Sources and impacts of inorganic and organic fine sediment in salmonid spawning gravels in chalk rivers

by

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Abstract

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Poor salmonid spawning habitat due to excessive fine sediment inputs has been identified as a major factor limiting survival in chalk rivers. A lack of knowledge about the complex processes and factors affecting survival was the driver for this study and gaps in the research were identified concerning the sources of fine sediment and the impact organic material had on salmonid survival in chalk streams. Consequently the main objectives of this study were to characterise spawning habitat quality of a chalk catchment, assess the sources of sediments accumulating within artificial redds, describe the composition of organic sediments using emerging technology and to create a novel method to assess the sediment oxygen consumption of those sediments. Methods were based around a catchment wide field based monitoring programme, consisting of artificially constructed spawning gravels which allowed hyporheic measurements to be taken, and sediment analysis and sediment oxygen consumption methods were carried out using different laboratory methods. Spawning habitat characteristics of the chalk catchment were found to exhibit; low sediment accumulation rates although original levels of fine sediment were high, high organic matter content, variable intra-gravel flow and intra-gravel oxygen concentrations and groundwater influences. Primary sources of fine sediment accumulating in spawning gravels and suspended sediments were found to be attributed to catchment surface sources, namely pasture (50-68%) and arable (32-50%) using inorganic and organic parameters. Organic composition of redd gravels was found to be dominated by protein material rather than humic substances, the more commonly found fluorescent compound in freshwater systems and the sediment oxygen consumption of sediments varied throughout the catchment and was found to consume the greatest oxygen in <63µm size fraction. Application of sediment oxygen consumption rates to existing parameter based models that predict salmonid survival, highlighted the need to address the sensitivity of current models to rivers experiencing low sediment accumulation rates. Outcomes of this study further the knowledge of the sources, organic composition and sediment oxygen consumption capacity of fine sediments accumulating in spawning gravels which can lead to appropriate mitigation on chalk rivers to improve salmonid spawning habitat.
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DECLARATION OF AUTHORSHIP

I, Samantha Lynn Bateman

declare that the thesis entitled

Sources and impacts of inorganic and organic fine sediment in salmonid spawning gravels in chalk streams

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

• this work was done wholly or mainly while in candidature for a research degree at this University;
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Signed: …………………………………………………………………………….
Date:…………………………………………………………………………….
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Chapter 1. Introduction

1.1 Research evolution

The declining productivity of spawning gravels within UK rivers has been the preoccupation of many fisheries scientists and managers aiming to promote successful incubation of salmonid embryos (Chapman 1988; Crisp 2000; Hendry and Cragghine 2005). There is a wealth of research linking the accumulation of fine sediments in spawning gravels with decreasing salmonid survival, dating back to the 1920s where a laboratory study noted salmonid survival decreased with high proportions of fine sediment (Harrison 1923). Research conducted in the 40s and 50s hypothesised the link between fine sediment impeding dissolved oxygen by blocking water exchange within redds (Krough 1941; Hayes et al. 1951) and field studies conducted on natural redds confirmed that this was indeed the case (Wickett 1954; Cooper 1965).

The oxygen requirements of salmonids were investigated as the next logical step after discovering the link between low dissolved oxygen levels and reduced survival. Laboratory studies looked at the oxygen consumption rate of salmonid eggs at different developmental stages. Conclusions were made that consumption of oxygen was limited by the supply rate of oxygen and threshold levels of oxygen concentration (Alderdice et al. 1958; Silver et al. 1963; Hamor and Garside 1978). Based on these laboratory studies, theoretical models of oxygen consumption by embryos were developed around the theories of diffusion (Hayes et al. 1951; Wickett 1954) and mass transport (Daykin, 1965; Chevalier and Carson 1985).

The processes governing the transfer of fine sediments to the river-bed environment was another area of active research that influenced today’s knowledge of the impact of fine sediment on spawning success. A number of laboratory and field studies were carried out in this field (Beschta and Jackson 1979; Lisle 1980; Carling 1984; Frostick et al. 1984). Observations from these studies suggested that the dominant mechanisms controlling the distribution and nature of fine sediments in river gravels were, 1) water-column based; where sediment is deposited on top of river beds and 2) river-bed based; where sediments are moved from the surface to other areas of the bed (Sear et al. 2008). Delivery of fine sediment to river beds and thus spawning gravels from suspended sediment loads results from a mix of gravitational settling and turbulence (Beschta and Jackson 1979; Carling 1984). Frostick et al. (1984) noted that the size and shape of fine sediment and the river bed gravels were instrumental in
determining the depositional size of the fine material. Typically smaller particles (clay and silt) were found to deposit from the bottom up in salmonid redds and larger sand particles were found to deposit on the top of redds at the gravel surface (Beschta and Jackson 1979; Frostick et al. 1984). Chapman (1988) inferred from these studies that the consequences for incubating salmon embryos was that accumulation of sands within redds could form a surface cap inhibiting fry emergence at the end of incubation and the smaller clay and silt particles would block the passage of oxygenated water through pore space in the gravels.

Salmonid stocks were declining rapidly by the mid to late 1980s and scientists focussed on a number of key areas to mitigate effects of the decline, one of them being spawning habitat quality leading to poor embryonic survival (Lotspeich and Everest 1981; Peterson and Metcalfe 1981; Chapman 1988; Meehan 1991). The first tools to predict salmonid survival based on the quality of spawning gravels were created by government agencies in the United States (USDA and USFS). Two types of tool which predict egg survival were created; those based on intra-gravel oxygen concentrations (Turnpenny and Williams; Maret et al. 1993; Ingendhal 2001) and those based on physical characteristics of the gravel bed (Lotspeich and Everest 1981; Cedarholm et al. 1981; Chapman 1988; Young et al. 1991).

In recent years other factors affecting survival have been researched, such as thermal regimes in the hyporheic environment (Chadwick et al. 1982; Malcolm et al. 2002), groundwater and surface water interactions, including redds located in areas of upwelling groundwater (Soulsby et al. 2001; Malcolm et al. 2003, 2004, 2006), sub-lethal effects of low dissolved oxygen after emergence (Cada et al. 2003; Portz et al. 2006) and oxygen consumption of sediments within redds (Chevalier and Carson 1984; Greig 2004). Indeed the number of factors affecting salmonid survival in redds and the complex interactions between them warrant further attention and recent studies continue to add to the growing base of research about factors affecting survival (Lapointe et al. 2004; Greig et al. 2005a; Greig et al. 2007; Heywood et al. 2007; Burke 2011).

The complex interplay of multiple factors influencing spawning success has created the need for multi-factor analysis of embryo survival and driven the creation of conceptual models in order to provide outcomes for survival (Alonso et al. 1996; Wu 2000). These models depend on high quality field data and would be improved by standardised methods of data collection and analysis to allow comparison between sites and catchments. Whilst it should be noted that models cannot represent the
natural environment precisely, they increase knowledge of the interactions between natural processes and expose areas that are currently lacking information. It is not sufficient to focus on only one factor affecting spawning success or one measure of survival, studies must address the multi-faceted process driven problem in a holistic way (Greig 2004).

Catchment scale research is a new concept being used by river scientists and managers. This requires all aspects of a river catchment be it chemical, geomorphological, hydrological and biological to be considered together and links between these areas to be defined (Vaughan et al. 2007). The interfaces between disciplines are often where gaps in understanding need bridging to improve river management; for example, the feedback system of the interactions between vegetation and sediment and how this influences flow characteristics and channel morphology which in turn affects sediment transport and vegetation growth (Vaughan et al. 2007).

Greig (2004) completed a study looking at multiple effects on embryo survival including sediment accumulation, sediment oxygen demands, intra-gravel dissolved oxygen concentration and flux and intra-gravel flow velocity. This study highlighted the importance of following this holistic, catchment- scale approach to elucidate factors affecting salmonid survival.

Greig (2004) developed the research avenue put forward by Chevalier and Carson (1984) concerning the consumption of oxygen by organic material within sediments accumulating within spawning gravels. Greig (2004) in his study looking at four catchments created sediment oxygen demand values for accumulating sediment in redds, based on traditional biochemical oxygen demand (BOD) methods and calculated the total percentage organic material contained within fine sediments. However due to the broad, holistic nature of the research, an in-depth study of the characteristics and impact of these organic sediments was not carried out and a future recommendation of this work was to further investigate at higher temporal and spatial resolution the organic component of sediments infiltrating spawning gravels (Greig 2004). Petticrew and Arocena (2003) completed a study monitoring the organic composition of salmonid rivers in the USA and noted the production of biofilms over river gravels at certain times of year which have the potential to consume dissolved oxygen and physically block gravel pore spaces. Sear et al. (2008) reviewed the research to date on organic sediments. Such research is complicated due to the ubiquitous, changeable nature of organic matter. Therefore there is a need to investigate the role organic
matter plays in defining spawning habitat quality of salmonid spawning gravels in more detail (Greig et al. 2005; Sear et al. 2008).

1.2 **Aims and Objectives**

In summary, the overarching aim of the study is to assess the sources and impact of inorganic and organic fine sediment accumulating in salmonid spawning gravels. Previous research has focussed on oxygen availability, intra-gravel flow and sediment accumulation in relation to survival and the links between these factors (Chevalier and Carson 1984; Acornley and Sear 1999; Greig et al. 2005a; Greig et al. 2007b; Heywood et al. 2007). However the lack of knowledge about the composition and source of accumulated inorganic sediments and organic sediments within spawning gravels has been overlooked. Organic sediment accumulation within spawning gravels has the potential to have an impact on the oxygen availability and intra-gravel flow regimes within the hyporheic environment, due to the oxygen consuming nature of these materials (Chevalier and Carson 1984; Greig et al. 2007b; Sear et al. 2008). In view of this fact, two broad aims were identified within the research.

The first aim was to characterise spawning habitat quality in a chalk river catchment in terms of inorganic fine sediment accumulation and to determine the source of inorganic fine sediment inputs in the catchment using the sediment fingerprinting approach. The second aim was to characterise organic sediments accumulating within spawning gravels and investigate the oxygen consuming capacity of these sediments. This second aim attempts to address the gap in the research concerning the organic fraction of sediments and its source, impact and behaviour in spawning gravels. The quantity, quality and potential sources of organic matter discovered within the redd environment are little known and need quantifying to understand the nature of its effects on incubating salmonids. Questions such as what is the composition of the organic matter, where is this organic matter coming from and how does the oxygen consumption of this organic matter affect the oxygen available for incubating salmon in the redd environment are addressed in this research.

1.2.1 **Research objectives and hypotheses**

Four specific research objectives have been developed considering the aims described above and hypotheses about the expected outcomes of the studies are set out;
1. **To characterise spawning habitat quality on the River Itchen over different spatial and temporal scales during the incubation period**

By using artificial redds to monitor variables such as dissolved oxygen levels and sediment accumulation throughout the catchment, spawning habitat was characterised. Spatial and temporal scales were investigated to ensure the whole catchment was represented in terms of spawning habitat quality and this gave an insight into the quality of salmonid spawning in chalk streams in general. The main hypotheses derived from this objective were; does variation in spawning habitat quality exist in the Itchen catchment and can variability be explained by sediment dynamics? Chapter four reports the findings of this objective.

2. **To assess the impact of sediment oxygen demand (SOD) of organic sediments on the availability of oxygen within the interstitial environment and the impact on oxygen consumption by incubating salmon embryos.**

By creating an experimental method to measure infiltrated fine sediment oxygen consumption and determining SOD rates at different spatial and temporal scales there was potential to explore the impact of these organic sources on the oxygen available to embryos within the hyporheic environment. This research aim is addressed in chapter five. Hypothesis; can sediment oxygen demand be quantified in accumulating fine sediments in redds using laboratory techniques?

3. **To trace inorganic sediment sources throughout the catchment using sediment fingerprinting technology and the use of a composite fingerprint suite to determine where mitigation of sediment control policy might be effective on the Itchen and similar rivers.**

Potential sediment sources will be identified and compared with accumulated sediment within the redd environment. Statistical methods were employed to determine composite fingerprint suites that can identify key source areas in the catchment and links to the accumulated material within the redd environment. The hypothesis resulting from this aim was; can the main catchment and in-stream fine sediment sources accumulating within spawning gravels be differentiated between on the Itchen? Chapter six addresses this research objective.
4. To identify and describe organic matter sources within the redd environment and explore the potential for creating a composite fingerprint suite for sourcing organic matter throughout the catchment to investigate the impact on salmonid spawning habitat quality

Spectrophotometric techniques were used to determine organic matter sources and the composition of redd sediment materials, alongside macro-plant and macro-invertebrate data and total organic content. There may be potential to use these properties to create a generic composite fingerprint suite for sourcing organic material within freshwater ecosystems in other chalk rivers in the UK. Chapter seven focuses on this objective. Hypothesis: is it possible to discern the composition of organic sediments found in spawning gravels using spectrophotometric techniques and is there any potential to use this information to successfully define the source of these materials?

1.3 Thesis structure and integration

The thesis begins with a basic introduction to the research, including research evolution leading to the aims and objectives identified and addressed within the main text (Chapter one). Chapter two then gives a general literature review of previous research, including factors affecting spawning habitat and specifically sedimentation of spawning habitat. Following on, Chapter three introduces the study catchment and methods used in the majority of analysis chapters. Chapters 4-7 of the thesis are concerned with addressing each of the four objectives in separate but linked and overlapping studies. The following section discusses the whole catchment approach that led to the rationale behind the structure of the thesis.

1.3.1 Thesis structure rationale

The two main aims identified, which led to the development of the four specific research objectives, were based on the acknowledged problem of increased sedimentation of salmonid spawning habitat in the UK. The four objectives of the research thesis were created based on the gaps of knowledge identified within the research field and to encompass potential issues and current issues that affect salmonid spawning habitat. In order to assess the spawning habitat of salmonids on the Itchen with a view to providing mitigation options for increasing salmonid populations a holistic approach was adopted. This comprehensive approach meant that
spawning habitat issues were identified at different spatial scales. Starting with the largest spatial scale of catchment level, the sources of fine sediment both inorganic and organic that were likely to impact on the spawning habitat quality of salmonids were identified. These catchment scale input sources of fine sediment are then linked via sedimentological, hydrological and ecological processes to the reach scale factors that impact on salmonid spawning habitat. Reach scale factors studied included, available oxygen, suspended sediment load, discharge and sediment deposition that are interlinked and impact on the quality of spawning habitat provided. Finally at the patch or redd scale the fine sediment deposited and infiltrated into the redd was characterised in terms of its inorganic and organic nature, intra-gravel flow through the redd and dissolved oxygen availability and finally the sediment oxygen demand imparted by infiltrated sediment surrounding eggs within the redd was studied. This whole catchment approach attempted to address sedimentation of spawning habitat on the Itchen from its source at catchment scale through the reach scale processes governing the degree of impact that sediment has on a reach to the finer magnitude redd scale processes and ultimately the final outcome of salmonid embryo survival. Figure 1.1 provides a conceptual representation of the different scales that the study objectives and therefore thesis chapters address and the links present between the different chapters.
Figure 1.1. Conceptual diagram describing the research objectives and links between the different chapters at three different spatial scales.
Chapter 2. Literature Review

This chapter reviews the relevant literature that underpins the background and reasoning behind this research. The life history, adaptations and declining conservation status of salmonids and specifically Atlantic salmon (Salmo salar) will be introduced. A review of previous literature stating spawning habitat preference and factors influencing success will be discussed.

2.1 Salmonid life history and adaptations

The salmonidae family are composed of sixty six species in eight genera; charr (Salvelinus), taimen (Hucho), grayling (Thymallus), inconnus (Stenodus), whitefishes (Coregonus and Prosopium) and Pacific and Atlantic salmon and trout (Salmo and Onchorhynchus) (Dewey 2006). Species within this family are found throughout the Northern hemisphere, usually anadramous, predate on other smaller fish species and are commercially an important sport fish species (Dewey 2006). They have a large geographical range being distributed widely across Europe as far south as Portugal and as far north as Iceland and the Barents Sea above Norway and Sweden (WWF 2008; Crisp 2000). Salmonid species are found across the United States of America and Canada (Montgomery 1999). The main salmonid species, indigenous to the UK are Atlantic salmon (Salmo salar), Sea/brown trout (Salmo trutta), Grayling (Thymallus thymallus), Arctic Char (Salvelinus alpinus). Figure 2.1 shows the distribution of the Annex II Habitats Directive protected species, Atlantic salmon in the UK.
The life cycles of salmonids are complex due to the fact that their life stages have differing habitat requirements. Anadromous salmonids utilise freshwater environments for reproductive and nursery life stages and then migrate to the marine environment to mature and grow which is possible due to an abundance of food (Mills 1991). They begin their lives as eggs, which are laid in nests known as redds, created by the female (Crisp 1996) in the headwater streams of river systems between autumn and winter time (Thorpe et al. 1998). Embryos develop within the redd environment for approximately 4-7 months in UK rivers. Development is entirely dependent on temperature (Crisp 2000). For Atlantic Salmon the incubation period within the gravels is typically 440 degree days (Hendry and Cragg-Hine 2003a). Once hatched the young salmonids (alevins) develop further in the gravels sustained by consumption of their yolk sac reserves (Danie et al. 1984). After the yolk sac has been fully absorbed the fish begin to emerge from the gravels as swim-up fry (approximately 25mm), where
they create territories within the stream environment (Danie et al. 1984). The territories created correspond with feeding stations utilised by juvenile fish for the consumption of benthic invertebrates (Hendry and Cragg-Hine 2003). Juvenile fish (fry and parr) remain in rivers for up to a year before they begin to smolt, undergoing morphological and physiological adaptations which enable them to migrate out to the marine environment (Crisp 1996). Often fish will spend between 1-5 years at sea in feeding grounds in the North Atlantic, gaining body weight and reaching maturity before returning to their natal rivers to spawn (Crisp 2000). Figure 2.2 describes the life cycle of the Atlantic salmon (*Salmo salar*).

