The role of methane-derived carbon as an energy subsidy to benthic invertebrates in streams

by

Aurora Sampson

Submitted in partial fulfilment of the requirements of the Degree of

Doctor of Philosophy

School of Biological and Chemical Sciences

Queen Mary, University of London

2015

Declaration

I, Aurora Sampson confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

Signature: A. Sampson

Date: 31st December 2014

Abstract

Recent evidence suggests that methane-derived carbon can provide an energy source to freshwater food webs. Cased caddis (Trichoptera) larvae, such as *Agapetus fuscipes* and *Silo nigricornis,* show consistently depleted carbon stable isotope ratios, suggesting a reliance on methane-derived carbon rather than the organic matter fixed by plants either in the stream or imported from the land. These two invertebrate species can be very abundant and thus there is considerable potential for trophic transfer of methane-derived carbon further up the food web. Hitherto, the evidence for this link between cased caddis and methane has been restricted to streams and rivers on permeable chalk geology across southern Britain.

This thesis examines the geographical distribution of this phenomenon and specifically whether it occurs elsewhere in catchments on different geology. It also examines the potential magnitude and importance of this methane-derived carbon source to these cased caddis populations. Twenty-nine sites on varying geology across Britain were sampled from April to November 2011. The results suggest that the use of methane-derived carbon by cased caddis and other primary consumers is more widespread than first thought.

To assess the proportion of methane-derived carbon contributing to cased caddis larvae, secondary production in the focal caddis taxa (*Agapetus fuscipes* and *Silo nigricornis*) was measured regularly, using the size-frequency method, at eight permanent sites selected from the various geologies. This, combined with stable carbon isotope measurements, suggests that methane-derived carbon may form a considerable subsidy in these freshwater systems and indeed may be widespread across the UK.

Table of Contents

List of Figures

List of Tables

Acknowledgements

This thesis was funded by NERC. Funding for conference attendance was provided by Queen Mary University.

Firstly I wish to thank my supervisors Alan Hildrew, Guy Woodward and Jon Grey for all the training and guidance they have given me throughout my time at Queen Mary.

A big thank you to my small army of fieldwork volunteers who came so willingly to help me, your help was much appreciated in what sometimes could be challenging weather conditions, especially when the midges were about! Thanks go to Nicki Ings, Flick Shelley, Sarah Tuffin, Jodie Simmons, Mike Cuzner, Danny Sheath, Susanna Maanoja, Gareth Jenkins, Vicky Warren, Michaela Radl, Matteo Dossena, Chris Hulatt, Maite Guignard, Valentine Seymour and anyone else I have forgotten.

Thank you to everyone who took time to help me in the laboratory, lent me various bits of equipment and helped me run temperamental machines such as the mass spectrometer. Thanks are in order to: Flick Shelley, Sarah Tuffin, Susanna Maanoja, Katrina Lansdown, Jimmy Pritchard, Nicki Ings, Matteo Dossena, Philip Sanders, Mrysini Chronopoulou, Ian Sanders, Vicky Warren and Louise Olde.

Thank you to Paul Fletcher for finding me various bits of fieldwork equipment and for helping me to successfully store more than a chest freezers worth of Surber samples! And an equally a gracious apology to

anyone who had to help me move the Surber samples at various times when the freezers needed more space!

Thank you to all the people who contributed useful discussion about my PhD, Nicki Ings for many a corridor or office chat that led me down interesting routes of exploration with my research, Philip Donkersley and Ali Birkett of Lancaster University for providing encouragement, reading through drafts and providing statistics help. Thank you to Eoin O'Gorman and Dan Perkins of Imperial College for helping me with statistics and R code.

Thank you to members of the R-space group Naiara OMahony, Paul Ha, Natasha McGowan and Mike Whitfield for providing helpful advice about R code.

On a personal note, thank you to all my friends who have motivated and encouraged me. A special thanks is in order to Joanne, Greg, and Vicky for all their encouragement.

A big thank you is in order to my boyfriend Mike who has encouraged me throughout the years as well as a big thank you to Ann for providing an awesome 'retreat' for writing.

Finally thank you to my parents and family who have encouraged me every step of the way.

For Nicki

"...there is scarcely a patch of standing water, ditch or veriest trickle where Caddis larvae of some kind may not be found"

~ Norman E. Hickin, 1952

1 General Introduction

1.1.1 Methane as a greenhouse gas

Methane is well known for its properties as a greenhouse gas (Owens *et al.* 1991; Walter *et al.* 2006; Murrell & Jetten 2009). Greenhouse gases in the atmosphere absorb and re-emit radiation at specific wavelengths within the thermal infrared spectrum. Infrared radiation is emitted by the earth's surface, the atmosphere and the clouds. This property of the gases creates the 'greenhouse effect' in which heat, that would, in the absence of these gases be radiated back out into space, is trapped in the surface-trophosphere system, the lowest part of the atmosphere (IPCC 2007). In recent decades this warming phenomenon has received increased attention because it has been linked to rising sea levels, shrinkage of the sea ice extent, increased precipitation and frequency of drought events as well as more frequent heat waves and extreme weather events, all of which have serious consequences for ecosystems and human populations (IPCC 2007). Methane (CH_4), is one of the most abundant greenhouse gases in the atmosphere after water vapour (H_2O) and carbon dioxide (CO_2), with 25 times the global warming potential of carbon dioxide by mass (IPCC 2007), thus methane is one of the most potent green house gases driving global warming. Natural and anthropogenic emissions of methane have been widely studied in order to understand how methane is produced and released (Stern & Kaufmann 1996; Bastviken *et al.* 2004; Walter *et al.* 2006; Bousquet *et al.* 2006; Miller *et al.* 2007, 2013; Prairie & del Giorgio 2013).

1.1.2 Methane in freshwater environments

Methane is common and widespread in many freshwater environments such as lakes, wetlands, bogs, marshes, swamps and rice paddy fields (Rudd & Hamilton 1978; Moore & Knowles 1990; Krüger *et al.* 2002; Huttunen *et al.* 2006). More recently, rivers and streams have attracted attention because they too can contain methane, and are often supersaturated relative to the amount expected under equilibrium with the atmosphere (Jones & Mulholland 1998; Anthony, Prahl & Peterson 2012; Campeau *et al.* 2014). Rivers and streams were once considered to have negligible methane emissions and were not expected to contribute significantly to global methane emissions. However, it is now known that these freshwater environments are important conduits for gas exchange of methane with the atmosphere (Moore & Knowles 1990; Bastviken *et al.* 2010, 2011; Baulch *et al.* 2011).

1.1.3 Methane as an energy source

Methane in fresh waters is now receiving attention for another reason; because of its potential to be used as an energy source by freshwater organisms (Bastviken *et al.* 2003; Trimmer *et al.* 2009; Shelley, Grey & Trimmer 2014). Until recently, it was thought that freshwater organisms derived most of their carbon from photosynthetic processes within the system (autochthonous), or from imported terrestrial production from the catchment (allochthonous) (Hynes 1970; Vannote *et al.* 1980; Thorp *et al.* 1998). Food

webs and the trophic pathways supporting secondary production in rivers and streams have been very widely studied and are believed to be based on allochthonous detritus (both particulate and dissolved) derived from terrestrial photosynthetic plants, and on autochthonous photosynthetic primary production from attached algae and cyanobacteria and to some (lesser) extent mosses and higher plants (Hynes 1970; Vannote *et al.* 1980; Wallace *et al.* 1995; Thorp *et al.* 1998; Mulholland *et al.* 2001; Trifonova *et al.* 2002; Allan & Castillo 2007). The relative contributions of the two sources of autochthonous and allochthonous is still contentious, with debate as to how much autochthonous or allochthonous material contributes to food webs respectively (Hein *et al.* 2003; Carpenter *et al.* 2005; Doucett *et al.* 2007; Risse-Buhl *et al.* 2012).

Dissolved methane can be imported into a river or stream from groundwater and as such can be considered 'allochthonous'. However, methane can also be produced *in-situ* in rivers and streams and therefore, production stemming from this source is 'autochthonous'. Most methane in ground water is the product of either bacterial or thermal mediation (Schoell 1988), and considered to be biogenic or thermogenic in origin. Methane oxidising bacteria are the main consumers of any methane that is produced in freshwater systems (Hanson & Hanson 1996; Trimmer *et al.* 2012).

1.1.4 Biogenic and thermogenic methane

Biogenic methane is formed by methanogenic bacteria via the process of methanogenesis, a chemosynthetic pathway in which reduced carbon compounds are produced through the oxidation of substrates using chemical energy instead of light energy (Woltemate, Whiticar & Schoell 1984). Biogenic methane can be transported into streams via groundwater or be produced in the river itself where anoxic conditions exist, for example in river beds clogged with fine sediment (Jones & Mulholland 1998; Whiticar 1999; Hlaváčová, Rulík & Čáp 2005; Gebert, Kothe & Grongroft 2006; Conrad *et al.* 2007). The process of methane production can be classified into different pathways based on the starting substrate, which, include carbon dioxide, acetate and formate (Whiticar 1999). Two main pathways of methanogenesis occur in freshwater environments: the 'carbonate-reduction' pathway (1), and the acetate fermentation pathway (2) (Koyama 1955; Takai 1970).

1. $CO_2 + 8H^+ + \rightarrow CH_4 + 2H_2O$

2. $*CH_3COOH \rightarrow CH_4 + CO_2$

*****indicates the intact transfer of the methyl position to CH_4.

Acetate fermentation is thought to be the most important pathway of methanogenesis in freshwater environments (Koyama 1955; Takai 1970; Beliaev, Finkelstein & Ivanov 1975; Winfrey *et al.* 1977). The carbonate

reduction pathway becomes more important if acetate is in short supply (Whiticar 1999). The production of biogenic methane can be considered a form of 'bacterial chemosynthesis' as it is the synthesis of organic compounds by bacteria using energy released by inorganic chemical reactions. Thermogenic methane is linked to natural gas fields, petroleum reservoirs and coal reserves (Kvenvolden 1995; Head, Jones & Larter 2003; Moore 2012), and is likely to be important in river basins that are associated with reserves of these resources (Bates *et al.* 2011).

1.1.5 Isotopic values of methane

The process of methane formation leads to the heavier stable isotope of carbon, ^{13}C, being 'discriminated against' (i.e. used less) in biochemical reactions in favour of the lighter isotope, ^{12}C. This leads to very low $\delta^{13}C$ values for methane (the methane may be described as '^{13}C-depleted') and subsequently in the bacteria consuming this methane (Summons, Jahnke & Roksandic 1994). The isotopic composition of methane reflects the formation process (Blair & Carter 1992; Sugimoto & Wada 1993). In freshwater sediments, methane that is generated from the carbonate-reduction pathway has a lower ^{13}C content than methane generated by the acetate-fermentation pathway (Whiticar, Faber & Schoell 1986; Sugimoto & Wada 1993).

Biogenic methane that is produced in sediments under anaerobic conditions can have a $\delta^{13}C$ value of around -80‰ to -60‰ (Whiticar *et al.* 1986;

Jędrysek 2005; Conrad *et al.* 2007), and is depleted in ^{13}C compared to thermogenic methane which can range between -50 to -20‰ (Whiticar 1999). The stable isotopes in organic samples can be measured using isotope ratio mass spectrometry and $\delta^{13}C$ in the tissues of an organism can be used to evaluate the carbon in its diet, especially when the various potential sources have distinct and temporally robust $\delta^{13}C$ ratios (DeNiro & Epstein 1978). The $\delta^{13}C$ of carbon remains relatively conserved from resource to consumer with a trophic fractionation factor of ~1‰ (Peterson & Fry 1987), allowing resources to be traced through food chains. Low $\delta^{13}C$ values for consumers can indicate reliance on a chemosynthetic energy pathway.

1.1.6 Chemosynthesis in deep sea vent communities

In 1976 the first benthic hydrothermal vent community was discovered at the Galapagos Rift in the East Pacific Ocean. This community included mussels, anemones and crabs (Lonsdale 1977) and was too far below the ocean surface to receive energy directly from photosynthesis; instead the community relied on organic compounds produced by chemosynthetic microorganisms at temperatures of 150 – 300°C. These chemosynthetic microorganisms were using the hydrogen sulphide and methane that existed in large concentrations around the vent and converting these to organic compounds that the community could use (Childress & Fisher 1992). Since this first discovery, more than 200 vent fields have been discovered with varying properties and chemical conditions (Wilcock *et al.* 2004). A later vent system that was discovered was the Lost City hydrothermal field located

15km from the Mid Atlantic Ridge, with physical and chemical characteristics that differed from the Galapagos Rift, with notably lower temperatures of around 40-90°C. Unlike the Galapagos Rift, microorganisms obtain their energy from methane and pure hydrogen as opposed to hydrogen sulphide (Kelley, Karson & Früh-Green 2005).

Several studies have investigated the isotopic composition of organisms at these deep sea vents. Rau & Hedges (1979) discovered a colony of deep sea mussels *Bathymodiolus thermophilus*, inhabiting the Galapagos vent system that had average $\delta^{13}C$ values of ~ -33‰, well outside the range of -20 to -15‰ that would be expected if the mussels had been exploiting solely organic material deposited from the euphotic zone. These values were consistent with the $\delta^{13}C$ of organic matter produced by bacterial chemoautotrophic production. The conclusion was that these mussels were dependent on this form of production. It has since been found that some colonist invertebrates in the hydrocarbon seep communities of the Gulf, such as the sea star *Sclerasterias* cf. *tanneri* and the predatory snail *Buccinum canetae*, have greatly depleted $\delta^{13}C$ and $\delta^{34}S$ values indicating almost 100% reliance on carbon and sulphur from seep production (MacAvoy *et al.* 2002). These combined discoveries led the way to methane being recognised as a feasible energy source in systems where photosynthetic input is minimal or absent.

1.1.7 Freshwater organisms displaying depleted $\delta^{13}C$ values

The first report of freshwater organisms displaying low $\delta^{13}C$ values was for chironomid larvae in Australian billabongs (Bunn & Boon 1993). They reported values as low as -38‰, with POM values of -25 to -30‰. Nearly a decade later, Grey (2002) reported values of -41‰ for the chironomid *Sergentia* spp. from Loch Ness and -53.5‰ for *Chironomus* spp. from Esthwaite Water. These values were far below that of the POM, the presumed food source for the chironomid species in these lakes. Similarly, Kiyashko, Narita & Wada (2001) measured a value of -64‰ for a sample of the midge *Strictochironomus* sp. from the profundal zone of Lake Biwa in Japan, where POM $\delta^{13}C$ was not below -30‰. Chironomids collected from five lakes in Southern Finland in summer 2002 by Jones & Grey (2004) also exhibited low $\delta^{13}C$ values, with some as low as -55‰. They suggested that the low $\delta^{13}C$ values seen for chironomid biomass was attributable to the incorporation of carbon derived from biogenic methane.

Conditions favourable for methane production and consumption are often present in lakes (Bastviken, Ejlertsson & Tranvik 2002; Bastviken *et al.* 2004; Kankaala *et al.* 2006b; Juutinen *et al.* 2009; Jones & Grey 2011). Hypolimnetic oxygen depletion can exist in the lower water layers of lakes due to stratification and a lack of mixing in the water column (Bastviken *et al.* 2002; Jones *et al.* 2008; Müller *et al.* 2012). Biogenic methane can be formed in lake sediments as imported allochthonous material and autochthonous material is degraded under anaerobic conditions, creating the conditions

necessary for methanogenesis. Methane oxidation by aerobic bacteria at the oxic/anoxic interfaces may then provide an important source of carbon for lake food webs (Bastviken *et al.* 2003; Jones *et al.* 2008; Sanseverino *et al.* 2012). There is evidence in lakes that methane-derived carbon can reach higher levels than primary consumers in food webs and support fish production. Thus, lower $\delta^{13}C$ values than expected were reported for large bream from a Kettle lake in Germany (Harrod & Grey 2006), with up to 20% of the fish's diet potentially comprised of isotopically light chironomid larvae. Similarly, in an urban lake in Finland, the ruffe species *Gymnocephalus cernuus*, was found, through the use of mixing models, to have incorporated methane-derived carbon by feeding on chironomids that had in turn fed on methanotrophic bacteria. About 17% of the ruffe biomass was calculated to be derived from methane-derived carbon (Ravinet *et al.* 2010).

1.1.8 Evidence for methane-derived carbon uptake in streams

More recently, research has indicated that some cased caddis also assimilate methane-derived carbon. Trimmer *et al.* (2009) found that two species of cased caddis *Agapetus fuscipes* and *Silo nigricornis* were consistently ^{13}C-depleted (mean -41.2 and -40.4‰, respectively) relative to their putative food resources in the River Lambourn, a chalk stream in East Berkshire. The primary food sources of these two species were believed to be epilithon and some detritus, food sources that are based on photosynthetically derived material, either in the stream or the terrestrial catchment (Douglas 1958; Elliott 1982; Arens 1990; Nijboer 2004). Trimmer

et al. (2009) calculated that methane-oxidising bacteria could provide *Agapetus fuscipes* with up to 30% of its carbon.

One conundrum associated with this finding is that chalk streams have previously been thought of as conventionally very productive systems, with high autochthonous primary production by algae and macrophytes, such as *Ranunculus* spp. (Marker 1976a; Berrie 1992; Wright 1992). Chalk streams also lack the stratified structure of lakes and, presumably, the conditions needed for methane to be produced, being well-oxygenated due to the flowing water. It is, therefore, initially surprising that cased caddis assimilate methane-derived carbon in these streams, when the other resources that they are presumed to feed on are so abundant. However, chalk streams are consistently supersaturated with dissolved methane relative to the amount expected under equilibrium conditions with the atmosphere (Sanders *et al.* 2007; Shelley *et al.* 2014). The groundwater supplying chalk aquifers often contains relatively high concentrations of methane (Gooddy & Darling 2005). In addition the growth of macrophytes in 'stands', and the consequent reduction in water velocity, can lead to sediment retention, particularly beneath the macrophytes themselves (Sand-Jensen 1998). These sediments can produce the anoxic conditions needed for methanogenesis (Jones & Mulholland 1998; Sanders *et al.* 2007).

Hitherto, all of the evidence for this methane-carbon pathway into caddis larvae has come from chalk streams (Tuffin 2014), a globally rare habitat with

>90% being found in southern Britain. So far, no research has considered whether the phenomenon of caddis larvae depleted in ^{13}C occurs on other geological types, or whether it is simply restricted to chalk. If this ^{13}C-depletion of caddis larvae occurs on other geologies elsewhere in Britain, it would indicate that methane-carbon uptake in freshwater systems is comparatively widespread. This would have important considerations for the way resources are viewed in freshwater food webs, with the traditional allochthonous and autochthonous inputs accompanied by a 'third way'; carbon derived from methane via chemosynthetic processes (Trimmer *et al.* 2012).

1.1.9 Life history of 'armoured' grazing caddis

So far the majority of the evidence suggesting that methane-derived carbon is taken up by cased caddis has come from two 'armoured' grazing species; *Agapetus fuscipes* and *Silo nigricornis* (Trimmer *et al.* 2009, 2010). In most of the streams surveyed for the work contained within this thesis, *A. fuscipes*, *S. nigricornis* and *Silo pallipes* (a species closely related to *S. nigricornis*) were the most common and abundant species found from the Glossomatidae and Goeridae families. Thus for these two reasons, this thesis focuses on these three species in preference to other species within the Glossosomatidae and Goeridae.

Agapetus fuscipes is a member of the trichopteran family Glossosomatidae. In Britain, this family comprises seven species, with *Agapetus delicatulus* and *Agapetus ochripes* often coexisting with *Agapetus fuscipes* in streams. Morphologically, these three species are very similar (Wallace, Wallace & Philipson 1990). More recently, *Synagapetus dubitans*, another morphologically very similar species, was discovered for the first time in Britain in a Yorkshire stream and has since been discovered in further streams in this area (Crofts 2012a b).

Silo nigricornis is in the trichopteran family Goeridae, of which there are three species in Britain. *Silo nigricornis* and *Silo pallipes* (also considered here) are easily distinguished from each other with relative ease by their distinctive pronotum shape (the 'saddle' of the caddis larvae), and by distinctive markings on the pronotum for both species.

Glossosomatid and goerid larvae are considered to be 'armoured grazers' because they have strong rigid cases made of sand grains and, in the case of *Silo nigricornis*, fragments of rock attached laterally as ballast stones thought to provided increased protection against drift (Koenig & Waringer 2008).

Glossosomatidae and Goeridae larvae are often numerically dominant in streams which have not undergone extensive anthropogenic change (Becker 1990; Poff & Ward 1995; Nijboer 2004; Alvarez & Pardo 2005; Nakano, Kuhara & Nakamura 2007; Morris & Hondzo 2013). If species of these caddis larvae were found to be ^{13}C-depleted in wider range of streams with varying geological types, the potential for methane-derived carbon to reach predators at higher trophic levels, would be considerable.

There are however, some aspects of the life history of these armoured grazers that are unclear. The number of instars that *Agapetus fuscipes* undergoes in Britain is unknown. It is currently thought that *A. fuscipes* is likely to undergo seven instars, as it does in the Breitenbach, a small well-studied stream in Germany (Becker 2005).

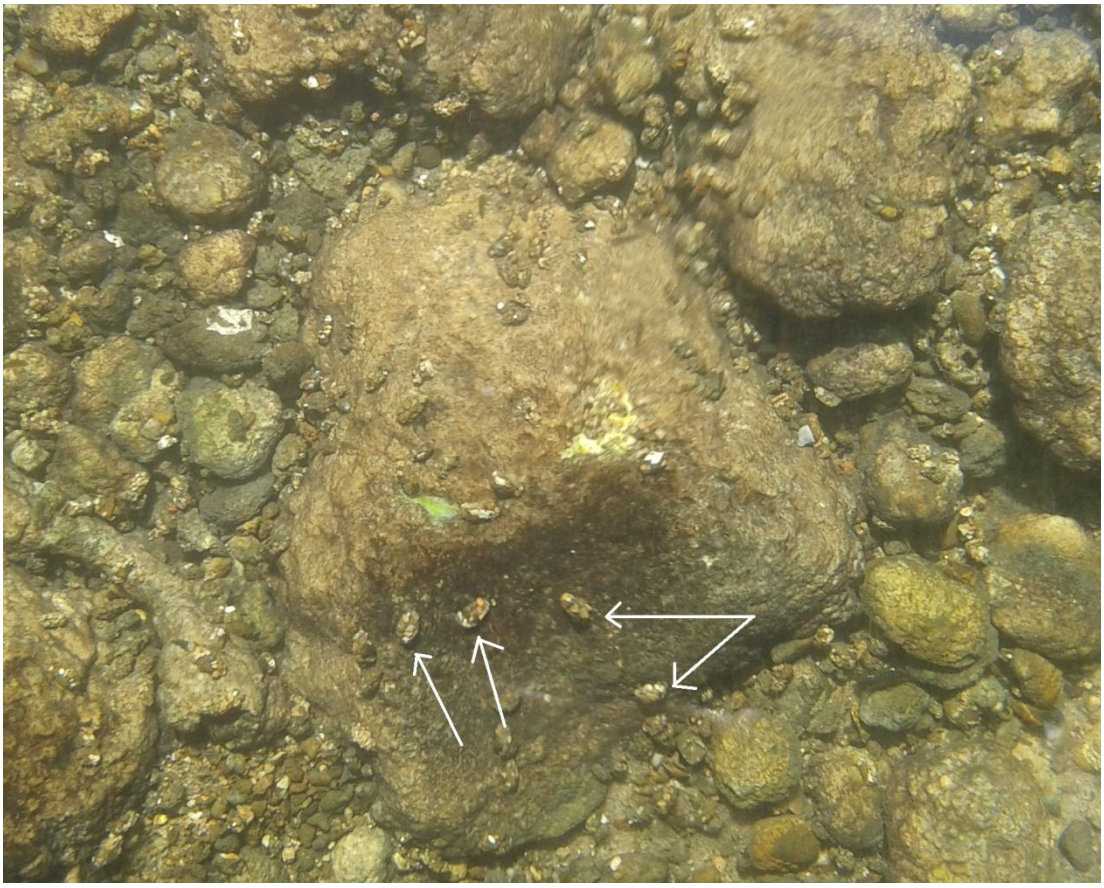

Figure 1.1 Armoured grazing caddis larvae such as *A. fuscipes* (highlighted with white arrows) are often abundant in streams such as the Cray (above). Photograph taken by author.

Most glossosomatid, and indeed most caddis species have five instars and it is relatively unusual for a caddis species to have more. *Sericostoma personatum*, another cased caddis, is the only species in Britain known to have more than five instars (with six; Elliott 1969). There is also uncertainty for *A. fuscipes* and *Silo* spp. in the number of generations per year that they have in Britain (Sangpradub, Giller & O'Connor 1999). *Agapetus fuscipes* is generally regarded as univoltine (Nielsen 1942; Benedetto Castro 1975; Sangpradub *et al.* 1999; Becker 2005), although Recasens & Murrillo (1986) reported it to be bivoltine in Spain and Iverson (1976) reported two separate cohorts in Denmark.

1.1.10 Uptake and assimilation of methane-oxidising bacteria by caddis larvae

The mechanism of uptake of methane-derived carbon by caddis is presumed to be via consumption of methane-oxidising bacteria (Trimmer *et al.* 2009), although this remains unsubstantiated. Trimmer *et al.* (2009) found that methane oxidation took place on both stream gravel and caddis cases and speculated that caddis could either passively ingest methane-oxidising bacteria (MOB), as a consequence of grazing epilithon containing MOB, or that the larvae actively graze and maintain a biofilm containing MOB on their own cases (or graze off those on conspecifics). There is some evidence that caddis larvae do, at times of resource limitation, graze from their own cases or those of other larvae. *Glossosoma intermedium* were observed grazing periphyton from conspecific cases when periphyton was scarce (Cavanaugh, Haro & Jones 2004). In an earlier observational study, larvae of *Agapetus fuscipes* survived longer in the laboratory in cases covered in algal growth than did larvae in 'clean' cases with no algal growth (Cox & Wagner 1989). More recent research by Ings, Hildrew & Grey (2010) found that the caddis larvae of a gallery-building caddisfly *Tinodes waeneri* fertilise this retreat via their nitrogenous excretions on which algae and other micro-organisms can grow and be exploited as a food resource by the caddis larvae.

1.1.11 Possible benefits of consumption of MOB

Ingesting and assimilating methane-derived carbon may provide life history benefits for the caddis larvae. When larval density is high, mortality is high (Marchant & Hehir 1999). Trimmer *et al.* (2009) suggested that larvae may survive when epilithon is reduced by ingesting methane-oxidising bacteria instead. Currently it is unknown whether grazing methane-oxidising bacteria confers any benefits to specific larval instars or life history stages.

1.1.12 Secondary production

Hitherto, population estimates of cased caddis have not been combined with stable isotope analysis of their $\delta^{13}C$ to quantify how much methane-derived carbon is assimilated by the caddis larvae over the life cycle. This is an important step in beginning to understand how much methane-derived carbon is taken up into freshwater food webs this way.

Establishing life-history parameters of the larvae in question (*A. fuscipes* and *Silo* spp.), will help pave the way for secondary production estimates to be calculated. Secondary production is the formation of heterotrophic biomass of a population, or a group of populations, over a period of time and provides a broad measure of success for that population (Benke 1993). Annual production can be measured in units of mass (grams of carbon/grams of dry mass) and is normally expressed per unit area per unit time (e.g. grams m^{-2} $year^{-1}$). Methods for the estimation of secondary production can be divided

into those requiring the presence or not of clear cohorts (groups of individuals born at the same time and developing in synchrony). Cohort methods are appropriate when such groups of larvae can be distinguished and followed through time. Non-cohort methods are appropriate for when a species undergoes asynchronous development or a cohort cannot be easily followed through time (Benke & Huryn 2006). The size-frequency method (Hynes & Coleman 1968; Hamilton 1969; Benke 1979) is often the most appropriate non-cohort method for field samples collected consecutively over the duration of one year.

Estimates of annual carbon production can then be combined with the $\delta^{13}C$ data of the larvae to further estimate how much of annual carbon production is methane-derived. This thesis assesses the extent of these potential methane subsidies to cased caddis secondary production in a range of stream systems in Britain.

1.1.13 Thesis aims

The overall aim of this thesis was to investigate the role of methane-derived carbon as an energy subsidy to cased caddis in streams and rivers across the UK. To achieve this aim, this thesis is split into four chapters:

Chapter 2

The aims of this chapter were to examine whether the phenomenon of ^{13}C-depletion seen in cased caddis larvae (relative to the epilithon) is widespread

in streams and rivers draining various geological types and whether ^{13}C-depletion of cased caddis correlates with stream methane concentration. Caddis $\delta^{13}C$ and stream methane concentration data are presented from a summer 2011 survey of 29 stream sites concentrated on three main geology types; chalk, limestone and sandstone. Some data collected in a summer survey by Tuffin 2014 was included to increase the number of chalk streams in the dataset. Relationships between geology, methane concentration and caddis $\delta^{13}C$ were examined using linear regression techniques.

Chapter 3

This chapter addresses gaps in the knowledge of key life history parameters of the caddis larvae studied within this thesis. The aims of this chapter were to identify whether caddis larvae of *Agapetus fuscipes*, *Silo nigricornis* and *Silo pallipes* can be separated into instars by easily identifiable body characteristics such as pronotum length, head width and body length, and whether generation times of the larvae can be identified. To answer these questions caddis larvae were collected quantitatively from eight streams chosen from the initial 29 streams surveyed in summer 2011 over a period of roughly one year. Body size characteristics were measured using a microscope and where possible larvae were assigned to instars using size-frequency histograms. Size-mass regressions were also calculated. Larval incidence of the three caddis species across the year was plotted in order to estimate generation times.

Chapter 4

This main aim of this chapter was to identify the streams in which caddis larvae have the highest secondary production and to examine the potential contribution to secondary production from methane-derived carbon. In order to achieve this aim, the size-frequency method (Hynes & Coleman 1968; Hamilton 1969; Benke 1979) was employed together with the size-mass regressions calculated in Chapter 3. Production estimates were combined with two-source stable isotope mixing models assuming two main resources of biofilm and methane-oxidising bacteria. ANCOVA models were used to ascertain whether chlorophyll *a*, month or stream influenced the amount of methane-derived carbon assimilated by caddis larvae. Finally linear regressions testing whether % MOB assimilation was correlated with the annual production of the caddis larvae were carried out.

Chapter 5

The first aim of this chapter was to examine whether methane-derived carbon becomes more critical at a specific point in life history for cased caddis. A secondary aim was to find out whether caddis larvae are more ^{13}C-depleted at certain times in the year and if this correlates with monthly stream methane concentration. In order to achieve the first aim larvae of *A. fuscipes* were sorted into instars for stable isotope analysis of each instar. The resulting $\delta^{13}C$ of the larvae was analysed using linear mixed effects models and differences in $\delta^{13}C$ of instars tested using a Tukey-Kramer HSD test. A further linear mixed effects model tested whether whether caddis larvae were influenced by the time of year and/or stream methane concentration.

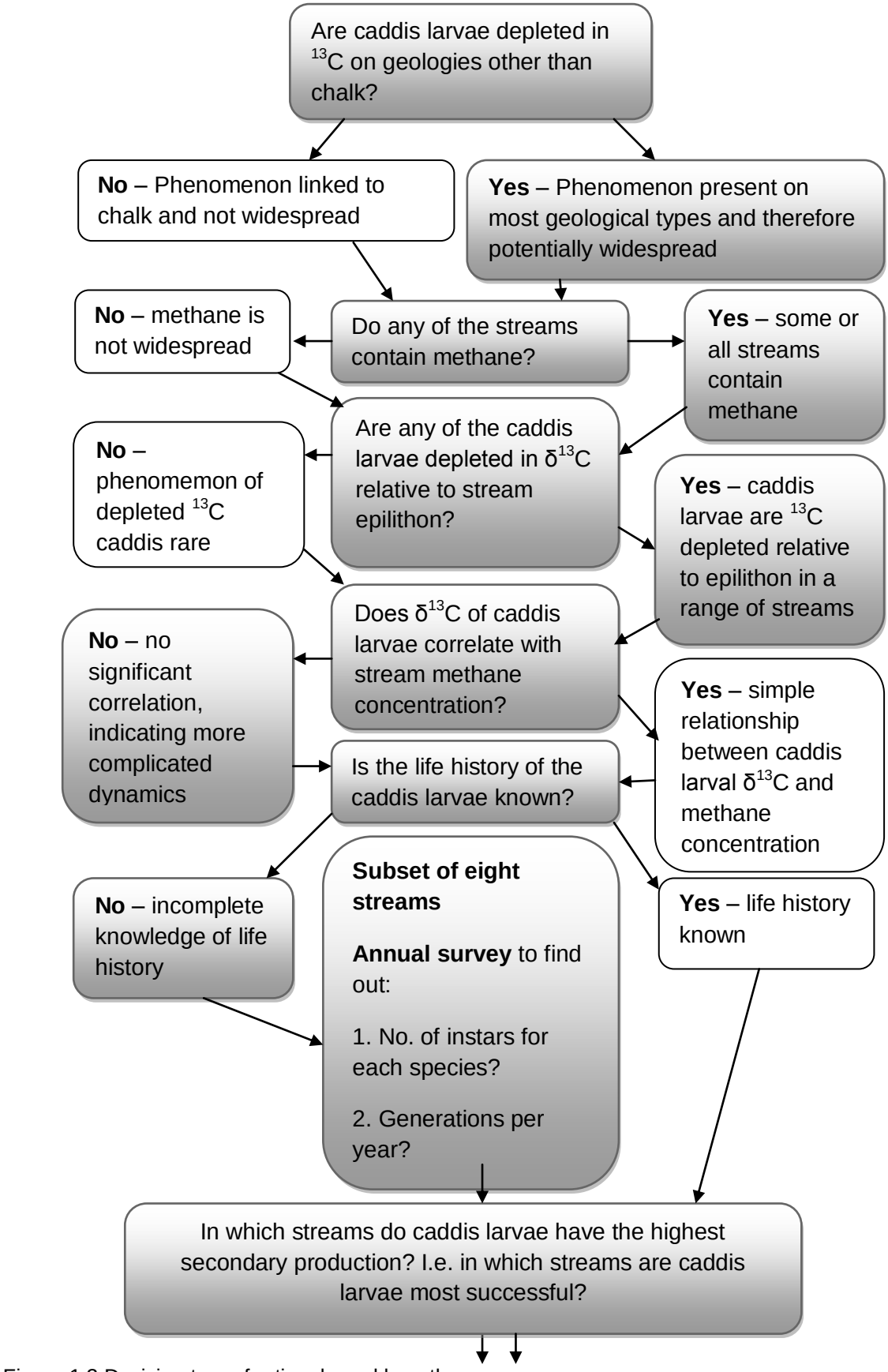

Figure 1.2 Decision tree of rationale and hypotheses

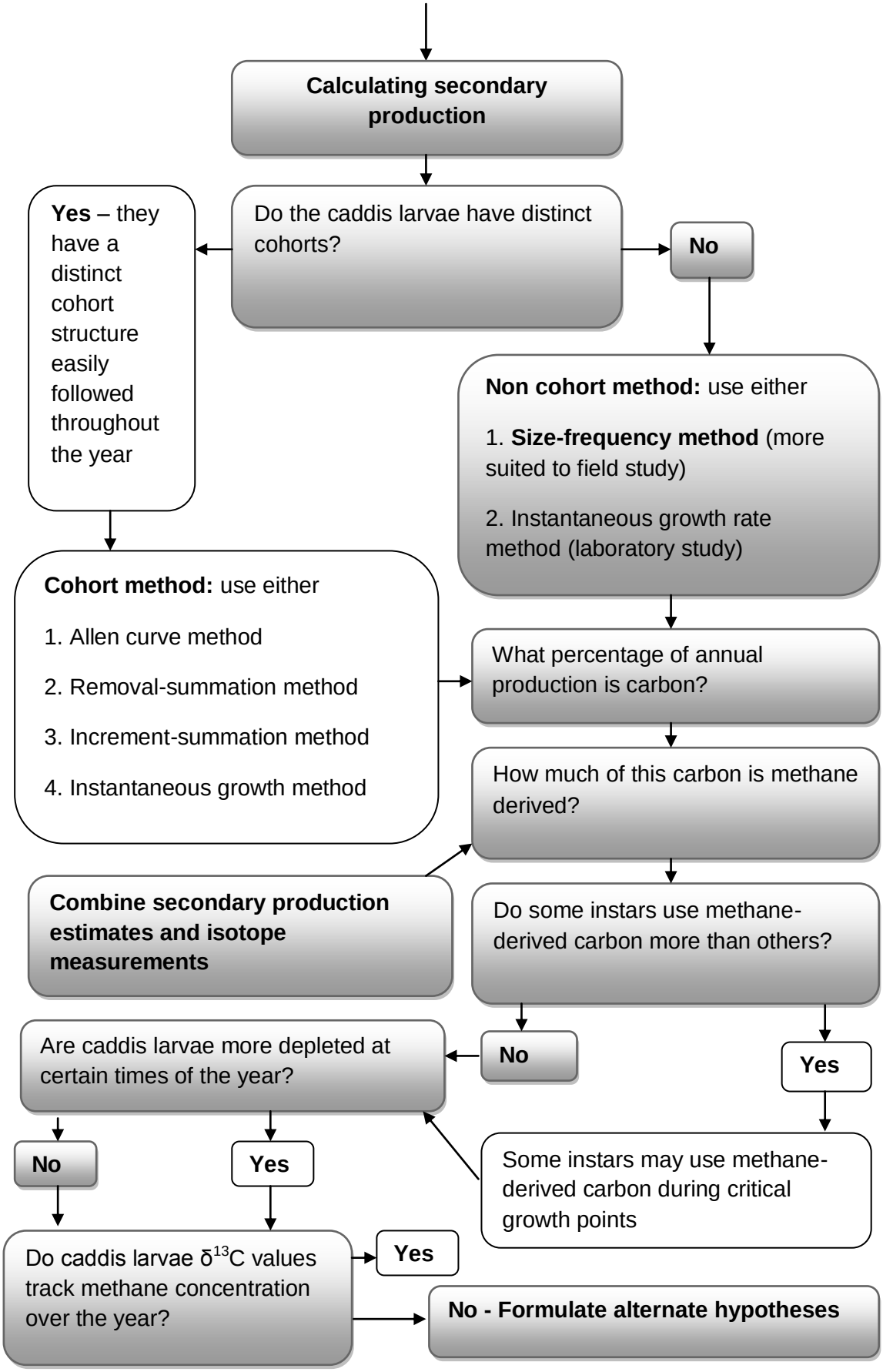

Figure 1.2 Decision tree of rationale and hypotheses continued.

2 Geology and the distribution of $\delta^{13}C$ depletion in cased caddis

2.1 Introduction

The first aim of this study was to investigate the geographical distribution of depletion of $\delta^{13}C$ in stream communities and, particularly, in the cased larvae of caddis from the families Glossosomatidae and Goeridae. They are most common on permeable geologies so sampling was concentrated on sandstone and limestone, as well as on chalk. A secondary aim of this study was to assess whether, as might be expected, any depletion in $\delta^{13}C$ of the caddis larvae from various sites would be correlated with methane concentration in the stream water. This latter expectation, if verified, would suggest that the importance of methane to the benthic community might be limited by its gross supply (either from chemosynthesis in the river itself or imported from elsewhere).

Food webs and the pathways of production in rivers and streams have been widely studied and are believed to be based overwhelmingly on organic carbon fixed by photosynthesis, either produced within the system by aquatic plants, algae and photosynthetic bacteria (autochthonous) or imported terrestrial production from the catchment (allochthonous) (Hynes 1970; Vannote *et al.* 1980; Thorp *et al.* 1998). The relative importance of this conventional allochthonous and autochthonous production is widely debated.

According to the River Continuum Concept (RCC) of Vannote et al (1980), allochthonous inputs are expected to be more important for small forested streams, where light availability to the stream channel is limited by shading (Fisher & Likens 1973; Webster & Meyer 1997). Autochthonous inputs are expected to be more important in fourth to sixth order un-shaded streams, where more light can reach the stream channel and terrestrial detritus inputs are lower (Vannote *et al.* 1980; Naiman *et al.* 1987; Finlay 2001). Allochthonous inputs are thought to become more important, once again, for rivers for order six or greater, as they are expected be light limited because of the increasing channel depth and turbidity, as well as having a plentiful supply of fine particulate organic matter transported from upstream (Vannote *et al.* 1980).

Some rivers seem to conform to the RCC; for example, Benke & Wallace (2014), found that, for a sixth order south-eastern U.S. coastal plain river, terrestrial organic matter was the main basis of production. However, other studies contradict the RCC and suggest that autochthonous inputs to large rivers have been underestimated. In the Ohio River, one of the largest rivers in the U.S., phytoplankton and detritus of autochthonous origin were found to be more important to consumers than organic matter from upstream (Thorp *et al.* 1998). Similarly, Bunn, Davies & Winning (2003) found that the food web was supported mainly by filamentous algal production, in a so-called 'bathtub' ring around the margins of turbid Australian floodplain rivers. These had been expected to be light-limited and dependent on terrestrial riparian carbon.

More recently, and adding to the controversy over the importance of allochthonous and autochthonous inputs to rivers, evidence has been discovered that suggests a third, chemosynthetic carbon source may be entering freshwater food webs, mainly in the form of methane-derived carbon (Trimmer *et al.* 2009). Methane is known to be present in many freshwater systems such as lakes, fens and bogs (Moore & Knowles 1990; Bastviken *et al.* 2004), with associated microbial communities made up of methanogens and methanotrophs often being detectable (Murrell & Jetten 2009; Gentzel *et al.* 2012). Methanogens produce methane via methanogenesis, a form of bacterial chemosynthesis which requires anoxic conditions to produce reduced carbon compounds using chemical energy (Woltemate *et al.* 1984; Jannasch & Mottl 1985; Jones & Grey 2011). Methanotrophy, is the process of metabolising methane, usually via oxidation, thus releasing the chemosynthetic energy created by methanogenesis (Rudd, Hamilton & Campbell 1974; Zeikus & Winfrey 1976; Boon & Mitchell 1995; Schubert *et al.* 2011).

The importance of methane in rivers and streams is poorly understood. Methane is a potent greenhouse gas and, at present, little is known about how much is emitted from rivers and streams to the atmosphere (Prairie & del Giorgio 2013). However, methane gas is a prominent trace constituent of the water in many rivers and streams, and can often be at concentrations well in excess of equilibrium with the atmosphere (Jones & Mulholland 1998; Darling & Gooddy 2006). Methane has been previously found to be in the

water of streams draining basalt, shale and dolomite (De Angelis & Lilley 1987; Jones & Mulholland 1998).

The methane contained within streams and rivers can be biogenic (formed from organic matter broken down by microbes under anoxic conditions) (Jones & Mulholland 1998; Hlaváčová *et al.* 2005; Gebert *et al.* 2006; Jones & Grey 2011) or thermogenic origin (methane from gas fields, petroleum reservoirs or coal) (Kvenvolden 1995; Head *et al.* 2003; Moore 2012) or a mixture of both. Most of the methane contained within rivers and streams is usually of biogenic origin (Jones & Grey 2011). Biogenic methane is often very C^{13} depleted (-80 to -60‰) due discrimination against C^{13} during the process of methanogenesis (Whiticar *et al.* 1986; Jędrysek 2005; Conrad *et al.* 2007).

Depleted values of $\delta^{13}C$ for carbon derived from biogenic methane are often quite distinct from the $\delta^{13}C$ of carbon derived from autochthonous or allochthonous sources fixed by photosynthesis. Imported allochthonous terrestrial C3 plant material has a value around -28‰ (Rau 1980; Rounick, Winterbourn & Lyon 1982; France 1995b; Finlay 2001). The $\delta^{13}C$ of autochthonous carbon produced by photosynthesis is more variable ranging from -50‰ to -10‰ (Osmond *et al.* 1981). Aquatic plants, for example, can vary more widely in $\delta^{13}C$ than terrestrial plant material, because they may use DIC which can also be very variable in $\delta^{13}C$ (Osmond *et al.* 1981), and atmospheric CO_2 can be used when leaves are above water (Hamilton &

Lewis 1992). Phytoplankton can sometimes also show large variation in $\delta^{13}C$ (France 1995; Finlay, Power & Cabana 1999).

The thickness of the "boundary layer" in streams can also affect the $\delta^{13}C$ of aquatic organisms. A boundary layer is created when stream water decreases in velocity with depth when it approaches the bottom of the stream bed. This profile in decreasing velocity is known as the boundary layer, the upper limit of the boundary layer is where the speed of the current is no longer influenced by the stream bed. Very close to the bed the flow is greatly reduced (Allan & Castillo 2007). The boundary layer controls the diffusion of inorganic molecules such as CO_2 and HCO_3^- to aquatic phototrophic organisms. Stream current can reduce the thickness of the boundary layer (Trudeau & Rasmussen 2003). If the boundary layer is thick, this limits the supply rate of inorganic carbon to phototrophs. When the supply of carbon is limited in this way, phototrophs utilise the heavier carbon isotope of C^{13} which they would normally discriminate against in favour of the lighter C^{12}. Plants in moving water tend to be more enriched in C^{13} than plants in still water (Osmond *et al.* 1981; France 1995a). Similarly, the $\delta^{13}C$ of algae decreases under conditions of high turbulence which promote mixing and diffusion of CO_2 to algal cells (France & Holmquist 1997; Finlay *et al.* 1999; Trudeau & Rasmussen 2003).

The $\delta^{13}C$ of carbon is often used as a "tracer" in isotope studies of food webs to assess which species consume each other and to track how carbon moves

to higher trophic levels. The $\delta^{13}C$ of carbon remains relatively conserved as it passes up through the food web, increasing only by around +1‰ for each new step (Peterson & Fry 1987). The increase in $\delta^{13}C$ between a source and its consumer is known as an "isotope discrimination factor" (Perga & Grey 2010). The conservation of the $\delta^{13}C$ ratio from source to consumer, as well as the large difference in $\delta^{13}C$ between methane-derived carbon and that derived from photosynthesis, should allow methane-derived carbon to be traced relatively straightforwardly through the food web. However there is uncertainty associated with the fractionation of carbon as it passes from source to consumer and this must also be taken into account. A variety of factors affect the isotope discrimination factors between organisms in food webs such as temperature, dietary protein content and excretion (Power, Guiguer & Barton 2003; Vanderklift & Ponsard 2003). Several studies have investigated isotope discrimination factors for aquatic invertebrates. Vander Zanden & Rasmussen (2001) discovered that aquatic herbivores displayed significantly lower $\delta^{13}C$ fractionation than non-herbivores (mean ± 1 SD; -0.41 ± 1.14‰ vs 0.91 ± 1.04‰). Post (2002), combined measurements of $\delta^{13}C$ fractionation from grazing snails and filter feeding mussels in lakes to obtain a $\delta^{13}C$ fractionation value that was representative of basal consumers (mean ± 1 SD; 0.39 ± 1.3‰). More recently Moore & Semmens (2008) incorporated a $\delta^{13}C$ fractionation value for aquatic organisms (mean ± 1 SD, 0.4 ± 1.20‰, McCutchan *et al.* 2003) into a Bayesian mixing model that can incorporate fractionation uncertainty.

Mass spectrometry has recently been used to study basal consumers in chalk stream food webs where, somewhat surprisingly, methane has been found at high concentrations in the water column of the streams (Trimmer *et al.* 2009; Shelley, Grey & Trimmer 2014). Chalk streams are highly productive, with very high autochthonous primary production (Berrie 1992). However, they have also often been found to contain high concentrations of dissolved methane in the water, particularly during the summer (Sanders *et al.* 2007; Trimmer *et al.* 2009). Anoxic conditions created by sediment retention around macrophyte stands can facilitate in-stream methanogenesis (Jones & Mulholland 1998; Sand-Jensen 1998; Sanders *et al.* 2007). This methane is available to naturally occurring methane oxidising bacteria and could thus be converted into particulate (methane-derived) carbon and incorporated into biofilms that could be grazed and assimilated by basal consumers, such as some cased caddis larvae.

Research carried out by Trimmer *et al.* (2009) in the River Lambourn, a chalk stream in East Berkshire, studied cased larvae of two species of armoured grazing caddis (Trichoptera), *Agapetus fuscipes* (Family Glossosomatidae) and *Silo nigricornis* (Goeridae). Both species are believed to consume mainly epilithon as well as some detritus (Douglas 1958; Elliott 1982; Arens 1990; Nijboer 2004). However, when the tissues of *A. fuscipes* and *S. nigricornis* were analysed using mass spectrometry they were found to be consistently $\delta^{13}C$ depleted (mean 41.2‰ and 40.4‰, respectively). These values were 2.7 – 11.1‰ lower than any of the putative autochthonous and allochthonous food sources sampled. Trimmer *et al.* (2009) had expected that both *A.*

fuscipes and *S.nigricornis* would, under fractionation of +0.5‰ (McCutchan *et al.* 2003), be similar in $\delta^{13}C$ relative to the epilithon of the river or one of the autochthonous or allochthonous resources sampled. This $\delta^{13}C$ depletion suggests assimilation of methane-derived carbon. The phenomenon of depleted $\delta^{13}C$ in *A. fuscipes* and *S. nigricornis*, relative to stream epilithon, has since been found in a wide range of chalk streams in the south of the UK (Tuffin, 2014). This is surprising given that the caddis larvaes' presumed food resource of algae is likely to be abundant within chalk streams (Marker 1976a; Shamsudin & Sleigh 1994).

Chalk streams, although widespread in much of southern and eastern Britain, are very rare globally (Biodiversity: the UK Steering Group Report 1995). Chalk streams stretch in a band from Norfolk in the east of the UK to Dorset in the south of the UK, with some also found in chalk outcrops in Lincolnshire and East Yorkshire. The geology of the British Isles is very varied, with permeable formations such as sandstone and limestone being well represented (Figure 2.1). Bedrock permeability is important because it can allow the transfer of methane present in groundwater to rivers and streams (Gooddy & Darling 2005). If methane exists at measurable concentrations in rivers and streams of other geological types, it would be inherent to suggest that this methane could be metabolised, in streams on other geologies, by naturally occurring methane oxidising bacteria, into a particulate form that could then, potentially, fuel basal consumers such as cased caddis. Armoured grazing caddis larvae, such as *Agapetus fuscipes* and *Silo nigricornis*, can be dominant and extremely numerous in streams and rivers

(Nijboer 2004; Alvarez & Pardo 2005). Thus, the potential biomass available for predators to exploit and for methane-derived carbon to reach higher trophic levels in the food web is substantial. Potential known riverine predators of *Agapetus* and *Silo* spp. that could take up this methane-derived carbon, include bullhead (*Cottus gobio*) and trout (*Salmo trutta*), (Otto & Johansson 1995) and predatory stonefly nymphs such as *Dinocras cephalotes* (Malmqvist 1992).

An obvious question is then to ask if this phenomenon is widespread in streams and rivers draining other geologies, as well as chalk, or draining chalk outside the south. Given that the pervasive paradigm is that lotic food webs are supported almost exclusively by autochthonous and allochthonous inputs, ultimately of photosynthetic origin, any evidence that a chemosynthetic carbon pathway is widespread and quantitatively substantial would mark a clear advance in our understanding. It would also require assessment of the magnitude of this contribution to secondary production and of any spatial and temporal patterns in its importance.

Figure 2.1 The bedrock geology of the UK is very varied. See the following page for a detailed key to the geologies. Reproduced with the permission of the British Geological Survey ©NERC. All rights reserved.


