.

Decomposition rates differed significantly among sites (LMEM $F$ $df_{N4}$, $df_{D49.981}$= 5.579, $P$ <0.001) and between months (LMEM $F$ $df_{N1}$, $df_{D49.889}$ = 11.752, $P$ <0.001) (Table 4.1). Total decomposition in coarse bags was significantly faster than in fine bags, highlighting the overriding influence of shredders (LMEM $F$ $df_{N1}$, $df_{D50.055}$ = 32.401, $P$ <0.001) (Table 4.1). There was a significant interaction between site × mesh type (LMEM $F$ $df_{N4}$, $df_{D\ 49.981}$ = 3.539, $P$ = 0.013), suggesting a significant difference between the rate and the relative dominance of the microbial and shredding components of leaf litter breakdown and their spatial position, but there were no two way or three way time interactions. This revealed that decomposition rates in June 2005, prior to the re-routing, were suppressed at the outfall with a concomitant recovery with distance downstream and that this pattern did not change after rerouting in July 2005 (Fig. 4.1). Further, overall decomposition rates were consistently higher at the three “upstream sites” (Up, Side, and WC) relative to the outfall pointing to it as the source of the perturbation.

Table 4.1. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per degree-day ($k_{dd}$) in June and July 2005. Sites: Up, Side, WC, Out and Down. Significant results at $P<0.05$ are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | $F$ – ratio | $P$ |
|---|---|---|---|---|
| Site | 4 | 49.981 | 5.579 | **0.001** |
| Mesh | 1 | 50.055 | 32.401 | **<0.001** |
| Time | 1 | 49.889 | 11.752 | **0.001** |
| Site x Mesh | 4 | 49.981 | 3.539 | **0.013** |
| Site x Time | 4 | 49.808 | 0.373 | 0.827 |
| Mesh x Time | 1 | 49.889 | 0.497 | 0.484 |
| Site x Mesh x Time | 4 | 49.808 | 0.517 | 0.724 |

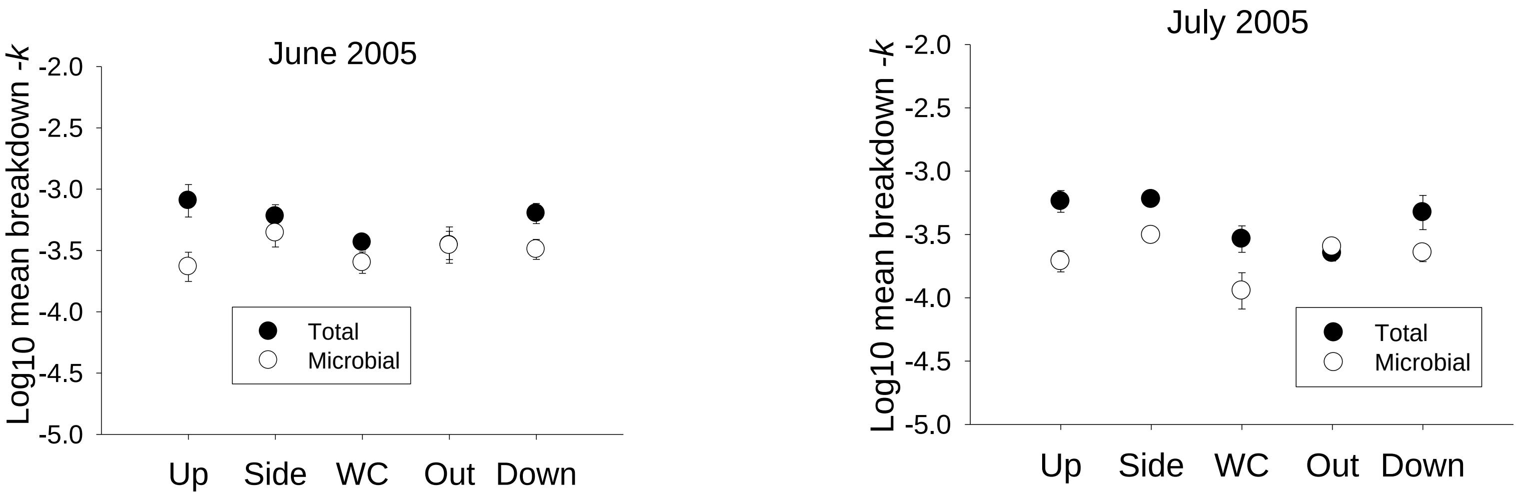


Figure 4.1. $\text{Log}_{10}$ mean leaf-litter decomposition rates per degree-day (*k* ± SE). ($k_{dd}$) in June and July 2005. White circles denote Microbial breakdown (fine mesh bags). Black circles denote Total breakdown (coarse mesh bags). June 2005 pre manipulation, July 2005 post manipulation. Sites: Up, Side, WC, Out and Down. N.B. June 2005 Out: Black dot behind white dot therefore Total = Microbial component.Out 2005 vs. Out 2006 no change Total = Microbial component.

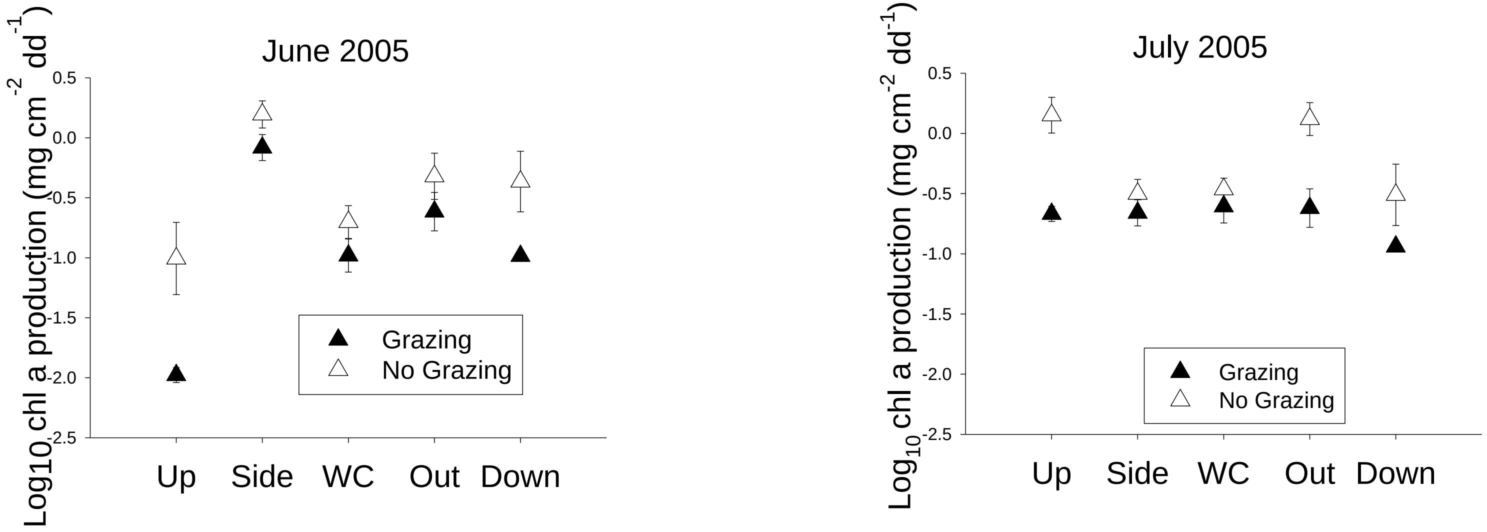
### *Functional measures b) algal production and herbivory.*

Analyses of the LMEM algal production rates per day and per degree-day revealed no significant difference between them, suggesting that the differences were not temperature related. This pattern was found throughout our analyses in this thesis (Chapter 4, 5 & 6). Therefore only the degree-day data are included in the results and analyses. Per day results appear as tables in the Appendix for the appropriate Chapter.

Algal accrual rates significantly differed in response to the main effects of site, herbivory (treatment) and time, with the “Upstream” sites being lower than the impacted “Outfall” and “Downstream” sites (Table 4.2, Fig. 4.2). Chlorophyll *a* biomass was higher on the grazer-excluded tiles than on the non-excluded tiles (LMEM $F\ df_{N1}, df_{D49.408} = 38.491$, $P < 0.001$) demonstrating the top-down effects of grazers across all sites. There was also a significant site × herbivory and site × time interaction, revealing that grazer effects were consistent over time and sites, but the lack of a three-way interaction revealed that grazer effects did not change in response to bioremediation (Table 4.2, Fig. 4.2).

Table 4.2. LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a* *(*mg cm-2) per degree-day (*dd*) June and July 2005. Sites: Up, Side, WC, Out and Down. Herbivory = V / NV. V = exclusion of grazers through use of Vaseline 'fence' and NV = non exclusion. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 4 | 49.182 | 8.900 | **<0.001** |
| Herbivory | 1 | 49.408 | 38.491 | **<0.001** |
| Time | 1 | 49.642 | 6.892 | **0.011** |
| Site x Herbivory | 4 | 49.182 | 2.575 | **0.049** |
| Site x Time | 4 | 49.431 | 13.092 | **<0.001** |
| Herbivory x Time | 1 | 49.642 | 0.035 | 0.852 |
| Site x Herbivory x Time | 4 | 49.431 | 0.544 | 0.704 |



June 2005

July 2005

Figure 4.2. Log$_{10}$ chlorophyll *a* production *(*mg cm$^{-2}$) per degree-day (*dd*) ± SE by site before manipulation in June 2005 and after manipulation, July 2005. Sites: Up, Side, WC, Out and Down. White triangles denote No Grazing (exclusion of grazers through use of Vaseline ‘fence’), black triangles denote Grazing (no exclusion of grazers).

### ***Structural measures: macroinvertebrates abundances in leaf litter bags and benthos.***

*G. pulex* (92%), and *A. aquaticus* (6%), were the most abundant shredding macroinvertebrates in the benthos and litter bags in this study (see Table 3 in Appendix 3). *S. personatum* (1%), were only found in the litter bags in July (Down) and the benthos (WC). Consequently *G. pulex* and *A. aquaticus* were identified as the likely drivers of macroinvertebrate-mediated decomposition, and were therefore the principal focus of our analyses in this Chapter. The results for the total abundance of shredders are included in the result tables and demonstrate that the individual species are important rather than the total.

*G. pulex* were the dominant shredder at the upstream sites (Up, Side, WC), becoming severely impoverished at the outfall, but increasing downstream, for both sample periods. In contrast, *A. aquaticus* was rarest at the upstream sites and at the outfall, but was the dominant shredder downstream, across both sample periods.

Leaf litter bag densities and benthic macroinvertebrate abundances both revealed statistically significant differences between sites for *G. pulex* and *A. aquaticus*. Whereas, there were no significant differences for the interaction of site × time in leaf bags, there were for the benthic abundance of *G. pulex* and *A. aquaticus.* This suggests change from the structural but not from the functioning measure in response to bioremediation. Clearly these two measures while being similar are not depicting exactly the same thing. In addition *G. pulex* abundance in the benthos differed significantly among the five sites (LMEM, benthos: $F\ df_{N4}, df_{D25} = 76.934$, $P < 0.001$; litter bags: $F\ df_{N4}, df_{D19.895} = 101.586$, $P < 0.001$) (Bonferroni pairwise comparisons for benthos & litter bags: Upstream = Side channel = Watercress beds > Outfall = Downstream) (Table 4.3 & 4.4; Fig 4.3 & 4.4). This suggests that PEITC may not be the causative agent, because the outfall and downstream sites are not like the upstream site i.e. rejecting hypothesis 2. Also the “Watercress beds”, “Side channel” and “Outfall” would all have been exposed to PEITC via the effluents from the watercress farm, this could therefore not account for high *G. pulex* numbers at the first two sites versus suppression at the latter. Detrimental impacts appear to be most profound at the outfall and downstream of it, suggesting that the cause of the problem is emanating from the outfall.

Table 4.3. LMEM results of comparisons of shredder coarse litter-bag abundance ($\log_{10}$ mean number per g leaf litter remaining) for *G. pulex* (G), *A. aquaticus* (A) and Total shredders (T). June and July 2005. Sites: Up, Side, WC, Out, Down Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Site | G | 4 | 19.895 | 101.586 | **<0.001** |
| | A | 4 | 25.042 | 91.504 | **<0.001** |
| | T | 4 | 21.236 | 48.925 | **<0.001** |
| Time | G | 1 | 20.304 | 0.954 | 0 .340 |
| | A | 1 | 25.311 | 0.122 | 0.730 |
| | T | 1 | 21.624 | 0.513 | 0.481 |
| Site x Time | G | 4 | 20.278 | 2.547 | 0.071 |
| | A | 4 | 25.278 | 0.740 | 0.574 |
| | T | 4 | 21.597 | 2.163 | 0.108 |

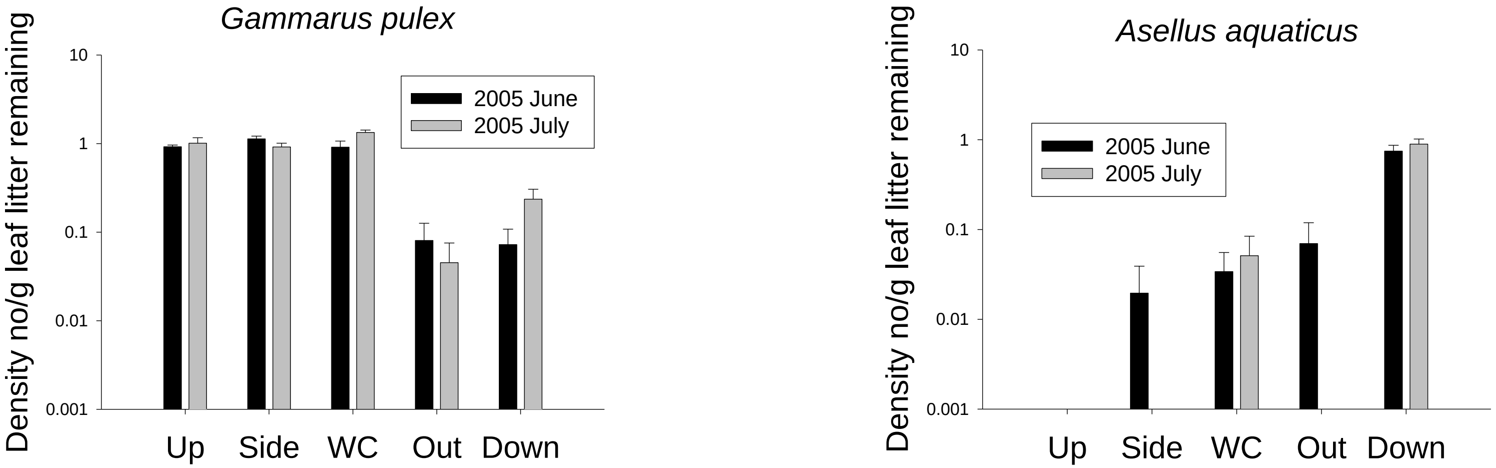


Figure 4.3. *G. pulex* and *A. aquaticus* abundance in coarse litter-bags ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE), before manipulation (June 2005) & after manipulation (July 2005). Sites: Up, Side, WC, Out and Down. N.B. This is a log scale.

Table 4.4. LMEM results of comparisons of benthic shredder abundance June, and July 2005. Sites: Up, Side, WC, Out and Down (nos. $m^{-2}$). *G. pulex* = *G, A. aquaticus* = A and Total shredders (T). Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Site | G | 4 | 25 | 76.934 | **<0.001** |
| | A | 4 | 25 | 81.071 | **<0.001** |
| | T | 4 | 25 | 53.174 | **<0.001** |
| Time | G | 1 | 25 | 1.233 | 0.277 |
| | A | 1 | 25 | 3.433 | 0.076 |
| | T | 1 | 25 | 7.169 | **0.013** |
| Site x Time | G | 4 | 25 | 4.634 | **0.006** |
| | A | 4 | 25 | 2.886 | **0.043** |
| | T | 4 | 25 | 1.415 | 0.258 |

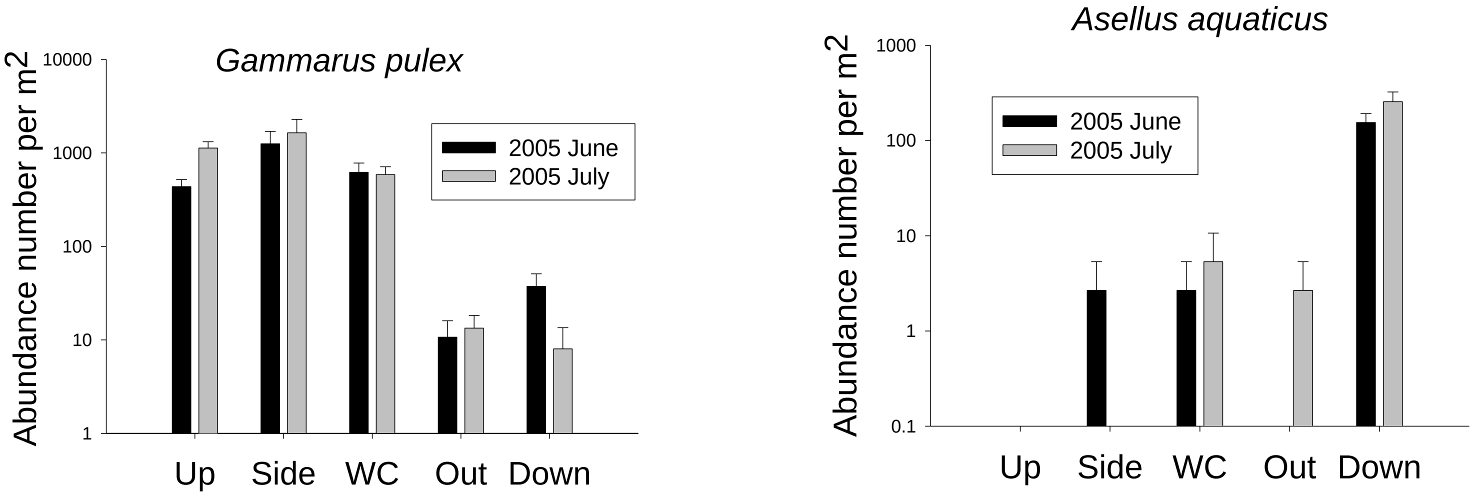


Figure 4.4. *G. pulex* and *A. aquaticus* benthic abundance (nos. $m^{-2}$ ± SE) before manipulation (June 2005) and after manipulation (July 2005). Sites: Up, Side, WC, Out and Down. N.B. This is a log scale.

### ***Ordination combining chemical data with functional and structural measures.***

PCA (Fig. 4.5) axis 1 accounted for 57.2% of the variation in the community data and was negatively associated with a gradient of nutrient pollution (BOD, Nitrite, Ammonia) and positively associated with microbial (*kF*) and shredder (*kC*) leaf litter breakdown and herbivory. Axis 2 accounted for 41.1% of the variation along a macroinvertebrate taxa gradient and followed a typical response to organic pollution. This separated the Up (unimpacted), the Out (impacted) and Down (recovery) sites (Fig. 4.5). The Up (unimpacted) sites were associated with the shredder *G. pulex* and the efficient grazer *Ancylus fluviatilis* (Müller). More pollution-tolerant taxa, such as Oligochaete worms, Chironomids (non predatory) and the leeches *Erpobdella octoculata* (L.), *Helobdella stagnalis* (L.), *Glossiphonia complanata* (L.) and *Piscicola geometra* (L.), were more characteristic of the impacted outfall site. The Down (recovery) site was associated with *A. aquaticus* and the dominant grazers (*Baetis spp*., *Planorbis leucostoma* (Millet) and *Seratella ignita* (Poda). Both axes together accounted for 98.3% of the variation, thus the majority of the variation was explained by these two axes. If there had been a positive response to the farms bioremediation, the July outfall site results would have been associated with the downstream (recovery) sites or the upstream (unimpacted) sites at the top and bottom of the ordination plot, respectively. However, our results suggest no positive change, as the July results remained closely associated with the pre-remediation June outfall site. Contrary to this, an RDA ordination where site and month were coded as dummy variables, suggests that the situation had deteriorated between June 05 and June 06 (Fig 4.6). Axis 1 and axis 2 accounted for 45.9% and 37.8% of the respective variation in the species data. Both axes accounted for 83.7% of the variation, suggesting that most of the variation was attributable to these two axes. This mirrored the PCA results, suggesting that the RDA and the PCA appear to be detecting very similar patterns within these data and therefore there were no important drivers left undetected.

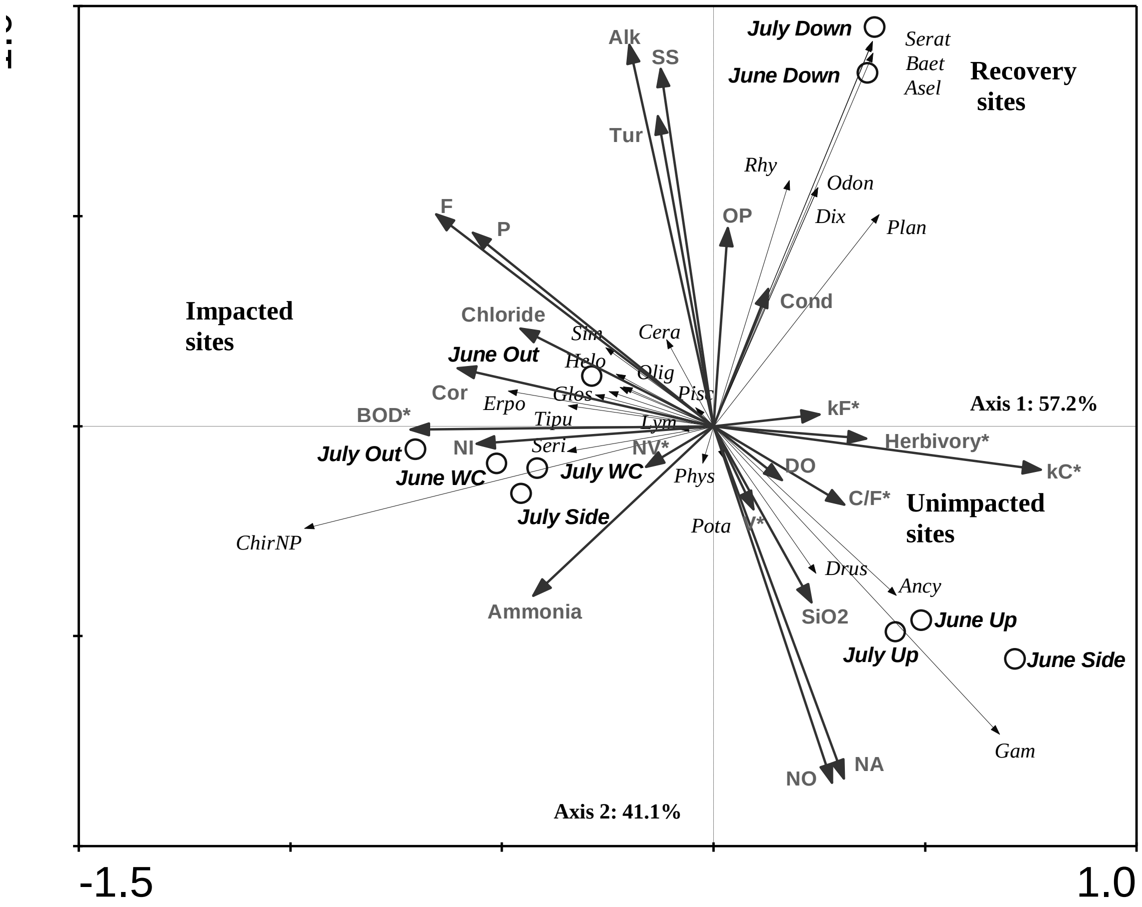


Figure 4.5. Principal components analysis (PCA) triplot of species scores vectors for benthic macroinvertebrate taxa (surber samples) (light arrows) environmental variables (dark arrows) with sites shown as centroids (open circles) recorded at St Mary Bourne watercress farm and the Bourne Rivulet in June and July 2005. Arrows display the correlation between species and the environmental variables and are oriented in the direction of maximum variation in value. Axis I (57.2%) & II (41.1%) accounted for 98.3% of the variation in the species data.

Abbreviations.
i) Sites:
Up = Upstream, Side = Side Channel, WC = Watercress Beds, Out= Outfall, Up = Upstream.

ii) Species codes:
Aga (*Agapetus fuscipes*); Ancy (*Ancylus fluviatilis*); Asel (*Asellus aquaticus* ); Baet (*Baetis rhodani* ); Cera (Ceratopogonidae); ChirNP (Chironomidae Non-predatory); ChirP (Chironomidae Tanypodinae);Chlor (*Chloroperla torrentium*); Crun (*Crunoecia irrorata*); Dix (*Dixella spp*); Drus (*Drusus annulatus*); Ecd (*Ecdyonurus venosus*); Elm (*Elmis aeanea*); Eph (*Ephemera danica*); *Eph* (Ephydridae); Erpo (*Erpobdella octoculata*); Gam

(*Gammarus pulex* ); Glos (*Glossiphonia complanata*); Hale (*Halesus radiatus* ); Helo (*Helobdella stagnalis* ); Hyd *Hydroptilla* spp); HydS (*Hydropsyche siltalai*); HydP (*Hydropsyche pellucidula*); Lasi (*Lasiocephala basalis*); Leuc (*Leuctra innernis*); Limn (*Limnephilus* spp); Lym (*Lymnaea peregra*); Myst (*Mystacides azurea*); Nem (*Nemurella pictetii*); Odon (*Odontocerum albicorne*); Olig (Oligochaeta); Ped (*Pedicia rivosa*); Pisc (*Piscicola geometra*); Phys (*Physa fontinalis*); Pisi (*Pisidium* spp); Plan (*Planorbis leucostoma*); Poly (*Polycentropus flavomaculatus*); Pota (*Potamophylax cingulatus*); Potam (*Potamopyrgus antipodarum*); Rhy (*Rhyacophila dorsalis*); Serat (*Seratella ignita_*); Seri (*Sericostoma personatum*); Silo (*Silo nigricornis*); Sim (*Simulium* spp); Tin (*Tinodes waeneri*); Tipu (*Tipula* spp);_Valv (*Valvata cristata*).

iii) Environmental variables:
Alky (Alkalinity); Ammonia; Chloride; COD (Chemical Oxygen Demand); Cond (Conductivity); Col (Colour); COrg (Organic Carbon); DO (Dissolved Oxygen); F (Fluoride); NOxid (Oxidised nitrogen); Nitrate; Nitrite; OP (Orthophosphate); SiO2; SS (Suspended Solids); P (Phosphate); Turb (Turbidity).

iv) Functional measures (denoted*):
BOD* (Biological Oxygen Demand); kC* (Decomposition in coarse litter bags per degree-day); kF* (Decomposition in fine litter bags per degree-day); C/F* (Ratio kC/Kf); NV* Chlorophyl *a* on Non Vaseline tile); V* (Chlorophyl *a* on Vaseline tile); Herbivory*V/NV (Ratio V/NV). (See Methods for details).

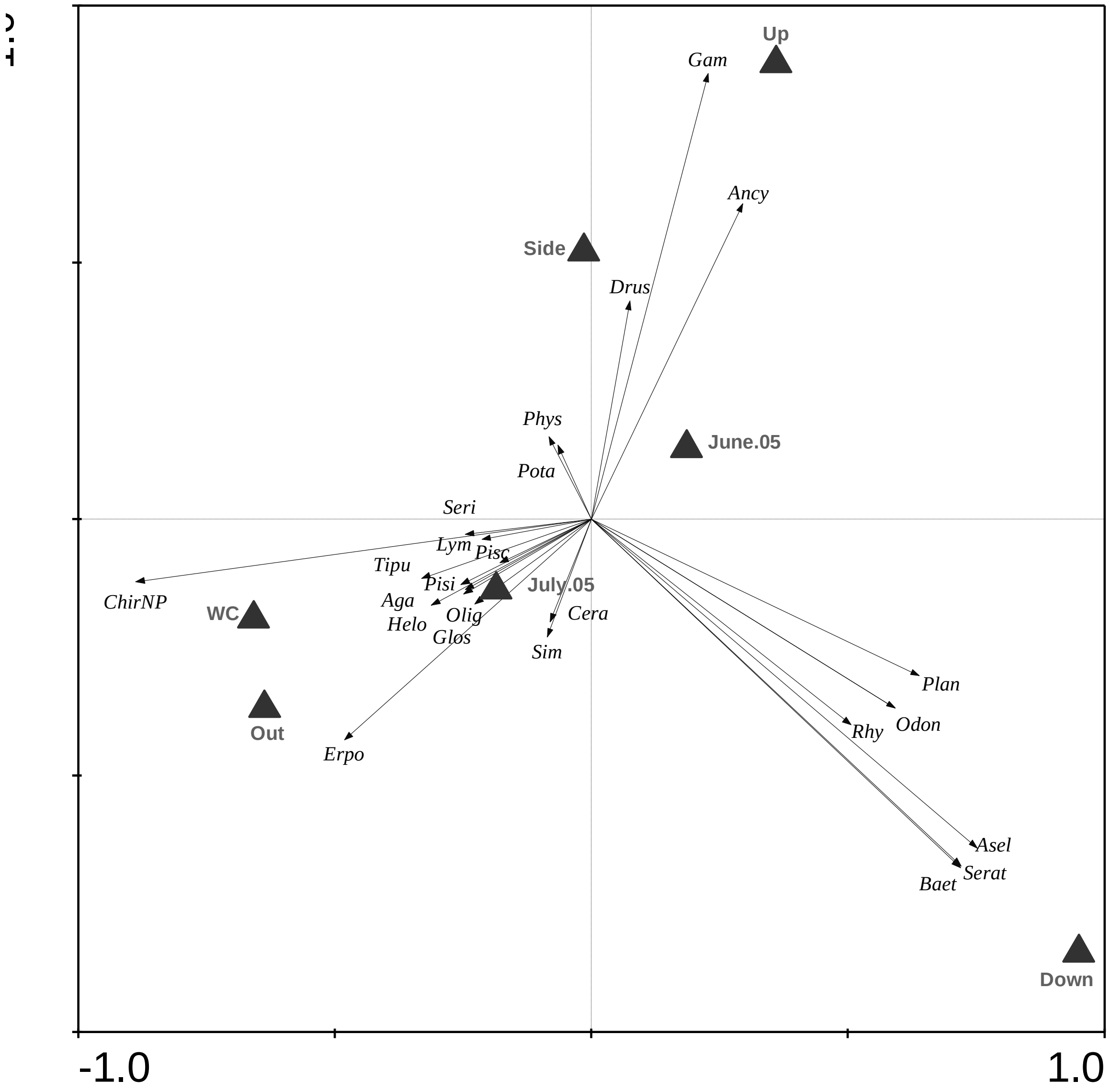


Figure 4.6. Redundancy analysis (RDA) biplot vectors of species scores for macroinvertebrate taxa (six surber samples / site) (light arrows), and month and sample sites (centroids). Site and month coded as dummy variables. St Mary Bourne watercress farm and the Bourne Rivulet over June and July 2005. Arrows display the correlation between species and sample sites and are oriented in the direction of maximum variation in value. Site and month were coded as dummy variables to determine the effect the treatment. Axis I (45.9%) & II (38.1.1%) accounted for 84.0% of the variation in the species data. Abbreviations: (see Fig 4.5 and Appendix 6 for full lists).

## Discussion.

Lotic ecosystem assessment has historically focused on measures of pattern (structure) rather than process (functioning) (Gessner & Chauvet 2002), despite their inclusion in legislative instruments e.g. EU WFD (EC 2003), US ACWA (EPA 2002) and their advocacy by freshwater biologists (e.g. Gessner & Chauvet 2002; Hladyz *et al*., 2011a, b; Woodward *et al.*, 2012). Few studies have included functioning measures and even fewer have examined both (Bonada *et al*., 2006; Friberg *et al.*, 2011) (but see Hladyz *et al*., 2011a, b). Despite their close association (Cardinale, Palmer & Collins 2002), changes in either structure or functioning are not necessarily mirrored by the other and vice versa (Gessner & Chauvet 2002; Palmer & Febria 2012; Woodward *et al.*, 2012). Both measures therefore complement each other and should as a consequence be included in the bioassessment of running waters (Woodward *et al.*, 2012), and this study adds further weight to this finding.

At the start of the study in June 2005 our measures of leaf litter breakdown together with shredding macroinvertebrate abundance, including *G. pulex,* were suppressed downstream of the watercress farms outfall in comparison to the upstream "reference" sites. However, there was no evidence of perturbation with the other measures of ecosystem functioning, primary production or herbivory. Subsequently in July 2005 the multiple measures of ecosystem functioning and structure were unable to detect any evidence of biological recovery, in fact the RDA ordination suggests that the situation worsens. This suggests that the bioremediation of the SMB watercress farm and factory, by re-routing its wastewaters back through the watercress beds to act as a wetland, was unsuccessful, at least over this timescale. The re-routing may need to be in place longer than a month for a change to occur and could be investigated in further studies. It may be possible that PEITC levels may also remain high despite the bioremediation. A viable means of accurately measuring

PEITC levels across the farm, which currently is not available, would have been useful in determining the effectiveness of the bioremediation, rather than attempting to infer this from indirect measures.

The evidence may also suggest that PEITC is not the principal stressor but rather an alternative, longer-term stressor such as chronic organic or toxic pollution is responsible. This is also evidenced by high BOD, nitrite, ammonia, nitrate, phosphate and algal production levels at the outfall and downstream sites relative to the control upstream sites (Table 2.2 pp. 66; Fig. 4.2), together with associated changes in macroinvertebrate abundance *(G. pulex)* and the deterioration between June and July 2005 seen in the RDA (Fig. 4.6). These field experiments are not in complete agreement with lab-based, single species ecotoxicological studies, suggesting that PEITC is the causative agent behind *G. pulex* declines (see Worgan & Tyrell 2006). PEITC has a short residence time of between 30 and 120 minutes in natural aquatic systems, due to its poor solubility in water, volatility and tendency to photodegrade (Ji, Kuo & Morris 2005; Environ 2008). Given this and that the residence time of water in a 100m section of watercress bed is approximately two hours (Cox 2006), it is highly unlikely that the effects of PEITC would be sufficiently profound or persistent on *G. pulex* populations over the larger spatiotemporal scales investigated in this field-based study, especially as *G. pulex* is highly mobile (Friberg & Jacobsen 1994). The abundance of *G. pulex* at the Side and WC sites, which are within areas exposed to high levels of effluents from the watercress beds (Fig. 4.3 & 4.4), lends further weight to this suggestion. Rather, our results suggest that organic pollution and perhaps chlorine use, its treatment and the possible persistence of monochloroamines in the factory process and downstream, could have been the principal stressors.

The suggestion that a number of stressors may be acting together, may account for the difficulties experienced in identifying the agent(s) responsible in previous studies, and provides a new means of devising an effective management-based solution (Marsden 2006; Worgan & Tyrell 2006). This field based case study was able to assess the stressed environment prior to and after bioremediation, while being subjected to a wide range of varying environmental factors, which is not possible under laboratory conditions (Persoone *et al.*, 1989; Adams 2003). That PEITC appeared to be the driver under laboratory conditions (Worgan & Tyrell 2006), but appeared to not be the case in the field, highlights the potential pitfalls of extrapolating from the laboratory to the field (Kimball 1985; Munkittrick & McCarty 1995; Vignati, Ferrari & Dominik 2007).

To summarise, the use of these multiple approaches enabled us to: i) demonstrate the role of these novel integrated measures as a more general tool for examination of ecosystems compromised by anthropogenic stressors ii) decrease the likelihood of PEITC being a principal driver under field conditions, and iii) widen the search for the involvement of other potential stressors at the VSL SMB watercress farm e.g. organic pollution and chlorine use. In the future, biomonitoring programmes will increasingly need to include multiple structural-functional measures if they are to deal with the complex array of both drivers and responses operating in natural ecosystems (Giller *et al.*, 2004; Woodward, Friberg & Hildrew 2009; Friberg *et al.*, 2011 Palmer & Febria 2012; Woodward et al., 2012), this study represents one of the first attempts to do so in running waters.

**References.**

Acreman, M., C., Adams, B., Birchall, P. and Connorton, B., (2000) Does Groundwater Abstraction Cause Degradation of Rivers and Wetlands? *Journal of the Chartered Institution of Water and Environmental Management*, **14**, 200-206.

Adams, S., M., (2003) Establishing causality between environmental stressors and effects on aquatic ecosystems. *Human and Ecological Risk Assessment,* **9**, 17–35.

Agnew, C., T., Clifford, N., J. & Haylett, S., (2000) Identifying and alleviating low flows in regulated rivers: the case of the Rivers Bulbourne and Gade, Hertfordshire, UK. *Regulated Rivers: Research & Management*, **16**, 245–266.

Armitage, P., D., Moss, D., Wright, J., F. & Furse, M., T., (1983) The performance of a new biological water-quality score system based on macroinvertebrates over a wide-range of unpolluted running-water sites. *Water Research*, **17**, 333–347.

Bonada, N., Prat, N., Resh, V., H., and Statzner, B., (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual review of Entomology,* **51**, 495–523.

Breen, P., F. & Seymour, B., S., (1994) The Combination of a Flood-Retarding Basin and a Wetland to Manage the Impact of Urban Runoff. *Water Science Technology*, **29**, 103-109.

Bunn, S., E. & Davies, P., M., (2000) Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia,* **422/423**, 61–70.

Casey, H., (1981) Discharge and chemical-changes in a chalk stream headwater affected by the outflow of a commercial watercress bed. *Environmental Pollution, Series B,* **2,** 373–385.

Casey, H. and Smith, S., M., (1994) The effects of watercress growing on chalk headwater streams in Dorset and Hampshire. *Environmental Pollution,* **85,** 2, 217–228.

Castella, E., Bickerton, M., Armitage, P., D. & Petts, G., E., (1995) The effects of water abstractions on invertebrate communities in UK streams. *Hydrobiologia,* **308**, 167– 182.

Cardinale, B., J., Palmer, M., A. and Collins, S., L., (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature,* **415,** 426-429.