![Life cycle of the Atlantic salmon (*Salmo salar*)](http://www.nefsc.noaa.gov/salmon/)

The variation in life history observed across the entire family *salmonidae* is extremely large for a vertebrate species (Hutchings and Jones 1998). Populations inhabiting specific rivers differ genetically and ecologically because of their responses to genetic thresholds and the conditions of the environment surrounding them (Thorpe et al. 1998). Atlantic and Pacific salmon sp. return to their rivers of birth due to a homing instinct, thought to be related to specific chemical memories formed during juvenile life stages (Mills et al. 2003), which leads to unique genetic populations, not only in
different river catchments but also within tributaries of the same systems (Garcia de Leaniz et al. 2007; Waples et al. 2009). The homing instinct of salmonid populations to return to their natal rivers is one of the main drivers behind these regional and local adaptations as populations are separated geographically and hence maintain their genetic integrity. These different phenotype-habitat responses have been studied over many years and are important to recognise when studying salmonids, as every geographical region and area contains fish with slightly varying life history characteristics (Moreby and Hendry 2008).

The most striking variations in life history characteristics include age and body size at maturity, fecundity, time spent at sea and when migration occurs (Hutchings and Jones 1998; Thorpe et al. 1998). A review of Atlantic salmon, which evaluated how life history characteristics influence optimal growth at sea, found that Quebec populations' average age at maturity was 6.27 years whilst in the UK populations’ average age at maturity was 4.58 years (Hutchings and Jones 1998). Greater parr growth rate was found to be the largest factor that determines smolt age and decreasing freshwater residency between populations (Hutchings and Jones 1998). There is a relationship between latitude, smolt growth and migration in Northern latitudes giving rise to slower growing smolts and delayed migration. This is not only thought to be due to temperature differences but also to the amount of daylight hours in different latitudinal regions (Metcalfe and Thorpe 1990). Atlantic salmon populations inhabiting North American rivers develop at a much slower rate to European Atlantic salmon which are mainly dependent on the contrasting temperature regimes in the two areas and the difference in latitude resulting in different daylight hours (Metcalfe and Thorpe 1990). Studies describing the difference in morphology of fish have showed that Atlantic salmon which live in high gradient, high velocity rivers tend to have more streamlined bodies and longer heads in comparison to salmon living in low lying rivers with low velocity profiles (Claytor et al. 1991). Adaptations to local environmental conditions are important survival traits of salmonid populations (Armstrong et al. 2003).

The physiological condition of female fish, along with age and size, determine the weight and size of deposited eggs and embryos. Danie et al. (1984) showed a positive correlation between diameter of eggs and age of female. The rate of embryo development is directly dependent on the temperature regime of each specific river (Crisp 1981); for example embryo development in groundwater fed rivers which typically have higher stable temperatures compared to development in freshet streams which display lower more variable temperatures have shorter incubation periods (Acornley 1999). Acornley (1999) demonstrated that the higher temperature chalk
stream regimes within gravel beds increase brown trout embryo development in comparison with development in other river types.

Variations within salmonid populations of certain river systems result from the dynamic nature of the river environment and anthropogenic impacts on habitats. Changes in the life history characteristics of Atlantic salmon (*Salmo salar*) populations have been observed for almost the entire geographical range of the species (Webb and Campbell 2000; Youngson et al. 2002). An example conducted on the River Dee in Wales found there to be a shift in run timing from spring-summer to late summer-autumn salmon and the proportion of multi-sea winter fish (residing at sea for more than one year) returning has declined since the 1950s (Aprahamian et al. 2008). Youngson et al. (2002) using rod and net catch information reported similar information for run times in Scottish rivers over a similar time period. In particular they noted a general decline in the abundance of salmon over the period 1952-1997 (Youngson et al. 2002).

### 2.2 Salmonid population importance and decline

Over the last 30 years, salmonid populations across the globe have been in decline. In the UK there is particular concern about the declining status of the Atlantic salmon (*Salmo salar*) and the species is now considered to be endangered in many countries (Bardonnet and Bagliniere 2000; WWF 2001). In Europe Atlantic salmon are protected under the EU Habitats Directive and are classed as a ‘sensitive’ species under the new Water Framework Directive (Walsh and Kilsby 2007). A combination of factors has led to the decline of salmonids throughout their various life stages. Anadromous salmonids are particularly vulnerable to multiple stressors because they have such wide ranging habitat requirements for their different life phases. Stressors in the freshwater as well as the marine environment cause considerable damage to populations globally (Greig 2004; Mills et al. 2003). For example the Environment Agency, the authority responsible for monitoring, maintaining and improving fisheries in England and Wales, expressed particular concern about the southern chalk stream salmon populations after seeing dramatic declines in their annual fish monitoring programme during the 1990s and observing downward trends in rod catches (Figure 2.3) (Environment Agency 2004).
Declining salmonid populations have an impact on the socio-economic and ecological status of many regions in the UK. Numerous policies and management techniques have been or are being employed to conserve population numbers e.g. salmon action plans (SAP) which are aimed at improving fish passage and spawning habitat in rivers, designation of special areas of conservation (SAC), closed fishing seasons and limits on net and rod licences (Environment Agency 2004). A number of government bodies are charged with monitoring fisheries stocks. Evidence from Scotland’s salmon and sea trout industry suggested that annual expenditure on salmon and sea trout fishing increased from £50.4 million in 1988 to £73.5 million in 2003 (Scottish Executive 2004) even though salmonid numbers throughout Scotland have been declining since the mid-1970s. In the 1990s the average catch was 29% of the 1960’s average (Scottish Executive 2004).

The state of salmonid stocks in the rest of the UK tells a similar story to those in Scotland. The Environment Agency reports annually on fish stocks throughout England and Wales. The 2007 report suggests that there are declines on annual rod and net catches based on a 5-year mean, with some increases in population numbers in northern rivers like the Tyne and Tees but with general declines in other rivers like the River Itchen in Hampshire (Environment Agency and CEFAS 2008). Catch and release schemes operate on all 64 principal salmon rivers in the UK with 10% fish released in 1993 to over 50% being released within the past five years (Environment Agency and CEFAS 2008). This is likely to increase brood stock in rivers and the amount of eggs available to the population for recruitment. The salmonid decline not only directly affects sport fishermen but also indirectly affects local businesses such as hotels, sport

Figure 2.3 Atlantic salmon (Salmo salar) rod catch declines in four southern England chalk streams (1954-2002). Adapted from source (Environment Agency 2004)
lodges, shops and local enterprises that rely on trade brought to the area by anglers (Radford 2007).

The ecological importance of salmonids to their environment and ecosystems has been documented in many studies. Salmonids are vital members of not one but two food webs; freshwater and marine. Their status as a ‘keystone’ species in numerous food chains should not be underestimated (Willson and Halpuka 1995). Many animals rely on salmonid population success to sustain their own populations. Willson and Halpuka (1995) described the amount of wildlife that consume Pacific salmon with no less than 13 mammal species, 25 bird species and 12 fish species. In UK freshwater systems salmon are a major food source for the common heron and cormorant as well as the merganser and goosander and in marine environments sharks, seals and large cod utilize salmon as a principal food source (Mills et al. 2003; Danie et al. 1984). The recovering otter population in the UK is causing some concern over the predation of Atlantic salmon (Hendry and Cragg-Hine 2003). The mass migration and mortality of salmonids in the Pacific North West have been shown to contribute substantially to the terrestrial nutrient inputs of carbon and nitrogen which could be vital to forest ecosystems (Cedarholm 1999; Drake et al. 2006; Reimchen et al. 2002). The role of marine-derived nutrients from the Atlantic salmon is less well understood but studies suggest net imports of nitrogen and phosphorus which may have been important in oligotrophic upland tributaries prior to human disturbance (Mills et al. 2000).

The major factors causing salmonid populations to decline globally and in the UK can be placed under four main headings. These headings are; over-exploitation, barriers to migration, loss and degradation of suitable habitat and the growth of the aquaculture industry (Sear and DeVries 2008; Mills et al. 2003).

In the marine phase of their life cycle salmonids come under pressure from anthropogenic factors such as over-fishing at feeding grounds in the North Atlantic and fishers using species indiscriminate fishing methods for their catches (Mills et al. 2003). Drift and set-gill nets in the high seas interceptor fisheries and coastal fisheries pick off fish that swim near to the surface such as Atlantic salmon; then are often discarded as by-catch as they are not the target fish for fishers (Danie et al. 1984; Hendry and Cragg-Hine 2003). Exploitation at mouths of estuaries using seine nets and poaching of spawning fish in the fresh water environment have also been highlighted as having a negative impact on UK populations although salmon and sea trout fishing effort as a whole has been decreasing since the 1980s (Environment Agency 2004).
In marine and fresh water eco-systems there is a growing threat to wild salmon populations from commercial salmon farms. Farmed salmonids now far outnumber wild salmonids in total population numbers (Fiske et al. 2006). The high density of these fish farms in certain regions has led to ever increasing direct and indirect threats to native wild salmonid populations. Direct threats to wild salmonids from farmed fish include disease, contamination of the wild gene pool (Clifford et al. 1998) and competition for resources from escaped fish. Contamination of the wild gene pool can ultimately lead to a reduction in fitness of the species (Fiske et al. 2006; Mills et al. 2003). Indirect effects such as poor water quality due to waste produced by farmed fish can cause benthic pollution and oxygen depletion for all aquatic organisms (Black et al. 2008). There are associated impacts on available food resources for wild fish as farmed fish consume large amounts of food. The majority of food comes from the ocean where large amounts of pelagic fish are removed in direct competition with wild fish to become fishmeal and fish oil products for use in the aquaculture and agricultural industries (Tacon 2005).

Barriers to migration are thought to have significantly contributed to declines in salmonid populations throughout the UK and Europe (Hendry et al. 2003). Weirs and sluices operate to regulate river flows and impound water. They often create physical barriers across rivers which returning mature fish find difficult and sometimes impossible to negotiate. Thorstad et al. (2008) reviewed the factors affecting upstream migration of fish and concluded that prolonged swimming speed versus burst swimming speed leave fish with an oxygen debt that could explain why fish do not navigate some barriers well and often decide to swim downstream in search of neighbouring rivers. As mitigation fish passes or ladders are often installed on migratory rivers across Europe and North America but there is still debate as to whether fish can find the entrance to passes and successfully navigate them once they have been located (Mills 1989).

Climate change could also cause a detrimental impact to salmonid populations with increasing sea levels and water temperatures potentially affecting food availability and disrupting migration pathways (Graham and Harrod 2009). Studies have shown that the shifting of thermal regimes within the oceans could lead to salmonids being excluded from the oceans altogether should CO$_2$ levels double in the next 50 years as climate modellers suggests (Welch 1998). Using models to predict the different scenarios that could occur as a result of climate change, Walsh and Kilsby (2007), discuss the implications of climate change on flow regimes and the effect on the life stages of salmonids in the UK. Climate change scenarios showing an increase of temperature (2.5-3 C), drier summers, wetter winters and increasing precipitation rates affect flows
required for fish to achieve upstream migration as low flows occur in summer and autumn months (Walsh and Kilsby 2007). Higher, flashier flows in winter at spawning time have the potential to detrimentally impact on populations as they could scour out incubating eggs (Walsh and Kilsby 2007).

Graham and Harrod (2009) hypothesised that increasing temperatures could exclude salmonids from some southern English rivers due to changing environmental variables and the timing of seasonal events which salmonids use to time migration. Actual observed climate change in alpine regions (increase in 1.1 °C between 1864-2000) found that emergence of trout fry advanced on average between 2.8-5.6 days which combined with higher flows at emergence time could have a potential impact on population dynamics. However further research is needed to substantiate this (Scheurer et al. 2009). Solomon and Sambrook (2004) discuss the relevance of thermal barriers to fish migration. They found that low flows and higher temperatures delayed fish in tidal waters and often led to failure of entering the river system (Solomon and Sambrook 2004).

Anthropogenic pollution causes the loss and degradation of suitable habitat for all different life phases (Hendry et al. 2003). Pollution causes deterioration of the quality of water within catchments. Poor water quality has negative impacts on salmonid populations that need good water quality to survive; hence they are often used as key indicators of a river's health (Environment Agency 2004). Problems associated with pollution degrading water quality and declining salmonid numbers within rivers is not a new issue and has been recognised by policy makers since the early nineteenth century (Ayton 1998). In 1825 a select committee to review the state of salmon fisheries in Scotland and the UK advised that people should "guard against discharges inimical to fish" (Anon 1825 sourced in Ayton 1998).

There are numerous types of chemical, biological and physical non-point and point source pollution that can degrade salmonid habitat including nutrient pollution, endocrine disruptors, invasive non-native species, thermal pollution and increasing sediment loads (Hendry et al. 2003). Most point source effluents such as domestic sewage outfall pipes and industrial effluent outfalls are rigorously controlled and monitored by government agencies so diffuse non-point source pollution such as endocrine disruptors and land surface run-off including nutrient and sedimentation inputs are perceived to be more of a threat to water quality and therefore salmonid population numbers (Environment Agency 2004; Hendry and Cragg-Hine 2003). Siltation of spawning gravels is a common occurrence in catchments afflicted with high levels of anthropogenic activity such as mining, forestry and intensive arable and
livestock agriculture (Milan et al. 2000; Greig et al. 2005a). Sedimentation of salmonid spawning gravels was highlighted as one of the major factors limiting incubation success and hence fry survival in UK rivers in the early 1980s.

2.3 General habitat for salmonids

Described below are some characteristics and general requirements of salmonids throughout their life stages. It is by no means meant to be an exhaustive list but demonstrates the wide variety of habitat needs salmonids require throughout their life stages. Juvenile salmonids, after emerging from their gravel nests (usually at night), occupy territories near to the substrate, where they feed on benthic invertebrates, that ideally have relatively low flows (Armstrong et al. 2003). Pacific juvenile salmonids were found to prefer areas with river flows <15 m s\(^{-1}\) and lots of wood cover when residing in large river systems (Beechie et al. 2005). Riley et al. (2006) investigated the habitat preference of salmonid fry in a southern English chalk stream and found that a large range of substrates were used by trout compared with salmon. Weed patches were utilised by fish in the night, whilst clear gravel areas with differing depths and flow were used during the day (Riley et al. 2006).

Natural pool/riffle sequences and sinuosity within channels encourage fish to create territories ideally with cover such as undercut banks, boulders and riparian vegetation (Crisp 1996). Larger fish occupy deeper and faster flowing reaches than smaller fish (Armstrong et al. 2003). Migrating adults have also been observed to shelter underneath boulders etc. hiding from predators (Crisp 1996). Night cover and stream discolouration are also thought to encourage upstream movement (Crisp 1996). Low temperatures impair the upstream migration of fish as do low flows within rivers (Armstrong et al. 2003; Hendry et al. 2003b). Spawning habitat requirements of salmonids and those which influence egg incubation success are discussed further in section 2.3.1.

2.3.1 Salmonid spawning habitat and preference

To manage salmonid fisheries effectively, it is vital to understand how the ecology of the species and the physical in-stream processes interact which define and shape their habitat (MacIsaac 2009). This is complex as salmonid species are known to spawn in a variety of habitats (e.g. rivers, lakes and inter-tidal areas) with a great diversity in
habitat characteristics (Morbey and Hendry 2008) e.g. within river ecosystems - freshet, upland, lowland and groundwater-fed streams all contain habitats that are utilised by spawning Atlantic salmon. It is important therefore to consider the multiple environmental controls that affect spawning habitat quality as opposed to looking at single factors (Lorenz and Eiler 1989; Lapointe et al. 2004; Greig et al. 2005a).

The broad focus of this research is based on the problem of salmonid habitat degradation in freshwater in the UK, specifically the reduction in suitable spawning grounds in headwater streams. There are many factors which affect salmonid spawning habitat suitability for example; flow rate, substrate, temperature, intra-gravel flow velocity, up-welling ground water and dissolved oxygen concentrations. All contribute to the conditions which influence egg survival within redd gravels (Greig 2004; Louhi et al. 2008; Malcolm et al. 2008). Other factors which affect the utilisation of habitat patches by individual spawners are; the size and number of pools, concretion of gravels, shading and cover from riparian vegetation, and competition with other fish (inter/intra species specific). Evolution of the salmonid family has led to the release of a smaller number of eggs compared with other family groups in the cyprinid order; so in terms of the energy expended in reproduction, it is comparatively high which means that the choice of spawning habitat is of great importance to the species in order to maximize the survival potential of the eggs (Bardonnet and Bagliniere 1997). Figure 2.4 describes the redd building process carried out by female fish at spawning grounds.

![Figure 2.4. The redd building process of salmonids in three main stages (Soulsby et al. 2001). A) sideways, full body digging action to create a depression in the river bed. B) hen and cock salmon depositing and fertilising eggs in depression. C) upstream digging of river bed to cover eggs with cleansed gravels for protection during development.](image)

2.3.1.1 Macro-scale and meso-scale controls on habitat

Larger macro habitat features have been revealed to influence salmonid spawning preference. Macro-habitat controls on spawning habitat quality are defined as ranging
from the catchment (several km) to the reach scale (~100m) and include catchment land-use, abstraction of groundwater, underlying geology, geomorphology and planform of rivers. Meso-scale controls on habitat can be defined as occurring from a patch (~1m) up to a channel reach (~100m) and include channel morphology, upwelling groundwater, in-stream vegetation patterns and sediment deposition. The development of a framework to investigate the potential temporal and spatial differences of distinct channel morphologies has been useful in determining preferential spawning habitat at the catchment, macro-scale and reach meso-scale (Montgomery and Buffington 1997). Montgomery (1999) observed that different species of Pacific salmon preferentially chose spawning sites in typical pool-riffle channel morphologies over other river types such as cascade or step pool reaches. Using the morphology classifications described in Montgomery and Buffington (1997), Atlantic salmon populations in a Scottish upland stream were also found to preferentially spawn in pool-riffle reaches (Moir et al. 2008). It should be noted that there are salmonid spawning rivers in the UK which do not adhere to the pool-riffle morphology described by previous studies as preferential locations for spawning salmonids (Sear et al. 2008). Figure 2.5 displays the typical pool/riffle sequence displayed in many rivers utilised by salmonids at the meso-scale.