APPIN GROUP
APPIN GROUP AND ARGYLL GROUP (UNDIFFERENTIATED)
APPLEBY GROUP
ARBUTHNOTT-GARVOCK GROUP
ARDVRECK GROUP
ARENIG ROCKS (UNDIFFERENTIATED)
ARGYLL GROUP
ARMAGH GROUP
ASHGILL ROCKS (UNDIFFERENTIATED)
BELFAST GROUP
BLACKCRAIG FORMATION AND GALDENOCH FORMATION (UNDIFFERENTIATED)
BORDER GROUP
BOUNDARY ZONE COMPLEX
BOWLAND HIGH GROUP AND CRAVEN GROUP (UNDIFFERENTIATED)
BRACKLESHAM GROUP AND BARTON GROUP (UNDIFFERENTIATED)
CAMBRIAN AND ORDOVICIAN ROCKS (UNDIFFERENTIATED)
CARADOC ROCKS (UNDIFFERENTIATED)
CLACKMANNAN GROUP
COAL MEASURES GROUP [OBSOLETE EXCEPT IN NORTHERN IRELAND: USE PCM, SWCM, CMSC]
CORALLIAN GROUP
CRAWFORD GROUP AND MOFFAT SHALE GROUP (UNDIFFERENTIATED)
CROSS SLIEVE GROUP
DEVONIAN ROCKS (UNDIFFERENTIATED)
DINANTIAN ROCKS (UNDIFFERENTIATED)
DUNNOTTAR-CRAWTON GROUP
DURNESS GROUP
ENLER GROUP
EOCENE TO MIOCENE ROCKS (UNDIFFERENTIATED)
FAULT ZONE ROCKS, UNASSIGNED
FINTONA GROUP
GALA GROUP
GAULT FORMATION AND UPPER GREENSAND FORMATION (UNDIFFERENTIATED)
GLENFINNAN GROUP
GRAMPIAN GROUP
GREAT OOLITE GROUP
GREY CHALK SUBGROUP
HAWICK GROUP
HIBERNIAN GREENSANDS FORMATION AND ULSTER WHITE LIMESTONE FORMATION (UNDIFFERENTIATED)
HIGHLAND BORDER COMPLEX [UNDER REVIEW; POSSIBLY OBSOLETE]
HOLSWORTHY GROUP
HOLYWOOD GROUP
INFERIOR OOLITE GROUP
INVERCLYDE GROUP
KELLAWAYS FORMATION AND OXFORD CLAY FORMATION (UNDIFFERENTIATED)
KILSKEERY GROUP
KIRKCOLM FORMATION
LAMBETH GROUP
LANARK GROUP
LEADHILLS SUPERGROUP
LEITRIM GROUP
LEWISIAN COMPLEX
LIAS GROUP
LLANDOVERY ROCKS (UNDIFFERENTIATED)
LLANVIRN ROCKS (UNDIFFERENTIATED)
LOCH EIL GROUP
LOCH MAREE GROUP
LOUGH NEAGH CLAYS GROUP
LOWER CAMBRIAN ROCKS (UNDIFFERENTIATED)
LOWER DEVONIAN ROCKS (UNDIFFERENTIATED)
LOWER GREENSAND GROUP
LOWER OLD RED SANDSTONE
LUDLOW ROCKS (UNDIFFERENTIATED)
MERCIA MUDSTONE GROUP
MESOPROTEROZOIC ROCKS (UNDIFFERENTIATED)
MIDDLE CAMBRIAN
MIDDLE DEVONIAN (UNDIFFERENTIATED)
MIDDLE JURASSIC ROCKS (UNDIFFERENTIATED)
MIDDLE OLD RED SANDSTONE (UNDIFFERENTIATED)
MILLSTONE GRIT GROUP [SEE ALSO MIGR]
MOINE SUPERGROUP
MORAR GROUP
NEOGENE ROCKS (UNDIFFERENTIATED)
OWENKILLEW SANDSTONE GROUP
PENNINE COAL MEASURES GROUP
PENNINE LOWER COAL MEASURES FORMATION AND SOUTH WALES LOWER COAL MEASURES FORMATION (UNDIFFERENTIATED)
PENNINE MIDDLE COAL MEASURES FORMATION AND SOUTH WALES MIDDLE COAL MEASURES FORMATION (UNDIFFERENTIATED)
PENNINE UPPER COAL MEASURES FORMATION
PERMIAN ROCKS (UNDIFFERENTIATED)
PORTLAND GROUP
PORTPATRICK FORMATION AND GLENWHARGEN FORMATION (UNDIFFERENTIATED)
PRIDOLI ROCKS (UNDIFFERENTIATED)
PURBECK LIMESTONE GROUP
QUEYFIRTH GROUP
RAVENSCAR GROUP
RED BAY FORMATION
RESTON GROUP
RICCARTON GROUP
ROE VALLEY GROUP
SCOTTISH COAL MEASURES GROUP
SHANMULLAGH FORMATION
SHERWOOD SANDSTONE GROUP
SHINNEL FORMATION AND GLENLEE FORMATION (UNDIFFERENTIATED)
SILURIAN ROCKS (UNDIFFERENTIATED)
SLEAT GROUP
SLIEVEBANE GROUP
SOLENT GROUP
SOUTH WALES UPPER COAL MEASURES FORMATION
SOUTHERN HIGHLAND GROUP
STEWARTRY GROUP
STOER GROUP
STONEHAVEN GROUP
STRATHCLYDE GROUP
STRATHEDEN GROUP
STRATHMORE GROUP
STRATHY COMPLEX
TAPPINS GROUP
TEIGN VALLEY GROUP
THAMES GROUP
THANET SAND FORMATION
TORRIDON GROUP
TREMADOC ROCKS (UNDIFFERENTIATED)
TRIASSIC ROCKS (UNDIFFERENTIATED)
TYRONE GROUP
UNNAMED EXTRUSIVE ROCKS, CAMBRIAN
UNNAMED EXTRUSIVE ROCKS, CARBONIFEROUS
UNNAMED EXTRUSIVE ROCKS, DEVONIAN
UNNAMED EXTRUSIVE ROCKS, DINANTIAN
UNNAMED EXTRUSIVE ROCKS, NEOPROTEROZOIC
UNNAMED EXTRUSIVE ROCKS, ORDOVICIAN
UNNAMED EXTRUSIVE ROCKS, PALAEOGENE
UNNAMED EXTRUSIVE ROCKS, PALAEOPROTEROZOIC
UNNAMED EXTRUSIVE ROCKS, PERMIAN
UNNAMED EXTRUSIVE ROCKS, SILESIAN
UNNAMED EXTRUSIVE ROCKS, SILURIAN
UNNAMED EXTRUSIVE ROCKS, SILURIAN TO DEVONIAN
UNNAMED IGNEOUS INTRUSION, CAMBRIAN TO ORDOVICIAN
UNNAMED IGNEOUS INTRUSION, CARBONIFEROUS TO PERMIAN
UNNAMED IGNEOUS INTRUSION, DEVONIAN
UNNAMED IGNEOUS INTRUSION, LATE SILURIAN TO EARLY DEVONIAN
UNNAMED IGNEOUS INTRUSION, NEOPROTEROZOIC
UNNAMED IGNEOUS INTRUSION, ORDOVICIAN TO SILURIAN
UNNAMED IGNEOUS INTRUSION, PALAEOGENE
UNNAMED IGNEOUS INTRUSION, PALAEOPROTEROZOIC
UNNAMED METAMORPHIC ROCKS, NEOPROTEROZOIC
UNNAMED METAMORPHIC ROCKS, PRE-CALEDONIAN TO CALEDONIAN
UNNAMED METASEDIMENTARY ROCKS, NEOPROTEROZOIC
UNST PHYLLITE GROUP
UPPER CAMBRIAN, INCLUDING TREMADOC
UPPER CRETACEOUS TO PALAEOGENE ROCKS (UNDIFFERENTIATED)
UPPER DEVONIAN ROCKS (UNDIFFERENTIATED)
UPPER JURASSIC ROCKS (UNDIFFERENTIATED)
UPPER OLD RED SANDSTONE
WARWICKSHIRE GROUP
WEALDEN GROUP

2.2 Methods

2.2.1 Site selection

Cased larvae of the caddis families Glossosomatidae and Goeridae are usually restricted to stony headwater streams and springs (Elliott 1982; Nijboer 2004). Methane is more likely to be present in streams where the underlying bedrock is permeable, in particular on chalk, sandstone and limestone where methane present in groundwater can be transported through the permeable geology to surface stream water (Gooddy & Darling 2005). With the habitat requirements for these cased caddis and the underlying geology in mind, the main site selection criteria were to include streams on various permeable geological types with a large population of glossosomatid and (or) goerid larvae present. Streams were on one of three main geological types: chalk, limestone and sandstone. Additionally, one site on the coal measures of South Wales (Site 7. Dare Country Park) was sampled as an example of another permeable geology type likely to be associated with coal bed methane (Alderton & Bevins 1996; Ulrich & Bower 2008). One mafic lava site (Site 26. Carrock Beck Spring, Lake District) was sampled as an example of a spring on a generally impermeable geology type but with a particularly dense population of *Agapetus*.

Twenty-nine rivers and streams were surveyed over seven geographic regions between April 2011 and November 2011 (Figure 2.2). The majority of rivers and streams were sampled during the summer (Table 2.1). Two chalk

streams in Lincolnshire (Belleau, site 28 and Welton le Wold, site 29), however, were surveyed later in November 2011, to broaden the geographical coverage of chalk to include the northern outcrops and to see if caddis larvae were also $\delta^{13}C$ depleted there.

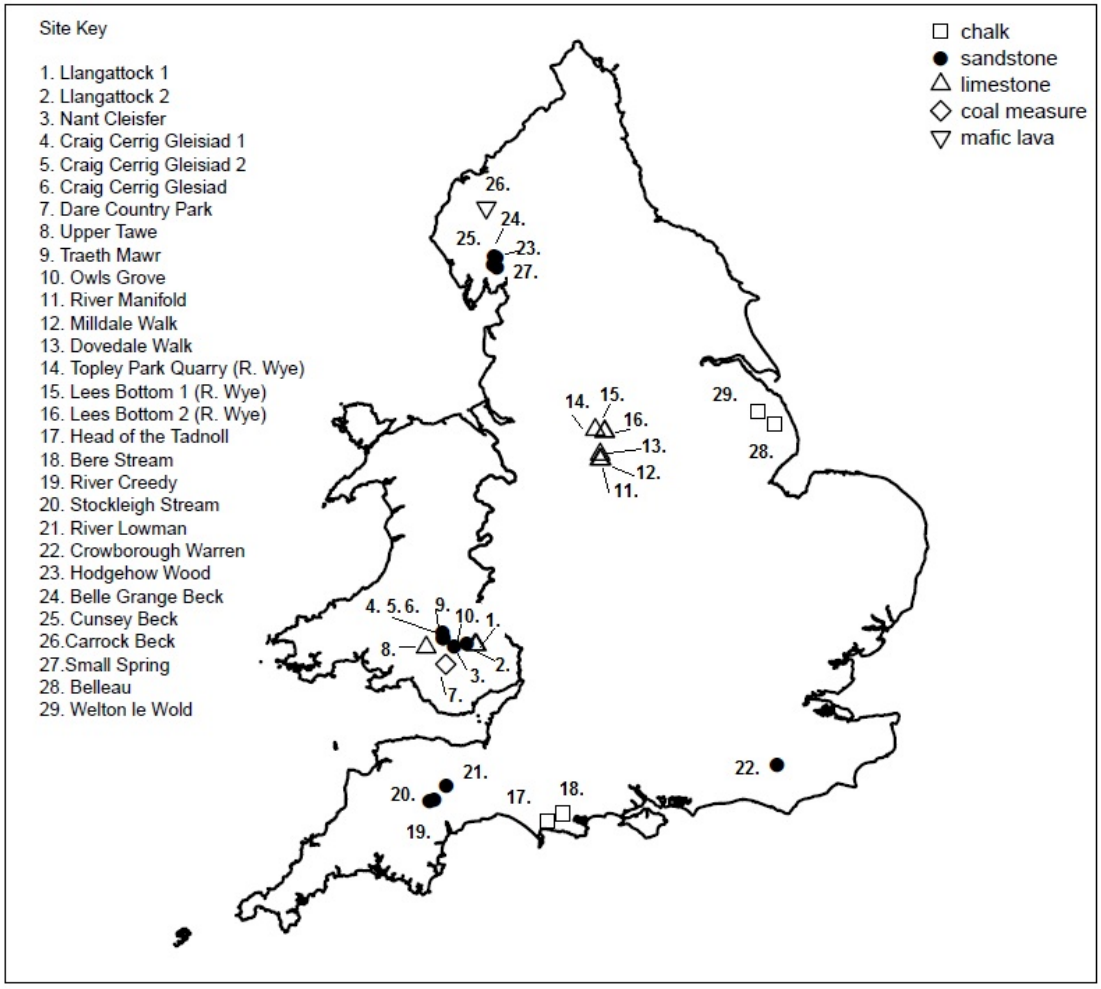

Figure 2.2 Map of the twenty-nine streams surveyed in summer 2011. Symbol denotes the underlying geology

Table 2.1 Sampling dates, grid references and bedrock classification of the 29 streams sampled in 2011.

Sampling Date	Site No.	Name	Area	Grid Reference	Bedrock
18-Apr-11	1	Llangattock 1	S. Wales	SO2065316850	Limestone
19-Apr-11	2	Llangattock 2	S. Wales	SO2171216512	Limestone
19-Apr-11	3	Nant Cleisfer	S. Wales	SO1441617174	Sandstone
19-Apr-11	4	Craig Cerrig Gleisiad 1	S. Wales	SN9713022240	Sandstone
19-Apr-11	5	Craig Cerrig Gleisiad 2	S. Wales	SN9671222010	Sandstone
19-Apr-11	6	Craig Cerrig Gleisiad 3	S. Wales	SN9680522050	Sandstone
20-Apr-11	7	Dare Country Park	S. Wales	SN9838002822	Coal Measure
20-Apr-11	8	Upper Tawe	S. Wales	SN8407015556	Limestone
20-Apr-11	9	Traeth Mawr	S. Wales	SN9636026068	Sandstone
21-Apr-11	10	Owls Grove	S. Wales	SO0481316263	Sandstone
05-May-11	11	River Manifold	Peak District	SK1346251313	Limestone
05-May-11	12	Milldale Walk	Peak District	SK1381455056	Limestone
05-May-11	13	Dovedale Walk	Peak District	SK1460351431	Limestone
05-May-11	14	Topley Park Quarry (R. Wye)	Peak District	SK1020872951	Limestone
06-May-11	15	Lees Bottom 1 (R. Wye)	Peak District	SK1691471407	Limestone
06-May-11	16	Lees Bottom 2 (R. Wye)	Peak District	SK1709971853	Limestone
31-May-11	17	Head of the Tadnoll	Dorset	SY7408987590	Chalk
31-May-11	18	Bere Stream	Dorset	SY8570693057	Chalk
01-Jun-11	19	River Creedy	Devon	SS8386402920	Sandstone
01-Jun-11	20	Stockleigh Stream	Devon	SS8765304452	Sandstone
01-Jun-11	21	River Lowman	Devon	SS9708013730	Sandstone
03-Jun-11	22	Crowborough Warren	E. Sussex	TQ4882230915	Sandstone
14-Jun-11	23	Hodgehow Wood	Lake District	SD4022199290	Sandstone
14-Jun-11	24	Belle Grange Beck	Lake District	SD3859599654	Sandstone
14-Jun-11	25	Cunsey Beck	Lake District	SD3780793931	Sandstone
14-Jun-11	26	Carrock Beck Spring	Lake District	NY3363135455	Mafic Lava
15-Jun-11	27	Small Spring	Lake District	SD4047791352	Sandstone
21-Nov-11	28	Belleau	Lincolnshire	TF4008075965	Chalk
22-Nov-11	29	Welton le Wold	Lincolnshire	TF2758888068	Chalk

2.2.2 Collection of caddis larvae and resources for stable isotope analysis

Three replicates of cased caddis larvae were collected directly by hand from three different gravel patches and stored in 50ml Falcon tubes. In some streams larvae were scarce so only one or two replicates were collected. Epilithon samples were taken by scraping rocks and the scrapings places in 50ml Falcon tubes. Samples were then immediately frozen in a 17L Engel portable freezer. In the laboratory, caddis larvae were removed from cases using tweezers. Fifteen larvae of all sizes (apart from pupae) from each original replicate were then placed in a 2ml eppendorf to make up a replicate for mass spectrometry (~2.5mg per eppendorf). Where possible three replicates of larvae (in some cases more and in some cases less depending on the abundance of larvae in each stream) were used for analysis with mass spectrometry (see Table 8.1 for the number of replicates used for each stream).

Caddis larvae and epilithon samples were then oven dried for 48 hours at 60°C and ground to a fine powder using an agate pestle and mortar. Epilithon was treated to get rid of any remaining carbonate residue by putting the samples in 20ml glass vials and adding a solution of 4% HCL to dissolve the carbonate. The vials were left until no more bubbles were seen rising from the solution, indicating that all the carbonate had been dissolved. In some cases 4% HCL was repeatedly added to dissolve all of the carbonate fully. The dried and ground epilithon samples were stored in 2ml Eppendorf

tubes. Samples were weighed out in ultra-clean tin capsules (0.5µg for caddis larvae and 0.8µg for epilithon) for stable isotope analysis. Epilithon samples that had a high C:N ratio were weighed out to 1.6µg, to increase the amount of nitrogen in the sample to obtain a reliable nitrogen isotope ratio. Samples were combusted using an elemental analyser coupled to a continuous flow isotope ratio mass spectrometer (CF/IRMS, Thermo-Finnigan, Delta Matt Plus). Isotope calibration was carried out using the international standard for carbon (Ref. 8542, sucrose -10.47‰ δ13C vs. Vienna-PeeDee Belemnite [VPDB], National Institute of Standards and Technology) and international standard for nitrogen (Ref. 8547, ammonium sulphate 0.4‰ vs. air, National Institute of Standards and Technology. Isotope values are expressed using the standard delta (δ) notation with units of per mille (‰):

$$\delta = [(R_{SAMPLE} / R_{STANDARD} - 1)] * 1000$$

For each run an initial calibration using urea was carried out to determine nitrogen and carbon content. Cicloesanone-2,4-denitrofenilildrazone ($C_{12}H_{14}N_4O_4$), was used as an internal standard every 10 samples to check the precision of the C and N stable isotope values.

2.2.3 Methane in river water

River methane concentration (n = 5 for each site) was measured by drawing up river water with a 60ml gastight syringe and a 150mm length of polytetrafluoroethylene tubing (after Pretty, Hildrew & Trimmer 2006). River water was pushed through the syringe through the tubing into a gas tight vial (12.5ml Exetainer, Labco). This water was allowed to overflow three times in order to avoid direct contact of the water with the atmosphere. Samples were fixed with 100µl $ZnCL_2$ 50% w/v; bactericide and the lid fastened. In the laboratory, a 2ml headspace of analytical grade helium was inserted into each vial using a two-way valve and gastight microlitre syringe. Samples were placed on rollers (Denley Spiramix, Thermo), for 24 hours to allow the gas inside the vials to equilibrate fully. After this, samples were analysed using a Gas Chromatograph in conjunction with a flame ionising detector (GC-FID). The methane concentration in the headspace of the vial was calculated from peak areas calibrated against known standards. The total amount of methane gas in each vial (headspace + water) was calculated using the ideal gas law and solubility coefficients (Yamamoto, Alcauskas & Crozier 1976).

The ideal gas law:

$$PV = nRT$$

Where P = pressure of the gas in the atmosphere (atm)

V = the volume of the gas in Litres (L)

n = the amount of gas in moles (mol)

T = the temperature of the gas in kelvins (K)

R = ideal gas constant (0.08206)

The Bunsen solubility constant for methane is expressed as follows:

$$\ln \beta = A_1 + A_2(100/T) + A_3 \ln (T/100) + S[B_1 + B_2(T/100) + B_3(T/100)^2]$$

Where ***β*** = the volume of gas (corrected to standard volume and pressure) absorbed in a unit volume of water at the measurement temperature when the partial pressure of the gas is 760mm.

A and ***B*** are constants:

A_1 = -67.1962 B_1 = -0.07291

A_2 = 99.1624 B_2 = 0.04167

A_3 = 27.9015 B_3 = -0.00646

T = temperature in degrees Kelvin (K)

S = salinity in parts per thousand

Using the ideal gas law and Bunsen coefficient to calculate the total CH_4 in nmol L^{-1} of streamwater:

1. CH_4 in 2ml headspace (nmol):

CH_4 (nmol/L^{-1}) = **(CH_4 mol / V) * 10^9**

CH_4 nmol in 2ml = **CH_4 (nmol/L^{-1}) * 0.002**

2. Total CH_4 in 12.5ml vial (nmol):

= **CH_4 (nmol/L^{-1}) * (0.002 + (*β* * 0.0105))**

3. CH_4 nmol L^{-1} stream water:

= **Total CH_4 in 12.5ml vial (nmol) / 0.0105**

2.2.4 Data analysis

All statistical analysis were completed in R version 3.1.1 (R Core Development Team 2014). Goerid larvae were only found at one stream site (8 Upper Tawe) with pupae found in 8 of the streams. Goeridae larvae and pupae were thus discounted from any analysis because of the low numbers found. Cunsey Beck (25) was discounted from any analysis involving larval $\delta^{13}C$ as only *Silo* spp. pupae were found and no other active larvae of either goeridae or glossosomatidae found. Glossosomatid larvae were found at 28 of the 29 streams. Thus glossosomatid larvae from 28 streams were used to investigate whether larvae were more depleted than the epilithon of the stream. The following equation was used to calculate the true isotope difference between the samples (after Fry 2006).

Where δ_1 = Caddis larval $\delta^{13}C$ (‰)

Where δ_2 = Epilithon $\delta^{13}C$ (‰)

$\delta_{1,2} = [(\delta_1 - \delta_2) / (\delta_2 + 1000)] * 1000$

Fractionation uncertainty around the $\delta^{13}C$ values of the caddis larvae was incorporated using a $\delta^{13}C$ discrimination factor of (mean ± 1 SD; 0.4 ± 1.20‰, McCutchan *et al.* 2003).

Methane concentration was first log transformed, as the data were found to be positively skewed. To test for differences in log mean methane concentration between the three main geologies (chalk, limestone and sandstone) an ANOVA was carried out. Individual linear regressions were carried out between methane concentration and cased caddis $\delta^{13}C$ for sites on chalk, limestone and sandstone. The one sample of goerid larvae measured (site 25 Cunsey Beck), was included in this analysis along with the other glossosomatidae larval $\delta^{13}C$ values. Initial diagnostic plotting revealed a possible positive relationship between the $\delta^{13}C$ of the caddis larvae and log methane concentration for the sandstone sites. Diagnostic plotting of chalk and limestone sites indicated a possible negative correlation between $\delta^{13}C$ and log methane concentration. To increase the replication of chalk sites, data obtained using identical methods, were also available from a related project (Tuffin, 2014) for a further 11 sites on southern chalk streams and are included here (Table 2.2). Finally, a linear regression of caddis $\delta^{13}C$ and log methane concentration at all 39 sites was carried out.

Table 2.2 Methane concentration data and *Agapetus fuscipes* larval $\delta^{13}C$ collected in summer 2010 by Tuffin (2014).

Name	Area	Grid Reference	CH_4 (nmol L^{-1}) (± SE)	Log CH_4 (nmol L^{-1}) (± SE)	Larvae $\delta^{13}C$ mean (± SE)
Darent	Kent	TQ4685755165	400.83 ± 3.67	2.60 ± 0.56	-34.95 (NA)
Itchen	Hampshire	SU5588232192	178.32 ± 1.96	2.25 ± 0.29	-37.54 ± 0.07
Test	Hampshire	SU3611236980	212.0 ± 2.29	2.33 ± 0.36	-39.12 ± 0.12
Wool	Dorset	SY8499286881	72.13 ± 1.71	1.86 ± 0.23	-40.40 ± 0.10
Lambourn	E. Berkshire	SU4275171367	101.66 ± 1.18	2.01 ± 0.07	-39.37 ± 0.07
Ver	Hertfordshire	TL1195110707	72.0 ± 5.06	1.86 ± 0.70	-42.15 (NA)
Cray	Kent	TQ4867172605	172.25 ± 1.13	2.24 ± 0.05	-38.35 ± 0.08
Till	Wiltshire	SU0714038545	225.87 ± 2.47	2.35 ± 0.39	-41.56 ± 0.02
Wylye	Wiltshire	SU0827535485	132.83 ± 1.93	2.12 ± 0.29	-36.83 (NA)
Wye	Buckinghamshire	SU8718792695	92.60 ± 1.18	1.97 ± 0.07	-39.09 (NA)
Chess	Buckinghamshire	TQ0163198782	290.23 ± 2.62	2.46 ± 0.42	-38.79 ± 0.02

2.3 Results

2.3.1 Glossosomatidae $\delta^{13}C$ depletion and geology

The $\delta^{13}C$ of the Glossosomatid caddis larvae ranged between -43.3 and -27.0‰ whilst the $\delta^{13}C$ of the epilithon ranged between -39.5 and -22.8‰ (Table 2.3). Glossosomatids were thus depleted in ^{13}C relative to their putative food, assuming a mean isotopic shift of +0.4 ± 1.2‰ with fractionation (mean + 1 SD, McCutchan *et al.* 2003). Under this range of fractionation, 95% of the values (if the larvae were only consuming epilithon) were expected to fall between -1.95 to 2.75‰ of the epilithon $\delta^{13}C$ baseline. Out of the 28 streams, 21 streams had glossosomatid larvae that exhibited $\delta^{13}C$ values that were more depleted in ^{13}C than -1.95‰ (Figure 2.3). Streams where the larvae were depleted were found on all geological types, apart from the one site on the coal measures. Caddis larvae were depleted in $\delta^{13}C$, relative to the stream epilithon, on chalk by an average of -8.68 ± 0.88‰ (n = 4), limestone -5.83 ± 1.59‰ (n = 9) and sandstone -4.63 ± 1.63‰ (n = 13). Glossosomatid larvae on the one stream draining mafic lava were depleted in $\delta^{13}C$ by -6.57‰ and, perhaps surprisingly, larvae from the single site on the coal measures were enriched in $\delta^{13}C$ relative to stream epilithon by +2.52‰, indicating little or no use of methane-derived carbon.

Table 2.3 *Agapetus fuscipes* larval $\delta^{13}C$ (‰) and epilithon $\delta^{13}C$ (‰). ǂ indicates the two sites where *Glossosoma conformis* was found instead of *Agapetus fuscipes*.

Site No.	Name	Lavae δ13C (‰) mean (±SE)	SE	*n* =	Epilithon δ13C (‰) mean (±SE)	SE	*N* =
1	Llangattock 1	-37.84	0.14	3	-27.56	0.09	3
2	Llangattock 2	-38.08	0.17	3	-29.35	0.30	3
3	Nant Cleisfer	-31.36 ǂ	0.55	3	-29.81	0.30	3
4	Craig Cerrig Gleisiad 1	-31.78	0.09	3	-31.57	0.82	3
5	Craig Cerrig Gleisiad 2	-30.87	0.39	3	-33.81	0.11	3
6	Craig Cerrig Gleisiad 3	-38.09	0.13	3	-34.07	-	1
7	Dare Country Park	-28.88	0.23	4	-31.32	-	1
8	Upper Tawe	-34.11	0.05	3	-39.53	-	1
9	Traeth Mawr Stream	-39.86	0.00	2	-22.78	0.34	3
10	Owls Grove	-28.42 ǂ	0.54	5	-29.23	-	1
11	River Manifold	-42.34	0.28	3	-35.68	-	1
12	Milldale Walk	-35.77	0.13	3	-31.46	-	1
13	Dovedale Walk Carpark	-38.94	0.11	3	-30.67	-	1
14	River Wye Topley Park Quarry	-39.65	0.42	3	-35.56	-	1
15	Lees Bottom1 (R. Wye)	-39.13	0.49	3	-32.66	-	1
16	Lees Bottom2 (R. Wye)	-37.16	0.11	3	-29.63	-	1
17	Head of the Tadnoll	-41.77	-	1	-33.41	0.62	3
18	Bere Stream	-39.56	-	1	-30.05	0.06	2
19	River Creedy	-35.4	-	1	-31.97	1.54	3
20	Stockleigh Stream	-40.13	-	1	-27.66	0.36	3
21	River Lowman	-37.44	0.25	3	-28.94	0.28	2
22	Crowborough Warren	-33.11	-	1	-27.41	0.63	3
23	Hodgehow wood	-34.51	0.14	3	-30.98	0.17	3
24	Belle Grange Beck	-27.01	0.17	3	-28.30	0.44	3
25	Cunsey Beck	NA	-	NA	-31.79	0.90	3
26	Carrock Beck Spring	-41.38	0.30	4	-35.04	1.02	3
27	Small Spring	-37.56	-	1	-30.48	0.47	3
28	Belleau	-43.32	0.34	3	-33.61	0.21	3
29	Welton le Wold	-40.09	0.20	3	-34.09	2.31	3

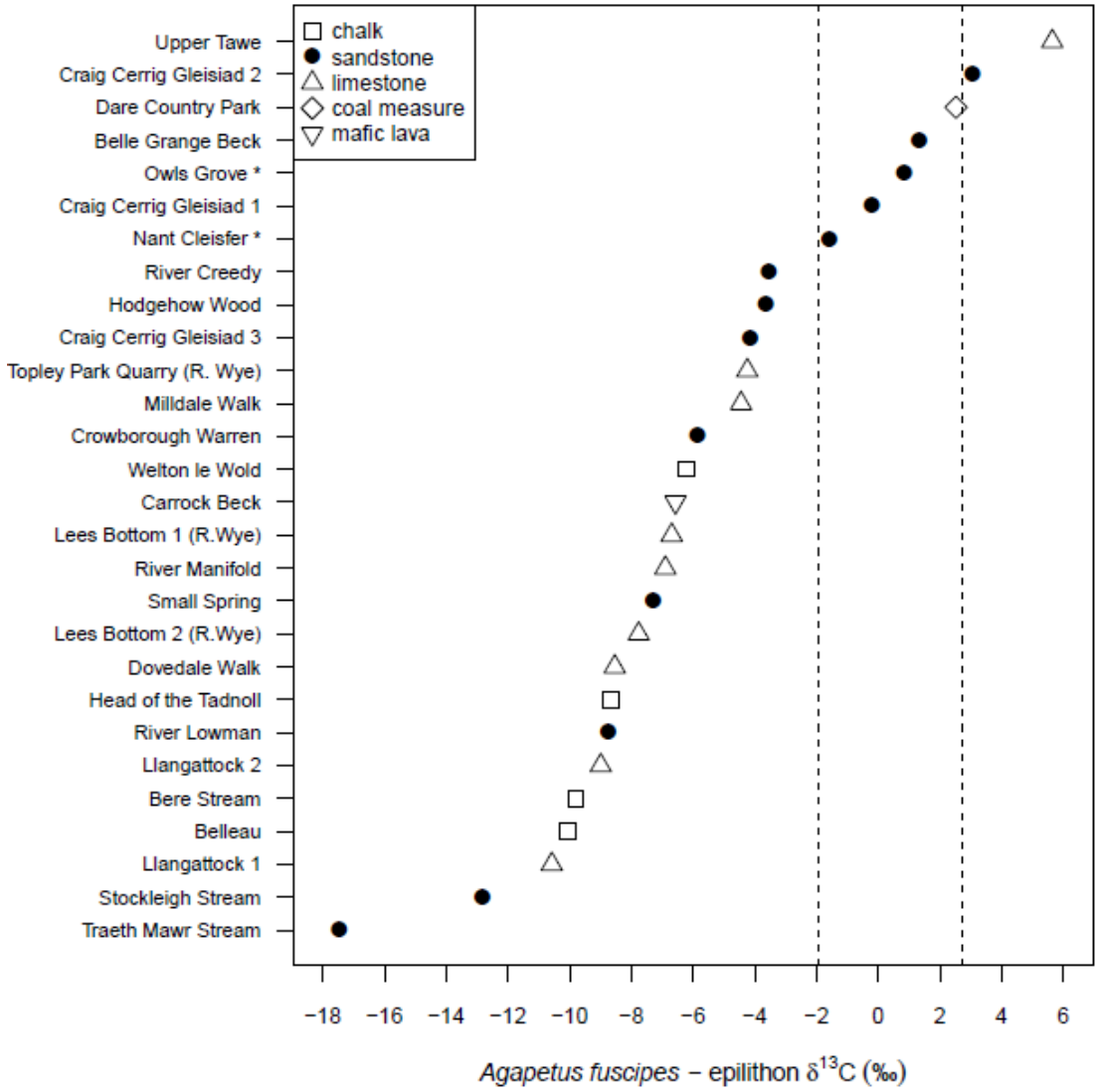

Figure 2.3 The $\delta^{13}C$ of glossosomatid larvae, relative to stream epilithon. The epilithon is set at zero to account for inter-site differences in the baseline. The area between the dashed lines indicates the expected larval $\delta^{13}C$ with a mean fractionation value of +0.4 ± 1.2‰ (mean + 1 SD, McCutchan et al, 2003) assuming that the epilithon was the primary food source. Under this fractionation with 95% confidence limits, values (assuming only epilithon is consumed) are expected to lie between -1.95 and 2.75‰ of the zero baseline.The main glossosomatid species was *Agapetus fuscipes*, excepting the only two streams marked with an asterisk (*) where *Glossosoma conformis* was found instead.

2.3.2 Stream water methane concentration

A wide range of methane concentration was observed across the 29 streams ranging from a minimum of 7.38 to a maximum of 574.9 nmol L^{-1} (Figure 2.4).

All 29 streams contained more methane than would be expected relative to equilibrium with the atmosphere.

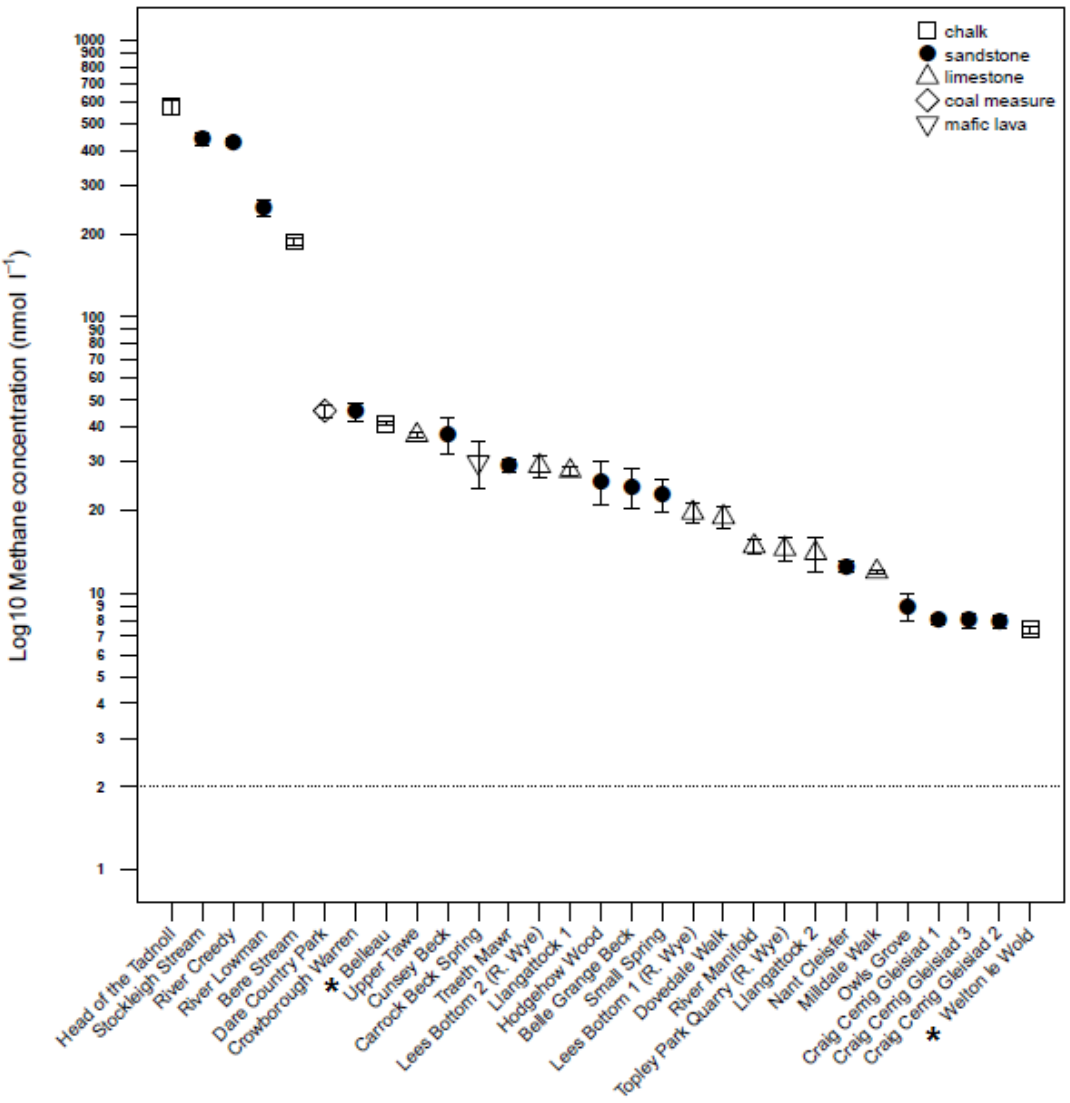

Figure 2.4 Rivers ranked by methane concentration, surveyed between April and August 2011 (n = 29). The two exceptions are Belleau and Welton le Wold, marked with an asterisk (*), that were sampled later in the year (November 2011). The dashed line indicates the concentration of methane in water when in equilibrium with the atmosphere.

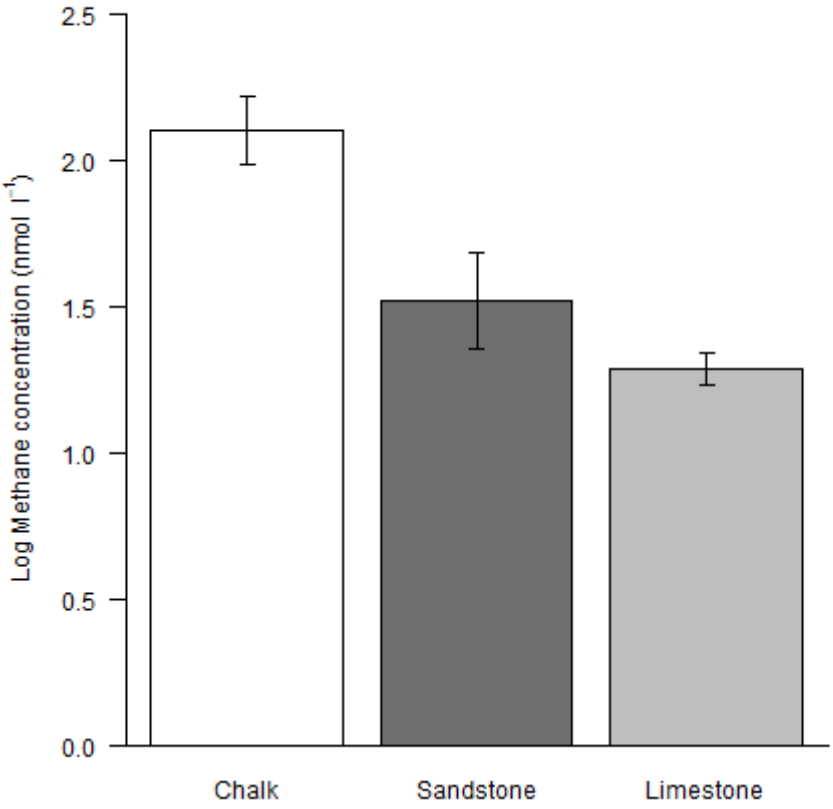

Figure 2.5 Mean (± 1 SE) methane concentration in nmol L^{-1} for the three main geological types; chalk (n = 15), sandstone (n = 14), limestone (n = 9). Data from eleven chalk streams (Table 2.2) surveyed in 2010 by Tuffin (2014) have been included.

Chalk rivers had a significantly higher log mean methane concentration (2.10 ± 0.12 nmol L^{-1}) than either sandstone (1.52 nmol L^{-1} ± 0.16, p = 0.002) or limestone (1.28 nmol L^{-1} ± 0.06, p = 0.0002, $F_{(2,\ 35)}$ = 9.585) (Figure 2.5). There was no significant difference in log mean methane concentration between limestone or sandstone geology.

2.3.3 Cased caddis δ13C, geology and methane concentration

There was no relationship between the $\delta^{13}C$ ratio of larvae and methane concentration for the sites on the chalk, sandstone or limestone, with the data for the different formations analysed separately. Caddis larvae for all (five) geologies combined for total 39 streams, also showed no significant correlation in $\delta^{13}C$ with increasing methane concentration (Figure 2.6).

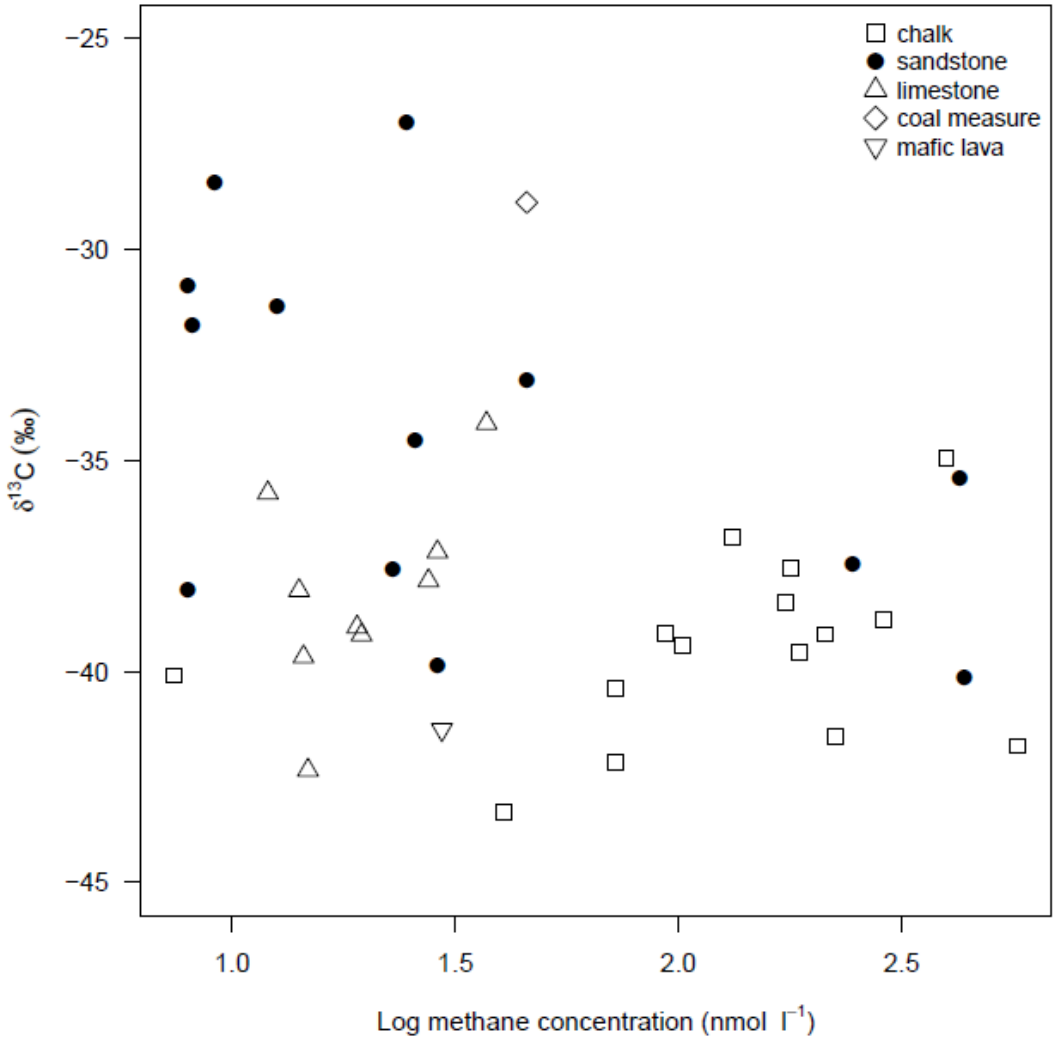

Figure 2.6 Log methane concentration and glossosomatid larval $\delta^{13}C$.

2.4 Discussion

2.4.1 Widespread $\delta^{13}C$ depletion in Glossosomatidae

The phenomenon of $\delta^{13}C$ depletion in some grazing cased caddis is evidently quite widespread across the UK, and was found not only on the chalk, but also in streams draining limestone, sandstone and mafic lava. This has important implications for our understanding of stream food webs, because it implies that methane-derived carbon is indeed supporting some of the secondary production in streams and that this is happening in a variety of streams, not just those on the southern chalk. Thus, two chalk streams in Lincolnshire (Belleau and Welton le Wold) also showed comparable $\delta^{13}C$ depletion to the chalk streams in south England, with the $\delta^{13}C$ of caddis larvae at -43.3 and -40.1‰, respectively. Carrock Beck Spring, a small spring tributary of Carrock Beck in the Lake District, also contained caddis larvae that were strongly $\delta^{13}C$ depleted (-41.4‰). This is somewhat surprising given that this is a relatively unimpacted spring with very little anthropogenic influence on generally impermeable geology (mafic lava). However, there are patches of blanket bog in this area (Grace *et al.* 2013). Blanket bogs are known to contain communities of both methanogenic and methane oxidising bacteria (Hales *et al.* 1996; McDonald *et al.* 1999) so it is conceivable that the larvae could be assimilating methane originating from upland blanket bogs in this area.

Somewhat surprisingly, the larvae collected from Dare Country Park, the coal measures site, were somewhat enriched in $\delta^{13}C$ relative to the epilithon of the stream, although a measurable concentration of methane was present in the water. One explanation for this may be that this site's methane could be thermogenic in origin. Thermogenic methane can sometimes be more enriched in $\delta^{13}C$ than biogenic methane, having a $\delta^{13}C$ between -50 to -20% (Whiticar, 1999).Thus in this stream caddis larvae may assimilate carbon derived from thermogenic methane, with a $\delta^{13}C$ similar to that of autochthonous or allochthonous resources. If this is indeed the case, it would not be possible to separate the relative contribution from methane-derived carbon or other sources to cased caddis biomass using the carbon stable isotope. Measuring both the $\delta^{13}C$ and the δD of the dissolved methane gas itself, collected in bulk from the stream channel, might elucidate the origin of the methane present in the stream water (Bao et al, 2014).

Another more general explanation for the enrichment of caddis larvae in a few sites by more than +0.4‰ relative to stream epilithon, is that the larvae could have been selectively feeding on and assimilating terrestrial detritus. The relative importance of terrestrial detritus and autochthonous algae to glossosomatid and goerid species has been debated, and it is known that *Agapetus fuscipes* and *Silo pallipes* can eat detritus as well as epilithon. Becker (1990) found that *A. fuscipes* and *S. pallipes* in the Breitenbach stream in Germany had higher proportions of detritus in their gut contents than algae. Becker argues that both species inhabit a restricted feeding space and, under competition with other species for high quality food

components such as diatoms, choose to consume detritus. The act of consuming detritus would also in turn remove it from settling and hindering the growth of algae and diatoms, food items which are of higher nutritional quality compared to detritus. Similarly, in a later experimental feeding study by Becker (1994) *A. fuscipes* and *S. pallipes* often chose detritus to consume rather than algae. Although fine particulate organic matter (FPOM) is not considered to be a high quality food resource (Becker 1994), previous work in the Tadnoll stream in Dorset revealed that 55% and 68% of gut fullness for *A. fuscipes* and *S. pallipes* consisted of FPOM (Jones, pers. Comm., 2015).

It is thus possible that in some streams, despite there being a supply of dissolved methane, that terrestrial detritus was particularly important. The conclusion must be that the larvae are not assimilating methane-derived carbon at such sites. Presumably, other food sources are abundant, or perhaps the methane is not being oxidised by the methane-oxidising bacteria present in the streamwater or the MOB are not particularly palatable to the glossosomatid larvae.

2.4.2 Dissolved methane in stream water

Dissolved methane was present in all of the 29 streams sampled. In every stream the measured concentration was much greater than would expected to be present in equilibrium with the atmosphere at saturation. This is important, as it suggests that supersaturated methane is likely to be found in

many freshwater streams, and is therefore a potential source of carbon and energy in freshwater food webs. The likelihood for methane to be consumed by methane oxidising bacteria, and subsequently for these bacteria to be grazed by primary consumers, is thus substantial. This finding of methane in stream water on all geological types supports the findings of Gooddy & Darling (2005), who found methane present in measurable amounts in various groundwaters across the UK, including sandstone, limestone and chalk geologies. Given that methane has also been found to be supersaturated in the water of streams draining basalt, shale and dolomite, (de Angelis & Lilley 1987; Jones & Mulholland 1998), it is likely that even more geological bedrock types have aquifers draining them that are saturated with dissolved methane.

All of the 29 streams sampled are supplied, at least partially, by groundwater. Thus it is not that unexpected that all the streams contained methane in measurable concentrations. Streams that are fed purely from surface water are unlikely to contain methane in such high concentrations, as there is not a groundwater input supplying 'allochthonous' methane from elsewhere.

Methane concentrations were highest in chalk streams, as suggested by earlier findings (Tuffin 2014).This is likely to be because chalk streams may receive a supply of methane from two sources; 'allochthonous' methane imported into the system via groundwater, and 'autochthonous' methane

produced *in-situ* in the stream (Gooddy & Darling 2005; Darling & Gooddy 2006; Sanders *et al.* 2007). The two chalk streams sites in Dorset (Site 17, Head of the Tadnoll and Site 18, the Bere) may have overlain oil reserves in this region. Previously oil has been found along the Dorset Coast at Osmington Mills as well as Kimmeridge and Wareham in the Isle of Purbeck and it is thought that oil reserves could underlie other areas in Dorset (Morris & Shepperd 1982; Watson, Hindle & Farrimond 2000; Selley 2012; Andrews 2013). Oil reserves are associated with methane gas emissions (Head *et al.* 2003) so it is possible that the high concentrations of methane recorded in the Tadnoll and Bere stream (574.9 and 187.1 nmol L^{-1} respectively) could have been supplemented by thermogenic methane from oil reserves underground. It was not possible to tell in this study whether this was the case as the $\delta^{13}C$ of the methane gas in stream water was not measured.

Methane concentration in the river water was not related to the $\delta^{13}C$ of the caddis larvae, which is perhaps surprising especially if bulk methane in the water column is indeed the source of the methane-derived carbon assimilated. However, this logic also infers that methane-derived carbon is the primary preferred resource of the caddis larvae rather than the 'conventional' resources of autochthnonous algae and allochthonous detritus. The $\delta^{13}C$ of glossosomatid larvae ranged widely across the 29 streams, and this presumably reflects a variable reliance on methane-derived carbon in the differing systems. Some populations may use methane-derived carbon variably depending on their requirements for energy and food. In streams where grazers are abundant, there may be competition for patchily

distributed resources (Kohler 1984, 1992); methane-derived carbon may then represent an important alternative source of fixed carbon for larvae.

The mechanism of uptake of methane-derived carbon by caddis is presumed to be via consumption of methane oxidising bacteria (Trimmer *et al.* 2009), although so far this remains unsubstantiated. In an earlier observational study, larvae of *A. fuscipes* survived longer in the laboratory in cases covered in algal growth that did larvae in 'clean' cases with no algal growth suggesting that the larvae were ingesting algae from their cases in times of resource limitation (Cox & Wagner 1989). Recent research by Ings, Hildrew & Grey (2010) found that the caddis larvae of a gallery-building caddisfly *Tinodes waeneri* fertilise this retreat via their nitrogenous excretions. Algae and other micro-organisms are then able to grow on the retreat and be exploited as a food resource by the caddis larvae.

Methane oxidation can take place on caddis cases at rates comparable to that of gravel (Trimmer *et al.* 2009). Trimmer *et al.* (2009) speculated that caddis larvae such as *A. fuscipes* could passively ingest methane oxidising bacteria as a consequence of grazing an epilithon 'mixture' containing MOB, or like *T. waerneri* actively maintain a biofilm containing MOB on their own cases (or graze off those of conspecifics).

Although the $\delta^{13}C$ values of the caddis suggest assimilation of methane derived carbon, other explanations cannot be ruled out at this stage. One is that caddis larvae may be selectively feeding on a food resource wasn't sampled or included in analysis such as filamentous algae or moss. Low $\delta^{13}C$ values have previously been reported for caddis larvae. A study by Rounick & James (1984), found two caddis species in a cold spring site in New Zealand, *Hudsonema amabilis* (stony cased caddis), and *Oxyethira albiceps* (micro-caddis), with $\delta^{13}C$ values of -43.6 and -40.3‰ respectively. However unlike the study of Trimmer *et al.* (2009), two potential food sources, *Melosira* spp. (algae) and *Fissidens* sp. (moss), were more $\delta^{13}C$ depleted than the caddis, with $\delta^{13}C$ of -46.3 and 45.3‰, respectively. The authors suggested that biogenic CO_2 from soil respiration in the source region of the spring, affects the $\delta^{13}C$ of the inorganic carbon in the spring that is available to the algae and moss. Finlay (2001), proposed that the $\delta^{13}C$ of algae is determined partially by the $\delta^{13}C$ of the DIC available and by the degree of discrimination against ^{13}C during carbon uptake and assimilation. In systems where carbon is limited, there will be less discrimination against ^{13}C, leading to more enriched $\delta^{13}C$ for algae. However, in systems where carbon is not limited, for example those with a high $CO_{2\ (aq)}$, algae can discriminate against ^{13}C because there is a large supply of the lighter ^{12}C readily available. This can lead to depleted $\delta^{13}C$ values for algae.

The biofilm is a complex matrix, including algae, bacteria, fungi, protozoa, metazoa, detritus and enzymes, as well as exudates and metabolic products (Allan & Castillo 2007). The $\delta^{13}C$ of the epilithon collected, therefore, will be

the average $\delta^{13}C$ value of all of these components combined. Therefore, algae (on which the caddis larvae are presumed to feed) may be much more depleted in $\delta^{13}C$ than the other biofilm components. If these caddis larvae preferentially digest and/or assimilate algal carbon they may actually reflect the true $\delta^{13}C$ of the algae, which cannot be separated from the bulk biofilm, and not necessarily the uptake of methane-derived carbon. There is sparse research on the possible preferential assimilation of biofilm components in freshwater animals. However, Rounick *et al.* (1982), found the mayfly *Deleatidium* to be relatively $\delta^{13}C$ depleted and proposed that, although the larvae ingest materials non-selectively, preferential assimilation of diatoms was occurring. Raikow & Hamilton (2001) also suggested that bivalves in a mid-western stream in the U.S. could preferentially assimilate the living component of the bulk organic material that they consumed. Interestingly another study by McNeely, Clinton & Erbe (2006) found the $\delta^{13}C$ of *Glossosoma* spp. to be ^{13}C-depleted. They analysed gut contents and concluded larvae were feeding on ^{13}C-depleted alage. Since gut contents were not collected in this study it cannot be ruled out that larvae may also have been eating ^{13}C-depleted algae from the biofilm matrix.

Overall, this survey of streams shows quite clearly that isotopic depletion of larvae is widespread, if not quite ubiquitous, and is not confined to chalk, while large concentrations of methane are also widespread in groundwater-fed streams. However, the lack of congruence between the larval signature and the concentration of methane raises some fundamental problems and challenges to the view that there is a link between the two.

3 Life histories of the grazing caddis

3.1 Introduction

The overall objective of this chapter was to underpin estimates of secondary production for the caddis species suspected of assimilating methane-derived carbon. Thus, the first aim was to investigate whether *A. fuscipes*, as in the Breitenbach in Germany (Becker 2005), has seven instars in streams in the UK and whether these instars can be successfully separated on the basis of easily assessed features such as pronotum length (a mid-ecdysial line down the 'saddle' of the pronotum of the larvae), body length or head width (see Figure 3.5). The second aim was to investigate whether instars (including instar I) of *S. nigricornis* and *S. pallipes* could be distinguished by larval head width. The third aim was to identify the generation time, and thus the number of generations per year, of the three species of caddis.

A knowledge of the life history of an organism is very important in order to interpret how it relates to its environment. In holometabolous insects, there are both larval and pupal stages, the former with a variable number of progressively larger instars. Generation time also varies between and within species and determines the number of generations per year (voltinism). Such basic information on the life history of organisms, along with data on population and body size, ultimately allows the calculation of their secondary production (Downing 1984). Secondary production is the formation of heterotrophic biomass of a population, or a group of populations, over a

period of time (Benke 1993). Quantifying the density and production of a population of organisms is particularly important when trying to quantify how much of a particular food resource is being consumed and/or assimilated by the population (Choy *et al.* 2009; Benke 2011; Brett *et al.* 2012).

Armoured grazing caddis larvae, such as *Agapetus fuscipes* (Glossosomatidae) and *Silo nigricornis* (Goeridae) (Figure 3.4), have recently been suggested to assimilate a novel source of energy in the form of methane-derived carbon (Trimmer *et al.* 2009). The larvae of these two families of caddis fly are often dominant in the benthos of streams and can reach high densities (Becker 1990; Poff & Ward 1995; Nijboer 2004; Alvarez & Pardo 2005; Nakano *et al.* 2007; Morris & Hondzo 2013). However, although these species are often numerous, relatively little is known about their life histories. We also do not know 'why' these species in particular might be assimilating methane-derived carbon, apparently in addition to the algae or detritus, derived from photosynthetic processes, which the larvae of both species are known to consume (Douglas 1958; Elliott 1982; Arens 1990; Nijboer 2004). Trimmer *et al.* (2009) calculated that *Agapetus fuscipes* larvae may derive up to 30% of their carbon from methane oxidising bacteria. They speculated that the larvae may be incorporating methane oxidising bacteria into their diet at times of food shortage, perhaps supporting the extremely high densities of larvae that can occur.

The quantity of methane-derived carbon assimilated by a population of caddis larvae could be estimated using life history parameters and data on body and population size along with stable isotope techniques. This would then allow an assessment of how much carbon from methane is potentially available to predators, parasites and decomposers and thus to the wider food web. If this amount of carbon was non-negligible, for example >5% of total production, then this would indicate, for the first time, a substantial role for chemosynthetic energy in the food webs of 'clean' streams.

In the initial extensive survey, the aim was to investigate the geographical and geological 'range' of depletion of the natural isotope of ^{13}C in grazing caddis, as an indication of the incorporation of methane-derived carbon. The next step was to study a sub-set of these sites in more detail, and to estimate the fraction of methane-derived carbon supporting secondary production, not just biomass, and the schedule with which it is incorporated during the life cycle. This chapter details the choice of the sites to be sampled and some basic biological details of the caddis life cycles which are necessary for estimating their secondary production.