Cox, J., Associates (2006) *Watercress growing and its environmental impacts on chalk rivers in England.* Report by Jonathan Cox Associates. English Nature Contract No. EIT-05-020 p 56.

Downes, J., D., Barmuta, L., A., Fairweather, P., G., Faith, D., P., Keough, M., J., Lake, P., S., Mapstone, B., D. and Quinn, G., P., (2002) Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. University Press, Cambridge

Environ (2008) *Literature review of the behaviour of mustard oils in aquatic ecosystems.* Report for Vitacress Salads Limited by Environ UK. Ltd.

Environment Agency (2004) *The State of England's Chalk Rivers*.

Environment Agency (2010) River Ecosystem (RE) Classification System. Retrieved 20.03.2010. http://www.environment-agency.gov.uk/research/planning/40297.aspx Retrieved 20.03.2010. http://www.environmentagency.gov.uk/static/documents/Research/river_e1.doc Retrieved 28.03.2012. http://www.ashford.gov.uk/pdf/Appendix%203.2%20-%20River%20Ecosystem%20Classification%20System.pdf

EPA (2002) Environmental Protection Agency. American clean water act (EPA ACWA). Available from: Retrieved 28.08.2012. http://epw.senate.gov/water.pdf

European Commission (2003) *The new EU Water Framework Directive*. Available from: Retrieved 28.08.2012. http://ec.europa.eu/environment/water/water-framework/index_en.html

Fewings, S., (1999) *Survey of Operational Practice on Watercress Farms in Hampshire*. Collaborative Project of the Environment Agency, Horticultural Development Council and Watercress Growers Association.

Friberg, N. & Jacobsen, D., (1994) Feeding plasticity of two detritivore-shredders. *Freshwater Biology*, **32**, 133-142.

Friberg, N., Bonada, N., Bradley, D., C., Dunbar, M., J., Edwards, F., K., Grey, J., Hayes, R., B., Hildrew, A., G., Lamouroux, N., Trimmer, M., and Woodward, G., (2011) Biomonitoring of human impacts in freshwater ecosystems: the good, the bad, and the ugly. *Advances in Ecological Research*, **44**, 2-68.

Gessner, M., O. & Chauvet, E., (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications,* **12**, 498–510.

Giller, P., S., Hillebrand, H., Berninger, U., G., Gessner, M., O., Hawkins, S., Inchausti, P. *et al*., (2004) Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos*, **104**, 423–436.

Graca, M., A., S., Cressa, C., Gessner, M., O., Feio, M., J., Callies, K., A. & Barrios, C., (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology,* **46**, 947–957.

Hardman, D., J., McEldowney, S. and Waite, S., (1993) Pollution: Ecology and Biotreatment, Longman Scientific and Technical, London, England.

Harrison, S., S., C. & Harris, I., T., (2002) The effects of bank side management on chalk stream invertebrate communities. *Freshwater Biology,* **47**, 2233-2245.

H.C.C., (1998) Biodiversity Action Plan for Hampshire: Volume One, Hampshire Biodiversity Partnership.

H.C.C. (2000) Biodiversity Action Plan for Hampshire: Volume Two, Hampshire Biodiversity Partnership. Chalk streams. Retrieved 28.08.2012. from http://www.hampshirebiodiversity.org.uk/pdf/PublishedPlans/ChalkStreamsHAPjjDTP2.pdf

Herricks, E., E. & Cairns, J., J., (1982) Biological monitoring. Part III – receiving system methodology based on community structure. *Water Research*, **16**, 141–153.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M., O., Giller, P., S., Gulis, V., Hutton, S., A., Lacoursie`re, J., O., *et al.*, (2011a) Stream ecosystem functioning in an agricultural landscape: The importance of terrestrial–aquatic linkages. *Advances in Ecological Research*, **44**, 211–276.

Hladyz, S., Abjornsson, K., Giller, P., S., and Woodward, G., (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology,* **48**, 443–452.

H.M.S.O., (1995) Biodiversity: *The UK Steering Group Report***,** Volume 1 and 2.

Holdich, D., M, Sibley, P, and Peay, S., (2004) The White-Clawed Crayfish – a decade on. *British Wildlife,* **15**, 153–164.

Holmes, N., T., H., (2006) The importance of long-term data sets in science and river management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **16**, 329 – 333.

Ji, Y., Kuo, Y. & Morris, M., E., (2005) Pharmacokinetics of dietray phenethyl isothiocyanate in rats. *Pharmaceutical Research,* **22**, 1658-1666.

Kadlec, R., H., Knight, R., L., Vymazal, J., Brix, H., Cooper, P. & Haberl, R., (2000) Constructed wetlands for pollution control - processes, performance, design and operation. IWA Scientific and Technical Report No. 8. IWA Publishing, London, UK.

Kimball, K., D. & Levin, S., A., (1985) Limitations of laboratory bioassays: the need for ecosystem level testing. *Ecoscience*, **35**, 165–171.

Mainstone, C., (1999) *Chalk rivers; nature conservation and management*. English Nature and Environment Agency.

Maltby, L., (1994) Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: *Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organisation* (Editors: Sutcliffe, D.W.). Freshwater Biological Association, Ambleside, UK.

Maltby, L., Clayton, S., A., Wood, R., M. & McLoughlin, N., (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. *Environmental Toxicology and Chemistry*, **21**, 361–368.

Mann, R., H., K., Blackburn, J., H. & Beaumont, W., R., C., (1989) The ecology of brown trout *Salmo trutta* in the English chalk streams. *Freshwater Biology*, **21**, 57-7

Marsden, C., D., (2006) *Combining chemistry, bioassay and biotic data to investigate the invertebrate decline in the Bourne Rivulet*. Report for Vitacress Salads Ltd by the Centre for Ecology and Hydrology (CEH), Dorset.

Martin, D., (2007) Environmental Agency Report: *A review of water quality in the upper reaches of the Bourne Rivulet between November 2004 and December 2006.*

Medgett, S., (1998) Environmental Agency Report: *The impact of St. Mary Bourne Cress Farm on the Bourne Rivulet.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Neal, C., Whitehead, P., G., (2002) Nutrient studies within the Thames Basin: biological and sediment-water interactions and the effects of point source reductions on water quality functioning. *Science of the Total Environment Total Environ*, 282/283, 3-7.

Newman, R., M., (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. *Journal of the North American Benthological Society,* **10**, 89–114.

Newman, R., Hanscom, Z. and Kerfoot, C., W., (1992) The watercress glucosinate-myrosinase system: a feeding deterrent to caddisflies, snails and amphipods. *Oecologia,* **92**, 1-7.

Newman, R., N., Kerfoot, W., C. and Hanscom, Z., (1990) Potential chemical defence in a spring stream macrophyte. *Journal of Chemical Ecology*, **16**, 245-259.

Newman, R., N., Kerfoot, W., C. and Hanscom, Z., (1996) Watercress allelochemcial defends high nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology*, **77,** 2312-2323.

Palmer, M., A. & Febria, C., M., (2012) The Heartbeat of Ecosystems, *Science.* **336**, 1393 - 1394.

Persoone, G., Van de Vel, A., Van Steertegem, M.. and De Nayer, B., (1989) Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions. *Aquatic Toxicology,* **14,** 149–166.

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P., & Dobson, M. (2010) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology*, **55**, 346–359.

Sandin, L. & Solimini, A., G., (2009) Freshwater ecosystem structure-function relationships: from theory to application. *Freshwater Biology*, **54**, 2017–2024.

Scott, A. and Beaumont, W., R., C., (1994) Improving the survival rates of Atlantic salmon (*Salmo salar* L.) embryos in a chalk stream. *Institute of Fisheries Management Annual Study Course 1993 Cardiff.* Nottingham: Institute of Fisheries Management, 20 pp.

ter Braak, C., J., F. & Prentice, I., C., (1988) A theory of gradient analysis. *Advances in Ecological Research*, **18**, 271-313.

Underwood, A., J., (1994) On beyond baci – sampling designs that might reliably detect environmental disturbances. *Ecological Applications,* **4**, 3–15.

Vignati, D., A., L., Ferrari, B., J., D. and Dominik, J., (2007) Laboratory-to-field extrapolation in aquatic sciences. *Environmental Science and Technology*, **15**, 1067-1073.

Vymazal J., (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological Engineering*, **25**, 478–90.

Vymazal, J., (2006) Removal of nutrients in various types of constructed wetlands. *Science of The Total Environment*, **380**, 48-65.

White, S. & Medgett, S., (2006) Environmental Agency Report: *Bourne Rivulet Invertebrate Survey.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Whitehead, P., G., Wilby, R., L., Butterfield, D. and Wade, A., J., (2006) Impacts of climate change on in-stream nitrogen in a lowland chalk stream: an appraisal of adaptation strategies, *Science of the Total Environment*, **365,** 260 –273.

Worgan, A., & Tyrell, R., (2006) *Monitoring behavioural responses of Gammarus pulex to watercress oils.* Report for Vitacress Salads Ltd by the Centre of Ecology and Hydrology, (CEH), Dorset.

Woodward, G., Gessner, M., O., Giller, P., S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B., G., Tiegs, S., D., Cariss, H., Dobson, M., Elosegi, A. ,Ferreira, V., Graça, M., A.,,S., Fleituch, T., Lacoursiere, J., O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L., B., M. and Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science*, **336**, 1438-1440.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R., B., (2008) Trophic trickles and cascades in complex food web: impacts of keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Woodward, G., Friberg, N. & Hildrew, A., G., (2009) Science and non-science in the biomonitoring and conservation of fresh waters. Chapter in *Freshwater Ecosystems: Biodiversity, Management and Conservation*. Nova Science Publishers, Inc., New York, U.S.A.

Wright, J., F., (1992) Spatial and temporal occurrence of invertebrates in a chalk stream, Berkshire, England. *Hydrobiologia*, **248**, 11–30.

Wright, J., F. & Berrie, A., D., (1987) Ecological effects of groundwater pumping and a natural drought on the upper reaches of a chalk stream. Regulated Rivers: Research & Management **1**, 145–160.

Wright, J., F., Armitage, P., D., Furse, M., T., Moss, D., (1984) The classification of sites on British rivers using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie,* **22**, 1939–1943.

# Chapter 5.

## *Stream ecosystem functioning and structure metrics to assess a surrogate wetlands bioremediation of complex discharges at Europe's largest watercress farm.*

### Introduction.

The EU WFD (European Commission 2003) is widely seen as the most radical and progressive environmental protection legislation in the world (e.g. Gessner & Chauvet 2002; Young, Matthaei & Townsend 2008; Hopkins, Marcarelli & Bechtold 2011; Hladyz *et al*., 2011a, b). The EU member states must achieve 'good ecological status' for all their fresh waters by 2015 or they may incur substantial financial penalties (Friberg *et al.,* 2011). The WFD requires the bioassessment of both the structure and functioning of freshwater ecosystems; however most of the current methodologies predominantly focus on structure. Both are interrelated (Cardinale, Palmer & Collins 2002) but are not identical (Sandin & Solimini 2009) and need to be assessed simultaneously (Bonada *et al*., 2006), to fully characterise the impact of stressors (Gessner & Chauvet 2002). We sought to address this by integrating measures of both ecosystem structure and functioning, in a further case study at the largest watercress farm in Europe.

The earlier study in Chapter 4 demonstrated that our measures of ecosystem functioning and community structure were able to detect reduced leaf litter breakdown and reduced *G. pulex* abundance below the VSL SMB watercress farm compared to upstream. However, the subsequent farm management change (re-routing) implemented and then monitored one month later in our study were unable to detect any change to these same measures. This suggests that wastewater re-routing to bioremediate PEITC was unsuccessful over this timescale, and that other more persistent stressors might be responsible, with chronic organic and toxic pollution being prime candidates.

In this next study, we explored further the effects of watercress farming upon our measures of ecosystem functioning (leaf litter breakdown, algal accrual) and structure (leaf litter and benthic macroinvertebrate abundances), at SMB watercress farm, and the efforts made to resolve them. An initial pre-remediation dataset was taken in June 2005. In July 2005 the SMB watercress beds were used to act as a mini wetland (after Vymazal 2005), to bioremediate the possible effects of PEITC (Fewings 1999) by a simple re-routing of the settlement tank effluent through the farm watercress beds (S. Rothwell pers.comm.) (sensu Vymazal 2006). The re-routing continued unabated for two years and we sampled again in June 2006 and June 2007. Thus similar time periods and month of year were compared with one another. The internal dynamics of the food web, which may be responsible for a time lag between chemical and biological recovery, as has been observed for other stressors e.g. acidification (Hildrew 2009; Layer *et al*., 2010a & b) were thus given longer to act. A further major change followed the June 2006 sampling, one month later in July 2006, when chlorine use and its treatment ceased at SMB watercress farm. The impact that these farm management changes had upon our measures of ecosystem functioning (leaf litter breakdown, algal accrual) and structure (leaf litter and benthic macroinvertebrate abundances), were investigated 12 months later in the June 2007 sampling.

The samples taken prior to farm management changes (June 2005) and subsequently after further farm management changes (June 2006 & June 2007) enabled a comparison with which to test our general hypothesis that farm management changes designed to remediate the effects of PEITC at SMB would be successful. The experimental hypotheses we tested were:

1) The upstream “control” site to be unaltered by the presence of the farm in both 2006 and 2007. The outfall site should show the strongest responses to the watercress farm, with evidence of at least partial recovery downstream.
2) Post manipulation in 2006 and 2007, if the farm management changes had been successful, the response of the outfall and downstream sites should resemble those of the upstream site.
3) If the manipulation was successful in 2006, there may have been a lagged recovery from PEITC the perceived stressor.
4) If the manipulation was not successful in 2006, but it is in 2007, it suggests that some other stressor(s) may be responsible rather than PEITC.

## Methods.

Refer to Chapter 2 & 3 – Sites & General Methods for a detailed description.

Three study sites “Up”, “Out” and “Down” were selected from the five sites originally surveyed intensively in Chapter 4. This simplified the experimental design by omitting the “Side” and “WC” sites and data, which were replicate spatial controls. These sites were based on their hydrological connectivity to the SMB watercress farm and to fulfil the experimental requirements for our BACI design.

The June 2005 data provided pre-manipulation baseline data, prior to the re-routing of the settlement tank effluent back through the watercress beds. Subsequent, post-manipulation data, one year later during June 2006 and two years later during June 2007, were able to test for the effects of the re-routing of the settlement tank wastewaters and the cessation of chlorine use respectively, This also accounted for potential background between-year differences among and within sites i.e. site x time interactions.

## Results.

### *Physico-chemical parameters.*

EQS levels were not exceeded upstream for any parameter except ammonia, which exceeded its EQS in each year. The nitrite and ammonia levels were also exceeded at all other sites and phosphate levels were elevated at the outfall and downstream sites relative to the upstream site, in each year. BOD levels were also high at 1.84, 4 & 2.2 in 2005, 2006 & 2007 respectively. Downstream nitrite and ammonia EQS's were exceeded in every year, but phosphate only in 2007 (Table 2.2, 2.3 & 2.4 pp.66, 67 & 68).

### *Functional measures: a) leaf litter decomposition rates.*

Litter decomposition rates differed significantly among sites, being elevated at the outfall and downstream relative to the upstream reference section (LMEM $F\ df_{N2}, df_{D30.528} = 18.413$, $P < 0.001$). They also changed over time, with a general decline over the three years of the study (LMEM $F\ df_{N2}, df_{D60.142} = 25.388$, $P < 0.001$) (Table 5.1; Fig. 5.1). Rates in coarse bags were considerably faster than in fine bags, highlighting the overriding influence of shredding macroinvertebrates as the main agents of decomposition (LMEM $F\ df_{N1}, df_{D30.566} = 27.507$, $P < 0.001$) (Table 5.1). These effects varied among sites and over time, however, as revealed by the significant two-way interactions for site × mesh type (LMEM $F\ df_{N2}, df_{D30.528} = 6.934$, $P = 0.003$) and mesh type × time (LMEM $F\ df_{N2}, df_{D60.142} = 4.594$, $P = 0.014$): essentially, microbial leaf-litter breakdown rates decreased in June 2007 compared to June 2005 and 2006, with a concomitant compensatory increase in shredder leaf-litter breakdown rates giving rise to similar overall decomposition rates. This revealed that as the agents of decomposition altered in June 2007 leaf-litter decomposition recovered. This suggests that the surrogate wetland (June 2006) did not improve this aspect of ecosystem functioning, and that something else could be suppressing recovery. Chlorine use was a possible choice as it ceased to be used in July 2006, after which recovery was evident in the June 2007 dataset. However, we can not be sure of this as chlorine was not specifically measured. A significant site × time (LMEM $F\ df_{\mathrm{N}4},\ df_{\mathrm{D}60.097} = 4.031$, $P = 0.006$) interaction, revealed rates at the downstream site that were relatively consistent over the three years, whereas there was a general decrease over time at the upstream and outfall sites. There was no significant three-way interaction, however, which suggested that full recovery of shredder activity was not attained at the outfall or downstream, even after two years of bioremediation. However the separation of both components (macroinvertebrate vs. microbial) (Fig. 5.1) may represent the beginning of a recovery of the macroinvertebrate contribution.

Table 5.1 LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per degree-day ($k_{dd}$) at sites Up, Out, Down, and in June 05, June 06 and June 07. Significant results at $P < 0.05$ are highlighted in bold.

| Comparison | d.f.$_{N}$ | d.f.$_{D}$ | $F$ – ratio | $P$ |
|---|---|---|---|---|
| Site | 2 | 30.528 | 18.413 | <**0.001** |
| Mesh | 1 | 30.566 | 27.507 | <**0.001** |
| Time | 2 | 60.142 | 25.388 | <**0.001** |
| Site x Mesh | 2 | 30.528 | 6.934 | **0.003** |
| Site x Time | 4 | 60.097 | 4.031 | **0.006** |
| Mesh x Time | 2 | 60.142 | 4.594 | **0.014** |
| Site x Mesh x Time | 4 | 60.097 | 1.050 | 0.389 |

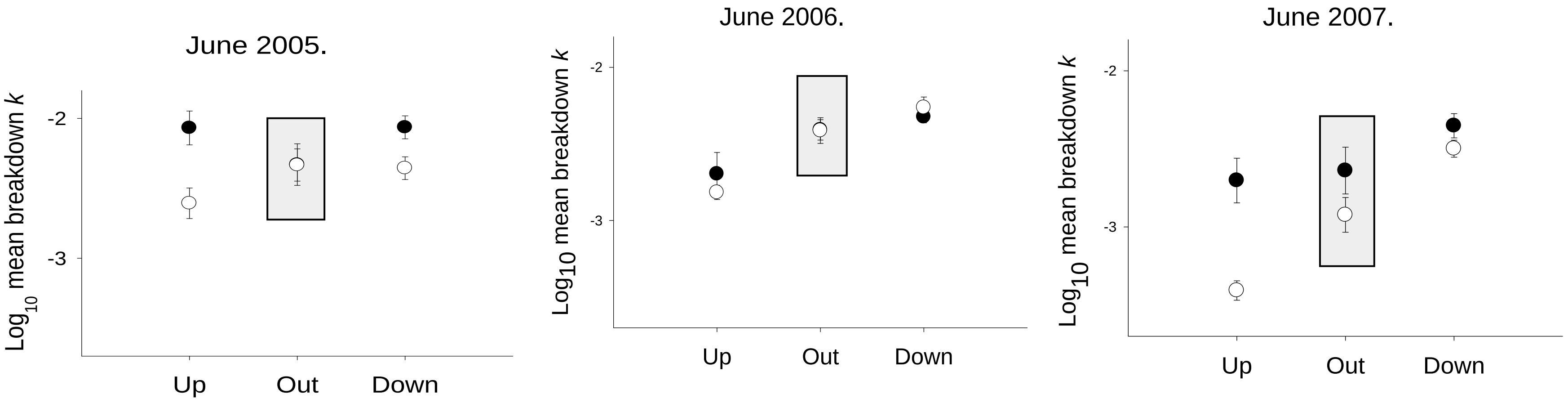


Figure 5.1. $Log_{10}$ mean leaf-litter decomposition rates per degree-day ($k$ ± SE). ($k_{dd}$) in June 2005, 2006 & 2007. Sites: Up, Out, Down. White circles denote Microbial breakdown (fine mesh bags). Black circles denote Total breakdown (coarse mesh bags). Shaded area denotes microbial dominance of breakdown at the outfall in 2005 and 2006, while 2007 demonstrates a separation of both components and the potential start of a recovery of the macroinvertebrate contribution.

### *Functional measures b) algal production and herbivory.*

Algal accrual rates differed significantly in response to all the main effects of Site, Time and Herbivory treatments, with the “Upstream” site having lowered algal production than the impacted “Outfall” and “Downstream” sites (Table 5.2). Marked top-down effects of grazers were evident across sites (LMEM $F\ df_{N1}, df_{D30.201} = 60.463$, $P < 0.001$), as was inter-annual variation (LMEM $F\ df_{N2}, df_{D59.837} = 7.221$, $P = 0.002$). There was also a significant time × site interaction (LMEM $F\ df_{N4}, df_{D59.711} = 5.016$, $P = 0.001$), but the lack of a three-way interaction revealed that grazer effects were consistent over both time and sites and thus did not change in response to bioremediation (Table 5.2, Fig. 5.2).

Table 5.2 LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a (*mg cm-2) per degree-day (*dd*) June 2005, 2006 and 2007. Sites: Up, Out, Down. Herbivory = V / NV. V = exclusion of grazers through use of Vaseline ‘fence’ and NV = non exclusion. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 2 | 30.084 | 21.157 | **<0.001** |
| Herbivory | 1 | 30.201 | 60.463 | **<0.001** |
| Time | 2 | 59.837 | 7.221 | **0.002** |
| Site x Herbivory | 2 | 30.084 | 0.850 | 0.437 |
| Site x Time | 4 | 59.711 | 5.016 | **0.001** |
| Herbivory x Time | 2 | 59.837 | 0.978 | 0.382 |
| Site x Herbivory x Time | 4 | 59.711 | 0.490 | 0.743 |

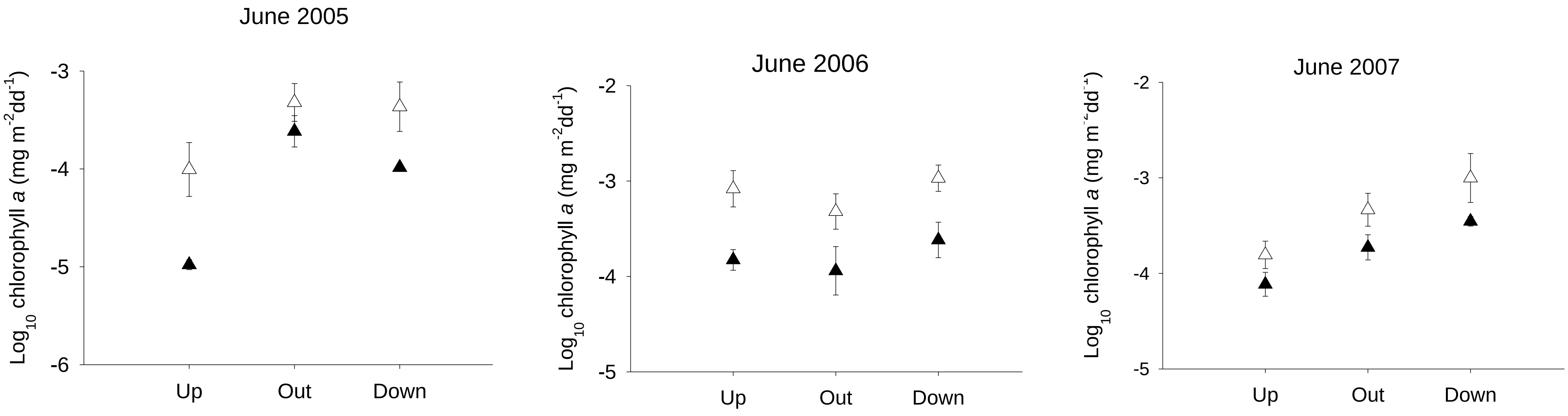


Figure 5.2. Log$_{10}$ chlorophyll *a* production *(*mg cm$^{-2}$) per degree-day (*dd*) ± SE by site before manipulation in June 2005, 2006 and 2007. Sites: Up, Out, Down. White triangles denote No Grazing (exclusion of grazers through use of Vaseline 'fence') black triangles denote Grazing (no exclusion of grazers).

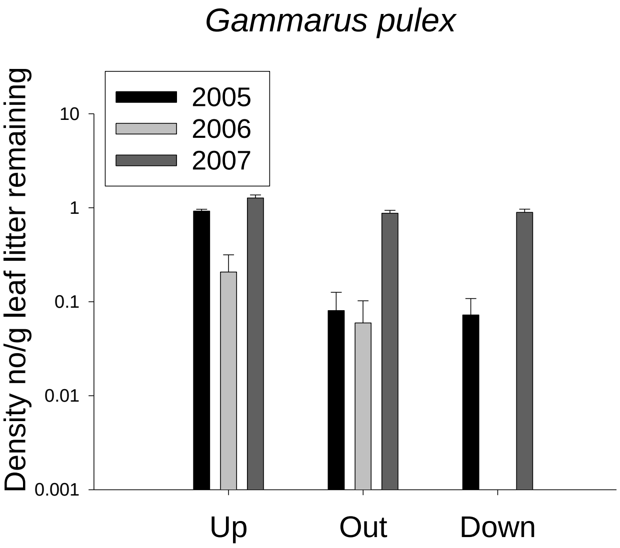
### *Structural measures: macroinvertebrates abundances in leaf litter bags and benthos.*

*G. pulex* and *A. aquaticus* were the most abundant shredding macroinvertebrates in the benthos and litter bags and therefore the principal focus of my analyses in this Chapter. *S. personatum* were only found in the benthos at the Up site in 2006.

The abundance of *G. pulex* in the benthos and in litter bags differed significantly over time (LMEM, benthos: $F\ df_{N2}, df_{D15} = 295.449$, $P < 0.001$; LMEM, bags: $F\ df_{N2}, df_{D29.575} = 138.868$, $P < 0.001$) and sites (LMEM, benthos: $F\ df_{N2}, df_{D15} = 16.707$, $P < 0.001$; LMEM, bags: $F\ df_{N2}, df_{D14.690} = 64.856$, $P < 0.001$). Significant time × site interaction terms revealed that *G. pulex* abundance was suppressed at the outfall & downstream sites in 2005 and 2006, but not in 2007 (LMEM, benthos: $F\ df_{N4}, df_{D30} = 39.652$, $P < 0.001$; LMEM, bags: $F\ df_{N4}, df_{D29.551} = 7.419$, $P < 0.001$) (Table 5.3 & 5.4; Fig 5.3 & 5.4). *G. pulex* were absent in the benthos and litter bags at the downstream site in 2006. *A. aquaticus* were rarest at the upstream site and at the outfall but were the dominant shredder downstream, in 2005 and 2006. However, in 2007 their abundance reduced and *G. pulex* recovered to become the dominant shredder downstream (LMEM, benthos: $F\ df_{N4}, df_{D30} = 6.747$, $P = 0.001$; LMEM, bags: $F\ df_{N4}, df_{D30.018} = 11.863$, $P < 0.001$) (Table 5.3 & 5.4; Fig 5.3 & 5.4).

Table 5.3 LMEM results of comparisons of shredder coarse litter-bags abundance June 2005, 2006 & 2007 Sites: Up, Out, Down ($\log_{10}$ mean number per g leaf litter remaining) for *G. pulex* (G), *A. aquaticus* (A) and Total shredders (T). Significant results at $P < 0.05$ are highlighted in bold.

| Comparison | | d.f.$_N$ | d.f.$_D$ | *F* – ratio | ***P*** |
|---|---|---|---|---|---|
| Site | G | 2 | 14.690 | 64.856 | **<0.001** |
| | A | 2 | 15.298 | 77.132 | **<0.001** |
| | T | 2 | 14.754 | 54.653 | **<0.001** |
| Time | G | 2 | 29.575 | 138.868 | **<0.001** |
| | A | 2 | 30.041 | 13.169 | **<0.001** |
| | T | 2 | 29.893 | 33.982 | **<0.001** |
| Site x Time | G | 4 | 29.551 | 7.419 | **<0.001** |
| | A | 4 | 30.018 | 11.863 | **<0.001** |
| | T | 4 | 29.872 | 9.495 | **<0.001** |



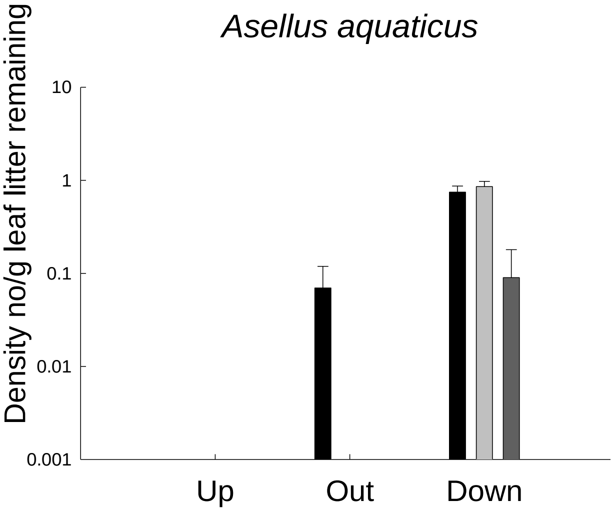
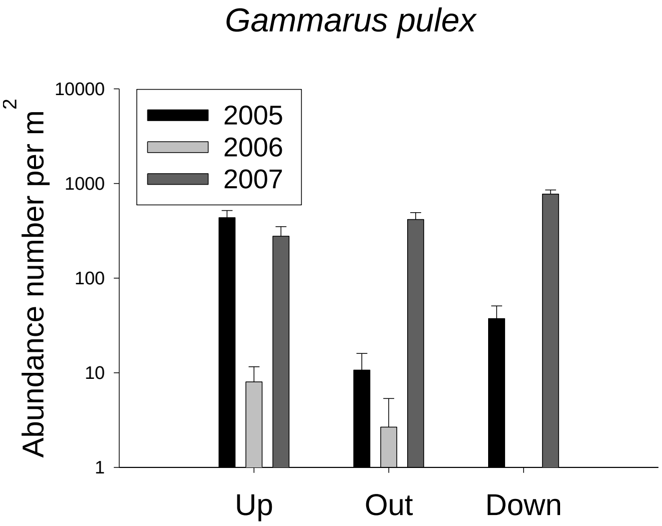


Figure 5.3. Shredder coarse litter-bag abundance June 2005, 2006 and 2007. Sites: Up, Out, Down ($\log_{10}$ mean number per g leaf litter remaining). *G. pulex* (G), *A. aquaticus* (A) Significant results at *P* <0.05 are highlighted in bold.

Table 5.4 LMEM results of comparisons of benthic shredder abundance (nos. $m^{-2}$) June 2005, 2006 & 2007 Sites: Up, Out, Down. *G. pulex* (G), *A. aquaticus* (A) and Total shredders (T). Significant results at $P < 0.05$ are highlighted in bold.

| Comparison | | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Site | G | 2 | 15 | 16.707 | **<0.001** |
| | A | 2 | 15 | 36.547 | **<0.001** |
| | T | 2 | 15 | 24.012 | **<0.001** |
| Time | G | 2 | 30 | 295.449 | **<0.001** |
| | A | 2 | 30 | 4.779 | **0.016** |
| | T | 2 | 30 | 126.223 | **<0.001** |
| Site x Time | G | 4 | 30 | 39.652 | **<0.001** |
| | A | 4 | 30 | 6.747 | **0.001** |
| | T | 4 | 30 | 20.459 | **<0.001** |



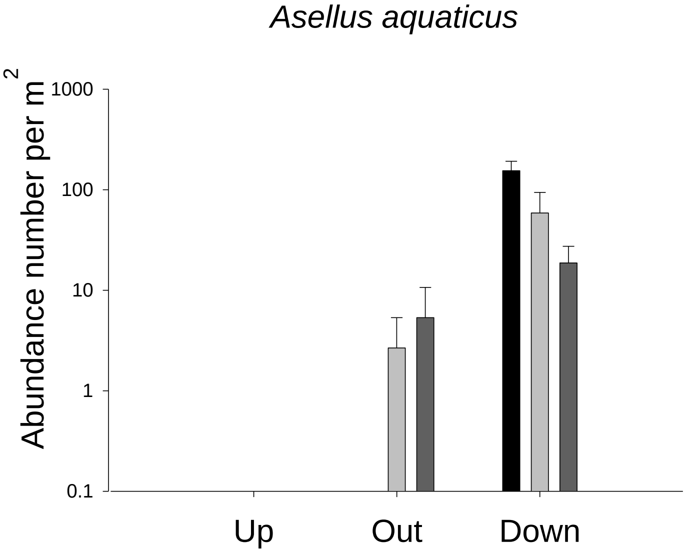


Figure 5.4. Shredder benthic abundance June, 2005, 2006 and 2007. Sites: Up, Out, Down (nos. $m^{-2}$). *G. pulex* = *G, A. aquaticus* = A Significant results at *P* <0.05 are highlighted in bold.

### ***Ordination of environmental data with functional and community-based structural measures.***

A PCA (Fig.5.5) revealed that declines in the relative abundance of *G. pulex* and the coarse to fine leaf litter bag breakdown ratio (C/F) were associated with axis 1, which separated the ‘‘impacted’’ Out site in 2005 and 2006 from the three 2007 sites (Fig. 5.5 shaded area) and accounted for 61.6% of the variation in the community data. Axis 1 was correlated with a suite of chemical variables associated with organic pollution (i.e., dissolved oxygen ($r$ = 0.88), nitrite ($r$ = 0.50), ammonia ($r$ = 0.48) and BOD ($r$ = 0.44). This suggests that the abundance and activity of shredding macroinvertebrates are suppressed, in particular *G. pulex*, in response to organic pollution at SMB. Axis 2 was positively associated with high algal production and herbivory, the 2005 and 2006 downstream sites and the absolute amount of chlorophyll *a* “consumed” was highest in the downstream recovery reach, where the dominant grazers (*Baetis* spp., *Planorbis leucostoma* (Millet) and *Seratella ignita* (Poda)) were most abundant. Both axes 1 & 2 together accounted for 93.8% of the variation in the community data.

If there had been a positive response to the farms bioremediation, the 2006 outfall site results would have been associated with the downstream (recovery) sites or the upstream (unimpacted) sites at the top and bottom left of the ordination plot, respectively. The results, however, suggest no change, as the 2006 results remained closely associated with the pre-remediation 2005 outfall site. In contrast, the 2007 outfall site results are associated with the upstream (unimpacted) sites at the bottom left of the ordination plot. In fact all the 2007 sites are closely associated (see shaded area) suggesting recovery not only at the impacted site but also downstream. An RDA (Fig. 5.6.) with site and year coded as dummy variables was tested with Monte Carlo permutations and forward selection. This revealed that the downstream recovery site was significantly different from the upstream and outfall sites (*F* = 3.323, *P* = 0.0320, Monte-Carlo n = 499) and that 2007 was significantly different from 2005 and 2006 (*F* = 3.971, *P* = 0.0180, Monte-Carlo n = 499), this may be due to the farm management practices changing and may suggest that the 2005 and 2006 changes (re-routing) had not been successful, but that further changes, possibly chlorine use cessation at SMB was the reason for the improvement evident in 2007. The RDA and the PCA appeared to be detecting very similar patterns within these data, suggesting that there may be no important ecosystem drivers left undetected. Axis I and axis 2 accounted for 49.4% and 23.4% of the respective variation in the species data and both axes accounted for 72.8% of the variation, suggesting that most of the variation was attributable to these two axes.

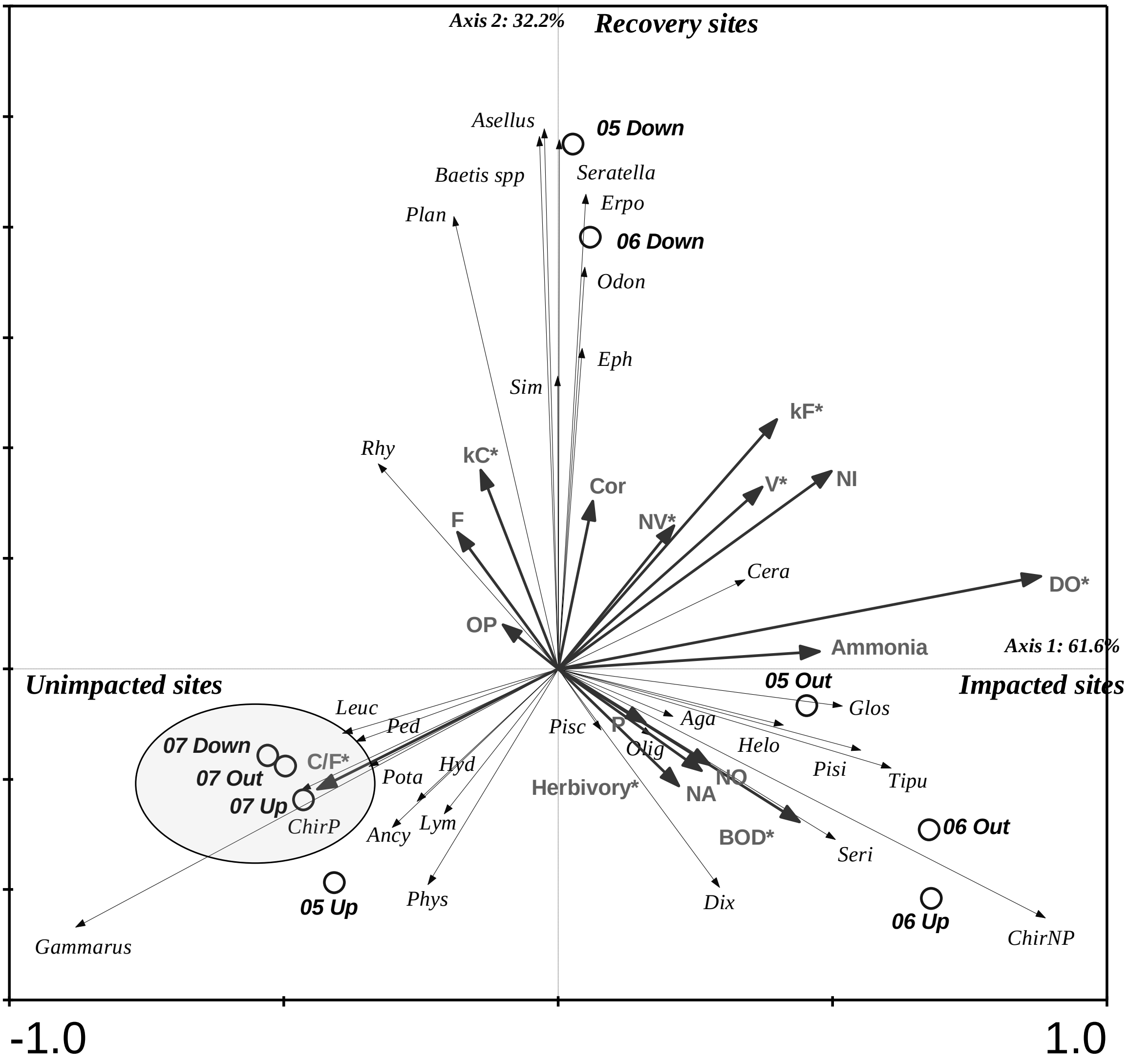


Figure 5.5. Principal components analysis (PCA) triplot of species scores vectors for macroinvertebrate taxa (six surber samples / site) (light arrows) environmental variables (dark arrows) with sites shown as centroids (open circles) recorded at St Mary Bourne watercress farm and the Bourne Rivulet in June 2005, 2006 and 2007. Arrows display the correlation between species and the environmental variables and are oriented in the direction of maximum variation in value. Axis I (61.6%) & II (32.2%) accounted for 93.8% of the variation in the species data. (Sites within the shaded ellipse highlight the increased homogenisation of community composition during June 2007).