![Diagram](image)

**Figure 2.5.** Diagrammatical representation of suitable spawning sites chosen by Atlantic salmon (*Salmon salar*) in rivers at meso-scale (Sear et al. 2008). The low pressure regions called riffles are preferential to spawning salmonids due to the exchange of surface and hyporheic water as defined by the thick black line.

A study carried out in Japan observed that there was a significant relationship between the sinuosity of channels and salmonid spawning; the greater the average sinuosity of
the river, the greater the abundance of salmonid redds (Fukushima 2001). Different salmonid species prefer different habitat areas at the catchment scale. Atlantic salmon (*Salmo salar*) were shown to prefer spawning within the main stem or larger tributaries of river networks, whereas brown trout (*Salmo trutta*) tended to choose spawning sites in the smaller headwater streams of river systems (Crisp 2000). High winter flows in larger systems can cause scour of river bed gravels. This is a particular problem in large North American river systems and Atlantic salmon and Pacific salmon alike have to contend with the problem of their eggs being scoured from the redds they inhabit if egg pocket depth is shallower than high flow scour events (MacIsaac 2009; Kondolf et al. 2008).

### 2.3.1.2 Micro-habitat controls

Micro-scale, local factors defined as occurring at the patch or redd scale (<3m) which include sediment accumulation within the redd, dissolved oxygen concentrations and intra-gravel flow pathways all play a role in salmonid spawning habitat preference. Three main physical factors and their interactions control spawning suitability in rivers; channel morphology, discharge and sedimentary characteristics (Moir et al. 2006).

In general, but not in all cases, spawning areas are characterised by shallow, fast flowing and broken water surface areas. Salmonids need to be able to hold position in the water to successfully deposit eggs and milt but there must be enough flow to carry fine sediment downstream (Beechie et al. 2008). These areas are more likely to be at the head or tail of pools or the crests of riffles as down-welling water is encouraged through the gravels at these areas (Crisp and Carling 1989; Mills 1989; Moir 1998). It has been observed that hydraulic features such as water velocity and depth tend to fall within a small range for ideal spawning habitat. Discharge and channel hydraulics have long been related to the habitat requirements of species within rivers (Moir et al. 2006). Riverine species need a certain discharge to maintain healthy populations. If there is no water available survival of aquatic species is nil. Suitability curves drawn up from many data sources suggest flow velocities for the most suitable spawning habitats range between 0.35 – 0.65 m s$^{-1}$ in some studies (Louhi et al. 2008) and 0.25-0.90 m s$^{-1}$ in others (Hendry and Cragg-Hine 2003a). Studies show that similar ranges of depth, velocity and sediment characteristics seem to be utilised by spawning salmonids whether they are using smaller tributaries or the main stem of the river (Moir et al. 2002). One way to describe the hydraulic characteristics of suitable salmonid habitat is to use the Froude number (Equation 2.1), proposed by Moir et al. (2002);
\[ Fr = \frac{v}{\sqrt{dg}} \]

Equation .2.1

where;
\begin{align*}
  v &= \text{velocity} \\
  d &= \text{depth} \\
  g &= 9.81 \text{ms}^{-1} \text{ gravitational acceleration}
\end{align*}

which gives the ratio velocity to depth found in habitats utilised by fish and creates a dimensionless number that allows comparison between river systems and species. The Froude number must satisfy the condition \(0.2 < Fr < 0.75\) for spawning habitat to be considered suitable (Moir et al. 2006). The minimum depth usable by spawning fish was found to be 0.125m (Armstrong et al. 2003). In general, water depths range from 20-50cm for Atlantic salmon to spawn (Louhi et al. 2008). Moir et al. (2006) looked into whether there was any relationship between channel morphology, discharge and hydraulic interactions at salmonid spawning sites. They discovered that salmonids spawn at many discharge levels, although optimum discharges were similar and hydraulic heterogeneity were not significant in the utilisation of spawning sites (Moir et al. 2006). In contrast the availability of suitable sediments seemed to control the use of sites for spawning (Moir et al. 2006).

Sediment characteristics are an important factor to consider when looking at suitable sites for spawning. Habitat requirements differ during actual redd construction, incubation and emergence (Kondolf et al. 2008). Spawning females need to be able to move the substrate to dig their redds, incubating eggs/embryos need adequate supplies of oxygen and adequate waste removal provided by flow through sediment pore spaces (Kondolf et al. 2008) and emerging fry must be able to surface from the gravels at emergence (Crisp 1993). Although the females displace a large percentage of the fine material within the redd during redd building (Chapman 1988), numerous studies have investigated the impacts of different sediment grain sizes and concentrations on spawning gravels (Crisp 1993; Levassuer et al. 2006; Lapointe et al. 2004; Greig 2004). Louhi et al. (2008) drew habitat suitability curves for substrate conditions for Atlantic salmon spawning gravels using data from many rivers and concluded that optimal sediment size ranged between 16 – 64mm. Composition of spawning gravels tends to be formed of a mix of cobbles (22->64mm), pebbles (2-22mm) and finer material (<2mm) (Hendry and Cragg-Hine 2003). Bardonnet and Bagliniere (1997) discovered that commonly river bed substrate found at spawning
sites of Atlantic salmon were composed of little sand particles and with a majority of coarser gravel and cobbles.

Threshold levels for fine sediment (<2mm) have been postulated from a number of historical studies aimed at allowing for 50% survival to emergence, see Table 2.1 (Milan et al. 2000). Recent examples of fine sediment thresholds allowing for greater than 50% survival are; 0.2 % and 0.3-0.4 % material <63μm reported by Levasseur et al. (2006) and Julien and Bergeron (2006) respectively in field studies using Atlantic salmon eggs. Lapointe et al. (2004) found a slightly higher threshold of 1.5% silt in laboratory experiments when examining the interaction between silt and sand particles within redds.

Fine sediment is thought to be more prevalent in certain river types. Sediment analysis carried out on three river typologies in southern England found that chalk rivers have the greatest loadings of fine sediment (<2mm) with an average of 48% by weight and all sites had above 32% matrix sediments (sand, silt and clay particles) in the substratum (Milan et al. 2000). Fine matrix material settles at different rates within gravel bed rivers, depending on the initial framework and size and density of particles, although matrix particles are more likely to stay nearer to the surface unless flood events force settled particles deeper into the bed (Sear et al. 2008). Armoured beds with stable gravel structure and low mobility due to stable flows allow fine material to penetrate the coarse surface particles until they reach the sub-surface layers where they become trapped and accumulate, filling pore space and decreasing intra-gravel flow from the bottom up (Sear et al. 2008).

<table>
<thead>
<tr>
<th>Source</th>
<th>Maximum per cent finer than grain size (mm)</th>
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<tbody>
<tr>
<td></td>
<td>0.83</td>
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<tr>
<td>Bjornn (1969)</td>
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<tr>
<td>Bjornn (1969)</td>
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<td>Cedarholm and Salo (1979)</td>
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<td>Hausle (1973)</td>
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<td>Hausle and Coble (1976)</td>
<td></td>
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<tr>
<td>Irving and Bjornn (1984)</td>
<td></td>
</tr>
<tr>
<td>Iwamoto et al. (1978)</td>
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<tr>
<td>Koski (1966)</td>
<td>21</td>
</tr>
</tbody>
</table>
Recent studies focussed on particle sizes <2mm to <63μm

<table>
<thead>
<tr>
<th>Source</th>
<th>Maximum per cent finer than grain size (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&lt;0.063</td>
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<tr>
<td>Lapointe et al. (2004)</td>
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</tr>
<tr>
<td>Levasseur et al. (2006)</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Julien and Bergeron (2006)</td>
<td>1.5</td>
</tr>
<tr>
<td>Heywood et al. (2007)</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td>0.6</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.6</td>
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Table 2.1. Threshold fine sediment allowing for 50% survival to emergence in previous literature (Milan et al. 2000) and updated with recent studies findings.

The act of redd building by spawning fish noticeably loosens matrix gravels as a result of disturbance of the framework material and re-deposits finer material to the base of the redd (Crisp 1996; Crisp and Carling 1989). The morphology and sediment stratigraphy of the redd influences the deposition of sediment in and around it. Coarser materials are deposited in the pot area and finer material is located in the tail spill area (Sear et al. 2008). Redd building also increases the flow of water into the pot and egg pocket area due to its morphology (Crisp and Carling 1989; Sear et al. 2008). Figure 2.6 describes the flow of water from the water column into the redd and the flow pathways through the redd within the interstitial environment.
Figure 2.6. Diagram of flow into and through a salmonid redd cross-sectional view (Sear et al. 2008). The isolines and numbers correspond to the flow velocity experienced at different areas within the redd. Near to the surface head of the redd experiences the greatest flow inducing surface water into river gravels.

Habitat quality described in terms of the discrete measurements of specific parameters mentioned above is a helpful and tried and tested approach to determine suitable spawning sites. However it is also useful to look at the size and the connectivity of these sites in relation to each other as this could influence spawning decisions (Issack et al. 2007). In North America a study to determine the relative importance of habitat quality, size and connectivity of spawning patches of Chinook salmon (Oncorhynchus tshawytscha) found that in terms of spawning density, the size and connectivity of patches were greater predictors of spawning density than habitat quality (Issack et al. 2007). It is important to consider these implications when managing salmonid habitats.

A factor which affects the development of eggs and embryos in the hyporheic environment is the temperature within the interstitial environment (Morbey and Hendry 2008). Dissolved oxygen levels required by fish increase with increasing temperature, in contrast to dissolved oxygen decreasing within surface water with increasing temperature (Crisp 2000). Different temperature regimes are found within spawning gravels which as mentioned earlier is another life history adaptation of salmonids to their habitats. When water temperatures fall below 2 °C, development of embryos stop and where temperatures exceed 12 °C, development is impeded and defects can form (Danner 2008). Temperature is the single most important control on the rate of development of incubating eggs and alevins (Acornley 1999). Temperature can also influence the size of alevins at hatch as lower temperature incubation induces slower metabolic development and hence gives rise to larger fry (Crisp 1996).
2.3.2 Sedimentation of spawning habitat and associated impacts

Sedimentation of spawning habitat has been shown to be one of the major factors contributing to declining salmonid populations within the UK (Chapman 1988; Greig et al. 2005a). Research has been carried out for many years into the sedimentation of salmonid spawning gravels and the degradation of the hyporheic zone, including field studies (Frostick et al. 1984; Sear 1993; Acornley and Sear 1999; Greig et al. 2005a) and controlled laboratory experiments (Beschta and Jackson 1979; Carling 1984; Greig et al. 2005b). Conclusions drawn from earlier research suggests that it is the amount of fine sediment available in a system that ultimately dominates accumulation rates into framework gravels. A regulated river study showed that infiltration of sediment was supply restrained until intense rainfall and high flows accessed new sources (Sear 1993).

The hyporheic zone is the zone of saturation within the stream bed (Boulton at al. 1998). Typically salmonids bury their eggs in the top 0.5-0.2m of this zone, depending on the size of the fish (Crisp and Carling 1989). The factors influencing this area of the river bed are significant in management and rehabilitation strategies for creating suitable spawning conditions for fish (Malcolm et al. 2008). Fine sediment intrusion and accumulation has been found to degrade the hyporheic zone in UK spawning habitats (Greig 2004). ‘Infiltration’ of fine material into the redd environment describes the process by which sediment moves into the redd and ‘accumulation’ explains the summation of fine material within redds over the incubation period (Sear et al. 2008). The depth of infiltration is dependent on the difference in pore size between the penetrating grains and the coarser bed material and boundary/critical shear stress limits (Diplas and Parker 1992) and accumulation is dependent on sediment supply and deposition rates.

2.3.2.1 Sediment size and impact on survival

Field and laboratory studies have shown that particle size has a large impact on the effect that sediment will have on egg survival and survival to emergence (Acornley and Sear 1999; Lapointe et al. 2004). Coarse sand can form a cap or seal over the redd and infill spaces from the top down (Carling 1984; Acornley and Sear 1999), hence inhibiting the emergence of fry from within the redd (Crisp 1993), a process known as ‘entombment’ (Sear et al. 2008). This mechanism is usually associated with accumulation throughout the incubation period. Very fine sands, silts and clay accumulate within pore spaces from the bottom to the top of a redd (Acornley and Sear
directly smothering eggs and indirectly causing a decline in intra-gravel flow velocity and decreasing dissolved oxygen levels within the redd environment (Soulsby et al. 2001; Greig et al. 2004; Julien and Bergeron 2006).

Sear et al. (2008) differentiated between the two mechanisms of redd sedimentation by suggesting that finer silt and clay particles are more relevant in the survival success of incubating salmon progeny whilst sand particles are related more to the trapping of emerging fry. The combination and concentration of different sized particles has marked effects on egg survival and has been shown in many studies (Phillips et al. 1975; Lapointe et al. 2004). Phillips et al. (1975) found an inverse relationship between increasing introduced concentrations of 1-3mm sand and emergent survival of coho salmon and steelhead trout in a controlled laboratory experiment. A small increase in percentage silt content within the mixture caused survival to emergence to decline rapidly. For example, a gravel mix with 15% sand found survival declining from 60% - 20% with an increase in silt content from 0%-4% (Lapointe et al. 2004).

Particles of <63μm are the most important size fraction in causing detrimental impacts on salmonid survival, as even a very small increase in loadings can lead to large declines in survival (Grieg et al. 2005a; Julien and Bergeron 2006). Similar findings were observed by Levasseur et al. (2006) who discovered that the proportion of silts and fine sands (<0.125mm) within redd gravels in Quebec explained 83% of the variation in embryo survival. However there is some debate as to whether research into just silt and clay particles (<63μm) is addressing the problem of sedimentation as larger particles (2mm) could be causing deleterious effects on spawning success (Evans et al. 2006). A recent study looking into the impact of sedimentation on Masou salmon (Onchorynchus masou) in Japan found that survival rate of embryos decreased exponentially with increasing <2mm fine sediment and when fines (<2mm) exceeded 60% most embryos died (Yamada and Nakamura 2009). They also found that decreasing permeability of the redd environment may be due to the grain size distribution, not just one specific grain size infiltrating the redd environment as has been suggested in previous studies (Yamada and Nakamura 2009).

Heywood et al. (2007) found that Atlantic salmon embryo survival dropped to 50% when redd infiltrated material comprised 9% (<2mm) and 8% (<1mm) material and survival dropped to 0% with a small increase of 14% (<2mm) and 12% (<1mm) in lowland agricultural chalk rivers. Sediment size information, collected in infiltration experiments carried out on the Newmills Burn in Scotland, showed that virtually all of the fine material collected over the incubation period was between 1-2mm with only 2% clay and silt content and egg mortality rates were above 20% in four of the redds.
measured (Soulsby et al. 2001). This study was classed as having very high infiltration rates for UK rivers and showed that sand particles (1-2mm) were the most detrimental to the survival of salmonid embryos in some redds (Soulsby et al. 2001). Supposedly in this case, entombment of emerging fry was the mechanism by which survival was most affected.

2.3.2.2 Dissolved oxygen levels and sedimentation

Infiltration of fine material into the redd not only directly physically inhibits emergence and smothers eggs but it also indirectly affects the amount of dissolved oxygen contained within stream water that will come into contact with the incubating salmon progeny. Incubation success relies on an adequate supply of oxygen to the eggs within the redd. This supply needs to be big enough to drive the oxygen gradient which forces oxygen across the egg membrane to supply the embryos with fresh oxygen for each development stage (Chevalier and Carson 1984; Greig et al. 2007).

Greig et al. (2005b) showed the direct impact that clay particles can have on the exchange of oxygen across the egg membrane. Laboratory experiments which involved Atlantic salmon ova respiration rates being recorded with increasing clay sediment loadings showed that 0.5g of introduced clay sediment produced an average reduction in oxygen consumption of 96% (Grieg et al. 2005b). This was most likely due to a thin film of clay settling on the eggs, suggesting that the clay physically blocked diffusion pathways on the chorion (outer layer) of the eggs (Greig et al. 2005b). This highlights the impact that very small, fine sediment particles (<63μm) have on the oxygen availability to incubating salmon young.

Indirect impacts of fine sediment on dissolved oxygen supply to incubating eggs include the physical blocking of interstitial pore spaces within the gravel framework (Chapman 1988; Sear et al. 2008). Figure 2.7 explains the factors and processes involved in controlling the availability of oxygen within river gravels.
The link between sedimentation and reduced dissolved oxygen levels within the hyporheic zone has been explored in a number of recent studies (Malcolm et al. 2005; Greig et al. 2007; Heywood and Walling 2007; Malcolm et al. 2008). The hyporheic zone is the active zone that connects surface stream water to ground water within a river bed (Boulton et al. 1998). The exchange of surface and hyporheic water is of great importance to incubating salmonid progeny because embryos require dissolved oxygen for growth and development and intra-gravel flow supplies this oxygen to the eggs and embryos (Malcolm et al. 2005). There is a need for high resolution spatial and temporal data in order to explain complex oxygen fluxes with intra-gravel flow interactions within the hyporheic zone. (Malcolm et al. 2008).