3.1.1 Rationale of site choice

Eight streams were chosen from the initial 27 surveyed in summer 2011 and the 11 surveyed in summer 2010 by Tuffin (2014), as described in Chapter 2. Sites 28 and 29, Belleau and Welton le Wold, were not part of the selection

process as these had not been surveyed at the time of choosing the streams. The initial aim was to include at least two or three streams draining each of the three main geology types of chalk, limestone and sandstone. These would be repeatedly surveyed over the duration of one year to enable estimates of production and to assess the quantitative importance of methane-derived carbon in a range of situations. For each of the three geological types of chalk, limestone and sandstone, two streams were chosen from each based on the $\delta^{13}C$ of the *A. fuscipes* larvae collected in the initial survey in 2011 (Chapter 2). One stream was chosen that had the most $\delta^{13}C$ depleted *A. fuscipes* and one stream that had the most enriched *A. fuscipes* (Figure 3.1). Three streams were on sandstone; Crowborough Warren (on the Ashdown sands of East Sussex), Craig Cerrig Gleisiad (one of the three sites previously sampled in 2011) and Traeth Mawr (both these latter draining the Old Red Sandstone of South Wales). Three streams were on chalk; the Tadnoll (Dorset), the Lambourn (Berkshire), the Darent (Kent). In addition, the Cray (a chalk stream on the south-eastern edge of London) was added as it has a very high density of *A. fuscipes* and can be accessed easily from Queen Mary Unversity. One stream, Upper Tawe, drained the carboniferous limestone of South Wales (Figure 3.2). Unfortunately the River Manifold, a limestone stream in the Peak District, had to be deleted as a sampling site, when the river was persistently in high spate during attempted sampling visits in November 2011 and April 2012.

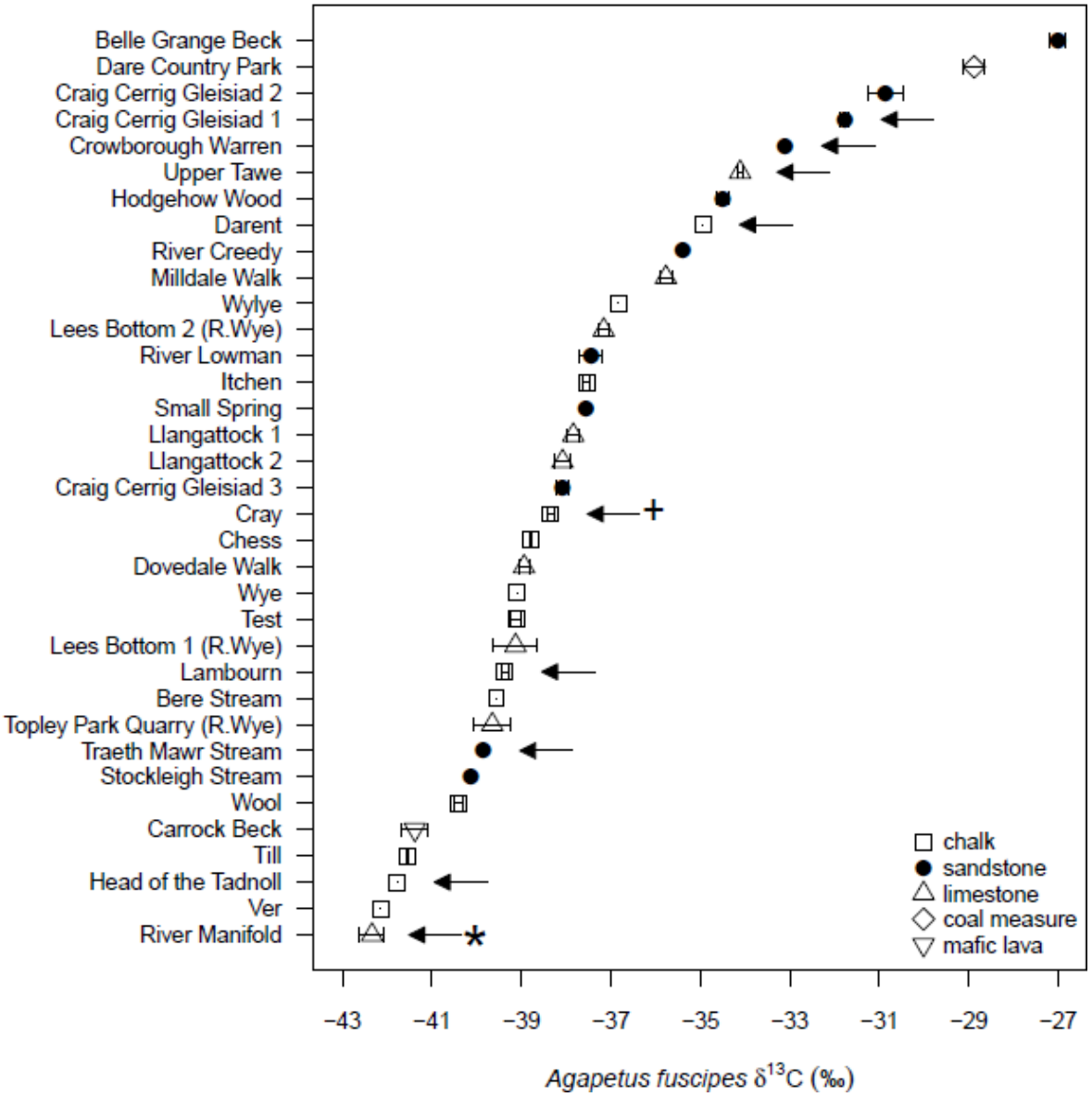

Figure 3.1 *Agapetus fuscipes* ranked by $\delta^{13}C$ (‰). Arrows show sites chosen from the initial 27 streams surveyed from Apr 2011 – Aug 2011. Belleau (Site 28) and Welton le Wold (Site 29) were surveyed after initial site selection and thus are not included here. Eleven chalk streams surveyed by Tuffin in summer 2010 are included (Table 2.2, Chapter 2). Arrows represent initial sites chosen for further study. * the River Manifold was found to be in flood and thus abandoned as a site for further study. + the Cray was an additional site sampled.

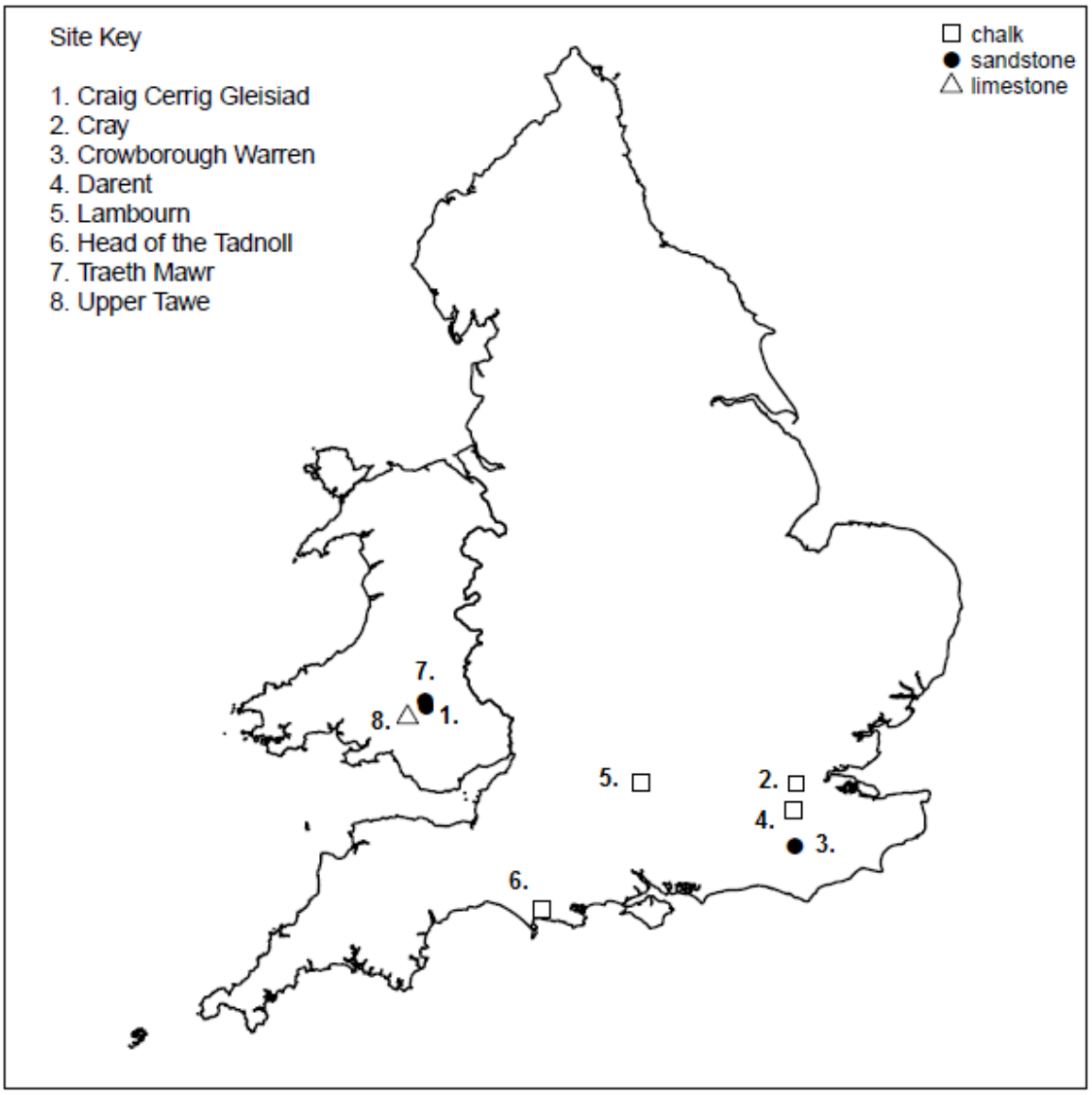

Figure 3.2 Map of eight streams sites surveyed repeatedly over the duration of one year from November 2011 to October 2012.

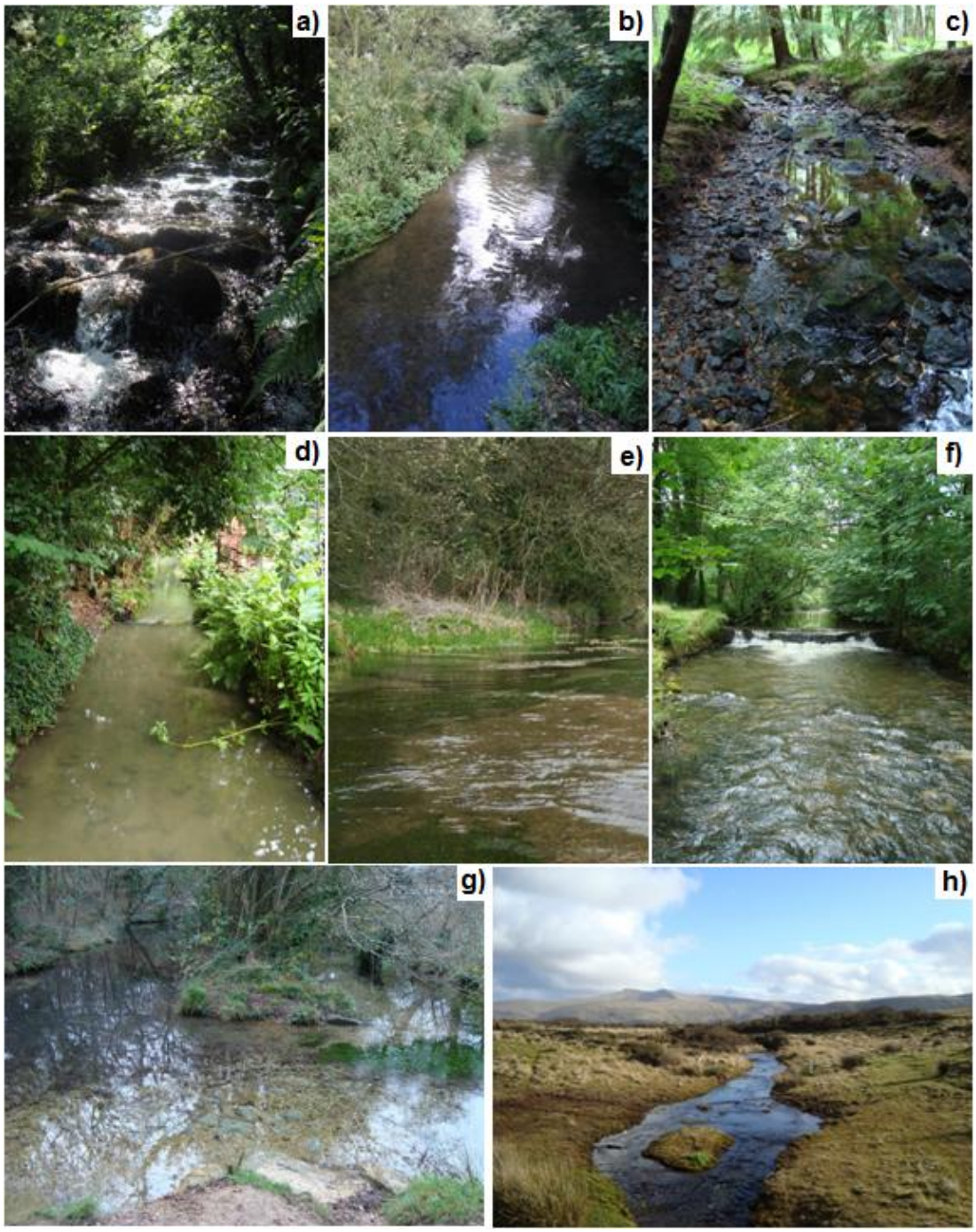

Figure 3.3 The eight streams a) Craig Cerrig Gleisiad, b) the Cray, c) Crowborough Warren, d) the Darent, e) the Lambourn, f) Upper Tawe, g) the Tadnoll and h) Traeth Mawr. Photographs taken by author.

3.1.2 Caddis life histories

Less is known about the life history of *Agapetus fuscipes* than *Silo* spp. *Agapetus fuscipes* is a member of the Glossosomatidae, a family in which species normally have five larval instars (Anderson & Bourne 1974; Irons 1988; Houghton & Stewart 1998; Alvarez & Pardo 2005), which is typical of most Trichoptera. However, *A. fuscipes* is unusual in that the species has more than five instars, with some debate as to how many. The only other caddis fly in the UK that has been documented as having more than five instars is *Sericostoma personatum*, a member of the Sericostomatidae (Elliott 1969). Nielsen (1942), documented seven instars for *A. fuscipes* in Denmark. However, (Benedetto Castro 1975), observed *A. fuscipes* in the laboratory and found the species produced eight instars. More recently, Sangpradub, Giller & O'Connor (1999), found *A. fuscipes* to have seven instars in an Irish stream and a separate study by Becker (2005) also found *A. fuscipes* to have seven instars in the Breitenbach, a well-studied small sandstone stream in Germany. Becker (2005) hypothesised that *A. fuscipes* had more instars than the typical five because of the high rate of mouthpart wear on the rough Bunter sandstone of the Breitenbach stream. It is unclear how many instars *A. fuscipes* has in the UK (Wallace *et al.* 1990). As well as the uncertainty regarding the number of instars that *A. fuscipes* has, *Agapetus* cannot be distinguished to species level before instar III to V (Wallace, Wallace & Philipson 2003).

Unlike the controversy surrounding *A. fuscipes*, *Silo* spp. are considered to have five instars, based on head width (Wallace *et al.* 1990). However, instars of *S.nigricornis* are currently differentiated on limited head width data from Nielsen (1942), which are not based on caddis larvae from Britain. For *S. pallipes* the current instar divisions are based on very limited head width data from 59 individual measurements (Wallace *et al.* 1990) . There are no head width data for the first instars of either *Silo* species based on UK specimens (Wallace *et al.* 1990). Head width divisions of the instars for *S. pallipes* have also been given by Grenier (1970) for continental Europe but these indicate smaller head widths for each instar than those given in the key for the UK by Wallace *et al.* (1990). Instars of *Silo pallipes* have also been differentiated by pronotum length (Elliott 1982).

3.1.3 Generations per year

Agapetus fuscipes is generally regarded as univoltine (Nielsen 1942; Benedetto Castro 1975; Sangpradub *et al.* 1999; Becker 2005), although Recasens & Murrillo (1986) reported it to be bivoltine in Spain. Iverson (1976) reported two separate cohorts in Denmark, with one cohort hatching in July and overwintering as instar VII and another cohort hatching in November and overwintering as instar IV. Other glossosomatid species have been found to be trivoltine. *Agapetus quadratus* was trivoltine in a spring-fed stream in Majorca (Alvarez & Pardo 2005), as was *Glossosoma nigrior* in two Alabama streams (Jin & Ward 2007). *Silo* spp. are thought to be univoltine. *Silo pallipes* has been found to be univoltine (Elliott 1982; Sangpradub *et al.*

1999) but, for *S. nigricornis*, there are few data to suggest whether this species is also univoltine although it was considered univoltine by Tod & Schmid-Araya (2009). In general, water temperature affects the growth rate of invertebrates (Sweeny & Vannote 1981; Hauer & Benke 1987), with multivoltine populations often being found in warmer streams (Fisher & Gray 1983; Barahona, Millan & Velasco 2005; Jin & Ward 2007).

It is thought that *A. fuscipes* completes its life cycle in one year in the UK. Mackereth (1960) presented size class data for *A. fuscipes* from a small stony stream in Windermere that suggested *A. fuscipes* had a life cycle of one year with adults emerging from June to October.

There have been differing views on whether *Silo* species have a one or two year life cycle. Whitehead (1945) suggested that *Silo pallipes* could have a two-year life cycle but did not provide any data to support this claim. Mackereth (1960) suggested that *S. pallipes* may have a life cycle of just one year. Later Elliott (1982) found *S. pallipes* had a life cycle of one year in a stream in Dartmoor (south-west England) and Sangpradub *et al.* (1999) also found *S. pallipes* to have mainly a ('complex') one year cycle in an Irish stream, though with some individuals requiring more than one year to emerge. *Silo nigricornis* was found to have one annual generation in two streams in Austria, the Lunzer Teichbach and the Traun (Malicky 1976). In a lake on the island of Brissago in Ticino, southern Switzerland, *S. nigricornis*

was found to have two generations per year, possibly in response to the warm summer lake temperatures (Malicky 1996).

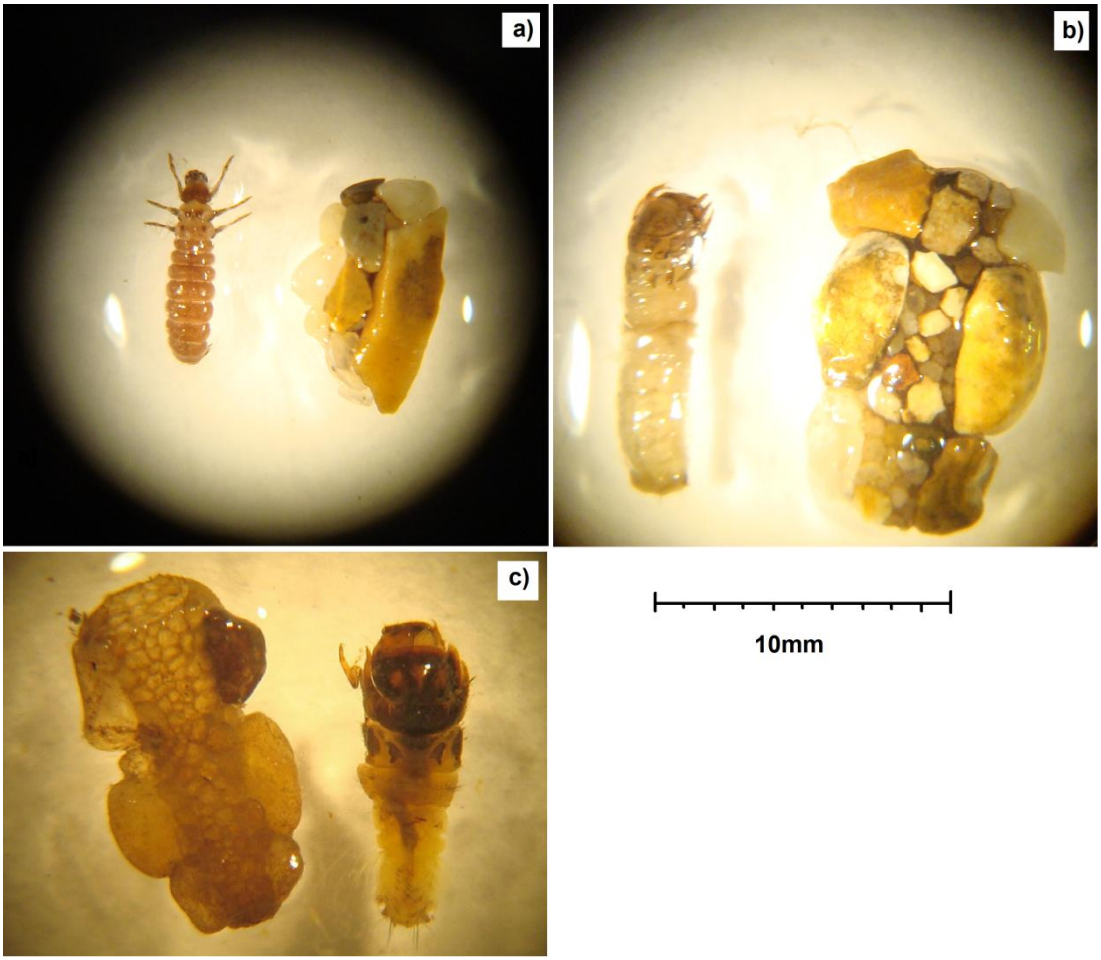

Figure 3.4 a) *Agapetus fuscipes* instar VII b) *Silo nigricornis* instar V c) *Silo pallipes* instar IV. Photographs taken by author.

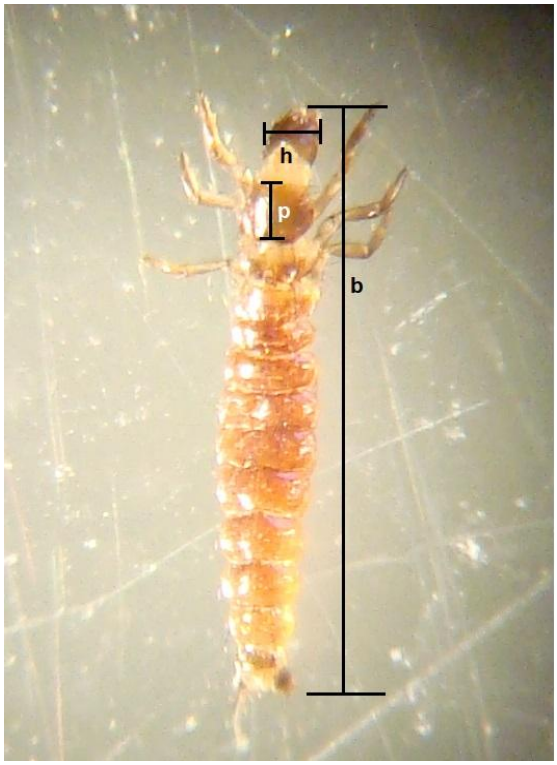

Figure 3.5. Body characteristics of *A. fuscipes* measured to try to separate individuals into instars; b = body length, p = pronotum length and h = head width. Larvae were measured using a Nikon SMZ 1000 microscope with an eyepiece graticule calibrated to the nearest µm. Photograph taken by author.

3.2 Methods

3.2.1 Measuring site characteristics

Channel width was measured in 2m intervals for a 10m stretch of each stream using a tape stretched across the wetted width of the stream. Channel depth was measured using a metre ruler for at least three equal distances along the two-metre intervals laid out for the width measurements. Habitat composition of the substrata of the stream bed for each 10m stretch was estimated visually as percentage cover. The substrate was classified into percent of bedrock, boulders, cobbles, gravel, sand and other. The macrophyte coverage of the stream channel on each of the six sampling occasions was also estimated visually. Likewise, the degree of shading (percentage tree cover) was estimated visually on each sampling occasion, thus indicating shading for each stream. Water temperature was measured on the six sampling occasions using a hand-held thermometer. Water velocity, 5cm above the bed, was measured using a hand-held flow meter (Geopacks, UK), with an impeller stick with propeller and electronic revolution counter. Five measurements were taken for each stream in the middle of each 2-m interval. Stream pH was measured in the field on the six sampling occasions using a portable pH probe (Hanna Instruments, UK). Water samples (n = 5) for nutrient analysis were taken on the six sampling occasions by collecting 30ml of the stream water in a plastic syringe and forcing the water through a pre-rinsed filter with a pore size of 25µl into acid rinsed Nalgene bottles. These were frozen using an Engel portable freezer

and later analysed with a segmented flow injection analyser (Skalar, San^{++}, Netherlands) using standard colorimetric methods to determine the concentration of five nutrients; ammonia (NH3), nitrate ($NO3^-$), nitrite ($NO2^-$), phosphate (PO_4^{3-}) and silicate (SiO2) (APHA, AWWA & WEF 2005). Ammonia was quantified using the Indophenol Blue method with absorbance measured at 630nm. Nitrite and nitrate were quantified via the Griess reaction (absorbance measured at 520nm), where nitrate was calculated by subtracting nitrite from the sum of nitrate and nitrite, which in turn, was quantified after reduction of nitrate to nitrate with an in line cadmium column. Phosphate was measured as soluble reactive phosphorus after reduction with ascorbic acid using the Molybdenum Blue method (absorbance was measured at 880nm). Silicate was quantified using the Molybdenum Blue method with absorbance measured at 660nm.

3.2.2 Sampling caddis larvae

On each of six occasions 15 randomly dispersed Surber sample units (15 x 15 cm quadrant, total area 225cm^2, mesh aperture 250µm) were taken in from a 10m stretch of each stream. Sampling periods were November/December 2011, February 2012, April 2012, May 2012, July 2012 and September/October 2012 (Table 3.1). For simplicity, hereafter, sampling occasions are labelled in Figures as November for the November/December and as September for September/October 2012. During each period sampling was undertaken as synchronously as possible. Samples were frozen in a 17 litre portable Engel freezer. For two of the streams, two

sampling time points were missed; Crowborough Warren was dry for November 2011 and the Upper Tawe was in flood in September 2012.

Table 3.1 Sampling dates for the six sampling occasions. ǂ indicates where a stream could not be sampled.

Stream	Nov/Dec	Feb/Mar	April	May	July	Sep/Oct
Craig Cerrig Gleisiad	14.11.2011	18.02.2012	14.04.2012	06.05.2012	31.07.2012	25.09.2012
Cray	28.11.2011	21.02.2012	16.04.2012	30.05.2012	19.07.2012	05.10.2012
Crowborough Warren	28.11.2011ǂ	01.03.2012	19.04.2012	23.05.2012	19.07.2012	02.10.2012
Darent	28.11.2011	21.02.2012	16.04.2012	30.05.2012	19.07.2012	02.10.2012
Lambourn	29.11.2011	28.02.2012	15.04.2012	31.05.2012	13.07.2012	26.09.2012
Tadnoll	10.12.2011	27.02.2012	17.04.2012	29.05.2012	20.07.2012	16.10.2012
Traeth Mawr	15.11.2011	18.02.2012	14.04.2012	06.05.2012	30.07.2012	25.09.2012
Upper Tawe	15.11.2011	19.02.2012	14.04.2012	06.05.2012	30.07.2012	25.09.2012ǂ

Table 3.2 The number of Surber samples sorted for each sampling occasion for each stream between November 2011 and October 2012. 'No data' indicates where Surber samples were not able to be collected due to drought or flood events.

Stream	Nov/Dec	Feb/Mar	April	May	July	Sep/Oct
Craig Cerrig Gleisiad	9	7	9	8	7	8
Cray	5	7	7	7	7	7
Crowborough Warren	No data	7	7	7	7	7
Darent	6	9	7	7	7	7
Lambourn	7	7	7	7	7	7
Tadnoll	7	7	7	8	7	7
Traeth Mawr	7	7	7	7	7	7
Upper Tawe	7	7	7	7	7	No data
Total	46	56	56	56	56	49
Cumulative total						328

3.2.3 Identification and instar separation

A total of 328 Surber samples (Table 3.2) were sorted and caddis larvae and pupae of the three species; *A. fuscipes*, *S. nigricornis* and *S. pallipes* were counted and identified under a Nikon SMZ 1000 microscope (magnification x80). For the measurement of dimensions, the eye-piece graticule of the microscope was calibrated to the nearest µm using a graduated slide. For all caddis larvae, where possible, total body length, pronotum length and head width were measured to the nearest (± 1µm). Total body length was taken as the length from the tip of the anal prolegs to the tip of the fronto-clypeus (front of the head). Pronotum length was taken to be the length of the 'saddle' of the caddisfly along the mid-dorsal ecdysial line (Figure 3.5). Frequency histograms of body length, pronotum length and head width were drawn for *A. fuscipes*. The frequency histogram of pronotum lengths was divided into the maximum of 59 size bins with a bin width of 12.5µm. Similarly the frequency histogram drawn for head widths was divided into the maximum of 46 size bins with a bin width of 12.5µm. The frequency histogram of body length was divided into 58 size bins with a bin width of 125µm. For *S. nigricornis* and *S. pallipes* the frequency histograms of head width were divided into 105 size bins with a bin width of 12.5µm. However instars could not easily be separated using these measurements so *A. fuscipes* larvae were assigned to one of seven instars using the frequency histograms in Becker (2005) since the range pronotum lengths observed was similar and the Breitenbach stream is of permeable sandstone geology and comparable to the streams in this study. *Silo* larvae head width frequency

histograms were drawn and larvae were assigned to instar based on peaks in the histograms.

3.2.4 Calculating density of caddis larvae

The mean number of *A. fuscipes, S. nigricornis* and *S. pallipes* was calculated for each sampling occasion for each stream. To obtain a value for the density of each species per metre squared of stream bed for each occasion, the mean number of each species per Surber sample (15cm by 15cm quadrant, 0.225m^2) for each sampling occasion was multiplied by 44.44 in order to obtain the mean number of each of the species per m^2.

3.2.5 Weighing of caddis larvae

Once the caddis larvae had been measured, a sample of whole, undamaged individuals were dried at 60°C for 48 hours in pre-weighed tin cups (Elemental Microanalysis). Once dry the caddis larvae were weighed to the nearest µg using a microbalance (Sartorius). For first instar larvae with low body mass, two to three individuals were placed in one tin cup and the average mass per larva used in further calculations. For second instar larvae and above, individual mass was used in further analysis. A total of 691 *A. fuscipes* larvae were weighed, yielding 643 individual mass measurements. For *S. nigricornis* a total of 122 larvae were weighed and 122 mass measurements obtained. For *S. pallipes* 17 larvae were weighed and 17 mass measurements were obtained. This was due to low numbers of *S.*

pallipes larvae in the Surber samples. Pronotum length was then plotted against mass for the three species for each stream and mass regression equations calculated.

3.2.6 Analysis of measurements

Based on the earlier instar divisions made for *A. fuscipes* and *Silo* spp., the proportion of each instar present for each species was calculated for each of the six sampling occasions. For each stream, mean pronotum length and range of *A. fuscipes* was calculated. For *S. nigricornis* and *S. pallipes*, mean head width and range were calculated. To assess the generation time of the larvae in the eight streams, larval instars and pupae were plotted by month and density, to assess when instar I larvae and pupae first appear. From these data minimum and maximum generation times were estimated. The following statistical analysis were completed in R version 3.1.1 (R Core Development Team 2014). Slopes of pronotum length-mass regressions for *A. fuscipes* for the eight streams were compared using the interaction term in an analysis of covariance (ANCOVA). The slopes of the head width-mass regressions for *S. nigricornis* for the Lambourn and Tadnoll were also compared using an interaction term in a separate analysis of covariance (ANCOVA). Mass was first transformed for both ANCOVA models by log10 to transform the exponential curve to a linear function.

3.3 Results

3.3.1 Site characteristics

Channel width ranged from 0.76 (Craig Cerrig Gleisiad) to 15.95m (the Tadnoll). The smallest mean channel widths were recorded for Crowborough Warren and Craig Cerrig Gleisiad (1.31 and 1.46m respectively). The largest mean channel widths were for the Upper Tawe and the Lambourn (6.84 and 7.98m respectively. Channel depth ranged from 0.5cm (Crowborough Warren and the Tadnoll) to 40cm in the Lambourn. The smallest mean channel depths were recorded in Crowborough Warren (4.9cm) and Traeth Mawr (8.7cm). The largest mean channel depths were recorded in the Darent (25.7cm) and the Cray (22.6cm). Streams contained varying compositions of habitat components and macrophyte coverage (see Table 3.3). The coldest stream water temperatures recorded were for Craig Cerrig Gleisiad (6°C in April) and the Darent (6°C in February and April) and the highest stream temperatures recorded were for Traeth Mawr and the Cray in May (20 and 23°C respectively). Mean stream velocity ranged from 0.17 (the Darent) to 0.66m/s (Upper Tawe). Stream pH remained relatively constant for the eight streams averaging between 7 to 8 (see Table 3.3). Shading varied between streams and seasonally with some streams, Craig Cerrig Gleisiad and the Darent, being as much as 90% shaded in the summer months.

Table 3.3 Site characteristics of the eight streams, with ranges in brackets, measured over the duration of one year from November 2011 to October 2012.

Parameter	Craig Cerrig Gleisiad	Cray	Crowborough Warren	Darent	Lambourn	Tadnoll	Traeth Mawr	Upper Tawe
Mean channel width (m)	1.46 (0.76 - 2.10)	5.23 (4.85 - 5.7)	1.31 (1.02 - 1.76)	3.33 (2.9 - 3.91)	7.98 (3.69 - 10.5)	6.72 (3.97 - 15.95)	2.15 (1.17 - 3.87)	6.84 (6.1 - 7.65)
Mean channel depth (cm)	9.3 (3.5 - 15)	22.6 (15.5 - 26.5)	4.9 (0.5 - 11)	25.7 (16.5 - 34)	20.9 (7.5 - 40)	19.6 (8 - 33.5)	8.7 (0.5 - 14.5)	20.5 (5 - 26)
Habitat composition (%)								
Bedrock	5	0	25	0	0	0	1	25
Boulder	75	3	24	45	2	8	20	35
Cobble	15	15	30	35	15	20	45	20
Gravel	4	75	20	12	65	70	25	10
Sand	1	7	1	6	18	2	9	10
Other	0	0	0	2	0	0	0	0
Macrophytes coverage (%)	30 – 35	0 - 1	1	0 - 1	15.1 - 45	5 – 38	45 - 60	40 – 45
Water temperature (°C)	(6 - 11.5)	(8.8 - 23)	(6.8 - 13)	(6 - 15)	(9.8 - 13)	(10.3 - 13)	(7 - 20)	(7 - 10)
Mean velocity (m/s)	0.58 ± 0.09	0.6 ± 0.03	0.27	0.17 ± 0.01	0.39 ± 0.08	0.27 ± 0.05	0.23 ± 0.02	0.66 ± 0.08
pH	7.9 (7.74 - 8.10)	8.02 (7.51 - 8.32)	7.2 (6.35 - 7.71)	7.77 (6.99 - 8.08)	7.79 (7.35 - 8.40)	7.21 (6.87 - 7.52)	7.81 (6.64 - 8.60)	7.89 (6.67 - 8.12)
Shading (%)	2 – 90	0 - 40	40 - 55	50 - 90	1 - 40	2.5 – 30	0	1 – 40

3.3.2 Stream nutrients

The three streams in Wales (Craig Cerrig Gleisiad, Traeth Mawr and Upper Tawe) had the lowest mean nitrate concentrations in stream water (102.7, 88.5 and 137.7µM respectively). Three of the southern chalk streams (the Darent, the Lambourn and the Tadnoll) contained the highest mean stream water concentrations of nitrate (717.9, 832.5 and 896.5µM respectively). Mean nitrite concentrations ranged between 0.3 to 2.4µM across the eight streams. Mean phosphate concentrations ranged between 0.3 to 1.5µM for seven of the eight streams. One stream, Crowborough Warren had high stream water phosphate concentrations recorded with a mean phosphate concentration of 6.5µM. Stream water mean silicate concentrations ranged from 76.5 to 295.2µM across the eight streams (Table 3.4).

Table 3.4 Mean nutrient concentrations for the eight streams from November 2011 to October 2012. Ranges are indicated in brackets. *Silicate was only measured for 3 sampling occasions (November, February and April) so the ranges displayed here consist of data from these three sampling occasions only.

Stream	Nitrate $NO3^-$ (µM)	Nitrite $NO2^-$ (µM)	Phosphate $PO4^{3-}$ (µM)	Ammonia NH3 (µM)	Silicate* SiO2 (µM)
Craig Cerrig Gleisiad	102.7 (22.5 – 188.6)	0.3 (0.05 – 0.6)	0.3 (0.07 – 1.2)	6.1 (0.4 – 12.6)	76.5 (72.7 – 83.6)
Cray	362.3 (99.8 – 543.2)	2.2 (1.0 – 3.6)	0.4 (0.1 – 0.9)	7.6 (3.7 – 12.4)	158.8 (74.6 – 297.3)
Crow. Warren	462.5 (202.8 – 795.4)	0.9 (0.2 – 1.7)	6.5 (5.6 – 7.3)	6.7 (2.9 -12.6)	132.9 (120.1 – 142.4)
Darent	717.9 (505.6 – 1168.2)	1.9 (1.0 – 3.1)	1.5 (0.7 – 2.2)	9.6 (5.4 – 12.6)	262.8 (217.8 – 286.3)
Lambourn	823.5 (522.8 – 1113.2)	2.4 (0.6 – 4.4)	0.6 (0.1 – 1.1)	7.0 (1.6 – 11.3)	295.2 (252.1 – 329.2)
Tadnoll	896.4 (475.5 – 1239.4)	3.7 (0.9 – 6.9)	0.5 (0.08 – 0.9)	11.0 (4.3 – 15.7)	199.6 (196.7 – 203.5)
Traeth Mawr	88.5 (14.0 – 226.9)	0.6 (0.1 – 1.1)	0.3 (0.06 – 0.5)	5.6 (0.8 – 11.0)	149.2 (193.7 – 129.0)
Upper Tawe	137.7 (23.7 – 248.7)	0.4 (0.06 – 0.6)	0.3 (0.09 – 0.4)	5.2 (0.5 – 8.8)	61.9 (54.6 – 66.0)

3.3.3 Caddis larvae collected

Agapetus fuscipes was present in all eight streams. *Silo nigricornis* was present in two of the chalk streams: The Lambourn and the Tadnoll. *Silo pallipes* was present in four of the eight streams: Craig Cerrig Gleisiad, Crowborough Warren, the Darent and Upper Tawe. A total of 10,070 *A. fuscipes*, 795 *S. nigricornis* and 150 *S. pallipes* larvae were identified from the Surber samples for the eight streams (Table 3.5).

Table 3.5 Caddis larvae counted from 328 Surber samples for each stream for each species with the total pronotum lengths (PL), head widths (HW) and body lengths (BL) measured and the total number of larvae weighed. Numbers in brackets indicate the actual total of larvae measured to obtain average measurements of instar I.

	Craig Cerrig Gleisiad	Cray	Crow. Warren	Darent	Lambourn	Tadnoll	Traeth Mawr	Upper Tawe	**Total**
A. fuscipes									
Total no.	160	4266	28	199	3121	96	143	2057	**10070**
Total PL	158	4250	28	193	3116	93	142	2027	**10007**
Total HW	81	1493	21	88	642	80	127	917	**3449**
Total BL	96	2438	22	158	1305	79	104	1079	**5281**
No. weighed	40	212	1	59	175 (221)	28	13	115 (117)	**643** (691)
S. nigricornis									
Total no.					178	617			**795**
Total HW					176	595			**771**
No. weighed					29	93			**122**
S. pallipes									
Total no.	10		15	38				87	**150**
Total HW	10		15	37				86	**148**
No. weighed	2			6				9	**17**

3.3.4 Agapetus fuscipes instar divisions

Body length and head width were discounted as characters to separate instars for *A. fuscipes* because frequency histograms showed no discernible peaks separating instars. The individual frequency histograms of pronotum length for the eight streams suggested that, for some streams (Craig Cerrig Gleisiad and Crowborough Warren), there were a few gaps possibly separating distinct instars (Figure 3.6), but histograms from both these sites were based on comparatively few individuals compared to the other streams surveyed (n = 158 and 28, respectively). For the six other streams, seven distinct larval instars based on pronotum length were not clear. Combining the data for all eight streams yielded no distinct separations between larval instars (Figure 3.8). The individual frequency histograms of head width also suggested that there were a few gaps separating some instars (Craig Cerrig Gleisiad and the Tadnoll, Figure 3.9 and Figure 3.10) but for the most part instars were not clearly identifiable from the peaks. Similarly for body length, the only streams with discernible peaks separating potential instars were Craig Cerrig Gleisiad (Figure 3.11) and the Tadnoll (Figure 3.12), although for the Tadnoll there were more than seven peaks and few individuals measured. Thus larvae were separated into size groups (presumed instars) using the pronotum frequency ranges identified by Becker (2005) and hereafter individuals of a size corresponding to these size groups will be referred to by the appropriate instar (Becker 2005).

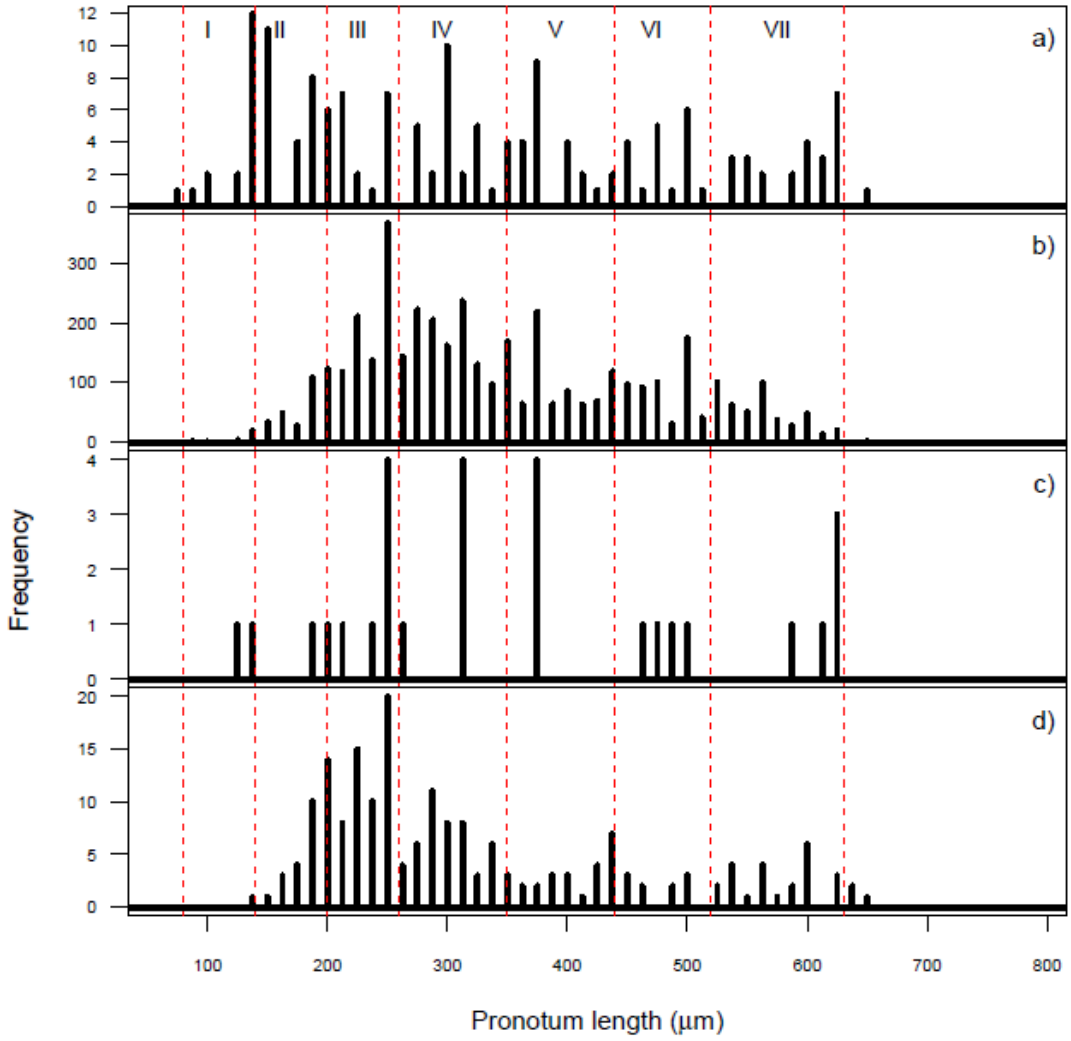

Figure 3.6 *Agapetus fuscipes* frequency distributions of pronotum length for a) Craig Cerrig Gleisiad (n = 158), b) the Cray (n = 4250), c) Crowborough Warren (n = 28), d) the Darent (n = 193). Dashed lines indicate the instar divisions of pronotum length of Becker (2005) (I – VII).

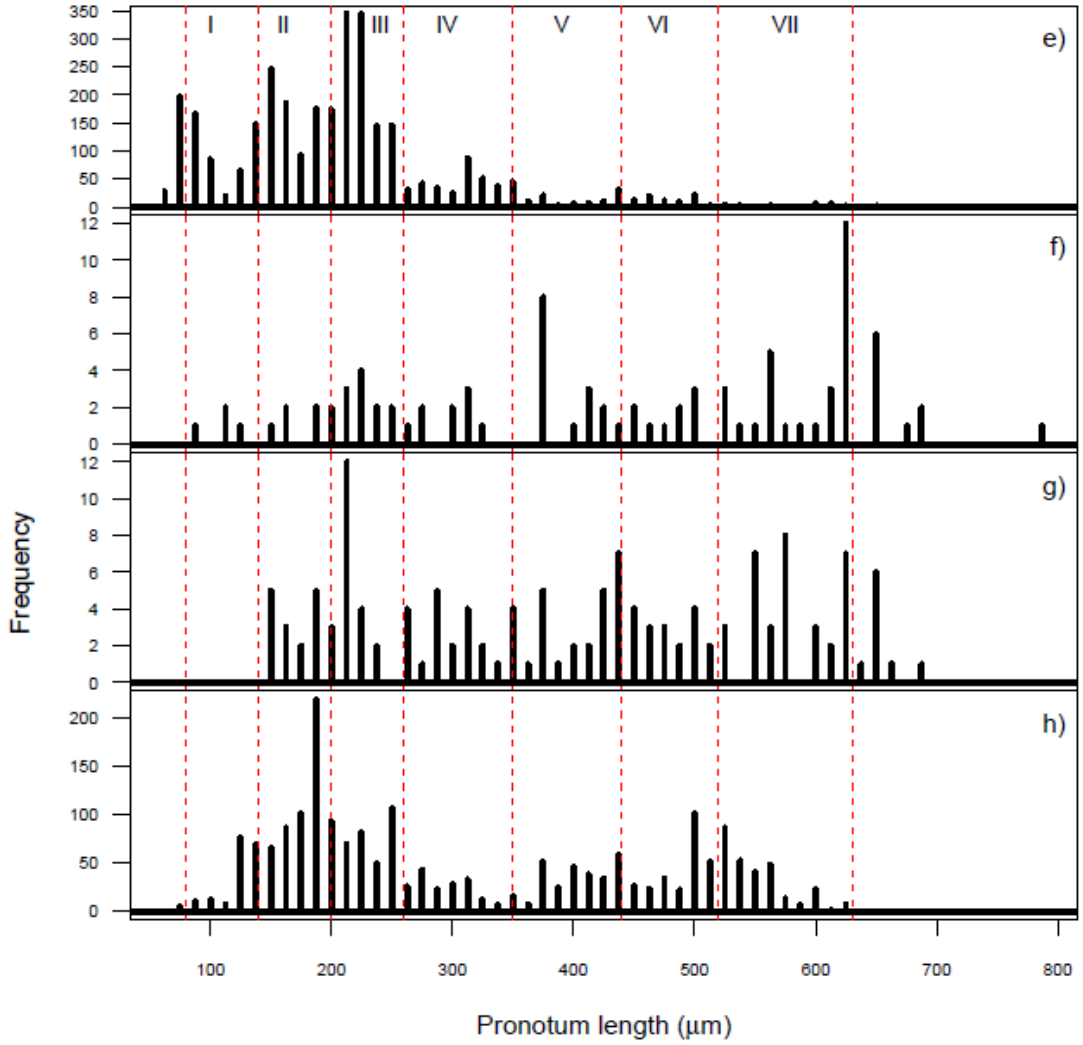

Figure 3.7 *Agapetus fuscipes* frequency distributions of pronotum length for e) the Lambourn (n = 3116), f) the Tadnoll (n = 93), g) Traeth Mawr (n = 142), h) Upper Tawe (n = 2027). Dashed lines indicate the instar divisions of pronotum length of Becker (2005) (I – VII).

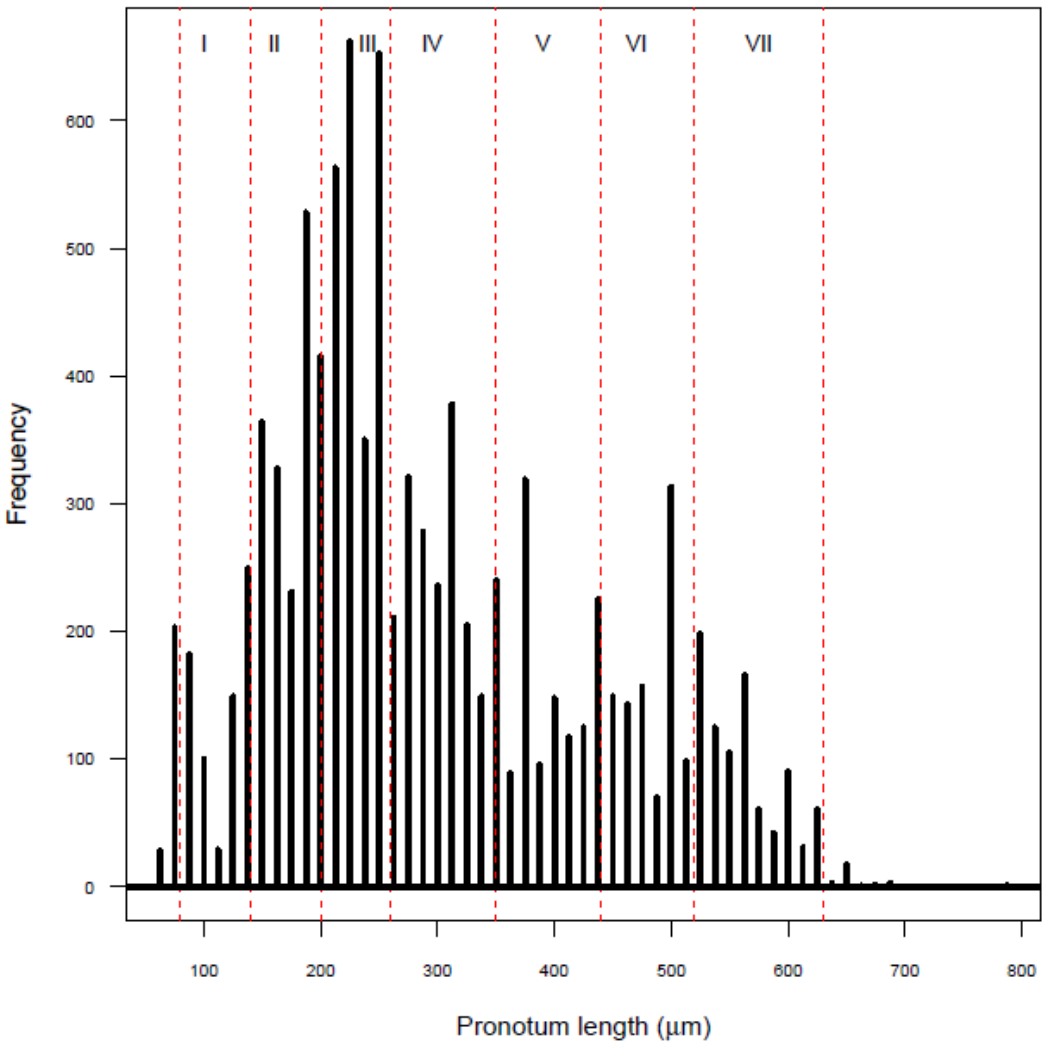

Figure 3.8 *Agapetus fuscipes* pronotum frequency distribution for the eight streams (n = 10007). Dashed lines indicate the instar divisions of pronotum length of Becker (2005) (I – VII).

For the eight streams *A. fuscipes* exhibited a range of pronotum lengths broadly similar to the range of 80 - 630µm reported by Becker (2005) (Table 3.6). The Lambourn contained some smaller first instar larvae with pronotum lengths measuring just 62.5µm. Three streams, the Lambourn, Tadnoll and Traeth Mawr, contained larvae with much larger pronotum lengths than those reported by Becker (2005) for instar VII (675, 787.5 and 687.5µm, respectively). No instar I larvae were found in the Darent and Traeth Mawr.

Table 3.6 Pronotum length (µm) of larval instars for *A. fuscipes* in the eight streams surveyed from November 2011 to October 2012. Larval instars were separated using the pronotum length histograms of Becker (2005).