Abbreviations.
i) Sites:
Up = Upstream, Out= Outfall, Down = Downstream. (e.g. 05 Up = June 2005 Up; 06 Out = June 2006 Out; 07 Down = June 2007 Down).

ii) Species codes:
Aga (*Agapetus fuscipes*); Ancy (*Ancylus fluviatilis*); Asel (*Asellus aquaticus* ); Baet (*Baetis rhodani* ); Cera (Ceratopogonidae); ChirNP (Chironomidae Non-predatory);

ChirP (Chironomidae Tanypodinae);Chlor (*Chloroperla torrentium*); Crun (*Crunoecia irrorata*); Dix (*Dixella spp*); Drus (*Drusus annulatus*); Ecd (*Ecdyonurus venosus*); Elm (*Elmis aeanea*); Eph (*Ephemera danica*); *Eph* (Ephydridae); Erpo (*Erpobdella octoculata*); Gam (*Gammarus pulex* ); Glos (*Glossiphonia complanata*); Hale (*Halesus radiatus* ); Helo (*Helobdella stagnalis* ); Hyd *Hydroptilla* spp); HydS (*Hydropsyche siltalai*); HydP (*Hydropsyche pellucidula*); Lasi (*Lasiocephala basalis*); Leuc (*Leuctra innernis*); Limn (*Limnephilus* spp); Lym (*Lymnaea peregra*); Myst (*Mystacides azurea*); Nem (*Nemurella pictetii*); Odon (*Odontocerum albicorne*); Olig (Oligochaeta); Ped (*Pedicia rivosa*); Pisc (*Piscicola geometra*); Phys (*Physa fontinalis*); Pisi (*Pisidium* spp); Plan (*Planorbis leucostoma*); Poly (*Polycentropus flavomaculatus*); Pota (*Potamophylax cingulatus*); Potam (*Potamopyrgus antipodarum*); Rhy (*Rhyacophila dorsalis*); Serat (*Seratella ignita*_); Seri (*Sericostoma personatum*); Silo (*Silo nigricornis*); Sim (*Simulium* spp); Tin (*Tinodes waeneri*); Tipu (*Tipula* spp); Valv (*Valvata cristata*).

iii) Environmental variables:
Alky (Alkalinity); Ammonia; Chloride; COD (Chemical Oxygen Demand); Cond (Conductivity); Col (Colour); COrg (Organic Carbon); DO (Dissolved Oxygen); F (Fluoride); NOxid (Oxidised nitrogen); Nitrate; Nitrite; OP (Orthophosphate); SiO2; SS (Suspended Solids); P (Phosphate); Turb (Turbidity).

iv) Functional measures (denoted*):
BOD* (Biological Oxygen Demand); kC* (Decomposition in coarse litter bags per degree-day); kF* (Decomposition in fine litter bags per degree-day); C/F* (Ratio kC/kF); NV* Chlorophyl *a* on Non Vaseline tile); V* (Chlorophyl *a* on Vaseline tile); Herbivory* V/NV (Ratio V/NV (See Methods for details).

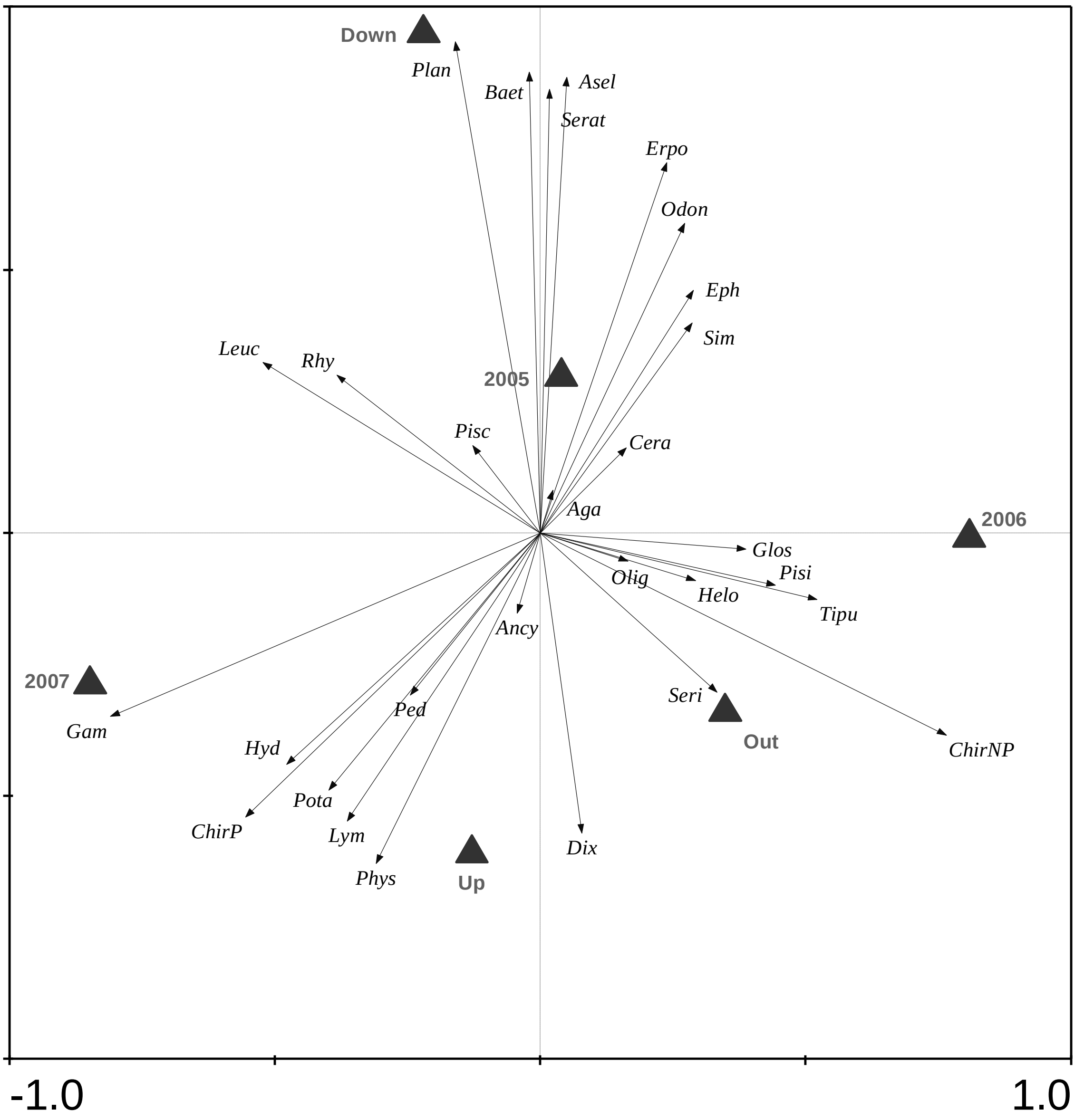


Figure 5.6. Redundancy analysis (RDA) biplot vectors of species scores for macroinvertebrate taxa (six surber samples / site) (light arrows and text) and year and sample sites (centroids). Site and year coded as dummy variables. (See Appendix 4 Fig. 1 for environmental variable plot). St Mary Bourne watercress farm and the Bourne Rivulet in June 2005, 2006 and 2007. Arrows display the correlation between species and centroids and are oriented in the direction of maximum variation in value. Site and year were coded as dummy variables. Axis I (49.4%) & II (23.4%) accounted for 72.8% of the variation in the species data. Abbreviations: (see Appendix 6 for full lists).

***Summary of results.***

There was clear evidence of a recovery at the outfall by the *G. pulex* abundance measures, evident as *G. pulex* suppression at the outfall during 2005 and 2006, but with a recovery in their abundance in 2007. The leaf litter breakdown measures of macroinvertebrate-mediated (coarse mesh) relative to microbially-mediated (fine mesh) decomposition rates, indicated the possibility of the commencement of a recovery, evident as separation of the two factions in 2007, but not in 2005 and 2006. However, upstream abundance in 2006 was suppressed compared to 2005, and comparable to the outfall *G. pulex* abundance in 2006, but both the upstream and outfall recovered in 2007. *G. pulex* abundance reduced to zero downstream in 2006 compared to the outfall for both leaf-litter and benthic abundance, possibly reflecting additional stress from the surrounding farm land e.g. diffuse pollution or simply reflecting a bad year for these macroinvertebrates. The EA has reported that 2006 was an unusually dry year and that flows were low at SMB, which can be reflected in deterioration in biological quality (White & Medgett 2006), which supports this. Overall decomposition rates were elevated at the outfall and the downstream sites, but revealed no evidence of recovery over time. A PCA and RDA revealed no change between 2005 and 2006 perhaps suggesting no effect of bioremediation (re-routing) after one year. However, 2007 was improved suggesting that this may be due to a reduction in organic pollution, but this was not supported by the physico-chemical parameters (BOD, nitrite, ammonia, phosphate & nitrite) which had remained above their Environmental Quality Standard levels (EQS). It may be that chlorine cessation and the toxic mix of chemicals e.g. chloramines, that may have formed in the settlement tank prior to its release at the outfall, were now no longer being produced and this was the reason for the improvement. This can only be inferred from our experiments as neither PEITC nor chlorine and its derivatives were specifically measured.

## Discussion.

Structural measures have dominated many stream bioassessment and monitoring schemes for decades, based on the differential sensitivities of a range of taxa to environmental gradients (Rosenberg & Resh 1993; Wright, Sutcliffe & Furse 2000; Woodward, Friberg & Hildrew 2009). Bioassessment and biomonitoring have evolved considerably over the past few decades, from a reliance on simple presence-absence scores of indicator species or diversity indices to more sophisticated multivariate analyses of community-level data (Herricks & Cairns 1982; Armitage *et al*., 1983; Wright *et al*., 1984; Bonada *et al*., 2006). Although there is a growing emphasis on the need to include measures of ecosystem functioning, such approaches are still relatively rare (Giller *et al*., 2004; Woodward 2009). However, they are becoming increasingly popular and have been advocated as providing potentially important insights into anthropogenic impacts on fresh waters (e.g. Riipinen *et al*., 2009; Hladyz *et al*., 2010).

Community structure and ecosystem functioning are closely entwined (Bunn & Davies 2000; Cardinale, Palmer & Collins 2002), but despite this interconnection, responses to stressors may be manifested as changes in structure only, in functioning only, or a combination of the two (Gessner & Chauvet 2002; Palmer & Febria 2012; Woodward *et al*., 2012). Consequently, if either structural or functional measures are investigated in isolation, potential complementary or synergistic effects could be overlooked; this is a potential shortcoming of many freshwater biomonitoring and bioassessment programmes, which almost never consider both approaches simultaneously (Bonada *et al*., 2006; Friberg *et al.,* 2011).

An amalgam of allochthonous and autochthonous energy inputs fuel the base of freshwater food webs and, ultimately, drive ecosystem functioning (Woodward 2009). Most studies, however, have focused only on a single ecosystem process (e.g. litter decomposition in streams) and although both pathways of energy flux are susceptible to environmental stressors, they may act in different ways, and at different rates (Woodward 2009). In running waters, autochthonous pathways are typically dominated by algal primary production (macrophytes generally entering the food web as detritus) and its consumption by grazers (Bernhardt & Likens 2004), whereas allochthonous detritus is processed by an array of bacteria, aquatic hyphomycetes, and macroinvertebrate shredders (Hieber & Gessner 2002). Consequently, when assessing overall ecosystem functioning, multiple process rates need to be quantified simultaneously and, ideally, in tandem with measures of community structure. This study highlighted this, by revealing that watercress farming had strong impacts on detrital processing via a key shredder species *G. pulex*, whereas microbially-mediated breakdown rates and algal production were elevated relative to the reference site. The functional and structural measures that were employed, although successfully registering significant impacts, differed in their detection of biological recovery following altered management practices designed to alleviate stressor impacts. The increase in *G. pulex* abundance at the previously most heavily impacted sites in 2007, represent the first evidence of recovery since negative impacts on the macroinvertebrate assemblage were initially recorded by the EA over a decade earlier (Medgett 1998). However, despite the recovery of this dominant shredder, there was no concomitant significant change in litter breakdown rates, suggesting that its functional role had still not been fully restored and that the system may not have completely recovered from stress.

Stressors can alter the proportions of algae that are palatable to grazing macroinvertebrates, and in response they may switch partly or totally between algal and detrital food resources (Chessman *et al.*, 2009). *G. pulex* may be utilising alternative food resources such as algae rather than leaf litter, which may be energetically preferable and more readily available. *G. pulex* is a generalist omnivore and it's `plasticity ' as an herbivore / predator allows it to persist in and colonising / invading disturbance prone ecosystems (MacNeil, Dick & Elwood 1997). There was evidence of recovery as the stressors altered, with an attendant change in the importance of the microbial fraction of breakdown at the impacted outfall site in 2007, a similar finding to that documented by Hladyz *et al.,* (2011a, b).

The lack of change after one month (2005) found in Chapter 4 and then after a further year of re-routing (2006) suggest that PEITC or a lagged recovery / hysteretic response to PEITC bioremediation may not be the agent responsible. It may also be that PEITC levels remain high despite the bioremediation. However, a positive response was detected by the functional (commencement of recovery) and structural measures in 2007, indicative of a recovery. This coincided with one year after the cessation of chlorine use and its treatment, which other than a continuation of the bioremediation, were the only major farm management practice changes made at SMB between 2006 and 2007 data collection, suggesting that chlorine may have had a role.

The field experiments both in this Chapter and in Chapter 4 at the SMB watercress farm did not concur with lab-based single species ecotoxicological studies that suggest PEITC may be the causative agent behind *G. pulex* declines (see Worgan & Tyrell 2006). The abundance of *G. pulex* within areas exposed to effluents from the watercress beds during this and my initial survey (see Fig.5.3 & 5.4 and Fig. 4.3 & 4.4 pp. 104 & 106) lends further weight to this suggestion. Rather, my data suggest that organic pollution e.g. as also evidenced by high BOD, nitrate, ammonia, nitrite, phosphate concentrations and algal production at the outfall, in addition to associated changes in macroinvertebrate abundance, and perhaps more importantly, chlorine use were the principal stressors. The potential role of chlorine use in suppressing *G. pulex* abundance is supported by an *in situ* study by Maltby *et al.*, (2000), which suggests that chlorine increases mortality and reduces detrital processing in *G. pulex*. In this study no clear evidence of recovery was evident after one year of re-routing (June 2006), whereas the first clear signs of recovery emerged in June 2007, 11 months after chlorine use had ceased entirely. The *G. pulex* species have highly sensitive chemosensory apparatus and even at very low concentrations, toxins can induce drift responses (*cf.* Lauridsen & Friberg 2005; Amiard-Triquet 2009). This might explain why *G. pulex* abundance was suppressed disproportionately relative to other members of the macroinvertebrate community.

This *in situ* study enabled me to assess the perturbed ecosystem whilst it was exposed to the full range of conditions manifested in the field. That different drivers appeared to be at work in the “real world” than suggested by ecotoxicological studies is perhaps not surprising (Woodward *et al.*, 2012), and highlights the potential pitfalls of extrapolating directly from the laboratory to the field (Kimball & Levin 1985; Vignati, Ferrari & Dominik 2007). Within any given ecosystem a range of often unknown drivers are likely to be operating, and potentially interacting with one another. This is opposed to the single isolated variables typically investigated in lab-based studies (Vignati, Ferrari & Dominik 2007), which also tend to focus on acute rather than chronic effects of stressors (e.g. $LD_{50}$ trials) (Forbes & Calow 2002). Thus the application of *in situ* ecosystem-based bioassays (e.g., Lauridsen & Friberg 2005) can therefore help to provide more realistic insights to help guide management decisions, with a view to aiding recovery (Marsden 2006; Worgan & Tyrell 2006), although the field is still in its embryonic stages (Woodward *et al.*, 2012; Palmer & Febria 2012).

In summary, this study confirmed that measuring key process rates in the field can provide valuable insight into ecosystem functioning (Gessner & Chauvet 2002), especially when combined with structural measures that provide additional complementary information (Woodward *et al.*, 2012). The use of these multiple approaches enabled us to: i) identify the likely stressors involved (organic pollution and chlorine use); ii) discount the likelihood of PEITC being a principal driver under field conditions; iii) demonstrate differential responses to anthropogenic impacts among a range of bioassessment measures; iv) detect the onset of ecological recovery, after a decade of the impact of watercress farming (Medgett 1998). Based on this case study, we advocate the wider use of combined structural-functional measures in future studies, to provide a more comprehensive assessment of the complex array of drivers and responses at play in natural ecosystems (Giller *et al*., 2004; Woodward *et al*., 2009; Woodward *et al.*, 2012; Palmer & Febria 2012).

**References.**

Agnew, C., T., Clifford, N., J. & Haylett, S., (2000) Identifying and alleviating low flows in regulated rivers: the case of the Rivers Bulbourne and Gade, Hertfordshire, UK. *Regulated Rivers: Research & Management*, **16**, 245–266.

Amiard-Triquet, C., (2009) Behavioral disturbances: the missing link between sub-organismal and supra-organismal responses to stress? Prospects based on aquatic research. *Human and Ecological Risk Assessment: An International Journal*, **15**, 87 – 110.

Armitage, P., D., Moss, D., Wright, J., F. & Furse, M., T., (1983) The performance of a new biological water-quality score system based on macroinvertebrates over a wide-range of unpolluted running-water sites. *Water Research*, **17**, 333–347.

Berrie, A., D., (1992) The chalk-stream environment. *Hydrobiologia*, **248**, 3-9.

Bernhardt, E., S. & Likens, G., E.. (2004) Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology*, **49**, 14-27.

Bonada, N., Prat, N., Resh, V., H. and Statzner, B., (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual review of Entomology*, **51**, 495–523.

Bunn, S., E. & Davies, P., M., (2000) Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia*, **422/423**, 61–70.

Casey, H. and Smith, S., M., (1994) The effects of watercress growing on chalk headwater streams in Dorset and Hampshire. *Environmental Pollution*, **85,** 2, 217–228.

Cardinale, B., J., Palmer, M., A. and Collins, S., L., (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, **415,** 426-429.

Chessman, B., C., Westhorpe, D., P., Mitrovic, S., M., Hardwick, L., (2009) Trophic linkages between periphyton and grazing macroinvertebrates in rivers with different levels of catchment development. *Hydrobiologia*, **625**, 135-150.

Downes, J., D., Barmuta, L., A., Fairweather, P., G., Faith, D., P., Keough, M. J. Lake, P., S., Mapstone, B., D. and Quinn, G., P., (2002) Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. University Press, Cambridge.

Environment Agency (2004) *The State of England's Chalk Rivers*.

European Commission (2003) *The new EU Water Framework Directive*. Available from: Retrieved 28.08.2012 http://ec.europa.eu/environment/water/water-framework/index_en.html

Fewings, S., (1999) *Survey of Operational Practice on Watercress Farms in Hampshire*. Collaborative Project of the Environment Agency, Horticultural Development Council and Watercress Growers Association.

Forbes, V., E., and Calow, P., (2002) Species sensitivity distributions revisited: a critical appraisal. *Human and Ecological Risk Assessment*, **8**, 473–492.

Friberg, N., Bonada, N., Bradley, D., C., Dunbar, M., J., Edwards, F., K., Grey, J., Hayes, R., B., Hildrew, A., G., Lamouroux, N., Trimmer, M. and Woodward, G., (2011) Biomonitoring of human impacts in freshwater ecosystems: the good, the bad, and the ugly. *Advances in Ecological Research*, **44**, 2-68.

Gessner, M., O. & Chauvet, E., (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications*, **12**, 498–510.

Giller, P., S., Hillebrand, H., Berninger, U., G., Gessner, M., O., Hawkins, S., Inchausti, P. *et al*., (2004) Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos*, **104**, 423–436.

Harrison, S., S., C. & Harris, I., T., (2002) The effects of bank side management on chalk stream invertebrate communities. *Freshwater Biology*, **47**, 2233-2245.

H.C.C. (2000) Biodiversity Action Plan for Hampshire: Volume Two, Hampshire Biodiversity Partnership. Chalk streams. Retrieved 28.08.2012 from http://www.hampshirebiodiversity.org.uk/pdf/PublishedPlans/ChalkStreamsHAPjjDTP2.pdf

Hieber, M. & Gessner, M., O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026-1038.

Herricks, E., E. & Cairns, J., J., (1982) Biological monitoring. Part III – receiving system methodology based on community structure. *Water Research*, **16**, 141–153.

Hildrew, A., G., (2009) Sustained Research on Stream Communities: A model system and the comparative approach. *Advances in Ecological Research*, **41**, 175–312.

Hladyz, S., Tiegs, S., D., Gessner, M., O., Giller, P., S., Risnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. and Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology*, **55**, 1916-1929.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M., O., Giller, P.S., Gulis, V., Hutton, S., A., Lacoursie`re, J., O., *et al*., (2011a) Stream ecosystem functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages. *Advances in Ecological Research.* **44**, 211–276.

Hladyz, S., Abjornsson, K., Giller, P., S., and Woodward, G. (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology*, **48**, 443–452.

Holmes, N., T., H., (2006) The importance of long-term data sets in science and river management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **16**, 329–333.

Hopkins, J., Marcarelli, A. & Bechtold, H., (2011) Ecosystem Structure and Function are Complementary Measures of Water Quality in a Polluted, Spring-Influenced River n. *Water, Air, & Soil Pollution*, **214,** 409-421.

Kimball, K., D. & Levin, S., A., (1985) Limitations of laboratory bioassays: the need for ecosystem level testing. *Ecoscience*, **35**, 165–171.

Lauridsen, R., B. and Friberg, N., (2005) Stream macroinvertebrate drift response to pulsed exposure of the synthetic pyrethroid lambda-cyhalothrin. *Environmental Toxicology*, **20**, 513–521.

Layer, K., Hildrew, A., G., Monteith, D. and Woodward, G., (2010a) Long-term variation in the littoral food web of an acidified mountain lake. Global Change Biol. **11**, 3133–3143.

Layer, K., Riede, J., O., Hildrew, A., G., and Woodward, G., (2010b). Food web structure and stability in 20 streams across a wide pH gradient. Advances in Ecological Research, **42**, 265–301.

MacNeil, C., Dick, J. T. A., and Elwood, R. W., (1997) The trophic ecology of freshwater *Gammarus* (Crustacea: Amphipoda); problems and perspectives concerning the Functional Feeding Group concept. Biological Reviews (Cambridge), **72**, 349–364.

Maltby, L., (1994) Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: *Water Quality and Stress Indicators in Marine and Freshwater*

*Systems: Linking Levels of Organisation* (Editors: Sutcliffe, D.W.). Freshwater Biological Association, Ambleside, UK.

Maltby, L., Clayton, S., A., Yu, H., McLoughlin, N., Wood, R., M., Yin, D., (2000) Using single-species toxicity tests, community-level responses, and toxicity identification evaluations to investigate effluent impacts. *Environmental Toxicology and Chemistry*, **19**, 151–57.

Mann, R., H., K., Blackburn, J., H. & Beaumont, W., R., C., (1989) The ecology of brown trout *Salmo trutta* in the English chalk streams. *Freshwater Biology*, **21**, 57-7.

Maltby, L., Clayton, S., A., Wood, R., M. & McLoughlin, N., (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. *Environmental Toxicology and Chemistry*, **21**, 361–368.

Medgett, S., (1998) Environmental Agency Report: *The impact of St. Mary Bourne Cress Farm on the Bourne Rivulet.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Palmer, M., A. & Febria, C., M., (2012) The Heartbeat of Ecosystems, *Science,* **336**, 1393 -1394.

Prenda, J., Armitage, P., D. & Grayston, A., (1997) Habitat use by the fish assemblages of two chalk streams. *Journal of Fish Biology*, **51**, 64–79.

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P., & Dobson, M. (2010) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology*, 55, 346–359.

Rosenberg, D., M. and Resh, V., H., (eds.), (1993) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Sandin, L. & Solimini, A., G., (2009) Freshwater ecosystem structure-function relationships: from theory to application. *Freshwater Biology*, **54**, 2017–2024.

Sear, D., A., Armitage, P., D. & Dawson, F., D., H., (1999) Groundwater dominated rivers. *Hydrological Processes*, **11,** 255-276.

Shamsudin, L. & Sleigh, M., A., (1994) Seasonal changes in composition and biomass of epilithic algal floras of a chalk stream and a soft water stream with estimates of production. *Hydrobiologia,* **273,** 131-146.

ter Braak, C., J., F., &. Prentice, I., C., (1988) A theory of gradient analysis. *Advances in Ecological Research*, **18**, 271-313.

Underwood, A., J., (1994) On beyond baci – sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3–15.

Vignati, D., A., L., Ferrari, B., J., D. and Dominik, J., (2007) Laboratory-to-field extrapolation in aquatic sciences. *Environmental Science and Technology*, **15**, 1067-1073.

Vymazal J., (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecological Engineering **25** (5), 478–90.

Vymazal, J., (2006) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, **380**, 48-65.

White, S. & Medgett, S., (2006) Environmental Agency Report: *Bourne Rivulet Invertebrate Survey.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Whitehead, P., G., Wilby, R., L., Butterfield, D. and Wade, A., J., (2006) Impacts of climate change on in-stream nitrogen in a lowland chalk stream: an appraisal of adaptation strategies, *Science of the Total Environment,* **365,** 260 –273.

Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshwater Biol*ogy, **54**, 2171–2187.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R., B., (2008) Trophic trickles and cascades in complex food web: impacts of keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Woodward, G., Friberg, N. and Hildrew, A., G., (2010b) Science and non-science in the biomonitoring and conservation of fresh waters. In: *Freshwater Ecosystems and Aquaculture Research* (Ed. by F. de Carlo and A. Bassano). 978-1-60741-707-1. Nova Science Publishing, New York, USA.

Woodward, G., Jones, J., I. & Hildrew, A., G., (2002) Community persistence in Broadstone Stream (UK) over three decades. *Freshwater Biology*, **47**, 1419–1435.

Woodward, G., Gessner, M., O., Giller, P., S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B., G., Tiegs, S., D., Cariss, H., Dobson, M., Elosegi, A. ,Ferreira, V., Graça, M., A.,,S., Fleituch, T., Lacoursiere, J., O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L., B., M. and Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science*, **336**, 1438-1440.

Worgan, A. & Tyrell, R., (2006) *Monitoring behavioural responses of Gammarus pulex to watercress oils*. Report for Vitacress Salads Ltd by the Centre of Ecology and Hydrology, (CEH), Dorset.

Wright, J., F., (1992) Spatial and temporal occurrence of invertebrates in a chalk stream, Berkshire, England. *Hydrobiologia*, **248**, 11–30.

Wright, J., F., Armitage, P., D., Furse, M., T. and Moss, D., (1984) The classification of sites on British rivers using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie*, **22**, 1939–1943.

Wright, J., F., Sutcliffe, D., W. and Furse, M., T., (Eds.) (2000) Assessing the biological quality of fresh waters: RIVPACS and other techniques. *Freshwater Biological Association*, Ambleside, UK.

Young, R., G., Matthaei, C., D. and Townsend, C., R., (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society,* **27**, 605-625.

## Chapter 6.

## *The temporal and spatial responses of ecosystem functioning and community structure to watercress farming across Hampshire and Dorset.*

### Introduction.

Ecosystem functioning measures are now the rising star in the assessment of freshwater ecosystems, driven by the EU Water Framework Directive (WFD) (European Commission 2003), the American Clean Water Act (ACWA) (EPA 2002) and their freshwater ecologist proponents (e.g. Gessner & Chauvet 2002; Young, Matthaei & Townsend 2008; Hopkins, Marcarelli & Bechtold 2011; Haldyz *et al.*, 2011a, b). These measures are still not widely used or integrated into current bioassessment protocols, which rather rely upon structural measures, where the composition of macroinvertebrate assemblages are assessed (Bonada *et al.*, 2006; Woodward, 2009; Statzner & Bech 2010). We sought to address this, in a series of spatiotemporal studies, at six watercress farms across Hampshire and Dorset.

The focus of our first two data chapters has been the largest watercress farm in Europe, at St Mary Bourne (SMB) in Hampshire, sited on the headwaters of the Bourne rivulet. Where, despite the highest chemical quality of its outfall waters, EA surveys have revealed long-term impacts on key chalk stream macroinvertebrate taxa, including *G. pulex* (Fig 1.2 pp. 29). Recent farm management changes designed to reduce these impacts were assessed using a set of functional and structural measures, in a new integrated approach to biomonitoring. It was suggested that the farms watercress beds could bioremediate, the possible negative effects of PEITC, an allellochemical found in watercress, due to the watercress beds close structural resemblance and action to a constructed wetland. The first study (Chapter 4) demonstrated the efficacy of these measures in detecting their impacts, but no response was found after re-routing the farm and factory wastewaters back through the farms watercress beds, one month later. The second study (Chapter 5) found that after a year of re-routing there was still no response, and that this lack of change one month and then after a further year of re-routing, suggest that some other stressors were responsible rather than PEITC. This was highlighted when a positive response was detected by our functional and structural measures, one year after the cessation of chlorine use and its treatment at SMB, albeit that this response may have been coincidental. Nevertheless, this represents the first evidence of recovery at SMB since these perturbations were first detected over a decade before.

The earlier trials conducted at SMB (Chapters 4 and 5) were replicated at other watercress farms and over time (seasons: autumn, winter, spring, summer) to test for the generality of the effects of watercress farming, that were found at SMB. Specifically, we sought to investigate the impact of watercress farming on the ecology of their watercourses, in a seasonal study at three watercress farms (temporal study) and a single season study (summer) at six watercress farms (spatial study), both of which included SMB. This enabled a comparison with which to test the general hypothesis, that watercress farming adversely affects the ecosystem functioning and community structure of chalk streams.

The experimental hypotheses tested here were that:

1) The reduction in leaf litter breakdown rates and *G. pulex* abundance seen at SMB would be general across other watercress farms.
2) Overall, the upstream “control” sites would be unaltered by the presence of the farm. The outfall sites should show the strongest responses if any to the

watercress farm, with evidence of recovery from these effects at the downstream “recovery” sites.

3) The outfall sites would be impacted and therefore have slower decomposition rates than the upstream and downstream reference sites. The separation between the total and microbial breakdown, which corresponds to the shredding macroinvertebrate component, would also be reduced.
4) Impacts at the outfall would be reflected by a reduction in algal accrual and herbivory, in comparison to the upstream and downstream reference sites.
5) The outfall impacts to be evident as reduced abundance of *G. pulex* in comparison to the upstream sites.
6) These impacts may be dependent upon, or exacerbated by, season e.g. an increase in breakdown due to a seasonal rise in temperature and they may be scale dependent, such that they are more evident in larger watercress farms.

## Methods.

Refer to Chapter 2 & 3 – Sites & General Methods for a detailed description.

### *Temporal Study.*

Repeated, replicated samples were taken during each of four seasons, autumn 2006 (December 2006), winter 2006/7 (February 2007), spring 2007 (April 2007) and summer 2007 (June 2007) at three watercress farms.

### *Spatial Study.*

Replicated samples were taken during the summer of 2007 (June 2007), at six watercress farms across Hampshire and Dorset.

## Results: Temporal Study.

### *Physico-chemical parameters.*

Nitrite and ammonia EQS's were exceeded for every month, site and stream except for nitrite at the upstream site at SMB. At Abbots Ann the phosphate EQS was exceeded in the spring and summer, but only at the outfall and downstream sites, this was similar at SMB but only downstream in summer. Perhaps due to the addition of fertilisers at both farms during this peak growing season, and diffuse pollution downstream of SMB. BOD was elevated at every site and stream in spring, except the upstream site at SMB. BOD was also elevated in the summer, but only at the outfall at SMB (Tables 2.1-2.5 pp.65-71).

### *Functional measures: a) litter decomposition rates.*

Litter decomposition rates differed significantly among sites (LMEM $F$ $df_{N1}$, $df_{D90}$ = 29.210, $P$ <0.001), streams (LMEM $F$ $df_{N1}$, $df_{D90}$ = 39.393, $P$ <0.001), seasons (LMEM $F$ $df_{N1}$, $df_{D270}$ = 10.751, $P$ <0.001) and mesh types (LMEM $F$ $df_{N1}$, $df_{D90}$ = 269.074, $P$ <0.001) (Table 6.1; Fig 6.1). Total decomposition in coarse bags was significantly faster than in fine bags, (LMEM $F$ $df_{N1}$, $df_{D90}$ = 269.074, $P$ <0.001) highlighting the overriding influence of shredders (Table 6.1; Fig 6.1). The significance of all of the three way interactions: stream x season x site (LMEM $F$ $df_{N12}$, $df_{D270}$ = 6.212, $P$ <0.001); stream x season x mesh (LMEM $F$ $df_{N6}$, $df_{D270}$ = 2.866, $P$ =0.010); stream x site x mesh (LMEM $F$ $df_{N4}$, $df_{D90}$ = 4.281, $P$ = 0.003) and season x site x mesh (LMEM $F$ $df_{N6}$, $df_{D270}$ = 2.976, $P$ = 0.008) (Table 6.1), revealed that the impacts of watercress farming varied over time, between streams, sites and consumer groups (macroinvertebrate shredders versus microbial decomposers) and that these effects were contingent upon season, watercress farm and site (Fig. 6.1). For instance, in all seasons, no reduction was evident at the outfall at Abbotts Ann and Fobdown, but, there was evidence of a decline at the outfall at St Mary Bourne. Upstream at Abbotts Ann a decline was evident, but only in autumn. A downstream decline was evident at Abbotts Ann for all seasons, but not at St Mary Bourne, and only in the summer at Fobdown (Fig. 6.1).