Low dissolved oxygen levels in spawning gravels can be attributed to the accumulation of fine sediment within redds (Heywood & Walling 2007; Levasseur et al. 2005; Greig 2004). Heywood and Walling (2007) observed that reduced dissolved oxygen levels within the interstitial environment reflected the decreased permeability of the redd, with increasing fine sediment accumulation. Greig (2004) collated empirical data demonstrating the relationship between dissolved oxygen supply to incubating embryos and fine sediment accumulation across a number of different river types and he observed that with increasing sediment infiltration and accumulation, oxygen levels decreased. The variability seen within the data from different redds and rivers here
demonstrates the dynamic and complex processes involved with oxygen supply to eggs which needs further definition in future research (Sear et al. 2008). Oxygen flux to incubating eggs has been suggested as the best indicator for incubation success (Greig et al. 2005a) so it is an important factor to consider when looking into the effect of sedimentation on spawning success. Grieg et al. (2007) gives a comprehensive review of factors influencing dissolved oxygen availability to salmonid embryos.

2.3.2.3 Oxygen thresholds within spawning gravels

Oxygen-supply related thresholds have been proposed that could help when assessing incubation habitat quality (Greig et al. 2007). Low habitat quality in terms of oxygen availability would be 0-6mg l\(^{-1}\) and high habitat quality would be >9mg l\(^{-1}\) (Greig et al. 2007). Oxygen concentrations and related embryo survival from previous studies are summarised in Table 2.2. These estimates of oxygen thresholds are likely to help inform policies that aim to protect spawning habitats across the UK. However it is worth noting that there are many other factors including temperature, groundwater inputs, intra-gravel flow velocity and dissolved oxygen concentration that affect the quality of salmonid spawning habitats. Setting threshold levels for dissolved oxygen levels are useful in quantifying habitat quality within redd environments which is currently lacking in management options and tools. However threshold levels are likely to differ between different rivers, species and hydraulic regimes. Chapman (1988) suggests that any reduction in dissolved oxygen levels from initial status is likely to reduce survival to emergence. Dissolved oxygen availability and supply to incubating eggs is highly dependent on the levels already contained within surface and ground water and also the associated intra-gravel flow velocities which transport the oxygen down through the redd environment. The large body of research discussing survival related to dissolved oxygen thresholds mentions numerous critical and sub-lethal levels relating to many different rivers and species (Malcolm et al. 2008).
<table>
<thead>
<tr>
<th>Source</th>
<th>Dissolved Oxygen (mg/l) related to embryo survival</th>
<th>Life stage and species</th>
<th>Other environmental variables measured</th>
</tr>
</thead>
</table>
| Turnpenny and Williams (1980) | 4.8 mg/l = 0% survival 6.5 mg/l = 50% survival 8 mg/l = 100% survival | Rainbow trout eggs to hatch | Apparent velocity  
<2 cm h\(^{-1}\) = 0% survival  
5 cm h\(^{-1}\) = 50% survival  
>100 h\(^{-1}\) = 100% survival |
| Malcolm et al. (2003)          | <7.6 mg/l embryo survival to hatch was negligible | Atlantic salmon and brown trout survival of Eggs to hatch | GW-SW interactions e.g. temperature, alkalinity Sedimentation |
| Heywood et al. (2007)          | 4.2 mg/l – 5.2 mg/l = low embryo survival  
>8.9 mg/l = higher survival | Atlantic salmon eggs and alevins | IG flow  
Sedimentation rates |
| Grieg et al. (2007)            | Habitat quality  
Low = 0-6 mg/l  
Med = 6-9 mg/l  
High = >9 mg/l | Atlantic salmon eggs and alevins | IG Flow  
Low = <1 cm h\(^{-1}\)  
Med = 2-15 cm h\(^{-1}\)  
High = >15 cm h\(^{-1}\)  
Sedimentation rates |

Table 2.2. Dissolved oxygen levels within the redd environment in previous studies
2.3.2.4 Intra-gravel flow and sedimentation

The nature and development of the redd structure encourages flow through the gravels due to its topography and structure (Sear et al. 2008). The hump of the redd, containing the egg pocket, increases surface flow over it and a shear zone forms where water is forced down into the surface gravels (Sear et al. 2008). Geist (2000) observed that hyporheic water discharged into Chinook salmon spawning patches was larger in volume and had higher dissolved oxygen concentrations and lower specific conductance than discharges into non-spawning patches. This suggests that utilised spawning gravels have higher permeability than non-spawning gravels, highlighting the importance of redd building to embryo survival, as cut gravels are shown in this study to be more permeable than uncut gravels.

Dissolved oxygen-rich water flowing through the redd is the key to maintaining a good supply of oxygen to the incubating eggs (Grieg 2004). Intra-gravel flow is important for two main reasons. Firstly, for replenishing the supply of dissolved oxygen to the incubating eggs and secondly, to remove toxic metabolic wastes produced by the eggs through their development (Crisp 2000; Greig et al. 2005c). Sediment infilling into pore spaces within the redds impedes the interchange of surface water and ground water due to reduced porosity and permeability of the river bed gravels (Soulsby 2000; Heywood and Walling 2007) which can lead to poor intra-gravel flow velocities. At the redd scale interstitial velocities were shown to decrease in storm events when more than 7 kg m\(^{-2}\) of sand was deposited on experimental redds (Zimmerman and Lapointe 2005).

Intra-gravel flow measurements are used to describe incubation success (Greig et al. 2005a) however they are not necessarily always linked. Previous research suggests that intra-gravel flow velocity measures are secondary to the effects of low dissolved oxygen for embryo survival and development (Malcolm et al. 2008). The River Test, a chalk stream in Hampshire, suffered a sharp decline in intra-gravel flow velocities that did not correspond with a related increase in sediment accumulation indicating within gravel processes are having an effect rather than accumulating sediment (Grieg et al. 2005a). Lapointe et al. (2003) suggested that in high sand loading systems there is no threshold intra-gravel flow velocity that can increase survival to emergence, although survival was shown to increase with flow velocity. This laboratory study suggests that regardless of flow strength, fine sediment particularly sand (coarse 0.5-2mm and fine 0.063mm-0.5mm), is likely to impede intra-gravel flow, resulting in little flushing effect within redds (Lapointe et al. 2003). Intra-gravel flow velocities have been suggested for
spawning habitat quality as follows; <1 cm h\(^{-1}\) for low habitat quality, 2-15 cm h\(^{-1}\) for medium habitat quality and >15 cm h\(^{-1}\) (Grieg et al. 2007) and are a good general indicator of the range of flows for suitable spawning habitat. Greig et al. (2005a) showed that fine sediment accumulation over the incubation period was directly related to declining intra-gravel flow velocity. This work shows that there is potential for measurements of the composition of sediment at specific sites to provide information about the potential intra-gravel flow velocities experienced within the redd environment (Malcolm et al. 2008).

Intra-gravel flow velocities are difficult to measure accurately as they are complex and follow three-dimensional pathways within riffles (Soulsby 2001). Exchange of river surface water and the underlying hyporheic zone water naturally follows horizontal and vertical pathways (Boulton et al. 1998). Earlier work has created methodologies to measure intra-gravel flow effectively (Carling and Boole 1986; Zimmerman and Lapointe 2005; Carling et al. 2006).

2.3.3 Effect of up-welling groundwater on salmonid spawning

Recent research has highlighted the importance of water quality within the hyporheic zone in relation to salmonid embryo survival (Boulton et al. 1998; Soulsby et al. 2001 Moir et al. 2002; Malcolm et al. 2003; Malcolm et al. 2004; Malcolm et al. 2005). The processes which control hyporheic water quality depend on the residence time and flow pathways. These are unique to their own hydrological and geomorphological conditions within specific rivers or catchments (Malcolm et al. 2008). Long residence (>5 years) ground water contains negligible dissolved oxygen and high solute concentration compared with short residence surface water, characterised by high dissolved oxygen concentrations and low solutes (Malcolm et al. 2008). This has obvious implications for salmonid embryo success. The effects of up-welling groundwater have been investigated in detail at many spatial and temporal scales (Malcolm et al. 2003, 2004, 2005). Catchments dominated by permeable, porous rock (e.g. chalk and limestone) often have substantial aquifer stores of groundwater that influence river flows and potentially impact on the incubation success of salmonids. A simple conceptual model of the hyporheic zone and its inputs is shown in Figure 2.8.
Figure 2.8. A conceptual model of the hyporheic mixing zone, where surface waters and ground waters interact (Soulsby et al. 2001). Redds can be found in both areas of upwelling and downwelling water sources, particularly within the high flow region as a pose to lower flow regions.

Lorenz and Eiler (1989) noted that up-welling groundwater regions provide three advantages for spawning Sockeye salmon; the increased hydraulic action of groundwater up-wellings loosen substrates which allows for easier spawning and emergence of fry and warmer temperatures than surface water increase development and reduce time spent in the gravels. It should be noted however that sockeye salmon can tolerate lower levels of dissolved oxygen from groundwater sources during incubation than other salmonid species (<3mg/l), highlighting the importance of considering life history variation between species when exploring habitat requirements.

Groundwater dominated streams have stable thermal regimes which could be particularly important in winter incubation times as warmer temperatures increase the rate of development of salmonid eggs (Lorenz and Eiler 1989; Crisp 2000). However up-wellings of groundwater tend to be low in dissolved oxygen and typically have high conductivity and high alkalinity (Malcolm et al. 2003). Hyporheic and surface water quality were measured in the Girnock burn catchment, Scotland and the spatial variability of water quality within the hyporheic zone was found to be much greater than that observed in surface waters (Malcolm et al. 2005). Dissolved oxygen levels were found to fluctuate from 28.3% - 94.1% and alkalinity and conductivity varied greatly over the incubation period (Malcolm et al. 2005). Groundwater dominated areas within the catchment contained the highest density of redds, possibly due to upstream glacial moraines which promoted favourable hydraulic and sedimentary
characteristics at these sites (Malcolm et al. 2005). This study highlights the importance of catchment influences on spawning patches and embryo survival (Malcolm et al. 2008).

Malcolm et al. (2003 and 2004) suggest that in redds where groundwater inputs dominate the hyporheic environment, egg survival was reduced compared to redds where the dominant input to the hyporheic zone was from surface water. Looking at factors influencing the reach scale, Malcolm et al. (2004) described the varying spatial and temporal dissolved oxygen concentrations and sources of water in the hyporheic zone of a particular riffle when storm events occur. Base flow rates increased as the water table and stream stage increased, bringing low dissolved oxygen concentration water into the hyporheic environment (Malcolm et al. 2004). This seems to contradict previous research which has highlighted the preferential use of up-welling groundwater by spawning salmonids (Lorenz and Eiler 1989; Curry and Noakes 1995), suggesting that these conditions are unique to small, upland streams where there are little sediment inputs.

A study carried out that compared up-welling groundwater spawning sites with spawning sites that had no groundwater inputs found that pre-emergent fry survival was better in the redds that contained groundwater dominating characteristics (84%) than survival observed in the redds with no groundwater characteristics observed (64%) (Garret et al. 1998). Conditions at both sites were very different with temperatures at the groundwater dominated sites exceeding surface water temperatures by 2.4-2.6°C compared with 0.2°C in surface water dominated sites (Garret et al. 1998). Fine sediment was greater in the groundwater dominated redds and surface water velocities were much lower where these redds were constructed (Garret et al. 1998). Temperature appears to be the largest factor influencing spawning preference and fry survival in this stream, as embryo development rates increase with increasing temperature reducing incubation time within the gravels. This is in agreement with Curry and Noakes (1995) who observed groundwater up-welling brought higher temperature water and oxygen concentrations to redd sites during the incubation period.

Most of these earlier observations were concerned with much larger catchments and stable up-welling, generally associated with much larger river bed features, not akin to the small localised up-welling found in small agricultural and upland streams. A study that supports this theory was carried out in a large Canadian catchment where Chinook salmon appeared to preferentially choose areas to spawn where there were areas of up-welling water from the hyporheic zone (Geist 2000). Dissolved oxygen levels were
found to be higher (9mg l$^{-1}$) at these up-welling areas than in other areas of the river not characterised by these up-welling regions (7mg l$^{-1}$) (Geist 2000). However no significant difference in temperature, dissolved oxygen level or hydraulic conductivity were observed between groundwater and surface water at these sites. This could potentially be due to peak events causing fluctuating river levels (average 2m day$^{-1}$) during the study period and rapidly increasing hyporheic exchange. This highlights the importance of not generalising between different river systems but taking into account catchment specific characteristics and understanding the dynamics of hyporheic exchange and water inputs in different rivers.

Soulsby et al. (2001) concluded that poor water quality within redds in a degraded agricultural stream may not be a significant limiting factor for egg survival as high survival rates were recorded, although they were variable. It was also remarked that dissolved oxygen concentrations did reach sub-lethal limits in the hyporheic zone but the temporal resolution was not sufficient enough to examine dynamics within the hyporheic zone (Soulsby et al. 2001). There is still considerable debate as to the relative importance of ground water up-welling and spawning preference in the literature and among fisheries scientists and managers.

2.3.4 Fine sediment sources and delivery and transport within river

The major sources of sediment within catchments result from mechanisms such as hill slope erosion, gully erosion and within channel processes such as channel incision and channel bank erosion (Prosser et al. 2001). Sediment supply and sediment transport capacity of rivers and catchments denote the type of river, either bedrock or alluvial (Montgomery et al. 1996). Rivers are transport mechanisms for catchment sediment deposits which are initially created by the natural chemical, biological and physical weathering of rock deposits and soil. There are three main ways that rivers transport sediment; bed-load transport, suspended load and solute load (Leeder 1999).

The transport of fine sediment in river systems and its deposition into gravel river beds has been studied by many researchers (Beschtia and Jackson 1979; Lisle 1980; Frostick et al. 1984; Carling 1984; Peloutier 1998; Cuthbertson 2001). The deposition of fine sediment into river beds can be defined as the vertical transfer of sediment from the near-bed surface flow to the gravel bed (Peloutier 1998). It is controlled mainly by the surface of the river bed and the near-bed turbulent flow conditions (Peloutier 1998). Gravity and near-bed hydraulics in the form of turbulent flows generated by eddies are the major factors that affect deposition rates of sediment, with gravity having a greater
effect on settling larger particles and turbulent flows on smaller particles forcing them into river beds. Carling (1984) found that turbulent forces re-suspended fine particles at the bed surface of open gravel frameworks in a surface layer of gravel with thickness equal to the mean grain size of the gravel. Deposition rates showed a strong positive relationship with the concentration of suspended sediment in these flume experiments (Carling 1984). Beschta and Jackson (1979) hypothesised that particle size affected the infiltration of deposited material to a much greater extent than the hydrological conditions experienced in flume studies. The depth that particles infiltrate into gravel beds is widely reported to be constrained by the geometric nature of the fine sediment grain size to framework pore size ratio (Frostick et al. 1984; Lisle 1989). The deposition and consequent infiltration of fine sediment increases the amount of matrix sediments in gravel beds which are trapped in place and can then only be removed, dependent on the flow conditions experienced within the river (Cuthbertson 2001). After deposition, particles generally infiltrate deeper in sub-surface layers of gravel beds, primarily via gravitational settling (Cuthbertson 2001).

The rate of infiltration of fine sediment into redds decreases as gravel-bed pore spaces fill from the bottom up by previously deposited fine particles (Carling 1984; Sear 1993; Heywood and Walling 2007). The amount and pattern of sediment infiltration into redds will therefore depend upon the geometric size and shape of particles, the concentration of suspended sediment and the size and shape of pore spaces available to settling material. Evidence to support this hypothesis in natural systems found that the siltation rate monitored at numerous sites in a chalk stream was often found to relate to the concentration of the suspended sediment in the surface water (Acornley and Sear 1999).

Figure 2.9 shows the main different filtration mechanisms of fine sediment to the river bed surface noted by McDowell-Boyer et al. (1986). Looking at Figure 2.9, surface filtration is mostly concerned with sediment larger than or equal to surface sediment sizes, settling on the surface rather than infiltrating; straining describes particles forced into pore spaces until other particles blocking routes halt movement and physical-chemical filtration mainly describe clay particles with negative charges being attracted to the surface of framework gravels (McDowell-Boyer et al. 1986).
Sediment inputs into the interstitial environment are complex and changeable and are driven by erosion mechanisms, such as the physical weathering of soil by rain and other climatic factors and are entirely reliant on climate conditions (Collins and Walling 2007). The magnitude of sediment supply for a specific catchment is influenced by many factors; slope of landscape, nature and location of sources, drainage patterns, vegetation cover and land-use (Walling 1983). Suspended and bed-load sediments within river systems are active both as sinks and sources of environmental contaminants and organic nutrient inputs which correspond to the specific environmental conditions imposed within a system (Prosser et al. 2001).

It is known that only a fraction of the sediment supplied to rivers from catchment sources reaches the outlet of systems, known as the sediment yield, and therefore the storage of transported sediment occurs within channels and on catchment slopes (Walling 1983). Spatial variation of in-channel storage of fine sediment in river bed gravels is common in small low lying agricultural catchments and temporal differences in storage can occur, for example, during different seasons (Walling and Collins 2006). Sediment storage within natural river systems has increased due to increasing sediment supply caused by agricultural practises, urban run-off and industry (Prosser et al. 2001). This is likely to result in changes to the biological entities contained within the river and river bed, the physical form of the river and chemical processes such as redox and anaerobic reactions (Prosser et al. 2001). It is therefore important to source sediment in catchments where increased loads from anthropogenic factors are occurring and causing ecological and morphological changes to the river environment.
In order to create effective management solutions to the problem of sedimentation, it is important to realise the major inputs and pathways of sediment into a system. Recent studies have sought to explain the sources of sediment within different catchments (Collins et al. 1997; Russell et al. 2001; Pierre et al. 2002; Walling et al. 2003; Walling 2005; Collins and Walling, 2007). Fine sediment is difficult to track through catchments as there are many possible pathways and routes to travel. A diffuse non-point source pollutant can stay near its source or can travel many miles downstream depending on sediment transport and settling rates. Flume studies have described a negative relationship between the downstream siltation rate of fine sediments and the source of sediment (Carling 1984), however this controlled environment is not representative of all dynamic natural systems. Many methods of sourcing sediment have been employed over time including aerial photographs or visually assessing sources (Wilson et al. 1993; Erikson et al. 2003), or using erosion pins or plots to measure bank and land surface loss (Foster 1995). Other methods of sourcing inorganic sediments using natural and manmade tracers such as caesium-137 ($^{137}$Cs) and lead-210 ($^{210}$Pb) (Foster 1995; Walling et al. 2003) and artificial seston counterparts such as corn pollen, brewer’s yeast or certain bacteria can be used to trace the deposition and transport of organic sediments (Hunken and Mutz 2007).