Pronotum length (µm)	Instar	Mean	SD	Range	Min	Max	N	Total
Craig Cerrig Gleisiad	I	102.1	20.0	50	75	125	6	
	II	148.1	12.8	37.5	137.5	175	27	
	III	213.7	23.6	62.5	187.5	250	31	
	IV	300.0	17.3	50	275	325	24	
	V	376.5	22.6	87.5	337.5	425	25	
	VI	475.6	23.8	75	437.5	512.5	20	
	VII	593.0	34.4	112.5	537.5	650	25	158
Cray	I	107.8	18.8	37.5	87.5	125	8	
	II	158.3	12.2	37.5	137.5	175	130	
	III	227.2	21.5	62.5	187.5	250	1066	
	IV	293.4	20.1	62.5	262.5	325	1100	
	V	375.1	26.4	87.5	337.5	425	831	
	VI	472.3	25.0	75	437.5	512.5	654	
	VII	560.5	29.4	125	525	650	461	4250
Crowborough Warren	I	125.0	-	-	125	125	1	
	II	137.5	-	-	137.5	137.5	1	
	III	229.7	25.8	62.5	187.5	250	8	
	IV	302.5	22.4	50	262.5	312.5	5	
	V	375.0	0.0	0	375	375	4	
	VI	481.3	16.1	37.5	462.5	500	4	
	VII	615.0	16.3	37.5	587.5	625	5	28
Darent	I	-	-	-	-	-	0	
	II	163.9	13.2	37.5	137.5	175	9	
	III	222.4	22.2	62.5	187.5	250	77	
	IV	293.4	17.9	62.5	262.5	325	40	
	V	376.0	32.5	87.5	337.5	425	24	
	VI	459.6	25.2	62.5	437.5	500	17	
	VII	582.7	37.6	125	525	650	26	193
Lambourn	I	89.0	17.1	62.5	62.5	125	561	
	II	154.1	12.1	37.5	137.5	175	671	
	III	217.7	18.4	62.5	187.5	250	1331	
	IV	299.1	21.2	62.5	262.5	325	269	
	V	363.2	27.1	87.5	337.5	425	141	
	VI	466.4	24.5	75	437.5	512.5	111	
	VII	587.1	40.5	150	525	675	32	3116
Tadnoll	I	109.4	15.7	37.5	87.5	125	4	
	II	158.3	7.2	12.5	150	162.5	3	
	III	219.2	20.0	62.5	187.5	250	15	
	IV	297.2	21.4	62.5	262.5	325	9	
	V	392.0	21.1	50	375	425	14	
	VI	475.0	23.6	62.5	437.5	500	10	
	VII	613.5	52.8	262.5	525	787.5	38	93
Traeth Mawr	I	-	-	-	-	-	0	
	II	158.8	10.3	25	150	175	10	
	III	210.1	14.6	50	187.5	237.5	26	
	IV	292.4	21.5	62.5	262.5	325	18	
	V	386.3	30.1	87.5	337.5	425	21	
	VI	467.0	26.5	75	437.5	512.5	25	
	VII	595.2	42.9	162.5	525	687.5	42	142
Upper Tawe	I	115.5	15.6	50	75	125	111	
	II	158.5	14.1	37.5	137.5	175	321	
	III	211.9	23.6	62.5	187.5	250	617	
	IV	290.2	19.8	62.5	262.5	325	164	
	V	392.4	23.6	87.5	337.5	425	223	
	VI	480.0	27.2	75	437.5	512.5	314	
	VII	550.3	25.9	100	525	625	277	2027

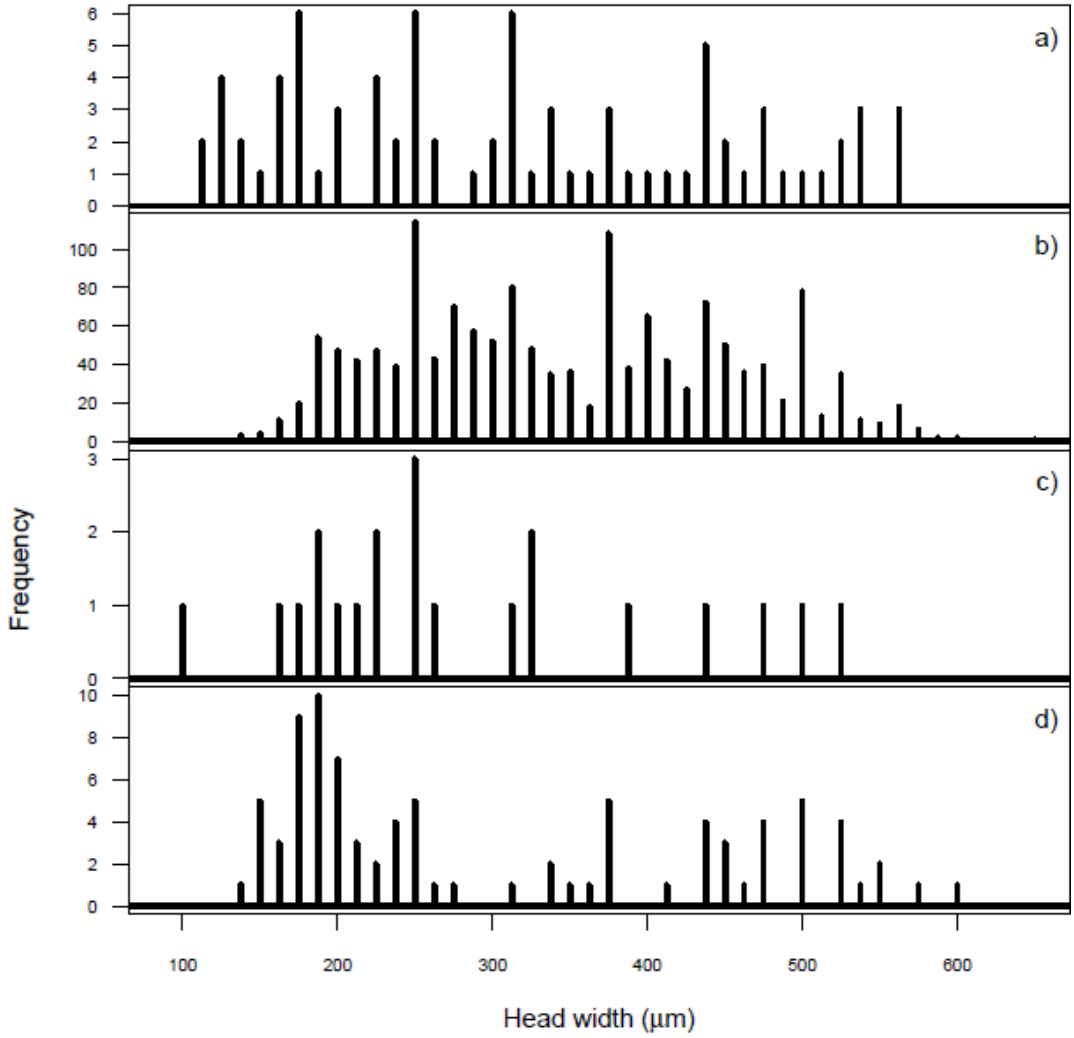

Figure 3.9 *Agapetus fuscipes* frequency distributions of head width for a) Craig Cerrig Gleisiad (n = 81), b) the Cray (n = 1493), c) Crowborough Warren (n = 21), d) the Darent (n = 88).

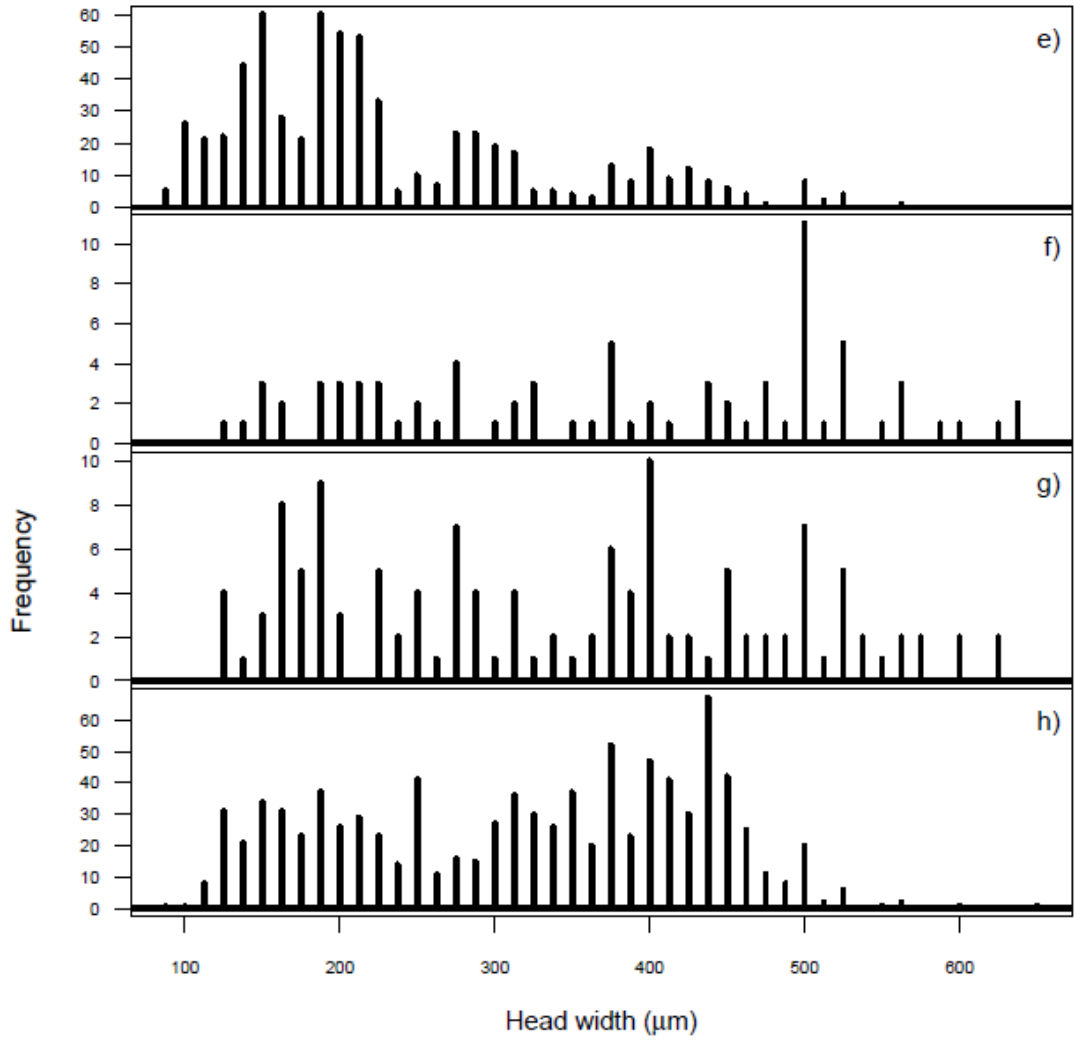

Figure 3.10 *Agapetus fuscipes* frequency distributions of head width for e) the Lambourn (n = 642), f) the Tadnoll (n = 80), g) Traeth Mawr (n = 127), h) Upper Tawe (n = 917).

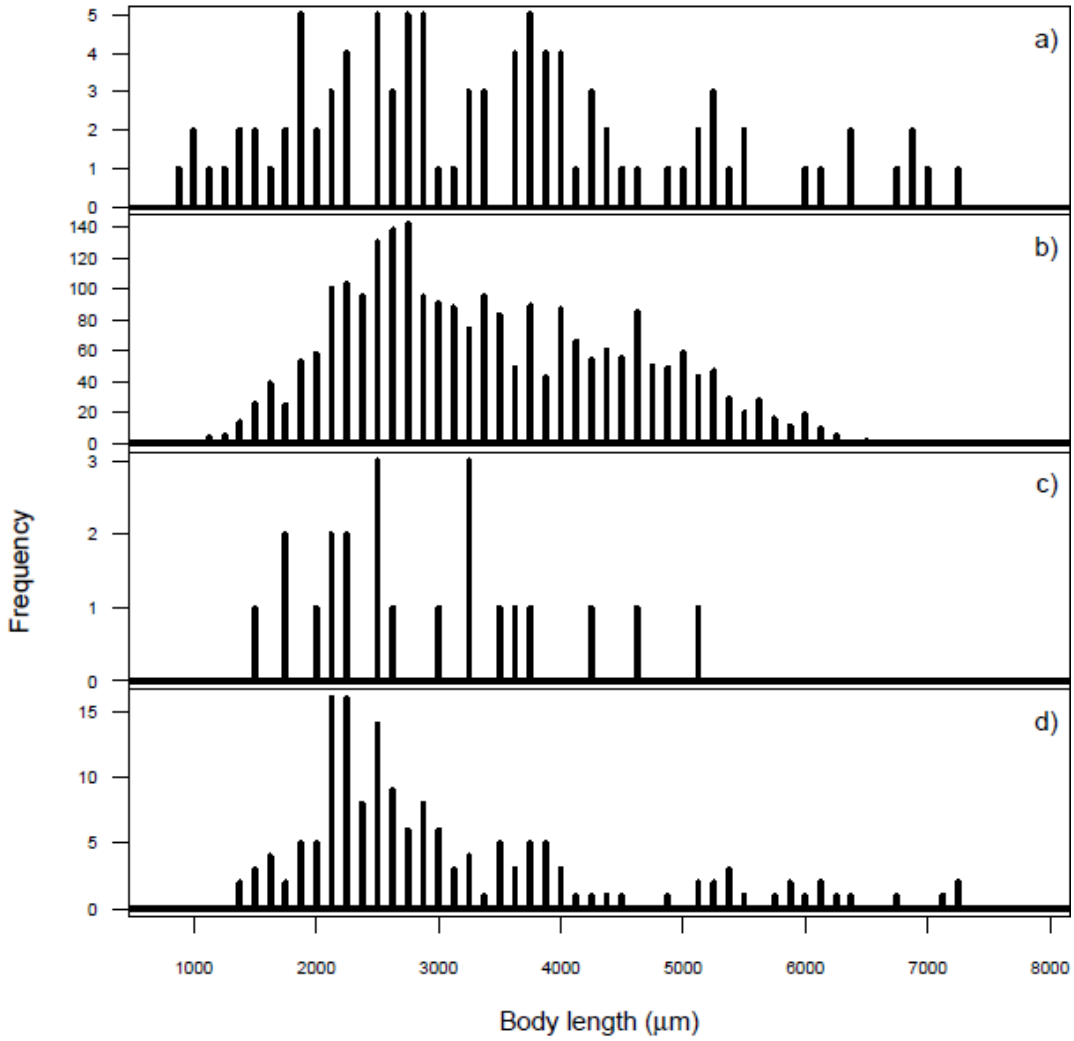

Figure 3.11 *Agapetus fuscipes* frequency distributions of body length for a) Craig Cerrig Gleisiad (n = 96), b) the Cray (n = 2438), c) Crowborough Warren (n = 22), d) the Darent (n = 158).

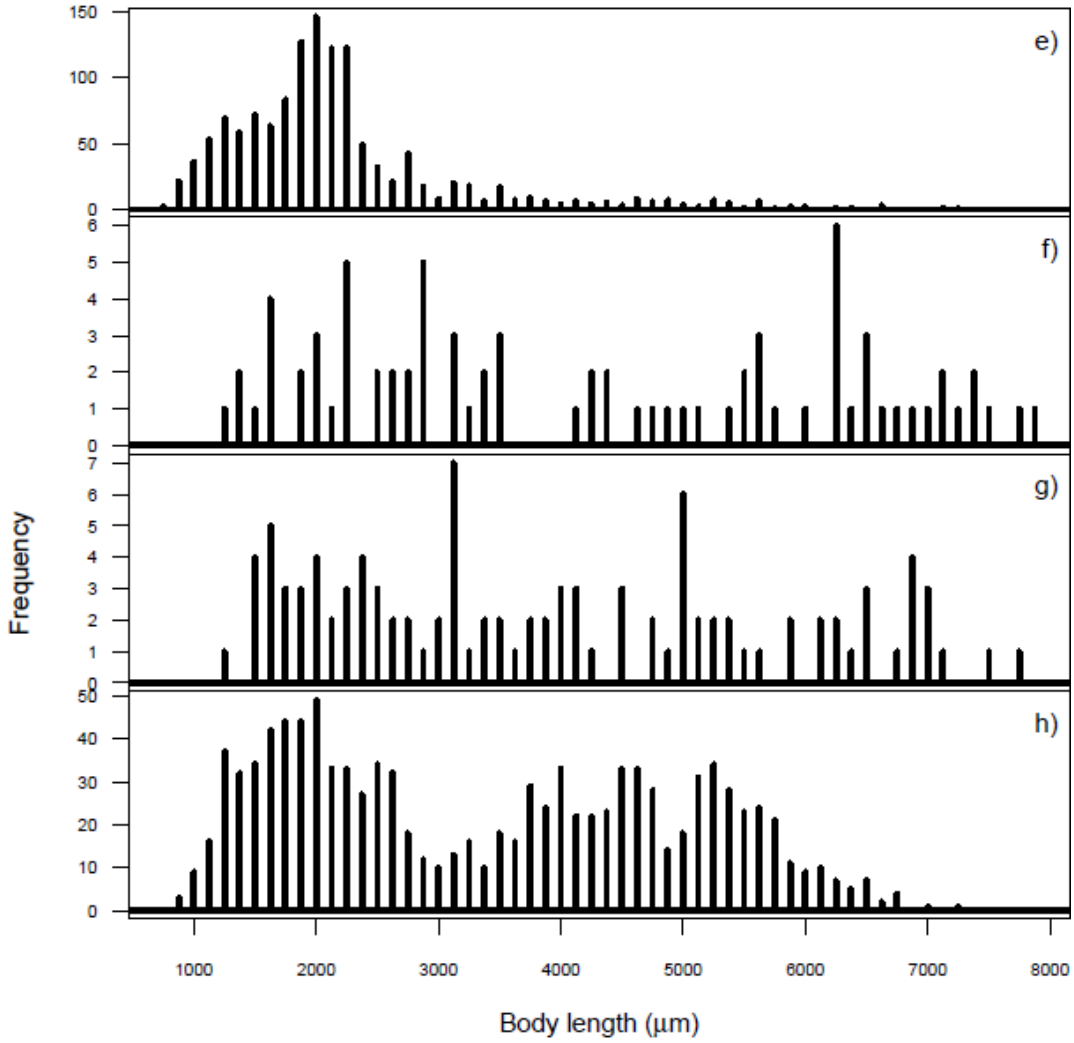

Figure 3.12 *Agapetus fuscipes* frequency distributions of body length for e) the Lambourn (n = 1305), f) the Tadnoll (n = 79), g) Traeth Mawr (n = 104), h) Upper Tawe (n = 1079).

3.3.5 Silo spp. instar divisions

Both *S. nigricornis* and *S. pallipes* exhibited distinct peaks on the frequency histograms of head width (Figure 3.13 and Figure 3.14), and five instars were easily distinguished from these peaks. These peaks fitted the range given previously by Nielsen (1942), although more total head width frequencies for each instar were recorded here. For both species of *Silo*, first instar larvae were found.

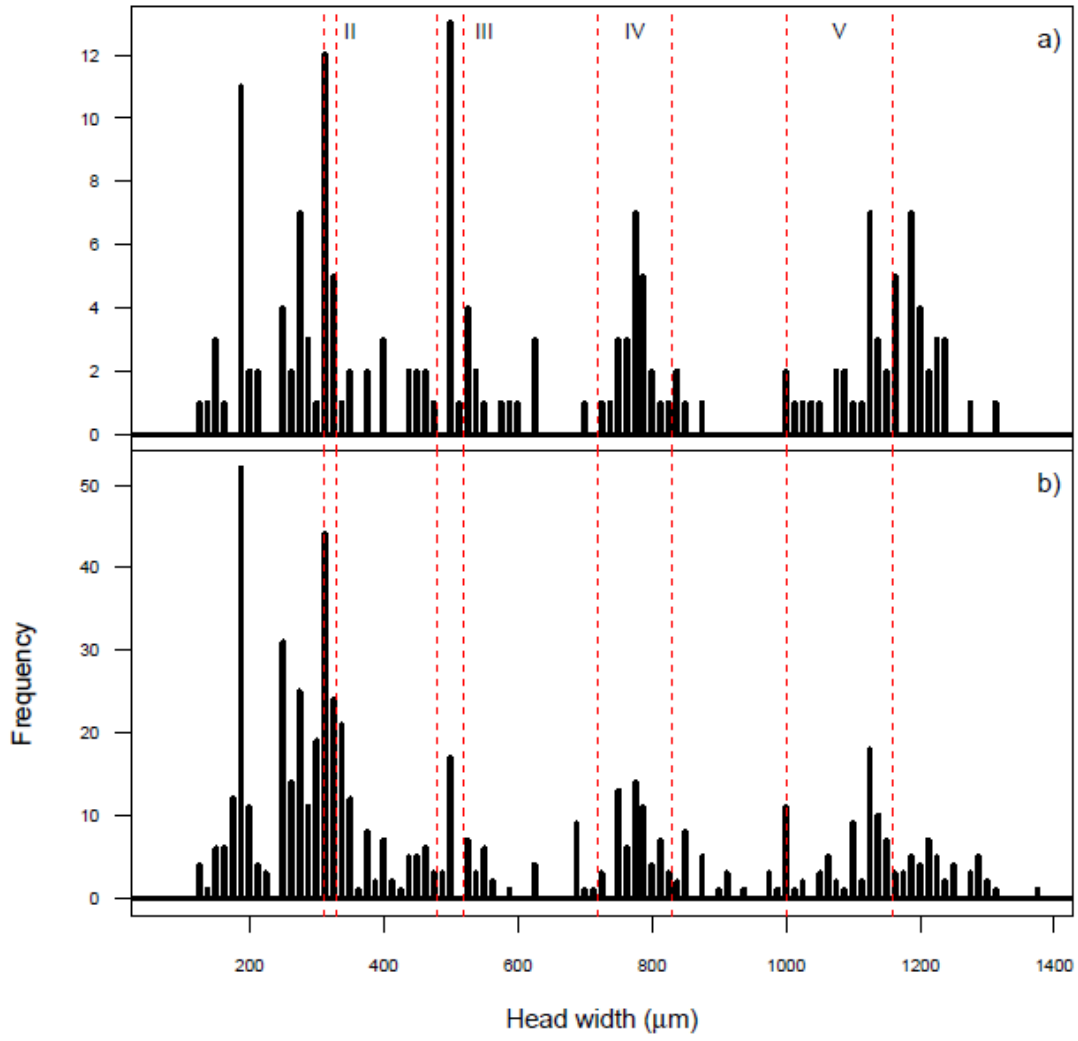

Figure 3.13 *Silo nigricornis* head width frequency histograms for a) the Lambourn (n = 176) and b) the Tadnoll (n = 595). Dashed lines indicate the instar divisions made by Nielsen (1942).

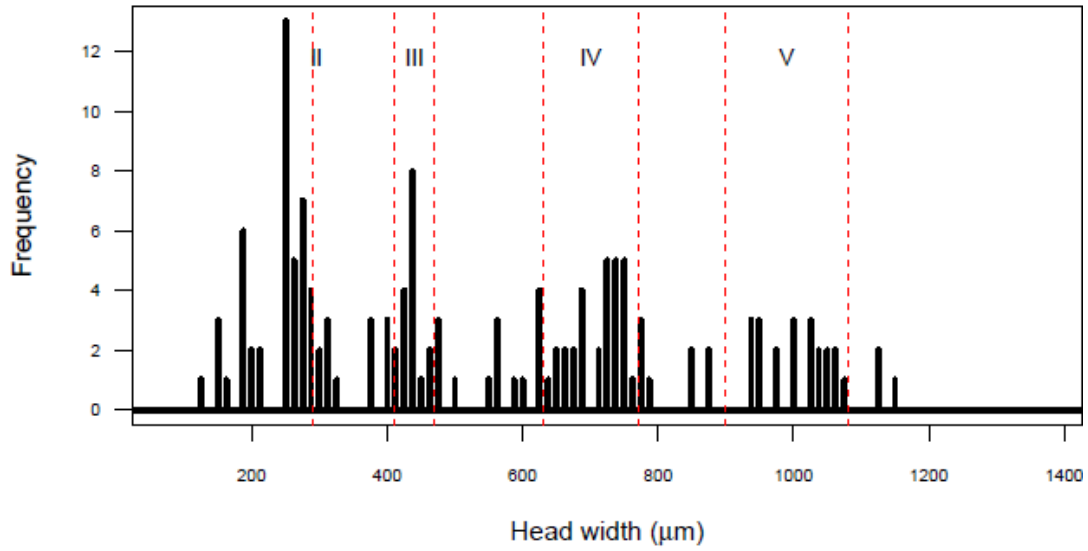

Figure 3.14 *Silo pallipes* head width frequencies for four streams combined; Craig Cerrig Gleisiad (n = 10), Crowborough Warren (n = 15), Darent (n = 38) and Upper Tawe (n = 87). Dashed lines indicate previous instar head width divisions from Wallace *et al.* (1990).

Table 3.7 Head width (µm) of larval instars collected for *S. nigricornis* for the Lambourn and the Tadnoll for November 2011 to October 2012.

Head width (µm)	Instar	Mean	SD	Range	Min	Max	N	Total
Lambourn	I	179.2	23.8	87.5	125	212.5	21	
	II	308.9	40.4	150	250	400	42	
	III	516.2	50.9	187.5	437.5	625	34	
	IV	782.8	37.6	175	700	875	29	
	V	1151.5	70.0	312.5	1000	1313	50	176
Tadnoll	I	182.7	19.8	100	125	225	99	
	II	305.7	66.3	150	250	400	219	
	III	502.3	50.8	212.5	412.5	625	65	
	IV	787.0	57.9	250	687.5	937.5	92	
	V	1140.5	87.9	400	975	1375	120	595

Table 3.8 Head width (µm) of larval instars for *S. pallipes* for four streams combined; Craig Cerrig Gleisiad, Crowborough Warren, Darent and Upper Tawe from November 2011 to October 2012.

Head width (µm)	Instar	Mean	SD	Range	Min	Max	N	Total
	I	179.2	25.7	87.5	125	212.5	15	
	II	271.4	22.4	125	250	325	35	
	III	431.5	31.8	125	325	500	27	
	IV	689.5	66.3	237.5	550	787.5	43	
	V	996.9	80.1	300	850	1150	28	148

3.3.6 Agapetus fuscipes instar distributions

Instar I larvae of *A. fuscipes* appeared in a five month period between July and November for most of the streams. No instar I larvae were found in the Cray or at Traeth Mawr, so the duration of the emergence period is unclear for these streams. In Crowborough Warren, instar I larvae appeared only in July. For most streams, pupae started to appear from April and were found in the streams for a further six to eight months suggesting asynchronous development of larvae throughout the year and that adult caddis were perhaps emerging continuously during this period. In Craig Cerrig Gleisiad pupae appeared slightly earlier (from February onwards). The total generation time for most larvae in the streams is thus deemed to be approximately one year, based on the first incidence of instar I larvae and pupae (Table 3.9). For the calculation of secondary production the two cohort intervals used will be 10 and 12 months to take into account variation in the time taken to develop from the first instar to the pupal stage.

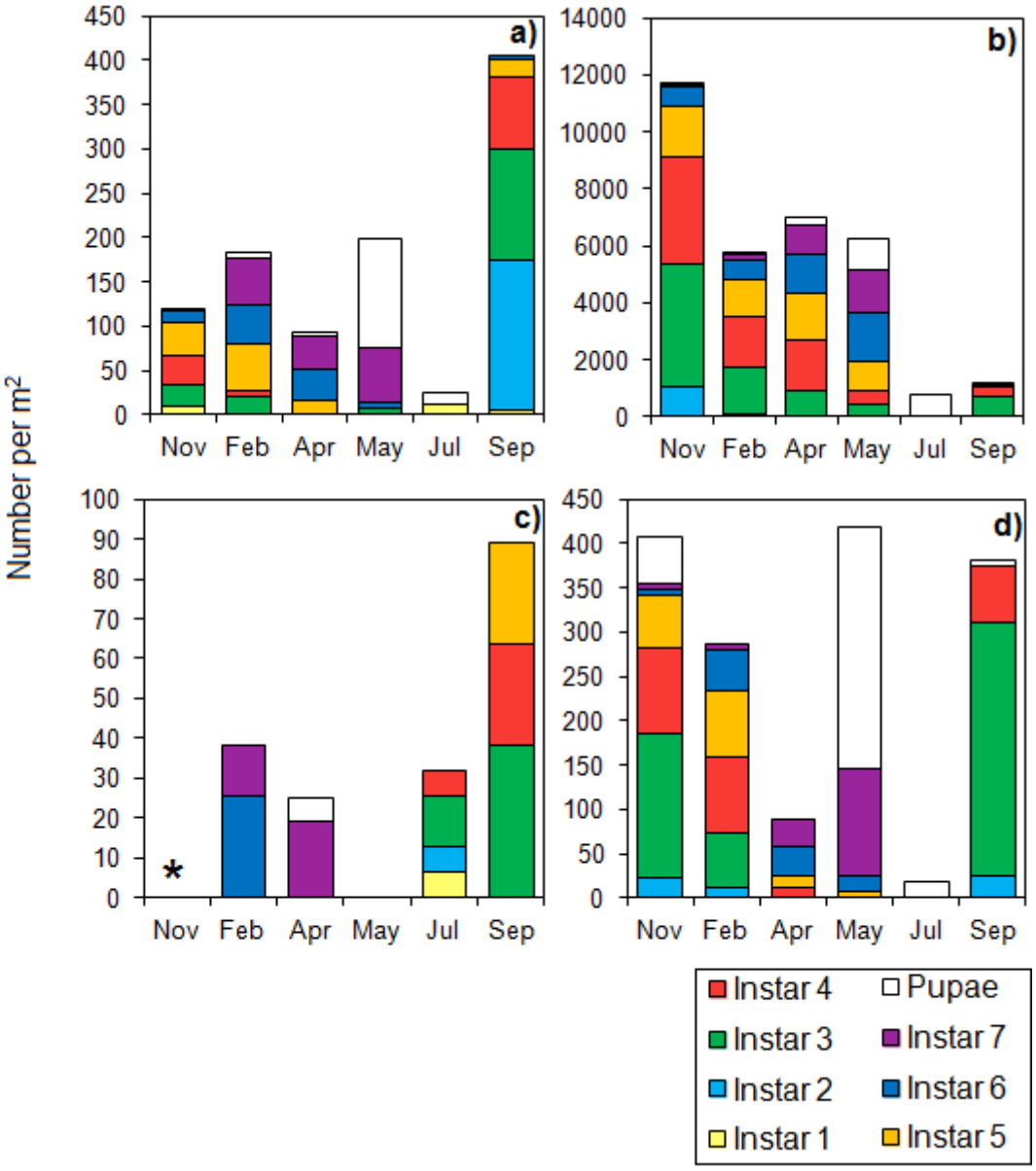

Figure 3.15 Number of *A. fuscipes* larvae per m^2 separated by instar in a) Craig Cerrig Gleisiad, b) Cray, c) Crowborough Warren, d) Darent, from November 2011 to October 2012. * represents where there are no data.

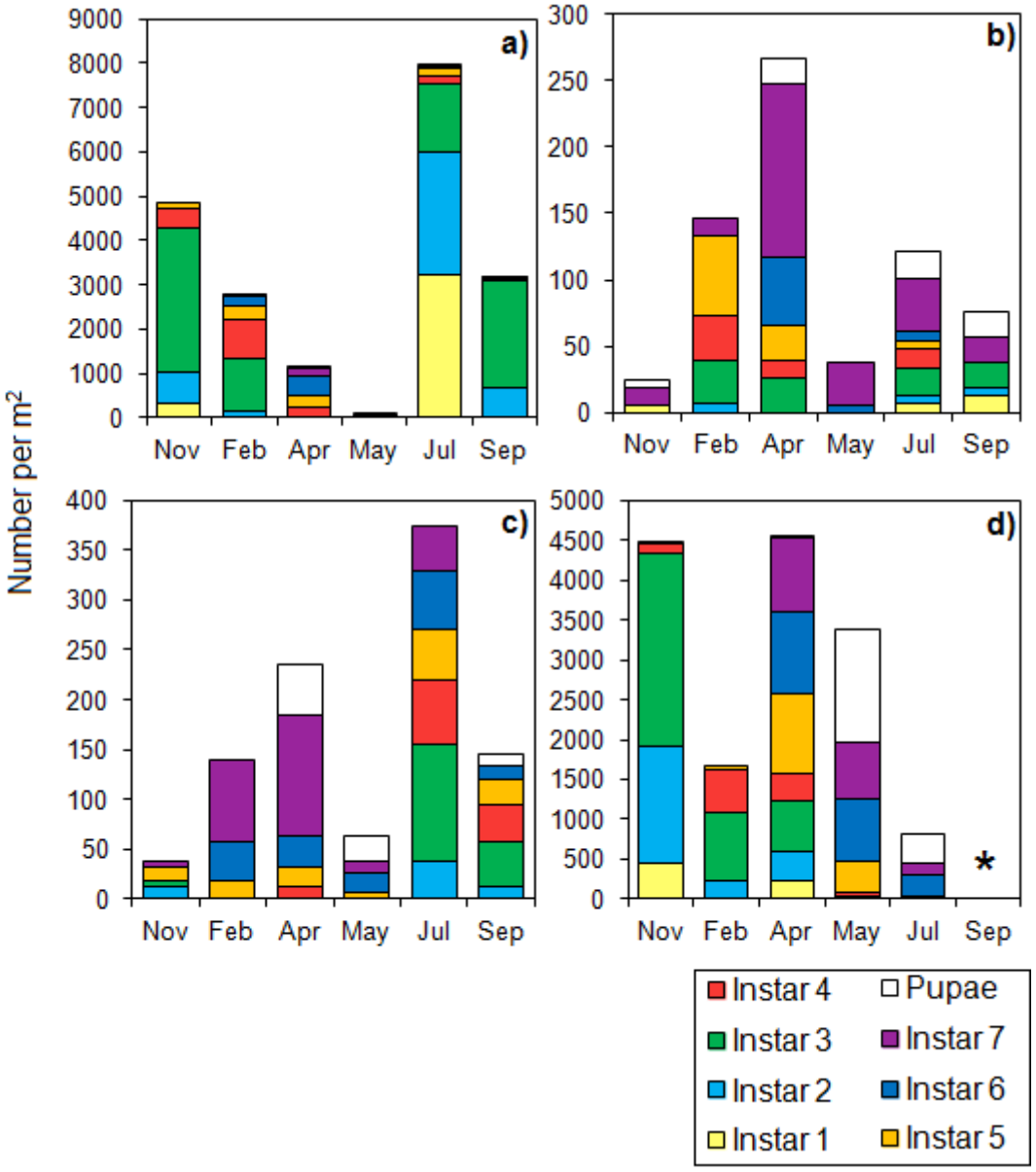

Figure 3.16 Number of *A. fuscipes* larvae per m^2 separated by instar in a) Lambourn b) Tadnoll, c) Traeth Mawr, d) Upper Tawe, from November 2011 to October 2012. * represents where there is no data.

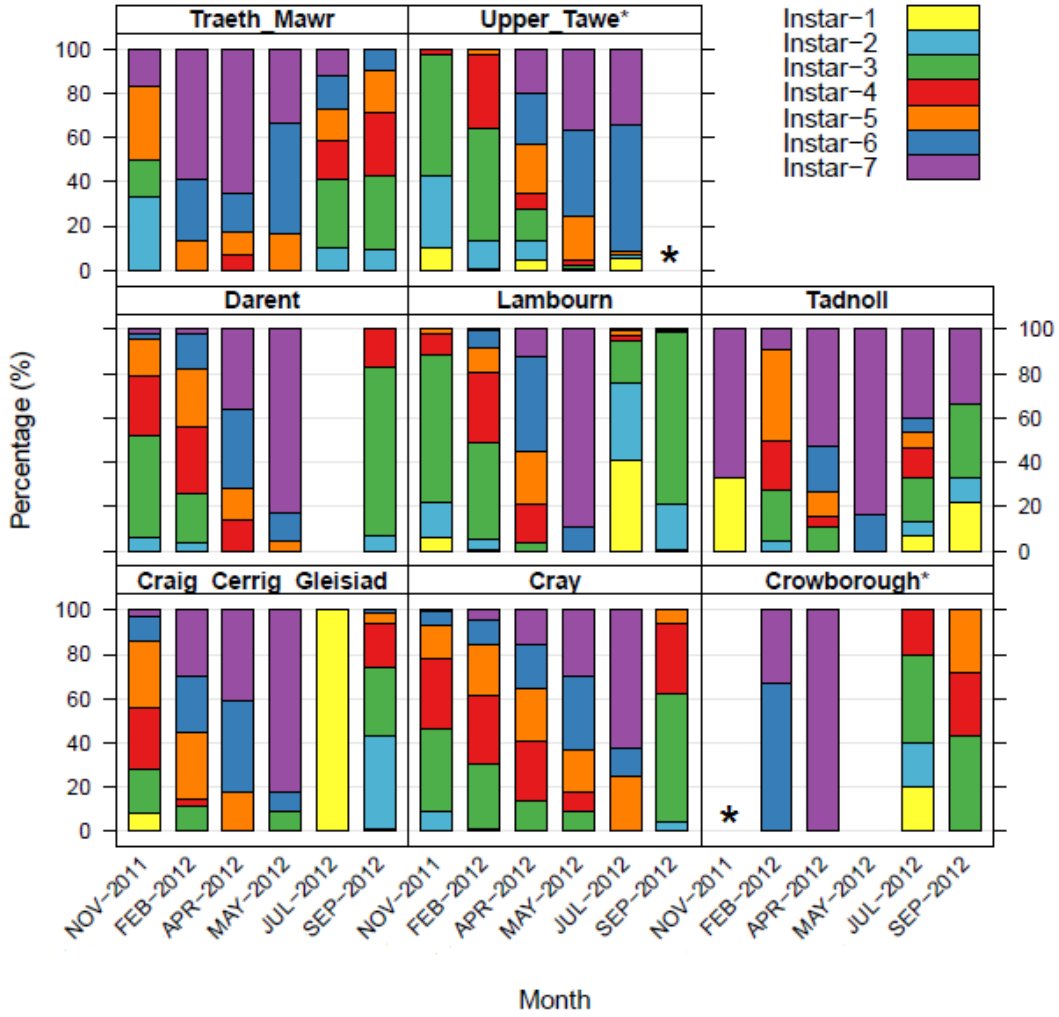

Figure 3.17 Instar distributions of *A. fuscipes* by sampling time points from November 2011 to October 2012 for the eight stream sites. * represents where there are no data.

Table 3.9 Summary of the months where first instars and pupae of *A. fuscipes*, *S. nigricornis* and *S. pallipes* were present in-stream and the estimated time it took larvae to reach the pupal stage from the first instar. Assumptions based on missing data or months are represented by a ?.

Stream	First instar emergence	Months that pupae were found	Time taken to reach the pupal stage
		A. fuscipes	
Craig Cerrig Gleisiad	Jul - Nov (5 months)	Feb - Jul (6 months)	10 – 12
Cray	Sep - Nov?	Apr - Jul (4 months)	10 – 12
Crowborough Warren	Jul	Apr	10
Darent	Jul - Nov (5 months)	Apr - Nov (8 months)	10 – 12
Lambourn	Jul - Nov (5 months)	Apr - Sep (6 months)	10 – 12
Tadnoll	Jul - Nov (5 months)	Apr - Nov (8 months)	10 – 12
Traeth Mawr	Jun - Nov?	Apr - Sep (6 months)	10 – 12
Upper Tawe	Sep - Nov?	May - Sep? (5 months)	10 – 12
		S. nigricornis	
Lambourn	Jul - Nov (5 months)	Feb - Jul (6 months)	10 - 12
Tadnoll	Sep - Nov (3 months)	Feb - Jul (6 months)	12 – 18
		S. pallipes	
Craig Cerrig Gleisiad	Nov	May	10 -12
Crowborough Warren	Jul - Sep (3 months)	Feb - May (4 months)	10 – 12
Darent	Jul - Sep (3 months)	Apr - Jul (4 months)	10 – 12
Upper Tawe	Jul - Feb (6 months)	May - Jul (3 months)	10 – 12

3.3.7 Silo nigricornis instar distributions

In the Lambourn *S. nigricornis* first instar larvae began appearing in a five month period between July to November. For the Tadnoll, first instar larvae appeared over a shorter period of three months between September and November. In both streams pupae were found for a six month period between February and July. Generation time for larvae in the Lambourn and the Tadnoll was deemed to be about one year. There is some indication that

there is a non-synchronous life cycle as larger individuals of instar 4 and 5 were found all year round. This may indicate that some individuals take one to two years to complete their life cycle. Two cohort production intervals will thus be used for subsequent calculations of production, 12 months and 18 months to reflect that some individuals may require a longer period of time to develop from the first instar to the pupal stage.

3.3.8 Silo pallipes instar distributions

Instar I larvae at Craig Cerrig Gleisiad were found in November and pupae were found in May. In Crowborough Warren and the Darent, instar I larvae were found between July and September with pupae appearing in February and April respectively. Instar I larvae were found for a longer period for Upper Tawe, appearing for a six month period from July to February. Pupae appeared from May to July. Similarly to *S. nigricornis*, generation time for *S. pallipes* was deemed to be about one year with some individuals perhaps needing two years to develop to adulthood. As with *S. nigricornis*, two cohort production intervals of 12 and 18 months will be used in subsequent calculations of production in the next chapter to reflect that some individuals may take longer to develop to the pupal stage.

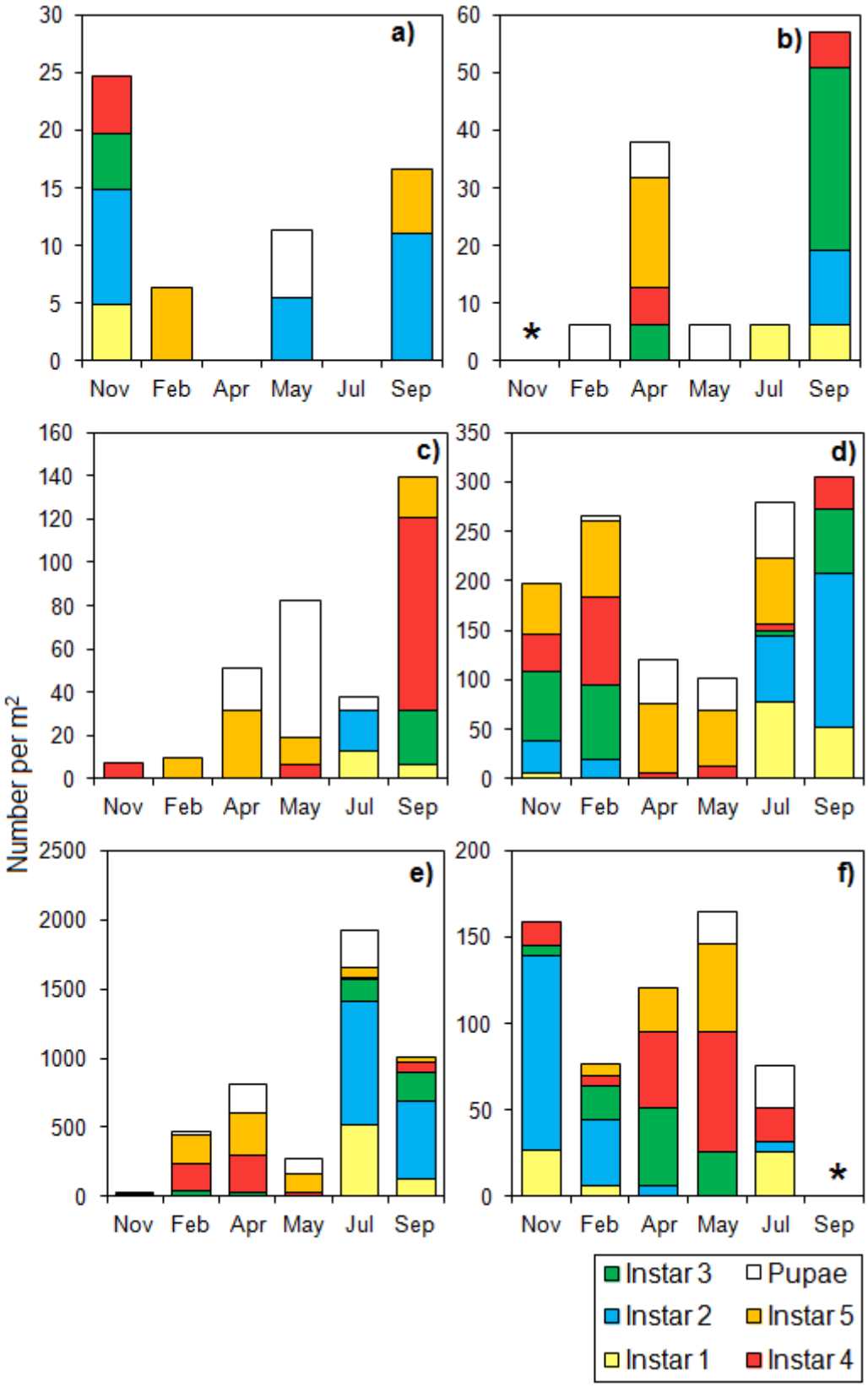

Figure 3.18 Number of *Silo* larvae per m^2 separated by instar in a) Craig Cerrig Gleisiad (*S. pallipes*), b) Crowborough Warren (*S. pallipes*), c) Darent (*S. pallipes*), d) Lambourn (*S. nigricornis*), e) Tadnoll (*S. nigricornis*) and f) Upper Tawe (*S. pallipes*), from November 2011 to October 2012. * represents where there are no data.

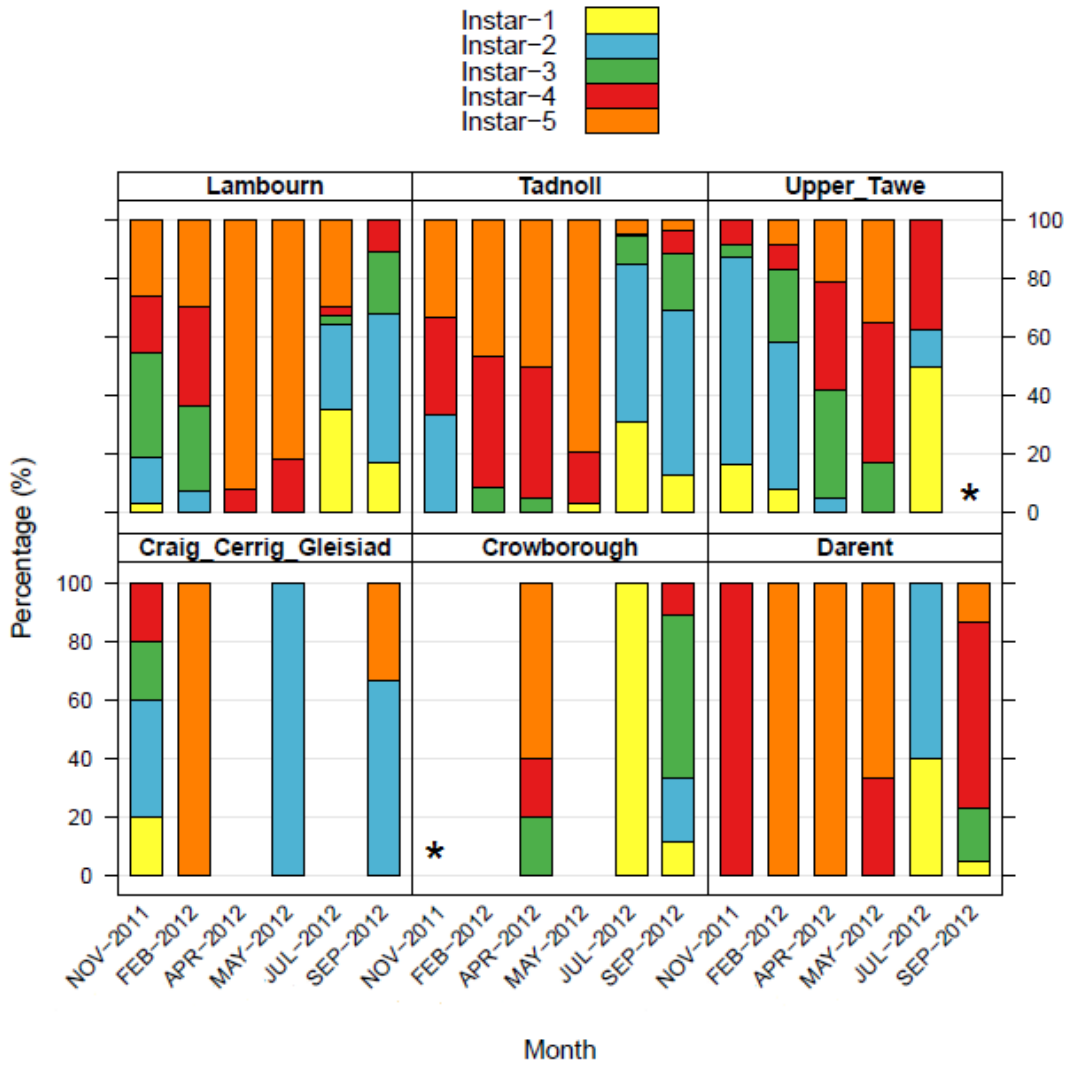

Figure 3.19 Instar distributions of *Silo* species by sampling time points from November 2011 to October 2012. The Lambourn and the Tadnoll contained *Silo nigricornis* and Craig Cerrig Gleisiad, Crowborough Warren, the Darent and the Upper Tawe contained *Silo pallipes*. * represents where there are no data.

3.3.9 Pronotum length-mass regressions

Pronotum length-mass regressions were calculated for seven out of eight streams for *A. fuscipes* (Figure 3.20). Due to low numbers of larvae, only one individual was weighed for Crowborough Warren, so an individual pronotum length-mass regression could not be calculated for this stream. No significant differences were found between pronotum length-mass regressions of 5 streams (Craig Cerrig Gleisiad, the Cray, the Darent, the Tadnoll and Traeth

Mawr). Dry mass increased significantly faster with pronotum length for the Upper Tawe, and significantly slower with pronotum length for the Lambourn ($F_{(2, 628)}$ = 258.6, p = 0.05). For *S. nigricornis*, head width-mass regressions were calculated for both the Lambourn and the Tadnoll. No significant differences were found between these two head-width mass regressions. One head width-regression was calculated for *S. pallipes* based on individuals from Craig Cerrig Gleisiad, Crowborough Warren, the Darent and Upper Tawe (Figure 3.22).

Table 3.10 Pronotum length (µm) or head width (µm) and mass (µg) regressions for the eight streams. Only one individual larvae was measured for mass from Crowborough Warren so a regression equation could not be calculated. ǂ equation for mass for the eight streams combined. ǂǂ Equation for five streams combined (Craig Cerrig Gleisiad, Cray,Darent, Tadnoll and Traeth Mawr). ǂǂǂCombined streams include Craig Cerrig Gleisiad, Darent and Upper Tawe.

Stream	Regression equation	Sig dif	R^2
	Agapetus fuscipes		
Craig Cerrig Gleisiad	Mass (µg) = 0.0012 e $^{0.01 \text{ PL (µm)}}$		0.77
Cray	Mass (µg) = 0.0015 e $^{0.0095 \text{ PL (µm)}}$		0.89
Crowborough Warren	-		-
Darent	Mass (µg) = 0.0023 e $^{0.0088 \text{ PL (µm)}}$		0.76
Lambourn	Mass (µg) = 0.0048 e $^{0.0094 \text{ PL (µm)}}$	*	0.62
Tadnoll	Mass (µg) = 0.0022 e $^{0.0086 \text{ PL (µm)}}$		0.85
Traeth Mawr	Mass (µg) = 0.0023 e $^{0.0092 \text{ PL (µm)}}$		0.81
Upper Tawe	Mass (µg) = 0.0009 e $^{0.0115 \text{ PL (µm)}}$	*	0.88
Combined (data from 8 streams)ǂ	Mass (µg) = 0.0016 e $^{0.0098 \text{ PL (µm)}}$		0.85
Combined (data from 5 streams)ǂǂ	Mass (µg) = 0.0017 e $^{0.0094 \text{ PL (µm)}}$		0.86
	Silo nigricornis		
Lambourn	Mass (µg) = 0.0126 e $^{0.0045 \text{ HW (µm)}}$		0.89
Tadnoll	Mass (µg) = 0.0112 e $^{0.0046 \text{ HW (µm)}}$		0.83
	Silo pallipes		
Combinedǂǂǂ	Mass (µg) = 0.0091 e $^{0.0049 \text{ HW (µm)}}$		0.88

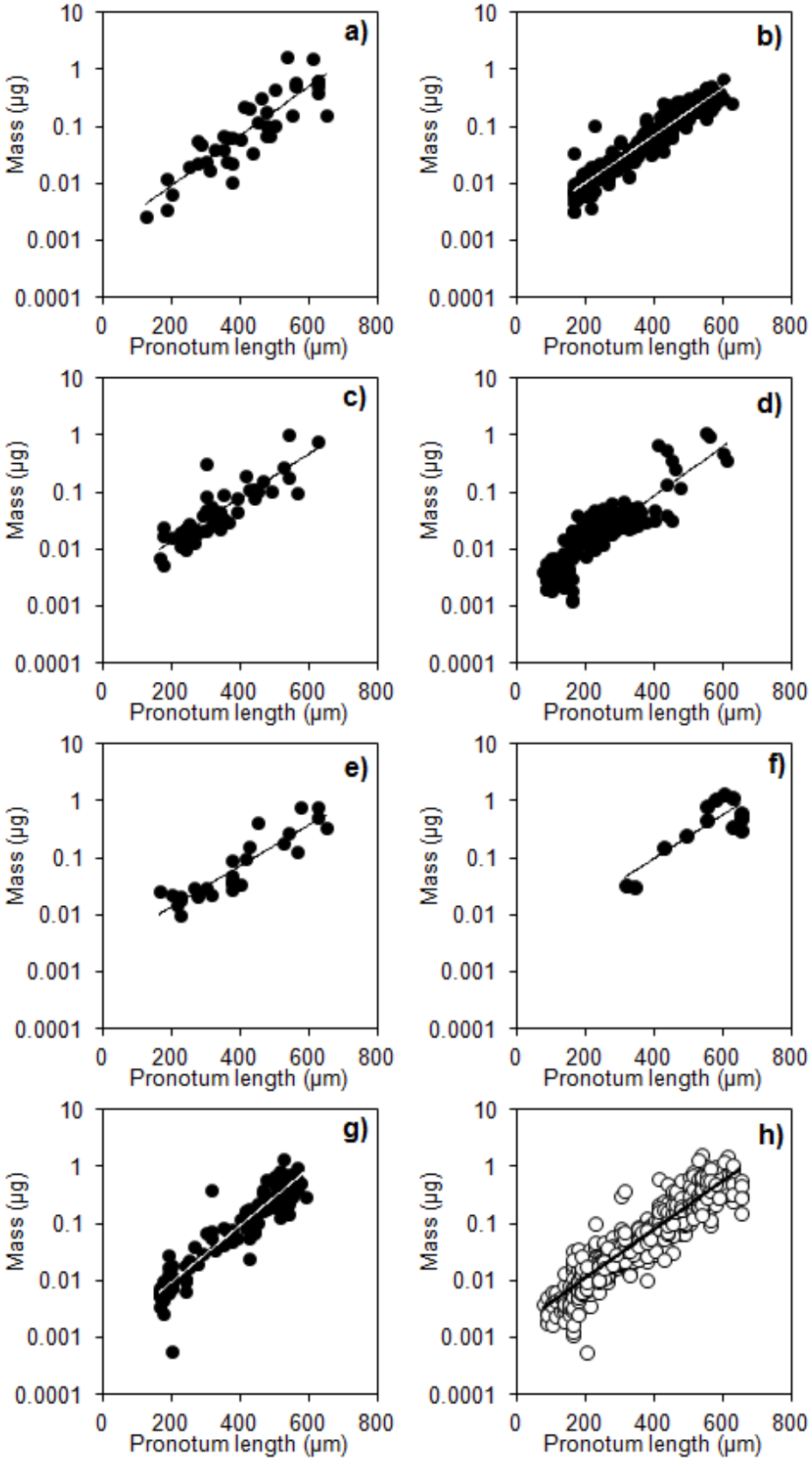

Figure 3.20 Pronotum length (µm) and mass (µg) regressions for *Agapetus fuscipes* a) Craig Cerrig Gleisiad, b) Cray, c) Darent, d) Lambourn, e) Tadnoll, f) Traeth Mawr, g) Upper Tawe and h) all sites combined.