Table 6.1. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per degree-day ($k_{dd}$. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Up, Out, Down. Significant results at $P<0.05$ are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | $F$ – ratio | $P$ |
|---|---|---|---|---|
| Stream | 2 | 90 | 39.393 | **<0.001** |
| Season | 3 | 270 | 10.751 | **<0.001** |
| Site | 2 | 90 | 29.210 | **<0.001** |
| Mesh | 1 | 90 | 269.074 | **<0.001** |
| Stream x Season | 6 | 270 | 4.099 | **0.001** |
| Stream x Site | 4 | 90 | 13.452 | **<0.001** |
| Stream x Mesh | 2 | 90 | 9.722 | **<0.001** |
| Season x Site | 6 | 270 | 8.833 | **<0.001** |
| Season x Mesh | 3 | 270 | 0.930 | 0.427 |
| Site x Mesh | 2 | 90 | 1.355 | 0.263 |
| Stream x Season x Site | 12 | 270 | 6.212 | **<0.001** |
| Stream x Season x Mesh | 6 | 270 | 2.886 | **0.010** |
| Stream x Site x Mesh | 4 | 90 | 4.281 | **0.003** |
| Season x Site x Mesh | 6 | 270 | 2.976 | **0.008** |
| Stream x Season x Site x Mesh | 12 | 270 | 1.026 | 0.425 |

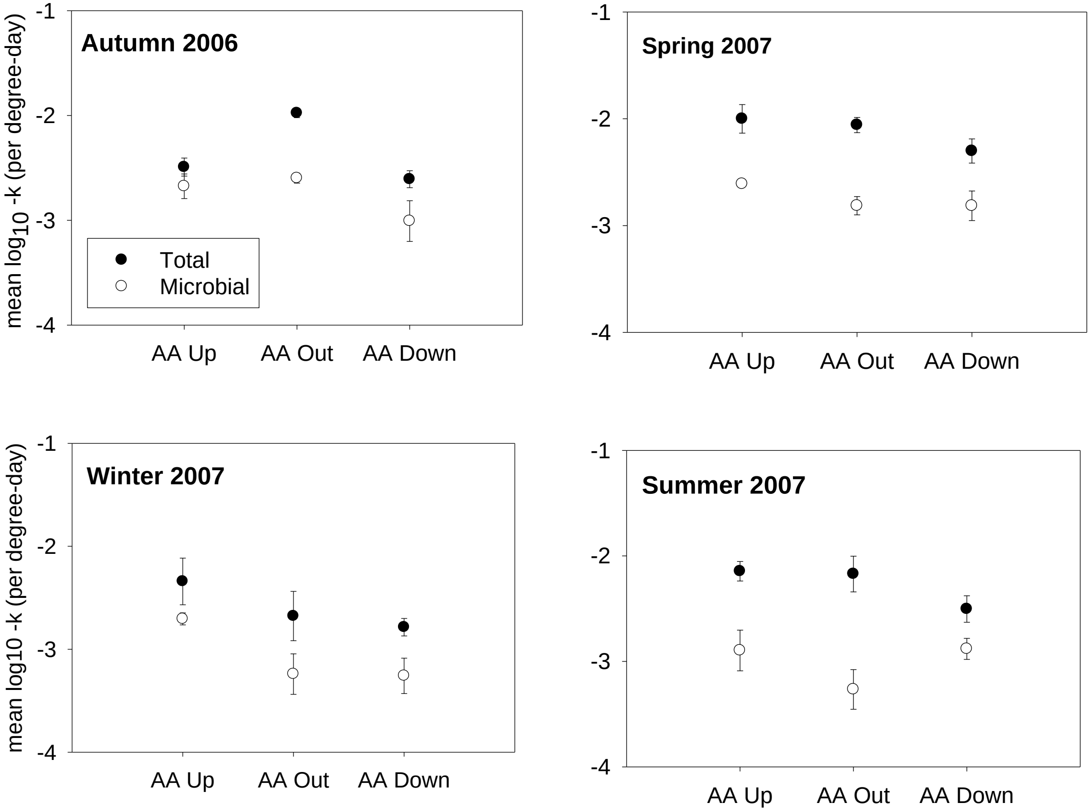


Fig. 6.1a. . Abbotts Ann. $\text{Log}_{10}$ mean leaf-litter decomposition rates per degree-day ($k_{dd}$) ($k$ ± SE). The closer together the separation of total (shredder + microbial) compared to the microbial breakdown data points suggests impacts and suppression of the activity of shredding macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

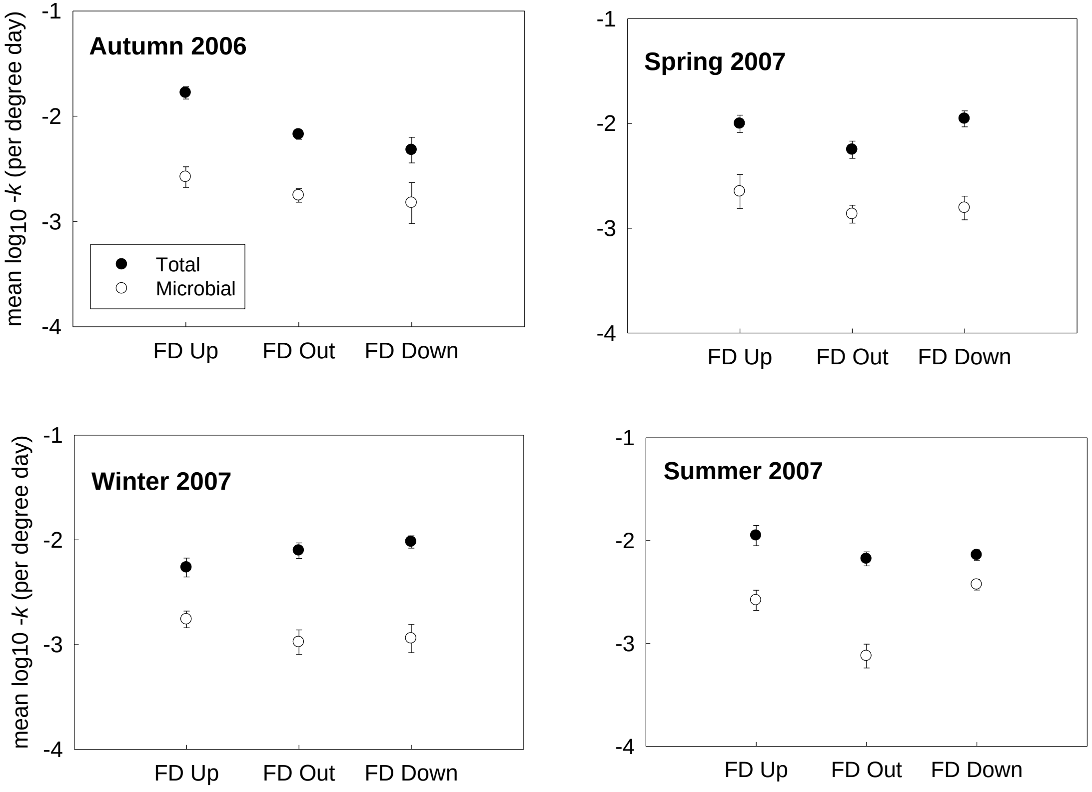


Fig. 6.1b. . Fobdown. Log$_{10}$ mean leaf-litter decomposition rates per degree-day ($k_{dd}$) ($k$ ± SE). The closer together the separation of total (shredder + microbial) compared to the microbial breakdown data points suggests impacts and suppression of the activity of shredding macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

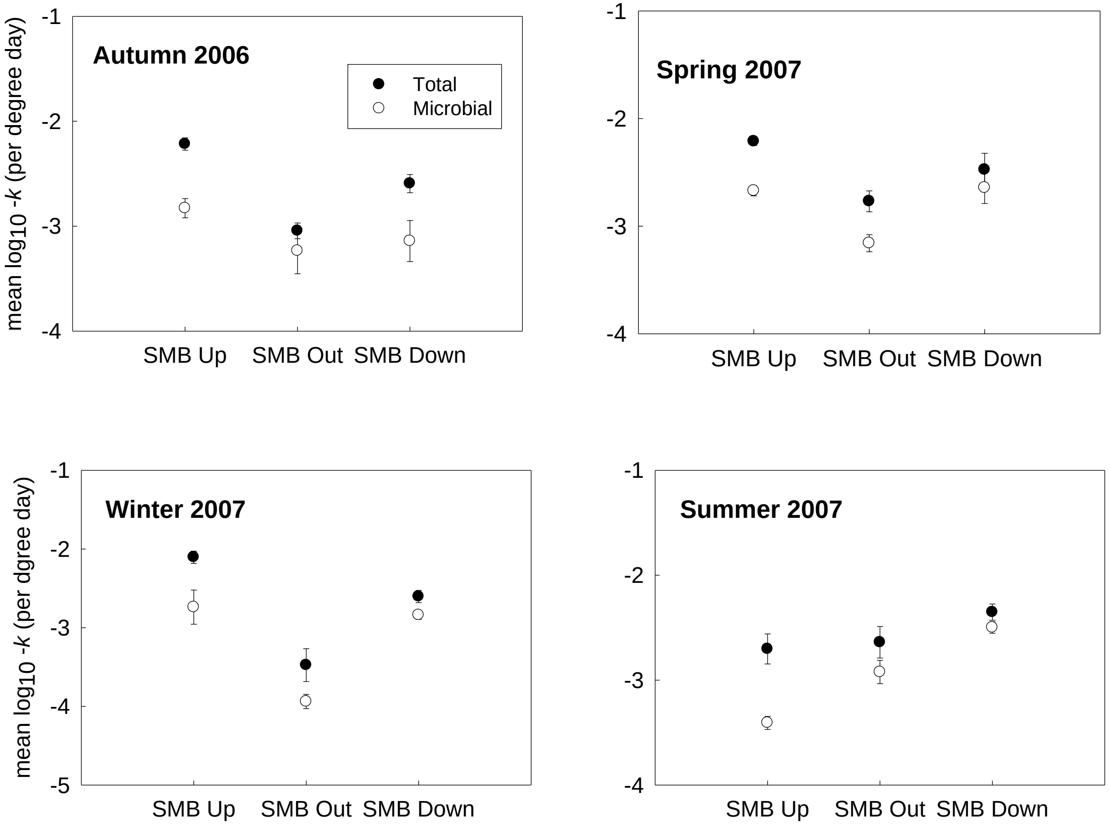


Fig. 6.1c. . St Mary Bourne. Log$_{10}$ mean leaf-litter decomposition rates per degree-day ($k_{dd}$) ($k$ ± SE). The closer together the separation of total (shredder + microbial) compared to the microbial breakdown data points suggests impacts and suppression of the activity of shredding macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

### *Functional measures b) algal production and c) herbivory.*

Overall there was no clear response to watercress farming, from the algal accrual and herbivory measures, and no consistent impact at the outfall compared to upstream. However, the downstream sites have the strongest separation of grazed compared to ungrazed tiles, and the responses in the spring were the most consistent among sites. Algal accrual was significantly different for the three way interaction of: season, stream and site (LMEM $F\ df_{N12}, df_{D267.043} = 15.560$, $P < 0.001$) revealing that the effects of watercress farming were not consistent but rather contingent upon season, farm and site (Table 6.2, Fig 6.2). Overall the top-down effects of grazers (herbivory) was evident, as chlorophyll *a* biomass was higher on the ungrazed tiles than on the grazed tiles across all sites (LMEM $F\ df_{N1}, df_{D90.323} = 72.324$, $P < 0.001$) (Table 6.2, Fig 6.2).

Table 6.2. LMEM results of comparison of stream algal colonisation tiles, log10 chlorophyll *a (*mg cm-2) per degree-day (*dd*). Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Up, Out, Down. Herbivory = NV / V. V = exclusion of grazers through use of Vaseline 'fence' and NV = non-exclusion. (Significant results at *P* <0.05 in bold).

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 2 | 90.308 | 49.424 | **<0.001** |
| Season | 3 | 268.015 | 19.233 | **<0.001** |
| Site | 2 | 90.319 | 79.296 | **<0.001** |
| Herbivory | 1 | 90.323 | 72.324 | **<0.001** |
| Stream x Season | 6 | 267.989 | 10.617 | **<0.001** |
| Stream x Site | 4 | 90.301 | 26.149 | **<0.001** |
| Stream x Herbivory | 2 | 90.308 | 0.062 | 0.940 |
| Season x Site | 6 | 267.989 | 7.055 | **<0.001** |
| Season x Herbivory | 3 | 268.015 | 0.379 | 0.768 |
| Site x Herbivory | 2 | 90.319 | 1.076 | 0.345 |
| Stream x Season x Site | 12 | 267.943 | 15.560 | **<0.001** |
| Stream x Season x Herbivory | 6 | 267.989 | 0.605 | 0.726 |
| Stream x Site x Herbivory | 4 | 90.301 | 0.177 | 0.950 |
| Season x Site x Herbivory | 6 | 267.989 | 0.177 | 0.983 |
| Stream x Season x Site x Herbivory | 12 | 267.943 | 0.302 | 0.989 |

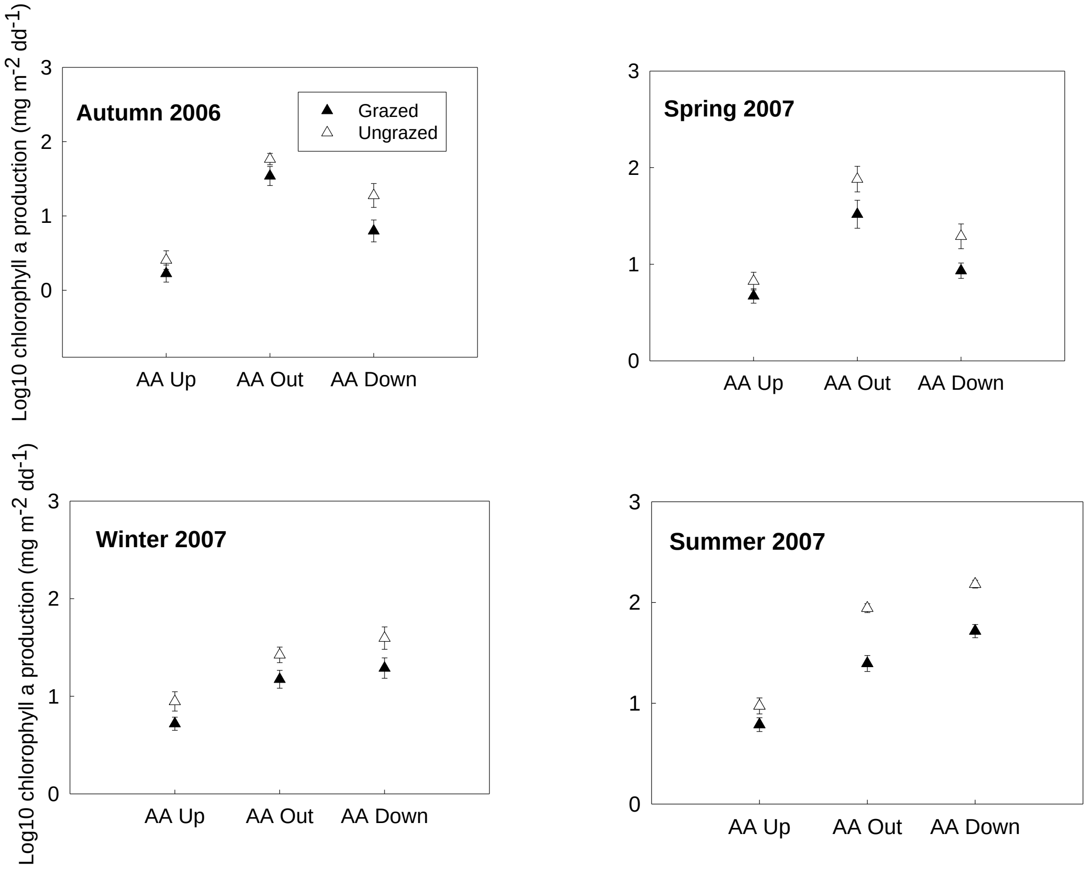


Fig. 6.2a. Abbotts Ann. Log$_{10}$ chlorophyll a production (mg cm-2) per degree-day (*d*) (± SE). White triangles denote No Grazing (exclusion of grazers through use of Vaseline ‘fence’), black triangles denote Grazing (no exclusion of grazers). The closer together the separation of the grazed compared to the ungrazed tiles suggests impacts and suppression of the activity of grazing macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

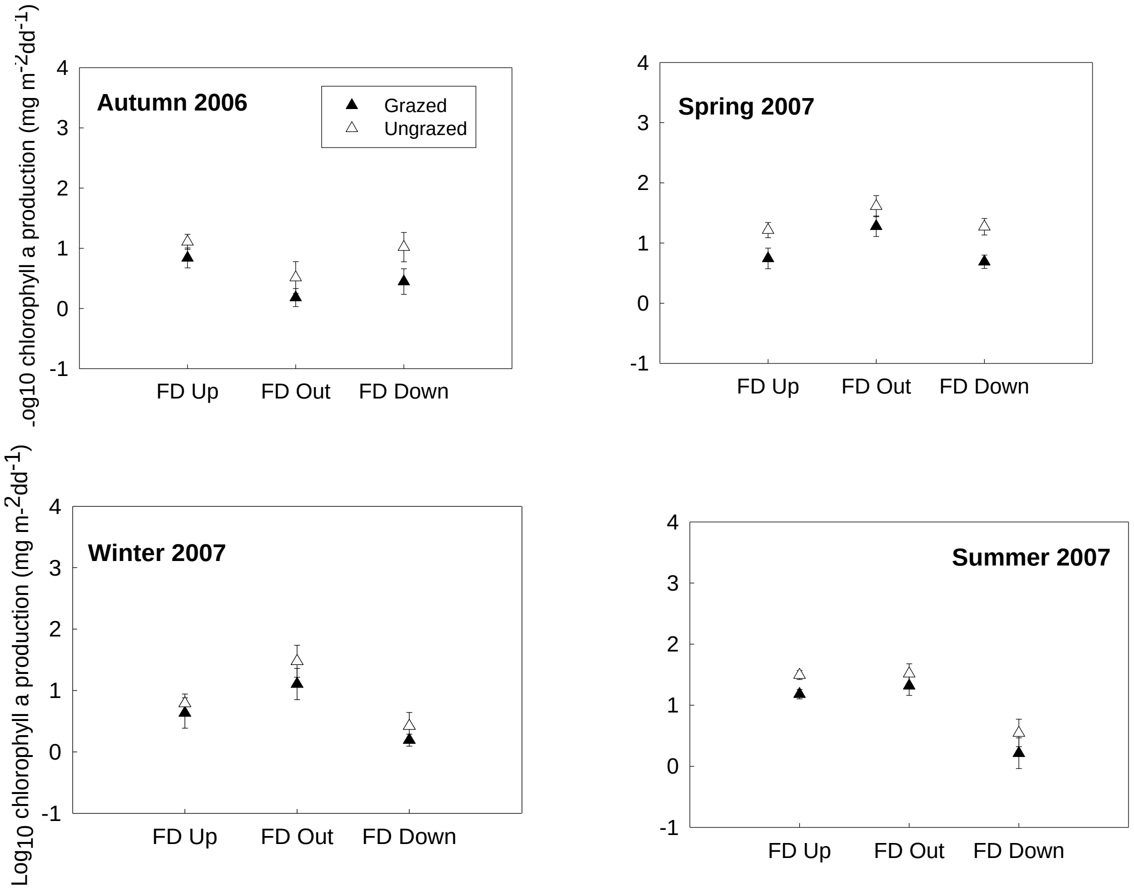


Fig. 6.2b. Fobdown. $Log_{10}$ chlorophyll a production *(*mg cm-2) per degree-day (*d*) (± SE). White triangles denote No Grazing (exclusion of grazers through use of Vaseline ‘fence’), black triangles denote Grazing (no exclusion of grazers). The closer together the separation of the grazed compared to the ungrazed tiles suggests impacts and suppression of the activity of grazing macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

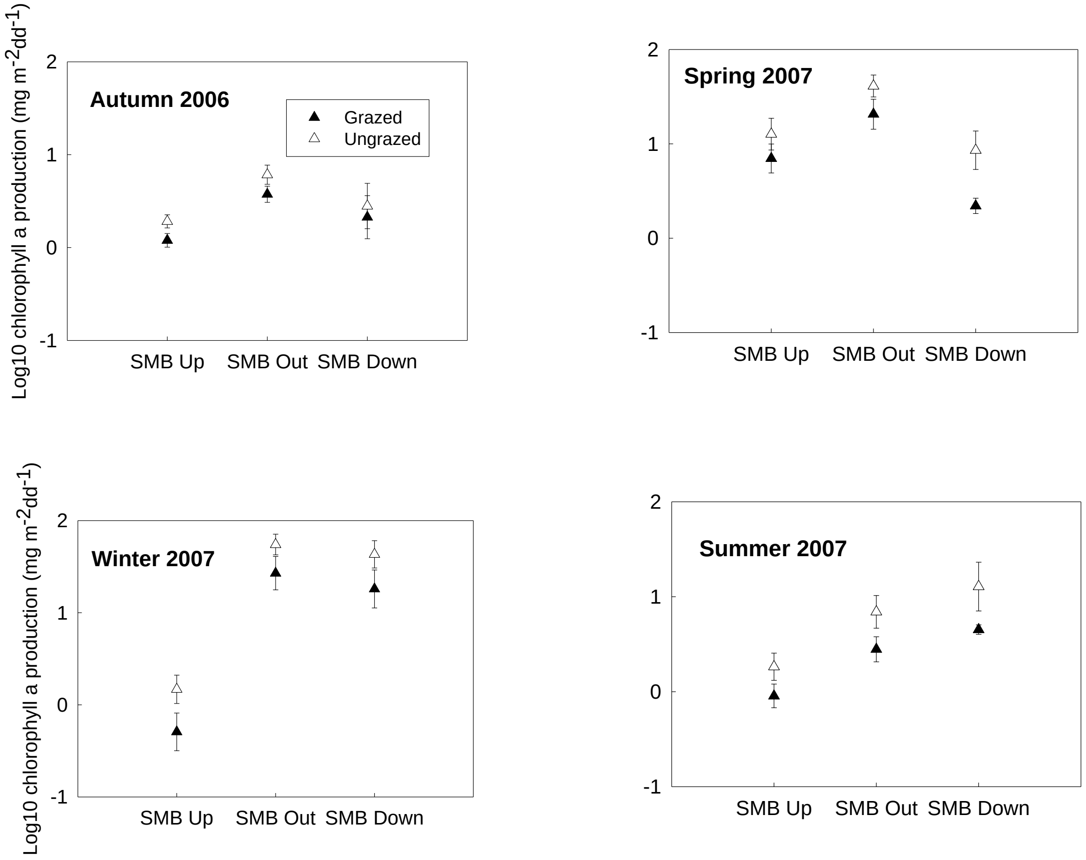


Fig. 6.2c. St Mary Bourne. $Log_{10}$ chlorophyll a production *(*mg cm-2) per degree-day (*d*) (± SE). White triangles denote No Grazing (exclusion of grazers through use of Vaseline 'fence'), black triangles denote Grazing (no exclusion of grazers). The closer together the separation of the grazed compared to the ungrazed tiles suggests impacts and suppression of the activity of grazing macroinvertebrates. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

### *Structural measures: a) macroinvertebrates in litter bags b) benthic macroinvertebrates.*

*G. pulex, A. aquaticus* and *Sericostoma personatum* (Spence) accounted for over 97% of shredders in the benthos and litter bags and were therefore the main focus of the analyses.

*G. pulex* leaf litter abundance differed significantly among sites (LMEM $F\ df_{\mathrm{N}2}, df_{\mathrm{D}45} = 152.466$, $P = <0.001$); streams (LMEM $F\ df_{\mathrm{N}2}, df_{\mathrm{D}45} = 7.096$, $P = 0.002$); and seasons (LMEM $F\ df_{\mathrm{N}3}, df_{\mathrm{D}135} = 29.012$, $P = <0.001$) (Table 6.3). *G. pulex* benthic abundance differed significantly among sites (LMEM $F\ df_{\mathrm{N}2}, df_{\mathrm{D}45} = 29.474$, $P = <0.001$) and seasons (LMEM $F\ df_{\mathrm{N}3}, df_{\mathrm{D}135} = 43.190$, $P = <0.001$) (Table 6.4). In general for both leaf bags and the benthos, *G. pulex* were not consistently rarer at the outfalls of the study farms, revealing that *G. pulex* was not affected by watercress farm outfalls. However, *A. aquaticus* and *S. personatum* were less abundant at SMB than at Abbots Ann and Fobdown, suggesting that conditions may have not have completely improved at SMB. Impacts were evident downstream, revealed by reduced *G. pulex* abundance compared to the outfall (Fig. 6.3 & 6.4). The interaction of season, stream and site (Leaf bags: LMEM $F\ df_{\mathrm{N}12}, df_{\mathrm{D}135} = 12.682$, $P <0.001$; Benthos: LMEM $F\ df_{\mathrm{N}12}, df_{\mathrm{D}135} = 10.482$, $P <0.001$) (Table 6.3 & 6.4) revealed that the effects of watercress farming were contingent upon season and watercress farm. Overall *A. aquaticus* were often missing from the upstream sites, were in greater numbers at the outfall, but were rarer downstream. *S. personatum* abundance show no clear general pattern in response to watercress farming, except that both *S. personatum* and *A. aquaticus* are rarer than *G. pulex* and this is more pronounced in the benthos than in litter bags (Fig. 6.3 & 6.4).

Table 6.3. LMEM results of comparisons of shredder densities in coarse litter-bags ($\log_{10}$ mean number per g leaf litter remaining) for *G. pulex* (G), *A. aquaticus* (A), *S. personatum* (S) and Total shredders (T). Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Up, Out, Down. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Stream | G | 2 | 45. | 7.096 | **0.002** |
| | A | 2 | 45 | 17.092 | **<0.001** |
| | S | 2 | 45 | 57.807 | **<0.001** |
| | T | 2 | 45 | 3.939 | **0.027** |
| Season | G | 3 | 135 | 29.012 | **<0.001** |
| | A | 3 | 135 | 6.450 | **<0.001** |
| | S | 3 | 135 | 0.824 | 0.483 |
| | T | 3 | 135 | 19.260 | **<0.001** |
| Site | G | 2 | 45 | 152.466 | **<0.001** |
| | A | 2 | 45 | 46.955 | **<0.001** |
| | S | 2 | 45 | 24.113 | **<0.001** |
| | T | 2 | 45 | 83.737 | **<0.001** |
| Stream x Season | G | 6 | 135 | 17.218 | **<0.001** |
| | A | 6 | 135 | 10.741 | **<0.001** |
| | S | 6 | 135 | 3.338 | **0.004** |
| | T | 6 | 135 | 15.678 | **<0.001** |
| Stream x Site | G | 4 | 45 | 28.880 | **<0.001** |
| | A | 4 | 45 | 41.770 | **<0.001** |
| | S | 4 | 45 | 6.505 | **<0.001** |
| | T | 4 | 45 | 32.101 | **<0.001** |

| | | | | | |
|---|---|---|---|---|---|
| Season x Site | G | 6 | 135 | 8.587 | **<0.001** |
| | A | 6 | 135 | 2.956 | **0.010** |
| | S | 6 | 135 | 5.799 | **<0.001** |
| | T | 6 | 135 | 6.770 | **<0.001** |
| Stream x Season x Site | G | 12 | 135 | 12.682 | **<0.001** |
| | A | 12 | 135 | 5.086 | **<0.001** |
| | S | 12 | 135 | 7.285 | **<0.001** |
| | T | 12 | 135 | 10.060 | **<0.001** |

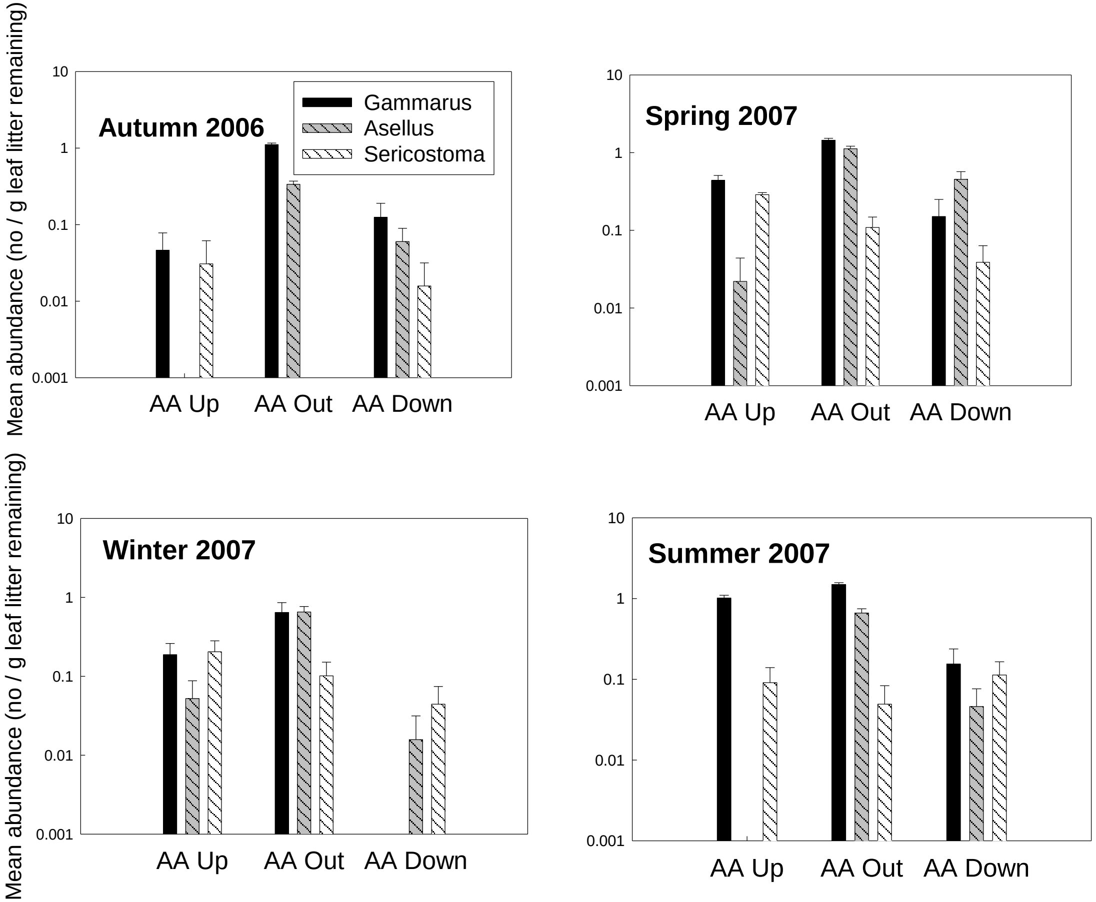


Fig. 6.3a. *G. pulex, A. aquaticus* and *S. personatum* coarse litter-bag abundance ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE). Abbotts Ann. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

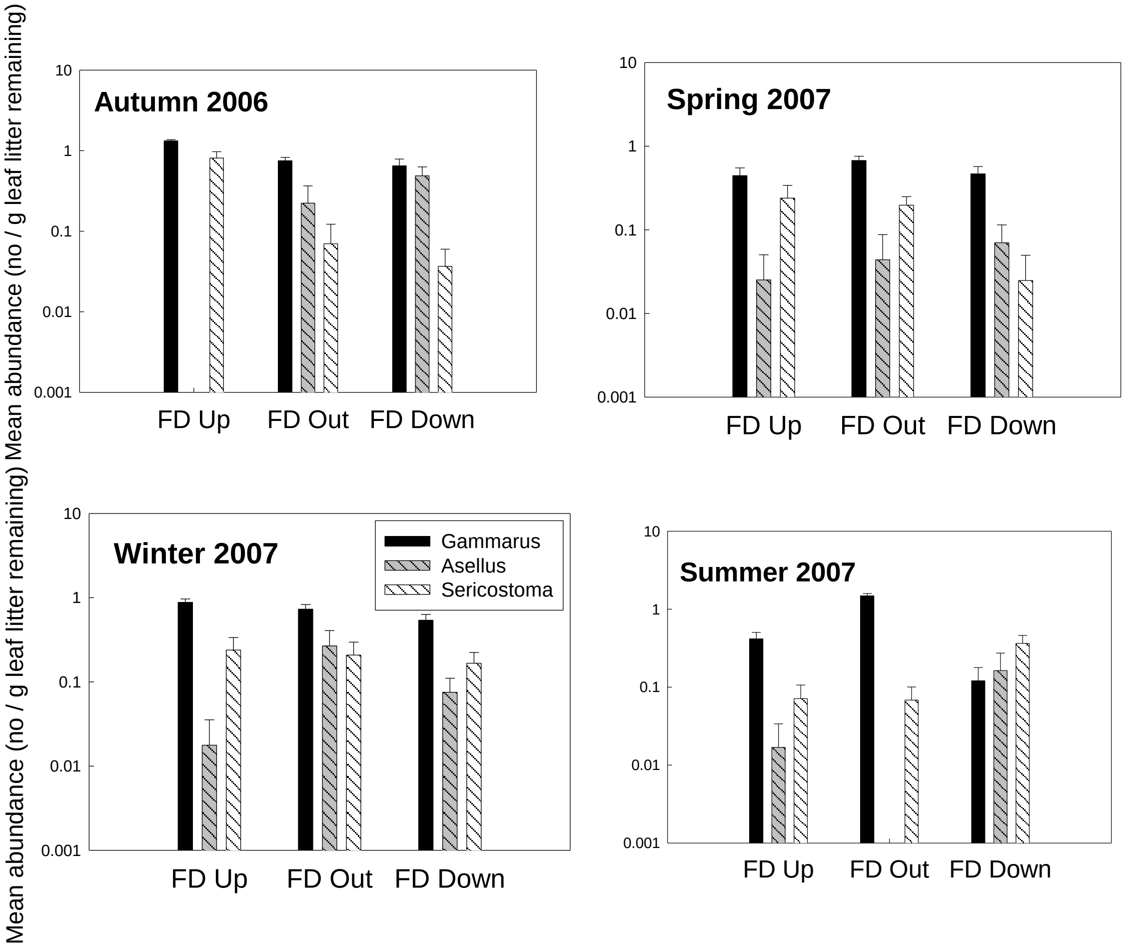


Fig. 6.3b. Fobdown. *G. pulex, A. aquaticus* and *S. personatum* coarse litter-bag abundance ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE). Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

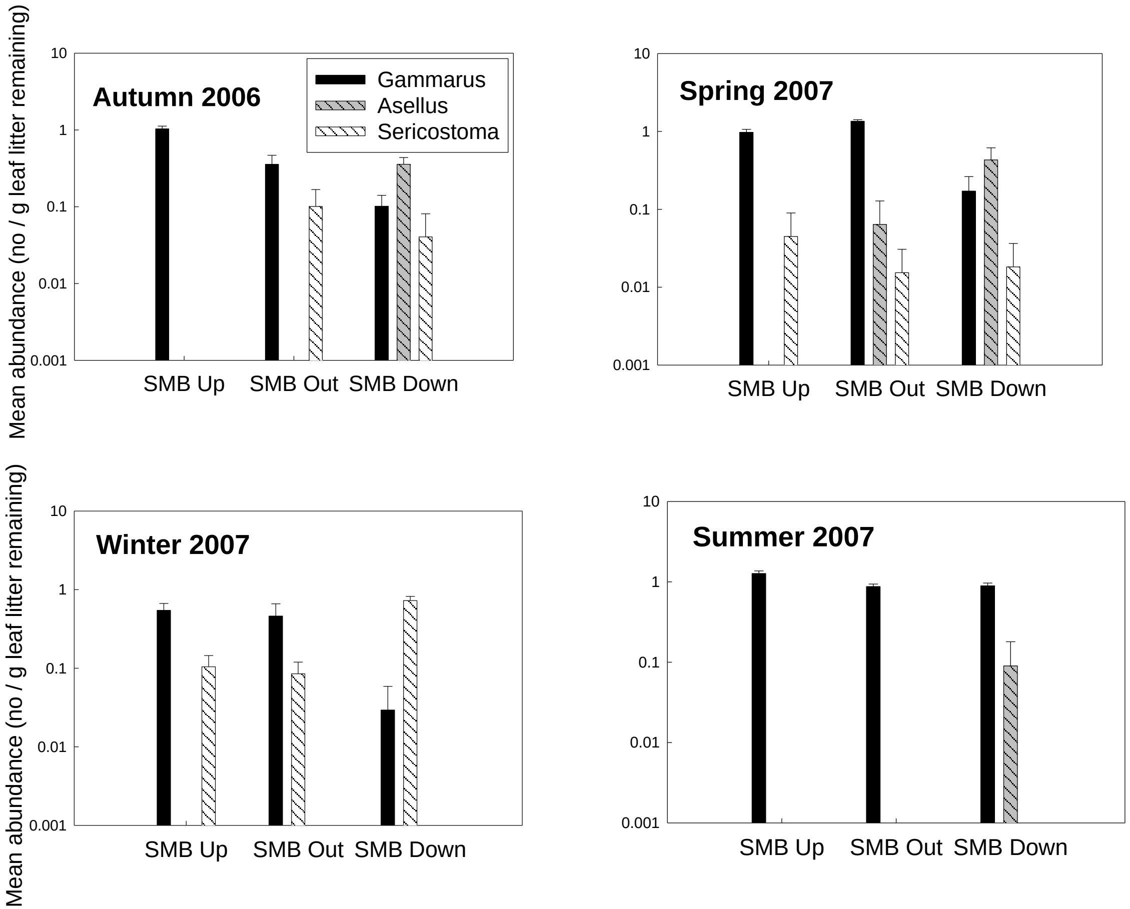


Fig. 6.3c. St Mary Bourne. *G. pulex, A. aquaticus* and *S. personatum* coarse litter-bag abundance ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE). Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

Table 6.4. LMEM results of stream comparisons of benthic shredder abundance (nos. $m^{-2}$). *G. pulex* (G), *A. aquaticus* (A), *S. personatum* (S) and Total shredders (T). Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007); Up, Out, Down. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Stream | G | 2 | 45 | 2.598 | 0.086 |
| | A | 2 | 45 | 31.521 | **<0.001** |
| | S | 2 | 45 | 6.861 | **0.003** |
| | T | 2 | 45 | 1.158 | 0.323 |
| Season | G | 3 | 135 | 43.190 | **<0.001** |
| | A | 3 | 135 | 1.968 | 0.122 |
| | S | 3 | 135 | 3.242 | **0.024** |
| | T | 3 | 135 | 32.332 | **<0.001** |
| Site | G | 2 | 45 | 29.474 | **<0.001** |
| | A | 2 | 45 | 44.296 | **<0.001** |
| | S | 2 | 45 | 4.591 | **0.015** |
| | T | 2 | 45 | 20.801 | **<0.001** |
| Stream x Season | G | 6 | 135 | 5.692 | **<0.001** |
| | A | 6 | 135 | 7.282 | **<0.001** |
| | S | 6 | 135 | 2.620 | **0.020** |
| | T | 6 | 135 | 5.916 | **<0.001** |
| Stream x Site | G | 4 | 45. | 16.359 | **<0.001** |
| | A | 4 | 45 | 46.193 | **<0.001** |
| | S | 4 | 45 | 4.401 | **0.004** |
| | T | 4 | 45 | 13.570 | **<0.001** |

| | | | | | |
|---|---|---|---|---|---|
| Season x Site | G | 6 | 135 | 7.560 | **<0.001** |
| | A | 6 | 135 | 5.276 | **<0.001** |
| | S | 6 | 135 | 4.459 | **<0.001** |
| | T | 6 | 135 | 5.771 | **<0.001** |
| Stream x Season x Site | G | 12 | 135 | 10.482 | **<0.001** |
| | A | 12 | 135 | 3.923 | **<0.001** |
| | S | 12 | 135 | 1.399 | 0.174 |
| | T | 12 | 135 | **<0.001** | **<0.001** |