However, the major problem with most techniques is that they can not necessarily link the source of sediment on the land surface to the sediment found within a river system and the ultimate yields of sediment. Assumptions should not be made about the effect and magnitude of a source on the sediment loading of a particular river (Foster 1995). Erosion plots and pins give a good indication of the amount of erosion occurring in a particular area and hence may give some information on the amount of surface/bank material that is entering a river system. But then there is no information about particular routes and also the efficient delivery of this sediment and fate within the river system after it has left the land (Walling 2005).

Problems also occur because spatial and temporal scales cannot be defined using earlier methodologies. Erosion of land within a catchment differs over space and time and it is unlikely that erosion plots or pins would give precise information about exactly where most erosion is occurring and at what time, except in a very small catchment (Peart and Walling, 1988). Nevertheless they are still a useful tool in identifying potential sources of suspended sediment load in the first instance. When using newer techniques such as sediment fingerprinting some assumptions are still made as to where sources are coming from and about the likelihood of potential inputs
to river systems, based on general categories such as geology, land-use or anthropogenic inputs.

2.3.4.1 Sediment Fingerprinting

Sediment fingerprinting is one of the most widely used methodologies for identifying potential sources of fine sediment (<63μm) through a river system. It was developed in response to the absence of methods that quantify sedimentation rates or sediment transfer within catchments (Collins et al. 1997). It involves the use of composite fingerprints which use an array of different chemical and physical sediment properties to link accumulated sediments within river systems to their catchment sources (Russell et al. 2001). The method has evolved from using just one property, e.g. carbon content, to discriminate between sources, to using a whole suite of different properties to differentiate between source materials (Collins et al. 1997; Russell et al. 2001; Walling and Collins, 2003; Collins and Walling 2007). Sediments from different sources have been found to have differing and unique chemical, biological and physical properties.

Some of the first fingerprint properties used to identify sediment sources included geochemical, mineralogic and mineral magnetic properties (Walling 2005). Jenkins et al. (2002) used the mineral magnetism approach to determine the sources of terrestrial and marine sediments in the Tay estuary in Scotland with a view to informing dredging and flood risk operations in the area. Russell et al. (2001) used geochemical (heavy metal and nutrient content), radiometric and mineral magnetic properties to determine suspended sediment sources in two lowland catchments in the UK. Other properties that have been used to discriminate between sources are sediment colour, isotopic signatures and fallout radionuclides (Walling 2005). It has been suggested that fingerprinting studies that do not include radionuclides as one of the properties to discriminate between sources should not be given much credence (Walling 2005).

The two main steps within the composite fingerprinting method are the statistical selection and verification of sediment signatures to determine their potential for discriminating between source types and the second being the comparison of composite fingerprints with the potential source types and sediment load within the river. To determine the contributions of each source type to the load in the river, multivariate analysis is used (Collins et al. 1997). Figure 2.10 describes the sediment fingerprinting concept from supply of sediment to rivers to the comparison of source and river sediment.
Figure 2.10. Conceptual model of the sediment fingerprinting method to establish sources of fine sediment within salmonid spawning gravels (adapted from Walling et al. 2003). Highlighted boxes describe the processes by which sediment enters spawning gravels.
So it is possible to separate out different sources from each other, hence giving an indication of the source of that particular type of sediment. It is also possible to attribute different sources with their relative abundance and importance in terms of their supply to that particular river. Evans et al. (2006) was able to recommend several sediment management strategies for rivers in Ireland by using the sediment fingerprinting technique. The results of their study showed that in terms of importance, drainage maintenance accounted for ~60% of suspended sediment load in the River Bush catchment and bank erosion accounted for ~58% of bed load sediment (Evans et al. 2006).

However it is worth noting that the characteristics and properties used within composite fingerprints are found to vary regionally and within different catchments so there is no universal method for all rivers (Walling et al. 2003). This is recognised by Collins and Walling (2002) who attempt to address this issue by determining properties that are capable of discriminating between sources in a number of different catchments. The findings show that the use of several different groups of properties will give the strongest discrimination between potential sources and this has been echoed in post-fingerprinting studies (Collins and Walling 2002; Evans et al. 2006; Collins and Walling 2007). Composite sediment fingerprints are thought to reduce the sources of error and increase reliability of the method to reduce the uncertainty associated with faulty source-sediment matches (Collins and Walling 2007).

One study that has used composite fingerprints gives estimates of the potential fine sediment sources within the Frome and Piddle catchments in Dorset, UK. Cultivated land was the largest contributor to fine sediment loading within the interstitial environment, with pasture being a secondary source of sediment (Collins and Walling 2007b). Channel bank and subsurface sources were also found to be contributing to the overall sediment load within the two catchments. However in the Frome catchment the banks were dominated by low gradient chalk banks that are well vegetated so the bank sources were thought to be originating from non-calcareous, disturbed banks elsewhere in the catchment (Collins and Walling 2007b). Groundwater-fed systems are particularly vulnerable to fine sediment accumulation, due to stable seasonal flows and increasing pressure from water abstraction by water companies and industry (Collins and Walling 2007a) so discovering the sources of fine sediment within these catchments are of great importance to biota. A UK wide study carried out by Walling et al. (2003) used the composite sediment fingerprinting methodology to discover the sources of fine sediment in eighteen principal Atlantic salmon rivers. Significant regional differences in sources were found between rivers and highlighted the usefulness of the method in relation to spawning success (Walling et al. 2003).
Sediment fingerprinting has tended to identify the source of the inorganic fraction of sediment within catchments as this is generally the more stable component of sediment that can be more easily traced through a system. Organic components of sediment are often overlooked in these types of studies and it would be beneficial to look into the organic sources of the sediment in terms of spawning habitat quality and the effect the organic matter fraction may have in the redd environment, particularly in groundwater dominated systems which have a higher organic matter content than other river typologies (Greig et al. 2005a).

2.3.5 Organic matter within sediment

River bed sediments are composed of inorganic and organic fractions which have different properties and oxygen consuming capacity. The inorganic fraction, in terms of its geochemical composition and size, shape and colour, has been widely studied in the context of the effects on incubating salmon ova (Lapointe et al. 2004; Greig et al 2005a; Levasseur et al. 2006; Heywood and Walling 2007). The organic fraction is less studied within the redd environment and river sediments in general (Greig et al. 2005a; McConnachie and Petticrew 2006). The two major forms of organic matter are particulate organic matter (POM) and dissolved organic matter (DOM) (McConnahie and Petticrew 2006). Particulate organic matter sources can originate from autochthonous (in-stream macrophyte, bryophyte and invertebrate material) or allochthonous sources (riparian leaf litter, animal faeces and effluent discharges) (Greig et al. 2007a).

Nitrogen and carbon, the most common elements found in biological organisms, can be transported as POM or DOM within inorganic sediments and their supply to river reaches is associated with the availability, transport and transformations that sediments undergo within river systems (Prosser et al. 2001). Recent organic matter research has focused on how organic material can influence sediment transport and stabilise bed sediments (Lundkvist et al. 2007; Sierra and Gomez 2007; Droppo 2009) and sourcing organic material through natural systems (Stedmon et al. 2003; McConnachie and Petticrew 2006; Baker and Spencer 2004).

Organic matter by nature is dynamic and variable and is susceptible to change in natural environments due to being composed of bio-degradable materials such as amino acids, carboxylic acids, carbohydrates, sterols and alcohols (Liu et al. 2007). It can be retained, transported or processed within rivers, depending on the amount of micro-organisms, the channel hydraulics and organic material supply (Wipfli et al. 2007). There has been a lot of work carried out on dissolved organic matter (DOM), which is the general term applied to all organic compounds that are found in soil
water, ground water and fresh water (Thacker 2005). DOM originates naturally from soils, terrestrial plants and riverine organic matter and typically is the largest source of organic carbon in running waters (Greig et al. 2007a). The main research focus for freshwater environments has been to attempt to characterise and source DOM with a view to understanding the role it plays in ecosystem functioning, mobilising organic and inorganic pollutants, carbon budgeting and waste water tracing through river systems (Baker 2004). However, because of its complex nature, only 25% of DOM is satisfactorily characterised leaving a further 75% to be characterised within the aquatic ecosystems (Baker 2004).

In terms of the role played by organic matter within the redd environment, dissolved oxygen consumption can be assumed to be one of the major factors played in any degrading interstitial environment. Grieg et al. (2007) describes two mechanisms by which organic material could influence oxygen availability to incubating salmon eggs; first the direct competition for oxygen from material being decomposed by microorganisms and secondly the potential growth of biofilms which affect the intra-gravel flow velocity and available oxygen concentration. Organic matter compounds can often cause colloidal flocculates as they are capable of interacting with other components (inorganic and organic) in the aquatic environment due to the large number of binding sites found on their typically large surface areas (Liu et al. 2007). This could mean that pollutants such as heavy metals could enter the redd environment and contaminate the eggs, reducing survival.

Biofilms are defined as microbial populations of micro-organisms such as bacteria, fungi and protozoans which are embedded in a matrix of their own secretions of extracellular polymeric substances (EPS) and inhabit bed sediments and other surfaces (Sierra and Gomez 2007; Lundkvist et al. 2007). Droppo (2009) discussed the bed stabilising capacity of biofilms. He found that erosion decreased with increasing bio-stabilisation and that EPS causes much of the infilling of interstitial pore spaces (Droppo 2009). Lundkvist et al. (2007) discussed the importance of bio-stabilisation of bed sediments which increased stability of sediments by 150% over physico-chemical mechanisms alone.

If there are higher quantities of organic material present within the interstitial redd environment then there is a possibility that pore spaces could become blocked at a faster rate due to increasing biofilm growth. This stabilises bed sediments (Droppo 2009; Lundkvist et al. 2007) and reduces the flushing effect of intra-gravel flow (Petticrew and Arocena 2003) and erosion capacity, hence decreasing dissolved oxygen concentration within the hyporheic environment. This has implications for salmonid
eggs residing in gravels which need high levels of dissolved oxygen to survive and develop and for adequate waste removal. Increased organic matter loading to streams and rivers will create more chance for bed sediment storage of sediment, as organic compounds aggregated with inorganic sediments create flocs with differing settling rates (Droppo 2001). Petticrew and Arocena (2003) observed distinct biofilm structures in salmon rivers in Canada, a web and film-like structures covering mineral sediments. The implications of this study suggest that different types of organic matter decomposition create different biofilm structures that have differing rates of bio-stabilisation, film-like structures infilling pore spaces faster and more effectively than web-like structures (Petticrew and Arocena 2003).

Biofilms created by excessive organic matter inputs can increase the sediment oxygen demand (SOD) of bed sediments, especially in anthropogenic disturbed systems where sewage outfall inputs and land-use run-off increase organic matter loading (Sierra and Gomez 2007). Sediment oxygen demand (SOD) which is also known as benthic oxygen demand can be described as the rate at which dissolved oxygen is removed from the water column by different processes in the stream bed sediments (Hatcher 1980). The rate at which oxygen is transferred is controlled by physical, chemical and biological processes. These include microbial and chemical degradation of organic material within sediments, where carbon is removed by a series of oxidation reactions and either stored in the sediments or released as soluble CO$_2$ back into the water column (Rauch et al. 2008). SOD rates are an important consideration when relating the detrimental impact of organic matter sources to salmonid embryo survival. They allow the quantification of oxygen consumption within redd sediments that could impact on salmonid survival. To date SOD has largely been overlooked within salmonid spawning habitat quality research.

It is therefore an important and appropriate research topic to investigate the catchment sources, composition and inputs of organic matter into river systems to understand fully the impacts that sedimentation could be having on the success to emergence of salmonids and the impact that increased organic matter loading has on oxygen supply to incubating eggs. In terms of sediment management issues it is crucial to be targeting the right problem areas within catchments and some organic matter sources have the potential to be easily regulated and controlled e.g. cattle poaching, effluent discharges and fertilizer application to fields.

McConnachie and Petticrew (2006) described tracing organic matter sources contained within suspended sediment using carbon to nitrogen (C:N) ratios and their associated stable isotopic signals in salmon rivers in Canada. They showed that there are seasonal
differences between the proportions of sources from autochthonous sources (salmon, algae etc.), contributing little during spring melt but increasing later in the year and allochthonous sources (pine needles, birch etc.) comprising the majority of organic material within suspended sediment during spring melt and declining thereafter.

It is possible to characterise organic matter sources within freshwater systems from different catchments using fluorescence spectroscopy (Baker 2004). Ultraviolet light causes electrons within organic matter components to excite and fluoresce, giving off light at certain intensities depending on the concentration and type of the particular substance in question, e.g. amino acids, fulvic or humic acids (Baker 2001). Fluorescence analysis has been used in studies to observe the number and species of different microbial communities (Hudson et al. 2008). It also can show the difference between plant derived organic matter and microbial derived organic matter as their fluorophores show differing peak intensities of emitted light (Hudson et al. 2008). Anthropogenic sources of dissolved organic matter and natural sources can be distinguished using a fluorescence spectrophotometer as can the different organic matter derived from terrestrial plant and animal manures (Hunt and Ohno 2007).

These studies show that fluorescence spectroscopy can be a useful tool to discriminate between certain sources of DOM, which is encouraging for the sourcing of organic matter within salmonid spawning gravels. There are still some uncertainties and errors involved with this method though as the exact relationship between fluorescent properties and the biogeochemical structure of organic matter is not known (Baker 2004). There is estimated to be only 40-60% of organic substances that actually fluoresce (Baker 2001) and the majority of DOM characteristics are still unknown (Baker 2004). Nevertheless to be able to get an idea of possible sources and the role that organic matter plays within the redd environment would be helpful to inform incubation success and spawning habitat quality models such as SIDO and would be useful knowledge to have for fisheries managers in terms of rejuvenating spawning habitat throughout systems.

2.3.6 Sediment management strategies

Sourcing sediment transported by rivers is important when designing sediment control or management strategies for catchments (Russell et al. 2001) because reducing the sources will ultimately lead to a reduction in sediment supply to the system, enhancing habitat quality for biota. Fisheries managers use many methods to alleviate the problem of fine sediment within spawning gravels, most being reach based rather than
looking at the catchment as a whole. These include; gravel imports, channel narrowing, bank revetment and stabilisation, weed cutting, gravel cleaning and in some cases the artificial stocking of rivers with either swim-up fry or eggs (Walling et al. 2003).

One of the major ways to tackle the problem of fine sediment within spawning gravels is to clean the area prior to the returning fish appearing, with high powered pressure jets that blast out the fine sediment from areas and also loosen gravels making it easier for the hen fish to cut their redds. A study to look at the effectiveness of gravel cleaning showed that at all cleaned sites there was a reduction in the fine sediment and dissolved oxygen levels stayed above the critical threshold limit (5mg l⁻¹) for the entire salmonid incubation period (Meyer et al. 2008). Gravel cleaning is routinely carried out by the Environment Agency across the UK and particularly in groundwater fed systems found mostly in the south of England.
Chapter 3. Research Catchment and Methodology

3.1 Chapter synopsis

This chapter provides detailed information about the study site and general methodological approach adopted to address the aims of the thesis. Contextual background information about the River Itchen including history, ecology and catchment characteristics are presented. Study sites within the catchment are identified and specific site choices are described. The main monitoring strategy employed to investigate the aims of the study is also set out and summary descriptions of each separate analysis is carried out in response to the main objectives which are briefly explained; each analysis chapter provides detailed methodology within it for coherency. Finally the SIDO-UK model is also introduced and its use within the context of this study is rationalised. The funding body for this research, the Environment Agency, specified the Special Area of Conservation (cSAC) and Site of Special Scientific Interest (SSSI) River Itchen as the study catchment for this research due to the decline in salmonid populations in recent years; the likely cause of this being the excessive amount of fine sediment present in the river highlighted by previous reports (Environment Agency 2004a; River Itchen Sustainability Report 2004; Greig et al. 2005).

3.2 Underlying methodological rationale

The central purpose of this investigation, as stated in section 1.2 is to characterise and assess accumulated inorganic and organic sediments and their associated impacts within salmonid spawning gravels on the River Itchen, Hampshire. As previously stated in the literature review there is a wealth of previous research relating salmonid survival success with the intrusion of fine sediment. There is still a specific need to study chalk streams in more depth however, as they have been identified as having particularly high levels of fine sediment which is compounded by naturally low flows (Acornley and Sear 1999; Potter and Dare 2003; Greig 2004; River Itchen Sustainability Project 2004;
Heywood et al. 2007). Previous research has focused on discrete reaches within river systems or comparisons between river typologies and there has been little, if any, whole catchment investigations carried out to investigate fine sediment infilling and its associated impacts in relation to salmonid spawning success, in particular on chalk streams (Acornley and Sear 1999; Zimmerman and Lapointe 2005; Greig et al. 2005; Heywood et al. 2007).

This study of the River Itchen is representative of many smaller UK chalk stream catchments found in the south of England so the outcomes of the research have relevance to other southern chalk streams. Grieg (2004) recognised the necessity of assessing multiple factors in order to address the complexity of the numerous environmental variables that can influence salmonid spawning habitat. Therefore a large number of pre-existing variables and new variables (sediment oxygen demand) that were known to and hypothesised to affect spawning habitat success were measured using a variety of field and laboratory methods. The four specific research objectives arrived at under the main aim stated above use different methodological approaches to address them:

1. To characterise spawning habitat quality on the Itchen over different spatial and temporal scales during the incubation period.