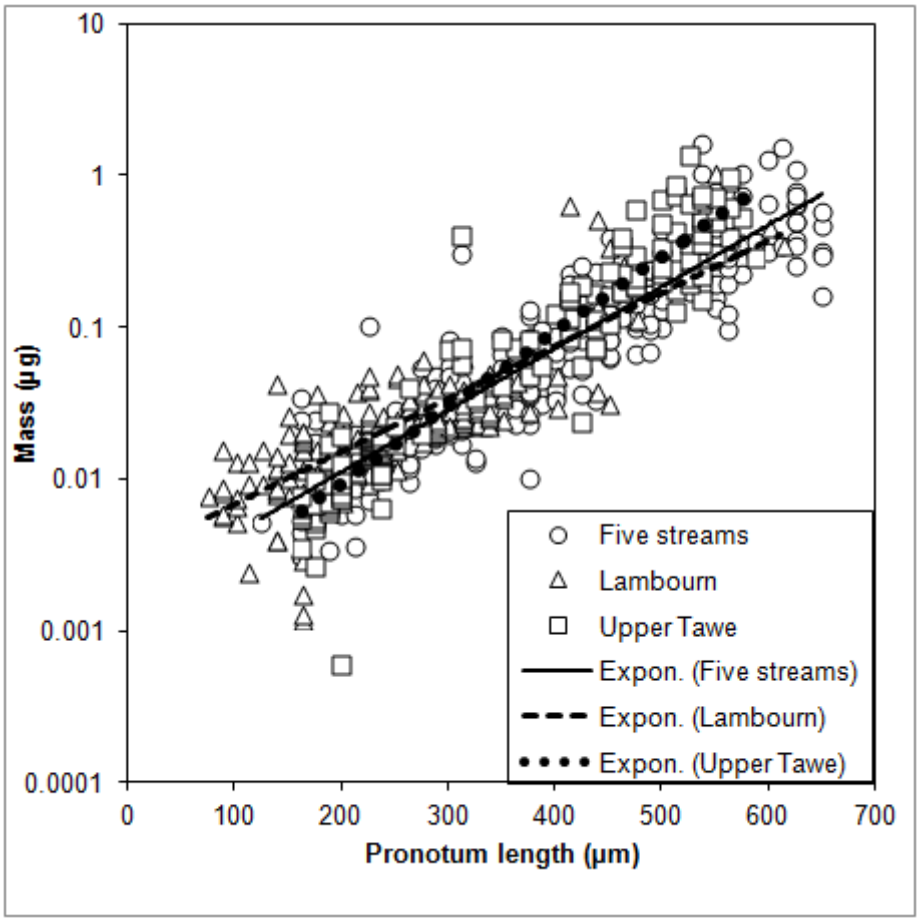

Figure 3.21 Pronotum length-mass regressions for five streams (Craig Cerrig Gleisiad, Cray, Darent, Tadnoll and Traeth Mawr), the Lambourn and Upper Tawe. For the Lambourn and Upper Tawe, slopes of best fit lines differed significantly from the other five streams ($F_{(14,628)}$ = 258.6, p = 0.05). The regression equation for the five streams is $y = 0.0017e^{0.0094x}$ R^2 = 0.86, the Lambourn $y = 0.0048e^{0.0094x}$, R^2 = 0.62 and Upper Tawe $y = 0.0009e^{0.0115x}$, R^2 = 0.88.

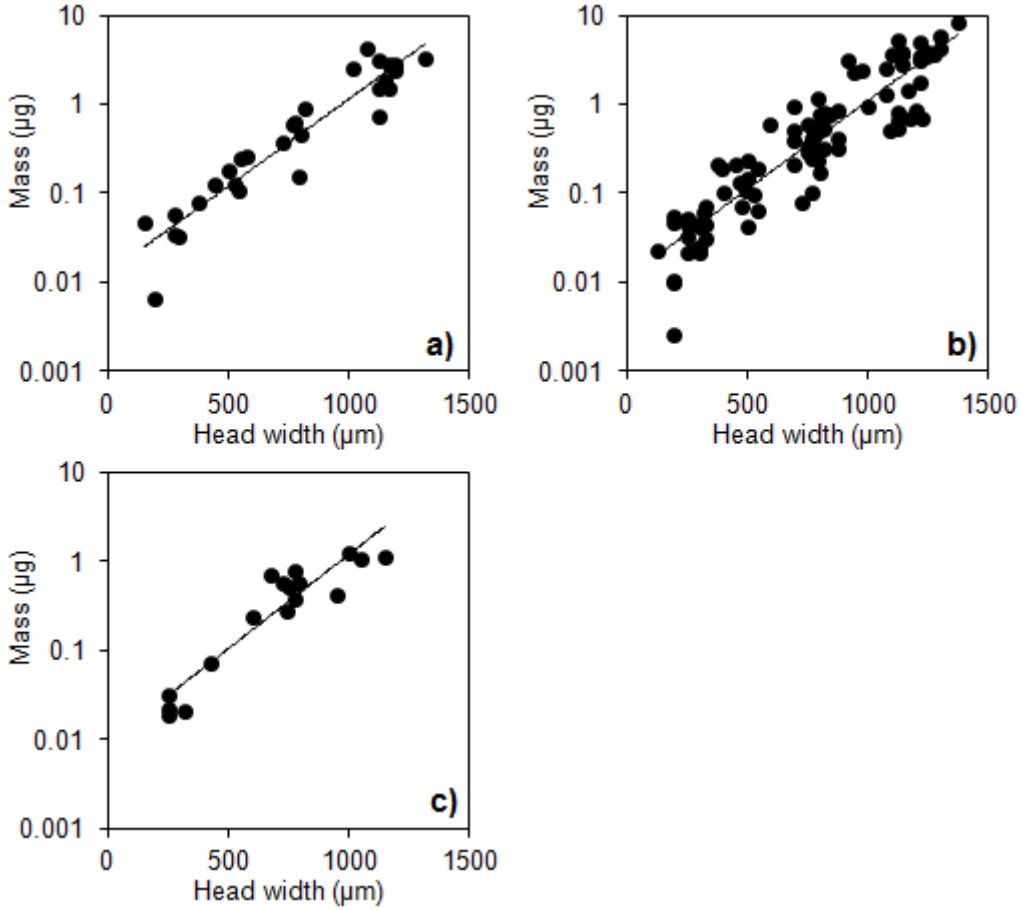

Figure 3.22 Head width (µm) and mass (µg) for *Silo* spp. a) Lambourn – *Silo nigricornis*, b) Tadnoll – *Silo nigricornis* c) Craig Cerrig Gleisiad, Darent and Upper Tawe – *Silo pallipes*.

3.4 Discussion

3.4.1 Separating instars

The primary finding of this study was that *Agapetus fuscipes* instars could not simply be separated by either body length, head width or pronotum length alone. Pronotum length produced partial histogram peaks for some streams and for some instars, but for most streams, peaks did not clearly separate instars. Generally, the range of pronotum lengths observed was similar to that of Becker (2005), although one stream, the Lambourn, contained smaller first instars. Three streams, the Lambourn, Tadnoll and Traeth Mawr contained several individuals with larger pronotum lengths that were outside the range observed for the Breitenbach population. The frequency histograms of head width and body length did not produce clearly defined instar peaks for most streams. The number of instars that *A. fuscipes* has in Britain is thus still unclear.

Although care was taken to measure caddis larvae accurately using the microscope and eyepiece scale, it is possible that instars could not be separated due to measurement error and the error magnification associated with this method. The size bin widths of the histograms can also influence the separation of data (Scott 1979) and thus the interpretation of the instars based on size characteristics. For *A. fuscipes* pronotum lengths and head widths the bars were separated to the maximum extent that the data would allow in order to spot gaps in size between the instars. If the bars are not

separated enough, it will be difficult to separate the instars for streams where there are lots of individuals measured. However, if few individuals are collected from a stream, a large number of bin widths may not group the data adequately to separate instars.

It is not possible to distinguish *Agapetus* to species below instar III – V or below 0.3mm in head width (Wallace *et al.* 2003) so some individuals of the morphologically similar *A. ochripes* and *A. delicatulus* could have been measured and inadvertently included in analysis. These species may have slightly different sized instars to *A. fuscipes* and trying to separate out the early instars by body size characteristics may not be possible where species coexist in the streams. There was considerable variation between streams for the mean pronotum lengths for each instar. Individual *Agapetus* larvae were assigned to an instar according to the frequency histograms of Becker (2005). Few pronotum lengths were found in most streams corresponding to instar 1 so the mean pronotum length for this instar for most streams will have been affected the most by a few values whereas in the Lambourn and Upper Tawe, more individuals corresponding to this instar were found and the mean is more likely to represent the mean pronotum length of first instar individuals in these two streams. There was also variation between streams for mean pronotum length for the last instar, instar 7. As individuals were not sexed and females are known to be considerably larger than males it is likely that this variation may come from the number of females and males in each stream. As already stated, instars of other *Agapetus* species could have inadvertently measured and included in the calculation of mean pronotum

length for each instar. *Agapetus fuscipes* as a species is also known to be variable in size with individuals from mountain streams being smaller than individuals from lowland streams (Wallace *et al.* 2003).

Despite not being able to separate *A. fuscipes* into instars in this study, the calculation of secondary production does not depend on being able to successfully separate species into instars. Secondary production can also be calculated by separating species into progressively larger size classes (Benke & Huryn 2006). *A. fuscipes* can thus be separated into seven size classes based on the divisions of Becker (2005) since the range of pronotum frequencies observed is very similar.

In contrast to *A. fuscipes*, both *S. nigricornis* and *S. pallipes* could be successfully separated into five instars based on frequency histogram peaks. This agrees with the previous findings of Nielsen (1942), Elliott (1982) and Wallace, Wallace & Philipson (1990). Instar I larvae were found for both *S. nigricornis* and *S. pallipes*, allowing a range of head widths corresponding to these larvae to be calculated for the first time. It is especially important to be able to accurately quantify the number of instar I larvae for secondary production calculations, as underestimating the first size-class or instar leads to underestimates of secondary production (Morin, Mousseau & Roff 1987; Bowles 1990). Apparent density of instar I individuals can often appear low because invertebrate species often pass through this stage very quickly (Benke & Huryn 2006).

Few other studies report not being able to separate insect instars by one of the three measures of body length, head width or pronotum length. A study of *Helicopscyche borealis* in a coastal California stream found that instars could not be separated by head width, despite over 1100 individuals being measured over a 14 month period (Resh, Lamberti & Wood 1984). In my study there could be a mix of cryptic species that confound the pronotum length frequency histograms. Morphologically cryptic species of Trichoptera can be found coexisting together in some streams. For example, Jackson & Resh (1998) studied two species of Sericostomatidae, *Gumaga nigricula* and *Gumaga griseola*, that were taken from streams and springbrooks in northern California. Earlier studies had indicated that the species were extremely variable or that morphologically cryptic species were unwittingly being included in studies. Allozyme electrophoresis was used to examine the genetic relationships of the species and discovered six genetically distinct groups. These groups, despite existing and feeding in close proximity to each other, maintained their reproductive isolation, possibly by pre-mating mechanisms.

Recent research on the genus *Agapetus* in America and Australia has uncovered more species than previously thought. Etnier *et al.* (2010), described 12 new species of *Agapetus* that had been discovered in central and eastern America, between 1998 to 2005. A few of these newly described species were cryptic. A review of the genus of *Agapetus* in Australia found,

similarly, that among the 23 species recognised, 13 were newly described (Wells 2010). In Britain, another glossosomatid species was recently discovered in 2010. *Synagapetus dubitans*, a species that is morphologically very similar to *Agapetus fuscipes*, was discovered near a woodland stream in Masham, North Yorkshire (Crofts 2012a). This species has since been found in more streams in North Yorkshire, often coexisting with *A. fuscipes* and work is ongoing to discover more sites where the species might exist (Crofts 2012b).

There is likely to be intraspecific variation within *A. fuscipes* with some individuals of a developmental stage being larger or smaller than others. There can be different larval phenotypes present in populations of caddis larvae that can take advantage of different environmental conditions and dietary items. For example in the Flathead River Basin in Montana, the caddisfly *Arctopsyche grandis* has two types of larvae that are morphologically different and can take advantage of different sized interstitial spaces; Type I and Type II. Type I larvae are common in compacted, small interstitial spaces. In contrast Type II larvae grow faster, are larger in their final instar and exhibit a preference for larger interstitial spaces (Hauer & Stanford 1981). Size variation in developmental stages of *A. fuscipes* may play a function in the species ability to successfuly colonise the river bed and acquire food resources when there is intraspecific competition between larvae.

3.4.2 Generation time

Agapetus fuscipes was found to be univoltine for streams, apart from Upper Tawe where first instar larvae were found in April. This finding agrees with the conclusions of Nielsen (1942), (Benedetto Castro 1975), Sangpradub *et al.* (1999) and Becker (2005), who also found *A. fuscipes* to be univoltine. *Silo pallipes* was found to be mostly univoltine in this study, agreeing with the findings of Sangpradub *et al.* (1999) and Elliott (1982). Similarly to *S. pallipes*, *S. nigricornis* was also found to be mostly univoltine.

Most streams contained a mixture of instars for *A. fuscipes*, *S. nigricornis* and *S. pallipes* for most months, with some streams having all instars present on a single sampling occasion. For the Darent and Traeth Mawr, no instar I individuals of *A. fuscipes* were found. This is likely to be because the small instars pass very quickly through this stage or that instar I larvae for these streams were deep in the hyporheic zone (Winterbourn & Wright-Stow 2002). Instar I larvae for all species were present on a number of sampling occasions, probably reflecting extended asynchronous development. Asynchronous development can be viewed as a 'bet-hedging' strategy in unpredictable environments (Winterbourn, Rounick & Cowie 1981; Sangpradub *et al.* 1999). Iverson (1976), also recorded asynchronous development for *A. fuscipes*, with two cohorts of larvae, one appearing in July and another in November. Similarly to this finding, the Upper Tawe exhibited two 'pulses' of first instar larvae; one in November and April. Asynchronous development is common for Trichoptera. González & Graça

(2003) found that the larvae of *Sericostoma vittatum* exhibited asynchronous development in a stream in central Portugal and suggested this was advantageous because it allowed larvae to persist throughout bouts of flooding. Similarly, Zwick (1996), suggested that, for the stone fly *Dinocras* spp., asynchronous egg development may play a crucial role in recolonization of rivers after scouring events caused by flooding. Alternatively, asynchronous development in freshwater environments can sometimes be a response to inter-specific and intra-specific competition (Tsurim *et al.* 2013). An assortment of different-sized larvae may reduce competition for food or space and enhance larval growth and production (Edgerly & Livdahl 1992).

One stream, Crowborough Warren, contained very few individuals of both *A. fuscipes* and *S. pallipes,* with 28 and 15 individuals collected respectively by the Surber sampling method. This may have been because the stream had experienced an intense drought in the period preceding sampling. This stream was found to be dry when inspected in November 2011 and was flowing again on the February sampling occasion. Although *Agapetus* and *Silo* spp. have adaptations to survive drought, a period of sustained drought can cause population numbers to decline (Nijboer 2004; Hille *et al.* 2014). This stream also contained high stream water concentrations of phosphorus throughout the year. Phosphorus concentrations can sometimes increase in rivers during periods of drought (Boar, Lister & Clough 1995; van Vliet & Zwolsman 2008) as ions become concentrated in the remaining stream

water. Under stagnant conditions the sediment can also release phosphate into the stream (Parr & Mason 2003; van Vliet & Zwolsman 2008).

When estimating secondary production, asynchronous development is important to consider when choosing the most appropriate method for calculation. Where a cohort of individuals cannot be followed easily through time a non-cohort method such as the size-frequency method is required (Benke & Huryn 2006). The main assumption of this method is that a mean size-frequency distribution determined from samples collected over the duration of a year provides a good approximation of a mortality curve for an average cohort (Hynes & Coleman 1968; Hamilton 1969; Benke 1979).

This study has established that *A. fuscipes* has a similar range of pronotum lengths to those found by Becker (2005) in the Breitenbach stream, although instars cannot be distinguished by body length, head width or pronotum length. Further research is needed to elucidate whether, as elsewhere, there is a mixture of morphologically cryptic species in the streams. In the future, the use of DNA barcoding and other genetic techniques may shed light on how to differentiate species successfully for this genus. For both *S. nigricornis* and *S. pallipes*, head width ranges including instar I have been observed and described which may help future studies on these species. All three species of caddis larvae have a degree of asynchronous development, which is an important consideration when choosing a suitable method for estimating secondary production. The most appropriate method is likely to be

a non-cohort method, such as the size-frequency method, and is the subject of the next chapter.

4 Secondary production in grazing caddis and its support by methane-derived carbon

4.1 Introduction

The first aim here was to measure secondary production for *A. fuscipes*, *S. nigricornis* and *S. pallipes* in eight streams sites and allocate how much production is from methane-derived carbon and carbon produced by photosynthesis. The second aim was to examine whether the chlorophyll *a* content of the biofilm on stream gravels correlates with the percentage of algae assimilated by the caddis larvae based on two source mixing models of larval $\delta^{13}C$. If this is the case it would suggest that caddis larvae are limited by the amount of algae that is available to them and that when algae is in short supply methane-derived carbon is assimilated as a 'top-up' or 'emergency' resource. The third aim of this chapter was to assess whether streams with high annual production (g m^{-2} yr^{-1}) are those with larvae that incorporate the highest percentage of methane-derived carbon. If true, this would suggest that methane-derived carbon is able to fuel higher annual production than could be achieved by caddis populations with only conventional autochthonous and allochthonous resources available to them.

4.1.1 Secondary production

Secondary production is the formation of heterotrophic biomass, for instance by benthic invertebrates in rivers and streams. Secondary production of an

population is an overall broad measure of its 'success' in pre-empting resources (Benke 1993). Secondary production can also be calculated for entire communities or smaller assemblages of species (such as guilds of grazers or predators) and represents the collective growth of that entire group. Estimates of production are expressed as mass or its energy equivalent (e.g. grams of carbon, grams of dry mass or joules) and is usually calculated per unit area per unit time (i.e. grams m^{-2} yr^{-1}). Biomass may be considered to be a 'static variable' as it represents one point in time where mass per unit area is measured, whereas secondary production is a 'functional variable' because it measures an ecological process over a period of time (Benke 1984, 2010).

Annual secondary production is the sum of all the biomass produced by heterotrophs over the course of one year, including biomass lost to predation, disease, parasitism and cannibalism (or any other source of mortality) plus the loss of tissue reserves due to moulting, silk production or starvation and the value if any of emigrants (Benke 1984; Benke & Huryn 2006).

4.1.2 History of secondary production

The first to measure the production of aquatic invertebrates was Boysen-Jensen (1919), who used a form of removal-summation method in the Limfjord, a shallow fjord in Denmark. Later, Borutski (1939), studied

production of the midge *Chironomus plumosus* in Lake Beloie, Russia. Despite methodological developments, by the 1960's published invertebrate production estimates were still unusual (Waters 1977), and only started to increase in the 1970's, after the development of what is now known as the size-frequency method paved the way for production estimates to be more easily calculated (Hynes & Coleman 1968; Hamilton 1969; Benke 1979).

4.1.3 The size-frequency method

The size-frequency method is useful when the 'cohort method' for measuring secondary production cannot be applied to a population, because of asynchronous development and the absence of a clear cohort structure (Benke 1984). It calculates the average survivorship of a hypothetical average cohort: samples are taken over one year to derive an average size-frequency distribution. A key initial assumption is that the life span of the species in question is about one year (Hamilton 1969; Benke & Waide 1977), with a 'cohort production interval' correction (CPI) applied where life cycles are known to be shorter (or longer) than one year (Benke 1979): i.e. this essentially doubles the estimate of annual production when there are two generations per year or halves it where the life cycle takes two years. The size frequency method is particularly useful for calculating the secondary production of Trichoptera, as many species lack a clear cohort structure and often have overlapping generations or asynchronous development (Marchant & Hehir; Georgian & Wallace 1983; Mackay & Waters 1986; Scrimgeour 1991).

4.1.4 Applications of secondary production

The estimation of secondary production is a requirement for quantifying the 'energy-flow concept', addressing energy flow through trophic levels, and can be useful for comparing two or more populations of a single species (Benke 1984; Benke & Huryn 2010). Estimates of secondary production for entire stream communities have been calculated (Krueger & Waters 1983; Strayer & Likens 1986), typically exploring how energy-flow differs between streams. Estimates of annual secondary production for entire stream communities vary worldwide over several orders of magnitude from 10^0 to 10^2 g dry mass m^{-2}, with some of the highest values reported for filter feeders in temperate streams (Wotton 1988; Grubaugh & Wallace 1995; Grubaugh, Wallace & Houston 1997; Huryn & Wallace 2000).

In recent decades, the estimation of secondary production has been used in many other applications, particularly in the study of the ecology of freshwater benthic invertebrates (Benke & Huryn 2010). Some of these applications include experimental and tracer-based studies of the trophic basis of production (Wallace *et al.* 1997, 1999; Webster & Meyer 1997; Hall, Wallace & Eggert 2000; Tank *et al.* 2010), the influence of non-native species (Johannsson *et al.* 2000; Hall, Tank & Dybdahl 2003; Hall, Dybdahl & VanderLoop 2006), assessing the effects of land use change (Grubaugh & Wallace 1995; Kedzierski & Smock 2001) and of the importance of

meiofauna relative to the macrofauna (Stead, Schmid-Araya & Hildrew 2005; Tod & Schmid-Araya 2009).

4.1.5 Trophic basis of production

In this chapter the relative contribution of two carbon sources (carbon derived from algae and carbon derived from methane) for three species of armoured grazing caddis larvae, *A. fuscipes*, *S. nigricornis* and *S. pallipes*, is investigated using stable isotope analysis combined with secondary production estimates. Gut contents analysis of consumers used to be the primary way to trace pathways of organic matter across trophic levels in food webs (Georgian & Wallace 1983; Benke *et al.* 2001). However, although gut contents analysis is useful in identifying food web links, it does not measure quantitatively what material is actually assimilated by an organism and can lead to some food sources being over- or under-estimated in their importance (Rosenfeld & Mackay 1987).

One of the first studies to estimate the relative contribution of different food resources to annual production, assessed six species of filter-feeding caddisflies by combining production calculations with gut contents analyses and food-specific assimilation efficiencies (Benke & Wallace 1980). The primary finding was that, although analysis of gut contents indicated detritus as the most important food resource, 80% of all caddisfly production was actually attributable to the assimilation of animal prey. Other studies

investigating the contribution of resources to caddisfly assemblages soon followed (Haefner & Wallace 1981; Ross & Wallace 1981, 1983). Some more recent studies of the trophic basis of production have focussed on the contribution of various available resources to individual taxa (Johnson, Tarter & Hutchens 2000; Alvarez & Pardo 2005; Yan & Li 2007).

4.1.6 Using stable isotopes to identify the trophic basis of production

The relative contribution of a resource to a consumer can be assessed by combining secondary production analysis with stable isotope analysis of consumer tissues, as long as the resources in question have distinct and temporally robust $\delta^{13}C$ ratios (DeNiro & Epstein 1978; Peterson & Fry 1987). Stable isotope ratios, unlike gut contents analysis, provide information on what resources have been incoporated into the tissues of an organism. Mixing models use the $\delta^{13}C$ values of the potential resources and consumers to calculate the relative contributions of the specific resources (Fry 2006).

One of the earliest studies to combine stable isotope analysis and production estimates was that by Peterson & Howarth (1987), which assessed whether detritus from a grass species (*Spartina alterniflora*) or algae supported most estuarine secondary production in the salt marshes of Sapelo Island in Georgia. They found that consumers intake a mixture of *S. alterniflora* detritus and alage. Similarly, Angradi (1994) used stable isotopes to identify trophic linkages in the Lower Colorado river and to identify which primary

producers were most likely to support secondary production in the river. It was suggested that benthic algae and phytoplankton form the basis of secondary production in one part of the system; the Glen Canyon Dam and that in other parts; the tributaries, terrestrial plants are likely to be more important. Other studies incorporating production estimates and stable isotope analysis have been used for coastal ecosystem studies and lakes (Duggins, Simenstad & Estes 1989; Sauriau & Kang 2000; Vander Zanden *et al.* 2006).

4.1.7 Quantifying the contribution of the 'third way'

Most previous studies that have measured the trophic basis of production for organisms in fresh waters have been based on the assumption that carbon contributing to annual production originates from photosynthesis, either autochthonous or allochthonous (Wallace *et al.* 1995; Jones *et al.* 1998; Thorp *et al.* 1998; Grey, Jones & Sleep 2001; Mulholland *et al.* 2001; Trifonova *et al.* 2002; Allan & Castillo 2007; Cole *et al.* 2011). However, it is now known that carbon can enter food webs via a 'third way', chemosynthesis, and in this case carbon derived from methanogenesis (Deines 2006; Trimmer *et al.* 2009; Jones & Grey 2011). Biogenic production of methane is driven by the bacterial conversion of substances such as acetate, formate, hydrogen and carbon dioxide rather than by light energy and the process of photosynthesis (Woltemate *et al.* 1984; Whiticar *et al.* 1986).

4.1.8 Lake food webs and assimilation of methane-derived carbon

Recent studies of lake food webs have shown that in some cases profundal chironomid larvae can assmilate methane-derived carbon. Previously, these chironomid larvae had been thought to ingest the 'rain' of detrital organic matter originating from the epilimnion and settling on the surface of the bed (Jonasson 2004). However a study of chironomid $\delta^{13}C$ values from 87 lakes suggested that, in at least a third of the lakes, there was ^{13}C-depletion in profundal larvae relative to the corresponding surface sediment, suggesting consumption of methane-oxidising bacteria (MOB), with some chironomids potentially deriving up to 70% of their carbon via their ingestion (Jones *et al.* 2008). Profundal chironomids depleted in ^{13}C are often associated with eutrophic stratified lakes, where anoxic/oxic interfaces exist either at the sediment-water interface, or at the oxycline in the stratified water column, and at which methane can potentially be oxidised by MOB (Grey, Kelly & Jones 2004a; Grey *et al.* 2004b; Kelly, Jones & Grey 2004; Hershey *et al.* 2006; Agasild *et al.* 2014). Importantly, there is also evidence to suggest that methane-derived carbon assimilated by chironomids reaches higher consumers such as fish (Harrod & Grey 2006; Ravinet *et al.* 2010).

Evidence has also suggested that methane-derived carbon is assimilated by armoured grazing caddis in chalk streams. Trimmer *et al.* (2009), through the use of isotopic mixing models, calculated that *A. fuscipes* in the River Lambourn may derive up to 11% of its carbon from MOB, rising to 20-30%

during autumn when the caddis larvae are most ^{13}C-depleted relative to the epilithon of the stream.

Evidence presented in Chapter 2 now suggests that the assimilation of methane-derived carbon by armoured grazing caddis is a relatively widespread phenomenon and is not just restricted to streams on chalk (where the phenomenon was initially discovered), but instead is present on other geological formations such as limestone and sandstone. The life history of three key species of caddis fly larvae involved in the assimilation of methane-derived carbon have been assessed and cohort production intervals estimated for the three species. Thus, the next step here was to calculate annual production values of three species of armoured grazers, *Agapetus fuscipes*, *Silo nigricornis* and *Silo pallipes*, in a sample of streams spanning a range of geologies.

This study combines secondary production estimates, calculated via the size-frequency method, with stable carbon isotope data in order to quantify how much methane-derived carbon is incorporated annually in the production of these caddis larvae.

4.2 Methods

4.2.1 Stream selection

Eight streams were chosen from an initial survey of 29, draining three main geological types; chalk, limestone and sandstone, Two streams were chosen from each geology, based on the $\delta^{13}C$ of the caddis larvae (one stream ^{13}C-depleted and one not) (Chapter 3).

4.2.2 Collection of caddis larvae and biofilm for stable isotope analysis

Caddis larvae were collected over six sampling occasions between November 2011 and October 2012 using the sampling methods laid out in Chapter 3, 3.2.2 *Sampling caddis larvae.* Biofilm samples (n = 3) were collected by scraping rocks and the scrapings placed in 50mL Falcon tubes. Samples were then immediately frozen in a 17L Engel portable freezer. Caddis larvae of *A. fuscipes*, *S. nigricornis* and *S. pallipes* were collected from the stream gravel by hand. Where caddis larvae were rare in some streams, they were later picked out from defrosted Surber samples (body characteristics measured) and then added to Eppendorf tubes and dried immediately at 60°C for 48 hours. For a full description of the stable isotope procedure for epilithon and the caddis larvae see Chapter 2, 2.2.2 *Collection of caddis larvae and resources for stable isotope analysis.*

4.2.3 Chlorophyll a content of gravel biofilm

On each of the six sampling occasions, the chlorophyll *a* content of biofilm on the top layer of river-bed gravel was measured as a proxy of algal biomass. The 10m section of river being sampled was split into five 2m width sections using a measuring tape. From each 2m width section, one sample of the top layer of river bed gravel was collected using a square scoop with an area of 25cm^2 (5 x 5 x 1cm depth). The five samples were stored in separate zip-lock bags and stored in a portable chiller at 5°C for transport back to the laboratory. In the laboratory chlorophyll *a* was extracted using 30ml of acetone (90% v/v with ultra-pure water) in sealed bottles that were stored for 24 hours in a dark refrigerator. Absorbance was measured for two wavelengths, 665nm, as a measure of chlorophyll extinction and 750nm as a measure of clarity. The chlorophyll *a* content was calculated with the following equation modified from Dalsgaard *et al.* (2000):

$$\textbf{Chlorophyll } \textit{a} \textbf{ µg/m}^2 = \frac{(665_A - 750_A) \times A \times V}{a * l}$$

where **A** = absorption coefficient of chlorophyll *a*, 11.0

V = volume of acetone extract (ml)

a = area of river bed sampled in m^2

l = path length of the cuvette (cm)

4.2.4 C:N ratio of biofilm

The molar carbon-to-nitrogen ratio of biofilm from the eight streams was obtained from the elemental analyser coupled to the mass spectrometer. This data was collected as an indicator of the biofilm palatability in the eight streams. The mean C:N ratio of the biofilm was calculated using the monthly mean C:N values from the streams. Low C:N ratios (4:1 to 8:1) for a food source indicate that it is a high quality food resource. Higher C:N ratios of resources (above 17:1) are considered to be of poor food quality (Gregory 1983).

4.2.5 C:N ratio of caddis larvae and lipid correction

The molar carbon-to-nitrogen ratio of the three species of caddis larvae was obtained from the elemental analyser coupled to the mass spectrometer. The mean C:N ratio for the three species of larvae was calculated in order to check whether the larval $\delta^{13}C$ was adversely affected by lipid accumulation rather than ^{13}C-depletion due to methane-derived carbon. Lipids are often more depleted in ^{13}C relative to proteins and carbohydrates because discrimination occurs during the biochemical processes preceding fatty acid biosynthesis (DeNiro & Epstein 1977; Melzer & Schmidt 1987). If the C:N ratio is much higher than four, mathematical correction of the $\delta^{13}C$ value can be applied (McConnaughey & McRoy 1979; Kiljunen *et al.* 2006).

The mean percentage of elemental carbon in caddis larvae dry mass was also calculated for *A. fuscipes* (n = 349), *S. nigricornis* (n = 107) and *S.pallipes* (n = 69) to enable calculation of secondary production in carbon units (see below).

4.2.6 Calculating the density of larvae

Larval density was calculated as the mean number of larvae per m^2 from seven Surber sample-units (225 cm^2) for each sampling occasion for each stream. The exception to this is where less Surber samples were collected for example in the Cray and the Darent in November 2011, when five and six Surber samples were collected, respectively.

4.2.7 Calculating secondary production

Secondary production was calculated for the three species of caddis fly using the size-frequency method (Hynes & Coleman 1968; Hamilton 1969; Benke 1979). An example of the calculations can be found in Table 4.1. The individual (specific to each stream) pronotum length-mass regressions, as calculated in Chapter 3, for each stream for each species were applied (see Chapter 3, Table 3.10). For *A. fuscipes* in Crowborough Warren, the overall pronotum length-mass regression for *A. fuscipes (*based on individuals from all eight streams) was used. For *S. pallipes* in the four stream sites of Craig Cerrig Gleisiad, Crowborough Warren, the Darent and Upper Tawe, an overall head width-mass regression was used. *Agapetus fuscipes* was

divided into seven size classes ('instars') based on the instars made by Becker (2005). The mean pronotum length or head width for each size class (*A. fuscipes*) or instar (*Silo* spp.) for each stream was calculated based on these equations. The average mass of the size groups or instars was then multiplied by the density of that size class or instar for each stream for each sampling occasion. The cohort production intervals of 10 and 12 months were applied to the secondary production calculations of *A. fuscipes*, based on the findings in Chapter 3. For *Silo nigricornis* and *Silo pallipes*, the cohort production intervals of 12 and 18 months were applied, to express the range of life spans indicated from the instar-distribution (see Chapter 3).

4.2.8 Mixing models

A two-source isotope mixing model (Fry 2006) was applied to estimate the percentage contribution of methane-derived carbon to the body tissues of the caddis larvae (see Figure 4.1). A key assumption of the model was that larval $\delta^{13}C$ simply comprised a mixture of two basal resources: biofilm and methane oxidising bacteria (MOB). As MOB $\delta^{13}C$ was not measured, MOB $\delta^{13}C$ values were calculated from methane gas values measured by Ings and Shelley (unpubl. data) and accounting for two different fractionation scenarios of the methane; 16‰ and 0‰, based on Summons (1994). As methane gas from stream water was, on average, -45‰, this gave values of -61 and -45‰ for MOB. A trophic fractionation factor of carbon by caddis larvae of +0.4‰ (McCutchan *et al.* 2003) was subtracted from the $\delta^{13}C$ values of the caddis larvae before input into the mixing model. The potential

dietary assimilation of MOB was derived for each of the six months in which samples were taken. This was then averaged to calculate two annual dietary percentages of MOB for each stream (under the different fractionation scenarios).These minimum and maximum percentages of methane-derived carbon incorporation were then multiplied (expressed as proportions) by the total carbon production to calculate the contribution of methane-derived carbon (in mass units, g) to the total production of the three caddis flies in the eight streams. An example of the calculations can be found in Table 4.2.

$$f_1 = (\delta_{SAMPLE} - \delta_{SOURCE2}) / (\delta_{SOURCE1} - \delta_{SOURCE2})$$

$$f_2 = 1 - f_1$$

where:

f_1 = proportion of source 1 that contributes to caddis diet

f_2 = proportion of source 2 that contributes to caddis diet

δ_{SAMPLE} = $\delta^{13}C$ (‰) of caddis larvae

$\delta_{SOURCE1}$ = $\delta^{13}C$ (‰) biofilm

$\delta_{SOURCE2}$ = $\delta^{13}C$ (‰) MOB

Figure 4.1 Two-source mixing model used in calculations (Fry 2006) to estimate the contribution of methane-derived carbon to caddis body tissue. A trophic fractionation factor of 0.4‰ (McCutchan *et al.* 2003) was first subtracted from caddis larvae $\delta^{13}C$ (‰) before incorporation into the mixing model. Proportions of sources (f_1 and f_2) were multiplied by 100 to obtain percentage contributions of sources to caddis larvae body tissue.

4.2.9 Analysis

The following statistical analysis were completed in R version 3.1.1 (R Core Development Team 2014). To investigate if the percentage of algae assimilated by the caddis larvae was related to a) the chlorophyll *a* content of the biofilm on stream gravel, b) month sampled or c) the stream, two ANCOVA models were carried out applying the two fractionation scenarios (i.e. 16 and 0‰).

A series of linear regression models were calculated to investigate whether the mean annual chlorophyll *a* content of biofilm (ug/m^2) gravel or (and) the mean annual MOB assimilation of the caddis larvae correlated with annual production (dry mass g m^{-2} yr^{-1}) (Table 4.3).

The key methods and aims of this chapter are summarised in Figure 4.2.

Table 4.1 Example calculation of annual production for *A. fuscipes,* using the size-frequency method applied to data for the Lambourn from November 2011 to October 2012. The density column is the mean value from samples taken throughout the year. P = Production; B = Biomass; Mean W = mean individual mass of each instar. [a] Negative values at the top of the table (right column) are not included since these are probably artifacts caused by under sampling or rapid growth through the first two instars. If negative values were found beneath a positive value these were included in the summation for Production. [b] Final 'mass at loss' is equal to the individual mass of the largest instar. [c] A cohort production interval has been applied to account for a 10 month period for larval growth (a shorter) life cycle. [d] Proportion of carbon in the larvae (a constant). [e] Uncorrected annual carbon production for a 12 month life cycle [f] Annual carbon production accounting for a 10 month CPI.

Instar	Density (No./m^2) N	Individual mass (mg) W	No. lost (No./m^2) N	Biomass (mg/m^2) N x W	Mass at loss (mg) mean W = $(W_1 + W_2)/2$	Biomass lost (mg/m^2) mean WΔN	Times no. size classes mean WΔN x 7
I	3556.7	0.005		16.2			
			-585.4		0.006	-3.7	(-25.8)[a]
II	4142.2	0.008		33.2			
			-3121.5		0.011	-34.3	(-240.2)[a]
III	7263.6	0.014		101.4			
			6444.2		0.021	136.3	953.9
IV	819.5	0.028		23.2			
			222.2		0.039	8.6	60.5
V	597.3	0.049		29.6			
			114.8		0.085	9.8	68.7
VI	482.5	0.121		58.6			
			292.0		0.234	68.4	479.0
VII	190.5	0.347		66.1			
			190.5[b]		0.347	66.1	462.8
			Biomass (ug)	328.3		Production (ug)	2024.9
			Biomass (g)	0.3		Production (g)	2.0
						Corrected production (g) (Prod. X 12/10)	2.4[c]
			Cohort P/B	6.2		Min carbon production (g)	2.0 * 0.48[d] = 0.96[e]
						Max carbon production (g)	2.4 * 0.48 = 1.15[f]

Table 4.2 Example calculation of the mean annual proportion of biofilm and MOB in the diet of *Agapetus fuscipes* in the Lambourn using mixing models. MOB are assumed to have a $\delta^{13}C$ value of -45‰, based on measurements of the $\delta^{13}C$ of methane gas taken from stream water (Ings and Shelley, unpubl. data). Fractionation was taken to be between 16 – 0‰ based on (Summons, Jahnke & Roksandic 1994). *A trophic fractionation factor of 0.4‰ (McCutchan *et al.* 2003) has been applied to caddis larval values (caddis $\delta^{13}C$ – 0.4‰).

	Sample	Source 1	Source 2a MOB	Source 2b MOB	**Mean annual proportion of biofilm in larval diet**		**Mean annual proportion of MOB in larval diet**	
					Mixing model A (w/o fract.)	Mixing model B (w fract.)		
Month	Mean larval $\delta^{13}C$ (‰)*	Mean biofilm $\delta^{13}C$ (‰)	$\delta^{13}C$ 0‰	$\delta^{13}C$ 16‰	$(\delta_{SAMPLE} - \delta_{SOURCE\ 2a}) / (\delta_{SOURCE\ 1} - \delta_{SOURCE\ 2a})$	$(\delta_{SAMPLE} - \delta_{SOURCE\ 2b}) / (\delta_{SOURCE\ 1} - \delta_{SOURCE\ 2b})$	Mixing model A (w/o fract.)	Mixing model B (w fract.)
Nov	-38.61	-33.20			(-38.61 - -45) / (-33.20 - -45) = **0.54**	(-38.61 - -61) / (-33.20 - -61) = **0.81**	0.46	0.19
Feb	-38.88	-32.56			(-38.88 - -45) / (-32.56 - -45) = **0.49**	(-38.88 - -61) / (-32.56 - -61) = **0.78**	0.51	0.22
Apr	-37.49	-32.81	**-45**	**-61**	(-37.49 - -45) / (-32.81 - -45) = **0.62**	(-37.49 - -61) / (-32.81 - -61) = **0.83**	0.38	0.17
May	-37.58	-31.21			(-37.58 - -45) / (-31.21 - -45) = **0.54**	(-37.58 - -61) / (-31.21 - -61) = **0.79**	0.46	0.21
Jul	-36.64	-32.88			(-36.64 - -45) / (-32.88 - -45) = **0.69**	(-36.64 - -61) / (-32.88 - -61) = **0.87**	0.31	0.13
Sep	-40.19	-33.55			(-40.19 - -45) / (-33.55 - -45) = **0.42**	(-40.19 - -61) / (-33.55 - -61) = **0.76**	0.58	0.24
Average	-38.23	-32.70			**0.55**	**0.80**	**0.45**	**0.20**

Table 4.3 Linear regression models for chlorophyll *a* content and percentage MOB assimilated as explanatory variables for caddis larvae annual production (dry mass g m^2 yr^{-1}).

Model	CPI (months)	Linear regressions
		A. fuscipes
M1	10	annual production (dry mass) ~ mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
M2	12	annual production (dry mass) ~ mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
		***Silo* spp. (*S. nigricornis* and *S. pallipes* combined)**
M3	12	annual production (dry mass) ~ mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
M4	18	annual production (dry mass) ~ mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
		A. fuscipes
M5	10	annual production (dry mass) ~ mean annual MOB assimilation (%) (0‰ fract.)
M6	12	annual production (dry mass) ~ mean annual MOB assimilation (%) (16‰ fract.)
		***Silo* spp. (*S. nigricornis* and *S. pallipes* combined)**
M7	12	annual production (dry mass) ~ mean annual MOB assimilation (%) (0‰ fract.)
M8	18	annual production (dry mass) ~ mean annual MOB assimilation (%) (16‰ fract.)
		A. fuscipes
M9	10	annual production (dry mass) ~ mean annual MOB assimilation (%) (0‰ fract.) * mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
M10	12	annual production (dry mass) ~ mean annual MOB assimilation (%) (16‰ fract.) * mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
		Silo* spp. *(S. nigricornis and S. pallipes combined)
M11	12	annual production (dry mass) ~ mean annual MOB assimilation (%) (0‰ fract.) * mean annual chlorophyll content of biofilm on stream gravel (ug/m^2)
M12	18	annual production (dry mass) ~ mean annual MOB assimilation (%) (16‰ fract.) * mean annual chlorophyll content of gravel biofilm on stream gravel (ug/m^2)

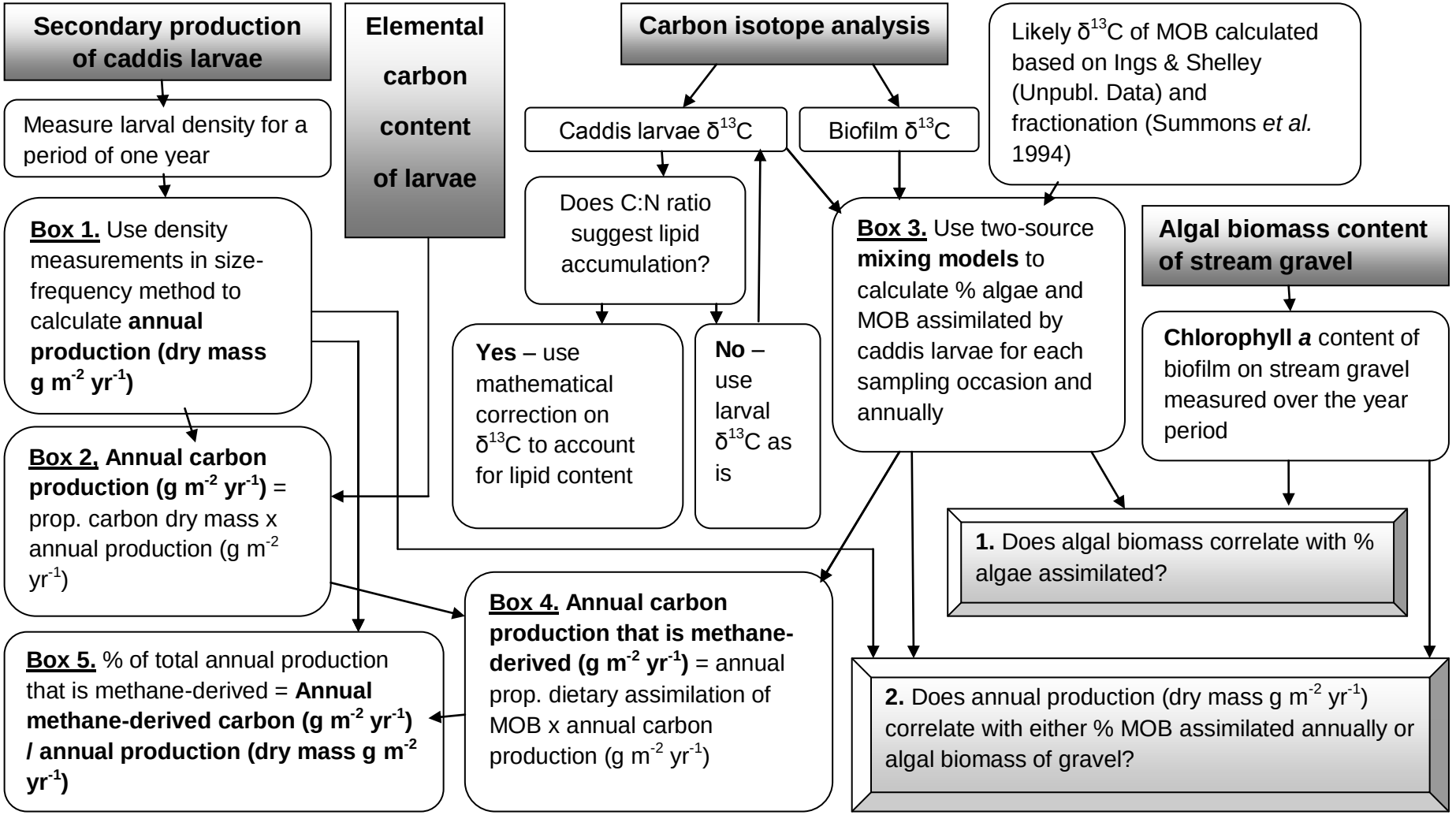

Figure 4.2 Summary of measurements and calculations

4.3 Results

4.3.1 Chlorophyll a content of gravel

Streams contained a range of chlorophyll *a* in gravel biofilm over the year ranging from 60.7 – 7723.0 ug/m^2 (Figure 4.3). The Cray contained by far the highest mean annual concentration of chlorophyll *a* (5313.3 ug/m^2) and Crowborough Warren contained the lowest mean annual concentration of chlorophyll *a* (207.0 ug/m^2).

4.3.2 C:N ratio of biofilm

The mean annual C:N ratio of the biofilm in the eight streams ranged from 5.48 ± 1.06 (mean ± 1 SD) in Crowborough Warren to 8.12 ± 1.27 (mean ± 1 SD) in Traeth Mawr.

Table 4.4 Mean annual biofilm C:N ratios for the eight streams. The means were calculated from the monthly C:N ratios(Nov, Feb, Apr, May, Jul and Sep).

Stream	Mean C:N ratio	St dev	St error	N
Craig Cerrig Gleisiad	6.73	1.05	0.43	6
Cray	7.02	1.27	0.57	5
Crowborough	5.48	1.06	0.47	5
Darent	6.25	1.23	0.50	6
Lambourn	6.89	0.54	0.22	6
Tadnoll	5.82	1.05	0.43	6
Traeth Mawr	8.12	1.27	0.52	6
Upper Tawe	6.16	0.82	0.36	5

4.3.3 C:N ratio of caddis

Mean annual molar C:N ratios for the three species of caddis larvae ranged between 4.37 ± 0.09 and 5.61 ± 0.16 (Table 4.5) and thus do not suggest lipid accumulation: thus caddis larval $\delta^{13}C$ values were not lipid corrected for further calculations.

Table 4.5 Mean annual molar carbon to nitrogen ratio for *A. fuscipes*, *S. nigricornis* and *S. pallipes* for the eight streams ± 1 SE. N is the number of samples contributing to the mean.

	A. fuscipes		***S. nigricornis***		***S. pallipes***	
Stream	C:N ratio	N	C:N ratio	N	C:N ratio	N
Craig Cerrig Gleisiad	5.04 ± 0.13	35			4.74 ± 0.26	6
Cray	5.43 ± 0.08	66				
Crowborough Warren	5.44 ± 0.21	17			5.34 ± 0.41	13
Darent	5.00 ± 0.18	26			4.66 ± 0.13	18
Lambourn	5.01 ± 0.10	56	4.97 ± 0.12	40		
Tadnoll	4.82 ± 0.14	30	4.91 ± 0.09	50		
Traeth Mawr	5.61 ± 0.16	47				
Upper Tawe	4.80 ± 0.10	51			4.37 ± 0.09	17

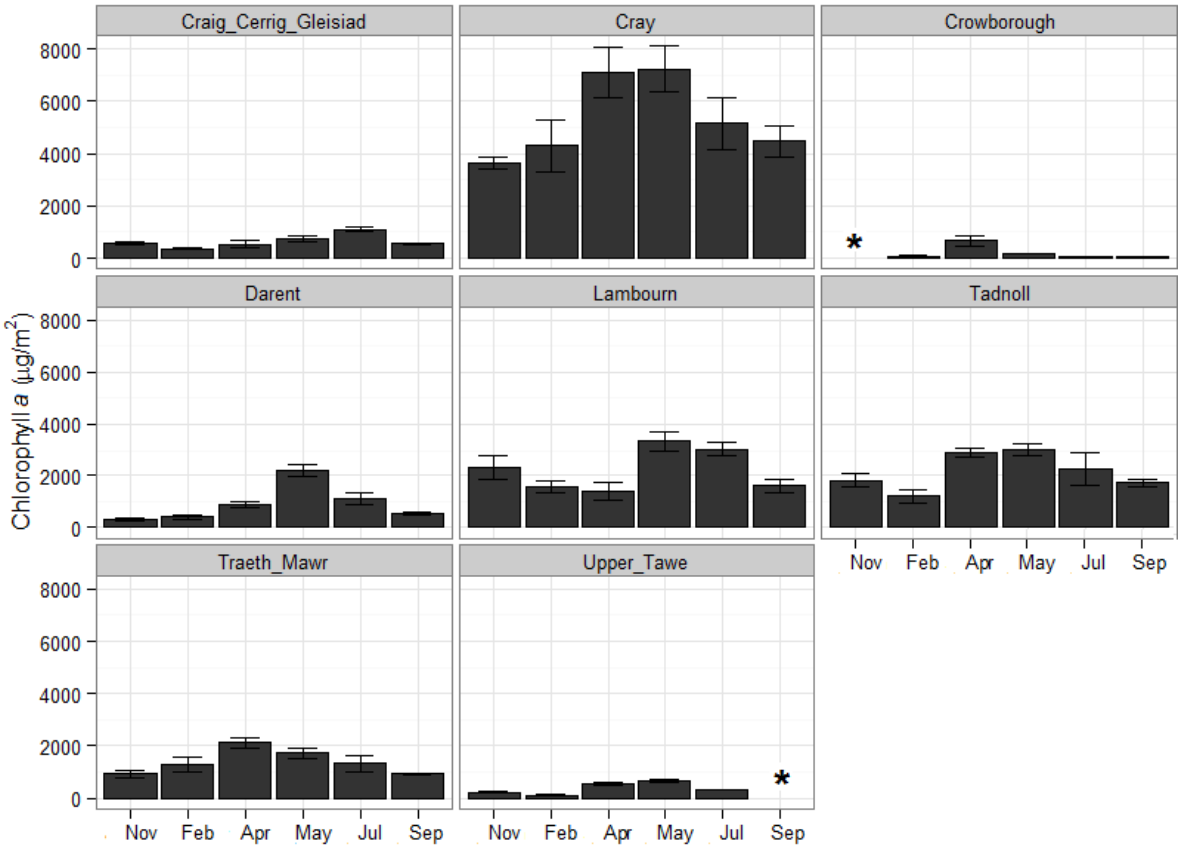

Figure 4.3 Chlorophyll *a* content of biofilm per m^2 of river bed for the eight streams from November 2011 to October 2012 ± 1 SE. * represents where there are no data.

4.3.4 Percentage of carbon in biomass

The dry mass of *A. fuscipes* was found to contain 48.0 ± 0.6% of carbon, with *S. nigricornis* and *S. pallipes* containing 46.1 ± 0.6% and 46.4 ± 0.8% respectively.

4.3.5 Larval densities

A wide range of densities of *A. fuscipes*, *S. nigricornis* and *S. pallipes* were observed over the eight streams and six sampling occasions (Figure 4.4 and Figure 4.5). *Agapetus fuscipes* was generally much more numerous than *S. nigricornis* and *S. pallipes*. The Cray, the Lambourn and Upper Tawe contained very high mean annual densities of *A. fuscipes* with 5070, 3301 and 2613 individuals per m^2, respectively. *Silo nigricornis* had the highest mean annual density in the Tadnoll with 652 larvae per m^2.

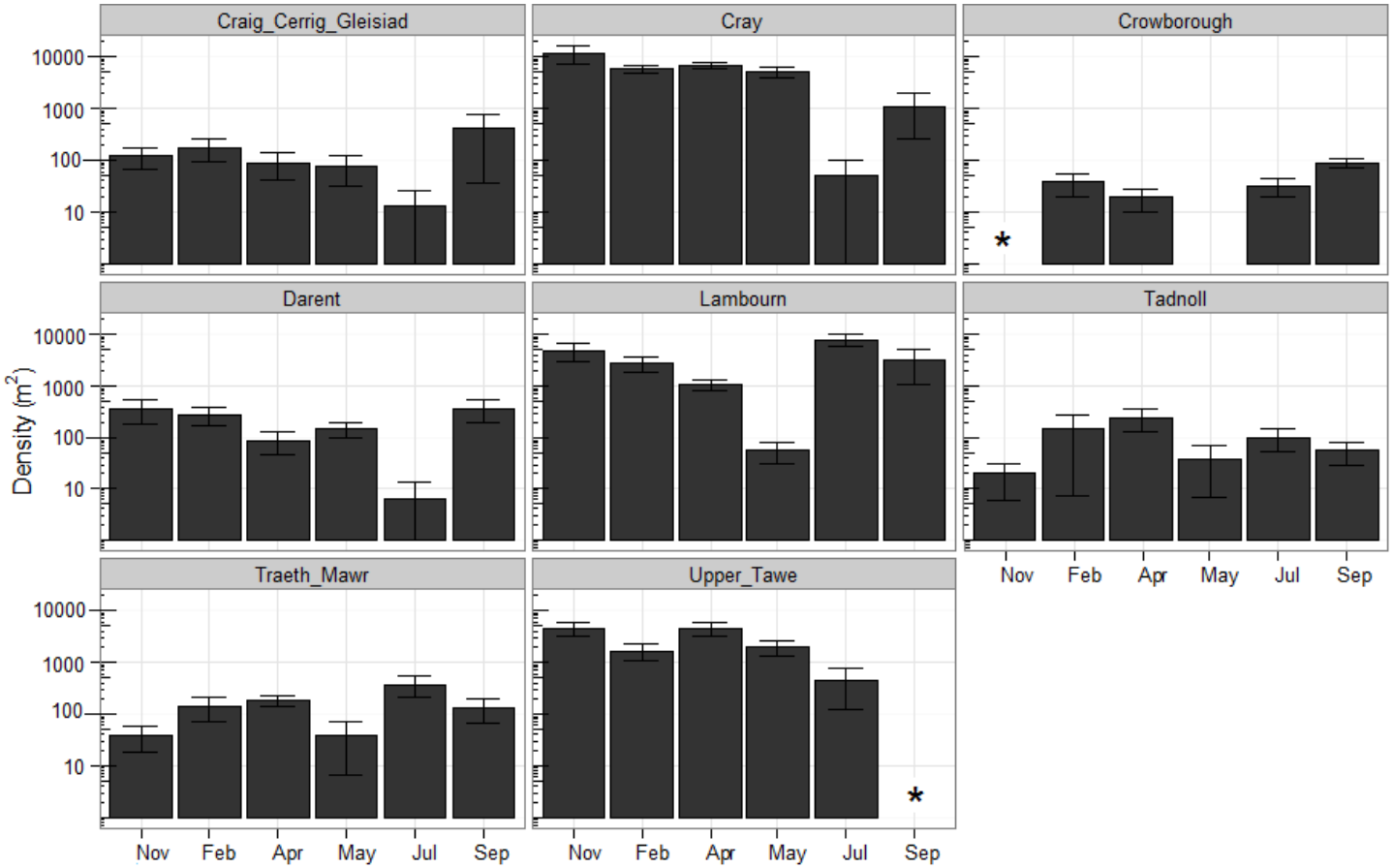

Figure 4.4 Mean density of *A. fuscipes* per m^2 for the eight streams from November 2011 to October 2012 ± 1 SE. *Indicates no data.