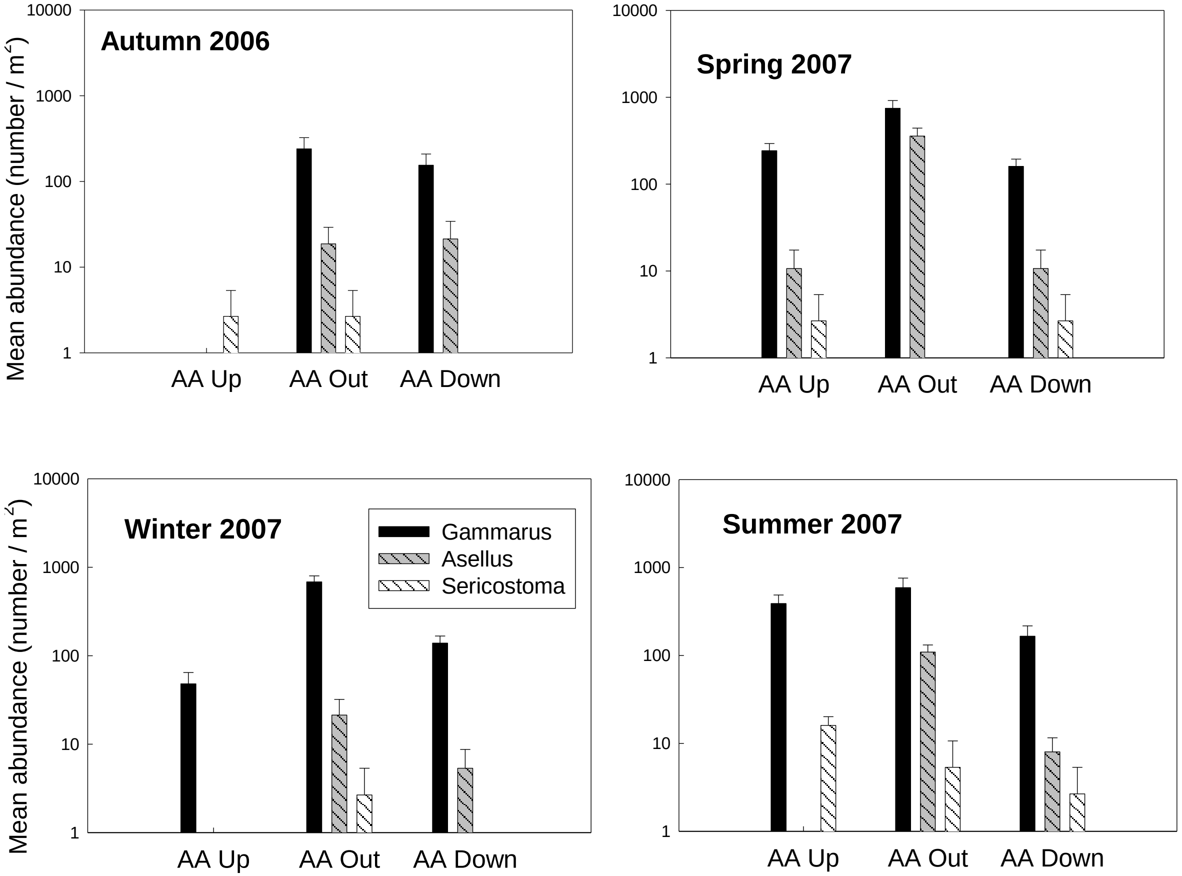


Fig. 6.4a. Abbotts Ann. Benthic abundance (nos. m$^{-2}$ ± SE) of *G. pulex*, *A. aquaticus* and *S. personatum*. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

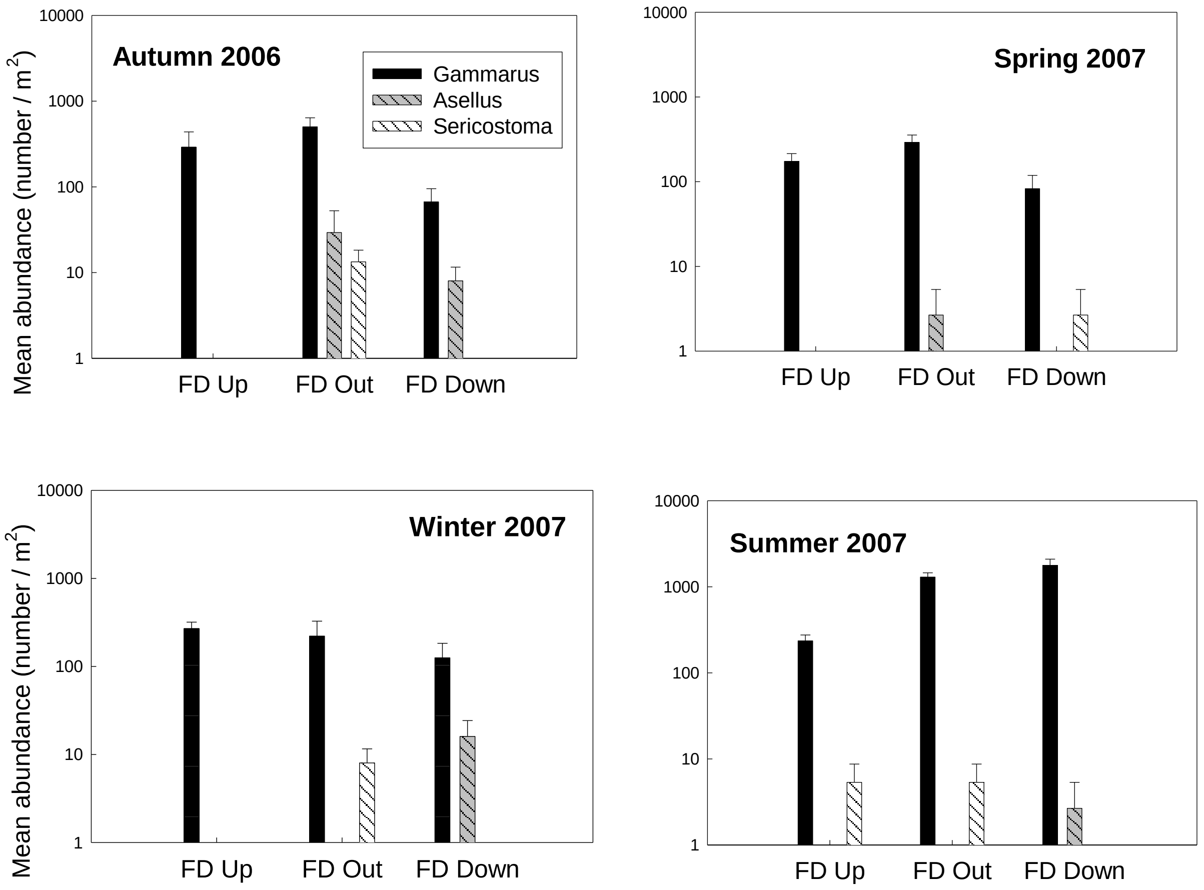


Fig. 6.4b. Fobdown. Benthic abundance (nos. $m^{-2}$ ± SE) of *G. pulex*, *A. aquaticus* and *S. personatum*. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

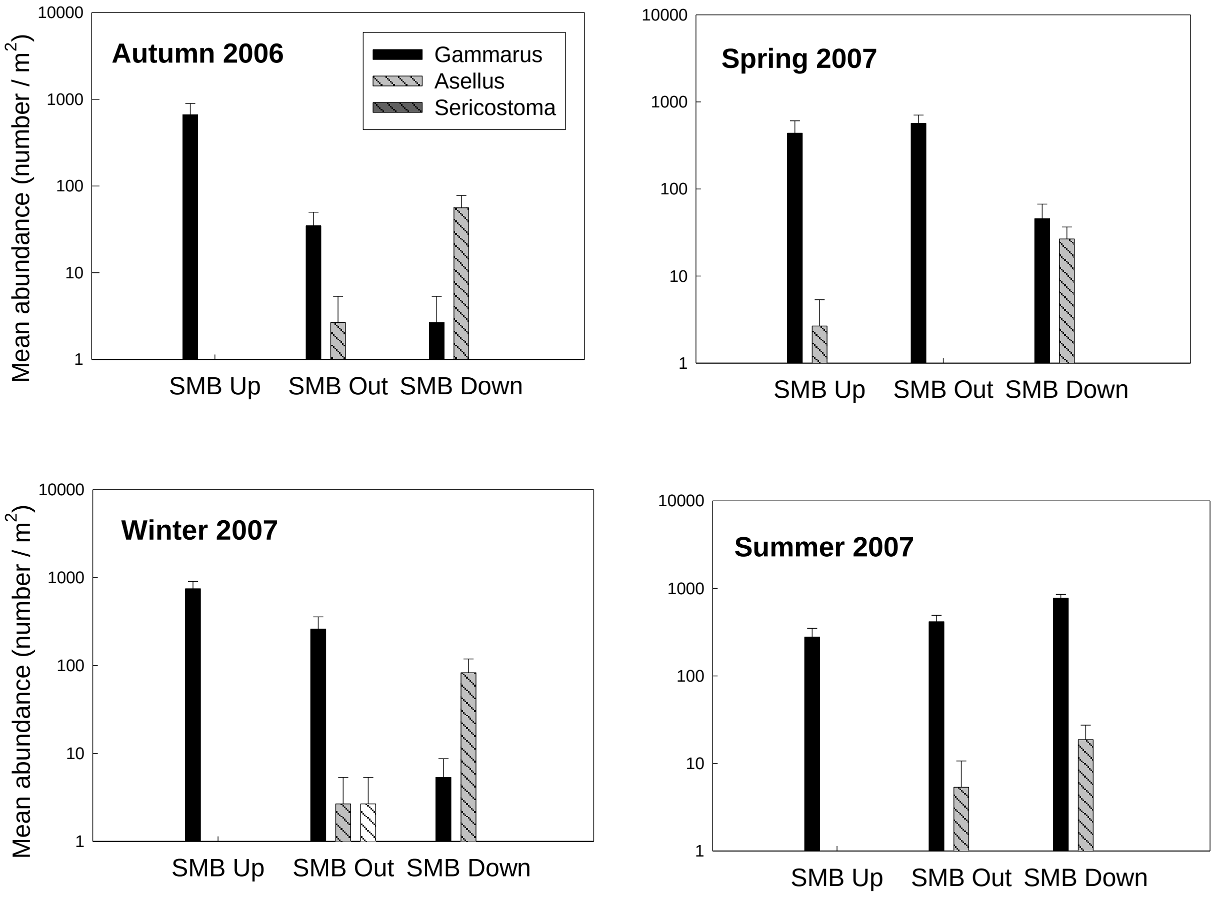


Fig. 6.4c. St Mary Bourne. Benthic abundance (nos. m$^{-2}$ ± SE) of *G. pulex*, *A. aquaticus* and *S. personatum*. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

***Ordination combining chemical data with functional and structural measures.***

*G. pulex* was driving the first axis of the PCA (Fig. 6.5a & 6.5b), and was positively correlated with chloride, dissolved oxygen, organic carbon, oxidised nitrogen and nitrate, the latter may be attributable to the application of chemical fertilisers even though this is strictly controlled within the farm confines. The dominant grazers *Baetis rhodani* (Pictet) and *Seratella ignita* (Poda) were strongly negatively associated with the second axis, which was positively associated with ammonia, nitrite, phosphate and biological oxygen demand (BOD), reflecting an organic pollution / nutrient enrichment gradient. Axis 1 separated the streams and sites while axis 2 separated the seasons from one another. Axis 1 (56.1%) and axis 2 (19.2%) together accounted for 75.3% of the species variation. An RDA with sites, season and stream dummy coded, revealed a similar pattern to the PCA, suggesting that there were no important drivers left undetected (Fig. 6.6a & 6.6b). Axis 1 (20.3%) and axis 2 (28.3%) accounted for 48.6% of the species variation.

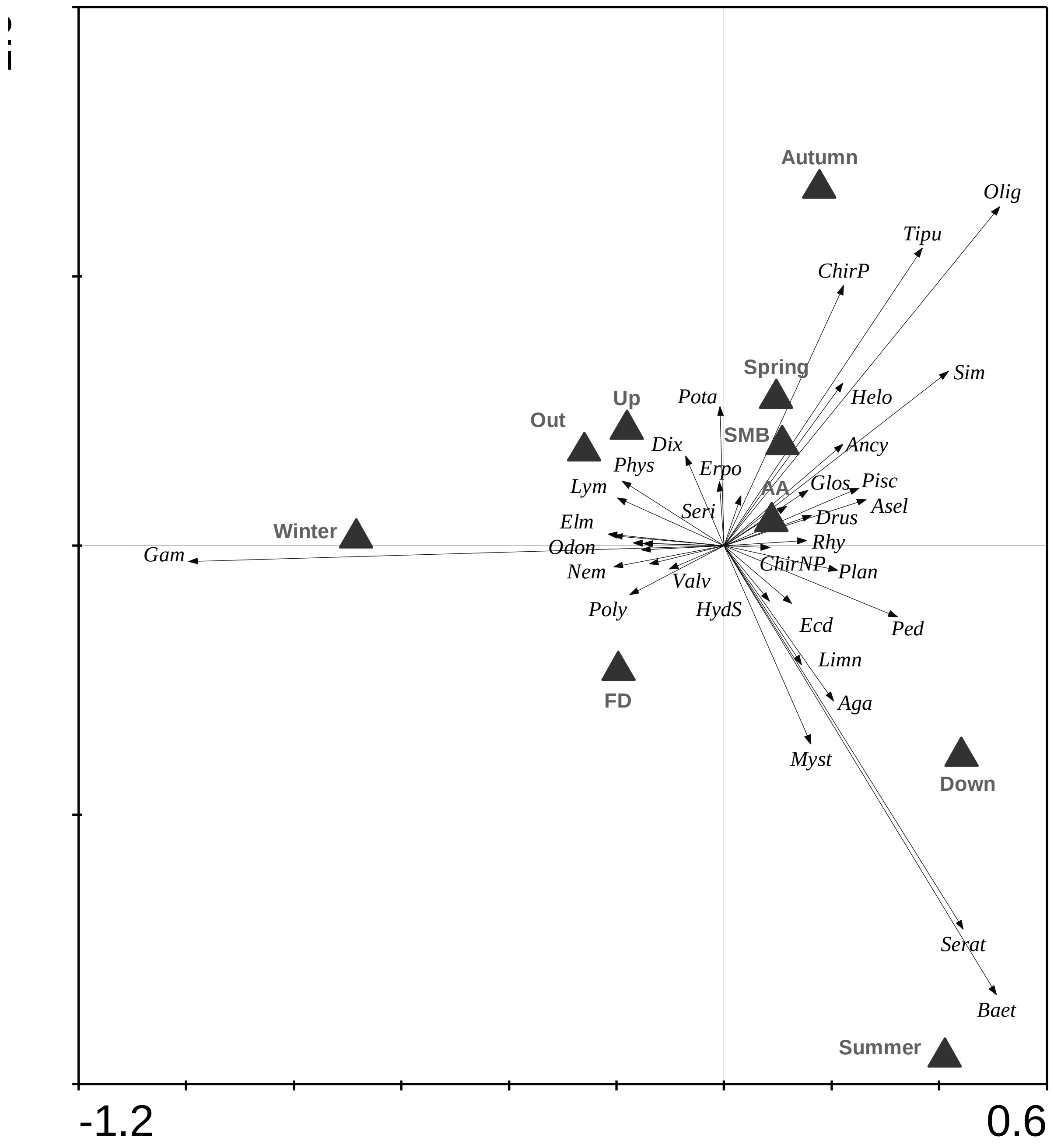


Figure 6.5a (and see 6.5b) PCA biplot of macroinvertebrate species (six surber samples / site) (arrows and small text) with site, stream and season (centroids and large text). Site, stream and season coded as dummy variables. N.B. two separate biplots used to display the species, environmental variables and centroids. Arrows display the correlation between species and the centroids which are oriented in the direction of maximum variation in value**.** Axis I (56.1%) & II (19.2%) accounted for 75.3% of the variation in the species data. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007).

Abbreviations.

i) Sites:
AA = Abbots Ann, FD = Fobdown, SMB = St Mary Bourne; Up = Upstream, Out= Outfall, Down = Downstream.

ii) Species codes:
Aga (*Agapetus fuscipes*); Ancy (*Ancylus fluviatilis*); Asel (*Asellus aquaticus* ); Baet (*Baetis rhodani* ); Cera (Ceratopogonidae); ChirNP (Chironomidae Non-predatory); ChirP (Chironomidae Tanypodinae);Chlor (*Chloroperla torrentium*); Crun (*Crunoecia irrorata*); Dix (*Dixella spp*); Drus (*Drusus annulatus*); Ecd (*Ecdyonurus venosus*); Elm (*Elmis aeanea*); Eph (*Ephemera danica*); *Eph* (Ephydridae); Erpo (*Erpobdella octoculata*); Gam (*Gammarus pulex* ); Glos (*Glossiphonia complanata*); Hale (*Halesus radiatus* ); Helo (*Helobdella stagnalis* ); Hyd *Hydroptilla* spp); HydS (*Hydropsyche siltalai*); HydP (*Hydropsyche pellucidula*); Lasi (*Lasiocephala basalis*); Leuc (*Leuctra innernis*); Limn (*Limnephilus* spp); Lym (*Lymnaea peregra*); Myst (*Mystacides azurea*); Nem (*Nemurella pictetii*); Odon (*Odontocerum albicorne*); Olig (Oligochaeta); Ped (*Pedicia rivosa*); Pisc (*Piscicola geometra*); Phys (*Physa fontinalis*); Pisi (*Pisidium* spp); Plan (*Planorbis leucostoma*); Poly (*Polycentropus flavomaculatus*); Pota (*Potamophylax cingulatus*); Potam (*Potamopyrgus antipodarum*); Rhy (*Rhyacophila dorsalis*); Serat (*Seratella ignita*_); Seri (*Sericostoma personatum*); Silo (*Silo nigricornis*); Sim (*Simulium* spp); Tin (*Tinodes waeneri*); Tipu (*Tipula* spp); Valv (*Valvata cristata*).

iii) Environmental variables:
Alky (Alkalinity); Ammonia; Chloride; COD (Chemical Oxygen Demand); Cond (Conductivity); Col (Colour); COrg (Organic Carbon); DO (Dissolved Oxygen); F (Fluoride); NOxid (Oxidised nitrogen); NA (Nitrate); NI (Nitrite); OP (Orthophosphate); SiO2; SS (Suspended Solids); P (Phosphate); Turb (Turbidity).

iv) Functional measures (denoted*):
BOD* (Biological Oxygen Demand); kC* (Decomposition in coarse litter bags per degree-day); kF* (Decomposition in fine litter bags per degree-day); C/F* (Ratio kC/Kf); NV* Chlorophyl *a* on Non Vaseline tile); V* (Chlorophyl *a* on Vaseline tile); V/NV* = Herbivory (Ratio V/NV). (See Methods for details).

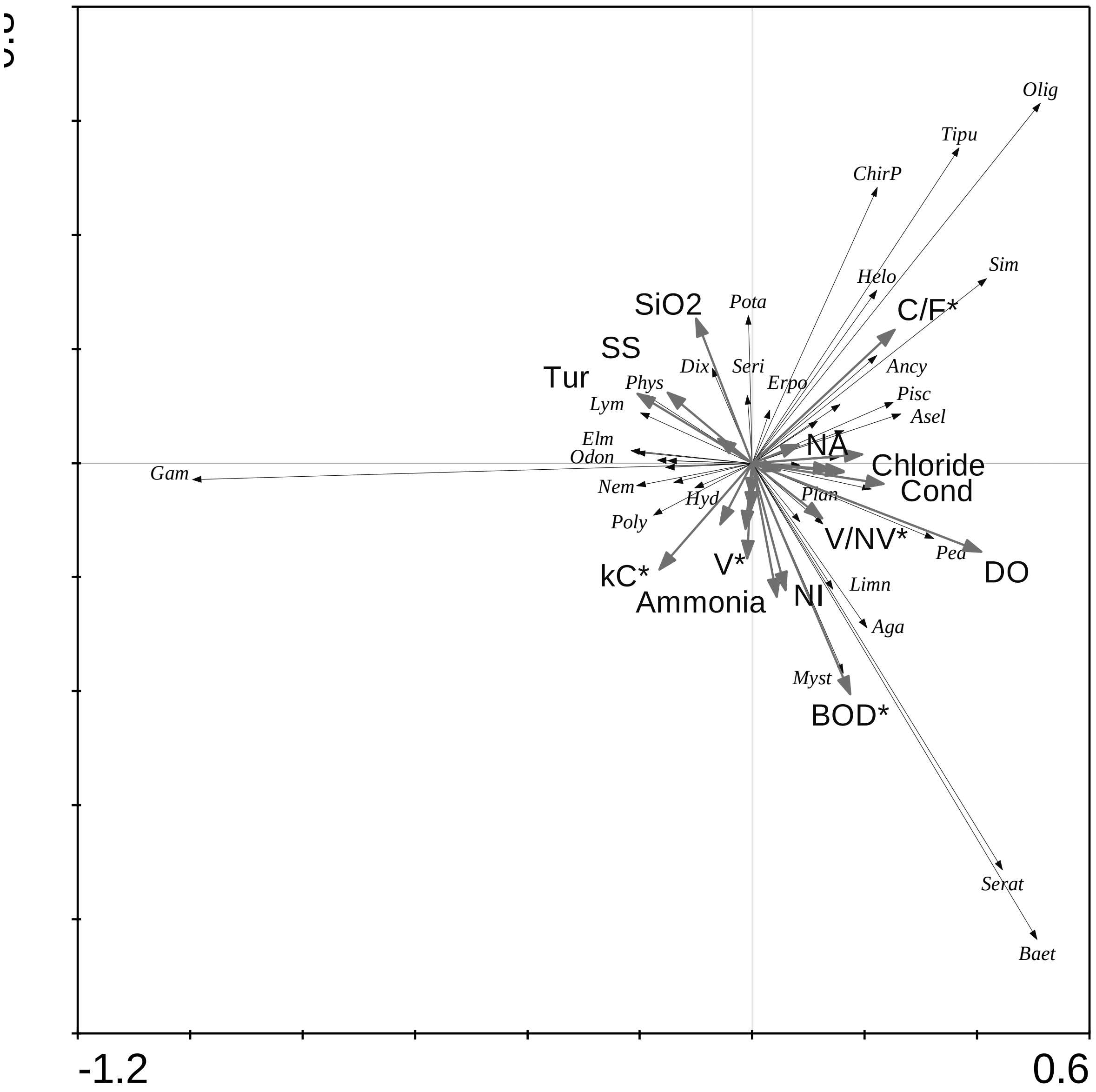


Figure 6.5b. PCA biplot of macroinvertebrate species (six surber samples / site) (small arrows and text) with environmental variables (large arrows and text). Site, stream and season coded as dummy variables. N.B. two separate biplots used to display the species, environmental variables and centroids see Fig. 6.5a. Arrows display the correlation between species and the environmental variables which are oriented in the direction of maximum variation in value**.** Axis I (56.1%) & II (19.2%) accounted for 75.3% of the variation in the species data. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Abbreviations: (see Fig 6.5 for abbreviations key).

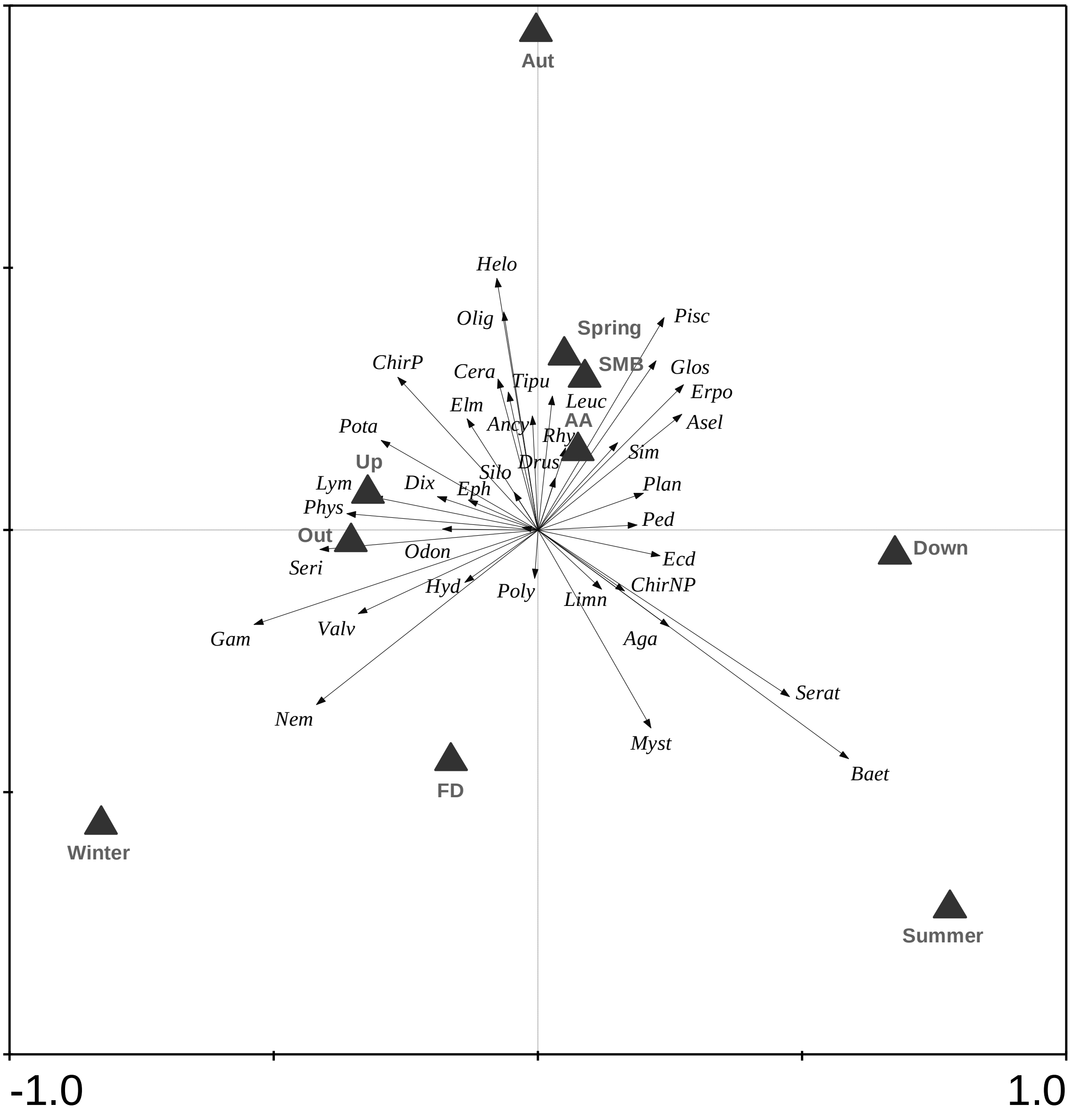


Figure 6.6a. (and see 6.6b.) RDA biplot of macroinvertebrate species (six surber samples / site) (small arrows and text) with season, stream and site (centroids and large text). Season, stream and site coded as dummy variables. N.B. two separate biplots used to display the species, season and site variables and centroids. (See Fig. 6.6b for environmental variable plot) Arrows display the correlation between species and the centroid which are oriented in the direction of maximum variation in value**.** Axis I (20.3%) & II (28.3%) accounted for 48.6% of the variation in the species data. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Abbreviations (See Appendix 6 for full lists).

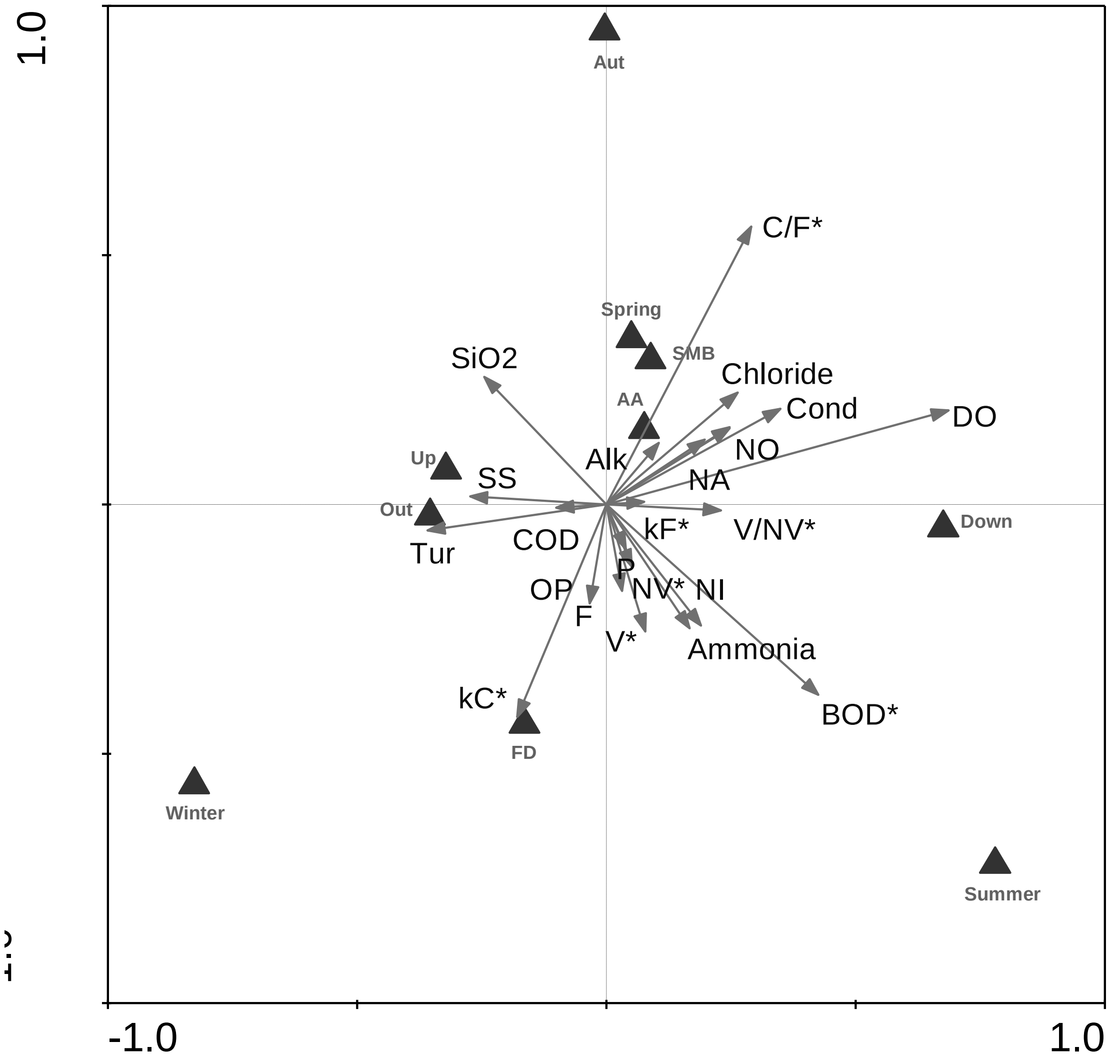


Figure 6.6b. RDA biplot of environmental variable (large arrows and large text) with season, stream and site (centroids and text). Arrows display the correlation between environmental variable and the centroid which are oriented in the direction of maximum variation in value**.** Axis I (20.3%) & II (28.3%) accounted for 48.6% of the variation in the species data. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). Abbreviations (See Appendix 6 for full lists).

***Summary of Temporal results (see Table 6.5).***

Leaf litter decomposition, leaf litter and benthic abundance revealed that the effects of watercress farming were contingent upon season and watercress farm. Specifically the results revealed no impacts of watercress farming at Abbots Ann and Fobdown outfalls. In contrast, leaf litter decomposition detected impacts at the SMB outfall, this was similar for leaf litter abundance but only in autumn, and for benthic abundance but in autumn and winter only. Downstream impacts were detected at Abbotts Ann and SMB by the decomposition measures, while Fobdown was impacted in summer only. Leaf litter and benthic abundance revealed impacts at Abbots Ann, but at SMB these impacts had recovered by the summer, as they had at Fobdown, but only for benthic abundance, while leaf litter abundance revealed impacts in summer only (Fig. 6.5 & 6.6). *G. pulex* were not consistently rarer at the outfalls of the study farms, revealing that *G. pulex* were not affected by watercress farm outfalls. However, *A. aquaticus* and *S. personatum* were less abundant at SMB than at Abbots Ann and Fobdown, suggesting that conditions may have not have completely improved at SMB. *G. pulex* abundance at SMB improved with each subsequent season at the outfall, but this effect is lagged downstream for leaf bags. A similar but more enhanced effect is seen in the benthos, but this is not reflected by leaf litter breakdown, which detected no improvement. The effects were inconsistent for algal accrual and herbivory at the farm outfalls. However, the overall top-down effects of grazers (herbivory) was evident (Fig 6.2). The shredding macroinvertebrate *G. pulex* was driving axis 1 of the PCA. A positive association with ammonia, nitrite, phosphate and BOD suggests that an organic pollution / nutrient enrichment gradient is driving axis 2. Axis 1 separated the streams and sites, while axis 2 separated the seasons.

|  | Abbots Ann |  | Fobdown |  | St Mary Bourne |  |
|---|---|---|---|---|---|---|
|  | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream |
| Leaf litter breakdown (Functional measure) |  |  |  |  |  |  |
| Autumn 2006 | X | ✓ | X | X | ✓ | ✓ |
| Winter 2006/7 | X | ✓ | X | X | ✓ | ✓ |
| Spring 2007 | X | ✓ | X | X | ✓ | ✓ |
| Summer 2007 | X | ✓ | X | ✓ | ✓ | ✓ |
|  | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream |
| Leaf litter macroinvertebrate abundance (Structural measure) |  |  |  |  |  |  |
| Autumn 2006 | X | ✓ | X | X | ✓ | ✓ |
| Winter 2006/7 | X | ✓ | X | X | X | ✓ |
| Spring 2007 | X | ✓ | X | X | X | ✓ |
| Summer 2007 | X | ✓ | X | ✓ | X | X |
|  | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream |
| Benthic macroinvertebrate abundance (Structural measure) |  |  |  |  |  |  |
| Autumn 2006 | X | ✓ | X | ✓ | ✓ | ✓ |
| Winter 2006/7 | X | ✓ | X | ✓ | ✓ | ✓ |
| Spring 2007 | X | ✓ | X | ✓ | X | ✓ |
| Summer 2007 | X | ✓ | X | X | X | X |

Table 6.5. Summary of results of Temporal watercress farms by measures deployed depicting if impacts are detected at the watercress (WC) farm or at the downstream site. Autumn 06 (December 2006); Winter 06 (February 2007); Spring 07 (April 2007); Summer 07 (June 2007). X denotes no impact detected; ✓ denotes impact detected.

## Results: Spatial study.

### *Physico-chemical parameters.*

Ammonia was in excess of its EQS in all streams and sites, perhaps indicative of the current ubiquitous nature of nitrogen pollution. The phosphate EQS was only exceeded at the Bere outfall, and at the SMB and Abbotts Ann outfall and downstream sites. Nitrite was in excess of EQS in all streams except upstream in the carrier stream at SMB and at all sites at the Meon. At the Meon these results maybe due to low levels of fertiliser application within the farm and low diffuse pollution. BOD was only elevated at the SMB outfall (Tables 2.1-2.5 pp. 65-71).

### *Functional measures: a) litter decomposition rates.*

Litter decomposition rates differed significantly among sites (LMEM $F\ df_{N2}, df_{D180} = 8.103$, $P = <0.001$); streams (LMEM $F\ df_{N5}, df_{D180} = 8.127$, $P = <0.001$) and by mesh type (LMEM $F\ df_{N1}, df_{D180} = 209.746$, $P = <0.001$). Total decomposition was significantly faster than microbial decomposition, (LMEM $F\ df_{N1}, df_{D180} = 209.746$, $P = <0.001$) emphasising the predominant influence of shredders (Table 6.6; Fig 6.7). The overall effects of watercress farming varied from site to site and were also not consistent across the study farms. This was revealed by the significant three-way interaction of stream x site x mesh (LMEM $F\ df_{N10}, df_{D180} = 4.863$, $P < 0.001$) and the significant interactions of stream x site (LMEM $F\ df_{N10}, df_{D180} = 6.513$, $P < 0.001$) and stream x mesh type (LMEM $F\ df_{N5}, df_{D180} = 8.241$, $P < 0.001$). However, there was no interaction for site x mesh type, which revealed the consistent effects of shredders versus microbes in response to the perturbation of watercress farming and suggests that shredders are not disproportionately affected by the perturbation. At Bere, Ilsington and SMB shredder impacts relative to microbial decomposition were suppressed at the outfall site. These negative impacts of watercress farming recovered downstream at Bere and Ilsington, but remained suppressed at SMB. While at Abbotts Ann, Fobdown and Meon, shredder relative to microbial impacts were comparable at the outfall suggesting that watercress farming was not negatively impacting these farms, but suppression was evident downstream at Abbotts Ann and Fobdown (Table 6.6 & Fig 6.7).