Artificial redds containing sediment infiltration baskets and standpipes and continuous monitoring probes alongside spot sampling were used. Spatial and temporal scales are investigated to ensure the whole catchment is represented in terms of spawning habitat quality. The first analysis chapter therefore explores the quality of spawning habitat and the variability exhibited within the catchment. Characterising the spawning habitat quality in terms of spawning habitat variables identified from within the literature using artificial redds can be found in Chapter 4.

2. To trace inorganic sediment sources throughout the catchment.

Potential catchment fine sediment sources were identified and collected from around the catchment. These were then compared with accumulated sediment within the redd environment using sediment fingerprinting technology developed by Collins et al. (1997). Composite fingerprint suites were created using laboratory techniques to devolve elemental composition of source and redd sediment and statistical methods were employed to determine key catchment sediment source areas in the catchment and link these back to the accumulated material within the redd environment with the use of a sediment mixing model employing Monte Carlo simulation techniques. An
investigation into the sources of the fine sediment accumulating within salmonid redds logically followed on in Chapter 5.

3. To identify and describe organic matter sources within the redd environment and explore the potential for creating a composite fingerprint suite for sourcing organic matter throughout the catchment.

Whilst previous researchers have found direct and indirect links between sediment infilling and other factors such as low dissolved oxygen levels and decreased intra-gravel flow affecting salmonid embryo survival, there has been little research into the effect organic sediments have on survival or indeed their interaction with other factors in the redd environment (Greig 2004; Sear and DeVries 2008). Briefly organic sediments are hypothesised to consume intra-gravel oxygen entering the redd and are thus in direct competition with salmonid embryos for dissolved oxygen, potentially affecting survival. By employing recent laboratory based spectrophotometric methods utilized in water quality research (Baker 2001; Hudson et al. 2007; Henderson et al. 2009), the determination of the organic matter composition of redd sediment, source and suspended sediment was addressed. In order to investigate the impact of organic sediments within spawning gravels two main aspects were identified as the starting point for considering organic sediments in redds: first an attempt to delineate the composition of organic sediments found within spawning gravels and secondly whether there was any scope for sourcing those organic sediments (Chapter 6).

4. To assess the impact of sediment oxygen demand (SOD) of these organic sediments on the availability of oxygen within the interstitial environment and the possible impact on oxygen consumption by incubating salmon progeny.

An experimental method was created to measure fine sediment oxygen consumption and determine SOD rates at different spatial and temporal scales. There is potential to explore the impact of these sediment oxygen demands on the oxygen available to embryos within the hyporheic environment using survival metrics already proposed. The sediment oxygen demand that is exhibited by accumulated sediments within salmonid redds can be found in Chapter 7.


3.3 Research Catchment

The River Itchen is a groundwater-fed chalk stream situated in Hampshire on the southern coast of the United Kingdom. It covers an area of approximately 400 km$^2$ and is internationally renowned as a first class example of a chalk stream (Environment Agency 2000). The river’s source is locally described as the Cheriton or Tichbourne stream which is joined by two tributaries, the river Arle and Candover stream, before forming the main body of the River Itchen above Ovington. The Itchen’s true source springs from the underlying chalk aquifer which recharges surface waters and gives rise to the river’s stable nature in terms of hydrochemistry, flow and temperature (Berrie 1992; Environment Agency & English Nature 1999; Acornley 1999). The chalk based aquifer supplies large amounts of nutrients which contribute to the prolific macrophyte communities that colonise the river. Because of this many plant and animal species are indigenous to the chalk stream environment such as the fine-lined pea mussel, *Pisidium tenuilineatum* and abundant river water crowfoot, *Ranunculus penicillatus* var *pseudofluitans*, and high biodiversity is one of the most celebrated features of the Itchen (Environment Agency 2006).

The Itchen, similar to other lowland river systems in the UK, is classed as heavily modified and requires a substantial amount of management to maintain its restricted multi-channel network (Environment Agency & English Nature 1999; Riley et al. 2009b). Modifications to support a variety of different needs have shaped the river, including: irrigation in the form of water meadows, energy to drive mill wheels and navigation in the form of canals and locks (Riley et al. 2009b). Figure 3.1 describes the elevation of the catchment which ranges from approximately 200m above sea level in the upper reaches of the catchment to 1m below sea level near the mouth of the river.

As a groundwater-dominated stream, the Itchen is characterised by stable flows, temperature and nutrient input regimes (Sear et al. 1999). This is due to the catchments underlying pervious and porous chalk geology. Figure 3.2 describes the geology found in the catchment which mostly consists in the upper and middle regions of Cretaceous chalk with small amounts of overlain tertiary clays, sands and silts dominating in the lower regions of the catchment. Mean annual discharge of the Itchen is approximately 5 cumecs, based on Environment Agency records from Allbrook and Highbridge gauging stations in the lower reaches of the catchment (Figure 3.4).

Land use is dominated by rough pasture and arable fields in the upper catchment; the lower catchment is highly urbanised, running through the cities and suburbs of
Winchester and Southampton. Figure 3.3 shows the major land-use designations in the catchment. Soils are generally described as shallow well drained calcareous, silty soils over chalk with smaller areas in the lower part of the catchment dominated by less permeable seasonally waterlogged clay soils with brown subsoil (NSRI, 2012). The Itchen River flows over alluvium, calcareous deposits that contain deep peaty soils (NSRI, 2012). Table 3.1 describes general catchment characteristics for the Itchen.

<table>
<thead>
<tr>
<th><strong>National Grid Reference (source)</strong></th>
<th>SU 5863 2757</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catchment Area</strong></td>
<td>400 km²</td>
</tr>
<tr>
<td><strong>Altitude (source)</strong></td>
<td>83 m</td>
</tr>
<tr>
<td><strong>Discharge at Gaters Mill (over the incubation period 2008-2010)</strong></td>
<td>Average: 7.16 m³s⁻¹&lt;br&gt;Minimum: 4.6 m³s⁻¹&lt;br&gt;Maximum: 14.2 m³s⁻¹</td>
</tr>
<tr>
<td><strong>Precipitation (annual mean 1910-2012) (Met Office 2012)</strong></td>
<td>733.75 mm</td>
</tr>
<tr>
<td><strong>Temperature (annual mean 1910-2012) (Met Office 2012)</strong></td>
<td>10.1 °C</td>
</tr>
<tr>
<td><strong>Nature conservation designations</strong></td>
<td>SSSI (Site of Special Scientific Interest)&lt;br&gt;cSAC (Special Area of Conservation)</td>
</tr>
</tbody>
</table>

Table 3.1. Summary of general Itchen catchment characteristics
Figure 3.1 Elevation of the Itchen catchment (CEH)

Figure 3.2 Geology of the Itchen catchment (CEH)
Figure 3.3. Land use as defined by the Land Cover Map 2000 (LCM2000, CEH). LCM2000 gives thematic classification of spectral data from satellite images and external datasets add context to help refine the spectral classification.
3.3.1 Historical context of the Itchen and flow management

The natural ecological base line of the river valley, prior to human disturbance, would have been a densely forested floodplain with dominant deciduous species such as oak, lime, ash, elm and hazel (River Itchen Sustainability Project 2004). The river would have had many small braided channels, lakes and marshy regions formed by woody debris dams. Human impact on the area began in Neolithic times (4500 – 2600 BC) when the hunter gatherer lifestyle moved over to agriculture. The growth of agriculture from the Iron Age onwards (750 BC - 43 AD) led to large scale deforestation and modification of the river channel (River Itchen Sustainability Project 2004; Sear et al. 2009).

Channel widening, the creation of water meadows and the use of sluice gates and hatches to regulate flow have been used in the past to exploit the river for agriculture and industry (Environment Agency 2004). The water meadow system, adopted in the 17th Century by British farmers to feed livestock throughout the winter, is thought to have had the single largest impact on the shape of the Itchen in its present form (Glasspool 2007). The system, now a relic, diverted river water via a series of channels and hatches to surrounding pastures to promote early grass growth to feed livestock. The water meadows naturally acted as silt traps as they slowed flow down and suspended sediment would settle before the river was diverted back to the water course (Environment Agency 2004; Walling et al. 2003). Sluice gates or hatches, as they are known on the Itchen, are used throughout the year to retain flow in periods of low flows often to maintain the salmonid fishery. Suspended sediment is more likely to drop out of suspension when there is less velocity as the river has less energy to maintain transport (Sear et al. 1999; Charlton 2008). Sear et al. (1999) reported that groundwater dominated rivers exhibit low rates of sediment transport which results in less fine sediment deposition on the river bed.

Berrie (1992) suggested that the primary control on the stable annual hydrological regime of groundwater rivers is the aquifer groundwater flux. Bed sediment transport is limited in groundwater dominated streams and suspended loads are generally less peaky than flashy river systems due to these stable flow regimes, low gradient and low drainage density (Walling and Amos 1999; Sear et al. 1999). Based on this assessment of sediment transport, the likely residence time of settled sediment on the river bed is generally thought to be very long (Acornley and Sear 1999).
The maximum and minimum daily flows hydrograph of the Itchen shows that flows follow a cyclical pattern of low and slightly higher flows depending on seasonal variation (Figure 3.4). The hydrograph displays some correlation with rainfall measured in the area; where rainfall is higher in the winter months so are river flows. However river flow shows delayed responses to extended rainfall events as rainwater permeates into the underlying aquifer, limiting surface run-off to the river channels. The long term average from 1978 - 2007 (green line) shows that there is a cyclical pattern of low and high flow years which is similar to other chalk streams. This has obvious implications for low sediment transport on the Itchen and the quality of spawning gravels.

Figure 3.4. Long term hydrograph from Allbrook and Highbridge gauging station on the River Itchen. The mean historic flow rate on the River Itchen is approximately 5 cumeecs (Courtesy of Environment Agency - http://www.environment-agency.gov.uk/static/documents/Research)

The main factors thought to be causing an impact on river flows on the River Itchen are: land drainage for agricultural purposes, water abstraction for an increasing population and groundwater augmentation in headwater tributaries to remediate low flows. Urban development and the increasing pressure of population growth together with the associated impacts of pollution and strain on water resources are progressively impacting river catchment quality. The present day Itchen is characterised by man-made banks, artificially regulated flows and a lack of natural riparian zone vegetation (Environment Agency 1999; 2000; 2004).
The Itchen is designated a Site of Special Scientific Interest (SSSI) for its unique chalk stream habitat and a Special Area of Conservation (SAC) for the Southern Damselfly (Coenagrion mercuriale) and Ranunculus spp. Many indigenous and protected species of plants, reptiles, fish, mammals and invertebrates are found in the River Itchen. Table 3.2 identifies some of the main species present on the Itchen.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Fish</th>
<th>Reptiles</th>
<th>Mammals</th>
<th>Invertebrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Crowfoot (Ranunculus spp.)</td>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Great crested Newt (Triturus cristatus)</td>
<td>European Otter (Lutra lutra)</td>
<td>Southern Damselfly (Coenagrion mercuriale)</td>
</tr>
<tr>
<td>Water Starworts (Callitriche spp.)</td>
<td>Brown Trout (Salmo trutta)</td>
<td>Water Vole (Arvicola amphibious)</td>
<td>Azure Damselfly (Coenagrion puella)</td>
<td></td>
</tr>
<tr>
<td>Fools Watercress (Apium nodiflorum)</td>
<td>Sea and river Lamprey (Lampetra fluviatilis)</td>
<td></td>
<td>Common blue Damselfly (Enallagma cyathigerum)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bullhead (Cottus gobio)</td>
<td></td>
<td></td>
<td>White clawed Crayfish (Austropotamobius pallipes)</td>
</tr>
<tr>
<td></td>
<td>Eels (Anguilla anguilla)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2. Protected and native species present in the River Itchen (English Nature and Environment Agency 1999; Hampshire Wildlife Trust surveys 2008)

The River Itchen exhibits ideal conditions for macrophyte (higher aquatic plants) growth and at certain times of the year it is inundated with large amounts of weed (macrophytes) which are managed by river keepers. Weed cutting is an important aspect of chalk stream management not only for fishery management and maintaining a balanced habitat for species but for navigation (less important nowerdays) and flood risk management. Managing in-stream macrophytes by cutting
allows suitable habitat to be created and maintained (i.e. trout refuges and to open areas encouraging new growth and light penetration) and to avoid toxic events occurring in the river such as eutrophic reaches. *Ranunculus spp.* are actively encouraged because they are primary producers on the River Itchen, where other macrophytes such as *Callitriche spp.* are removed due to their closely packed growth patterns which trap silt. Invertebrate communities provide a food source for fish and give an indication of the health of the river. Within the river invertebrate populations are described as highly diverse with over 300 species identified in the main stem of the river indicating good water quality (Environment Agency 2004b). The endangered mammals, otters (*Lutra lutra*) and water voles (*Arvicola amphibious*) are also found on the banks of the River Itchen.

### 3.3.3 Fish populations past and present

The River Itchen has historically contained healthy populations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Historical reports from the 18th Century detail images of mill workers pitch-forking salmon off spawning redds as there was a glut on the river and they were the major source of food. The Environment Agency reports that declining populations have been observed in both trout and more notably salmon since the 1960s shown in Figure 2.3 (Environment Agency 2004b).

The Environment Agency monitors fish populations on the River Itchen. Figure 3.5 shows returning adult Atlantic salmon (*Salmo salar*) stocks on the River Itchen from 1988 – 2002. There is a clear downward trend in stock abundance over the period. An acute decline was observed from 1988 onwards which has been attributed to low flows and sediment accumulation within gravels, reducing spawning success (Environment Agency 2004a).
Figure 3.5. Returning adult salmon to three southern chalk rivers from 1988-2001, based on electronic fish counters (Environment Agency, CEH)

The River Itchen, like other Southern chalk streams, displays similar patterns to other UK rivers with regard to declining salmonid populations. Whilst there has been some recovery of salmon and sea trout populations on some UK rivers (e.g., Tyne), salmon stocks are still critically depleted (Environment Agency 2004b). Research has suggested that numerous factors have contributed to the population declines in UK rivers but generally survival at sea is thought to be the major contributing factor, as all river populations have experienced similar patterns of decline (Environment Agency 2004b; Greig et al. 2005). A number of factors have been identified that detrimentally affect salmonid stocks in the marine life phase, the major ones being disease, exploitation, predation and food availability (Hansen and Quinn 1998). The first few months at sea are thought to be critical for survival and climatic conditions are hypothesised to be indirectly related to mortality rates, where declining sea surface temperature appears to correlate with declining salmonid populations in the sea (Hansen and Quinn 1998; Jonsson and Jonsson 2004; Environment Agency 2004b).

3.3.4 Natural spawning on the River Itchen

Spawning occurs on the River Itchen between November and January and for the most part takes place downstream from the city of Winchester. The greatest density of salmon spawning takes place around Bishopstoke near Eastleigh, which is approximately 6 miles from the tidal limit of the river at Woodmill. Despite the
construction of a fish pass at Durngate Weir in Winchester in 2007, so far there is little evidence that salmon are navigating the structure and successfully spawning in the higher quality spawning gravels located upstream of Winchester (Dave Hunter, personal communication, EA). Figure 3.6 demonstrates areas of the River Itchen known to be utilised by spawning salmonids and describes observed nursery habitat for both trout and salmon. In low flow years spawning success is thought to be reduced on the River Itchen which is most likely to be due to a combination of interacting factors including low intra-gravel flow, low dissolved oxygen concentrations and pores clogged with fine sediment (Cragg-Hine 2002).

Figure 3.6. Diagram representing salmonid habitat and reaches where Atlantic salmon spawning has occurred in the past on the River Itchen. Modified from the River Itchen Steering Group, Sustainability Study, Final Report (2004).

Average gravel composition in UK salmonid rivers with typical pool-riffle habitat generally contain less than 12-15% fine material (Cragg-Hine 2002). However chalk rivers tend to have a smaller proportion of larger stones and higher proportions of
fine sediment. Some examples of sediment <4mm from chalk streams are: Frome (16-22%), Avon (18-22%) and the Itchen (27-38%) (Cragg-Hine 2002). Dissolved oxygen levels in the redd environment measured in earlier studies found that levels remain higher than critical threshold limits (>5mg l⁻¹) for the duration of the incubation period in other chalk rivers (Greig et al. 2005; Heywood and Walling 2007). Mortalities were thought to be based on the available supply of oxygen to developing embryos which could have been affected by the higher portion of fine sediment surrounding eggs (Greig 2004). Intra-gravel flow velocity was hypothesised to be the greatest limiting factor on salmonid embryo survival in the River Test (Greig et al. 2005a; 2005b).

A consequence of the high proportion of fine material found within chalk streams salmonid redds might be that there may be a reduction in the winnowing of the fine sediment during redd construction leading to a high proportion of fine sediment remaining in redds (Cragg-Hine 2002). Milan et al. (2000) found that chalk stream sites had >25% of <1mm sediment and that at lower depths (15-30cm) there was approximately twice the loading of fine sediment than at the river bed surface. These results support previous work that found that a minimum of 16% up to a maximum of 74% <1mm sediment was present in the bottom section of salmonid redds in Dorset chalk streams (Crisp and Carling 1989). The compacted gravels of chalk streams could also be reducing the potential for fish to move gravels, allowing fine sediment to be left undisturbed in the bottom of redds (Cragg-Hine 2002). Crisp and Carling (1989) noted that the main purpose of redd building was to loosen the gravels ready for egg deposition. In light of this it has been observed that fish tend to spawn in the same patches of habitat every year on the River Itchen, where gravels are significantly more disturbed than other areas of the river bed (Heb Leman, personal communication, EA). Overcutting of redds on chalk streams has been observed in research on the River Piddle. The report found that 22% of redds were utilised by two or more hen salmon (Cragg-Hine 2002). This suggests a lack of suitable spawning sites on chalk streams where bed sediments are known to be more cemented than other rivers. This is because of the large amount of matrix (fine) sediment present and the effect of calcium carbonate deposits binding material together (Crisp and Carling 1989).