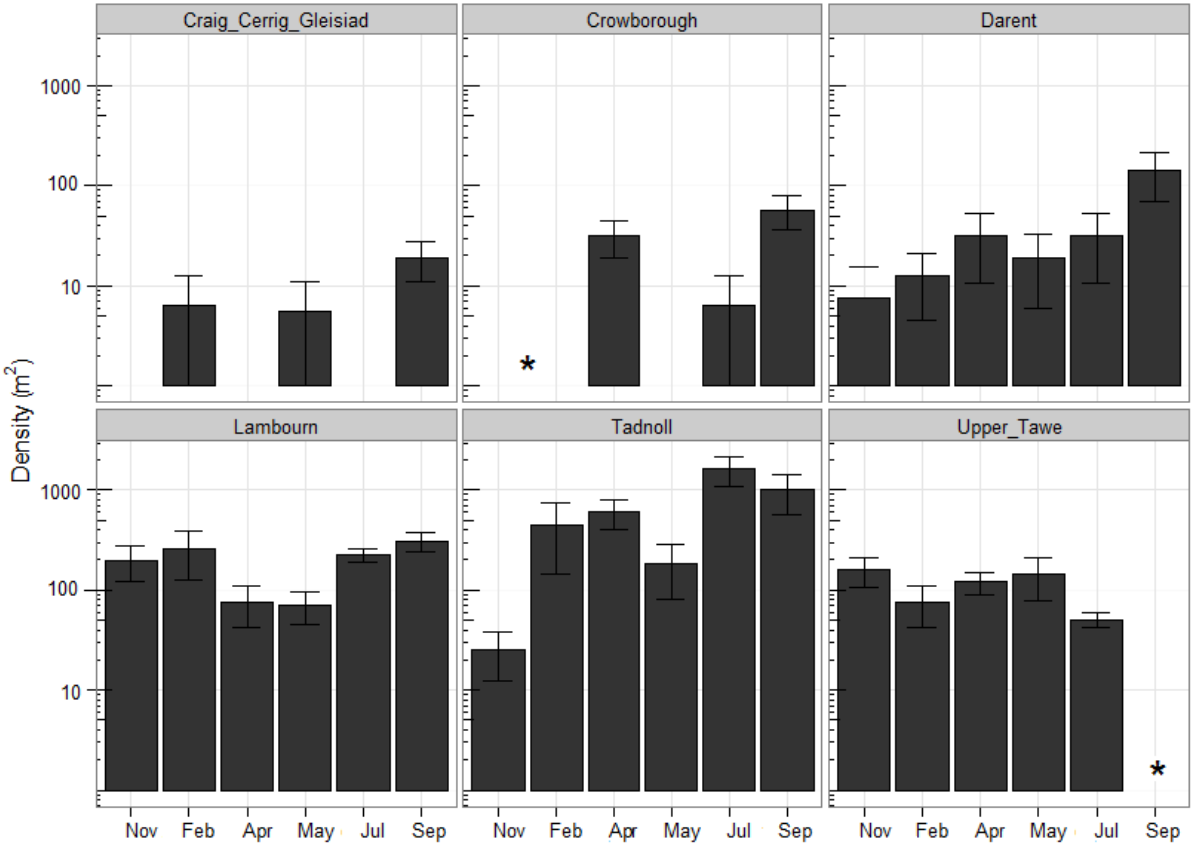

Figure 4.5 Mean density of *S. nigricornis* (Lambourn and Tadnoll) and *S. pallipes* (Craig Cerrig Gleisiad, Crowborough Warren, the Darent and Upper Tawe) from November 2011 to October 2012 ± 1 SE. *Indicates no data.

4.3.6 Stable isotope analysis

All three species of caddis larvae were consistently ^{13}C-depleted relative to stream epilithon for all of the eight streams. *Agapetus fuscipes* in Craig Cerrig Gleisiad was the least ^{13}C-depleted annually relative to the epilithon (-0.86‰), whereas for Traeth Mawr *A. fuscipes* was the most ^{13}C-depleted annually relative to the epilithon (-11.84‰). *Silo* spp. were also consistently ^{13}C-depleted annually relative to the epilithon (-6.10 to -4.05‰).

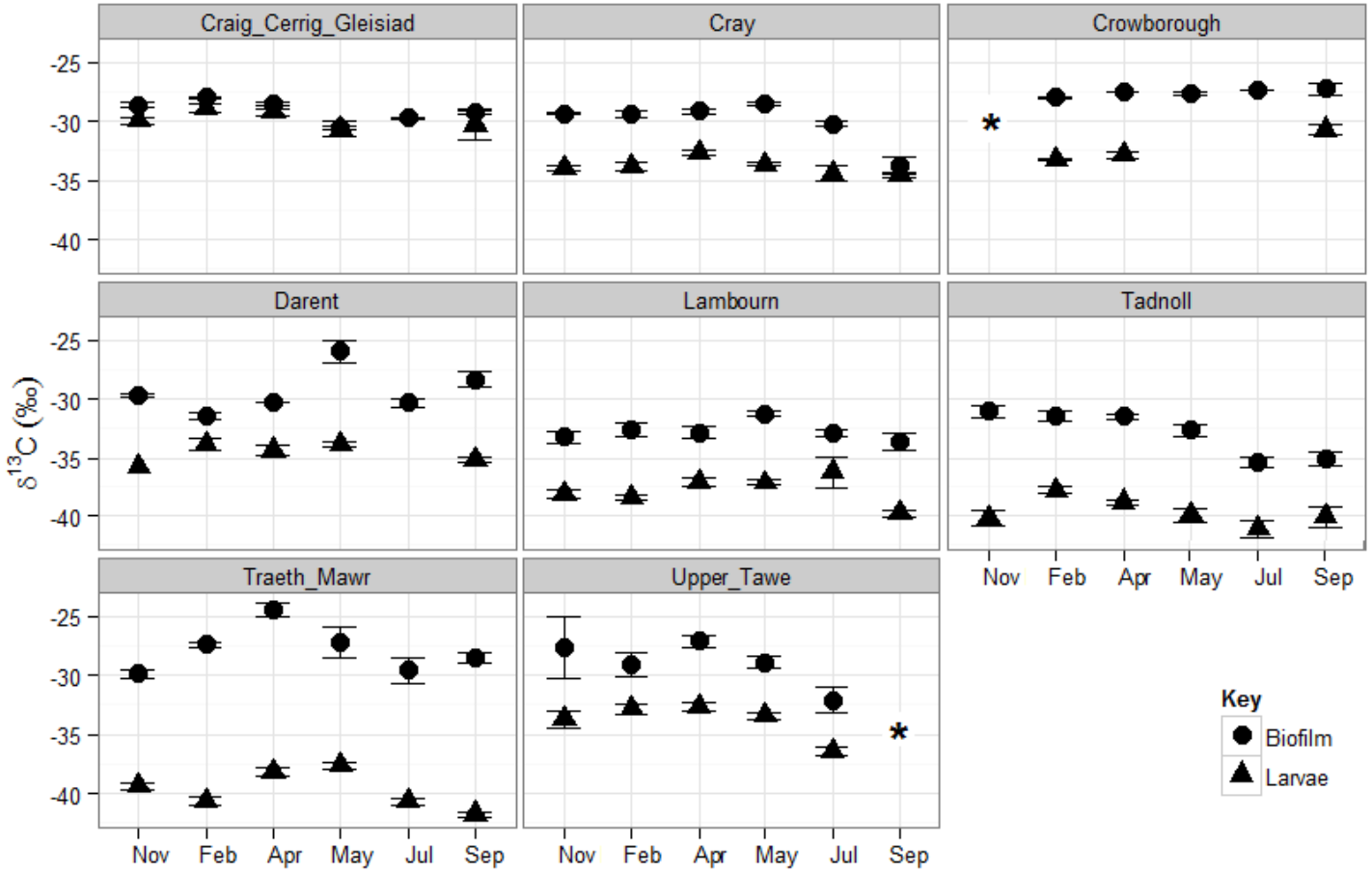

Figure 4.6 Mean $\delta^{13}C \pm 1$ SE for *A. fuscipes* and biofilm for the eight streams from November 2011 to September 2012. *Indicates no data. Some monthly larval $\delta^{13}C$ values are missing where there were too few caddis larvae for isotope analysis.

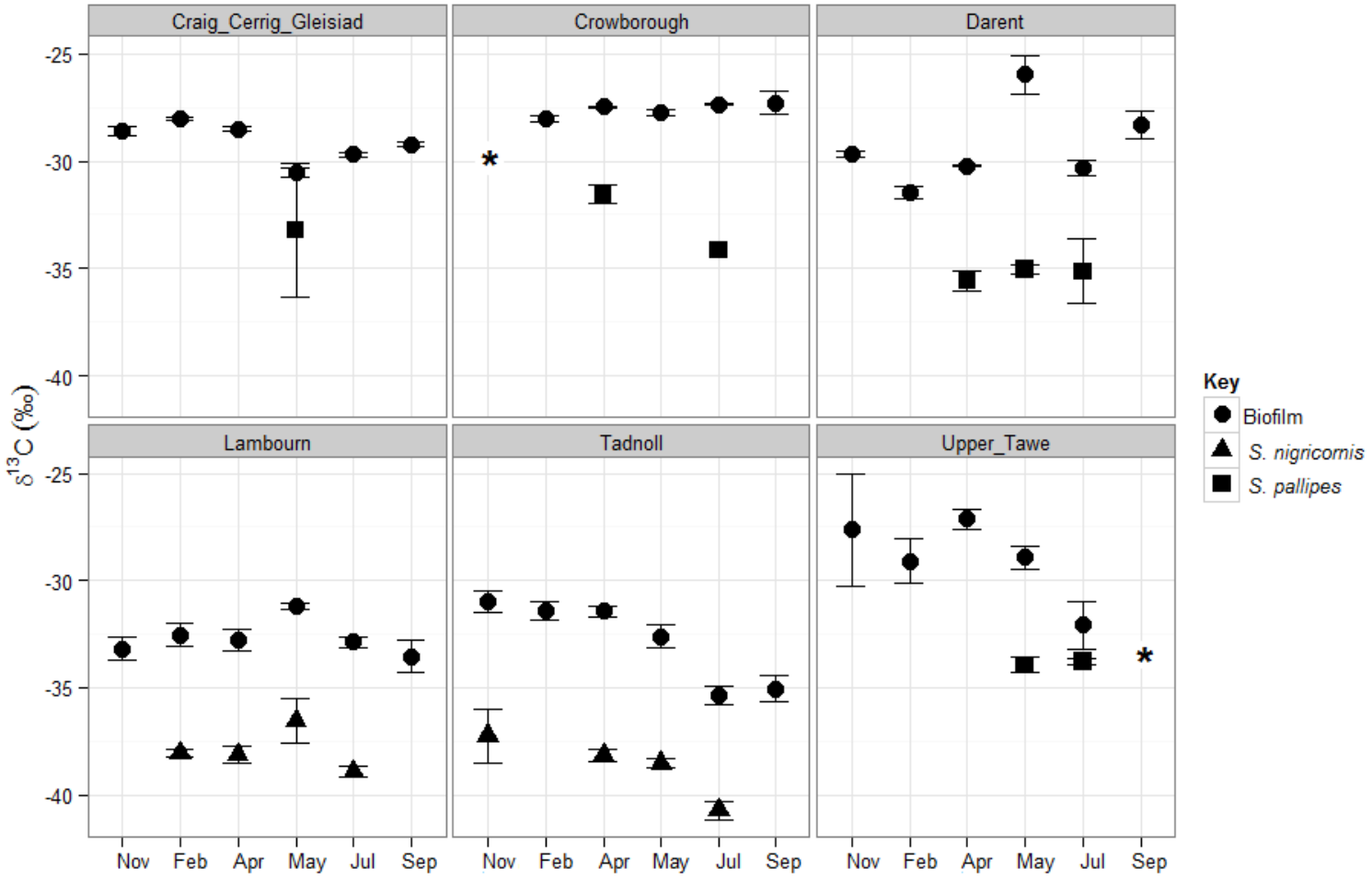

Figure 4.7 Mean $\delta^{13}C \pm 1$ SE for *S. nigricornis* (Lambourn and Tadnoll), *S. pallipes* (Craig Cerrig Gleisiad, Crowborough Warren, Darent and Upper Tawe) and biofilm from November 2011 to October 2012. *Indicates no data. Some monthly larval $\delta^{13}C$ values are missing where there were too few caddis larvae for isotope analysis.

4.3.7 Annual production

Annual production estimates for *A. fuscipes* for the eight streams, applying two CPI intervals of 10 and 12 months, ranged between 0.13 - 12.13 g^{-2} yr^{-1} (Table 4.6). Annual production estimates for *S. nigricornis* in the Lambourn and Tadnoll, applying two CPI intervals of 12 and 18 months, ranged from 0.46 – 7.51 g^{-2} yr^{-1}. For *S. pallipes*, with CPI intervals of 12 and 18 months, annual production ranged from 0.05 – 0.67 g^{-2} yr^{-1} in the four streams where it was present.

4.3.8 Percentage of methane-derived carbon assimilated

Mixing models showed that *A. fuscipes* in Traeth Mawr was likely to incorporate the highest mean methane-derived carbon of the eight streams annually (36.9 to 71.4%). *Agapetus fuscipes* in Craig Cerrig Gleisiad incorporated the least mean methane-derived carbon of the eight streams annually (3.5 to 7%). *Silo* spp. incorporated between 13.8 to 53.4% methane-derived carbon (Figure 4.8 and Figure 4.9).

4.3.9 Percentage contribution of methane-derived carbon to annual production

Methane-derived carbon was calculated to contribute between 1.7 to 3.3% to the total annual production (g m^{-2} yr^{-1}) of *A. fuscipes* in Craig Cerrig Gleisiad. This was the lowest amount calculated and for *A.*

fuscipes in the other seven streams methane-derived carbon contributed between 6.5 to 33.1% to the total annual production (g m^{-2} yr^{-1}) of the species. Similarly, for *S. nigricornis* and *S. pallipes*, methane-derived carbon was calculated to contribute between 8.5 to 24.6 and 7.0 to 17.7% to total annual production, respectively, in the eight streams (Table 4.8).

Table 4.6 A summary of biomass and production (dry mass) values for *A. fuscipes*, *S. nigricornis* and *S. pallipes* for the eight streams between November 2011 and October 2012. Production could not be calculated for *S. pallipes* for Craig Cerrig Gleisiad because of low numbers of larvae. Corrected production (B) is based on a CPI of 10 months for *A. fuscipes* and 18 months for both *S. nigricornis* and *S. pallipes*.

Stream	**A** Annual production g m^{-2} yr^{-1}	**B** Corrected production g m^{-2} yr^{-1}	**C** Biomass (g)	**D** Cohort P/B	**E** Cohort P/B corr.	**F** Min carbon Production g m^{-2} yr^{-1}	**G** Max carbon production g m^{-2} yr^{-1}	*Annual mean dietary carbon contribution from MOB (%)*		*Annual methane-derived carbon production g m^{-2} yr^{-1}*	
								H Min (16‰ fract.)	**I** Max (-0‰ fract.)	Min (= **F * H**)	Max (= **G * I**)
A. fuscipes											
Craig Cerrig Gleisiad	0.41	0.49	0.10	4.10	4.92	0.20	0.24	3.5	7.0	0.01	0.02
Cray	10.11	12.13	2.11	4.79	5.75	4.85	5.82	13.5	28.0	0.66	1.63
Crowborough Warren	0.13	0.16	0.03	4.33	5.20	0.06	0.07	15.2	29.2	0.01	0.02
Darent	0.45	0.54	0.10	4.50	5.40	0.22	0.26	17.9	36.2	0.04	0.09
Lambourn	2.02	2.42	0.33	6.12	7.35	0.97	1.16	19.5	45.0	0.19	0.52
Tadnoll	0.38	0.46	0.12	3.17	3.80	0.18	0.22	25.7	59.5	0.05	0.13
Traeth Mawr	0.70	0.84	0.19	3.68	4.42	0.34	0.40	36.9	71.4	0.12	0.29
Upper Tawe	6.90	8.28	1.66	4.16	4.99	3.31	3.97	16.4	32.8	0.54	1.30
S. nigricornis											
Lambourn	0.69	0.46	0.62	1.11	0.74	0.21	0.32	18.4	42.3	0.04	0.13
Tadnoll	7.51	5.01	2.03	3.70	2.47	2.30	3.45	23.1	53.4	0.53	1.85
S. pallipes											
Craig Cerrig Gleisiad	-	-	0.02	-	-	-	-	13.8	27.7	-	-
Crowborough Warren	0.07	0.05	0.03	2.33	1.56	0.02	0.03	15.2	29.2	<0.01	0.01
Darent	0.36	0.24	0.12	3.00	2.00	0.11	0.17	19.1	38.6	0.02	0.06
Upper Tawe	0.67	0.45	0.15	4.47	2.98	0.21	0.31	16.4	32.9	0.03	0.10

Table 4.7 Summary of previous life history and secondary production studies for Trichopteran families Glossosomatidae and Goeridae.

Reference	Species	Larval instars	Voltinism (CPI)	Annual Production (g m-2 yr-1)	Habitat	Location
Glossosomatidae						
Benedetto Castro (1975)	*Agapetus fuscipes*	8	Univoltine	0.62	Spring	Denmark
Iversen (1976, 1988)	*Agapetus fuscipes*	7	Univoltine (1 year)	0.08	Spring	Denmark
Marchant & Hehir (1999)	*Agapetus monticolus*	5	Univoltine (10 months)	0.04	Stream	SE Australia
Neves (1979)	*Agapetus pinatus*	n/a	n/a	0.001 - 0.003	Stream	NE USA
Marchant & Hehir (1999)	*Agapetus pontona*	5	Univoltine (5-6 months)	0.10	Stream	SE Australia
Alvarez & Pardo (2005)	*Agapetus quadratus*	5	Trivoltine (4 months)	4.80	Temporary Stream	E Spain
Georgian & Wallace (1983)	*Agapetus* sp.	5	Univoltine (44 days)	0.02	Stream	E USA
Tod & Schmid-Araya (2009)	*Agapetus* sp.	n/a	340.5 - 365 days	1.03 - 2.02	Lambourn Stream	SE England
Krueger & Waters (1983)	*Glossosoma intermedium*	n/a	Multivoltine	5.8	Stream	N USA
Jin & Ward (2007)	*Glossosoma nigrior*	5	Trivoltine (120 days)	0.04 - 0.25	Stream	SE USA
Jin & Ward (2007)	*Glossosoma nigrior*	5	Trivoltine (120 days)	0.46 - 1.23	Stream	SE USA
Neves (1979)	*Glossosoma nigrior*	n/a	n/a	0.30	Stream	NE USA
Georgian & Wallace (1983)	*Glossosoma nigrior*	5	Bivoltine (152 days - Winter)	0.11	Stream	E USA
Georgian & Wallace (1983)	*Glossosoma nigrior*	5	Bivoltine (192 days - Summer)	0.50	Stream	E USA
Robinson & Minshall (1998)	*Glossosoma nigrior*	5	Bivoltine	0.67	Stream	N USA
Robinson & Minshall (1998)	*Glossosoma nigrior*	5	Bivoltine	0.07	Stream	N USA
Krueger & Waters (1983)	*Glossosoma* sp.	n/a	Univoltine	0.2	Stream	N USA
Krueger & Waters (1983)	*Glossosoma* sp.	n/a	Univoltine	1.3	Stream	N USA
Goeridae						
Georgian & Wallace (1983)	*Goera fuscula*	n/a	Univoltine (215 days)	0.009	Stream	E USA
Avlyush (2013)	*Goera tungusenis*	n/a	Univoltine	0.95	Stream	Mongolia
Tod & Schmid-Araya (2009)	*Silo* sp.	n/a	340.5 days	0.24 - 0.54	Lambourn Stream	SE England

Table 4.8 Methane-derived carbon contribution (%) to total annual production in **dry mass** g m^{-2} yr^{-1} (total annual production in dry mass constitutes carbon + all other elements).

Stream	min %	max %
	A. fuscipes	
Craig Cerrig Gleisiad	1.7	3.3
Cray	6.5	15.5
Crowborough Warren	7.3	14.0
Darent	8.6	17.4
Lambourn	9.4	21.6
Tadnoll	12.3	28.6
Traeth Mawr	17.7	34.3
Upper Tawe	7.9	15.7
	S. nigricornis	
Lambourn	8.5	19.4
Tadnoll	10.6	24.6
	S.pallipes	
Crowborough	7.0	13.4
Darent	8.8	17.7
Upper Tawe	7.6	15.1

4.3.10 Contribution of methane-derived carbon to annual production

The contribution of methane-derived carbon to annual production for *A. fuscipes* was highest in the Cray, Upper Tawe and the Lambourn, ranging between 0.19 – 1.63 g m^2 yr^{-1}. For *S. nigricornis* the contribution of methane-derived carbon to annual production was highest in the Tadnoll ranging from 0.53 – 1.85 g m^{-2} yr^{-1} (Table 4.6 and Figure 4.10).

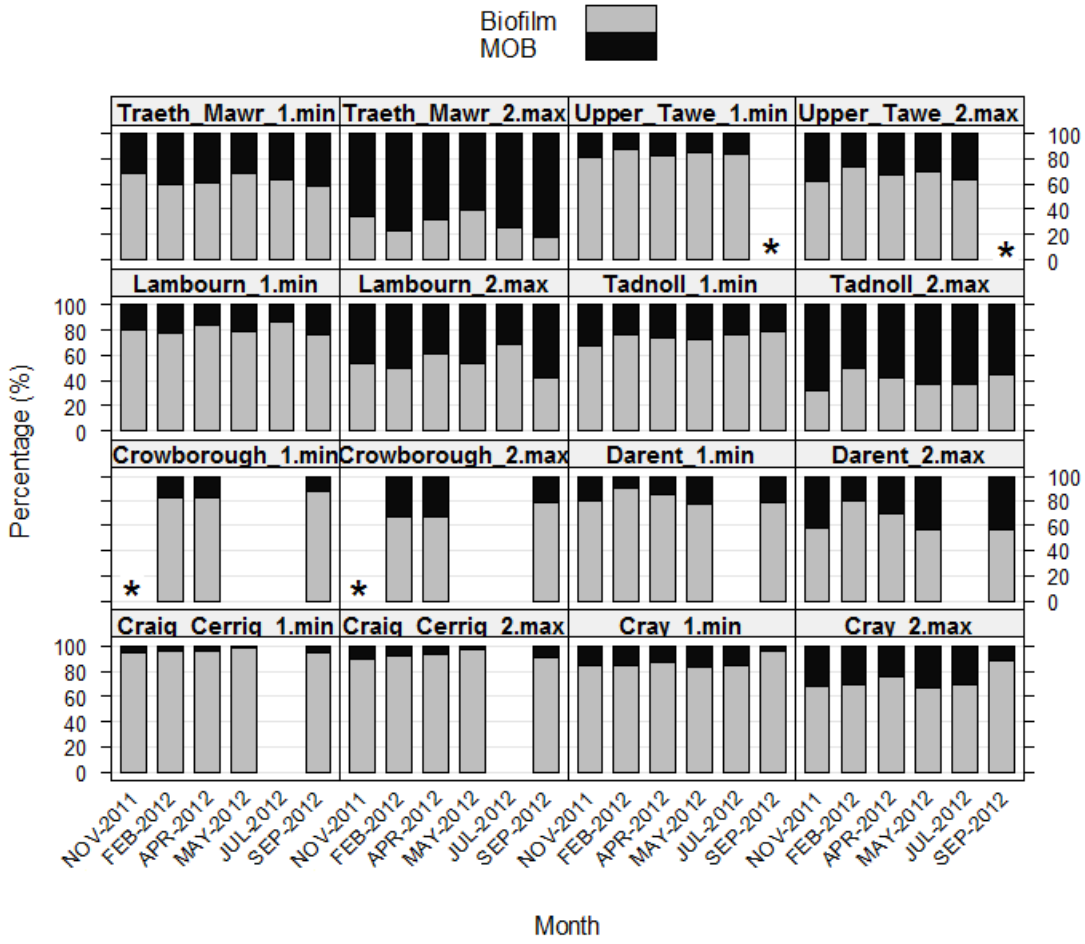

Figure 4.8 Potential MOB and biofilm % in *A. fuscipes* biomass from November 2011 to October 2012. Two scenarios are presented for each stream with the minimum amount of MOB dietary input based on a fractionation scenario of 16‰ from a starting value of -45‰. The maximum scenario is based on 0‰ fractionation. *represents where there is no data due to stream flood and drought events. Empty bars exist where there were too few caddis found for isotope analysis and further calculation of MOB assimilation by caddis.

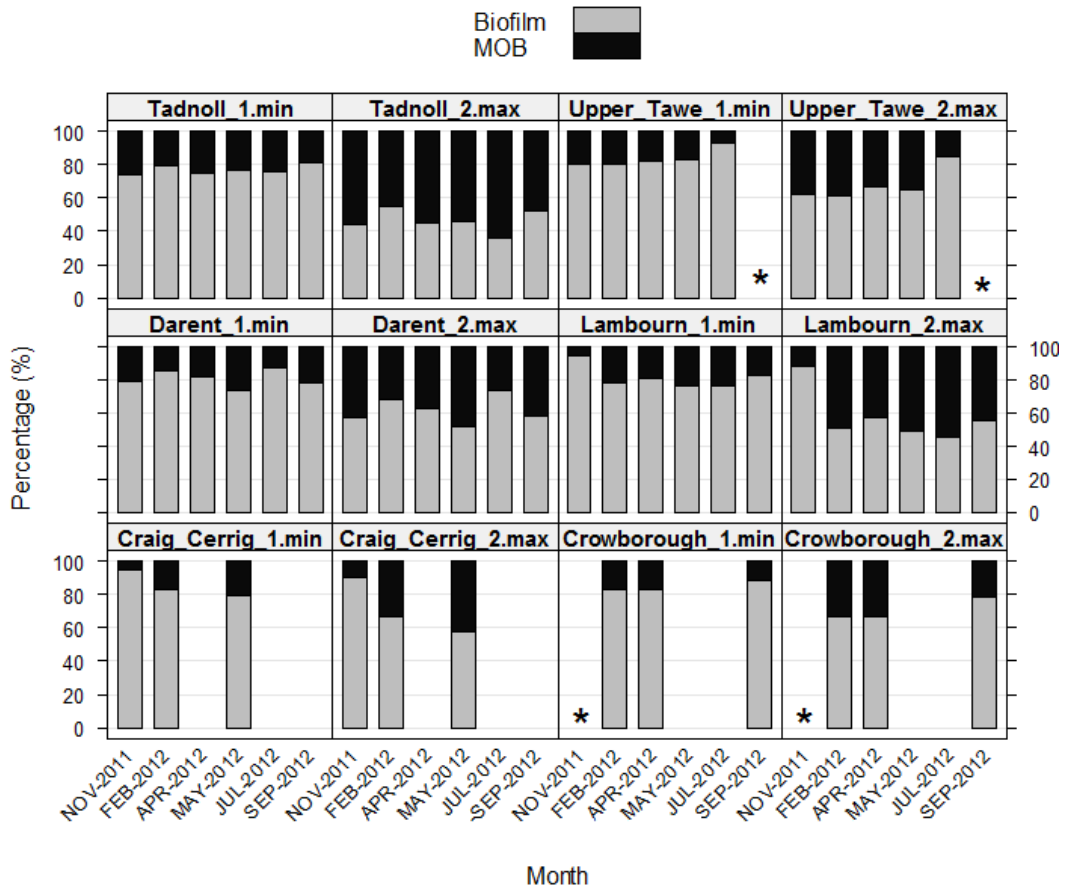

Figure 4.9 Potential MOB and biofilm % in *S. nigricornis* and *S. pallipes* biomass from November 2011 to October 2012. Two scenarios are presented for each stream with the minimum amount of MOB dietary input based on a fractionation scenario of 16‰ from a starting value of -45‰. The maximum scenario is based on 0‰ fractionation. *represents where there is no data due to stream flood and drought events. Empty bars exist where there were too few caddis found for isotope analysis and further calculation of MOB assimilation by caddis.

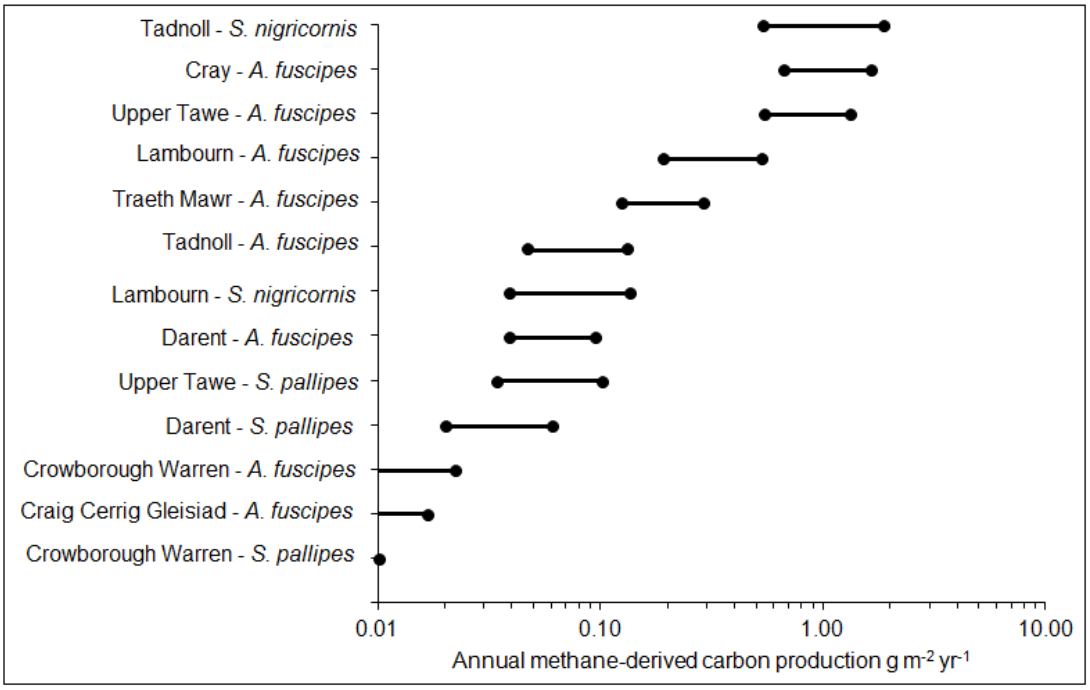

Figure 4.10 Range of methane-derived carbon incorporated into dry mass (g m^{-2} yr^{-1}) for each stream and caddis fly species, ranked by the maximum amount incorporated.

4.3.11 Chlorophyll a content and algal assimilation by caddis larvae

There was no significant relationship between the chlorophyll *a* content of the gravel and the percentage of algae assimilated by the caddis larvae (for either MOB fractionation scenario of 16‰ or 0‰). Month was not found to impact the percentage assimilation of algae by the larvae. Stream significantly impacted the percentage of algae assimilated by the caddis larvae ($p<0.001$, $F_{8,33} = 28.11$) and ($p<0.001$, $F_{8,33} = 33$), respectively.

4.3.12 Total annual production and its support by methane-derived carbon

There was no significant relationship between the total annual production (g m^{-2} yr^{-}) of the caddis larvae and the percentage of MOB or chlorophyll content incorporated under the two fractionation scenarios of 16‰ and 0‰ for either *A. fuscipes* or *Silo* spp. (*S. nigricornis* and *S. pallipes* combined) and there was also no significant relationship between the chlorophyll *a* content of the gravel and annual production (g m^{-2} yr^{-}) for either *Agapetus fuscipes* or *Silo* spp. combined.

4.4 Discussion

4.4.1 Annual production of caddis

Annual production of *A. fuscipes* ranged between 0.13 – 12.13 dry mass g m^{-2} yr^{-1} in the eight streams. Six of these streams were within the range previously calculated for species of the genus *Agapetus* with values of annual production ranging between 0.001 – 4.80 g m^{-2} yr^{-1} (Benedetto Castro 1975; Marchant & Hehir 1999; Alvarez & Pardo 2005; Tod & Schmid-Araya 2009). Two of the streams, Upper Tawe and the Cray, had much higher annual production than previously reported, with estimates ranging from 6.90 – 8.28 and 10.11 – 12.13 dry mass g m^{-2} yr^{-1}, respectively. The previous highest estimate for an *Agapetus* sp. was by Alvarez & Pardo (2005) who reported annual production of 4.8 g m^{-2} yr^{-1} for *Agapetus quadratus*, a trivoltine species in a temporary stream in Majorca. Alvarez & Pardo (2005) attributed the high annual production to the high stream temperatures, the stream being 'predator-free', food not being a limiting resource and the absence of other competing grazers. Similarly an annual production of 5.8 g m^{-2} yr^{-1} was reported for *Glossosoma nigrior* which was multivoltine in an Alabama stream in the north USA. This estimate is currently the largest estimate of annual production for a glossosomatid caddis species in published literature. Estimates for other univoltine glossosomatid species worldwide are lower and range from 0.001 to 2.02 g m^{-2} yr^{-1} (Benedetto Castro 1975; Iversen 1976, 1988; Neves 1979; Georgian & Wallace 1983; Krueger & Waters 1983; Marchant & Hehir 1999; Tod & Schmid-Araya

2009). Multivoltine species tend to exist in places where temperatures are warmer for longer and thus accrue biomass quickly and reach adulthood in a shorter space of time (Hauer & Benke 1987; Alvarez & Pardo 2005).

For two streams (the Darent and Traeth Mawr) no individuals corresponding to the instar 1 size range for *A. fuscipes* were found and in other streams (Craig Cerrig Gleisiad, the Cray, the Tadnoll and Upper Tawe) very few individual instar 1 larvae were found. This means that annual production estimates for these streams will have been underestimated as the size frequency method calculates the sum of all size classes in its working (Benke & Huryn 2006). There are potential ways to correct for the undersampling of the smallest size classes. For example exponential mortality curves can be fitted to density estimates of the larger size classes to back-calculate the starting density of smaller size classes (Freeman & Freeman 1985; Morin *et al.* 1987). Waters & Crawford (1973) calculated the secondary production of the mayfly *Ephemerella subvaria* using various methods including the size-frequency method. They also constructed a “catch curve” extrapolation on their data to correct for the low number of individuals in the smaller size classes. They estimated that secondary production was likely to have been underestimated by 10 - 20%.

Mixing models indicated that the highest percentage range (19.5 to 71.4%) of methane-derived carbon incorporated annually by *A. fuscipes* was in the three streams: Traeth Mawr, the Tadnoll and the Lambourn. This is similar to

the findings of Jones *et al.* (2008), who found that profundal chironomids in lakes could obtain up to 70% of their larval carbon from methanotrophic bacteria. Despite methane-derived carbon being taken up by caddis in such high percentages in these streams, methane-derived carbon contributed the most to total annual carbon production in streams where overall annual production was the highest, such as the Upper Tawe and the Cray where carbon from methane contributed between 0.5 – 1.30 and 0.66 – 1.63 grams per m^2 per year, respectively.

Silo nigricornis and *S. pallipes* (in the six streams where they were present) assimilated a lower percentage of methane-derived carbon than observed for *A. fuscipes*, ranging between 7.0 – 24.6%. In the Tadnoll methane-derived carbon was estimated to contribute between 0.53 – 1.85 g m^2 yr^{-1} to annual production (g m^{-2} yr^{-1}). This is similar to the values observed for *A. fuscipes* in the Upper Tawe and the Cray. Previous estimates of annual production for *Silo* spp. range between 0.009 and 0.95 g m^2 yr^{-1} (Georgian & Wallace 1983; Tod & Schmid-Araya 2009; Avlyush 2013), so this estimate of methane-derived carbon alone that contributes to the total annual production of *S. nigricornis* is large in comparison.

The Cray had the highest algal biomass annually of the eight streams indicating that there may be plentiful algae available to support the caddis larvae in this stream. As with the temporary stream surveyed by Alvarez & Pardo (2005), there were few other competitors with grazing caddis larvae,

mayflies, snails and limpets, all either absent or scarce in Surber samples. Although water temperature was not measured continuously, there was some indication from spot temperatures taken on sampling occasions that the Cray reached high water temperatures, on one occasion being recorded at 23°C which was much higher than any of the temperatures recorded from the other streams (Chapter 3). As discussed in Chapter 3, water temperature can affect the growth rates of benthic invertebrates, with high temperatures increasing annual production (Benke 1993; Marchant & Hehir 1999).

The mean molar C:N ratio (4.37 ± 0.09 – 5.61 ± 0.16) of the three caddis species does not suggest that the $\delta^{13}C$ values are due to lipid accumulation. These values agree with the findings of Trimmer *et al.* (2009), who observed very similar C:N ratios for *A. fuscipes* and *S. nigricornis.* The elemental carbon content of *A. fuscipes* and the two *Silo* spp. was found to be 48 and 46%, respectively. The elemental carbon content of freshwater invertebrates is usually about 50% but can fall within a range from 35 – 57% (Cross *et al.* 2003; Back & King 2013). It is often assumed that macroinvertebrates maintain a homeostatic elemental composition of nitrogen, phosphorus and carbon throughout their life span in order to have an adequate supply for the demands of metabolism, growth and reproduction (Sterner & Hessen 1994) and that nutrient ratios are specific to species (Elser *et al.* 1996). A previous study by Back & King (2013) ascertained that for six of eight aquatic taxa, %C was invariant across ontogeny. Previous to these findings, Veldboom & Haro (2011) found differences in body tissue carbon content through ontogeny for the caddisfly species *Brachycentridae occidentalis*. Although it

is assumed in this study that %C is constant throughout the life history of the caddis, potential differences in %C throughout ontogeny could lead to the calculation of annual carbon production being under- or over-estimated. This error would thus be propagated in the further calculations assessing the proportion of carbon that is methane-derived.

For all of the streams there was evidence that MOB were consumed by caddis, with caddis larvae being ^{13}C-depleted relative to the biofilm in all of the eight streams throughout the course of the year. This agrees with the findings of Trimmer *et al.* (2009) and Tuffin (2014), who also found larvae of *A. fuscipes* and *S.nigricornis* to be frequently ^{13}C-depleted year round. However other studies have attributed ^{13}C-depletion of grazing organisms to other reasons. McNeely, Clinton & Erbe (2006) found that *Glossosoma* larvae in headwaters of the South Fork Eel River, northern California, were markedly lower in $\delta^{13}C$ than the epilithon by ≥5‰. They studied the gut contents of these larvae and concluded that these larvae were selectively feeding on algae, despite algae being scarce. The $\delta^{13}C$ of algae is variable depending on boundary layer dynamics and the $\delta^{13}C$ of the inorganic carbon supplied. If a thin boundary layer exists around algae, algae are not CO_2 limited and can discriminate against the heavier ^{13}C isotope becoming ^{13}C-depleted (Finlay 2001; Finlay, Khandwala & Power 2002; Trudeau & Rasmussen 2003). If larvae selectively feed on algal cells in preference to other biofilm components it is likely they will show ^{13}C-depletion relative to the biofilm. In order to confirm that caddis larvae are feeding on MOB, stable isotope data could be used in conjunction with the assessement of bacterial

biomarkers that are present in MOB e.g. phospholipid fatty acids (PLFA's) in the body tissues of the caddis larvae. Deines, Bodelier & Eller (2007) were able to confirm chironomid ingestion of MOB by using a combined approach using the addition of ^{13}C-labelled methane to incubated sediment with chironomids in an experimental set up and detecting phospholipid fatty acids diagnostic for MOB in chironomid larval tissue.

The mixing models had a key assumption that the caddis larvae did not consume any other dietary items such as leaf litter, moss or filamentous algae. Although leaf litter and moss were not included in any analysis in this chapter, they were sampled for their $\delta^{13}C$ values (see the Appendix). For some months, for some streams moss values were more $\delta^{13}C$-depleted than caddis larval values suggesting that moss cannot be ruled out as another potential food resource that the caddis larvae could be feeding on. Streams where moss is abundant and could be a potential food source include Craig Cerrig Gleisiad, the Tadnoll and Upper Tawe. Filamentous algae, epiphytic algae and macrophytes were not sampled so also cannot be discounted as potential food resources.

The two-source mixing model was based on data from six sampling points throughout the year from the eight streams, with streams being sampled roughly every two months. Whilst this was an adequate number of sampling points to calculate secondary production using the size-frequency method, isotope data used in the mixing models may not have incorporated the full

range of larval or biofilm $\delta^{13}C$ that exists year round in these streams. Another key disadvantage was that biofilm could not be separated for isotope analysis and is a bulk measure of all of the components contained within it. Assuming that all of the biofilm was algae is a large assumption in the mixing model and it is extremely unlikely that this was the case. Another large assumption of the model was that MOB ranged from $\delta^{13}C$ from -61 to -45‰ (under 0 - 16‰ fractionation) and was based on a previous assumption that they were similar to the $\delta^{13}C$ of stream water (-45‰) previously measured by Ings and Shelley (Unpubl. data). If MOB were consuming methane that was less or more depleted than this, the potential range of $\delta^{13}C$ values they could exist at may be different to the range included in the model.

Despite methane-derived carbon contributing to annual production for *A. fuscipes* and *S. nigricornis* in all of the streams, there was no relationship between streams with a high annual production in dry mass and the percentage of methane-derived carbon incorporated for either *A. fuscipes* or *Silo* spp. (combined data for *S. nigricornis* and *S. pallipes*). Although streams with high annual production do not correlate with those where a lot of methane-derived carbon is used it does not necessarily mean that those with a lower annual production would be as productive if there was no methane-derived carbon available to them.

The C:N ratios of the biofilm in the eight streams indicated that the biofilm in these streams was of high quality and relatively palatable. The mean annual

C:N ratios of the eight streams' biofilms ranged from 5.48 ± 1.06 to 8.12 ± 1.27 (mean ± 1 SD) which is in agreement with Gregory (1983) who states that a C:N ratio ranging between 4 – 8 indicates a high quality resource.

There were no relationships seen when algal biomass and annual percentage incorporation of methane-derived carbon were modelled as predictors of overall annual production for the caddis larvae. Although algal biomass has previously been found to strongly affect growth and survival of grazing invertebrates (Lamberti & Resh 1983; Lamberti, Feminella & Resh 1987; Feminella & Resh 1990), the biofilm that was sampled here was simply a snapshot of the algae present in the stream at the time that the caddis larvae were sampled. This does not take into account the algae removed and consumed by grazing and assimilated by organisms. Ideally an estimate of the algae removed by grazing or the gross primary production (GPP) of the algae would be more useful. Under intense grazing, there can be a low algal biomass but high primary production by algae. There is some evidence that the grazing of algae can prevent the senescence of the assemblage thus maintaining productivity (Martin, Taylor & Barton 1991). Gross primary production of algae can be estimated by setting up river plots that exclude grazers and measuring the chlorophyll *a* over time. Logistic models can then be used to calculate the maximum growth rate and maximum density of algae without grazers. Other plots allowing grazers in can be established and the chlorophyll *a* measured to compare the amount of algae removed by grazing (Moulton *et al.* 2015). Grazing caddis can greatly reduce chlorophyll *a* concentrations in biofilm. McNeely, Finlay & Power (2007) removed

Glossosoma of natural densities (>1000 m^2) from stream sections from a small tributary of the South Fork Eel River in northern California and observed a doubling in chlorophyll *a.*

Incorporation of algal carbon did differ among the streams, suggesting that other variables contributed to determining the percentage algae assimilated by caddis. The difference between streams in how much algae is incorporated by caddis larvae might be explained by interactions with other grazers or variations in algal palateability (Hart 1985; Kohler & Wiley 1997). Algal communities can vary between streams and often highly dynamic with some types of algae peaking in abundance at different times of year (Marker 1976a b; Ledger *et al.* 2008). Factors such as light availability and the availability of dissolved gases and nutrients as well as stream water chemistry can influence total algal biomass as well as the type of algal species that settle (Marker 1976b; Ledger & Hildrew 2001). Pillsbury & Lowe (1999) found that in acidic lakes in northern Michigan, high light conditions favoured the growth of green filamentous algae over diatoms and desmids which preferred lower light conditions. Grazing organisms can also modify the species present and in what amount in algal communities (Colletti *et al.* 1987; Steinman *et al.* 1987; Hill & Knight 1988; Dudley & D'Antonio 1991; Rosemond 1993) . Rosemond (1993) investigated the grazing communites in a small forested stream in eastern Tennessee and found grazed algal communities were dominated by a chlorophyte and a cyanophyte whereas ungrazed communities were made up of diatoms regardless of nutrient and light conditions. Periodic disturbances and spates can also alter the algal

community (Ledger *et al.* 2008; Stevenson 2009). All of these factors can affect the type and amount of algae available for grazers to eat.

Although food limitation can be one of the strongest factors limiting annual production, many other factors (biotic and abiotic) can affect the annual production of freshwater invertebrates such as water temperature, water chemistry, habitat complexity and biological interactions (Benke 1984; Plante & Downing 1989). Factors limiting annual production may often be the same as those limiting life history (Benke 1984). For instance, the availability of key nutrients such as phosphorus and nitrogen can affect invertebrate production as these nutrients are needed for growth. If these nutrients are in short supply annual production can be reduced (Cross, Wallace & Rosemond 2007).

The amount of habitat available and the complexity of this habitat can affect annual production, for example the presence of macrophytes can increase annual production. Tod & Schmid-Araya (2009) surveyed annual production in the Lambourn for both gravel habitats and macrophyte stands and found annual production to be much higher in the latter than the former. This may partially explain why *A. fuscipes* in the Upper Tawe has high annual production as there is a high profusion of moss on which many *A. fuscipes* larvae were observed on the six sampling occasions. In small streams such as Craig Cerrig Gleisiad, Crowborough Warren and Traeth Mawr, there was less gravel substratum and gravel patches for the caddis larvae than in larger

streams such as the Lambourn, Upper Tawe and Cray. A lack of available particles of a suitable size can also limit case-building by caddis (Cummins 1964; Statzner, Mérigoux & Leichtfried 2005).

Although rare, parasitism of caddis larvae can limit density and thus annual production. Kohler & Wiley (1992) observed a microsporidian parasite *Cougourdella* sp. which reduced *Glossosoma nigrior* density from > 2000 m^2 to < 10 m^2 in three Michigan streams. Whilst it is not known whether *A. fuscipes* is parasitized in the UK, both *S. pallipes* and *S. nigricornis* are at risk from a parasitoid wasp, *Agriotypus armatus* which attacks larvae and pupae sometimes affecting as much as 10% of the population (Elliott 1982). *Agriotypus armatus* leaves a characteristic 'ribbon' (actually a 'breathing tube' bearing a spiracle) trailing from the case of the caddis larvae. No *Silo* cases were observed with attached ribbons, thus it seems unlikely that this parasitoid had much if any effect on the annual production of the *Silo* populations surveyed in the course of that year. In the Tadnoll *A. armatus* has previously been observed infecting 1% of Goeridae sampled there (J.I. Jones, pers. Comm., 2015). It is possible that *Agriotypus armatus* was present in the *S. nigricornis* population in the Tadnoll but was not observed due to low abundance within the *S. nigricornis* population in the stream.

The questions remain as to why and how methane-derived carbon is taken up by caddis larvae. This study examined $\delta^{13}C$ values generated from isotope samples containing all instars of *A. fuscipes, S. nigricornis* and *S.*

pallipes. There may be specific life history benefits to consuming methane-derived carbon at specific life ‘stages’ or instars. Although this study does not indicate that algal biomass is a limiting factor for production it may be that methane-derived carbon offers advantages, perhaps to later instars rapidly storing lipid reserves for pupation under intense intraspecific competition. As yet, methane-derived carbon and its uptake by the three caddis species remains an enigmatic feature of these larvae.

5 Methane-derived carbon at different developmental stages

5.1 Introduction

The first aim of this chapter was to investigate whether there was any evidence of a dietary switch during the life cycle of *Agapetus fuscipes*, in terms of the incorporation of methane-derived carbon. In particular, it was questioned whether larger instars (five, six and seven) are more ^{13}C-depleted than younger stages (i.e. instars one to four). The rationale is that there might be more competition for resources as instars get larger and less algal food is available *per capita* (the larvae are often conspicuously crowded), so that larvae are likely to rely on carbon derived from methane as a 'top-up' or 'emergency' resource at that time.

Previously, spot measurements taken from a wide survey of streams from across Britain have shown that stream methane concentration is not correlated with mean glossosomatid larval $\delta^{13}C$ (Chapter 2). However, the second aim was to assess whether *A. fuscipes* larvae were more ^{13}C-depleted on any of the six sampling occasions throughout the year and, if so, whether this correlates with monthly stream methane concentration (concentration is known to vary through the year). This would suggest that the uptake of methane-derived carbon depends on the availability of stream methane.

Larval insects pass through a number of progressively larger instars, moulting between each one, while the larval stage overall often accounts for the largest fraction of the life cycle (Butler 1984). The number of instars in aquatic insects can vary between three to fifty (generally fewer in holometabolous insects and more in hemimetabolous insects) and can be fixed or flexible depending on the species and environmental conditions (Butler 1984). Where a species has a fixed number of instars, these can be used as a categorical variable to give an indication of developmental age or how close an individual is to metamorphosis. The body mass of aquatic insect larvae increases as a function of linear dimensions, such as body length or head width, with mass increase usually being an exponential or power relationship (Martins *et al.* 2014).

Adult and larval insects often have profoundly different morphology and, as in most aquatic insects, occupy different habitats. Even different larval instars may have different behaviour and resource use, depending on their particular needs at each stage. Caddisflies for example may use different materials for case building depending on the instar and size. For instance, the early stages of two limnephilid species, *Pycnopsyche lepida* and *Pycnopsyche luculenta,* construct their larval cases out of disks cut from tree leaves. As the larvae increase in size, and when the availability of whole leaves decreases, *P. lepida* begins to use mineral and sand particles in case construction whilst moving to faster water, whereas *P. luculenta* starts to add twigs to its case and moves to the stream margins where the water is slower (Cummins 1964; Wallace *et al.* 1992). In the case of *P. lepida* it is likely that

the heavier mineral grains and sand used in case construction act as ‘ballast’; this enables them to occupy faster flowing areas in the centre of the channel where there is a stony bed, and they then switch to a diet of epilithic algae from allochthonous detritus (Cummins 1964).

Resource switching also occurs in other caddisfly species. Keiper & Foote (2000) investigated the feeding behaviour of three hydroptilid species; *Hydroptila consimilis*, *Ochrotrichia spinosa* and *Ochrotrichia wojcickyi*. They found that third instar *Ochrotrichia* larvae consumed more diatoms than other the instars/species tested (*Ochrotrichia* spp. fifth and *H. consimilis* first, third and fifth), with the other instars consuming *Cladophora*, a filamentous chlorophyte. They reasoned that this was a division of trophic resources that minimised the niche overlap between the two genera. Versatile feeding behaviour is especially useful in habitats which are regularly disturbed, when there are food shortages or when there is inter- or intra-specific competition. Larval mortality can occur at different life stages. For instance Hildrew (2009) indicated a mean survival rate of about 70% from egg to mid-instar (3) in the predatory, net-spinning larva of the caddis *Plectrocnemia conspersa* compared with an equivalent rates of less than 5% for larvae (also predatory) of the alderfly *Sialis fuliginosa* in the same stream. The latter situation with mortality loaded towards early instars is probably the more usual situation in aquatic insects.

The addition of particularly nutritious dietary items or simple resource switching, may help caddisfly larvae develop to the pupal stage in environments where overall food quality is poor. For instance, Wissinger *et al.* (1996) examined two (normally) detritivorous limnephilid species from the subalpine wetlands of Colorado and found that one species, *Asynarchus nigriculus*, sometimes preyed on another, *Limnephilus externus,* as well as being cannibalistic and using mob-based behavioural tactics to consume other *A. nigriculus* larvae. They suggested that this predatory behaviour provided a supplement to the main diet of detritus, allowing those larvae to complete the larval stage in time to pupate in that year.

As seen in Chapter 4, the amount of methane-derived carbon that contributes to total annual production for armoured grazing caddis larvae such as *Agapetus fuscipes*, *Silo nigricornis* and *Silo pallipes* is variable across streams. That is, there is spatial variation. The focus here is on potential sources of temporal variation. So far, it is unknown whether these armoured grazing caddis use methane-derived carbon at a critical point in their life history; for example, when mass is rapidly accumulated in the final instars. Previous work (Chapter 3) found that body mass increases exponentially with increasing pronotum length (*A. fuscipes*) and head width (*S. nigricornis* and *S. pallipes*), with *A. fuscipes* likely to have seven instars and both *S. nigricornis* and *S. pallipes* with five.

5.2 Methods

5.2.1 Separating instars for isotope analysis

Larvae were taken from three different patches of stream from a 10 m stretch at intervals of 2.5 metres along the 10 m length (2.5, 5 and 7.5 m) on each of the six sampling occasions and stored in 50ml Falcon tubes which each patch constituting a replicate (3 replicates). These were frozen immediately in an Engel portable freezer. In the laboratory individual larvae of *Agapetus fuscipes* were separated from cases and sorted into instars under a Nikon microscope (x80) using the ranges of pronotum length reported by Becker (2005). The three replicates of every instar and pupae present at each sampling occasion were stored in Eppendorf tubes and immediately dried in an oven at 60°C for 48 hours in these tubes. Where few larvae could be found, further individuals were picked out from Surber samples to increase the number of replicates for each stream for each sampling season to three. A replicate consisted of at least 0.1 ml worth of larvae per 2ml Eppendorf tube (~0.7 – 3mg depending on the instar). For smaller instars more larvae per replicate were required to make up the minimum mass needed to run the sample through the mass spectrometer. Thus for instar 1 ~250 individual larvae made up a replicate, instar 2 (~200), instar 3 (~100), instar 4 (~50), instar 5 to 7 and pupae (~15). In some cases where larvae or instars were rare, smaller numbers of replicates of 1 or 2 samples were used for mass spectrometry. Due to the small mass of instar 1 larvae, the only stream and month where there was enough body tissue to analyse the $\delta^{13}C$ of the larvae

was from the Lambourn stream in the month of July. See Chapter 2, section 2.2.2 *Collection of caddis larvae and resources for stable isotope analysis* for isotope preparation and analysis.

5.2.2 Methane in stream water

Methane concentrations in the eight streams were measured on each of the six sampling occasions using the protocol laid out in Chapter 2: *Methane in river water.*

5.2.3 Analysis

All analyses were undertaken in R version 3.1.1. (R Core Team, 2014). To investigate whether *Agapetus fuscipes* larval $\delta^{13}C$ was related to instar, a linear mixed effects model analysis was used via the *lme* function in the nlme package in R. Since replicate samples of *A. fuscipes* larvae of the various instars were taken sequentially (over six sampling occasions; Chapter 3) and repeatedly from eight streams, these multiple measurements constitute temporal pseudo-replication. Since some instars, particularly the first two, were absent in some months, the data structure was also unbalanced. Linear mixed effects models can take into account nested structures and unbalanced relationships between a response variable and its covariates. Instar and stream were fixed effect factors with month (categorical variable listed from A – F) and replicate as random effects. Tukey-Kramer HSD tests

(after Bray & Nieh 2014) was carried out to determine which instars differed significantly from each other in $\delta^{13}C$.