Table 6.6. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per degree-day (*$k_{dd}$*). Summer 2006 (June 2007), at sample sites, Up, Out, Down on six chalk streams. Significant results at *P*<0.05 are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 5 | 180 | 8.127 | **<0.001** |
| Site | 2 | 180 | 8.103 | **<0.001** |
| Mesh | 1 | 180 | 209.746 | **<0.001** |
| Stream x Site | 10 | 180 | 6.513 | **<0.001** |
| Stream x Mesh | 5 | 180 | 8.241 | **<0.001** |
| Site x Mesh | 2 | 180 | .264 | 0.768 |
| Stream x Site x Mesh | 10 | 180 | 4.863 | **<0.001** |

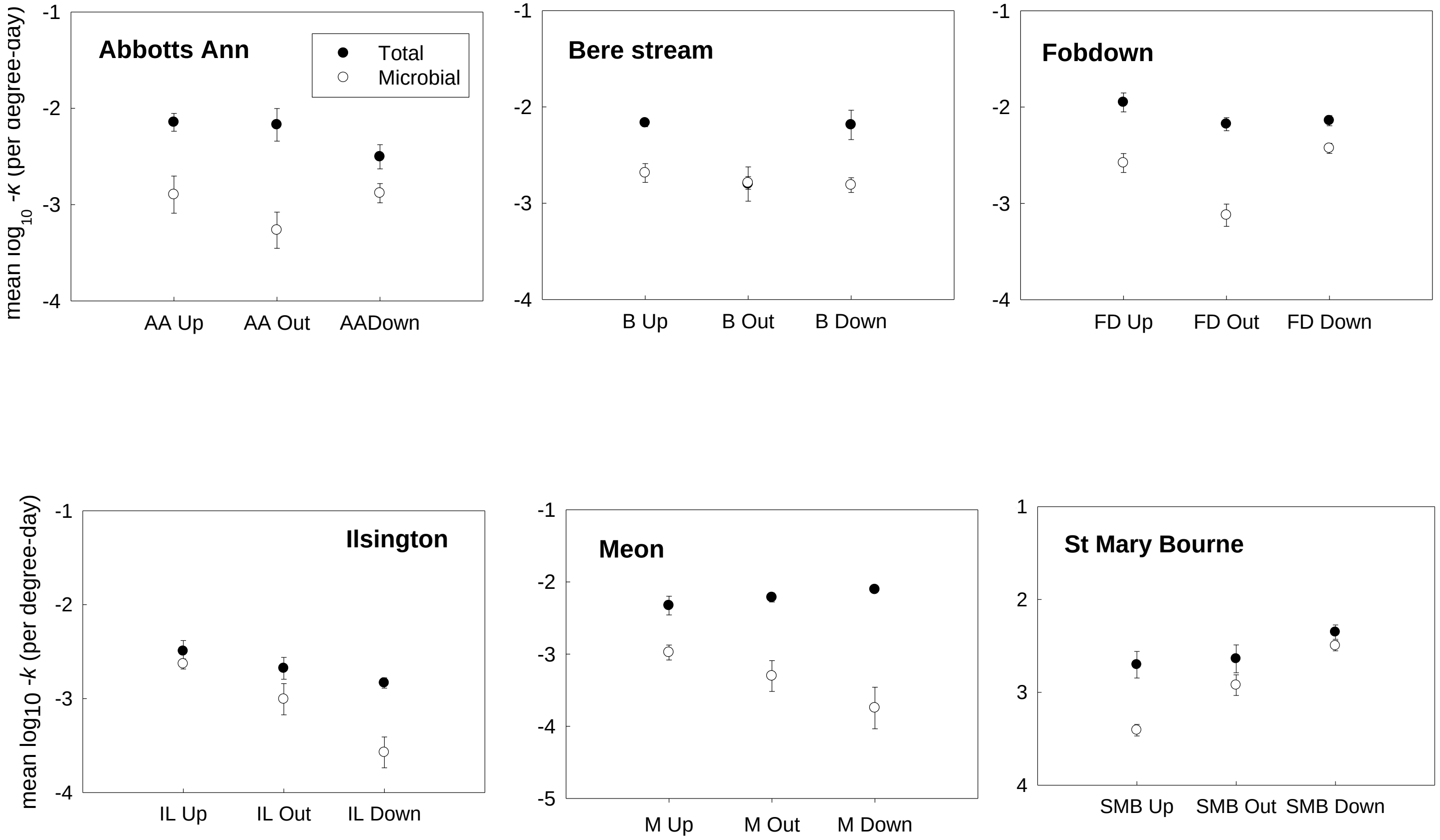


Figure 6.7. Log$_{10}$ mean leaf-litter decomposition rates per degree-day ($k_{dd}$) ($k$ ± SE). Summer 07 (June 2007), at sample sites, Up, Out, Down on six chalk streams. White circles denote microbial breakdown (fine mesh bags). Black circles denote total breakdown (coarse mesh bags). The closer together the separation of total (shredder + microbial) compared to the microbial breakdown data points, suggests sites were primarily microbially-driven, that the activity of shredding macroinvertebrates was suppressed, and that the sites were impacted, while dissimilar sites were shredder-driven.

***Functional measures b) algal production and c) herbivory.***

In general there was no distinct pattern in response to watercress farming from the algal accrual and herbivory measures. They reveal no consistent impact at the outfall compared to the upstream control sites, but rather they appear to be context-dependent and specific to each watercress farm. Algal accrual was significantly different for the two way interaction of: stream and site (LMEM *F df*N*10, df*D176 = 18.722, *P* < 0.001) revealing that the effects of watercress farming were not consistent but rather contingent upon farm and site. The top-down effects of grazers (herbivory) was evident as chlorophyll *a* biomass was higher on the ungrazed tiles than on the grazed tiles across all sites (LMEM *F df*N*1, df*D176 = 83.106, *P* < 0.001). The downstream sites have the strongest separation of grazed versus ungrazed tiles and there is evidence of an enrichment effect moving downstream (Table 6.7 & Fig 6.8).

Table 6.7. LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a (*mg cm-2) per degree-day (*dd*). Summer 2006 (June 2007), at sample sites, Up, Out, Down on six chalk streams. Herbivory = NV / V. V = exclusion of grazers through use of Vaseline ‘fence’ and NV = non exclusion. (Significant results at *P* <0.05 in bold).

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 5 | 176 | 30.426 | **<0.001** |
| Site | 2 | 176 | 5.734 | **0.004** |
| Herbivory | 1 | 176 | 83.106 | **<0.001** |
| Stream x Site | 10 | 176 | 18.722 | **<0.001** |
| Stream x Herbivory | 5 | 176 | 1.909 | 0.095 |
| Site x Herbivory | 2 | 176 | 0.087 | 0.917 |
| Stream x Site x Herbivory | 10 | 176 | 0.601 | 0.812 |

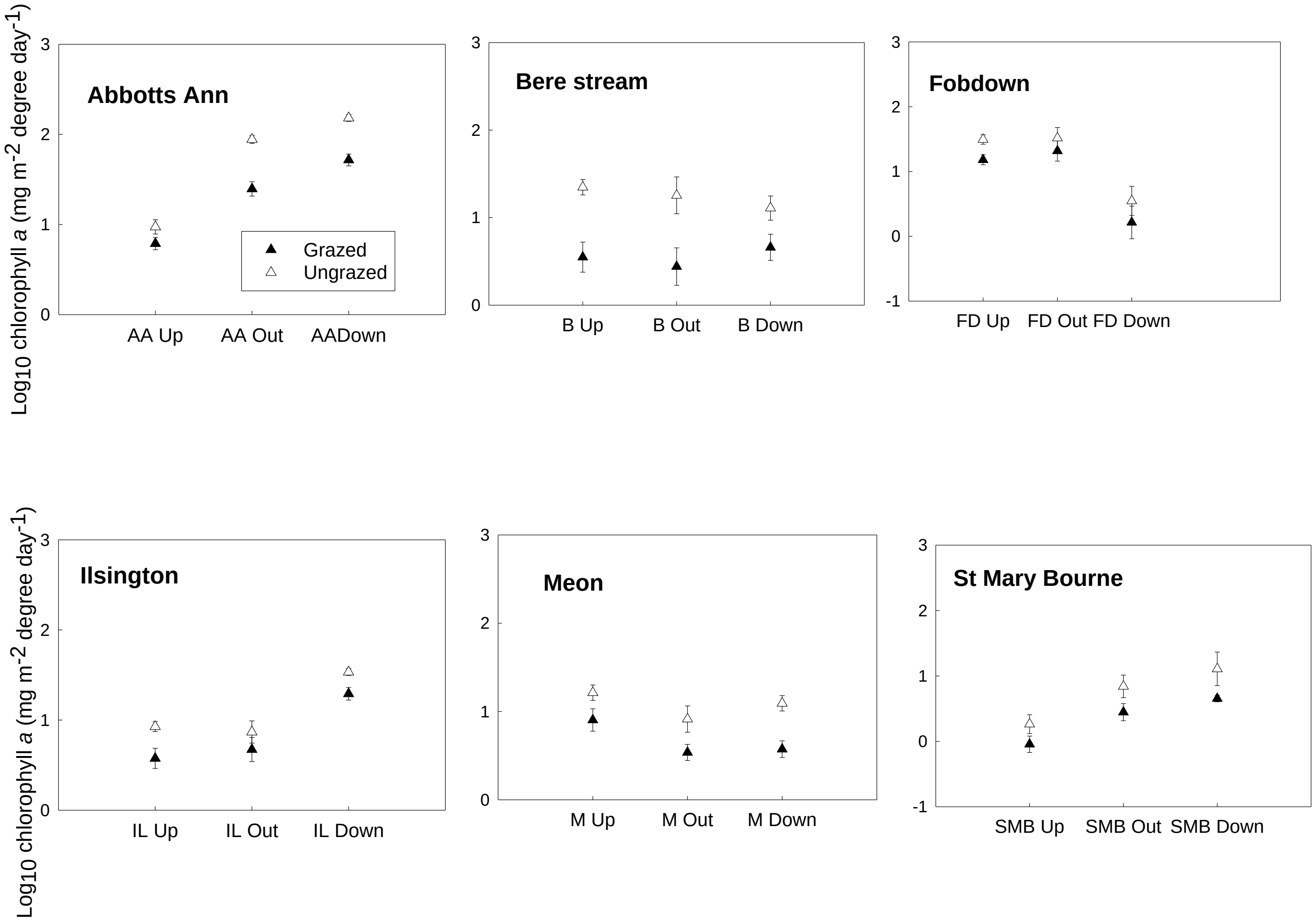


Figure 6.8. Log$_{10}$ chlorophyll *a* production *(*mg cm-2) per degree-day (*d*) (± SE). Summer 07 (June 2007), at sample sites, Up, Out, Down on six chalk streams. White triangles denote No Grazing (exclusion of grazers through use of Vaseline ‘fence’), black triangles denote Grazing (no exclusion of grazers)

### *Structural measures: a) macroinvertebrates in litter bags b) benthic macroinvertebrates.*

The most abundant shredding macroinvertebrates in the benthos and litter bags were *G. pulex*, *A. aquaticus* and *S. personatum* accounting for over 99% of all shredders and were therefore the main focus of the analyses.

*G. pulex* leaf litter abundance differed significantly among sites (LMEM $F\ df_{N2}, df_{D90} = 42.593$, $P = <0.001$) and streams (LMEM $F\ df_{N5}, df_{D90} = 35.760$, $P = <0.001$) (Table 6.8) and *G. pulex* benthic abundance differed significantly among streams (LMEM $F\ df_{N5}, df_{D90} = 11.673$, $P = <0.001$) (Table 6.9). Both abundance measures revealed that, *G. pulex* was not consistently rarer at the outfalls of the watercress farms than the other sites. To the contrary the opposite was true in certain cases. *G. pulex* were equally abundant at the outfall and upstream sites but became rarer downstream of the watercress farms (Fig 6.9 & 6.10). However, the interaction of stream and site (Leaf bags: LMEM $F\ df_{N10}, df_{D90} = 23.052$, $P < 0.001$; Benthos: LMEM $F\ df_{N10}, df_{D90} = 9.834$, $P < 0.001$) (Table 6.8 & 6.9) revealed that this did vary across streams, suggesting that where there was evidence of *G. pulex* suppression, it was context specific rather than a general feature and that the effects of watercress farming were contingent upon site and watercress farm. *A. aquaticus* and *S. personatum* exhibited no clear general pattern in response to watercress farming, but both were rarer than *G. pulex* and this effect is more pronounced in the benthos than in litter bags, where *S. personatum* are not necessarily rarer than *A. aquaticus* (see Table 6.8 & 6.9; Fig 6.9 & 6.10).

Table 6.8. LMEM results of comparisons of shredder densities in coarse litter-bags ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE), for *G. pulex* (G), *A. aquaticus* (A), *S. personatum* (S) and Total shredders (T). Summer 2007 (June 2007), at sample sites, Up, Out, Down on six chalk streams. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Stream | G | 5 | 90 | 35.760 | **<0.001** |
| | A | 5 | 90 | 29.938 | **<0.001** |
| | S | 5 | 90 | 7.679 | **<0.001** |
| | T | 5 | 90 | 23.543 | **<0.001** |
| Site | G | 2 | 90 | 42.593 | **<0.001** |
| | A | 2 | 90 | 47.771 | **<0.001** |
| | S | 2 | 90 | 5.169 | **0.008** |
| | T | 2 | 90 | 52.180 | **<0.001** |
| Stream x | G | 10 | 90 | 23.052 | **<0.001** |
| Site | A | 10 | 90 | 18.138 | **<0.001** |
| | S | 10 | 90 | 3.217 | **0.001** |
| | T | 10 | 90 | 21.165 | **<0.001** |

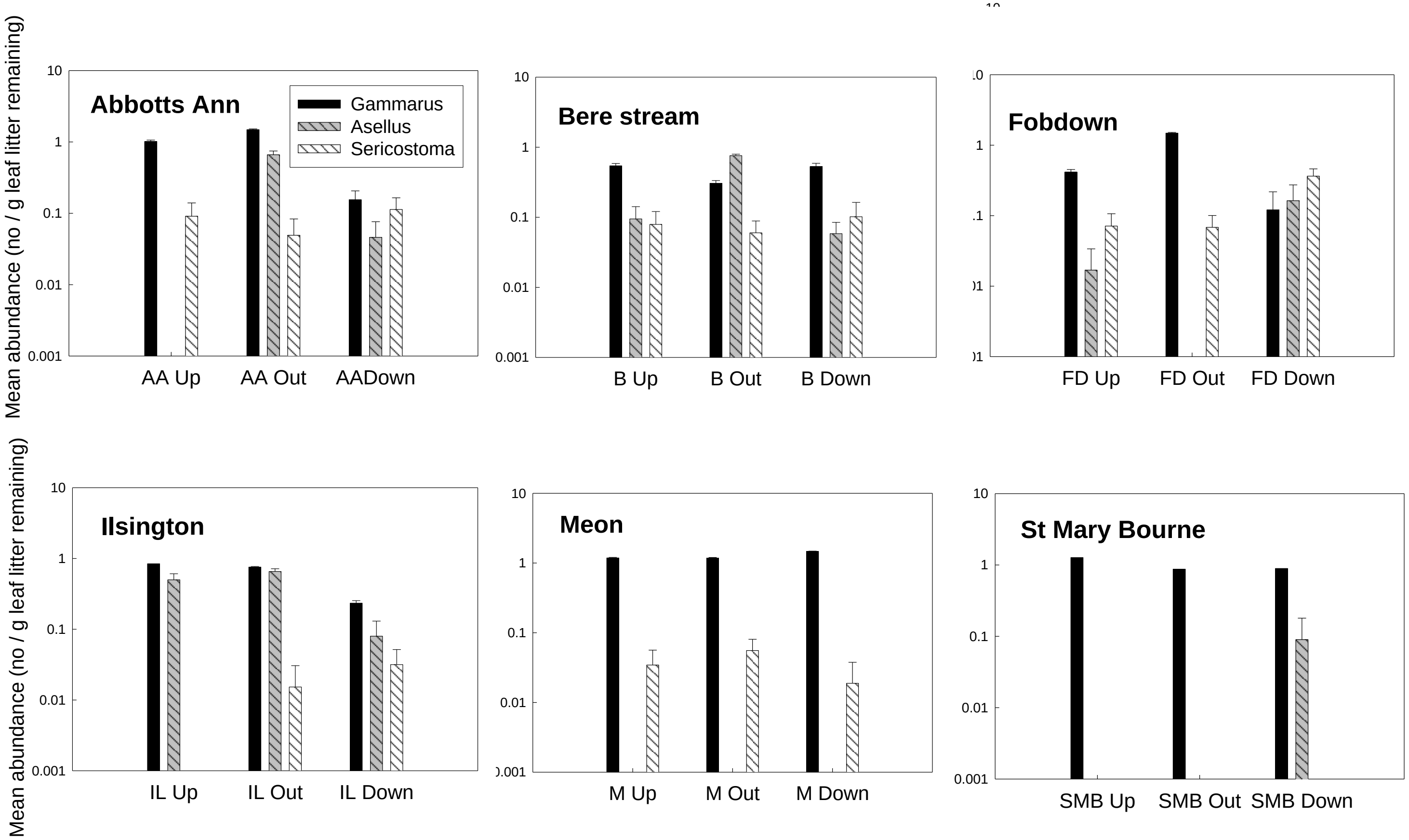


Figure 6.9. Shredder abundance in coarse litter-bags ($\log_{10}$ mean number $g^{-1}$ leaf litter remaining ± SE). *G. pulex*, *A. aquaticus* and *S. personatum.* Summer 07 (June 2007), at sample sites, Up, Out, Down on six chalk streams.

Table 6.9. LMEM results of comparisons of benthic shredder abundance (nos. m$^{-2}$) *G. pulex (G), A. aquaticus* (A), *S. personatum (S)* and Total shredders (T). Summer 2006 (June 2007) at sample sites, Up, Out, Down on six chalk streams. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|---|
| Stream | G | 5 | 90 | 11.673 | **<0.001** |
| | A | 5 | 90 | 13.716 | **<0.001** |
| | S | 5 | 90 | 4.627 | **0.001** |
| | T | 5 | 90 | 10.384 | **<0.001** |
| Site | G | 2 | 90 | 1.977 | 0.144 |
| | A | 2 | 90 | 31.642 | **<0.001** |
| | S | 2 | 90 | 2.580 | 0.081 |
| | T | 2 | 90 | 3.969 | 0.022 |
| Stream x Site | G | 10 | 90 | 9.834 | **<0.001** |
| | A | 10 | 90 | 9.042 | **<0.001** |
| | S | 10 | 90 | 2.728 | **0.006** |
| | T | 10 | 90 | 9.733 | **<0.001** |

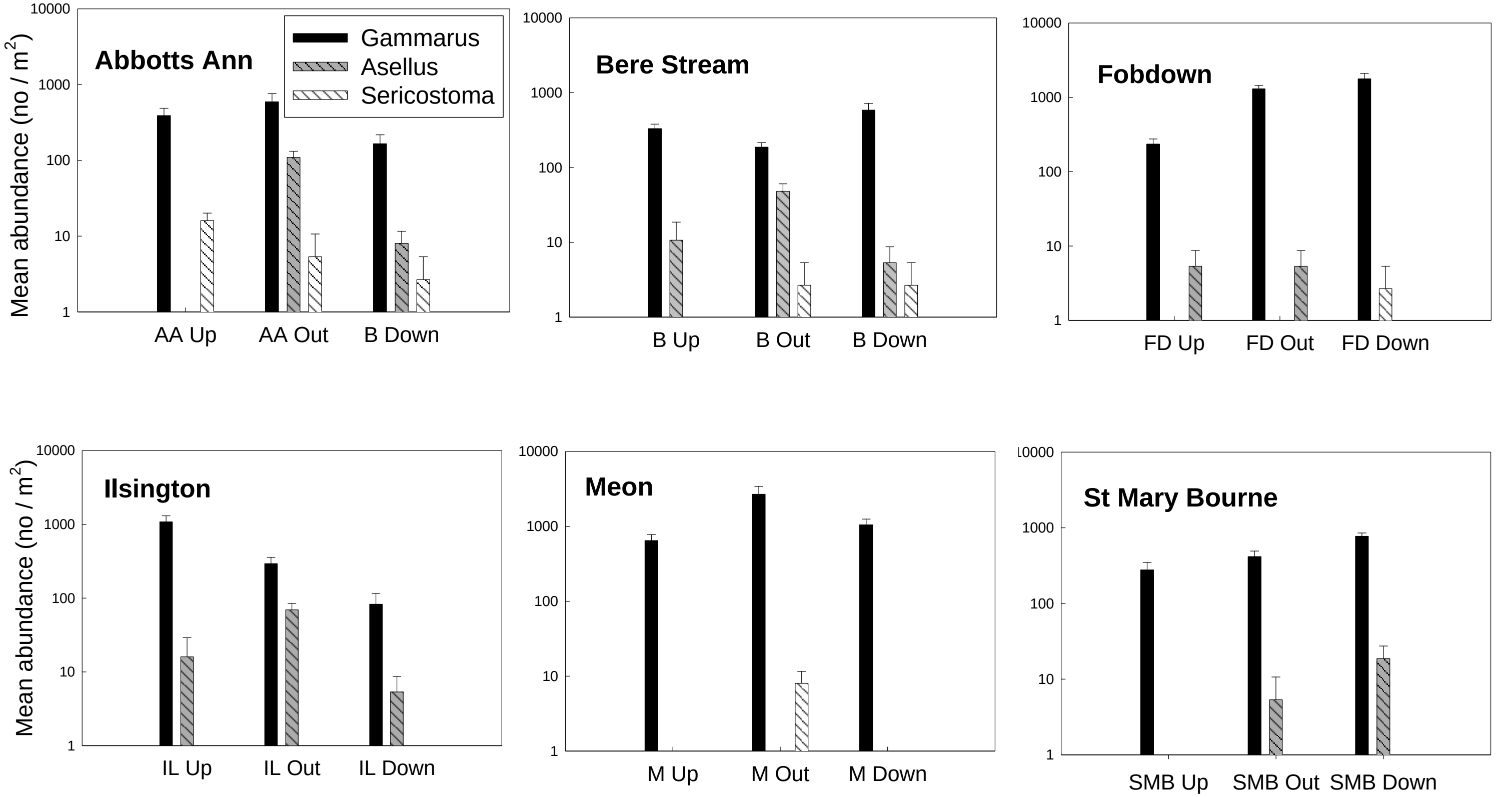


Figure 6.10. Benthic abundance (nos. m$^{-2}$ ± SE) of *G. pulex*, *A. aquaticus* and *S. personatum*, Summer 07 (June 2007); at sample sites Up, Out, Down on six chalk streams.

***Ordination combining chemical data with functional and structural measures.***

The PCA (Fig. 6.11) revealed that the relative abundance of *G. pulex* decreased strongly, along axis 1, this negative association with organic loading is well known (e.g. Friberg *et al.*, 2010). This accounted for the main differences and separated the sites, whereas axis 2 separated the streams from one another reflecting an organic pollution / nutrient enrichment gradient through its association with nitrate, ammonia, phosphate and organic carbon and the strong negative association of *B. rhodani* and *S. ignita*. Axis 1 (69.1%) and axis 2 (22.3%) together accounted for 91.4% of the total variation. A further PCA with sites and stream coded as dummy variables (Fig.6.12) highlights the overall varying, but more positive condition of the other watercress farms compared to SMB. An RDA with sites and streams fitted as the constraining variables (dummy coded) accounted for 29.5% of the total variation (axis 1: 13.4% and axis 2: 16.1%) revealing a similar pattern to that derived from the PCA, thus the direct and indirect ordinations were similar therefore all the important drivers were accounted for (Fig. 6.13).

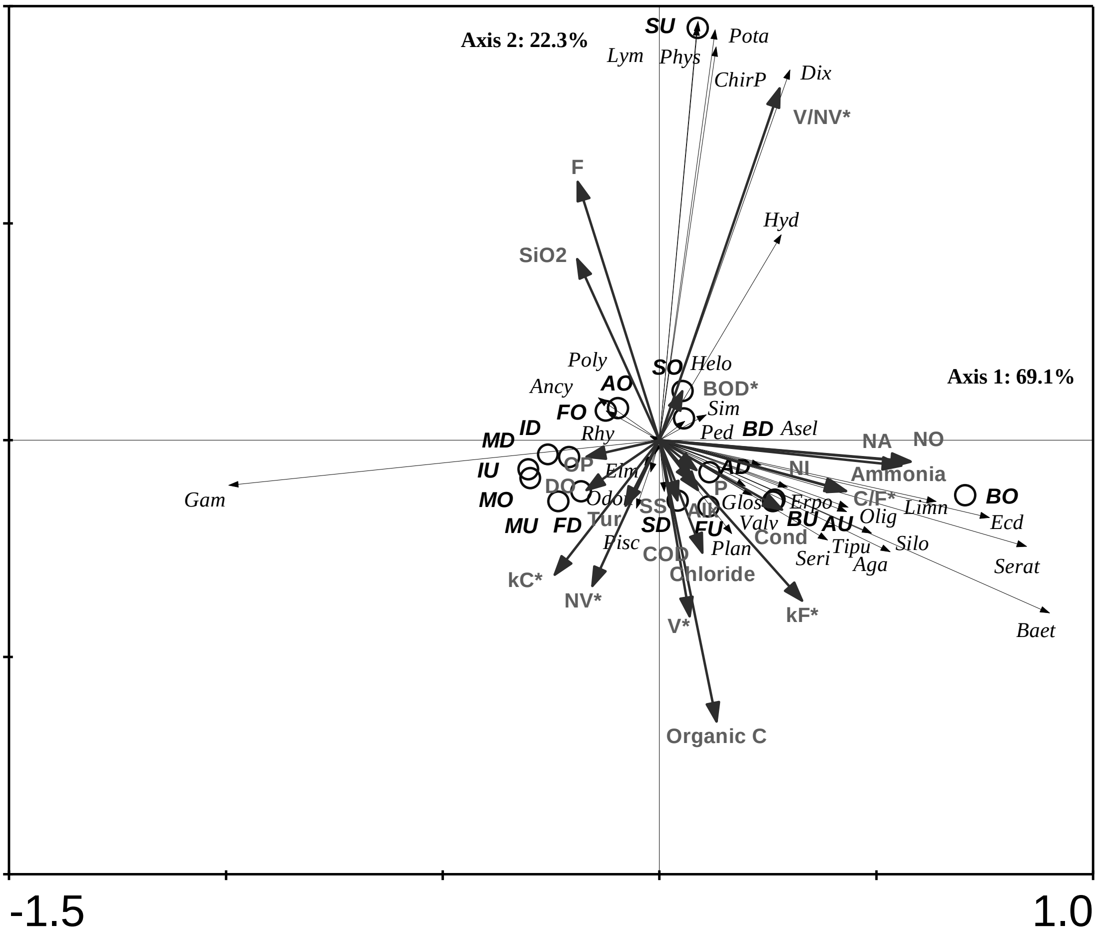


Figure 6.11. PCA triplot of macroinvertebrate species (six surber samples / site), environmental variables (vectors / arrows) and sites (Up, Out, Down) at six watercress farms (centroids). (Outlier site IO removed). Summer 2007 (June 2007). Arrows display the correlation between species and the environmental variables and are oriented in the direction of maximum variation in value**.** Axis I (69.1%) & II (22.3%) accounted for 91.4% of the variation in the species data.

Abbreviations:
i**)** Sites: A = Abbots Ann, B = Bere, F = Fobdown, I = Ilsington, M = Meon, S = St Mary Bourne; U = Upstream, O= Outfall, U = Upstream. (e. g. AU = Abbotts Ann, Upstream). (See Fig 6.7 for abbreviations key).

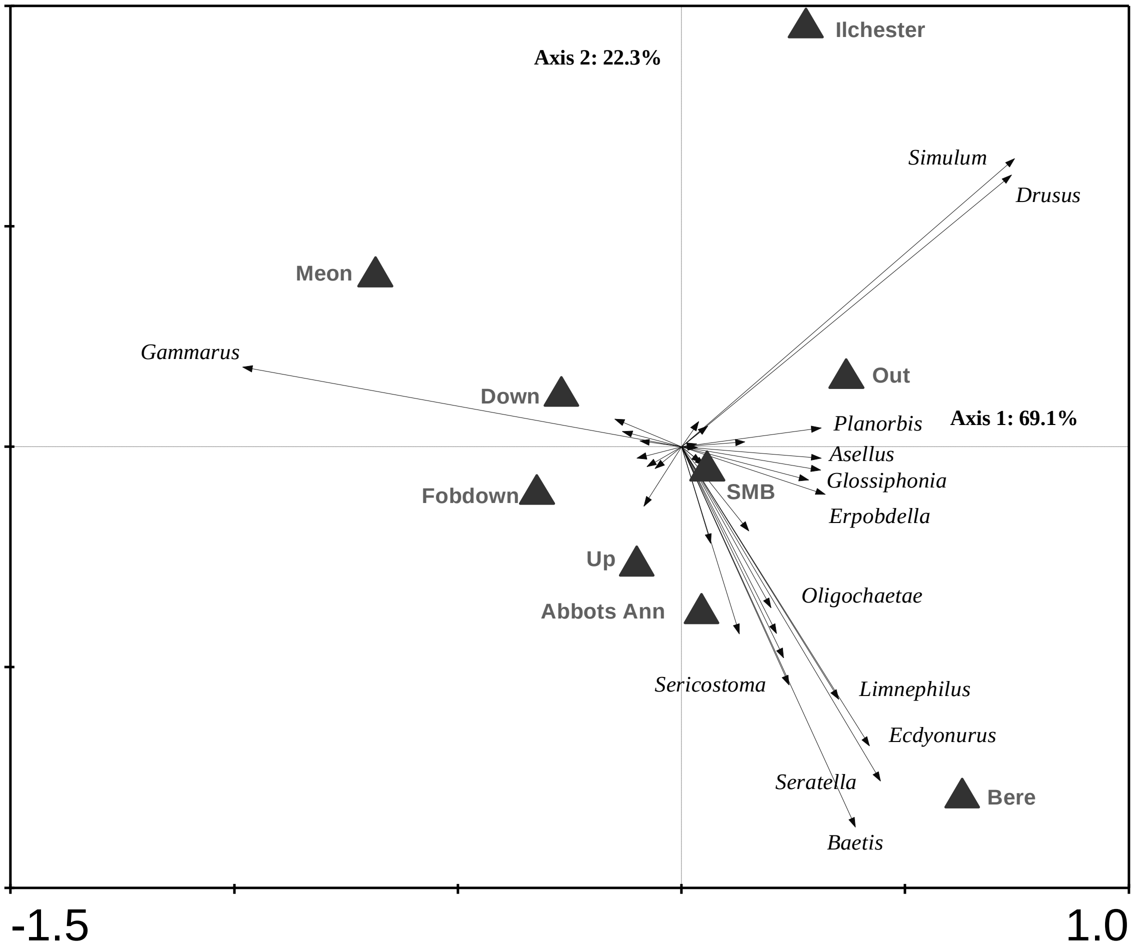


Figure 6.12. PCA biplot of macroinvertebrate species (six surber samples / site) (vectors / arrows) and sites (Up, Out, Down) at six watercress farms (centroids). Sites and stream coded as dummy variables. N.B. two separate biplots used to display the species, season and site variables and centroids (see Appendix 5 Fig. 1 for environmental variable plot) (Summer 2007 (June 2007). Arrows display the correlation between species and the site and stream variables and are oriented in the direction of maximum variation in value**.** Axis I (69.1%) & II (22.3%) accounted for 91.4% of the variation in the species data. Abbreviations see Appendix 6 for full lists).

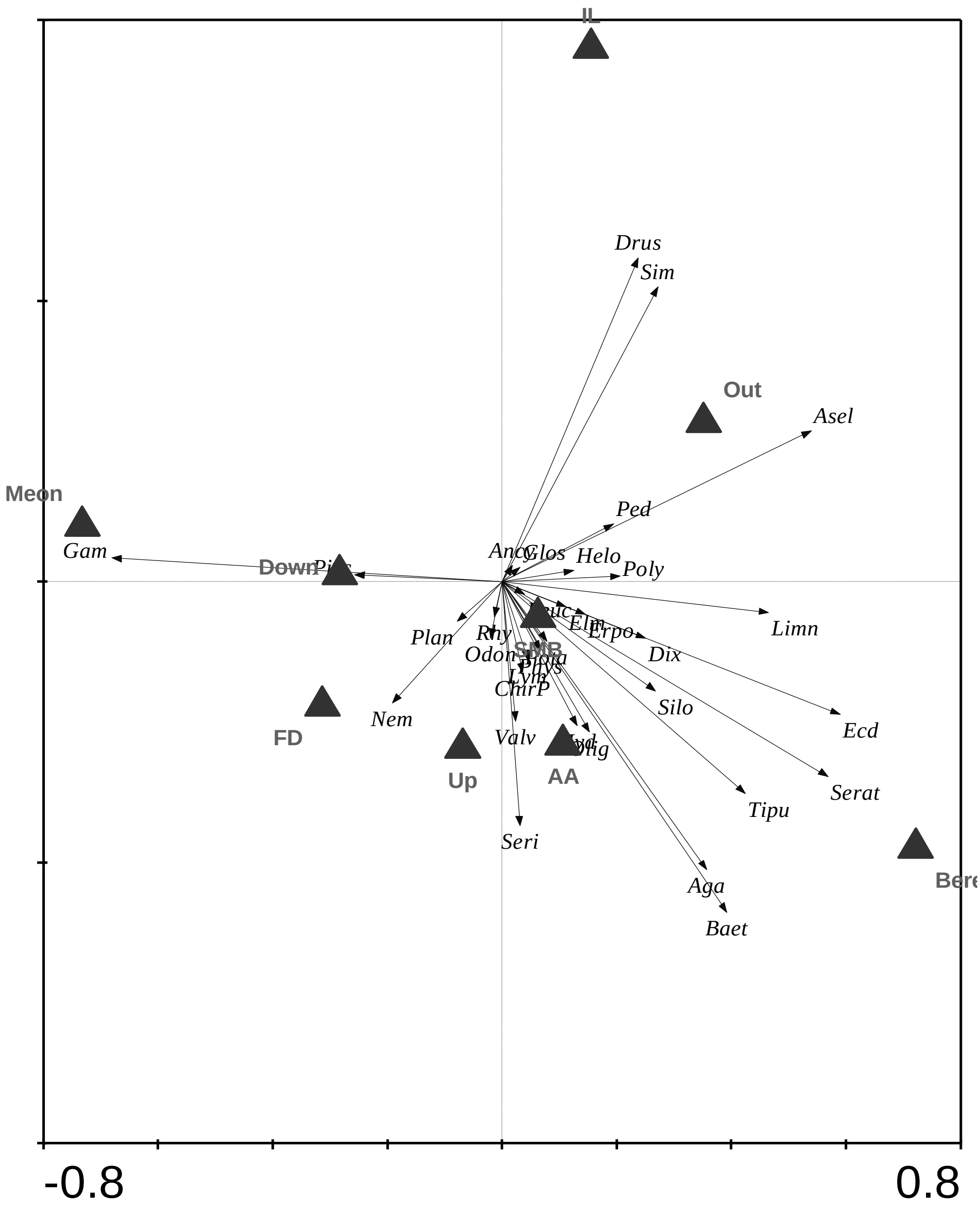


Figure 6.13. RDA biplot of macroinvertebrate species (six surber samples / site) (large arrows and text), and sites (Up, Out, Down) at six watercress farms (centroids). Summer 2007 (June 2007). Sites and stream coded as dummy variables. N.B. two separate biplots used to display the species, season and site variables and centroids. (See Appendix 5 Fig. 2 for environmental variable plot) Arrows display the correlation between macroinvertebrate species and environmental variables and are oriented in the direction of maximum variation in value. Axis I (29.7%) & II (11.9%) accounted for 41.6% of the variation in the species data. (Abbreviations See Appendix 6 for full lists).

***Summary of Spatial results (see Table 6.10).***

The leaf litter breakdown results suggest that Bere, Ilsington and SMB watercress farms were impacting their associated chalk stream with recovery evident downstream of Bere and Ilsington but not at SMB.  However, Abbotts Ann, Fobdown and Meon watercress farms were not impacting their associated chalk stream, but downstream effects were evident at Fobdown and Abbots Ann, but that these impacts were not attributable to watercress farming. Leaf litter abundance results revealed no impacts of watercress farming as did benthic abundance, but with the exception of Ilsington. Downstream impacts were revealed by leaf litter and benthic abundance at Abbots Ann and Ilsington, but only by leaf litter abundance at Fobdown. The other functioning measures algal accrual and herbivory were inconclusive with respect to the impact of watercress farming. Revealing no consistent impact at the outfall compared to the upstream sites, but suggests more intense macroinvertebrate grazing activity downstream compared to the outfall and upstream sites. PCA axis 1 was driven by *G. pulex* abundance and separated the sites, whereas axis 2 suggests an organic pollution / nutrient enrichment gradient, which separated the streams from one another. The PCA and RDA suggest that the extent of the negative impacts evident at SMB watercress farm are not reflected at other farms.

| | Breakdown | | Leaf litter abundance | | Benthic abundance | |
|---|---|---|---|---|---|---|
| Watercress farm | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream | Impact WC farm | Impact downstream |
| Abbotts Ann | X | ✓ | X | ✓ | X | ✓ |
| Bere | ✓ | X | X | X | X | X |
| Fobdown | X | ✓ | X | ✓ | X | X |
| Ilsington | ✓ | X | X | ✓ | ✓ | ✓ |
| Meon | X | X | X | X | X | X |
| St Mary Bourne | ✓ | ✓ | X | X | X | X |

Table 6.10. Summary of Spatial study results, at six watercress farm, in summer 2007 (June 2007), by metric, showing if impact was detected at the watercress (WC) farm or at the downstream site. X denotes no impact detected; ✓ denotes impact detected.

## Discussion.

Freshwater streams are energetically underpinned by a combination of, algal primary production and herbivory (autochthonous pathway), and detrital consumption mediated by bacteria, aquatic hyphomycetes, and macroinvertebrate shredders (allochthonous pathway). These basal energy inputs, their linkages (Hieber & Gessner 2002; Bernhardt & Likens 2004) and the flux of energy through them (Rooney 2006) are susceptible to environmental stressors, which may cause them to react in different ways and rates (Woodward 2009). In addition, changes in either pathway are not necessarily mirrored by the other and vice versa (Gessner & Chauvet 2002; Palmer & Febria 2012; Woodward *et al.*, 2012). The EU WFD (European Commission 2003) and US ACWA (EPA 2002) are legislative instruments that explicitly focus on the ecological status and integrity of waters, through the assessment of ecosystem structure and ecosystem functioning. Despite this and advocates within the research community (e.g. Gessner & Chauvet 2002; Hladyz *et al*., 2011a, b; Palmer & Febria 2012; Woodward *et al.,* 2012) the majority of lotic bioassessment still concentrates on structural measures. Bioassessment infrequently includes ecosystem structure together with either of the ecosystem functioning pathways (primary production or detrital) (e.g. Gulis, Ferreira & Graca 2006) and almost never with them all together (but see Hladyz *et al.,* 2011a, b). This study has addressed that imbalance in a series of comparative studies that included Europe’s largest watercress farm, the focus of earlier intensive study, within an extensive survey of other watercress farms. The combination of intensive (model systems) and extensive research (the comparative approach) used here, is a powerful one for population, community and ecosystems ecologists (Hildrew 2009). The comparative approach is essential for making empirical predictions, whereas model systems are useful for understanding the mechanistic basis of these predictions. Thus, by placing SMB in the context of a range of similar but contrasting systems, we can understand more than would have been apparent from simply studying it even more intensively, but in isolation (Hildrew 2009).