3.4 Specific study site locations and maps

In order to address the research aims set out in section 3.2 and create a catchment wide data set that would provide a representative assessment of spawning habitat
quality on the River Itchen, sites were chosen at various locations along the river which were known to have been utilised by salmonids for spawning habitat in the past. Specific factors affected the choice of suitable sites including the river-bed was clear from weed and movable by spawning salmon, the depth of overlying water was less than 50cm and that the site was accessible. Some of these points relate to the likely spawning behaviour of wild salmonids and others were necessary to accurately measure parameters within the artificial reds and obtain high quality data. It should be noted that despite there being little evidence of salmon spawning upstream of Winchester, sites were chosen upstream based on the premise that there is historical evidence that salmon used to spawn in the upper reaches. There have been recent sightings of salmon navigating the fish pass opened in 2007 at Durngate Mill and heading to the upper River Itchen (Adrian Fewings, personal communication, EA). This naturally gave rise to the ‘upper’ and ‘lower’ catchment characterisation of sites; the latter being below Winchester and the former above.

3.4.1 2008 and 2009 field seasons

The main aims of the 2008 field season were to explore the spatial and temporal variation in spawning habitat quality exhibited in the Itchen catchment to address the first objective outlined in section 3.2. It was important to choose sites that represented salmonid spawning habitat throughout the entire catchment. Figure 3.7 shows the distribution of sites chosen for the 2008 and 2009 field seasons. Table 3.3 describes the site specific characteristics of the 2008 field sites. Detailed maps of site choice can be found in Table 3.4.
Based on site specific information from data collected in the 2008 field season, representative sites were chosen to explore further the spawning habitat quality in terms of sediment oxygen demand, organic composition and groundwater influence. The distribution of sites chosen for the 2009 field season can be found in Figure 3.7.

The sites were chosen using similar criteria as the 2008 sites. Data collected from the 2008 sites informed site choice in 2009 to ensure that contrasting and representative sites of catchment spawning habitat quality were monitored in the 2009 field season. Table 3.3 describes the site specific characteristics of the 2009 field sites. Photographs provide an upstream and downstream view of the sites in Table 3.5.
<table>
<thead>
<tr>
<th>Site Characteristic</th>
<th>Arle</th>
<th>Abbotstone</th>
<th>Cheriton</th>
<th>Ovington</th>
<th>Martyr Worthy</th>
<th>Winchester</th>
<th>Shawford House</th>
<th>Bishopstoke</th>
<th>Gaters Mill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grid Reference</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
<td>SU 57399 32578</td>
</tr>
<tr>
<td>Channel width (m)</td>
<td>7.1</td>
<td>3.9</td>
<td>4.5</td>
<td>12</td>
<td>11.5</td>
<td>8</td>
<td>7.3</td>
<td>15.8/6.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Spawning flow depth (m)</td>
<td>0.20</td>
<td>0.18</td>
<td>0.17</td>
<td>0.40</td>
<td>0.34</td>
<td>0.27</td>
<td>0.30</td>
<td>0.24/0.48</td>
<td>0.30</td>
</tr>
<tr>
<td>D₅₀ (uncut mm)</td>
<td>0.5</td>
<td>1.7</td>
<td>1.2</td>
<td>0.7</td>
<td>0.4</td>
<td>0.8</td>
<td>2.4</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>% &lt;1mm (uncut)</td>
<td>21.13</td>
<td>7.89</td>
<td>23.12</td>
<td>17.99</td>
<td>19.44</td>
<td>20.39</td>
<td>3.15</td>
<td>8.82</td>
<td>12.60</td>
</tr>
<tr>
<td>% &lt;2mm (uncut)</td>
<td>23.68</td>
<td>9.04</td>
<td>27.13</td>
<td>20.47</td>
<td>20.77</td>
<td>23.46</td>
<td>4.25</td>
<td>10.99</td>
<td>15.50</td>
</tr>
<tr>
<td>Land use</td>
<td>Rough pasture</td>
<td>Rough pasture</td>
<td>Arable/rough pasture</td>
<td>Rough pasture/urban</td>
<td>Rough pasture/arable</td>
<td>Rough pasture/urban</td>
<td>Improved pasture/urban</td>
<td>Urban</td>
<td>Rough pasture/urban</td>
</tr>
</tbody>
</table>

Table 3.3 Describes the general site characteristics of 2008 field sites and 2009 field sites (in red). Note slight change in Bishopstoke site in 2009 gives two widths and depth measurements. The proximity of the sites meant that the same sedimentary characteristics were thought sufficient.
<table>
<thead>
<tr>
<th>Site</th>
<th>Detailed Map</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovington</strong></td>
<td>![Ovington Map]</td>
</tr>
<tr>
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<td>SU 57399</td>
</tr>
<tr>
<td>32578</td>
<td>32578</td>
</tr>
</tbody>
</table>

| **Martyr Worthy** | ![Martyr Worthy Map] |
| SU 57399         | SU 57399      |
| 32578            | 32578         |

<p>| <strong>Winchester</strong>   | ![Winchester Map] |
| SU 57399        | SU 57399       |
| 32578           | 32578          |</p>
<table>
<thead>
<tr>
<th>Site</th>
<th>Detailed Map</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shawford House</strong></td>
<td>SU 57399 32578</td>
</tr>
<tr>
<td><strong>Bishopstoke</strong></td>
<td>SU 57399 32578</td>
</tr>
<tr>
<td><strong>Gaters Mill</strong></td>
<td>SU 57399 32578</td>
</tr>
</tbody>
</table>

Table 3.4. Detailed maps of 2008 field sites. Red circles indicate sites.
<table>
<thead>
<tr>
<th>Site</th>
<th>Upstream</th>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td><img src="image1" alt="Arle Upstream" /></td>
<td><img src="image2" alt="Arle Downstream" /></td>
</tr>
<tr>
<td>Ovington</td>
<td><img src="image3" alt="Ovington Upstream" /></td>
<td><img src="image4" alt="Ovington Downstream" /></td>
</tr>
<tr>
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<td><img src="image5" alt="Winchester Upstream" /></td>
<td><img src="image6" alt="Winchester Downstream" /></td>
</tr>
<tr>
<td>Bishopstoke</td>
<td><img src="image7" alt="Bishopstoke Upstream" /></td>
<td><img src="image8" alt="Bishopstoke Downstream" /></td>
</tr>
</tbody>
</table>

Table 3.5. Photographs at 2009 field sites, upstream and downstream view point
3.5 **Methodological approach: Artificial redds and standpipes**

Previous research on salmonid spawning habitat has employed a number of approaches to explore the hyporheic environment. Observations of natural redds have added to researchers' knowledge regarding the dimensions and location of redds (Carling 1984; Crisp and Carling 1989) and have been used to assess grain size distribution and survival rates (Chambers et al. 1954; Ringler 1970). The use of emergence traps on natural redds gives survival data for salmonids (Chapman 1988; Meyer 2005). Laboratory flume studies have explored flow rates and sediment loading capacity within redds (Carling et al. 2006) and man-made, artificial redds have been built in rivers to investigate factors influencing the interstitial zone most recently to avoid interfering with natural redds (Acornley and Sear 1999; Heywood et al 2007; Greig et al. 2007).

In order to assess spawning habitat quality on the River Itchen, artificial redds were constructed. Artificial redds cannot provide an accurate assessment of conditions present in natural redds (Chapman 1988) but disturbance of natural Atlantic salmon redds in the UK is prohibited due to its protected species status. So therefore the best alternative method to assess the hyporheic environment was to build a series of artificial redds which would not affect natural salmonid recruitment. There are certain advantages when using artificial redds to assess spawning habitat quality because multiple factors can be measured and continuous monitoring probes can be placed directly in the pot section of the redd where eggs are placed, enabling intimate monitoring of the environment surrounding the eggs (Greig 2004). An example of an artificial redd constructed in this study at Martyr Worthy can be found in Figure 3.8.
In order to ensure that the artificial redds were as close to real salmonid redds as possible, guidance was sought from the literature (Crisp and Carling 1989; Armstrong 2003). A twisting, digging action was used with both feet and spades to create the redd depression or pot areas where eggs and sediment baskets were later to be deployed. During the cutting process, disturbed gravel was washed further downstream creating the tail spill of the redd. After placing the eggs and monitoring equipment into the pot section, the river bed upstream was cut to create the tell-tale morphological clean gravel ‘hump’ and upstream depression, characteristic of salmon redds. Figure 3.9 depicts the morphology of a salmon redd and the notable components.
Figure 3.9. A typical salmonid redd. Note the morphology, depression at the front, the hump of the egg pocket/pot section and the tail spill (wdfw.wa.gov 31/03/12).

The general aim and subsequent aims and objectives of the research relate to and assess the impact of sedimentation together with other factors on salmonid spawning gravels (section 3.2). In order to measure sediment accumulation and associated factors, sediment bags and infiltration baskets were inserted into the pot section of the artificial redds. The method used in Greig (2004), originally deployed by Lisle (1989), Sear (1993) and Acornley and Sear 1999, was adopted as a template for the setup in this study. Wire mesh sediment baskets (diameter 85mm, height 230mm) and polyethylene rip-stop bags were deployed within redds at the same time as construction. Gravel was truncated to >4mm and placed within the sediment basket to ensure the starting sediments included no fine material that would be removed in natural redds by the action of redd building (Crisp and Carling 1989).

Stainless steel standpipes (diameter 40mm height 350mm) similar to those used by Greig (2004) were situated in the pot section upstream of the sediment baskets. These allowed for sampling of the redd environment throughout the incubation period. The standpipes consisted of one metal tube inside another; the inner tube had drilled holes (4mm apart) to allow intra-gravel water to flood the tube at 0.15 – 0.2m depth below the gravels in the egg zone (Crisp and Carling 1989) when opened for monitoring purposes (Greig 2004). Figure 3.10 illustrates the sediment baskets, bags and standpipes in the context of the artificial redds.
Figure 3.10. Diagram describing the field equipment and site set-up. A. Sediment basket and infiltration bag. B. Metal standpipe, closed and open. C. Visual representation of the sediment baskets, bags and standpipes installed in an artificial redd.

Standpipes remained in their closed position with a rubber bung inserted to limit sediment intrusion when monitoring was not taking place and were opened and allowed to settle for 10 minutes prior to measurements being taken. A PVC plastic tube extension attached to the opening of the standpipe allowed accurate measurements of the hyporheic environment without interference from surface waters. Variables measured in the standpipes included dissolved oxygen, temperature, conductivity and
intra-gravel flow velocity. A full description of the methods of assessment of spawning habitat quality is given in Chapter 4 section 4.3.

3.6 Laboratory analysis

Brief descriptions of laboratory analysis carried out to address each separate objective are given within each section. Each analysis chapter contains its own methods section where detailed laboratory procedures can be found.

Particle size distribution in redds and sediment assessment of cut and uncut redds and infiltrated material were created by using a two stage laboratory method. Wet and dry sieving techniques were used to separate fractions >63µm and coulter counter laser particle sizing was used for size fractions <63µm. Chapter four section 4.3 gives detailed methods pertaining to the collection of granular spawning habitat quality data.

Chapter five describes an in-depth evaluation of the sediment oxygen consumption and demand of accumulated redd sediments collected at sites over the incubation period. Sealed glass chambers were used to infer the loss of oxygen in the gas headspace to the water/sediment mixture below to measure the oxygen consumption rates and ultimately the sediment oxygen demand of fine infiltrated redd sediment. For a detailed description of the method used see Chapter five section 5.6.

A sediment sourcing study was carried out to determine the provenance of accumulated sediments within the redd environment (Chapter six). Fine sediment collected from sites was analysed to determine the concentration and composition of certain elements. ICP-MS (inductively coupled plasma mass spectrometry) and AAS (atomic absorption spectroscopy) were used to measure the sediment properties. Chapter six describes laboratory methods in more detail.

Analysis of organic material accumulated in redd sediments involved many different laboratory procedures and methods. Chapter seven describes the composition of likely organic sources of sediments in salmonid redds. Laboratory methods used included: fluorescence analysis, UV/vis absorbance, total carbon determination, loss on ignition experiments and macroflora and fauna percentage cover counts. Detailed accounts of the methodology can be found in chapter seven section 7.3.
3.7 Model application

Some existing models were used to support the research objectives. The following section details information about the relevant models used within preceding analysis chapters.

3.7.1 Sediment fingerprinting mixing model

In order to investigate the provenance of sediment accumulating within salmonid redds, a sediment fingerprinting method was employed. The major concepts underpinning the sediment fingerprinting method are: to identify sediment sources from discrete natural tracers; statistically define those tracers best at discriminating between sources and to model the contributions of each sediment source to fine sediment accumulating within the river (Collins et al. 1998; Davis and Fox 2009). A sediment mixing model was employed similar to the one described in Collins et al. (1997) to obtain results about the source of inorganic and organic sediments accumulating within redds. This model matches a composite suite of fingerprint properties from catchment and in-stream source material to the same properties measured in sediment accumulated within salmon redds. Objective functions in linear equation form were created for each fingerprint property in the composite suite and its corresponding source categories. These functions minimise the sum of squares of the combined relative errors of the source categories and estimates can then be made of the source category contribution relative to a redd sediment sample (Collins and Walling 2007a). Chapter six, section 6.5 gives a detailed description of the sediment mixing model used to determine the provenance of fine sediment in the Itchen catchment.

3.7.2 SIDO-UK spawning habitat quality model

There are a number of models created that predict spawning success of fish based on habitat quality variables. SIDO-UK is a model that uses measured environmental data from spawning habitats to give a prediction of salmonid egg survival for UK rivers. It was modified from its original form of SIDO (sediment intrusion and dissolved oxygen) to predict salmonid spawning success in UK rivers (Carling et al 2003; Sear 2010). The
original model was created by Alonso et al. (1996) by the United States Department of Agriculture (USDA). It was created to predict salmonid survival in relation to sediment intrusion and intra-gravel oxygen concentrations and aimed to be non-site specific or geographically limited (Havis et al. 1993). SIDO-UK is increasingly being used as a management tool to model the effects of sedimentation loads on salmonid spawning habitats and assess how changing land practices and management can impact on the likely sediment loading to rivers (Havis et al. 1993). Carling et al. (2003) modified SIDO and calibrated it for UK salmonid species (*Salmo salar* and *Salmo trutta*) and river types, creating SIDO-UK. SIDO-UK is a mathematical, deterministic model that comprises two coupled domains; the stream domain with associated hydrological inputs with parameters such as daily mean discharge and suspended sediment load separated into size fraction ratios and the redd site domain (with parameters such as cut and uncut gravel composition, cross section and slope (Carling et al. 2003). The model estimates salmonid egg survival based on simulations of dissolved oxygen consumption by eggs and sediment relating to the quality and quantity of infiltrating fine sediment (Havis et al. 1993). Linking micro-scale processes occurring at the egg and redd scale back to whole catchment processes, such as fine sediment delivery, is the real challenge that models like SIDO-UK attempt to address (Sear 2010).

Data collected during the two field campaigns can be used in SIDO-UK to calibrate the model for the Itchen and predict survival of salmonids. SIDO-UK was used within the context of this research to model the effect changing the sediment oxygen demand of infiltrated sediments based on laboratory results had on egg survival. Chapter five section 5.4.5 details the results obtained and gives a detailed description of the model set up. Future scenarios could also be modelled to assess the impact of changing sediment loads and possibly sources and other parameters on egg survival in the catchment; however the scope of this project did not stretch to carrying out this work.

### 3.7.3 Limitations of methodological approach

The first notable limitation which affects all of the analyses carried out in this study is the use of artificial redds to assess spawning habitat sedimentation and associated factors instead of using natural redds. The data obtained from sites is therefore recognised as only giving an approximation of spawning habitat on the River Itchen. The amount of published literature (Acornley and Sear 1999; Malcolm et al. 2003; Greig et al. 2005a; Heywood and Walling 2007) that uses the methodology of artificial redds is such that they can be now generally accepted as representing conditions found in natural salmon redds. As there was no opportunity of investigating natural
salmon redds it was thought that the use of artificial redds allowed a reasonable next best option for obtaining information about spawning on the River Itchen.

Some concern over the impact of sediment baskets and bags influencing flow and passage of fine sediment had been raised in other studies (Burke 2011). However, porosity of baskets was estimated to be ~90% so the influence on infiltrating sediments and intra-gravel flow was thought to be minimal. The pulling up of the infiltration bags prior to removal of the basket from the redd and the actual removal of the basket/bag from the redd allowed a small proportion of fine sediment to escape. The loss of fine sediment was minimised, however it should be noted that the results are likely to be a slight underestimate of sediment infiltration.

Occasionally during the field seasons it was not possible to take measurements from the standpipes when river flow drowned the top of the standpipe adaptor. A modification to the length of the adaptor was discussed. Towards the end of the field season compacted fine sediment and calcium carbonate deposits affected the opening of standpipes. This uncertainty was unquantified and measures were taken to ensure full opening of the standpipe. The 2009 field season saw a slight modification to the standpipes by the use of a stabilising platform (heavy duty plastic disk, surrounded by heavy stones) to hold the base of the standpipe more firmly in the redd environment. This allowed compacted sediment to be wiggled out from between the outer and inner casing.

It should be noted that little Atlantic salmon ova survival data was obtained from the two field seasons. Acquiring Atlantic salmon eggs from some rivers is particularly difficult because of Environment Agency protection of wild salmon stocks, which is the case for the River Itchen. This limitation is explained in further detail in a later section (4.3.4 and 4.3.6).

Further limitations to specific methodologies used in each separate analysis can be found within the relevant analysis chapters.
Chapter 4. Characterising spawning habitat quality on the Itchen

4.1 Chapter Synopsis

In order to explore the effect of inorganic and organic fine sediment on spawning habitat on the River Itchen, the spawning habitat quality on the Itchen was first investigated. This chapter details the spawning habitat quality currently found on the River Itchen in terms of the sedimentary characteristics and processes essential for the successful incubation of salmonid embryos.