To test whether the mean $\delta^{13}C$ of *A. fuscipes* larvae (data from all instars combined) was due to stream methane concentration, month or stream, a linear mixed effects model was applied as before using the *lme* function in the nlme package in R. Methane concentration and stream were fixed effect factors with month (a categorical variable listed from A – F) as a random effect.

Finally, to test whether the mean annual $\delta^{13}C$ of *A. fuscipes* larvae (data from all instars combined) was due to the mean annual methane concentration of the stream water, a linear regression was carried out. Stream methane concentrations were first log-transformed as the data were found to be highly skewed and then a mean annual methane concentration for each stream was then calculated from these logged values.

5.3 Results

5.3.1 Instar differences in $\delta^{13}C$

There were differences in larval $\delta^{13}C$ between the seven instars and pupae. The Tukey-Kramer HSD comparison test showed that instar 1 larvae (Lambourn, July) were significantly less ^{13}C-depleted than the other six instars and pupae ($p<0.001$). There was high variation in $\delta^{13}C$ between the three samples of instar 1 larvae. Instar 2 larvae were also significantly less ^{13}C-depleted than instars 3 and 5 ($p<0.001$) and instars 4 and 6 ($p<0.05$) (Figure 5.1, Figure 5.2, Figure 5.3).

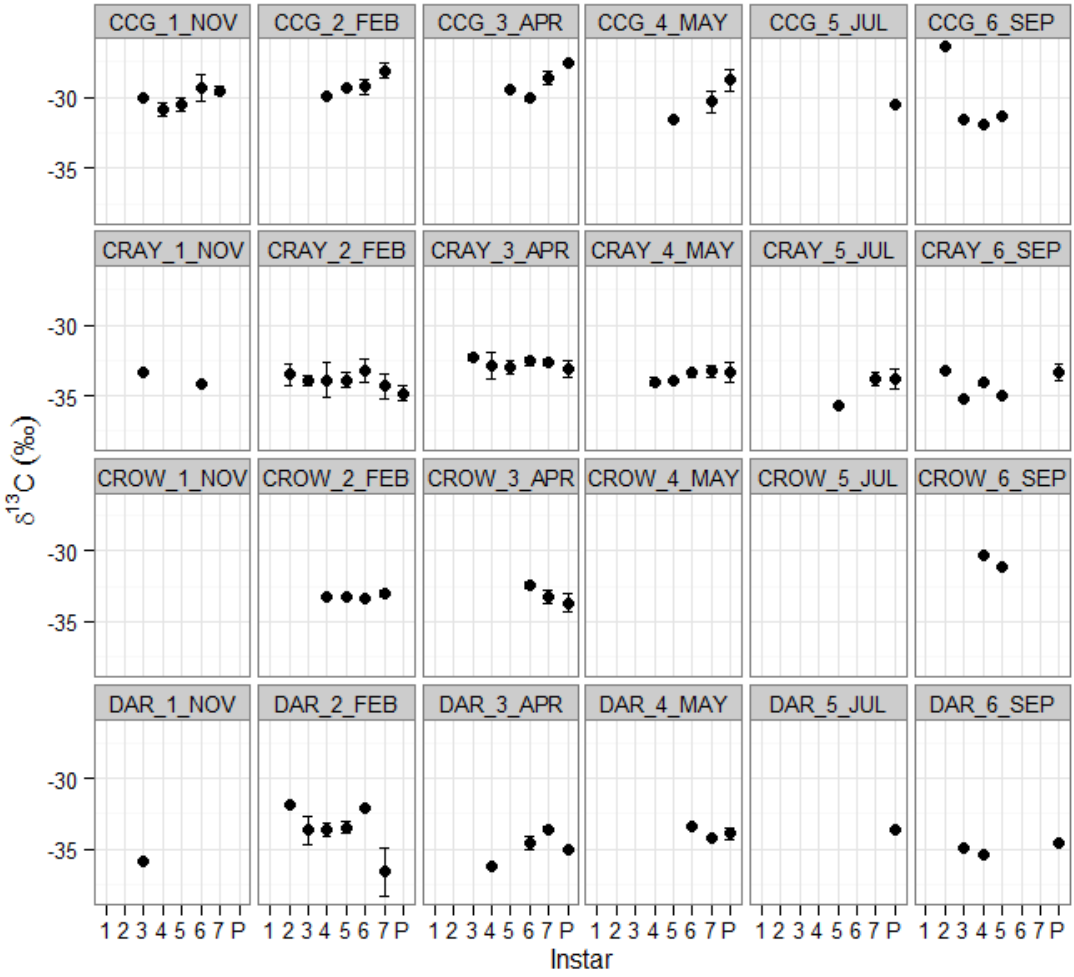

Figure 5.1 *Agapetus fuscipes* instars 1 to 7 and pupae (P), mean $\delta^{13}C$ values (± 1 SE) on the six sampling occasions (months, left to right) for four streams; Craig Cerrig Gleisiad (top row, CCG), the Cray (second row, CRAY), Crowborough Warren (third row, CROW) and the Darent (bottom row, DAR). Empty boxes denote no data.

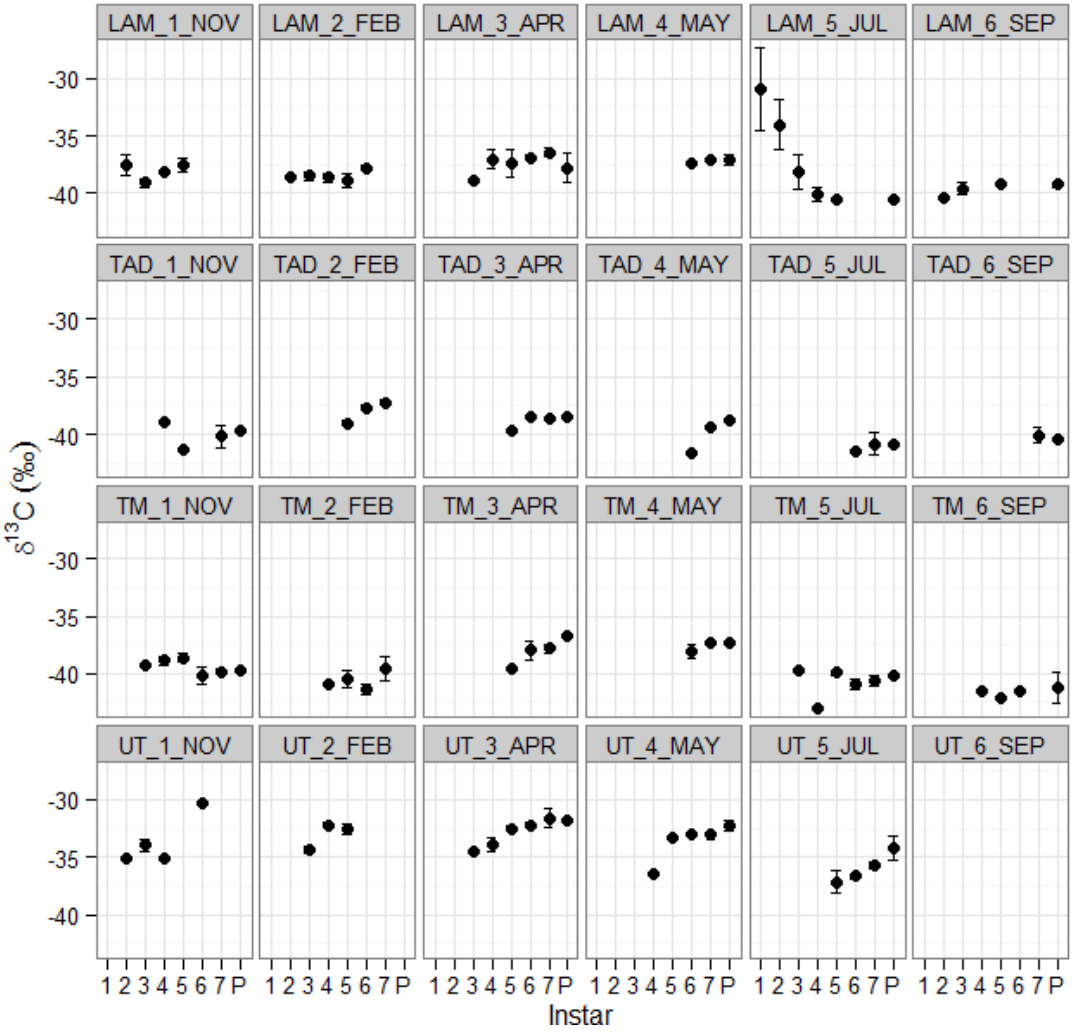

Figure 5.2 *Agapetus fuscipes* instars 1 to 7 and pupae (P), mean $\delta^{13}C$ values (± 1 SE) on the six sampling occasions (months, left to right) for four streams; the Lambourn (top row, LAM), the Tadnoll (second row, TAD), Traeth Mawr (third row, TM) and the Upper Tawe (bottom row, UT). Empty boxes denote no data.

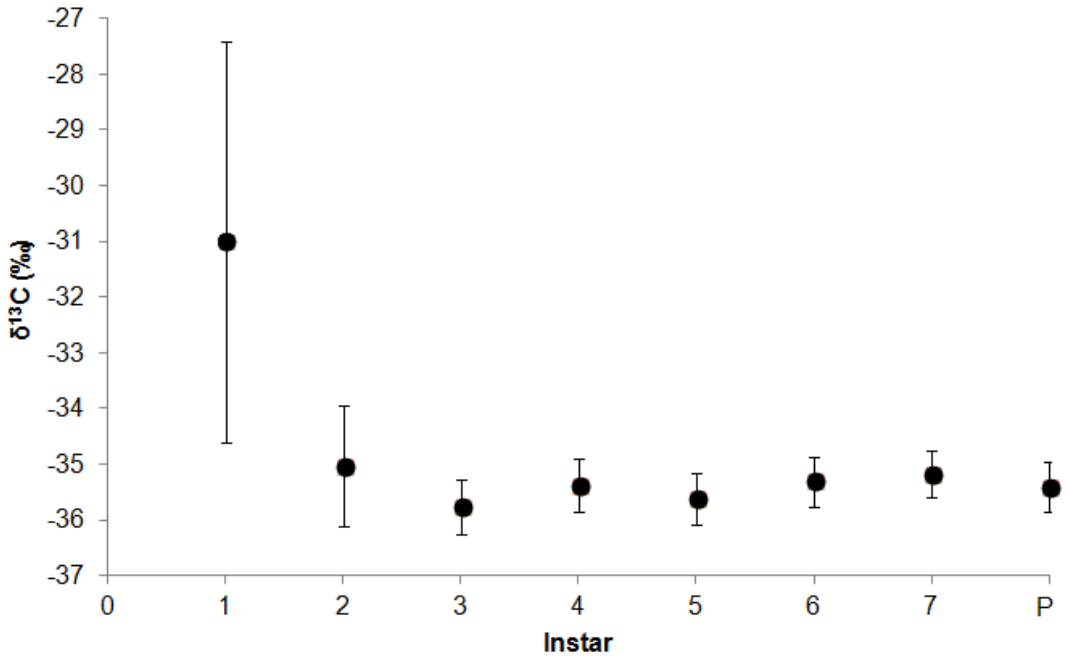

Figure 5.3 *Agapetus fuscipes* instars and pupae $\delta^{13}C$ ± 1 SE, instar 1 (n = 3), instar 2 (n = 14), instar 3 (n = 36), instar 4 (n = 47), instar 5 (n = 64), instar 6 (n = 70), instar 7 (n = 81) and pupae (n = 67), (data for eight streams combined).

5.3.2 Linear mixed effects models

The overall mean monthly larval $\delta^{13}C$ of *A. fuscipes* was not related to overall mean monthly methane concentration. Nor were the mean monthly values of larval $\delta^{13}C$ of *A. fuscipes* related to monthly methane concentrations in the individual streams. However, larvae were significantly more ^{13}C-depleted overall in the months of July and September ($p>0.02$) than November, February, April and May (Figure 5.4).

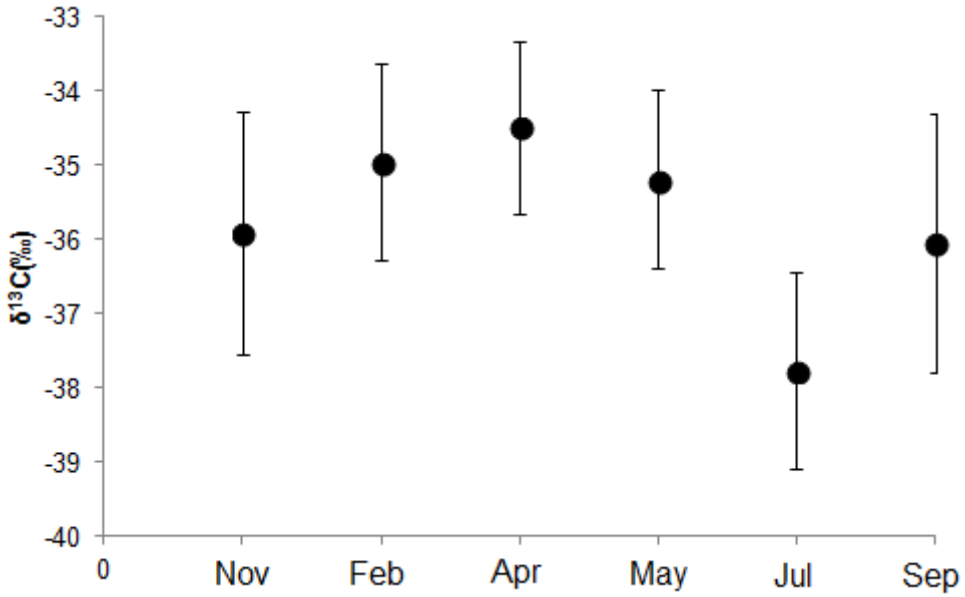

Figure 5.4 Mean *A. fuscipes* larval $\delta^{13}C$ ± 1 SE, Nov (n = 6), Feb (n = 8), Apr (n = 8), May (n = 7), Jul (n = 5) and Sep (n = 7) for the six sampling occasions from November 2011 to September/October 2012 (data for eight streams combined).

There was no significant correlation between overall mean (of the eight streams) annual larval $\delta^{13}C$ and overall mean annual log methane concentration (Figure 5.5).

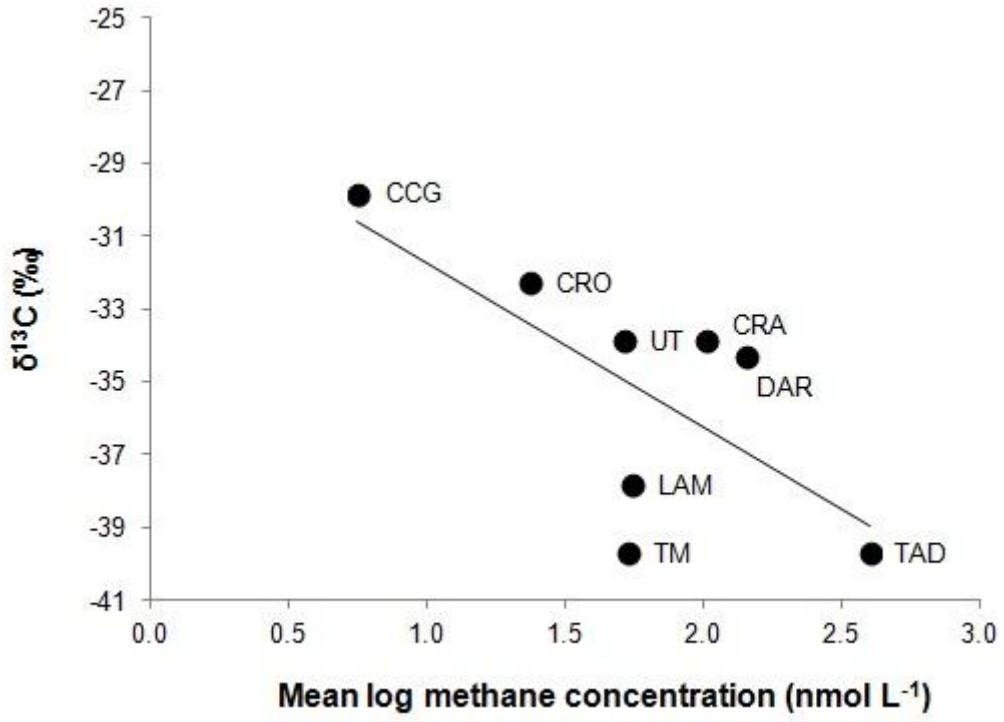

Figure 5.5 Annual (n = 8) mean log methane concentration and annual (n = 8) mean larval $\delta^{13}C$ for *A. fuscipes*: y = -4.6759x – 26.531, R^2 = 0.47. Data points are labelled as: Craig Cerrig Gleisiad (CCG), Crowborough Warren (CRO), the Cray (CRA), the Darent (DAR), the Lambourn (LAM), the Tadnoll (TAD), Traeth Mawr (TM) and Upper Tawe (UT).

5.4 Discussion

Methane-derived carbon starts begins to be incorporated in larval biomass from the second larval instar for *Agapetus fuscipes*. This suggests that methane-derived carbon is a consistently important resource (at least in some streams) for every instar beyond instar 1. The finding that instar 1 larvae are more enriched in ^{13}C than other larger instars is based only on three samples of larvae from the Lambourn in the month of July. This may not hold true for instar 1 larvae in every stream. Ideally, more samples of instar 1 larvae should be sampled from each stream to corroborate this finding. There was a high degree of variation between the three samples. This may have been down to difficulty separating larvae from small larval case particles for isotope analysis which may have affected the $\delta^{13}C$ of the larvae. Alternatively larvae may reflect spatial variation in $\delta^{13}C$ of the egg stage in different patches of stream bed.

Methane-derived carbon may be consumed as a 'top-up' or 'emergency' resource for all instars including the larger instars. There may be benefits to all instars in consuming methane-derived carbon, rather than specific benefits to certain developmental stages. Though the mechanism of uptake of methane-derived carbon by armoured grazers is still not fully understood, it is assumed, as in other organisms, that they ingest methane-oxidising bacteria directly (MOB) (Trimmer *et al.* 2009).

There is some laboratory evidence that consuming methanotrophic bacteria benefits zooplankton. The $\delta^{13}C$ of *Daphnia* has been found to be significantly more ^{13}C-depleted when feeding on microbial suspensions that were enriched with biogenic methane. Growth rate, survival and reproduction was equal or more than in *Daphnia* in non-enriched cultures (Kankaala *et al.* 2006a). Ingestion of MOB by caddis larvae may improve overall dietary quality. Methane oxidising bacteria are unusual among prokaryotes in that they are known to contain sterols and sterol-like compounds in large amounts (Bird *et al.* 1971; Summons *et al.* 1994; Cvejic *et al.* 2000). Arthropods cannot synthesise sterols *de novo* but rely on obtaining sterols and/or their pre-cursor through their diet (Goad 1981; Grieneisen 1994). Sterols are important to arthropods because they are part of the make-up of steroid hormones, such as ecdysteroids, which control processes such as moulting and the creation of cell membrane components (Grieneisen 1994).Thus, ingesting MOB might benefit armoured grazing caddis larvae by providing them with sterols and sterol-like compounds that are needed in the moult which occurs at the end of each larval instar. This could explain why MOB and, consequently, methane-derived carbon is ingested from the second instar of *A. fuscipes* onwards through all larval instars to pupation. Armoured grazing caddis larvae could also presumably derive dietary sterols from ingesting algae. However it is known that the sterol content of phytoplankton (and by analogy, other algae) can decrease if either phosphorus or light is limiting (Piepho, Martin-Creuzburg & Wacker 2010). Thus, ingesting MOB might provide an 'insurance policy' to the caddis larvae when the sterol content of algae is low. The sterol content of phytoplankton is

known to decrease in summer when light and phosphorus is limited (Piepho *et al.* 2010). Intriguingly mean caddis larval $\delta^{13}C$ was the most ^{13}C-depleted in July and September, but was not related to the methane concentration of the stream water of this period. This may indicate that caddis larvae were taking advantage of methane-oxidising bacteria when the sterol content of their algal food was reduced.

However, it remains a conumdrum as to why (if caddis larvae are ingesting MOB for their sterol content) the phenomemon of ^{13}C-depletion has, so far only been observed in armoured grazing caddis species. All arthropods moult and therefore presumably other grazing species in streams would exploit MOB for their sterol content and exhibit ^{13}C-depletion. The answer may lie in the niche occupied by these particular caddis species. *Agapetus fuscipes* and *S. pallipes* have been found to favour areas of the stream bed where the current velocity is low (0.05 – 0.2 m s^{-1}) (Becker 1994). In patches of the stream where current velocity is low, larvae remain in restricted feeding areas at high densities for day and night, with litte exploration to new feeding areas. Intensive grazing takes place which favours small-fast growing diatoms (Becker 1994). Diatoms have been found to be important to the diet of cased caddis larvae, being chosen over detritus and green algae in feeding trials (Becker 1990). Diatoms contain high amounts of unpolysaturated lipids and are considered to be of high nutritional quality (Becker 1994; Brett & Müller-Navarra 1997; Brett *et al.* 2009). Although intense grazing favours the growth of small diatoms, intra- and inter-specific competition can reduce the amount of diatoms available to the caddis larvae.

Seasonal variability in periphyton composition can also affect what cased caddis larvae consume. Diatoms for example have been recorded in gut contents of *A. fuscipes* from spring to summer in the Breitenbach stream in Germany with increasing particulate organic matter recorded in gut contents throughout autumn and winter (Becker 1990). When small diatoms are limited, MOB could potentially be consumed as replacement high quality food, especially if the larvae are constrained to feeding upon areas of the river bed where there is low velocity current.

More research is needed to deduce whether the sterol content of MOB is important in insect nutrition and whether *Agapetus* and *Silo* spp. are consuming MOB as a result of being limited to feeding under low velocity current conditions. For future research, larval gut contents data measured throughout the year could be combined with carbon isotope analysis and current velocity measurements. This may help elucidate whether diatoms are consumed by larvae as a key food source in these streams and subsequently whether the ^{13}C-depletion of larval tissues associated with the consumption of MOB occurs when diatoms are scarce. Investigation of the individual components of stream biofilm would also help to identify the fluctuation of specific food items such as diatoms throughout the year.

As discussed previously (Chapter 2), there may be an alternative explanation for the ^{13}C-depletion seen in the caddis larvae. Lennon *et al.* (2006) suggested that ^{13}C-depletion in *Daphnia* was due to their feeding on ^{13}C-

depleted phytoplankton, rather than the direct incorporation of carbon derived from biogenic methane. They proposed that phytoplankton increase their use of isotopically light CO_2 when terrestrial inputs of DOC are high. Similarly, Finlay (2001) also suggests that the $\delta^{13}C$ of algae is at least partially determined by the $\delta^{13}C$ of the DIC available. In systems where carbon is not limited, algae will discriminate against the heavier isotope of ^{13}C in preference to take up the lighter ^{12}C isotope, leading to very ^{13}C-depleted values for algae. It is possible that the larvae might have been feeding on photosynthetic biota with thin boundary layers that were not sampled or included in analysis, for example moss and other aquatic macrophytes such as *Ranunculus* spp., filamentous algae or epiphytic algae. If streams are supersaturated with CO_2, and organisms have thin boundary layers and low rates of photosynthesis, organisms are likely to be ^{13}C-depleted because they receive a plentiful supply of inorganic carbon, namely CO_2, which under thick boundary layers has high diffusion resistance (Allen & Spence 1981; Osmond *et al.* 1981; France 1995c). Evidence of boundary layer effects on basal consumers has already been documented. Finlay *et al.* (1999) found that there was a negative relationship between water velocity and herbivore $\delta^{13}C$ which in turn reflected algal $\delta^{13}C$ ratios. Rasmussen & Trudeau (2010) also found strong relationships between the $\delta^{13}C$ of mayfly herbivores (Heptageniidae and Baetidae), collector-gatherers (Simuliidae), periphyton and water velocity.

However, boundary layer effects can only partially explain the low $\delta^{13}C$ values of these three species of caddis larvae. In order for the ingestion and

assimilation of highly depleted algae to explain the ^{13}C-depletion of caddis larvae relative to the biofilm, the larvae would have to be preferentially ingesting/assimilating algal carbon which is much more depleted than the rest of the biofilm (since the $\delta^{13}C$ of the biofilm measured is the mean $\delta^{13}C$ of the bulk biofilm). Although evidence for preferential ingestion/assimilation is limited, there is some indication that other aquatic invertebrates, such as mayflies, bivalves and chironomids can preferentially ingest/assimilate particular biofilm components (Rounick *et al.* 1982; Hall 1995; Raikow & Hamilton 2001).

Some species of algae have been reported to have very low $\delta^{13}C$ values. Rounick & James (1984) surveyed a cold spring site in New Zealand and found *Melosira* sp. and *Fissidens* sp. to have $\delta^{13}C$ values of -46.3 and -45.3‰, respectively, whilst two species of caddisfly; (*Hudsonema amabilis* and *Oxyethira albiceps*) were also ^{13}C-depleted, with $\delta^{13}C$ values of -43.6 and -40.3‰, respectively. The authors attributed the low $\delta^{13}C$ values in this particular cold spring site to the incorporation into animal biomass of organic matter synthesised from diverse sources of inorganic carbon, such as geothermal carbon and isotopically light inorganic carbon from the respiration of biogenic methane. Rosenfeld & Roff (1992) also reported ^{13}C-depleted algae (-37 ± 4.6‰) in forested sites in Ontario, Canada. In a Rocky Mountain river in the USA, two species of algae (*Cladophora glomerata* and *Hydrurus* sp.) were also $\delta^{13}C$ depleted, with values of -36.9 and 42.7‰, respectively (Angradi 1993). If ingestion of MOB and subsequent incorporation of methane-derived carbon is **not** the reason why these armoured grazing

caddis are ^{13}C-depleted but, rather their ^{13}C-depletion is down to selective ingestion/preferential assimilation of very depleted algae (values of $\delta^{13}C$ in the literature, are down to around -47‰) then it suggests that this preferential ingestion or assimilation could be a very efficient process that yields the $\delta^{13}C$ of c -42‰ in some streams.

Future work must endeavour to identify whether the low $\delta^{13}C$ values exhibited by these armoured grazing caddis larvae are due to the direct incorporation of methane-derived carbon or via isotopically light carbon from algae. To accept unequivocally that the low $\delta^{13}C$ values of these armoured grazing caddis are due to the incorporation of methane-derived carbon from MOB biomass, the presence of the specific fatty acids synthesised by MOB need to be identified within caddis larval biomass. Certain fatty acids, such as 16:1ω8c, 16:1ω8t, 16:1ω6c, 16:1ω5t, 18:1ω8c and 18:1ω8t, can be used as biomarkers for MOB (Sanseverino *et al.* 2012). Proteobacterial MOB contain very unusual fatty acids (Sundh, Bastviken & Tranvik 2005; Steger *et al.* 2011). Previously, the link between the ingestion of MOB by chironomids has been confirmed by the detection of phospholipid fatty acids (PLFA's) from MOB in the tissue of chironomids (Kiyashko *et al.* 2004).

A progressive approach using stable isotopes to test the $\delta^{13}C$ of grazing caddis larvae, as well as testing for the presence of fatty acids, is needed to confirm the incorporation of methane-derived carbon ascribed to the

ingestion of MOB and dismiss the hypothesis of preferential ingestion/assimilation of isotopically light algae.

The $\delta^{13}C$ of the larvae did not track the bulk methane concentration of the stream water over the course of the year. This corroborates the findings of chapter 2 in which the measurements of methane concentration did not correlate with caddis larvae $\delta^{13}C$. The assimilation of MOB and methane-derived carbon must be independent of the methane concentration of the water. Increasing stream water methane concentrations may not mean that there are more MOB present in the stream as part of the biofilm community or that caddis larvae will eat consume more MOB. For future work it might be better to try and estimate the "amount" or population size of the MOB available to the caddis larvae from the biofilm or, if they exist on caddis cases and assimilation of MOB is through grazing of conspecific cases the amount of MOB on caddis larvae cases. Phospholipid fatty acid techniques could firstly be used to determine if specific biomarkers indicative of MOB are present in stream gravel biofilm or on caddis larval cases.

To conclude, methane-derived carbon is incorporated continuously by *A. fuscipes* larvae from instar 2 onwards, suggesting that it represents a dietary supplement throughout development. It is proposed that this may be due to an ongoing need for sterols in moulting and in synthesising ecdysial hormones perhaps when high quality food resources such as diatoms are scarce. Larvae may be constrained to areas of stream where the current

velocity is low to feed and under intra- and inter-specific competition may consume MOB when other high quality food components of the biofilm matrix are in short supply. This evidently needs further research. Other explanations for the consistent $\delta^{13}C$ depletion of the larvae is that they are preferentially assimilating isotopically light algae (which themselves may have incorporated light inorganic carbon derived from methane oxidation). To distinguish between these two possibilities future work needs to focus on identifying the presence of fatty acids associated with MOB. There might be a link between the methane concentration of the streams and the $\delta^{13}C$ of the caddis larvae, but more work needs to be done to identify how stream water methane concentration affects the uptake of methane by MOB.

6 General Discussion

Armoured grazing caddis larvae, such as *Agapetus fuscipes* and *Silo nigricornis*, show consistently ^{13}C-depleted carbon isotope values at many sites, suggesting a widespread reliance on methane-derived carbon. Food webs and the pathways of production in rivers and streams were previously thought to be based overwhelmingly on organic carbon fixed by photosynthesis, either produced within the system (autochthonous) or imported terrestrial production from the catchment (allochthonous) (Hynes 1970; Vannote *et al.* 1980; Thorp *et al.* 1998). This proposition is clearly falsified to some extent.

The larvae of families Glossosomatidae and Goeridae can often be numerically dominant in streams relatively unaffected by anthropogenic change (Becker 1990; Poff & Ward 1995; Nijboer 2004; Alvarez & Pardo 2005; Nakano *et al.* 2007). Thus, there is considerable potential for methane-derived carbon to reach potential predators/parasites. Hitherto, the evidence for armoured grazers assimilating methane-derived carbon has been restricted to streams and rivers draining the chalk across southern Britain.

The first aim of this research was to assess the geographical distribution of ^{13}C-depleted caddis larvae. Caddis larvae were sampled from 29 stream sites, most of which were on three main geological types; sandstone, limestone and chalk. A secondary aim was to assess whether the ^{13}C-

depletion of the caddis larvae correlates with the methane concentration in the stream water.

The phenomenon of ^{13}C-depleted caddis larvae was found to be widespread in the survey area, with 21 of the 28 streams analysed containing caddis larvae depleted in ^{13}C relative to the epilithon of the stream (their purported food) under fractionation of 0.4 ± 1.2‰ (mean ± 1 SD, McCutchan *et al.* 2003). The phenomenon was not restricted to chalk but was also found in streams draining sandstone, limestone and mafic lava. In 7 out of the 29 streams caddis larvae were enriched in ^{13}C or were similar relative to the epilithon. Caddis larvae in these streams were presumed to be feeding on the epilithon or a detritus source with little influence from methane-derived carbon.

A wide range of methane concentrations was observed in the streams, with all 29 containing more methane than would be expected relative to equilibrium with the atmosphere. Concentrations ranged from 7.38 to a maximum of 574.9 nmol L^{-1}. This was a somewhat unexpected finding, given that most of the streams surveyed were relatively unimpacted by anthropogenic change. Streams draining the chalk contained significantly higher log mean methane concentrations than streams draining sandstone or limestone geologies. Two streams in Dorset with high stream methane concentrations; the Tadnoll and the Bere, may have received thermogenic methane from oil beds underneath the county (Morris & Shepperd 1982;

Watson *et al.* 2000; Selley 2012; Andrews 2013). Similarly another stream, Dare Country Park was likely to be supplied by thermogenic methane from coal beds nearby (Alderton & Bevins 1996; Ulrich & Bower 2008). The $\delta^{13}C$ of stream water methane was not measured so it is not possible in this case to identify whether the methane in rivers and streams was biogenic, thermogenic or a mixture of both.

Despite all of the streams surveyed containing methane, there was no relationship between the $\delta^{13}C$ signature of the caddis larvae and overall stream methane concentration. This finding is perhaps surprising, especially if bulk methane in the water column is indeed the source of the methane-derived carbon assimilated. However, it is unlikely that the caddis larvae are relying solely on methane-derived carbon as a primary food source, instead they are likely to be consuming a mixture of 'conventionally-derived' carbon from photosynthetic processes as well as well as methane-derived carbon. The $\delta^{13}C$ of glossosomatid larvae ranged widely across the 29 streams, and this presumably reflects a variable reliance on methane-derived carbon in the differing systems and populations' need for food and energy. Grazers, such as armoured caddis larvae often undergo competition for patchily distributed resources (Kohler 1984, 1992); methane-derived carbon may then represent an important alternative source of fixed carbon subsidy for the larvae.

The overall objective of this thesis was to quantify how much of annual secondary production for these species of caddis larvae is derived from

methane. In order to calculate secondary production estimates, life history parameters such the number of instars and number of generations per year need to be known (Benke & Huryn 2006). Thus another aim of this thesis was to identify crucial life history parameters for *Agapetus fuscipes, Silo nigricornis* and *Silo pallipes*, three key armoured grazing species suspected of assimilating methane-derived carbon. In particular, it was necessary to identify the number of larval instars in *A. fuscipes* and the generation time of all three species. This in turn required assessment of whether instars could be successfully separated using measurements of body features such as pronotum length, body length and head width.

A subset of eight streams from the earlier survey of 29 were chosen, based on the $\delta^{13}C$ values of the caddis larvae (most ^{13}C-depleted and most ^{13}C-enriched) for streams draining three main geologies: chalk, limestone and sandstone. Over the duration of one year, larvae of three species of caddis fly were collected.

Instars of *Agapetus fuscipes* could not be separated using pronotum length, body length or head width. This is in direct contrast to another study of *A. fuscipes* in the Breitenbach, a small well-studied stream in Germany, where larvae could be differentiated into instars using measurements of pronotum length (Becker 2005). It is possible that there is a mix of cryptic, morphologically similar species confounding measurements of body features. Instead larvae were separated into seven size groups (presumed instars)

based on the pronotum frequency histograms of Becker (2005). Recent research in Australia and America has uncovered more species in the *Agapetus* genus than were previously recognised, with some morphologically similar species now being recognised as distinct species (Etnier *et al.* 2010; Wells 2010). *Agapetus fuscipes* is often found coexisting with three other morphologically similar species, *A. ochripes* and *A. delicatulus* (Wallace *et al.* 1990). It has been noted that it is not possible to distinguish *Agapetus* to species below instar III to V (Wallace *et al.* 2003) thus it might be that individuals belonging to *A. ochripes* and *A. delicatulus* were inadvertently included in analysis masking potential size gaps in instars. More recently a new glossosomatid species, *Synagapetus dubitans*, has been found coexisting with *A. fuscipes* in streams in Yorkshire with research ongoing to see where else the species might exist (Crofts 2012a b). Few first instar larvae were found for *A. fuscipes* and there was considerable variation in mean pronotum lengths for size groups, presumed instars suggesting that *A. fuscipes* is variable in size between streams. Previously it has been noted that larvae are smaller in mountain streams compared to lowland streams (Wallace *et al.* 2003). In the future DNA barcoding and genetic techniques may be able to sort morphologically similar larvae into separate groups of species (Jackson & Resh 1998).

In contrast to *A. fuscipes*, *Silo nigricornis* and *S. pallipes* were successfully separated into five instars, including the first, on the basis of head width measurements. This is in agreement with previous findings by Nielsen (1942), Elliott (1982) and Wallace, Wallace & Philipson (1990).

Agapetus fuscipes was found to be mostly univoltine apart from in the Upper Tawe where first instar larvae were found in April. This finding is in agreement with other studies (Nielsen 1942; Benedetto Castro 1975b; Becker 2005). *Silo pallipes* and *S. nigricornis* were also found to be univoltine although a presence of large larvae all year round in some streams suggested that some larvae may take longer, say one to two years to reach pupation. There was highly asynchronous development for all three species in the streams with often five instars being present at any given time. Asynchronous development may be part of a 'bet-hedging' strategy in environments that can be unpredictable for example, at risk of flooding or drought events (Winterbourn *et al.* 1981; Zwick 1996; Sangpradub *et al.* 1999; González & Graça 2003). Alternatively asynchronous development can be a response to inter-specific and intra-specific competition (Tsurim *et al.* 2013) where an assortment of different-sized larvae may reduce potential competition for food and space (Edgerly & Livdahl 1992). Due to the highly asynchronous nature of development for the three caddis species in question, annual secondary production was calculated using the size-frequency method (Hynes & Coleman 1968; Hamilton 1969; Benke 1979).

Annual secondary production estimates for *A. fuscipes* ranged between 0.13 – 12.13 g m^{-2} yr^{-1}. Previously reported estimates for this genus have ranged from 0.001 – 4.80 g m^{-2} yr^{-1} (Benedetto Castro 1975; Marchant & Hehir 1999; Alvarez & Pardo 2005; Tod & Schmid-Araya 2009). Two streams had

extremely high estimates of secondary production, Upper Tawe and the Cray with estimates of 6.90 – 8.28 and 10.11 – 12.13 g m^{-2} yr^{-1} respectively. The Cray is a relatively 'predator-free' stream with few other grazing competitors and high algal biomass observed. Spot measurements of stream water temperature also revealed that the temperature can be high, on one sampling occasion being 23°C. High water temperatures can increase production and reduce the time needed for a generation to complete its life cycle (Benke 1993; Marchant & Hehir 1999; Alvarez & Pardo 2005). The Upper Tawe was dissimilar to the Cray in that it had a well established grazing community with other competitors such as mayflies, snails and limpets. Water temperatures were not unduly high compared to the Cray. However, there was habitat complexity with a high profusion of moss within the stream upon which many *A. fuscipes* were observed. Many factors can affect or limit annual production of aquatic invertebrates such as water temperature, water chemistry, habitat complexity and biological interactions such as competition and parasitism (Elliott 1982; Benke 1984; Plante & Downing 1989; Kohler & Wiley 1992; Cross *et al.* 2007).

Identifying the %C content of the caddis larvae was an important first step in beginning to calculate the amount of methane-derived carbon assimilated by the three caddis species. Generally it is assumed that nutrient ratios of important elements are specific to species (Elser *et al.* 1996) so it was important to have measures of %C for each of the caddis species. Carbon comprises 48% and 46% of the mass of *A. fuscipes* and both *Silo* spp. respectively. This is almost half of the total annual production (g m^{-2} yr^{-1}) of

the species. Of this carbon, a variable amount is potentially derived from methane for the eight streams. It was assumed for the calculation of secondary production that %C was invariant across ontogeny (Back & King 2013). Potential differences in %C throughout ontogeny could lead to the calculation of annual carbon production being under- or over-estimated.

All three species of caddis larvae investigated (*A. fuscipes*, *S. nigricornis* and *S. pallipes*) were consistently ^{13}C-depleted relative to the epilithon of the streams across the year. This is in agreement with previous findings by Trimmer *et al.* (2009) and Tuffin (2014). Further to this finding, the incorporation of carbon derived from methane is likely to provide between 1.7 to 34.3% of total annual production (g $^{-2}$ yr^{-1}) for *A. fuscipes* and between 7.0 to 24.6% for *Silo* spp. This is not inconsiderable, however, despite this, annual production of both *A. fuscipes* and *Silo* spp. (dry mass g m^{-2} yr^{-1}) did not correlate with the percentage methane-derived carbon incorporated into biomass by the larvae. This suggests that assimilation of methane-derived carbon from methane-oxidising bacteria is not fuelling higher annual production than could be achieved by assimilating carbon derived from only autochthonous and allochthonous resources. This leads to further interesting questions such as whether methane-derived carbon is a high quality nutritional resource for caddis larvae.

There was also no significant relationship between the annual production of the caddis larvae and the algal biomass of the streams suggesting that there is not a simple relationship between the amount of algae available to the larvae and annual production of the caddis larvae. Algal biomass is also not a good measure of algal production as it simply measures the amount of algal biomass on a particular sampling occasion rather than the total amount of algae produced for the year. Rather an estimate of the gross primary productivity would be more useful in assessing the potential amount of algae available to grazing consumers (Moulton *et al.* 2015). This can be achieved by setting up plots or tiles in streams, some which are available to grazing consumers and some where consumers are excluded to measure the difference in algal biomass after being grazed (McNeely *et al.* 2007). A large algal biomass may not directly correlate with the amount available to caddis larvae, for example some algal varieties may not be palatable or nutritious to the caddis larvae. There may also be interspecific or intraspecific competition that prevents caddis larvae from ingesting algae (Hart 1985; Kohler & Wiley 1997). Algal communities may vary between streams due to the light availability, dissolved gases and nutrients present as well as physical factors such as disturbance and flooding (Marker 1976b; Ledger & Hildrew 2001; Ledger *et al.* 2008; Stevenson 2009).

Methane-derived carbon was found to be incorporated in larval biomass of *A. fuscipes* from the second larval instar onwards throughout to the seventh instar. Thus, methane-derived carbon remains a consistently important resource throughout the larval stages of *A. fuscipes*. There may be benefits

to consuming methane-derived carbon to all size instars rather than specific benefits to certain instars. The mechanism by which armoured caddis larvae take up methane-derived carbon is still unclear, however it is assumed that, like other organisms, they ingest methane-oxidising bacteria (Trimmer *et al.* 2009). More research is needed to deduce how the caddis larvae take advantage of these bacteria. If the bacteria are part of the biofilm matrix they do not influence the $\delta^{13}C$ of the biofilm that much suggesting they are not present in a large quantity relative to other biofilm components. If this is the case the caddis may be selectively feeding on methane oxidising bacteria in the biofilm. Selective feeding of biofilm components by grazing aquatic invertebrates has been found previously in several studies (Rounick *et al.* 1982; Rezanka & Hershey 2003; McNeely *et al.* 2006). If caddis are not feeding selectively on MOB from the biofilm matrix, perhaps the MOB exist on caddis cases and caddis graze their own cases and those of conspecifics to obtain the bacteria. Grazing of conspecific cases has been observed before for *Glossosoma intermedium* (Cavanaugh *et al.* 2004). In order to address this question behavioral studies would be needed to see how frequently "case-grazing" occurs as well as investigation into whether MOB reside on cases or whether MOB exist as part of the biofilm.

Methane-oxidising bacteria are unusual prokaryotes in that they contain sterols and sterol-like compounds in relatively large amounts (Bird *et al.* 1971; Summons *et al.* 1994; Cvejic *et al.* 2000). Arthropods cannot synthesise sterols *de novo* but instead rely on obtaining sterols and related pre-cursor compounds from their diet (Goad 1981; Grieneisen 1994). Sterols

are important dietary components as they are needed in the production of steroid hormones such as ecdysteroids which control moulting processes and the creation of cell membrane components (Grieneisen 1994). Since each larval instar undergoes a moult to reach the next instar it is conceivable that MOB are consumed throughout larval stages for their high sterol content to help produce moulting hormones. The incorporation of methane-derived carbon into larval biomass may thus be an indirect consequence of the ingestion of MOB.

If MOB are consumed for their high sterol content it still remains a conundrum as to why, so far, only these cased caddis taxa have exhibited ^{13}C-depletion, when presumably other grazing taxa should be able to exploit MOB as food source if MOB are part of the biofilm matrix. It has been proposed that the answer may lie in the niche that these species of armoured grazing caddis occupy. Where current velocity is low, caddis graze in high densities with little exploration to new feeding areas often feeding on high quality food sources such as diatoms (Becker 1990, 1994). It has been proposed that grazing species such as caddis can maintain an assemblage of small, closely adhering fast-growing diatoms by feeding on larger or loosely attached microalgae that might otherwise prevent the diatoms from flourishing (Colletti *et al.* 1987; Steinman *et al.* 1987; Hill & Knight 1988). Diatoms contain high amounts of polyunsaturated fats (Brett & Müller-Navarra 1997; Brett *et al.* 2009). MOB may be consumed as a high quality food-resource containing lipids when diatoms are in short supply due to intra- or inter-specific competition in these grazing patches. To understand whether

this is the case, gut contents for these larvae as well as detailed current velocity data and carbon isotope measurements would be needed. As well as these data it would be useful to examine biofilm samples in more detail to see what components make up the majority of biofilm.

The overall mean monthly larval $\delta^{13}C$ of *A. fuscipes* larvae was not found to be related to the overall mean monthly methane concentration in the streams. However, larvae were more ^{13}C-depleted in the months of July and September. Previous research has shown that the sterol content of phytoplankton reduces in summer when light and phosphorus is limiting (Piepho *et al.* 2010). If the relationship between sterols and phosphorus seen in phytoplankton were upheld in algae, this could suggest that caddis larvae are exploiting MOB for their high sterol content when their algal food may be reduced in sterol content or of low palateability.

An alternative explanation for the ^{13}C-depletion observed in armoured caddis may be that the caddis larvae are preferentially ingesting/assimilating algal carbon that is much more ^{13}C-depleted than the rest of the biofilm. In systems where carbon is not limiting, algae are known to discriminate against the heavier isotope of ^{13}C in preference to take up the lighter ^{12}C isotope (France 1995a; France & Holmquist 1997; Finlay *et al.* 1999; Finlay 2001). Algae have previously, in the literature, been observed to have very low $\delta^{13}C$ values, (as low as -47‰) (Rounick & James 1984; Rosenfeld & Roff 1992; Angradi 1993). If this is the reason why armoured grazing caddis

larvae are so ^{13}C-depleted then this would mean that preferential ingestion/assimilation is an efficient process yielding similar observed larval $\delta^{13}C$ values of c -42‰ in some streams. Selective feeding by cased caddis has been observed before. McNeely, Clinton & Erbe (2006), used gut contents analysis combined with a stable isotope approach found that *Glossosoma* spp. sampled from the headwaters of the South Fork Eel River in northern California fed selectively on algae where it was scarce in the biofilms of forested streams. They also found that *Glossosoma* larval $\delta^{13}C$ values in small unproductive streams were markedly lower than the $\delta^{13}C$ of the epilithon by ≥ 5‰. This difference is comparable to the difference in $\delta^{13}C$ between caddis larvae and epilithon for some of the eight streams studied in this thesis.

Another question that remains unanswered is why ^{13}C-depletion has so far, been observed in armoured grazing caddis larvae and not other grazing taxa. It has been proposed in this thesis that this might be due to how they graze; i.e. grazing the cases of conspecifics (Cox & Wagner 1989; Cavanaugh *et al.* 2004), or a feature of the niche that they occupy; restricted areas of low current velocity (Becker 1994) where intraspecific competition for high quality food such as diatoms is intense (Becker 1994; Brett & Müller-Navarra 1997; Brett *et al.* 2009). Further research could incorporate testing for the presence of methane-oxidising bacterial DNA on caddis larvae cases and gravel as well as behavioural studies of caddis larvae to investigate if and how often they graze conspecific cases to obtain MOB.

More research is needed to comprehensively answer whether the low $\delta^{13}C$ values observed for these grazing caddis larvae are due to the incorporation of methane-derived carbon or the incorporation of isotopically light carbon from algae or another isotopically light food source. A progressive approach combining stable isotope analysis and fatty acid biomarkers could be used. Certain fatty acids are specific to MOB and can be used as biomarkers (Deines *et al.* 2007; Sanseverino *et al.* 2012). The presence of these biomarkers in caddis larval biomass could be tested. Previous research has confirmed the ingestion of MOB by chironomids by the detection of phospholipid fatty acids (PLFA's) in chironomid biomass (Kiyashko *et al.* 2004; Deines *et al.* 2007).

7 References

Agasild H., Zingel P., Tuvikene L., Tuvikene A., Timm H., Feldmann T., *et al.* (2014) Biogenic methane contributes to the food web of a large, shallow lake. *Freshwater Biology* **59**, 272–285.

Alderton D.H.M. & Bevins R.E. (1996) P-T conditions in the South Wales Coalfield: evidence from coexisting hydrocarbon and aqueous fluid inclusions. *Journal of the Geological Society* **153**, 265–275.

Allan J.D. & Castillo M.M. (2007) *Stream Ecology: Structure and function of running waters*, 2nd Edn. Springer.

Allen E.D. & Spence D.H.N. (1981) The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. *New Phytologist* **87**, 269–283.

Alvarez M. & Pardo I. (2005) Life history and production of Agapetus quadratus (Trichoptera: Glossosomatidae) in a temporary, spring-fed stream. *Freshwater Biology* **50**, 930–943.

Anderson N.H. & Bourne J.R. (1974) Bionomics of three species of glossosomatid caddis flies (Trichoptera: Glossosomatidae) in Oregon. *Canadian Journal of Zoology* **52**, 405–411.

Andrews I.J. (2013) *The Carboniferous Bowland Shale gas study: geology and resource estimation*. British Geological Survey for Department of Energy and Climate Change, London.

De Angelis M.A. & Lilley M.D. (1987) Methane in surface waters of Oregon estuaries and rivers. *Limnology and Oceanography* **32**, 716–722.

Angradi T.R. (1993) Stable carbon and nitrogen isotope analysis of seston in a regulated Rocky Mountain river, USA. *Regulated Rivers: Research & Management* **8**, 251–270.

Angradi T.R. (1994) Trophic linkages in the lower Colorado River: multiple stable isotope evidence. *Journal of the North American Benthological Society* **13**, 479–495.

Anthony S.E., Prahl F.G. & Peterson T.D. (2012) Methane dynamics in the Willamette River, Oregon. *Limnology and Oceanography* **57**, 1517–1530.

APHA, AWWA & WEF (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st Edn. (Eds A.D. Eaton & M.A.H. Franson), American Public Health Association, Washington, D.C.

Arens W. (1990) Wear and tear of mouthparts: a critical problem in stream animals feeding on epilithic algae. *Canadian Journal of Zoology* **68**, 1896–1914.

Avlyush S. (2013) *Life cycles and secondary production of Ephemeroptera, Plecoptera, and Trichoptera (Insecta) under an extreme continental climate (River Kharaa, Mongolia)*. Technische Universitat Dresden.

Back J.A. & King R.S. (2013) Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects. *Freshwater Science* **32**, 837–848.

Barahona J., Millan A. & Velasco J. (2005) Population dynamics, growth and production of Sigara selecta (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream. *Freshwater Biology* **50**, 2101–2113.

Bastviken D., Cole J., Pace M. & Tranvik L. (2004) Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles* **18**, 1–12.

Bastviken D., Ejlertsson J., Sundh I. & Tranvik L. (2003) Methane as a source of carbon and energy for lake pelagic food webs. *Ecology* **84**, 969–981.

Bastviken D., Ejlertsson J. & Tranvik L. (2002) Measurement of methane oxidation in lakes: a comparison of methods. *Environmental Science & Technology* **36**, 3354–3361.

Bastviken D., Santoro A.L., Marotta H., Pinho L.Q., Calheiros D.F., Crill P., *et al.* (2010) Methane emissions from Pantanal, South America, during the low water season: toward more comprehensive sampling. *Environmental Science & Technology* **44**, 5450–5455.

Bastviken D., Tranvik L.J., Downing J.A., Crill P.M. & Enrich-Prast A. (2011) Freshwater methane emissions offset the continental carbon sink. *Science* **331**, 50.

Bates B.L., McIntosh J.C., Lohse K.A. & Brooks P.D. (2011) Influence of groundwater flowpaths, residence times and nutrients on the extent of microbial methanogenesis in coal beds: Powder River Basin, USA. *Chemical Geology* **284**, 45–61.

Baulch H.M., Dillon P.J., Maranger R. & Schiff S.L. (2011) Diffusive and ebullitive transport of methane and nitrous oxide from streams: are bubble-mediated fluxes important? *Journal of Geophysical Research* **116**, G04028.

Becker G. (1990) Comparison of the dietary composition of epilithic trichopteran species in a first-order stream. *Archiv für Hydrobiologie* **120**, 13–40.

Becker G. (1994) Food preference by five trichopteran scrapers. *Hydrobiologia* **273**, 171–178.

Becker G. (2005) Life cycle of Agapetus fuscipes (Trichoptera, Glossosomatidae) in a first-order upland stream in central Germany. *Limnologica* **35**, 52–60.

Beliaev S.S., Finkelstein Z.I. & Ivanov M. V. (1975) Intensity of bacterial methane formation in ooze deposits of certain lakes. *Mikrobiologiia* **44**, 309–312.

Benedetto Castro L. (1975) Ökologie und Produktionsbiologie von Agapetus fuscipes Curt. im Breitenbach 1971-1972. *Archiv für Hydrobiologie, Supplement* **45**, 305–375.

Benke A.C. (1979) A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. *Limnology and Oceanography* **24**, 168–171.

Benke A.C. (1993) Concepts and patterns of invertebrate production in running waters. *Verhandlungen des Internationen Vereinigung Limnologie* **25**, 15–38.

Benke A.C. (2010) Secondary production. *Nature Education Knowledge* **1**, 1–7.

Benke A.C. (1984) Secondary production of aquatic insects. In: *Ecology of Aquatic Insects*. (Eds V.H. Resh & D.M. Rosenberg), pp. 289–322. Praeger, New York.

Benke A.C. (2011) Secondary production, quantitative food webs, and trophic position. *Nature Education Knowledge* **2**, 1–12.

Benke A.C. & Huryn A.D. (2010) Benthic invertebrate production—facilitating answers to ecological riddles in freshwater ecosystems. *Journal of the North American Benthological Society* **29**, 264–285.

Benke A.C. & Huryn A.D. (2006) Secondary production of macroinvertebrates. In: *Methods in Stream Ecology*, 2nd Edn. (Eds F.R. Hauer & G.A. Lamberti), pp. 691–710. Academic Press, London.

Benke A.C. & Waide J.B. (1977) In defence of average cohorts. *Freshwater Biology* **7**, 61–63.

Benke A.C. & Wallace J.B. (2014) High secondary production in a Coastal Plain river is dominated by snag invertebrates and fuelled mainly by amorphous detritus. *Freshwater Biology* **60**, 236–255.

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

Benke A.C., Wallace J.B., Harrison J.W. & Koebel J.W. (2001) Food web quantification using secondary production analysis: predaceous invertebrates of the snag habitat in a subtropical river. *Freshwater Biology* **46**, 329–346.

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

Biodiversity: the UK Steering Group Report (1995) *Volume 2: Action Plans*. London, HMSO.

Bird C.W., Lynch M., Pirt F.J., Reid W.W., Brooks C.J.W. & Middleditch B.S. (1971) Steroids and squalene in Methylococcus capsulatus grown on methane. *Nature* **230**, 473–474.

Blair N.E. & Carter W.D. (1992) The carbon isotope biogeochemistry of acetate from a methanogenic marine sediment. *Geochimica et Cosmochimica Acta* **56**, 1247–1258.

Boar R.R., Lister D.H. & Clough W.T. (1995) Phosphorus loads in a small groundwater-fed river during the 1989–1992 East Anglian drought. *Water Research* **29**, 2167–2173.