The reduced *G. pulex* abundance seen at SMB was not unique to this watercress farm, but this reduction was more pronounced at SMB, which suggests that different drivers operate at different farms and to a varying extent. This is important, as it undermines the practice in ecotoxicological studies of the extrapolation of optimised laboratory results to the field, which are conversely exposed to varying environmental conditions, one of the ongoing central problems in ecotoxicology (Kimball & Levin 1985; Vignati, Ferrari & Dominik 2007) (e.g.Worgan & Tyrell 2006).

The relative differences between macoinvertebrate and microbial mediated breakdown demonstrate the sensitivity of these detrital measures and their ability to discern low level impacts on these ecosystems, which is not a strong feature of the more commonly used structural measures e.g. macroinvertebrate abundance (Young & Collier 2009; Woodward *et al.,* 2012). These differential responses are indicative of the importance and need to include multiple measures of ecosystem pattern and process in the assessment of running waters (Woodward *et al.*, 2012; Palmer & Febria 2012). The pattern of detrital breakdown at the SMB watercress farm outfall suggests that the stressor responsible was affecting both the microbial and shredding macroinvertebrate detrital pathways in autumn, winter and spring, but that this agent or agents may have altered so as to affect only the shredding macroinvertebrate component in summer. Stream identity matters and the effects could be a reflection of the different management practices and farm size, manifest as a scale or a concentration effect induced by stressors. This is supported by the fact that SMB has an operating factory and is a considerably larger watercress farm at 6.9 hectares compared to the other farms at 3.2, 2.4, 1.2, 0.8 and 0.8 hectares, which may explain the effects seen at SMB compared to the other farms. Watercress annual output is related to size of the farm rather than any other factor (S. Rothwell pers.comm.). It might also be that only *G. pulex* are affected, but that other shredders "fill the gap" in terms of maintaining the leaf litter decomposition process rates, but this is not supported by the community data. However, what is probably more relevant, is that the SMB watercress farm also has a factory to pack and wash watercress and other salad leaves, and this is more likely be the defining difference and therefore the cause of the impacts from watercress farming at SMB. There may also have been farm and factory management practice changes made at SMB without our knowledge that could be attributable, but investigation has found no such activity, and all the changes that have been made, were made before this study in the summer of 2007. The change made in June 2006 to the sanitising process, when the use of chlorine and its treatment ceased within the factory, could offer another possible explanation. The cessation could have been exerting a lagged effect and may have taken until the following summer (2007) to fully register at the outfall. This is supported by the shredding macroinvertebrate litter bag and benthos abundance measures in this study, which detected an incremental improvement of these measures with each subsequent season. Further, the pattern affecting both the macroinvertebrate and the microbial detrital pathways, culminating in recovery at the outfall in the summer, discussed at the beginning of this section, also supports this explanation.

Streams within tracts of agricultural land can be subject to multiple anthropogenic stressors (Townsend, Uhlmann & Matthaei 2008), which can include raised nutrient concentrations, inputs of fine sediment and exposure to pesticides. These may modify community structure and biodiversity (Townsend, Uhlmann & Matthaei 2008) and alter ecosystem functioning, such as rates of algal accrual (Rasmussen, Friberg & Larsen 2008) or leaf breakdown (Young, Matthaei & Townsend 2008). The downstream impacts at SMB (breakdown suppression) may consequently be a response to diffuse pollution from the agricultural practices on the farm land either side of the stream, rather than the upstream watercress farm. This is supported by the elevated phosphate levels found only at the SMB downstream site in summer 2007 i.e. not at the upstream and outfall. The impacts here appear to only affect the shredding macroinvertebrate and not the microbial component of breakdown, a similar finding to other recent studies in response to stressors e.g. altered riparian vegetation (Hladyz *et al.,* 2011a, b), higher temperatures (Boyero *et al.,* 2011), pesticides (Schäfer *et al.,* 2012), pH (Riipinen *et al.,* 2010) and a similar pattern is also seen at both Fobdown and Abbotts Ann. All the farms have similar land use in their surrounding land; therefore it is a strong possibility that diffuse pollution, may well be responsible for the downstream impacts.  This reinforces the suggestion that river managers and freshwater ecologists need ‘‘to think outside of the stream’’ to consider the influences of the surrounding terrestrial environment, in an integrated approach taking into account the conditions and potential influences external to the river channel (Moss 2008; Woodward, 2009; Perkins *et al.,* 2010; Hladyz *et al.,* 2011a,  b). In a similar way that leaf breakdown is an integrative process, linking riparian vegetation with microbial and invertebrate activities (Young, Matthaei & Townsend 2008).

If SMB were being affected by multiple stressors, this could explain the impacts upon both the macroinvertebrate and microbial component within the farm, most likely caused by the factory and its processes, and the macroinvertebrate component only downstream, possibly due to diffuse pollution. This would also explain why other watercress farms are generally impact free at their outfalls, as they have no factory, and only exhibit macroinvertebrate component impacts downstream, possibly due to diffuse pollution from the agricultural land that bounds all the farms.

Upstream, in autumn at Abbots Ann, intriguingly breakdown and both abundance measures were impacted relative to the outfall, whereas we would have expected the opposite if watercress farming were responsible and these impacts recover with each subsequent season. This can be explained by the fact that in the summer and early autumn of 2006, in preliminary site visits prior to this study, the stream above the watercress farm, which was to be the upstream site, had temporarily dried. This is a common natural occurrence in winterbourne sections of chalk stream headwaters (Berrie 1992), however one likely to increase as a result of climate change (Edwards *et al.,* 2012), exacerbated by anthropogenic impacts e.g. over-abstraction (Agnew, Clifford & Haylett 2000), which can have a profound effect upon community structure (Matthaei, Piggott & Townsend 2010) and the functioning of aquatic ecosystems (Ledger *et al.,* 2011 & 2012). The only flows in the stream were downstream of the watercress farm, sourced from boreholes into the underlying aquifer for use within the farm; these were able to augment and at times such as this were the major source of flow at the outfall and downstream sites. Watercress farms while being a major abstractor do not actually consume all the water taken from the aquifer, the majority of the abstracted water is eventually returned to the carrier stream. It is a condition of watercress farms non-consumptive licence that at least 95% of the water is returned locally (Cox 2006). Importantly, they are able to maintain the flow in sections of chalk streams that might otherwise be susceptible to desiccation (see Casey 1981). Watercress farms thus can become the perennial head of the chalk stream and can potentially take the place of additional and costly augmentation schemes further downstream, e.g. the Candover and Arle augmentation schemes on the River Itchen (see Booker *et al.*, 2004).

Both Abbotts Ann and Meon watercress farms appear to not perturb leaf litter breakdown and if anything watercress farming enhances the shredder impacts relative to microbial decomposition, possibly as a result of the exclusion of fish from within the farms confines, but if this were the reason, then there would be evidence of this at the other farms too, however there is no evidence to support this. Further, downstream at Abbotts Ann shredder impacts relative to microbial decomposition were suppressed, while at the Meon they were enhanced. Fish predation might explain this reduction in shredder activity at Abbotts Ann, this is supported by the community measures evidenced as a decline in the abundance of all the major shredding macroinvertebrates progressing downstream through the farm. Predators can exert powerful indirect effects on primary production and herbivory (e.g. Power, 1990) and there is increasing evidence that fish can suppress leaf-litter decomposition rates by influencing the abundance and/or activity of shredders (e.g. Woodward *et al.*, 2008). Salmonids are voracious feeders and *G. pulex,* the major shredding macroinvertebrate found in this study, can form a large proportion of their diet in the wild (Maltby 1994). These headwater chalk streams, support large and extensive populations of trout (Mann, Blackburn & Beaumont 1989) and also *C. gobio* (bullhead) (Mann 1971) which favour *G. pulex* as prey (Mills & Mann 1990) (see Harrison, Bradley & Harris 2005). Fish predation may therefore also be a factor in the depletion of shredding macroinvertebrate populations downstream of the watercress farms. Perhaps the Pillhill brook being a tributary of the River Test famous for its sports fishing and that a trout fishery operates 1km downstream, may translate into there being more predatory fish in the stream below Abbotts Ann. Consequently the top-down effects of predation may not be as strong on the Meon where the community measures show high abundance of *G. pulex*. The general tendency across all streams for *G. pulex* to decline downstream might be a reflection of increased fish predation in the main stream. Fish population data and gut analysis studies, to include this higher predatory level, would be useful for testing this hypothesis and perhaps could be incorporated into future studies.

Leaf litter breakdown presents a different process orientated view of the effect of watercress farming than that from the community structure measures of benthic and litter bag abundance. These two structural measures also differ from each other in that the abundance of the shredding macroinvertebrates *A. aquaticus* and *S. personatum* were greater in leaf litter bags than in the benthos. Leaf litter is an important habitat for macroinvertebrates (Palmer *et al*., 2000) and this may be due to the attraction of the leaves as a food resource (Dobson 1994) and or as refugia during high flow events in the stream as a short term or seasonal response (Lancaster and Hildrew 1993). More importantly, however, this demonstrates that these two measures are similar, but are not measuring the same aspect of the same ecosystem. Algal accrual and herbivory, the other functioning measures reveal that the effects of watercress farming may not be as comprehensive as has been suggested by the other measures, it could be that these algal pathways are more robust to stressors (Odum 1985; Howarth 1991) (but see Niyogi, Lewis & McKnight 2002) suggesting that high algal biomass is a desirable state. This may be as a consequence of the fast energy flux and few linkages within algal food chains (Rooney *et al.,* 2006), which confer them with a higher degree of resilience to stressors, compared to the slower and more complex detrital pathways.

To summarise, the combination of multiple measures of ecosystem structure and functioning in this study revealed differential responses to watercress farming. Thus whilst they may be similar, they are not a substitute for one another, but are complementary, providing an alternative and more complete perspective, important in fully characterising ecosystem processes and their pattern (Palmer & Febria 2012; Woodward *et al.,* 2012). These were further enhanced in their explanatory ability when assessed in the context of a model system (SMB), within a range of similar but contrasting systems (temporal and spatial study) (Hildrew 2009). This study enabled us to i) demonstrate differential responses to anthropogenic impacts among a novel integrated range of ecosystem structure and functioning bioassessment measures ii) detect the development of ecological recovery, after decades of the impacts of watercress farming (Medgett 1998); iii) providing further evidence of organic pollution and suggesting the possible involvement of chlorine use iv) demonstrate that while there were impacts at certain farms, the magnitude and extent of the impacts of watercress farming were unique to SMB, and that these impacts were not consistent at the other watercress farms in this study.

The contrasting responses from these structural and functional measures can thus provide a synergy that would not have been evident from considering any one measure in isolation (Gessner & Chauvet 2002; Sandin & Solimini 2009). Consequently, this study highlights the value of integrated structural-functional approaches, which have yet to be actively adopted into current biomonitoring schemes, especially in lotic waters (Woodward 2009; Palmer & Febria 2012; Woodward *et al.*, 2012).

## References.

Agnew, C., T., Clifford, N., J. & Haylett, S., (2000) Identifying and alleviating low flows in regulated rivers: the case of the Rivers Bulbourne and Gade, Hertfordshire, UK. *Regulated Rivers: Research & Management*, **16**, 245–266.

Bernhardt, E., S. & Likens, G., E.. (2004) Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology,* **49**, 14-27.

Berrie, A., D., (1992) The chalk-stream environment. *Hydrobiologia,* **248**, 3-9.

Booker, D., J., Dunbar, M., J., Acreman, M., C., Akande, K. & Declerck, C., (2004) Habitat assessment at the catchment scale; application to the River Itchen, UK. In: Hydrology: Science and Practice for the 21st Century, Vol. II (Eds B. Webb, M.C. Acreman, C. Maksimovic, H. Smithers & C. Kirby) pp. 10– 18. Proceedings of the British Hydrological Society International Conference July 2004. British Hydrological Society, London.

Bonada, N., Prat, N., Resh, V., H. and Statzner, B., (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual review of Entomology,* **51**, 495–523.

Boulton, A., J. & Boon, P., I., (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over a new leaf? *Australian Journal of Marine and Freshwater Research*. **42**, 1–43.

Boyero, L., Pearson, R., G., Gessner, M., O., Barmuta, L., A., Ferreira, V. & Graca, M.,A.S., (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters,* **14**, 289–294.

Casey, H., (1981) Discharge and chemical-changes in a chalk stream headwater affected by the outflow of a commercial watercress bed. *Environmental Pollution, Series B,* **2,** 5, 373–385.

Casey, H. and Smith, S., M., (1994) The effects of watercress growing on chalk headwater streams in Dorset and Hampshire. *Environmental Pollution,* **85,** 2, 217–228.

Cox, J., Associates (2006) *Watercress growing and its environmental impacts on chalk rivers in England.* Report by Jonathan Cox Associates. English Nature Contract No. EIT-05-020 p 56.

Dobson M., (1991) An assessment of mesh bags and plastic leaf traps as tools for macroinvertebrate assemblages in natural leaf packs. *Hydrobiologia*, **222**, 19-28.

Edwards, F., Baker, R., Dunbar, M., & Laize, C. (2012) A review of the processes and effects of droughts and summer floods in rivers and threats due to climate change on current adaptive strategies. *NERC/Centre for Ecology & Hydrology,* Retrieved 28.08.2012 http://nora.nerc.ac.uk/18414/1/N018414CR.pdf

Environmental Agency (1992) *Invertebrate data Dorset / Wiltshire 1992*.

Environmental Agency (1995) *Invertebrate data Dorset / Wiltshire 1995*.

Environmental Agency (1997-1998) *Invertebrate data Dorset / Wiltshire 1997-1998.*

Environmental Agency (2004a) *Invertebrate data Dorset / Wiltshire 2004*.

Environment Agency (2004) *The State of England's Chalk Rivers*.

Environment Agency (2010). River Ecosystem (RE) Classification System. Retrieved 20.03.2010 http://www.environment-agency.gov.uk/research/planning/40297.aspx Retrieved 20.03.2010. http://www.environmentagency.gov.uk/static/documents/Research/river_e1.doc

Retrieved 28.03.2012 http://www.ashford.gov.uk/pdf/Appendix%203.2%20-%20River%20Ecosystem%20Classification%20System.pdf

European Commission (2003) *The new EU Water Framework Directive*. Available from: Retrieved 28.08.2012. http://ec.europa.eu/environment/water/water-framework/index_en.html

EPA (2002) Environmental Protection Agency. American clean water act (EPA ACWA). Available from: Retrieved 28.08.2012 http://epw.senate.gov/water.pdf

Friberg, N., Skriver, J., Larsen, S. E., Pedersen, M. L., Buffagni, A., (2010) Stream macroninvertebrate occurrence along gradients in organic pollution and eutrophication. *Freshwater Biology*, **55**, 1405–19.

Gessner, M., O. & Chauvet, E., (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications*, **12**, 498–510.

Gulis, V., Ferreira, V. & Graca, M., A., S., (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshwater Biology, **51**, 1655–1669.

Harrison, S., S., C., Bradley, D., C. & Harris, I., T., (2005) Uncoupling strong predator-prey interactions in streams: the role of marginal macrophytes. *Oikos*, **108**, 433-448.

H.C.C. (2000) Biodiversity Action Plan for Hampshire: Volume Two, Hampshire Biodiversity Partnership. Chalk streams. Retrieved 28.08.2012 from http://www.hampshirebiodiversity.org.uk/pdf/PublishedPlans/ChalkStreamsHAPjjDTP2.pdf

Hildrew, A., G., (2009) Sustained Research on Stream Communities: A model system and the comparative approach. *Advances in Ecological Research*, **41**, 175–312.

Hladyz, S., Tiegs, S., D., Gessner, M., O., Giller, P., S., Risnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. and Woodward, G., (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology*, **55**, 1916-1929.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M., O., Giller, P., S., Gulis, V., Hutton, S., A., Lacoursie`re, J., O., *et al.*, (2011a) Stream ecosystem functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages. *Advances in Ecological Research.* **44**, 211–276.

Hladyz, S., Abjornsson, K., Giller, P., S., and Woodward, G., (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology*, **48**, 443–452.

Holmes, N., T., H., (2006) The importance of long-term data sets in science and river management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **16**, 329 – 333.

Hopkins, J., Marcarelli, A. & Bechtold, H., (2011) Ecosystem Structure and Function are Complementary Measures of Water Quality in a Polluted, Spring-Influenced River n. *Water, Air, & Soil Pollution*, **214,** 409-421.

Howarth, R., W. (1991) Comparative responses of aquatic ecosystems to toxic chemical stress. In: Cole J, Lovett G, Findlay S, editors. Comparative analysis of ecosystems. New York: Springer-Verlag. p 169–95.

Kimball, K., D. & Levin, S., A., (1985) Limitations of laboratory bioassays: the need for ecosystem level testing. *Ecoscience*, **35**, 165–171.

Lancaster, J. & Robertson, A., L., (1995) Microcrustacean prey and macroinvertebrate predators in a stream food web. *Freshwater Biology*, **34**, 123–134.

Lamberti, G., A. & Resh, V., H., (1983) Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. *Ecology,* **64**, 1124–1135.

Ledger, M., E., Edwards, F., K., Brown, L., E., Milner, A., M. & Woodward, G., (2011) Impact of simulated drought on ecosystem biomass production: an experimental test in stream mesocosms. *Global Change Biology*, **17**, 2288–97.

Ledger, M., E., Brown, L., E., Edwards, K., E., Milner, A., M. & Woodward, G., (2012) Drought alters the structure and functioning of complex food webs. *Nature Climate Change,* 1-5. Retrieved 10.09.2012. from http://dx.doi.org/10.1038/NCLIMATE1684

Maltby, L., (1994) Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: *Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organisation* (Editors: Sutcliffe, D.W.). Freshwater Biological Association, Ambleside, UK.

Mann, R., H., K. (1971) The populations, growth and productions of fish in four small streams in southern England. *Journal of Animal Ecology,* **40**, 155-190.

Mann, R., H., K., Blackburn, J., H. & Beaumont, W., R., C., (1989) The ecology of brown trout *Salmo trutta* in the English chalk streams. *Freshwater Biology*, **21**, 57-7.

Martin, D., (2007) Environmental Agency Report: *A review of water quality in the upper reaches of the Bourne Rivulet between November 2004 and December 2006.*

Matthaei, C., D., Piggott, J., J. & Townsend, C., R., (2010) Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. *Journal of Applied Ecology,* **47**, 639-649.

McSwann, E., (2005) Environmental Agency Report: Invertebrate data for the Bourne rivulet 1989 - 2004.

Medgett, S., (1998) Environmental Agency Report: *The impact of St. Mary Bourne Cress Farm on the Bourne Rivulet.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Mills, C., A. and Mann, R., H., K. (1990) The bullhead Cottus gobio, a versatile and successful fish. 51st Annual Report Freshwater Biology Association pp.76-88.

Moss, B., (2008) The Water Framework Directive: total environment or political compromise? *Science of the Total Environment*, 32-41.

Niyogi, D., K., W., M. Lewis, Jr., and McKnight, D., M., **(**2002). Effects of stress from mine drainage on di-versity, biomass, and function of primary producers in mountain streams. *Ecosystems,* **5**, 554-567.

Odum, E., P., (1985) Trends expected in stressed ecosystems. *Bio-Science,* **35**, 419–22.

Palmer, M., A., Swan, C., M., Nelson, K., Silver, P. & Alvestad, R., (2000) Streambed landscapes: evidence that stream invertebrates respond to the type and spatial arrangement of patches. *Landscape Ecology,* **15**, 563–576.

Palmer, M., A. and Febria, C., M., (2012) The Heartbeat of Ecosystems, *Science.* **336**, 1393 -1394.

Perkins, D., M., Reiss, J., Yvon-Durocher, G., and Woodward, G., (2010) Global change and food webs in running waters. *Hydrobiologia,* **657**, 181-198.

Power, M., E., (1990) Effects of fish in river food webs. *Science,* **250**, 811–814.

Prenda, J., Armitage, P., D. & Grayston, A., (1997) Habitat use by the fish assemblages of two chalk streams. *Journal of Fish Biology,* **51**, 64–79.

Rasmussen, J., J., Friberg, N. & Larsen, S., E., (2008) Impact of lambda-cyhalothrin on a macroinvertebrate assemblage in outdoor experimental channels: implications for ecosystem functioning. *Aquatic Toxicology,* **90**, 228–234.

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P., & Dobson, M. (2010) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology,* 55, 346–359.

Roddie, B., Edwards, T. and Crane, M., (1992) Potential impact of watercress discharges on the freshwater amphipod Gammarus pulex L. *Bulletin of Environmental Contamination and Toxicol*ogy, **48**, 63-69.

Rooney, N., McCann, K., Gellner, G. and Moore, J., C., (2006) Structural asymmetry and the stability of diverse food webs. *Nature,* **442**, 265–269.

Sandin, L. & Solimini, A., G., (2009) Freshwater ecosystem structure-function relationships: from theory to application. *Freshwater Biology*, **54**, 2017–2024.

Schäfer, R., von der Ohe, P., Rasmussen, J., Kefford, B., J., Beketov, M., Schulz, R., Liess, M., (2012) Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environmental Science and Technology*, **46**, 5134−5142.

Sear, D., A., Armitage, P., D. & Dawson, F., D., H., (1999) Groundwater dominated rivers. *Hydrological Processes*, **11,** 255-276.

Shamsudin, L. & Sleigh, M., A., (1994) Seasonal changes in composition and biomass of epilithic algal floras of a chalk stream and a soft water stream with estimates of production. *Hydrobiologia,* **273,** 131-146.

Soulsby, P., G., (1975) Biological report; Summary report on cress farm investigations. Southern Water.

Statzner, B. & Bêche L., A. (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology,* **55,** 80-119.

Townsend, C., R., Uhlmann, S., S. & Matthaei, C., D., (2008) Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology,* **45**, 1810–1819.

ter Braak, C., J., F., &. Prentice, I., C., (1988) A theory of gradient analysis. *Advances in Ecological Research,* **18**, 271-313.

van Lanen, H., A., J., & Dijksma, R. (1999) Water flow and nitrate transport to a groundwater-fed stream in the Belgian- Dutch chalk region. *Hydrological Processes,* **13**, 295–307.

Vignati, D., A., L., Ferrari, B., J., D. and Dominik, J., (2007) Laboratory-to-field extrapolation in aquatic sciences. *Environmental Science and Technology*, **15**, 1067-1073.

Vymazal, J., (2006) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, **380**, 48-65.

White, S. & Medgett, S., (2006) Environmental Agency Report: *Bourne Rivulet Invertebrate Survey.* Environment Agency, Colvedene Court, Wessex Way, Colden Common, Winchester.

Whitehead, P., G., Wilby, R., L., Butterfield, D. and Wade, A., J., (2006) Impacts of climate change on in-stream nitrogen in a lowland chalk stream: an appraisal of adaptation strategies, *Science of the Total Environment*, **365,** 260 –273.

Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshwater Biol*ogy, **54**, 2171–2187.

Woodward, G., Jones, J., I. & Hildrew, A., G., (2002) Community persistence in Broadstone Stream (UK) over three decades. *Freshwater Biology,* **47**, 1419–1435.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R., B., (2008) Trophic trickles and cascades in complex food web: impacts of keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Woodward, G., Friberg, N. & Hildrew, A., G., (2009) Science and non-science in the biomonitoring and conservation of fresh waters. Chapter in *Freshwater Ecosystems: Biodiversity, Management and Conservation*. Nova Science Publishers, Inc., New York, U.S.A.

Woodward, G., Gessner, M., O., Giller, P., S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B., G., Tiegs, S., D., Cariss, H., Dobson, M., Elosegi, A. ,Ferreira, V., Graça, M., A.,,S., Fleituch, T., Lacoursiere, J., O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L., B., M. and Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science*, **336**, 1438-1440.

Worgan, A. & Tyrell, R. (2006) *Monitoring behavioural responses of Gammarus pulex to watercress oils.* Report for Vitacress Salads Ltd by the Centre of Ecology and Hydrology, (CEH), Dorset.

Wright, J., F., Armitage, P., D., Furse, M., T. and Moss, D., (1984) The classification of sites on British rivers using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie*, **22**, 1939–1943.

Wright, J., F., Gunn, R., J., M., Winder, J., M., Wiggers, R., Vowles, K., Clarke, R., T. & Harris, I., (2002b) A comparison of the macrophyte cover and macroinvertebrate fauna at three sites on the River Kennet in the mid 1970s and late 1990s. *Science of the Total Environment,* **282,** 121-142.

Young, R., G. &. Collier, K., J. (2009) Contrasting responses to catchment modification among a range of functional and structural indicators of river ecosystem health. *Freshwater Biology*, **54**, 2155–2170.

Young, R., G., Matthaei, C., D. and Townsend, C., R., (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society,* **27**, 605-625.

# Chapter 7.

## General Discussion.

This thesis has sought to develop the depth of understanding of the two ecosystem functioning pathways (allochthonous and autochthonous) (processes) when used alongside measures of structure (pattern). They demonstrate their differing, yet complementary responses to stressors in the field, which collectively can offer a more comprehensive, sensitive and cost effective approach than most other bioassessment schemes currently available.

Different responses from the measures reveal that the basal energy pathways are susceptible to environmental stressors, but that this is manifest in different ways and rates (Woodward 2009), which are not necessarily mirrored by the other and vice versa (Gessner & Chauvet 2002; Palmer & Febria 2012; Woodward *et al.*, 2012). It is therefore vital that multiple ecosystem process rates are quantified together with measures of community structure (Riipinen *et al.*, 2010; Hladyz *et al.*, 2011a, b), thus ensuring that all the contributory and explanatory features of an ecosystem are taken into consideration in their assessment, prior to arriving at any conclusions. Functional indicators can also be sensitive and thus have utility in discerning low levels of impacts, which is often problematic using conventional structural indicators (Young & Collier 2009; Woodward *et al.*, 2012). Thus they can detect the onset of recovery from degraded states (e.g. this study), and can also be useful following river restoration (Palmer *et al.*, 2005).

Specifically, initial reduced leaf litter breakdown rates and *G. pulex* abundance at the intensively studied watercress farm, the focus of this study, did not respond to bioremediation or its lagged effect, designed to treat the perceived effects of PEITC, a natural allelochemical present in watercress responsible for its characteristic peppery taste (Newman 1991), one month later or after continuing for a year. However, evidence of recovery was detected in response to altered farm management practices (Magbanua *et al*., 2010), principally the cessation of chlorine use, which improved with each subsequent season from autumn 2006 onwards. PEITC was therefore discounted as the stressor, but rather, chlorine use was determined to be the more likely choice as the source of the perturbation. Further comparative studies (sensu Hildrew 2009) at SMB together with other watercress farms, revealed that reduced leaf litter breakdown and *G. pulex* abundance were not all unique to SMB and that these impacts were not consistent across all the other watercress farms in the study. This suggests that different drivers operate at the different farms, and to a varying extent.

The field of ecotoxicology is experiencing a surge in attention among ecologists, as current research has led to a better understanding of how contaminants can impact natural ecosystems such as watercress farms. For example, the application of insecticides to aquatic ecosystems can reduce invertebrate diversity and abundance, decrease decomposition rates and increase primary productivity (Relyea 2005; Schafer *et al.*, 2007). The assessment of the relationship between ecosystem structure and functioning, and toxicity studies (Forbes & Forbes 1993) has been advocated for many years to enable a better understanding of the connection between fate and effect of contaminants (Forbes & Kure 1997). However, the focus of the majority of ecotoxicology research in lotic systems to date, has been to determine how community structure is altered (e.g. Relyea & Hoverman 2006; Muñoz 2009), rather than ecosystem functioning (e.g. Wallace *et al.*, 1997; but see Schafer *et al*,. 2007) and the measures demonstrated in this study may be useful in the development of this field, particularly when integrated within *in situ* studies.

The *in situ* studies in Chapters 4 and 5 manipulated and assessed the stressed ecosystem while at the same time as being exposed to the complete range of environmental conditions encountered in the field, which laboratory studies are unable to reproduce. The disparity between this study and those presented by earlier ecotoxicological studies into PEITC (Worgan & Tyrell 2006), highlights one of the ongoing central problems in ecotoxicology, that laboratory studies take place under optimal conditions but in nature are exposed to a wide range of varying environmental conditions (Persoone *et al.* 1989; Adams 2003), and emphasises the potential pitfalls of extrapolating directly from the laboratory to the field (Kimball & Levin 1985; Vignati, Ferrari & Dominik 2007). The practical application of *in situ* ecosystem-based bioassays (e.g. Lauridsen & Friberg 2005; Zhou *et al.*, 2008) can thus generate a more realistic representation with which to steer managers, albeit that this research area is still in its early stages (Woodward *et al* 2012; Palmer & Febria 2012).

Many fresh waters are exposed to multiple stressors (e.g. Niyogi *et al.*, 2007; Ormerod *et al*., 2010), which can act synergistically on important ecosystem processes (Rasmussen *et al.*, 2011) and the consequences of stressors are often predicted on the basis of knowledge of single effects (Townsend *et al.*, 2008). Therefore, if water resources managers only consider the effects of individual stressors, their assessment of risk may be higher or lower than in reality (Matthaei, Piggott & Townsend 2010).

The reduction and marked improvements in organic pollution, the dominant stressor of interest in biomonitoring programmes for decades, particularly in western european lotic waters (e.g. Hildrew & Statzner 2010), has led to secondary stressors, that were previously masked, revealing themselves. This presents a challenge to regulators and water managers if they are to maintain and improve the ecological quality status of freshwaters in line with legislation and to also achieve their environmental quality targets (Friberg *et al.,* 2011). The requirement for novel, sensitive and cost effective bioassessment tools is one that leaf-litter breakdown in conjunction with other functioning and structural measures could be ideally suited.

### ***Caveats and future directions.***

The exact role of chlorine is not clear in the perturbations at SMB, but what is clear is that the freshwater shrimp *G. pulex* has made a remarkable comeback to the waters of the Bourne rivulet, below the SMB watercress farm. Where for the previous thirty years or more, there had been severe disruptions to communities of the usually dominant and ubiquitous freshwater shrimp *G. pulex*. It would therefore be of ecological importance and a key research goal to reassess these waters with these measures to examine what effect the passage of time has had on these unique, valuable and iconic ecosystems, since these studies were concluded.

We were limited by financial, logistical, time and manpower constraints. Flows were not logged during the course of the study and water chemistry was confined to once during each sampling period. The fact that PEITC could not be measured and that chlorine and its derivatives were not measured left gaps in our ability to categorically identify their implication. However, we were able to infer negative impacts from our experiments.

The role and value of bioassessment schemes may now need to be reappraised and redesigned unless used alongside measures of ecosystem functioning. Structural measures are not sufficient on their own, particularly as biota can fulfil multiple trophic roles and with varying levels of intensity, and are thus unable to capture the dynamic properties of an ecosystem that represent its actual performance (Palmer & Febria 2012). Whereas, structure and functioning measures when applied together, encompass most of the contributory and explanatory features of an ecosystem, therefore these measures are not mutually exclusive. From a cost perspective, the leaf-litter and algal tile methods are relatively inexpensive and certainly less time consuming than community measures, particularly when taking the taxonomic expertise required into consideration. Another method that has the potential to be of use is whole system metabolism, which measures the combination of gross primary production (GPP) and ecosystem respiration (ER) (Yvon-Durocher *et al.,* 2012). GPP and ER require more sophisticated equipment than measurements of algal accrual and leaf breakdown, but improvements in technology have made these metabolism measurements relatively easy and cost effective (review Young, Matthaei & Townsend 2004; Young, Matthaei & Townsend 2008; Yvon-Durocher *et al.,* 2012). Other methods that quantify key biophysical processes include pollutant removal rates or sequestration and nutrient cycling or flux rates; these may be useful in ascertaining the best measure for a particular stressor and environmental conditions and will require further research to establish (Palmer & Febria 2012).

### ***Conclusions.***

The watercress farming industry under the stewardship of the EA and the action of pressure groups has made considerable efforts to minimise and control their impact upon chalk streams, to the extent that the majority of watercress farms now have little negative impact. The exception has been at the SMB watercress farm, which up until 2006 remained the only known farm where a lack of *G. pulex* abundance persisted directly below its outfall. *G. pulex* has now re-established its presence, but there is still a danger that this may change. At SMB, uniquely a factory exists alongside a watercress farm which processes (washes and packs) substantial volumes of watercress and other salad leaves. The potential threat is from the scale of the operation, the introduction of invasive species and pathogens from the washing and packing of salad products from overseas (Africa, North America and mainland Europe), the escape of toxic chemicals and substances used and stored on the site, the proximity of the Bourne rivulet to the site and their simple hydrological connection. All of which will need to be addressed to ensure that the substantial improvements made by VSL at SMB are maintained.

This study also highlights the lack of winterbourne and headwater studies, in particular chalk streams. These unique and rare ecosystems need to be characterised using metrics of ecosystem structure and functioning, preferably including examples of “pristine” chalk stream winterbournes such as the River Wissey in Norfolk, described as a benchmark chalk river by the EA (1998). A pristine winterbourne such as this could serve as a potential reference site, for comparison with and for river managers to benchmark against, within their river management plans.

Climate change will have a major effect upon rivers, including chalk rivers and streams (Whitehead *et al.,* 2006). Chalk streams will be particularly susceptible as up to 90% of their discharge is from groundwater (Casey, Smith, & Clarke 1990; Berrie 1992). Future global warming is predicted to increase and faster than at current rates, with a global rise of between 1.1 and 6.4 $^{0}$C by 2099 depending on the levels of greenhouse gas emissions (IPCC, 2007a). However, even at current levels of greenhouse gas emissions, past emissions could contribute to a further 0.5 $^0$C rise in global temperature (Wigley, 2005). Increases in air temperature directly equate to an increase in water temperature, for example French rivers have increased by 2.6 $^0$C between 1979 and 2003 (Daufresne & Boet, 2007), and Welsh streams by 1.4 $^0$C between 1981 and 2005 (Durance & Ormerod, 2007). It is predicted that the extent of drought affected areas will not only increase, but that heavy precipitation events, which are very likely to increase in frequency, will exacerbate the risk of flood (IPCC, 2007b). There is little data on how droughts and floods might impact ecosystem functioning, other than extrapolating from known effects to physical habitats and community structure (Edwards *et al.*, 2012). Further research is therefore needed to improve our understanding of the links and relationship between climate change, ecosystem structure, species composition and ecosystem functioning.

The development and integration of alternative, sensitive and cost effective bioassays such as those demonstrated in this study may provide the tools urgently required to continue to improve and maintain the health of running waters. Consequently given the current requirements of water legislation, that are presented to managers and researchers, there is an opportunity for the more formal incorporation and inclusion of ecosystem functioning measures, such as leaf decomposition and algal accrual bioassays in biomonitoring programmes, alongside and complemented by the more commonly used structural measures.

Lotic ecosystem assessment has historically focused on measures of pattern (structure) rather than process (functioning) (Gessner & Chauvet 2002), despite their inclusion in legislative instruments e.g. EU WFD (EC 2003), US ACWA (EPA 2002) and their advocacy by freshwater biologists (e.g. Gessner & Chauvet 2002; Hladyz *et* *al*., 2010; Woodward *et al.,* 2012). Few studies have included functioning measures and even fewer have examined both (Bonada *et al*., 2006; Friberg *et al.*, 2011). Despite their close association (Cardinale, Palmer & Collins 2002) and similarity, they are unable to replace each other as changes in either structure or functioning are not necessarily mirrored by the other and vice versa (see Gessner & Chauvet 2002; Palmer & Febria 2012; Woodward *et al.,* 2012). Both metrics therefore complement each other, with the ability to offer a more inclusive, holistic and accurate assessment than either could individually. Ecosystem structure and ecosystem functioning are not interchangeable and simply suitable replacements for one other, both measures should therefore be included in the future bioassessment of running waters (Woodward *et al.,* 2012).

## References.

Adams, S., M., (2003) Establishing causality between environmental stressors and effects on aquatic ecosystems. *Human and Ecological Risk Assessment,* **9**, 17–35.

Bonada, N., Prat, N., Resh, V., H. and Statzner, B., (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual review of Entomology,* **51**, 495–523.

Cardinale, B., J., Palmer, M., A. and Collins, S., L., (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, **415,** 426-429.

Casey, H., Smith, S.M. & Clarke, R.T. (1990). Trends and seasonality in the nitrate concentrations and loads of the Bere stream (Dorset) for the period 1966-1986. *Chemistry and Ecology,* **4**, 85-94.Daufresne, M. & Boet, P. (2007) Climate change impacts on structure and diversity of fish communities in rivers. *Global Change Biology,* **13,** 2467-2478.

Durance, I. & Ormerod, S., J. (2007) Climate change effects on upland stream macroinvertebrates over a 25-year period. *Global Change Biology*, **13,** 942-957.

Edwards, F., Baker, R., Dunbar, M., & Laize, C. (2012) A review of the processes and effects of droughts and summer floods in rivers and threats due to climate change on current adaptive strategies. *NERC/Centre for Ecology & Hydrology,* Retrieved 05.02.2013 http://nora.nerc.ac.uk/18414/1/N018414CR.pdf

Environmental Agency (1998) River Habitat Quality - the physical character of rivers and streams in the UK and Isle of Man. River Habitat Survey Report No. 2 May 1998. Retrieved 05.02.2013 http://www.riverhabitatsurvey.org/wp-content/uploads/2012/07/RHS.pdf

European Commission (2003) *The new EU Water Framework Directive*. Available from: Retrieved 28.08.2012 http://ec.europa.eu/environment/water/water-framework/index_en.html

EPA (2002) Environmental Protection Agency. American clean water act (EPA ACWA). Retrieved 28.08.2012. from http://epw.senate.gov/water.pdf

Forbes, T., L. and Forbes, V., E., (1993) A critique of the use of distribution based extrapolation models in ecotoxicology. *Functional Ecology,* **7**, 249–54.

Forbes, T., L. & Kure, L., K., (1997) Linking structure and function in marine sedimentary and terrestrial soil ecosystems: implications for extrapolation from the laboratory to the field. In: Ecological risk assessment of contaminants in soil, eds N., M., van Straalen & H., Løkke, Chapman & Hall, London, Ecotoxicology Series 5, 127-156.