Boon P.I. & Mitchell A. (1995) Methanogenesis in the sediments of an Australian freshwater wetland: Comparison with aerobic decay, and factors controlling methanogenesis. *FEMS Microbiology Ecology* **18**, 175–190.

Borutski E. V. (1939) Dynamics of the total benthic biomass in the profundal of Lake Beloie. *Proceedings of the Kossino Limnological Station* **22**, 156–195.

Bousquet P., Ciais P., Miller J.B., Dlugokencky E.J., Hauglustaine D.A., Prigent C., *et al.* (2006) Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* **443**, 439–443.

Bowles D.E. (1990) Life history and variability of secondary production estimates for Corydalus cornutus (Megaloptera: Corydalidae) in an Ozark stream. *Journal of Agricultural Entomology* **7**, 61–70.

Boysen-Jensen P. (1919) Valuation of the Limfjord: Studies on the fish-food in the Limfjord: 1909-1917: its quantity, variation and annual production. *Report of the Danish Biological Station* **16**, 1–44.

Bray A. & Nieh J. (2014) Non-consumptive predator effects shape honey bee foraging and recruitment dancing. *PLOS ONE* **9**, e87459.

Brett M.T., Arhonditsis G.B., Chandra S. & Kainz M.J. (2012) Mass flux calculations show strong allochthonous support of freshwater zooplankton production is unlikely. *PLOS ONE* **7**, e39508.

Brett M.T., Kainz M.J., Taipale S.J. & Seshan H. (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 21197–21201.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology* **38**, 483–500.

Bunn S.E. & Boon P.I. (1993) What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* **96**, 85–94.

Bunn S.E., Davies P.M. & Winning M. (2003) Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology* **48**, 619–635.

Butler M.G. (1984) Life Histories. In: *The Ecology of Aquatic Insects*. (Eds V.H. Resh & D.M. Rosenberg), pp. 24–55. Praeger, London.

Campeau A., Lapierre J.-F., Vachon D. & del Giorgio P.A. (2014) Regional contribution of CO2 and CH4 fluxes from the fluvial network in a lowland boreal landscape of Québec. *Global Biogeochemical Cycles* **28**, 57–69.

Carpenter S.R., Cole J.J., Pace M.L., Van de Bogert M., Bade D.L., Bastviken D., *et al.* (2005) Ecosystem subsidies: terrestrial support of aquatic food webs from 13C addition to contrasting lakes. *Ecology* **86**, 2737–2750.

Cavanaugh J.C., Haro R.J. & Jones S.N. (2004) Conspecific cases as alternative grazing surfaces for larval Glossosoma intermedium (Trichoptera: Glossosomatidae). *Journal of the North American Benthological Society* **23**, 297–308.

Childress J.J. & Fisher C.R. (1992) The biology of hydrothermal vent animals: physiology, biochemistry and autotrophic symbioses. *Oceanography and Marine Biology* **30**, 337–441.

Choy E.J., Richard P., Kim K.-R. & Kang C.-K. (2009) Quantifying the trophic base for benthic secondary production in the Nakdong River estuary of Korea using stable C and N isotopes. *Journal of Experimental Marine Biology and Ecology* **382**, 18–26.

Cole J.J., Carpenter S.R., Kitchell J., Pace M.L., Solomon C.T. & Weidel B. (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen.

Proceedings of the National Academy of Sciences of the United States of America **108**, 1975–1980.

Colletti P.J., Blinn W., Pickart A. & Wagner V.T. (1987) Influence of different densities of the mayfly grazer Hepfagenia criddlei on lotic diatom communities. *Journal of the North American Benthological Society* **6**, 270–280.

Conrad R., Chan O.-C., Claus P. & Casper P. (2007) Characterization of methanogenic Archaea and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany). *Limnology and Oceanography* **52**, 1393–1406.

Cox E.J. & Wagner R. (1989) Does Agapetus fuscipes cultivate algae in its case? *Hydrobiologia* **175**, 117–120.

Crofts S.M. (2012a) Synagapetus dubitans, a caddisfly new to Britain. *FBA News 56*, 10–11.

Crofts S.M. (2012b) *Synagapetus dubitans. 2012 study results*. Riverfly partnership, London.

Cross W.F., Benstead J.P., Rosemond A.D. & Wallace J.B. (2003) Consumer-resource stoichiometry in detritus-based streams. *Ecology Letters* **6**, 721–732.

Cross W.F., Wallace J.B. & Rosemond A.D. (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology* **88**, 2563–2575.

Cummins K.W. (1964) Factors limiting the microdistribution of larvae of the Caddisflies Pycnopsyche lepida (Hagen) and Pycnopsyche guttifer (Walker) in a Michigan stream (Trichoptera: Limnephilidae). *Ecological Monographs* **34**, 271–295.

Cvejic J.H., Bodrossy L., Kovács K.L. & Rohmer M. (2000) Bacterial triterpenoids of the hopane series from the methanotrophic bacteria Methylocaldum spp.: phylogenetic implications and first evidence for an unsaturated aminobacteriohopanepolyol. *FEMS Microbiology Letters* **182**, 361–365.

Dalsgaard T. (ed), Nielsen L.P., Brotos V., Viaroli P., Underwood G., Nedwell D.B., *et al.* (2000) *Protocol handbook for NICE-Nitrogen Cycling in Estuaries: a project under the EU research programme: Marine Science and Technology (Mast III)*. Silkeborg, Denmark.

Darling W.G. & Gooddy D.C. (2006) The hydrogeochemistry of methane: evidence from English groundwaters. *Chemical Geology* **229**, 293–312.

Deines P. (2006) *Methane as a source of carbon and energy for chironomid larvae*. Christian-Albrechts-Universität.

Deines P., Bodelier P.L.E. & Eller G. (2007) Methane-derived carbon flows through methane-oxidizing bacteria to higher trophic levels in aquatic systems. *Environmental Microbiology* **9**, 1126–1134.

DeNiro M.J. & Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

DeNiro M.J. & Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**, 261–263.

Doucett R.R., Marks J.C., Blinn D.W., Caron M. & Hungate B.A. (2007) Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* **88**, 1587–1592.

Douglas B. (1958) The ecology of the attached diatoms and other algae in a small stony stream. *Journal of Ecology* **46**, 295–322.

Downing J.A. (1984) Assessment of Secondary Production: The First Step. In: *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters*, 2nd Edn. (Eds J.A. Downing & F.H. Rigler), Blackwell Scientific Publications, Oxford.

Dudley T.L. & D'Antonio C.M. (1991) The effects of substrate texture, grazing, and disturbance on macroalgal establishment in streams. *Ecology* **72**, 297–309.

Duggins D.O., Simenstad C.A. & Estes J.A. (1989) Magnification of secondary production by kelp detritus in coastal marine ecosystems. *Science* **245**, 170–173.

Edgerly J.S. & Livdahl T.P. (1992) Density-dependent interactions within a complex life cycle: the roles of cohort structure and mode of recruitment. *Journal of Animal Ecology* **61**, 139–150.

Elliott J.M. (1969) Life history and biology of Sericostoma personatum Spence (Trichoptera). *Oikos* **20**, 110–118.

Elliott J.M. (1982) The life cycle and spatial distribution of the aquatic parasitoid Agriotypus armatus (Hymenoptera: Agriotypidae) and its caddis host Silo pallipes (Trichoptera: Goeridae). *The Journal of Animal Ecology* **51**, 923–941.

Elser J.J., Dobberfuhl D.R., Mackay N.A. & Schampel J.H. (1996) Organism size, life history, and N:P stoichiometry: toward a unified view of cellular and ecosystem processes. *BioScience* **46**, 674–684.

Etnier D.A., Parker C.R., Baxter J.T. & Long T.M. (2010) A review of the genus Agapetus Curtis (Trichoptera: Glossosomatidae) in eastern and central North America, with description of 12 new species. *Insecta Mundi* **149**, 1–78.

Feminella J.W. & Resh V.H. (1990) Hydrologic influences, disturbance, and intraspecific competition in a stream caddisfly population. *Ecology* **71**, 2083–2094.

Finlay J.C. (2001) Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology* **82**, 1052–1064.

Finlay J.C., Khandwala S. & Power M.E. (2002) Spatial scales of carbon flow in a river food web. *Ecology* **83**, 1845–1859.

Finlay J.C., Power M.E. & Cabana G. (1999) Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. *Limnology and Oceanography* **44**, 1198–1203.

Fisher S.G. & Gray L.J. (1983) Secondary production and organic matter processing by collector macroinvetebrates in a desert stream. *Ecology* **64**, 1217–1224.

Fisher S.G. & Likens G.E. (1973) Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs* **43**, 421–439.

France R.L. (1995a) Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. *Marine Ecology Progress Series* **124**, 307–312.

France R.L. (1995b) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 651–656.

France R.L. (1995c) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography* **40**, 1310–1313.

France R.L. & Holmquist J.G. (1997) d13C variability of macroalgae: effects of water motion via baffing by seagrasses and mangroves. *Marine Ecology Progress Series* **149**, 305–308.

Freeman B.J. & Freeman M.C. (1985) Production of fishes in a subtropical black-water ecosystem: the Okefenokee Swamp. *Limnology and Oceanography* **30**, 686–692.

Fry B. (2006) *Stable Isotope Ecology*, 1st Edn. Springer, New York.

Gebert J., Kothe H. & Grongroft A. (2006) Prognosis of methane formation by river sediments. *Journal of Soils & Sediments* **6**, 75–83.

Gentzel T., Hershey A.E., Rublee P.A. & Whalen S.C. (2012) Net sediment production of methane, distribution of methanogens and methane-oxidizing bacteria, and utilization of methane-derived carbon in an arctic lake. *Inland Waters* **2**, 77–88.

Georgian T. & Wallace J.B. (1983) Seasonal production dynamics in a guild of periphyton-grazing insects in a southern Appalachian stream. *Ecology* **64**, 1236–1248.

Goad L.J. (1981) Sterol biosynthesis and metabolism in marine invertebrates. *Pure and Applied Chemistry* **53**, 837–852.

González J.M. & Graça M.A.S. (2003) Conversion of leaf litter to secondary production by a shredding caddis-fly. *Freshwater Biology* **48**, 1578–1592.

Gooddy D.C. & Darling W.G. (2005) The potential for methane emissions from groundwaters of the UK. *Science of the Total Environment* **339**, 117–126.

Grace M., Dykes A.P., Thorp S.P.R. & Crowle A.J.W. (2013) *Natural England review of upland evidence - The impacts of tracks on the integrity and hydrological function of blanket peat (NEER002)*.

Gregory S. V (1983) Plant-herbivore interactions. In: *Stream Ecology*. (Eds J.R. Barnes & G.W. Minshall), pp. 157–190. Plenum Press, New York.

Grenier S. (1970) Biologie d'Argiotypus aramtusi Curtis (Hymenoptera: Agriotypidae), parasite de nymphes de trichopteres. *Annals de Limnologie* **6**, 317–361.

Grey J. (2002) A chironomid conundrum: queries arising from stable isotopes. *Verhandlungen des Internationen Vereinigung Limnologie* **28**, 102–105.

Grey J., Jones R.I. & Sleep D. (2001) Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography* **46**, 505–513.

Grey J., Kelly A. & Jones R.I. (2004a) High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnology and Oceanography* **49**, 239–244.

Grey J., Kelly A., Ward S., Sommerwerk N. & Jones R.I. (2004b) Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae

in relation to feeding and life cycle variability. *Freshwater Biology* **49**, 681–689.

Grieneisen M.L. (1994) Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. *Insect Biochemistry and Molecular Biology* **24**, 115–132.

Grubaugh J.W. & Wallace J.B. (1995) Functional structure and production of the benthic community in a Piedmont river: 1956-1957 and 1991-1992. *Limnology and Oceanography* **40**, 490–501.

Grubaugh J.W., Wallace J.B. & Houston E.S. (1997) Production of benthic macroinvertebrate communities along a southern Appalachian river continuum. *Freshwater Biology* **37**, 581–596.

Haefner J.D. & Wallace J.B. (1981) Production and potential sestone utilization by Parapsyche cardis and Diplectrona modesta (Trichoptera: Hydropsychidae) in two streams draining contrasting southern Appalachian watersheds. *Environmental Entomology* **10**, 433–441.

Hales B.A., Edwards C., Ritchie D.A., Hall G., Pickup R.W. & Saunders J.R. (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Applied & Environmental Microbiology* **62**, 668–675.

Hall R.O. (1995) Use of a stable carbon isotope addition to trace bacterial carbon through a stream food web. *Journal of the North American Benthological Society* **14**, 269–277.

Hall R.O., Dybdahl M.F. & VanderLoop M.C. (2006) Extremely high secondary production of introduced snails in rivers. *Ecological Applications* **16**, 1121–1131.

Hall R.O., Tank J.L. & Dybdahl M.F. (2003) Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Frontiers in Ecology and the Environment* **1**, 407–411.

Hall R.O., Wallace J.B. & Eggert S.L. (2000) Organic matter flow in stream food webs with reduced detrital resource base. *Ecology* **81**, 3445–3463.

Hamilton A.L. (1969) On estimating annual production. *Limnology and Oceanography* **14**, 771–781.

Hamilton S.K. & Lewis W.M. (1992) Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. *Geochimica et Cosmochimica Acta* **56**, 4237–4246.

Hanson R.S. & Hanson T.E. (1996) Methanotrophic bacteria. *Microbiological Reviews* **60**, 439–471.

Harrod C. & Grey J. (2006) Isotopic variation complicates analysis of trophic relations within the fish community of Plußsee: a small, deep, stratifying lake. *Archiv für Hydrobiologie* **167**, 281–299.

Hart D.D. (1985) Causes and consequences of territoriality in a grazing stream insect. *Ecology* **66**, 404–414.

Hauer F.R. & Benke A.C. (1987) Influence of temperature and river hydrograph on black fly growth rates in a subtropical blackwater river. *Journal of the North American Benthological Society* **6**, 251–261.

Hauer F.R. & Stanford J.A. (1981) Larval specialisation and phenotypic variation in Arctopsyche grandis (Trichoptera: Hydropsychidae). *Ecology* **62**, 645–653.

Head I.M., Jones D.M. & Larter S.R. (2003) Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **426**, 344–352.

Hein T., Baranyi C., Herndl G.J., Wolfgang W. & Schiemer F. (2003) Allochthonous and autochthonous particulate organic matter in floodplains of the River Danube: the importance of hydrological connectivity. *Freshwater Biology* **48**, 220–232.

Hershey A.E., Beaty S., Fortino K., Kelly S., Keyse M., Luecke C., *et al.* (2006) Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production. *Limnology and Oceanography* **51**, 177–188.

Hildrew A.G. (2009) *Chapter 4 Sustained Research on Stream Communities. A Model System and The Comparative Approach*, 1st edn. Elsevier Ltd.

Hill W.R. & Knight A.W. (1988) Concurrent grazing effects of two stream insects on periphyton. *Limnology and Oceanography* **33**, 15–26.

Hille S., Kristensen E.A., Graeber D., Tenna R., Jorgensen N.K. & Baattrup-Pedersen A. (2014) Fast reaction of macroinvertebrate communities to stagnation and drought in streams with contrasting nutrient availability. *Freshwater Science* **33**, 847–859.

Hlaváčová E., Rulík M. & Čáp L. (2005) Anaerobic microbial metabolism in hyporheic sediment of a gravel bar in a small lowland stream. *River Research and Applications* **21**, 1003–1011.

Houghton D.C. & Stewart K.W. (1998) Life history and case-building behavior of Culoptila cantha (Trichoptera: Glossosomatidae) in the Brazos River, Texas. *Annals of the Entomological Society of America* **91**, 59–70.

Huryn A.D. & Wallace J.B. (2000) Life history and production of stream insects. *Annual Review of Entomology* **45**, 83–110.

Huttunen J.T., Vaisanen T.S., Hellsten S.K. & Martikainen P.J. (2006) Methane fluxes at the sediment-water interface in some boreal lakes and reservoirs. *Boreal Environment Research* **11**, 27–34.

Hynes H.B.N. (1970) *The ecology of running waters*. Liverpool University Press, Liverpool.

Hynes H.B.N. & Coleman M.J. (1968) A simple method of assessing the annual production of stream benthos. *Limnology and Oceanography* **13**, 569–573.

Ings N.L., Hildrew A.G. & Grey J. (2010) Gardening by the psychomyiid caddisfly Tinodes waeneri: evidence from stable isotopes. *Oecologia* **163**, 127–139.

IPCC (2007) *Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. (Eds R.K. Pachauri & A. Reisinger), Geneva, Switzerland.

Irons III J.G. (1988) Life history patterns and trophic ecology of Trichoptera in two Alaskan (U.S.A.) subarctic streams. *Canadian Journal of Zoology* **66**, 1258–1265.

Iversen T.M. (1976) Life cycle and growth of Trichoptera in a Danish spring. *Archiv für Hydrobiologie* **78**, 482–493.

Iversen T.M. (1988) Secondary production and trophic relationships in a spring invertebrate community. *Limnology and Oceanography* **33**, 582–592.

Jackson J.K. & Resh V.H. (1998) Morphologically cryptic species confound ecological studies of the caddisfly genus Gumaga (Trichoptera: Sericostomatidae) in Northern California. *Aquatic Insects* **20**, 69–84.

Jannasch H.W. & Mottl M.J. (1985) Geomicrobiology of deep-sea hydrothermal vents. *Science* **229**, 717–725.

Jędrysek M.O. (2005) Depth of the water column in relation to carbon isotope ratios in methane in freshwater sediments. *Geological Quarterly* **49**, 151–164.

Jin H.-S. & Ward G.M. (2007) Life history and secondary production of Glossosoma nigrior Banks (Trichoptera: Glossosomatidae) in two Alabama streams with different geology. *Hydrobiologia* **575**, 245–258.

Johannsson O.E., Dermott R., Graham D.M., Dahl J.A., Millard E.S., Myles D.D., *et al.* (2000) Benthic and pelagic secondary production in Lake Erie after the invasion of Dreissena spp. with implications for fish production. *Journal of Great Lakes research* **26**, 31–54.

Johnson B.R., Tarter D.C. & Hutchens J.J. (2000) Life history and trophic basis of production of the mayfly Callibaetis fluctuans (Walsh)(Ephemeroptera: Baetidae) in a mitigated wetland, West Virginia, USA. *Wetlands* **20**, 397–405.

Jonasson P.M. (2004) Benthic invertebrates. In: *The Lakes Handbook Volume 1. Limnology and Limnetic Ecology*. (Eds P.E. O'Sullivan & C.S. Reynolds), pp. 341–416. Blackwell Publishing, Oxford.

Jones J.B. & Mulholland P.J. (1998) Influence of drainage basin topography and elevation on carbon dioxide and methane supersaturation of stream water. *Biogeochemistry* **40**, 57–72.

Jones R.I., Carter C.E., Kelly A., Ward S., Kelly D.J. & Grey J. (2008) Widespread contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae. *Ecology* **89**, 857–864.

Jones R.I. & Grey J. (2011) Biogenic methane in freshwater food webs. *Freshwater Biology* **56**, 213–229.

Jones R.I. & Grey J. (2004) Stable isotope analysis of chironomid larvae from some Finnish forest lakes indicates dietary contribution from biogenic methane. *Boreal Environment Research* **9**, 17–23.

Jones R.I., Grey J., Sleep D. & Quarmby C. (1998) An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. *Proceedings of the Royal Society B: Biological Sciences* **265**, 105–111.

Juutinen S., Rantakari M., Kortelainen P., Huttunen J.T., Larmola T., Alm J., *et al.* (2009) Methane dynamics in different boreal lake types. *Biogeosciences* **6**, 209–223.

Kankaala P., Grey J., Arvola L. & Jones R.I. (2006a) Experimental d13C evidence for a contribution of methane to pelagic food webs in lakes. *Limnology and Oceanography* **51**, 2821–2827.

Kankaala P., Huotari J., Peltomaa E., Saloranta T. & Ojala A. (2006b) Methanotrophic activity in relation to methane efflux and total heterotrophic bacterial production in a stratified, humic, boreal lake. *Limnology and Oceanography* **51**, 1195–1204.

Kedzierski W.M. & Smock L.A. (2001) Effects of logging on macroinvertebrate production in a sand-bottomed, low-gradient stream. *Freshwater Biology* **46**, 821–833.

Keiper J.B. & Foote B.A. (2000) Biology and larval feeding habits of coexisting Hydroptilidae (Trichoptera) from a small woodland stream in Northeastern Ohio. *Annals of the Entomological Society of America* **93**, 225–234.

Kelley D., Karson J. & Früh-Green G. (2005) A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science* **307**, 1428–1434.

Kelly A., Jones R.I. & Grey J. (2004) Stable isotope analysis provides fresh insights into dietary separation between Chironomus anthracinus and C. plumosus. *Journal of the North American Benthological Society* **23**, 287–296.

Kiljunen M., Grey J., Sinisalo T., Harrod C., Immonen H. & Jones R.I. (2006) A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology* **43**, 1213–1222.

Kiyashko S.I., Imbs A.B., Narita T., Svetashev V.I. & Wada E. (2004) Fatty acid composition of aquatic insect larvae Stictochironomus pictulus (Diptera: Chironomidae): evidence of feeding upon methanotrophic bacteria. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* **139**, 705–711.

Kiyashko S.I., Narita T. & Wada E. (2001) Contribution of methanotrophs to freshwater macroinvertebrates: evidence from stable isotope ratios. *Aquatic Microbial Ecology* **24**, 203–207.

Koenig E. & Waringer J. (2008) The ballast stones in Silo nigricornis cases (Insecta: Trichoptera): drift resistance and ecological benefits, investigated by acoustic Doppler velocimetry. *Archiv für Hydrobiologie Supplement* **166**, 311–328.

Kohler S.L. (1992) Competition and the structure of a benthic stream community. *Ecological Monographs* **62**, 165–188.

Kohler S.L. (1984) Search mechanism of a stream grazer in patchy environments: the role of food abundance. *Oecologia* **62**, 209–218.

Kohler S.L. & Wiley M.J. (1992) Parasite-induced collapse of populations of a dominant grazer in Michigan streams. *Oikos* **65**, 443–449.

Kohler S.L. & Wiley M.J. (1997) Pathogen outbreaks reveal large-scale effects of competition in stream communities. *Ecology* **78**, 2164–2176.

Koyama T. (1955) Gaseous metabolism in lake mud and paddy soils. *Journal of Earth Sciences Nagoya University* **3**, 65–76.

Krueger C.C. & Waters T.F. (1983) Annual production of macroinvertebrates in three streams of different water quality. *Ecology* **64**, 840–850.

Krüger M., Eller G., Conrad R. & Frenzel P. (2002) Seasonal variation in pathways of CH4 production and in CH4 oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biology* **8**, 265–280.

Kvenvolden K.A. (1995) A review of the geochemistry of methane in natural gas hydrate. *Organic Geochemistry* **23**, 997–1008.

Lamberti G.A., Feminella J.W. & Resh V.H. (1987) Herbivory and intraspecific competition in a stream caddisfly population. *Oecologia* **73**, 75–81.

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., Harris R.M.L., Armitage P.D. & Milner A.M. (2008) Disturbance frequency influences patch dynamics in stream benthic algal communities. *Oecologia* **155**, 809–19.

Ledger M.E. & Hildrew A.G. (2001) Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology* **46**, 1457–1470.

Lennon J.T., Faiia A.M., Feng X. & Cottingham K.L. (2006) Relative importance of CO2 recycling and CH4 pathways in lake food webs along a dissolved organic carbon gradient. *Limnology and Oceanography* **51**, 1602–1613.

Lonsdale P. (1977) Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep Sea Research* **24**, 857–863.

MacAvoy S.E., Carney R.S., Fisher C.R. & Macko S.A. (2002) Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. *Marine Ecology Progress Series* **225**, 65–78.

Mackay R.J. & Waters T.F. (1986) Effects of small impoundments on hydropsychid caddisfly production in Valley Creek, Minnesota. *Ecology* **67**, 1680–1686.

Mackereth J.C. (1960) Notes on the Trichoptera of a stony stream. *Proceedings of the Royal Entomological Society of London. Series A, General Entomology* **35**, 17–23.

Malicky H. (1996) Köcherfliegen von der Insel Brissago, Kanton Tessin (Trichoptera). *Entomologische Berichte Luzern* **36**, 77–94.

Malicky H. (1976) Trichopteren-Emergenz in zwei Lunzer Bächen. *Archiv für Hydrobiologie* **77**, 51–65.

Malmqvist B. (1992) Stream grazer responses to predator odour: an experimental study. *Nordic Journal Of Freshwater Research* **67**, 27–34.

Marchant R. & Hehir G. (1999) Growth, production and mortality of two species of Agapetus (Trichoptera: Glossosomatidae) in the Acheron River, south-east Australia. *Freshwater Biology* **42**, 655–671.

Marchant R. & Hehir G. How efficient is extraction of stream insect larvae from quantitative benthic samples? *Marine and Freshwater Research* **51**, 825–826.

Marker A. (1976a) The benthic algae of some streams in southern England: II. The primary production of the epilithon in a small chalk-stream. *The Journal of Ecology* **64**, 359–373.

Marker A.F.H. (1976b) The benthic algae of some streams in southern England: I. Biomass of the Epilithon in some small streams. *Journal of Ecology* **64**, 343–358.

Martin I.D., Taylor W.D. & Barton D.R. (1991) Experimental analysis of density dependent effects on two caddisflies and their algal food. *Journal of the North American Benthological Society* **10**, 404–418.

Martins R.T., Melo A.S., Gonçalves J.F. & Hamada N. (2014) Estimation of dry mass of caddisflies Phylloicus elektoros (Trichoptera: Calamoceratidae) in a Central Amazon stream. *Zoologia* **31**, 337–342.

McConnaughey T. & McRoy C.P. (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* **53**, 257–262.

McCutchan J.H., Lewis W.M., Kendall C. & McGrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**, 378–390.

McDonald I.R., Upton M., Hall G., Pickup R.W., Edwards C., Saunders J.R., *et al.* (1999) Molecular ecological analysis of methanogens and methanotrophs in blanket bog peat. *Microbial Ecology* **38**, 225–233.

McNeely C., Clinton S.M. & Erbe J.M. (2006) Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society* **25**, 787–799.

McNeely C., Finlay J.C. & Power M.E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology* **88**, 391–401.

Melzer E. & Schmidt H.-L. (1987) Carbon isotope effects on the pyruvate dehydrogenase reaction and their importance for relative carbon-13 depletion in lipids. *The Journal of Biological Chemistry* **262**, 8159–8164.

Miller J.B., Gatti L. V., D'Amelio M.T.S., Crotwell A.M., Dlugokencky E.J., Bakwin P., *et al.* (2007) Airborne measurements indicate large methane

emissions from the eastern Amazon basin. *Geophysical Research Letters* **34**, L10809.

Miller S.M., Wofsy S.C., Michalak A.M., Kort E.A., Andrews E.A., Biraud S.C., *et al.* (2013) Anthropogenic emissions of methane in the United States. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 20018–20022.

Moore J.W. & Semmens B.X. (2008) Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* **11**, 470–480.

Moore T.A. (2012) Coalbed methane: a review. *International Journal of Coal Geology* **101**, 36–81.

Moore T.R. & Knowles R. (1990) Methane emissions from fen, bog and swamp peatlands in Quebec. *Biogeochemistry* **11**, 45–61.

Morin A., Mousseau T.A. & Roff D.A. (1987) Accuracy and precision of secondary production estimates. *Limnology and Oceanography* **32**, 1342–1352.

Morris K.A. & Shepperd C.M. (1982) The role of clay minerals in influencing porosity and permeability characteristics in the Bridport Sands of Wytch Farm, Dorset. *Clay Minerals* **17**, 41–54.

Morris M.W.L. & Hondzo M. (2013) Double-averaged rough-bed open-channel flow with high Glossosoma (Trichoptera: Glossosomatidae) abundance. *Environmental Fluid Mechanics* **13**, 257–278.

Moulton T.P., Lourenço-Amorim C., Sasada-Sato C.Y., Neres-Lima V. & Zandonà E. (2015) Dynamics of algal production and ephemeropteran grazing of periphyton in a tropical stream. *International Review of Hydrobiology* **100**, 31–68.

Mulholland P.J., Fellows C.S., Tank J.L., Grimm N.B., Webster J.R., Hamilton S.K., *et al.* (2001) Inter-biome comparison of factors controlling stream metabolism. *Freshwater Biology* **46**, 1503–1517.

Müller B., Bryant L.D., Matzinger A. & Wuest A. (2012) Hypolimnetic oxygen depletion in eutrophic lakes. *Environmental Science & Technology* **46**, 9964–9971.

Murrell J.C. & Jetten M.S.M. (2009) The microbial methane cycle. *Environmental Microbiology Reports* **1**, 279–284.

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.

Nakano D., Kuhara N. & Nakamura F. (2007) Changes in size structure of macroinvertebrate assemblages with habitat modification by aggregations of caddisfly cases. *Journal of the North American Benthological Society* **26**, 103–110.

Neves R.J. (1979) Secondary production of epilithic fauna in a woodland stream. *The American Midland Naturalist* **102**, 209–224.

Nielsen A. (1942) Über die entwicklung und biologie der trichopteren mit besonderer berucksichtigung der quell Trichopteren Himmerlands. *Archiv für Hydrobiologie* **17**, 255–631.

Nijboer R. (2004) The ecological requirements of Agapetus fuscipes Curtis (Glossosomatidae), a characteristic species in unimpacted streams. *Limnologica* **34**, 213–223.

Nijboer R.C. & Verdonschot P.F.. (2004) Variable selection for modelling effects of eutrophication on stream and river ecosystems. *Ecological Modelling* **177**, 17–39.

Osmond C.B., Valaane N., Haslam S.M., Uotila P. & Roksandic Z. (1981) Comparisons of δ13C values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. *Oecologia* **50**, 117–124.

Otto C. & Johansson A. (1995) Why do some caddis larvae in running waters construct heavy, bulky cases? *Animal Behaviour* **49**, 473–478.

Owens N.J.P., Law C.S., Mantoura R.F.C., Burkill P.H. & Llewellyn C.A. (1991) Methane flux to the atmosphere from the Arabian Sea. *Nature* **354**, 293–296.

Parr L.B. & Mason C.F. (2003) Long-term trends in water quality and their impact on macroinvertebrate assemblages in eutrophic lowland rivers. *Water Research* **37**, 2969–2979.

Perga M.-E. & Grey J. (2010) Laboratory measures of isotope discrimination factors: comments on Caut, Angulo & Courchamp (2008, 2009). *Journal of Applied Ecology* **47**, 942–947.

Peterson B.J. & Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293–320.

Peterson B.J. & Howarth R.W. (1987) Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography* **32**, 1195–1213.

Piepho M., Martin-Creuzburg D. & Wacker A. (2010) Simultaneous effects of light intensity and phosphorus supply on the sterol content of phytoplankton. *PLOS ONE* **5**, e15828.

Pillsbury R.W. & Lowe R.L. (1999) The response of benthic algae to manipulations of light in four acidic lakes in northern Michigan. *Hydrobiologia* **394**, 69–81.

Plante C. & Downing J.A. (1989) Production of freshwater invertebrate populations in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1489–1498.

Poff N.L.R. & Ward J. V. (1995) Herbivory under different flow regimes: a field experiment and test of a model with a benthic stream insect. *Oikos* **72**, 179–188.

Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecological Society of America* **83**, 703–718.

Power M., Guiguer K.R.R.A. & Barton D.R. (2003) Effects of temperature on isotopic enrichment in Daphnia magna: implications for aquatic food-web studies. *Rapid Communications in Mass Spectrometry* **17**, 1619–1625.

Prairie Y.T. & del Giorgio P.A. (2013) A new pathway of freshwater methane emissions and the putative importance of microbubbles. *Inland Waters* **3**, 311–320.

Pretty J.L., Hildrew A.G. & Trimmer M. (2006) Nutrient dynamics in relation to surface–subsurface hydrological exchange in a groundwater fed chalk stream. *Journal of Hydrology* **330**, 84–100.

R Core Development Team (2014) R: a language and environment for statistical computing.

Raikow D.F. & Hamilton S.K. (2001) Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. *Limnology and Oceanography* **46**, 514–522.

Rasmussen J.B. & Trudeau V. (2010) How well are velocity effects on d13C signatures transmitted up the food web from algae to fish? *Freshwater Biology* **55**, 1303–1314.

Rau G.H. (1980) Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 742–746.

Rau G.H. & Hedges J.I. (1979) Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source. *Science* **203**, 648–649.

Ravinet M., Syva J., Jones R.I. & Grey J. (2010) A trophic pathway from biogenic methane supports fish biomass in a temperate lake ecosystem. *Oikos*, 409–416.

Recasens L. & Murrillo L. (1986) Life cycle of Agapetus fuscipes Curtis, 1834 and Hydropsyche siltalai Dohler, 1963 (Trichoptera) in Vallvidrera streams (Catalunya, N. E. Spain). *Jornadas de la Asociation Espaniola de Entomologica* **8**, 1224–1232.

Resh V.H., Lamberti G.A. & Wood J.R. (1984) Biological studies of Helicopsyche borealis (Hagen) in a coastal California stream. In: *Proceedings of the 4th International Symposium on Trichoptera*. (Ed. J.C. Morse), pp. 315–319. Dr W. Junk Publishers, The Hague.

Rezanka K.M. & Hershey A.E. (2003) Examining primary producer – consumer interactions in a Lake Superior tributary using 15 N-tracer, grazer-reduction , and nutrient-bioassay experiments. *Journal of the North American Benthological Society* **22**, 371–387.

Risse-Buhl U., Trefzger N., Seifert A.-G., Schönborn W., Gleixner G. & Küsel K. (2012) Tracking the autochthonous carbon transfer in stream biofilm food webs. *FEMS Microbiology Ecology* **79**, 118–131.

Robinson C.T. & Minshall G.W. (1998) Macroinvertebrate communities, secondary production, and life history patterns in two adjacent streams in Idaho, USA. *Archiv für Hydrobiologie* **142**, 257–281.

Rosemond A.D. (1993) Interactions among irradiance, nutrients and herbivores constrain a stream algal community. *Oecologia* **94**, 585–594.

Rosenfeld J.S. & Mackay R.J. (1987) Assessing the food base of stream ecosystems: alternatives to the P/R ratio. *Oikos* **50**, 141–147.

Rosenfeld J.S. & Roff J.C. (1992) Examination of the carbon base in southern Ontario streams using stable isotopes. *Journal of the North American Benthological Society* **11**, 1–10.

Ross D. & Wallace J. (1983) Longitudinal patterns of production, food consumption, and seston utilization by net-spinning caddisflies (Trichoptera) in a southern Appalachian stream (USA). *Holartic Ecology* **6**, 270–284.

Ross D.H. & Wallace J.B. (1981) Production of Brachycentrus spinae Ross (Trichoptera: Brachycentridae) and its role in seston dynamics of a southern Appalachian stream (USA). *Environmental Entomology* **10**, 240–246.

Rounick J.S. & James M.R. (1984) Geothermal and cold springs faunas: inorganic carbon sources affect isotope values. *Limnology and Oceanography* **29**, 386–389.

Rounick J.S., Winterbourn M.J. & Lyon G.L. (1982) Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in

some New Zealand streams: a stable carbon isotope study. *Oikos* **39**, 191–198.

Rudd J.W.M. & Hamilton R.D. (1978) Methane cycling in a eutrophic shield lake and its effects on whole lake metabolism. *Limnology and Oceanography* **23**, 337–348.

Rudd J.W.M., Hamilton R.D. & Campbell N.E.R. (1974) Measurement of microbial oxidation of methane in lake water. *Limnology and Oceanography* **19**, 519–524.

Sanders I.A., Heppell C.M., Cotton J.A., Wharton G., Hildrew A.G., Flowers E.J., *et al.* (2007) Emission of methane from chalk streams has potential implications for agricultural practices. *Freshwater Biology* **52**, 1176–1186.

Sand-Jensen K. (1998) Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology* **39**, 663–679.

Sangpradub N., Giller P.S. & O'Connor J.P. (1999) Life history patterns of stream-dwelling caddis. *Archiv für Hydrobiologie* **146**, 471–493.

Sanseverino A.M., Bastviken D., Sundh I., Pickova J. & Enrich-Prast A. (2012) Methane carbon supports aquatic food webs to the fish level. *PLOS ONE* **7**, e42723.

Sauriau P.-G. & Kang C.-K. (2000) Stable isotope evidence of benthic microalgae-based growth and secondary production in the suspension feeder Cerastoderma edule (Mollusca, Bivalvia) in the Marennes-Oleron Bay. *Hydrobiologia* **440**, 317–329.

Schoell M. (1988) Multiple origins of methane in the Earth. *Chemical Geology* **71**, 1–10.

Schubert C.J., Vazquez F., Lösekann-Behrens T., Knittel K., Tonolla M. & Boetius A. (2011) Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di Cadagno). *FEMS Microbiology Ecology* **76**, 26–38.

Scott D.W. (1979) On optimal and data-based histograms. *Biometrika* **66**, 605–610.

Scrimgeour G.J. (1991) Life history and annual production of a net spinning caddisfly Aoteapsyche colonica (Trichoptera: Hydropsychidae) in an unstable New Zealand river. *New Zealand Natural Sciences* **18**, 31–37.

Selley R.C. (2012) UK shale gas: the story so far. *Marine and Petroleum Geology* **31**, 100–109.

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.

Shelley F., Grey J. & Trimmer M. (2014) Widespread methanotrophic primary production in lowland chalk rivers. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20132854.

Statzner B., Mérigoux S. & Leichtfried M. (2005) Mineral grains in caddisfly pupal cases and streambed sediments: resource use and its limitation through conflicting resource requirements. *Limnology and Oceanography* **50**, 713–721.

Stead T.K., Schmid-Araya J.M. & Hildrew A.G. (2005) Secondary production of a stream metazoan community: does the meiofauna make a difference? *Limnology and Oceanography* **50**, 398–403.

Steger K., Premke K., Gudasz C., Sundh I. & Tranvik L.J. (2011) Microbial biomass and community composition in boreal lake sediments. *Limnology and Oceanography* **56**, 725–733.

Steinman A.D., McIntire C.D., Gregory S. V, Lamberti G.A. & Ashkenas L.R. (1987) Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. *Journal of the North American Benthological Society* **6**, 175–188.

Stern D.I. & Kaufmann R.K. (1996) Estimates of global anthropogenic methane emissions 1860–1993. *Chemosphere* **33**, 159–176.

Sterner R.W. & Hessen D.O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics* **25**, 1–29.

Stevenson R.J. (2009) Benthic algal community dynamics in a stream during and after a spate. *Journal of the North American Benthological Society* **9**, 277–288.

Strayer D. & Likens G.E. (1986) An energy budget for the zoobenthos of Mirror Lake, New Hampshire. *Ecology* **67**, 303–313.

Sugimoto A. & Wada E. (1993) Carbon isotopic composition of bacterial methane in a soil incubation experiment: contributions of acetate and CO2/H2. *Geochimica et Cosmochimica Acta* **57**, 4015–4027.

Summons R.E., Jahnke L.L. & Roksandic Z. (1994) Carbon isotopic fractionation in lipids from methanotrophic bacteria: relevance for interpretation of the geochemical record of biomarkers. *Geochimica et Cosmochimica Acta* **58**, 2853–2863.

Sundh I., Bastviken D. & Tranvik L.J. (2005) Abundance, activity, and community structure of pelagic methane-oxidizing bacteria in temperate lakes. *Applied & Environmental Microbiology* **71**, 6746–6752.

Sweeny B.W. & Vannote R.L. (1981) Ephemerella mayflies of White Clay Creek: bioenergetic and ecological relationships among six coexisting species. *Ecology* **62**, 1353–1369.

Takai Y. (1970) The mechanism of methane fermentation in flooded paddy soil. *Soil Science and Plant Nutrition* **16**, 238–244.

Tank J.L., Rosi-Marshall E.J., Griffiths N.A., Entrekin S.A. & Stephen M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* **29**, 118–146.

Thorp J.H., Delong M.D., Greenwood K.S. & Casper A.F. (1998) Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* **117**, 551–563.

Tod S.P. & Schmid-Araya J.M. (2009) Meiofauna versus macrofauna: secondary production of invertebrates in a lowland chalk stream. *Limnology and Oceanography* **54**, 450–456.

Trifonova I., Denisova I., Stanislavskaia E., Aphanasieva A. & Ulianova D. (2002) The relative contributions by phytoplankton and macrophytes to primary production of two shallow lakes (Karelian Isthmus, Russia). *Polish Journal of Ecology* **50**, 357–370.

Trimmer M., Grey J., Heppell C.M., Hildrew A.G., Lansdown K., Stahl H., *et al.* (2012) River bed carbon and nitrogen cycling: state of play and some new directions. *The Science of the Total Environment* **434**, 143–158.

Trimmer M., Hildrew A.G., Jackson M.C., Pretty J.L. & Grey J. (2009) Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnology and Oceanography* **54**, 1541–1547.

Trimmer M., Maanoja S., Hildrew A.G., Pretty J.L. & Grey J. (2010) Potential carbon fixation via methane oxidation in well-oxygenated riverbed gravels. *Limnology and Oceanography* **55**, 560–568.

Trudeau V. & Rasmussen J.B. (2003) The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology and Oceanography* **48**, 2194–2199.

Tsurim I., Silberbush A., Ovadia O., Blaustein L. & Margalith Y. (2013) Inter- and intra-specific density-dependent effects on life history and development strategies of larval mosquitoes. *PLOS ONE* **8**, e57875.

Tuffin S. (2014) *Methane-derived carbon in chalk river ecosystems*. Queen Mary University London.

Ulrich G. & Bower S. (2008) Active methanogenesis and acetate utilization in Powder River Basin coals, United States. *International Journal of Coal Geology* **76**, 25–33.

Vanderklift M.A. & Ponsard S. (2003) Sources of variation in consumer-diet d15N enrichment: a meta-analysis. *Oecologia* **136**, 169–182.

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.

Veldboom J.A. & Haro R.J. (2011) Stoichiometric relationship between suspension-feeding caddisfly (Trichoptera: Brachycentridae) and seston. *Hydrobiologia* **675**, 129–141.

Van Vliet M.T.H. & Zwolsman J.J.G. (2008) Impact of summer droughts on the water quality of the Meuse river. *Journal of Hydrology* **353**, 1–17.

Wallace I.D., Wallace B. & Philipson G.N. (1990) *A Key to the Case-Bearing Caddis Larvae of Britain and Ireland*. Freshwater Biological Association.

Wallace I.D., Wallace B. & Philipson G.N. (2003) *Keys to the case-bearing caddis larvae of Britain and Ireland*. Freshwater Biological Association, Ambleside.

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

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., Whiles M.R., Eggert S., Cuffney T.F., Lugthart G.J. & Chung K. (1995) Long-term dynamics of coarse particulate organic matter in three Appalachian Mountain streams. *Journal of the North American Benthological Society* **14**, 217–232.

Wallace J.R., Howard F.O., Hays H.E. & Cummins K.W. (1992) The growth and natural history of the caddisfly Pyncopsyche luculenta (Betten) (Trichoptera: Limnephilidae). *Journal of Freshwater Ecology* **7**, 399–405.

Walter K.M., Zimov S.A., Chanton J.P., Verbyla D. & Chapin F.S. (2006) Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. *Nature* **443**, 71–75.

Waters T.F. (1977) Secondary production in inland waters. *Advances in Ecological Research* **10**, 91–164.

Waters T.F. & Crawford G.W. (1973) Annual production of a stream mayfly population: a comparison of methods. *Limnology and Oceanography* **18**, 286–296.

Watson D.F., Hindle A.D. & Farrimond P. (2000) Organic geochemistry of petroleum seepages within the Jurassic Bencliff Grit, Osmington Mills, Dorset, UK. *Petroleum Geoscience* **6**, 289–297.

Webster J.R. & Meyer J.L. (1997) Organic matter budgets for streams: a synthesis. *Journal of the North American Benthological Society* **16**, 141–161.

Wells A. (2010) Australian species of the genus Agapetus (Trichoptera: Glossosomatidae), with descriptions of 13 new species. *Zootaxa* **2420**, 1–25.

Whitehead H. (1945) Notes on the biology of Agriotypus armatus. *Naturalist Hull* **815**, 123–126.

Whiticar M.J. (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* **161**, 291–314.

Whiticar M.J., Faber E. & Schoell M. (1986) Biogenic methane formation in marine and freshwater environments: CO2 reduction vs. acetate fermentation—Isotope evidence. *Geochimica et Cosmochimica Acta* **50**, 693–709.

Wilcock W.S.D., Delong E.F., Kelley D.S., Baross J.A. & Carey S.C. eds (2004) *The Subseafloor Biosphere at Mid-Ocean Ridges*. American Geophysical Union, Geophysical Monograph Series Volume 144, Washington, D.C.

Winfrey M.R., Nelson D.R., Klevickis S.C. & Zeikus J.G. (1977) Association of hydrogen metabolism with methanogenesis in lake Mendota sediments. *Applied & Environmental Microbiology* **33**, 312–318.

Winterbourn M.J., Rounick J.S. & Cowie B. (1981) Are New Zealand stream ecosystems really different? *New Zealand Journal of Marine and Freshwater Research* **15**, 321–328.

Winterbourn M.J. & Wright-Stow A.E. (2002) Depth distribution of stream invertebrates in the hyporheic zone: diel and life history effects. *New Zealand Natural Sciences* **27**, 15–26.

Wissinger S.C., Sparks G.B., Rouse G.L., Brown W.S. & Steltzer H. (1996) Intraguild predation and cannabalism among larvae of detritivorous caddisflies in subalpine wetlands. *Ecology* **77**, 2421–2430.

Woltemate I., Whiticar M.J. & Schoell M. (1984) Carbon and hydrogen isotopic composition of bacterial methane in a shallow freshwater lake. *Limnology and Oceanography* **29**, 985–992.

Wotton R.S. (1988) Very high secondary production at a lake outlet. *Freshwater Biology* **20**, 341–346.

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

Yamamoto S., Alcauskas J.B. & Crozier T.E. (1976) Solubility of methane in distilled water and seawater. *Journal of Chemical & Engineering Data* **21**, 78–80.

Yan Y. & Li X. (2007) Abundance, life cycle, production dynamics and trophic basis of a dominant caddisfly (Hydropsyche carbonaria McLachlan) in a subtropical stream in China. *Journal of Freshwater Ecology* **22**, 261–266.

Vander Zanden M.J., Chandra S., Park S., Vadeboncoeur Y. & Goldman C.R. (2006) Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 2608–2620.

Vander Zanden M.J. & Rasmussen J.B. (2001) Variation in d15N and d13C trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* **46**, 2061–2066.

Zeikus J.G. & Winfrey M.R. (1976) Temperature limitation of methanogenesis in aquatic sediments. *Applied & Environmental Microbiology* **31**, 99–107.

Zwick P. (1996) Variable egg development of Dinocras spp. (Plecoptera, Perlidae) and the stonefly seed bank theory. *Freshwater Biology* **35**, 81–100.

8 Appendix

Table 8.1 $\delta^{13}C$ values for caddisfly larvae and pupae collected in 2011 (n = number of samples contributing to the mean).

		A. fuscipes				***Silo* spp.**				***G. conformis***	
Site No.	Name	Lavae δ13C (‰) mean (±SE)	*n* =	Pupae δ13C (‰) mean (±SE)	*n* =	Larvae δ13C (‰) mean (±SE)	*n* =	Pupae δ13C (‰) mean (±SE)	*n* =	Larvae δ13C (‰) mean (±SE)	*n* =
1	Llangattock 1	-37.84 ± 0.14	3	-38.13	1						
2	Llangattock 2	-38.08 ± 0.17	3								
3	Nant Cleisfer									-31.36 ± 0.55	3
4	Craig Cerrig Gleisiad 1	-31.78 ± 0.09	3								
5	Craig Cerrig Gleisiad 2	-30.87 ± 0.39	3								
6	Craig Cerrig Gleisiad 3	-38.09 ± 0.13	3								
7	Dare Country Park	-28.88 ± 0.23	4								
8	Upper Tawe	-34.11 ± 0.05	3			-35.28	1			-35.29	1
9	Traeth Mawr Stream	-39.86 ± 0.00	2								
10	Owls Grove									-28.42 ± 0.54	5
11	River Manifold	-42.34 ± 0.28	3								
12	Milldale Walk	-35.77 ± 0.13	3								
13	Dovedale Walk Carpark	-38.94 ± 0.11	3	-39.38	1						
14	River Wye Topley Park Quarry	-39.65 ± 0.42	3								
15	Lees Bottom1 (R. Wye)	-39.13 ± 0.49	3								
16	Lees Bottom2 (R. Wye)	-37.16 ± 0.11	3								
17	Head of the Tadnoll	-41.77	1					-41.00 ± 0.14	6		
18	Bere Stream	-39.56	1					-39.14 ± 0.12	6		
19	River Creedy	-35.4	1	-35.29 ± 0.06	2						
20	Stockleigh Stream	-40.13	1	-35.81 ± 0.28	3						
21	River Lowman	-37.44 ± 0.25	3	-34.55	1			-32.46 ± 0.70	4		
22	Crowborough Warren	-33.11	1	-32.90 ± 0.14	2			-31.32 ± 0.63	4		
23	Hodgehow wood	-34.51 ± 0.14	3	-33.82 ± 0.24	3			-33.37 ± 0.47	3		
24	Belle Grange Beck	-27.01 ± 0.17	3	-26.75 ± 0.51	3			-27.16	1		
25	Cunsey Beck							-30.86 ± 1.37	3		
26	Carrock Beck	-41.38 ± 0.30	4	-41.50 ± 0.50	2						
27	Small Spring	-37.56	1	-36.33 ± 0.21	3			-36.02 ± 0.12	3		
28	Belleau	-43.32 ± 0.34	3								
29	Welton le Wold	-40.09 ± 0.20	3								

Table 8.2 $\delta^{13}C$ values of moss spp. and leaf litter collected from November 2011 to October 2012 from the eight streams (n = 3).* Samples could not be taken due to flood and drying events. ǂ Leaf litter was sampled in February instead of November.

	Moss							
	Craig Cerrig Gleisiad	**Cray**	**Crowborough Warren**	**Darent**	**Lambourn**	**Tadnoll**	**Traeth Mawr**	**Upper Tawe**
Nov	-34.61 ± 1.51	-	*	-	-	-28.89 ± 1.02	-	-39.55 ± 0.48
Feb	-28.95 ± 0.85	-30.34 ± 0.43	-29.08 ± 1.13	-28.42 ± 1.15	-	-39.17 ± 3.47	-26.18 ± 1.10	-28.46 ± 0.34
Apr	-28.19 ± 0.80	-	-29.51 ± 0.57	-	-	-27.81 ± 0.98	-26.64 ± 0.77	-31.55 ± 2.13
May	-33.74 ± 0.76	-	-28.51 ± 0.30	-	-	-28.41 ± 0.88	-30.74 ± 0.99	-33.52 ± 0.89
Jul	-34.24 ± 0.60	-	-29.34 ± 0.05	-	-	-35.91 ± 2.79	-	-38.96 ± 0.39
Sep	-27.83 ± 0.96	-	-29.64 ± 0.39	-	-	-29.03 ± 0.77	-25.96 ± 0.78	*
	Leaf Litter							
Nov	-28.54 ± 0.45	-28.07 ± 1.26	-28.1 ± 0.48ǂ	-27.83 ± 0.15	-27.41 ± 0.40	-27.47 ± 0.92	-26.33 ± 0.48	-28.47 ± 1.13

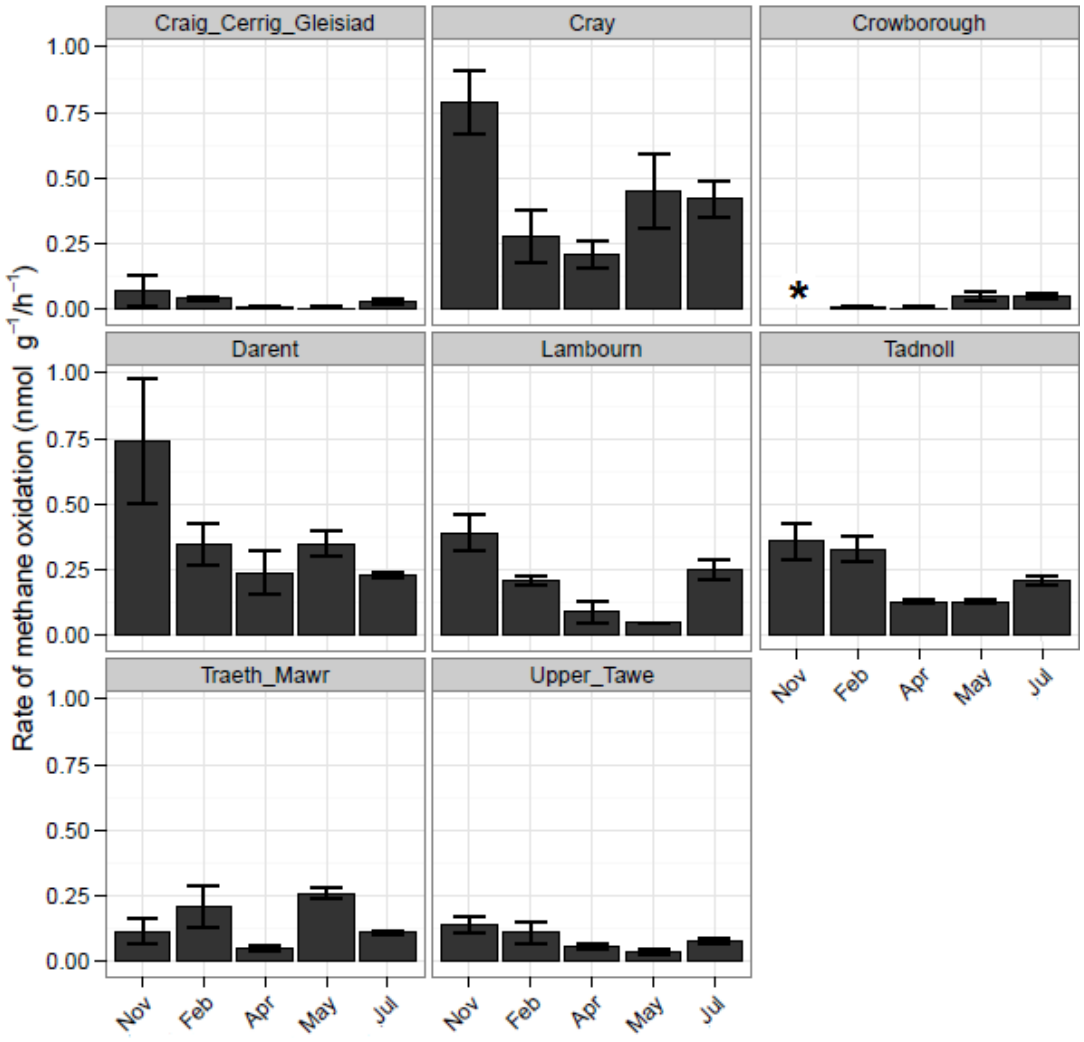

Figure 8.1 Rates of methane oxidation for stream gravel for five sampling occasions from November 2011 to July 2012 for Craig Cerrig Gleisiad (sandstone), the Cray (chalk), Crowborough Warren (sandstone), the Darent (chalk), the Lambourn (chalk), the Tadnoll (chalk), Traeth Mawr (sandstone) and Upper Tawe (limestone).