Friberg, N., Bonada, N., Bradley, D., C., Dunbar, M., J., Edwards, F., K., Grey, J., Hayes, R., B., Hildrew, A., G., Lamouroux, N., Trimmer, M. and Woodward, G., (2011) Biomonitoring of human impacts in freshwater ecosystems: the good, the bad, and the ugly. *Advances in Ecological Research*, **44**, 2-68.

Gessner, M., O. & Chauvet, E., (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications,* **12**, 498–510.

Hildrew, A., G., (2009) Sustained Research on Stream Communities: A model system and the comparative approach. *Advances in Ecological Research,* **41**, 175–312.

Hildrew, A., G., and Statzner, B., (2010) European rivers: Perspectives and prospects. In: European Rivers (Ed. by K. Tockner and C. Robinson). Elsevier, UK.

Hladyz, S., Tiegs, S.D., Gessner, M.O., Giller, P.S., Risnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. and Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology*, **55**, 1916-1929.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M.O., Giller, P.S., Gulis, V., Hutton, S.A., Lacoursie`re, J.O., *et al*., (2011a) Stream ecosystem functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages. *Advances in Ecological Research.* **44**, 211–276.

Hladyz, S., Abjornsson, K., Giller, P.S., and Woodward, G. (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology*, **48**, 443–452.

IPCC. (2007a) *Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge and New York.

IPCC (2007b) Summary for policy makers. In: Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press Cambridge and New York. 7-22 pp.

Kimball, K., D. & Levin, S., A., (1985) Limitations of laboratory bioassays: the need for ecosystem level testing. *Ecoscience*, **35**, 165–171.

Lauridsen, R., B. and Friberg, N., (2005) Stream macroinvertebrate drift response to pulsed exposure of the synthetic pyrethroid lambda-cyhalothrin. *Environmental Toxicology*, **20**, 513–521.

Magbanua, F., S., Townsend, C., R., Blackwell, G., L., Phillips, N. & Matthaei, C., D., (2010) Responses of stream macroinvertebrates and ecosystem function to conventional, integrated and organic farming. *Journal of Applied Ecology,* **47,** 1014-1025.

Matthaei, C., D., Piggott, J., J. & Townsend, C., R., (2010) Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. *Journal of Applied Ecology,* **47**, 639-649.

Moss, B., (2008) The Water Framework Directive: total environment or political compromise? *Science of the Total Environment,* 32-41.

Muñoz, I., López-Doval, J., C., Ricart, M., Villagrasa, M., Brix, R., Geszinger, A., Ginebreda, A., Guasch, H., López de Alda, M., Romaní, A.M., Sabater, S., Barceló, D., (2009) Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (NE Spain). *Environmental Toxicology and Chemistry.* doi:10.1897/08-486.1

Newman, R., M., (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. *Journal of the North American Benthological Society,* **10**, 89–114.

Niyogi, D., K., Koren, M., Arbuckle, C., A. & Townsend, C., R., (2007) Stream communities along a catchment land-use gradient: subsidy-stress responses to pastoral development. *Ecosystem Management,* **39**, 213–225.

Ormerod, S., J., Dobson, M., Hildrew, A., H. and Townsend, C., (Eds.) (2010) Multiple Stressors in Freshwater Ecosystems. *Freshwater Biology,* **55**, 1–269.

Palmer, M., A. and Febria, C., M., (2012) The Heartbeat of Ecosystems, *Science.* **336**, 1393 -1394.

Palmer, M., A., Bernhardt, E., S., Allan, J., D., Lake, P., S., Alexander, G., Brooks, S., Karr, J., Clayton, S., Dahm, C., N., Follstad, Shah, J., Galat, D., L., Loss, S., G., Goodwin, P., Hart, D., D., Hassett, B., Jenkinson, R., Kondolf, G., M., Lave, R., Meyer, J., L., O'Donnell, T., K., Pagano, L. & Sudduth, E., (2005) Standards for ecologically successful river restoration. *Journal of Applied Ecology,* **42**, 208-217.

Perkins, D., M., Reiss, J., Yvon-Durocher, G., and Woodward, G., (2010) Global change and food webs in running waters. *Hydrobiologia,* **657**, 181-198.

Persoone, G., Van de Vel, A., Van Steertegem, M, and De Nayer, B., (1989) Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions. *Aquatic Toxicology,* **14,** 149–166.

Rasmussen, J., J, Wiberg-Larsen, P., Baattrup-Pedersen, A., Friberg, N., Kronvang, B., (2012) Physical structure of stream microhabitats influences the response of stream macroinvertebrate communities to pesticide stress. *Environmental Pollution,***164**, 142–9.

Relyea, R., A., (2005) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* **15**, 618–627.

Relyea, R., A. and Hoverman, J., T., (2006) Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. *Ecology Letters,* **9**, 1157–1171

Riipinen, M., P., Fleituch, T., Hladyz, S., Woodward, G., Giller, P., & Dobson, M. (2010) Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology,* 55, 346–359.

Schäfer, R., von der Ohe, P., Rasmussen, J., Kefford, B., J., Beketov, M., Schulz, R., Liess, M., (2012) Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environmental Science and Technology,* **46**, 5134−5142.

Townsend, C., R., Uhlmann, S., S. & Matthaei, C., D., (2008) Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology,* **45**, 1810–1819.

Vignati, D., A., L., Ferrari, B., J., D. and Dominik, J., (2007) Laboratory-to-field extrapolation in aquatic sciences. *Environmental Science and Technology,* **15**, 1067-1073.

Wallace, J., B., Eggert, S., L., Meyer, J., .L. & Webster, J., R., (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102-104.Whitehead, P., G., Wilby, R., L., Butterfield, D. and Wade, A., J., (2006) Impacts of climate change on in-stream nitrogen in a lowland chalk stream: an appraisal of adaptation strategies, *Science of the Total Environment,* **365,** 260 –273.

Wigley, T., M., L. (2005) The Climate Change Commitment. *Science,* **307,** 1766-1769.

Woodward, G., Friberg, N. & Hildrew, A., G., (2009) Science and non-science in the biomonitoring and conservation of fresh waters. Chapter in *Freshwater Ecosystems: Biodiversity, Management and Conservation*. Nova Science Publishers, Inc., New York, U.S.A.

Woodward, G., Gessner, M., O., Giller, P., S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B., G., Tiegs, S., D., Cariss, H., Dobson, M., Elosegi, A. ,Ferreira, V., Graça, M., A.,,S., Fleituch, T., Lacoursiere, J., O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L., B., M. and Chauvet, E., (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning, *Science,* **336**, 1438-1440.

Worgan, A. & Tyrell, R. (2006) *Monitoring behavioural responses of Gammarus pulex to watercress oils.* Report for Vitacress Salads Ltd by the Centre of Ecology and Hydrology, (CEH), Dorset.

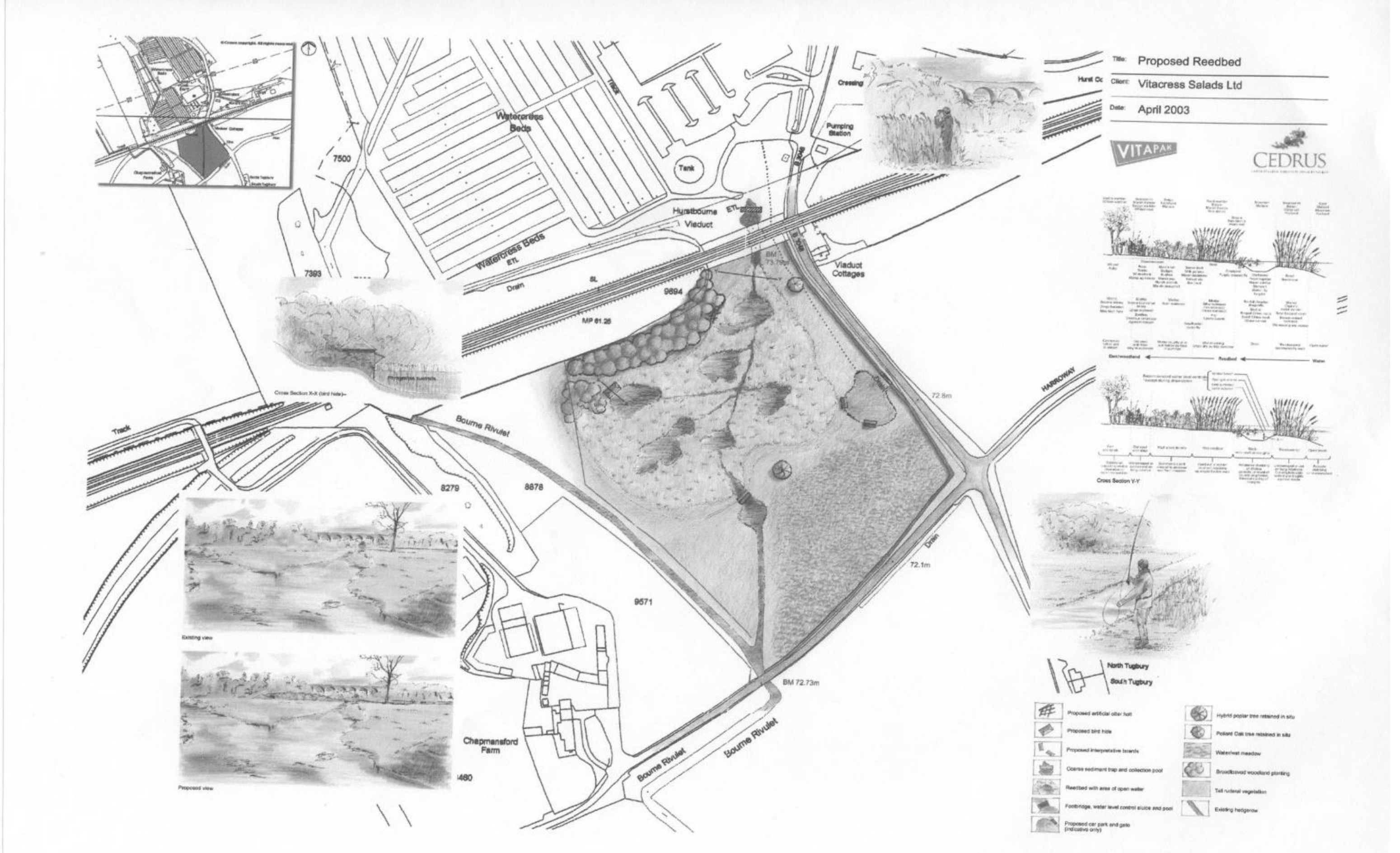
Young, R., G. &. Collier, K., J. (2009) Contrasting responses to catchment modification among a range of functional and structural indicators of river ecosystem health. *Freshwater Biology*, **54**, 2155–2170.

Young, R., G., Matthaei, C., D. and Townsend, C., R., (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society,* **27**, 605-625.

Young, R., Townsend, C. & Matthaei, C. (2004) *Functional indicators of river ecosystem health – an interim guide for use in New Zealand*. Prepared for Ministry for the Environment - Sustainable Management Fund Contract 2208. Retrieved 28.08.2012 from http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/functional-indicators-guide-final.pdf.

Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., (2008) Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica Chimica Acta*, **606**, 135-150.

# Appendix 1. Plans for a wetland below St Mary Bourne watercress farm on land specifically purchased for the project



## Appendix 2. List of identification keys used in the thesis.

Bass, J. (1998) *Last-Instar Larvae and Pupae of the Simuliidae of Britain and Ireland: a Key with Brief Ecological Notes.* Freshwater Biological Association Scientific Publication No. **55**. Titus Wilson and Son Ltd., Kendal, UK.

Brooks, S. (2004) *Field guide to the dragonflies and damselflies of Great Britain and Ireland*. British Wildlife Publishing, Gillingham, UK.

Edington, J.M. & Hildrew, A.G. (1995) *Caseless caddis larvae of the British Isles.* Freshwater Biological Association Scientific Publication No. **53**. Titus Wilson and Son Ltd., Kendal, UK.

Elliott, J.M., Humpesch, U.H. & Macan, T.T. (1988) Larvae of the British Ephemeroptera. Freshwater Biological Association Scientific Publication No. **49**. Titus Wilson and Son Ltd., Kendal, UK.

Friday, L.E. (1988) *A key to the adults of British water beetles*. Fields Studies, Vol 7 No.1, Dorset Press, Dorset, UK.

Harding, P. (Ed.) (2004) *Freshwater Fishes in Britain: the species and their distribution.* Harley Books, Colchester, UK.

Hynes, H.B.N. (1993) *Adults and nymphs of the British Stoneflies (Plecoptera).* Freshwater Biological Association Scientific Publication No. **17**. Titus Wilson and Son Ltd., Kendal, UK.

Macan, T.T. (1960) *A key to the British fresh- and brackish-water Gastropods.* Freshwater Biological Association Scientific Publication No. **13**. Titus Wilson and Son Ltd., Kendal, UK.

Maitland, P.S. (2004) *Keys to the Freshwater Fish of Britain and Ireland, with notes on their distribution and ecology.* Freshwater Biological Association Scientific Publication No. **62**. Titus Wilson and Son Ltd., Kendal, UK.

Wallace, I.D., Wallace, B. & Philipson, G.N. (2003) *Keys to the case-bearing caddis larvae of Britain and Ireland.* Freshwater Biological Association Scientific Publication No. **61**. Titus Wilson and Son Ltd., Kendal, UK.

Savage, A.A. (1989) *Adults of the British Aquatic Hemiptera Heteroptera: a key with ecological notes.* Freshwater Biological Association Scientific Publication No. **50**. Titus Wilson and Son Ltd., Kendal, UK.

## Appendix 3. Chapter 4 Supplementary tables and figures.

Table 1. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per day ($k_d$) in June and July 2005 Sites: Up, Side, WC, Out, Down June. Significant results at $P<0.05$ are highlighted in bold

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 4 | 49.981 | 5.958 | **0.001** |
| Mesh | 1 | 50.055 | 32.405 | **<0.001** |
| Time | 1 | 49.889 | 11.750 | **0.001** |
| Site x Mesh | 4 | 49.981 | 3.540 | **0.013** |
| Site x Time | 4 | 49.808 | 0.373 | 0.827 |
| Mesh x Time | 1 | 49.889 | 0.497 | 0.484 |
| Site x Mesh x Time | 4 | 49.808 | 0.517 | 0.723 |

Table 2. LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a* *(*mg cm-2) per day *(d)* June and July 2005 Sites: Up, Side, WC, Out, Down. Grazing = exclusion of grazers through use of Vaseline 'fence' versus non exclusion. Significant results at *P* <0.05 are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 4 | 49.182 | 11.539 | **<0.001** |
| Grazing | 1 | 49.408 | 38.491 | **<0.001** |
| Time | 1 | 49.642 | 6.892 | **0.011** |
| Site x Grazing | 4 | 49.182 | 2.575 | **0.049** |
| Site x Time | 4 | 49.431 | 13.092 | **<0.001** |
| Grazing x Time | 1 | 49.642 | 0.035 | 0.852 |
| Site x Grazing x Time | 4 | 49.431 | 0.544 | 0.704 |

Table 3. The % of the three shredders *G. pulex*, *A. aquaticus* and *S. personatum* found in the benthos and litter bags in June and July 2005.

|  | *G. pulex* | *A. aquaticus* | *S. personatum* | Total |
|---|---|---|---|---|
| Benthos | 90 | 9 | 0.5 | 99.5 |
| Litter bags | 62 | 37 | 0.5 | 99.5 |

## Appendix 4. Chapter 5 Supplementary tables and figures.

Table 1. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per day ($k_d$). Up, Out, Down, in June 05, June 06 and June 07. Significant results at $P < 0.05$ are highlighted in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 2 | 30.528 | 29.271 | **<0.001** |
| Mesh | 1 | 30.565 | 27.510 | **<0.001** |
| Year | 2 | 60.142 | 28.323 | **<0.001** |
| Site x Mesh | 2 | 30.528 | 6.934 | **0.003** |
| Site x Year | 4 | 60.097 | 3.309 | **0.016** |
| Mesh x Year | 2 | 60.142 | 4.594 | **0.014** |
| Site x Mesh x Year | 4 | 60.097 | 1.050 | 0.389 |

Table 2. LMEM results of comparison of algal colonisation tiles log10 chlorophyll *a* (mg cm-2) per day. Up, Out, Down, in June 05, June 06 and June 07. Grazing treatment (NV / V) = exclusion of grazers through use of Vaseline 'fence' (V) versus non-exclusion (NV). Significant results at $P < 0.05$ in bold.

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Site | 2 | 30.084 | 30.054 | <<br>**<0.001** |
| Grazing | 1 | 30.201 | 60.463 | **<0.001** |
| Year | 2 | 59.837 | 7.895 | **0.001** |
| Site x Grazing | 2 | 30.084 | .850 | 0.437 |
| Site x Year | 4 | 59.711 | 4.509 | **0.003** |
| Grazing x Year | 2 | 59.837 | 0.978 | 0.382 |
| Site x Grazing x Year | 4 | 59.711 | 0.490 | 0.743 |

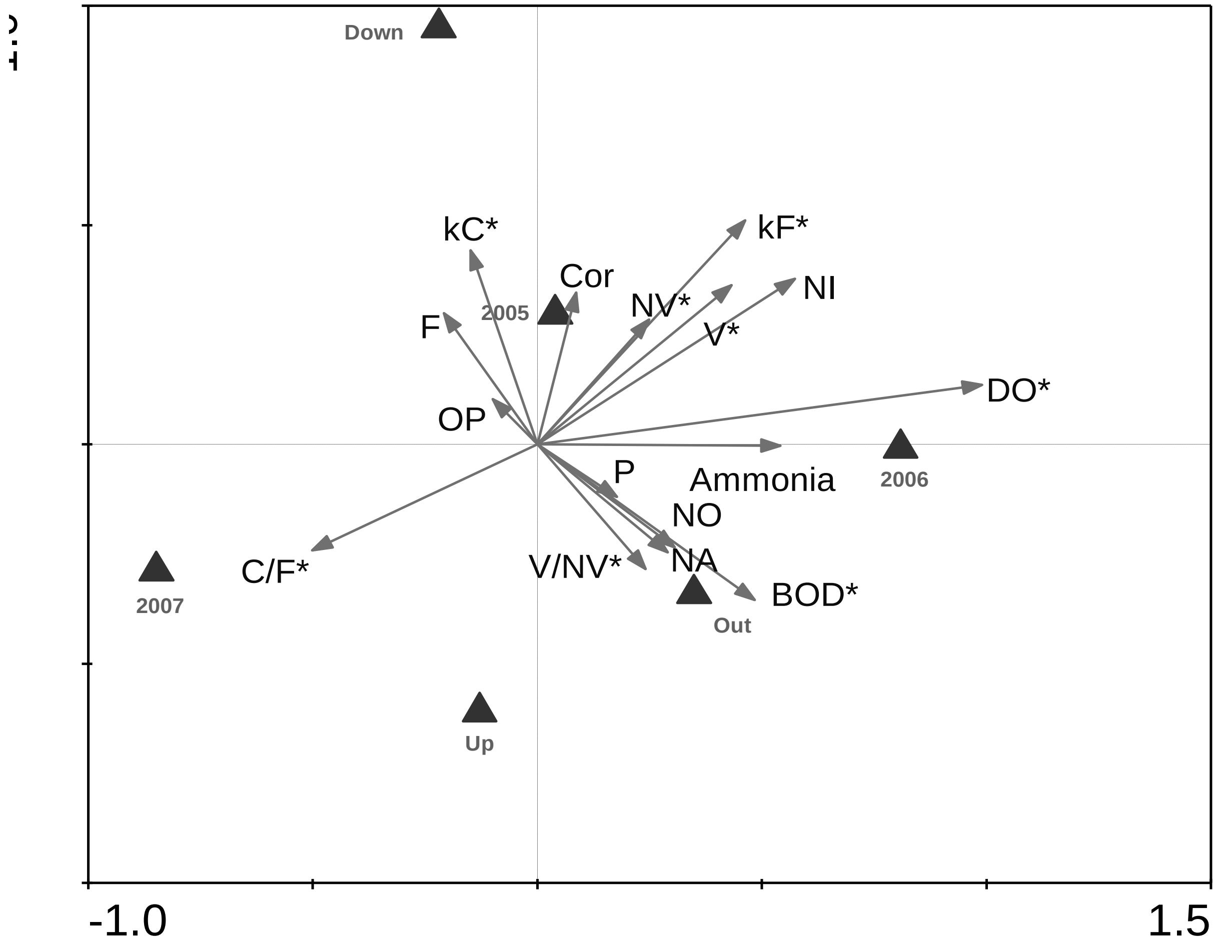


Figure 1. Redundancy analysis (RDA) biplot vectors of species scores for environmental variables (arrows) and year and sample sites (centroids) recorded at St Mary Bourne watercress farm and the Bourne Rivulet in June 2005, 2006 and 2007. Arrows display the correlation between the environmental variables and centroids and are oriented in the direction of maximum variation in value. Site and year were coded as dummy variables and environmental variable as supplementary files Axis I (49.4%) & II (23.4%) accounted for 72.8% of the variation in the species data. Abbreviations: (see Appendix 6 for full lists).

## Appendix 5. Chapter 6 Supplementary tables and figures.

Table 1. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per day ($k_d$) *in* autumn 06, winter 06, spring 07 and summer 07 Up, Out, Down.. Significant results at *P*<0.05 are highlighted in bold.

| Comparison | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 2 | 90 | 43.574 | **<0.001** |
| Season | 3 | 270 | 16.040 | **<0.001** |
| Site | 2 | 90 | 28.426 | **<0.001** |
| Mesh | 1 | 90 | 269.074 | **<0.001** |
| Stream x Season | 6 | 270 | 2.617 | **0.018** |
| Stream x Site | 4 | 90 | 12.886 | **<0.001** |
| Stream x Mesh | 2 | 90 | 9.722 | **<0.001** |
| Season x Site | 6 | 270 | 9.328 | **<0.001** |
| Season x Mesh | 3 | 270 | 0.930 | 0.427 |
| Site x Mesh | 2 | 90 | 1.355 | 0.263 |
| Stream x Season x Site | 12 | 270 | 7.805 | **<0.001** |
| Stream x Season x Mesh | 6 | 270 | 2.886 | **0.010** |
| Stream x Site x Mesh | 4 | 90 | 4.285 | **0.003** |
| Season x Site x Mesh | 6 | 270 | 2.976 | **0.008** |
| Stream x Season x Site x Mesh | 12 | 270 | 1.026 | 0.425 |

Table 2. LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a* (mg cm-2) per day (*d*) in autumn 06, winter 06, spring 07 and summer 07 Up, Out, Down. (NV / V = exclusion of grazers through use of Vaseline ‘fence’ V and non exclusion N V) (Significant results at *P* <0.05 in bold).

| Comparison | d.f.$_{N}$ | d.f.$_{D}$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 2 | 90.308 | 52.254 | <0.001 |
| Season | 3 | 268.015 | 26.186 | <0.001 |
| Site | 2 | 90.319 | 80.320 | <0.001 |
| NV /V | 1 | 90.323 | 72.324 | <0.001 |
| Stream x Season | 6 | 267.989 | 11.931 | <0.001 |
| Stream x Site | 4 | 90.301 | 26.773 | <0.001 |
| Stream x NV /V | 2 | 90.308 | 0.062 | 0.940 |
| Season x Site | 6 | 267.989 | 6.686 | <0.001 |
| Season x NV /V | 3 | 268.015 | 0.379 | 0.768 |
| Site x NV /V | 2 | 90.319 | 1.076 | 0.345 |
| Stream x Season x Site | 12 | 267.943 | 14.970 | <0.001 |
| Stream x Season x NV /V | 6 | 267.989 | 0.605 | 0.726 |
| Stream x Site x NV /V | 4 | 90.301 | 0.177 | 0.950 |
| Season x Site x NV /V | 6 | 267.989 | 0.177 | 0.983 |
| Stream x Season x Site x NV /V | 12 | 267.943 | 0.302 | 0.989 |

Table 3. The % of the three shredders *G. pulex, A. aquaticus* and *S. personatum* found in the benthos and litter bags in all seasons.

| | *G. pulex* | *A. aquaticus* | *S. personatum* | Total |
|---|---|---|---|---|
| Benthos | 92 | 5 | 0.5 | 97.5 |
| Litter bags | 79 | 14 | 5 | 98 |

Table 4. LMEM results of comparisons of leaf-litter $\log_{10}$ decomposition rates per day ($k_d$) *in* summer 2007; Up, Out, Down. Significant results at $P<0.05$ are highlighted in bold

| Comparison | $d.f._N$ | $d.f._D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 5 | 180 | 10.736 | **<0.001** |
| Site | 2 | 180 | 5.945 | **0.003** |
| Mesh | 1 | 180 | 209.744 | **<0.001** |
| Stream x Site | 10 | 180 | 6.857 | **<0.001** |
| Stream x Mesh | 5 | 180 | 8.242 | **<0.001** |
| Site x Mesh | 2 | 180 | 0.264 | 0.768 |
| Stream x Site x Mesh | 10 | 180 | 4.863 | **<0.001** |

Table 5. LMEM results of comparison of algal colonisation tiles, log10 chlorophyll *a* *(*mg cm-2) per day (*d*) in summer 2007; Up, Out, Down. (NV / V = exclusion of grazers through use of Vaseline 'fence' V and non exclusion N V) (Significant results at *P* <0.05 in bold).

| Comparison | d.f.$_N$ | d.f.$_D$ | *F* – ratio | *P* |
|---|---|---|---|---|
| Stream | 5 | 176 | 33.585 | **<0.001** |
| Site | 2 | 176 | 8.351 | **<0.001** |
| NV/V | 1 | 176 | 83.106 | **<0.001** |
| Stream x Site | 10 | 176 | 19.642 | **<0.001** |
| Stream x NV/V | 5 | 176 | 1.909 | 0.095 |
| Site x NV/V | 2 | 176 | 0.087 | 0.917 |
| Stream x Site x NV/V | 10 | 176 | 0.601 | 0.812 |

Table 6. The % of the three shredders *G. pulex*, *A. aquaticus* and *S. personatum* found in the benthos and litter bags in summer 2007 at all six watercress farms in Spatial study.

| | *G. pulex* | *A. aquaticus* | *S. personatum* | Total |
|---|---|---|---|---|
| Benthos | 97 | 2 | 0.5 | 99.5 |
| Litter bags | 89 | 8 | 2 | 99 |

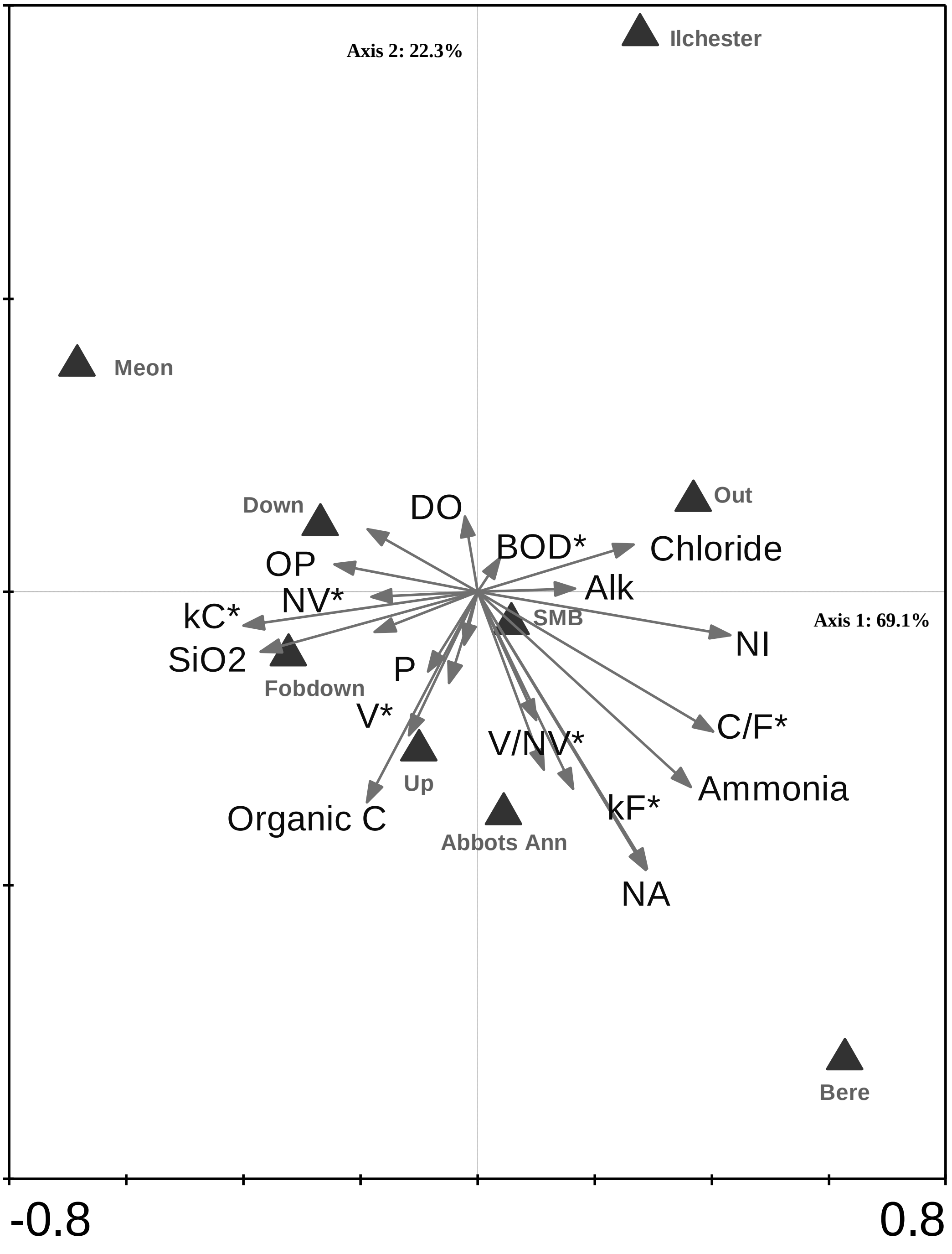


Figure 1. Spatial study. PCA biplot of environmental variables (vectors / arrows) and sites (Up, Out, Down) at six watercress farms (centroids). Arrows display the correlation between species and the environmental variables and are oriented in the direction of maximum variation in value**.** Axis I (69.1%) & II (22.3%) accounted for 91.4% of the variation in the species data. Abbreviations (See Appendix 6 for full lists).

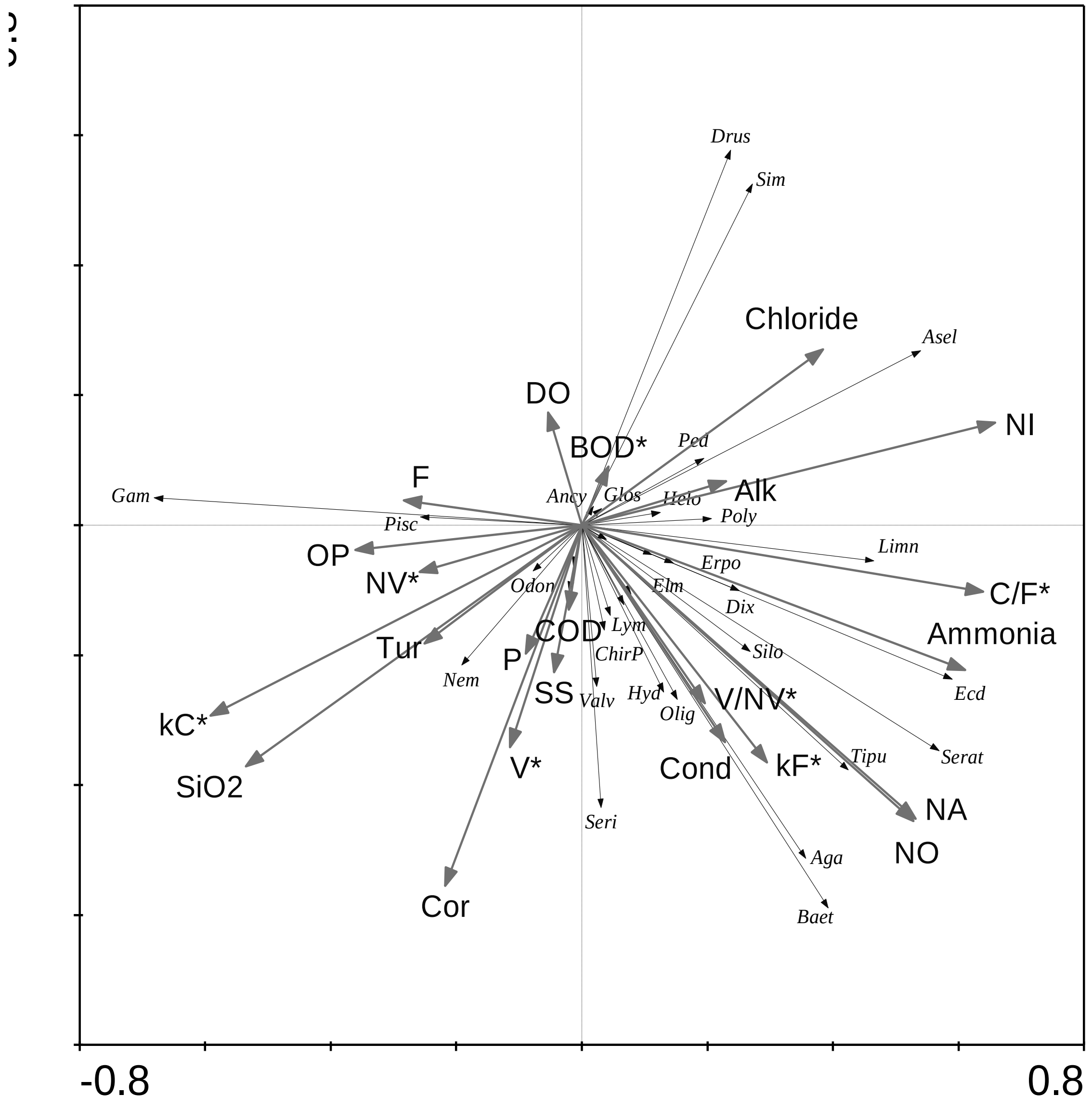


Figure 2. Spatial study. RDA biplot of macroinvertebrate species (six surber samples / site) (small arrows and text), environmental variables (large arrows and text) at six watercress farms (centroids). Arrows display the correlation between macroinvertebrate species and environmental variables and are oriented in the direction of maximum variation in value**.** Axis I (29.7%) & II (11.9%) accounted for 41.6% of the variation in the species data. Abbreviations (See Appendix 6 for full lists).

## Appendix 6. Species codes and Environmental variables used in ordinations.

i) Sites:
Chapter 4: Up = Upstream, Side = Side Channel, WC = Watercress Beds, Out= Outfall, Up = Upstream.

Chapter 5: Up = Upstream, Out= Outfall, Down = Downstream. (e.g. 05 Up = June 2005 Up; 06 Out = June 2006 Out; 07 Down = June 2007 Down).

Chapter 6, Temporal Study: AA = Abbots Ann, FD = Fobdown, SMB = St Mary Bourne; Up = Upstream, Out= Outfall, Down = Downstream

Chapter 6, Spatial Study: A = Abbots Ann, B = Bere, F = Fobdown, I = Isington, M = Meon, S = St Mary Bourne; U = Upstream, O= Outfall, U = Upstream. (e. g. AU = Abbotts Ann, Upstream).

ii) Species codes:
Aga (*Agapetus fuscipes*); Ancy (*Ancylus fluviatilis*); Asel (*Asellus aquaticus* ); Baet (*Baetis rhodani* ); Cera (Ceratopogonidae); ChirNP (Chironomidae Non-predatory); ChirP (Chironomidae Tanypodinae);Chlor (*Chloroperla torrentium*); Crun (*Crunoecia irrorata*); Dix (*Dixella spp*); Drus (*Drusus annulatus*); Ecd (*Ecdyonurus venosus*); Elm (*Elmis aeanea*); Eph (*Ephemera danica*); *Eph* (Ephydridae); Erpo (*Erpobdella octoculata*); Gam (*Gammarus pulex* ); Glos (*Glossiphonia complanata*); Hale (*Halesus radiatus* ); Helo (*Helobdella stagnalis* ); Hyd *Hydroptilla* spp); HydS (*Hydropsyche siltalai*); HydP (*Hydropsyche pellucidula*); Lasi (*Lasiocephala basalis*); Leuc (*Leuctra innernis*); Limn (*Limnephilus* spp); Lym (*Lymnaea peregra*); Myst (*Mystacides azurea*); Nem (*Nemurella pictetii*); Odon (*Odontocerum albicorne*); Olig (Oligochaeta); Ped (*Pedicia rivosa*); Pisc (*Piscicola geometra*); Phys (*Physa fontinalis*); Pisi (*Pisidium* spp); Plan (*Planorbis leucostoma*); Poly (*Polycentropus flavomaculatus*); Pota (*Potamophylax cingulatus*); Potam (*Potamopyrgus antipodarum*); Rhy (*Rhyacophila dorsalis*); Serat (*Seratella ignita_*); Seri (*Sericostoma personatum*); Silo (*Silo nigricornis*); Sim (*Simulium* spp); Tin (*Tinodes waeneri*); Tipu (*Tipula* spp); Valv (*Valvata cristata*).

iii) Environmental variables:
Alky (Alkalinity); Ammonia; Chloride; COD (Chemical Oxygen Demand); Cond (Conductivity); Col (Colour); COrg (Organic Carbon); DO (Dissolved Oxygen); F (Fluoride); NOxid (Oxidised nitrogen); Nitrate; Nitrite; OP (Orthophosphate); SiO2; SS (Suspended Solids); P (Phosphate); Turb (Turbidity).

iv) Functional measures (denoted*):
BOD* (Biological Oxygen Demand); kC* (Decomposition in coarse litter bags per degree-day); kF* (Decomposition in fine litter bags per degree-day); C/F* (Ratio kC/kF); NV* Chlorophyl *a* on Non Vaseline tile); V* (Chlorophyl *a* on Vaseline tile); Herbivory* V/NV (Ratio V/NV (See Methods for details).