The introduction describes the impact of fine sediment on salmonid gravels from the literature and the specific objectives of the chapter. It should be noted here that the literature review (Chapter 2) focussed a great deal on this so the reader is directed back to this chapter for more in-depth detail to avoid repetition. The methods section describes the monitoring strategy and main methods employed to measure at a range of spatial and temporal scales the habitat variables described in the literature as affecting spawning site quality. The results section details data collected during the field seasons and attempts to delineate the factors affecting survival of salmonids. The discussion section puts the River Itchen data in the context of other studies on chalk streams and other river types. Other factors affecting survival, apart from fine sediment intrusion, are discussed.
4.2 Introduction

Fine sediment delivery has been identified as one of the major driving factors limiting spawning success on the River Itchen (Environment Agency 2004). The Itchen catchment has been defined in the ‘at risk at present’ group for a sediment delivery mapping project carried out by the Environment Agency which has implications for salmonid populations (Environment Agency 2004).

When salmon stocks were found to be declining, one of the major factors influencing that decline was reported to be poor embryonic survival due to sedimentation of spawning gravels (Cragg-Hine 2002; CEFAS report, Potter and Dare 2003). A large amount of research shows the negative impact of fine sediment on the survival of salmonid embryos (Phillips et al. 1975; Lisle 1989; Chapman 1988; Acornley and Sear 1999; Greig et al. 2005a). A number of consequences were thought to be caused by infilling of redds by sediment including direct blocking of pore spaces causing inhibited alevin movement (Sternacker and Geist 2010), indirect decline in dissolved oxygen levels and intra-gravel flow levels and the consumption of oxygen by organic sediments within the redd environment (Greig et al. 2005a; Heywood et al. 2007; Sear et al. 2008). A range of empirical metrics were created to estimate embryo survival and spawning habitat quality. There have been two main areas of research in creating survival metrics; interstitial oxygen concentrations (Chapman 1988; Chevalier and Carson 1985) and river bed sedimentary grain size determinants, such as the Fredle index (Lotspeich and Everest 1981), % fine sediment below a certain threshold (Phillips et al. 1975) and median grain size (D9) based on cumulative particle distribution parameters, D16 and D84 (Shirazi and Seim 1979). Threshold limits for intra-gravel oxygen concentrations and per cent fine material within the redd environment were some of those proposed. Intra-gravel flow (IGF) velocity was another area highlighted as an important factor affecting survival, however due to difficulties in measuring it, there are only a few studies that created empirical IGF threshold levels (Turnpenny and Williams, 1980; Carling 1985).

Infiltrated fine sediment can influence survival within the redd in two ways via different mechanisms; the first being the sand sized fraction (2mm-63µm), which not only indirectly blocks pore spaces within the gravels from the bottom up, has been found to create a seal like cap on redds, impeding the emergence of fry from the nest (Phillips et al. 1975; Beschta and Jackson 1979; Crisp 1993; Kondolf 2000). The second is the finer clay and silt sized fraction (<63µm) which directly blocks the pore spaces of eggs, inhibiting oxygen transfer (Greig et al. 2005c) and directly clogs pore spaces within the
larger gravel framework, indirectly blocking the passageway of freshly oxygenated water to eggs (Chapman 1988; Acornley and Sear 1999; Grieg et al. 2007; Heywood et al. 2007).

Greig (2004) noted that looking at the effect of fine sediment on dissolved oxygen levels or intra-gravel flow velocity could give a site specific indication of embryo survival; however focusing on a single attribute would not provide the best possible metric to estimate survival. A holistic view of factors affecting spawning habitat quality would improve predictions of spawning success and provide more information about the interactions between the different factors. Figure 4.1 describes a conceptual model of the influence of fine sediment accumulation and sedimentation on survival to hatch.

Delineating the factors affecting survival was seen as the most effective way to tackle declining recruitment; however it is now clear that focusing on one area such as intra-gravel oxygen concentration or granular descriptors does not allow for contributing factors such as sediment accumulation and/or intra-gravel flow that can affect oxygen availability to incubating embryos simultaneously. Multifactor analysis is the most recent research area that is being developed to explore salmonid embryo survival (Greig 2004; Heywood et al. 2007). This type of analysis incorporates multiple factors.
influential factors that affect spawning success such as grain size characteristics, intra-gravel flow and oxygen concentrations in the redd environment. Multi-parameter models are one way to combine several factors to estimate survival. SIDO (sediment intrusion and dissolved oxygen model) is one such example of a multi-parameter model (Alonso 1996). High quality, spatial and temporal field data are the essential basis for incorporation into existing salmonid survival metrics and models. Previous studies have reported a wide degree of variation between fine sediment granular determinants and survival and the results are often contradictory between catchment types and methods used. This is most likely due to the complexity surrounding the factors affecting survival in the hyporheic environment and the catchment and site-specific nature of the redd environment. Differences in hydraulic forces (Tonina and Buffington 2005; Sear et al. 2008), composition of bed sediment (Lapointe et al. 2005; Greig et al. 2005a), gradient (Soulsby et al. 2001), sediment availability and mobility of gravels (Allan and Frostick 1999) all impact on the infiltration of fine sediment to the redd in different catchments. For greater detail of the mechanisms of fine sediment infiltration and the effects of fine sediment on spawning success see Chapter 2.

4.2.1 Specific Chapter Objectives

This chapter describes salmonid spawning habitat quality on the River Itchen and uses a number of factors to assess spawning habitat quality to ascertain what limits spawning success. Relationships are explored between habitat variables. The main objectives of this chapter are as follows:

- Collect high quality spawning habitat data at a number of different spatial and temporal scales to assess the spawning habitat quality using metrics provided by the literature
- Quantify the catchment variability in spawning habitat quality displayed on the River Itchen
- Define the factors contributing to spawning habitat quality and identify the main drivers affecting spawning habitat quality
- Assess and consider the relationships between spawning habitat quality variables
- Discuss other factors affecting spawning habitat quality on the River Itchen
4.3 Methods

In order to achieve the objectives set out above, salmonid spawning habitat variables were measured over the incubation seasons during 2007-2008 and 2008-2009. Based on recent research in the field (Greig 2004; Greig et al. 2005a; Heywood and Walling 2007; Sear and DeVries 2008) variables measured during the incubation period of salmonid embryos included: sediment accumulation, bulk suspended sediment load, dissolved oxygen concentration (interstitial and river), intra-gravel flow, temperature and salmonid embryo survival. Specific site choice and general monitoring strategy, including detailed information on artificial redd building, can be found in Chapter 3.

The 2008 field season was designed to give a wide ranging picture of the spawning habitat quality on the Itchen in terms of spatial and temporal scales at nine sites (Figure 3.7). Based on results from the 2008 period, the 2009 field season focused on four sites which were chosen as representative of spawning habitat quality on the River Itchen and evidence of wild salmonid spawning. Spatial and temporal trends in spawning habitat characteristics were explored as in the 2008 period, although in greater depth.

4.3.1 Monitoring strategy

The monitoring strategy adopted allowed the collection of high quality spatial and temporal hyporheic data from the catchment. Two different types of site were created which enabled this; sub and super sites. Both were based around the use of artificial redds with standpipes (Chapter 3, section 3.4) which allowed monitoring of the redd environment, avoiding damaging natural redds and their incubating salmonid progeny.

The major difference between the two types of site was the monitoring equipment installed at the sites. Sub-sites were used to assess spatial variation of factors which influence survival using standpipes and sediment baskets and weekly measurements and super sites contained logging equipment and accurate probes which enabled continuous monitoring of some of these factors to create detailed, temporal data sets. Detailed descriptions of the two different site types follow.

4.3.1.1 Sub-sites

Artificial redds were created to explore the spatial and temporal variation of spawning habitat available on the River Itchen. Detailed description of the artificial redd methodology can be found in Chapter 3. In 2008, nine sites were setup with two...
artificial redds per site, each containing standpipes and sediment baskets (Grieg, 2004). Weekly measurements were taken within the redd using the standpipes and also in the water column. Measurements taken included stage, dissolved oxygen concentration, temperature, conductivity and intra-gravel flow. Dissolved oxygen (mg l⁻¹) and temperature (°C) were measured using a YSI™ 250 oxygen probe. Conductivity (S) was measured using a WPA cm35 probe. Intra-gravel flow velocity (IGF) was measured using a conductimetric standpipe developed by Carling and Boole (1987). This method gauges intra-gravel flow by recording the dilution rate of a saline solution within the standpipe (Greig 2004). Greig et al. (2005b) refined the earlier method by creating two calibration curves which estimate flow velocity based on the exponent of the decay curve measured in the field. The lower limit of the probes response is 1 cm h⁻¹ and velocities < 200 cm h⁻¹ have a standard error of 18 cm h⁻¹ compared with higher velocities >200 cm h⁻¹ which exhibit a standard error of 97 cm h⁻¹ (Grieg et al. 2005b).

The same method and equipment was used to collect spatial data in 2009; however the sites were reduced to four as these sites were thought to be representative of spawning habitat on the Itchen for the variation displayed in the 2008 data.

**4.3.1.2 Super sites**

An upper and lower site in 2008 and 2009 were used to continuously monitor some of the spawning habitat variables to give a detailed temporal record over the incubation period. In 2008, one of the redds created at the Arle and Gators Mill sites was fitted with continuous monitoring probes measuring dissolved oxygen concentration in the redd (approx. 30cm depth), and in the water column, a pressure transducer to measure hydrostatic pressure together with a turbidity probe.

The 2009 monitoring season had the same equipment at the Arle site; however the downstream site was transferred to Bishopstoke. These sites were chosen as they were representative of upstream and downstream spawning habitat on the River Itchen. Bishopstoke was of particular interest to quantify the habitat quality in detail as the greatest density of wild salmonid spawning on the Itchen occurs in the small reaches in this area (Heb Leman (EA), personal communication, November 2007). The probes mentioned above were attached to a DL2e Delta-T data logger on the river bank that logged probe output every 10 minutes. Table 4.1 describes the detailed specifications of each probe deployed at sites. A diver and barometric pressure transducer was deployed within piezometers in 2009 at the continuously monitored sites to investigate the presence of upwelling groundwater. Appendix 1 details probe
calibration of oxygen probes as different probes were used to measure the same variable.

<table>
<thead>
<tr>
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<th>Measurement</th>
<th>Range measured</th>
<th>Error</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
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<td>Dissolved oxygen µM (µmol (^{-1}))</td>
<td>0 – 500 µmol l(^{-1})</td>
<td>&lt;8 µmol l(^{-1})</td>
<td>Optical measurement of dissolved oxygen</td>
</tr>
<tr>
<td></td>
<td>Temperature °C</td>
<td>0 – 40 °C</td>
<td>±0.1 °C</td>
<td></td>
</tr>
<tr>
<td>Analite turbidity probe (NEP 390)</td>
<td>Turbidity (FTU)</td>
<td>0 – 1000 NTU</td>
<td>&lt;1% - 3% for 1000 NTU</td>
<td>90° Infra –Red (ISO7027)</td>
</tr>
<tr>
<td>Druck pressure sensor</td>
<td>Depth (mm)</td>
<td>0 – 1500 mm</td>
<td>±0.1 %</td>
<td>Submersible pressure sensor for measuring hydrostatic liquids</td>
</tr>
<tr>
<td>Schlumberger Mini Diver (Piezometers)</td>
<td>Measuring hydraulic head (up-welling and down-welling)</td>
<td>0 – 100m</td>
<td>±1 cm H(_2)O</td>
<td>Pressure transducer to monitor groundwater level</td>
</tr>
<tr>
<td>Barometric pressure transducer</td>
<td>Measuring air pressure</td>
<td>0 – 1.5m</td>
<td>±0.5cm H(_2)O</td>
<td>Air pressure transducer to measure air pressure</td>
</tr>
</tbody>
</table>

Table 4.1. Continuous monitoring probes and specifications used over the 2008 and 2009 field seasons at the super sites.
4.3.2 Sediment accumulation and particle size analysis

Sediment accumulation was measured in all redds using wire mesh sediment baskets (diameter 85mm, height 230mm) and polyethylene rip-stop bags. These were deployed within redds at the same time as construction. Gravel was truncated to >4mm and placed within the sediment basket to ensure the starting sediments included no fine material that would be removed in natural redds by the action of redd building (Crisp and Carling 1989). It also allowed for a control starting point where all of the fine material collected would have been accumulated over the incubation period. Baskets were placed within bags that were retracted to allow through flow of sediment and water. At the end of the incubation period, plastic cord that was attached to the top of each bag was used to pull up the bag around the basket prior to removal from the redd to minimise the loss of finer particles (<2mm) from the basket (Greig 2004). In 2008, total sediment accumulation over the entire incubation period was measured to hatch and two sediment baskets were deployed in each redd. The reason for the shortness of the 2008 field season related to the eyed eggs planted in artificial redds at the beginning of January. Due to early hatch, the egg baskets were removed from the sites and the redds rebuilt minus the egg baskets. The 2009 accumulation experiments used three baskets in each redd and recorded temporal accumulation over the incubation period from green egg to emergence with baskets pulled out at eyeing, hatch and emergence development stages. Because newly fertilised salmon eggs could be supplied during the 2009 field season, the entire incubation period of salmonid eggs on the River Itchen could be monitored.

Particle size analysis was based on a two-tiered method (Greig 2004). Sediment was retained from accumulation baskets and bags for laboratory analysis where sediment was wet sieved using British standard brass sieves (410) to 4mm, 2mm and 63µm. Dry sieving of the entire range of particles was not undertaken so as to reduce the loss of the finer size fractions which were of greater importance to this study based on previous literature (Greig et al. 2005; Sear et al. 2008). Particle size distribution analysis was carried out on the <63µm size fraction using a LS130 Coulter Counter. The Coulter Counter measures the fine sand to clay range of particles contained within an electrolyte solution. An electrical current gauges the volume of that particular particle and the machine counts the number of that particle in solution. The particle size data from the Coulter Counter was combined with the wet sieved data to create a complete particle size distribution for redd sites.

Freeze coring of uncut bed sediments and also freshly cut redds was carried out to provide a base line estimate of the river bed prior to redd cutting and then as a
starting point estimate for the sediment accumulation experiments within the redd. Standard freeze-coring methodology using similar equipment and procedures was used to collect samples (Walkoten 1976; Evenson 2001). Liquid nitrogen was used as the freezing agent and poured for approximately 2 minutes into a 1.5m hollow steel standpipe (Figure 4.2) that was driven into the river bed to a depth of 30cm. Two freeze cores were taken per site for an uncut and a cut redd. A tripod and winch were used to extract the frozen cores from the river bed and samples were removed and stored in 10 litre buckets for further analysis. Full particle size distribution (31.5mm – 63µm) using dry sieving techniques for the material greater than 63µm was carried out using British standard brass sieves (410) placed on a mechanical shaker to sort and then material retained on the sieves was weighed and recorded. The finer particles of fine sand, silts and clays(<63µm) were analysed using the Coulter Counter and combined with the sieved data to provide full particle size distributions.

Figure 4.2. Example of the cores used to collect freeze core samples from cut and uncut redds (Evenson 2001).

Loss on ignition experiments were performed on subsamples of different class sized particles to determine percentage organic matter and carbonate content. The mass of
samples were recorded and after burning at 550°C for two hours, the weight was recorded followed by another burn at 950°C for four hours where the weight was noted (Lamb, 2004).

The particle size distribution was input into Gradistat (Blott 2000) which was used to characterise different sites on the Itchen by computing Method of Moment statistics for each sample. Table 4.2 shows the particle distribution statistics computed to enable comparisons between sites. The median (D_{50}) and other percentile values were calculated for each site.

<table>
<thead>
<tr>
<th>Particle size parameters</th>
<th>Equation</th>
<th>Explanation</th>
<th>Equation number</th>
</tr>
</thead>
</table>
| Geometric Mean (D_{g}) mm | \exp \left( \frac{\sum f \ln M_m}{100} \right) | f = frequency in per cent  
M_m = midpoint of each size class interval in metric (\mu m) (Blott 2000) | No. 1 |
| Sorting Coefficient (S_o) | S_o = \sqrt{D_{25}/D_{75}} | Trask (1932) | No. 2 |
| Skewness (SK_g) \mu m | \frac{\sum f(\ln M_m - \ln S_o)^3}{100 \ln \sigma^3} | f = frequency in per cent  
M_m = midpoint of each size class interval in metric (\mu m) (Blott 2000) | No. 3 |
| Kurtosis (K_g) \mu m | K = \frac{D_{75} - D_{25}}{2(D_{90} - D_{10})} | Trask (1932) | No. 4 |
| Fredle index (F_i) | F_i = \frac{D_g}{S_o} | Lotspiech and Everest (1981) | No. 5 |

Table 4.2. Descriptions of particle size distribution parameters. Geometric Method of Moment statistical equations were used to calculate, geometric mean, sorting coefficient, skewness and kurtosis.
4.3.3 Suspended sediment measurement

Suspended sediment was recorded using two methods. Estimates of the total suspended sediment load passing a redd site over time was recorded using an isokinetic sampler at all sites. The design of the sampler was modelled on those described in Phillips et al. (2000) and Russell et al. (2001). The morphology of the sampler induced slight retention of stream water within the main body, which allowed natural settling of suspended sediments within the chamber, before the stream water left via an outlet tube (Figure 4.3).

![Diagram of isokinetic sampler](image)

Figure 4.3. The cross-sectional view of the isokinetic suspended samples attached to stakes in the river bed (Phillips et al. 2000)

The use of these samplers permitted bulk samples to be collected over the incubation period allowing comparisons to be made between sites of the spatial and temporal variability in total suspended sediment load relative to the time period the sampler was collecting sediment. The collection of bulk samples also enabled further analysis to be carried out on the sediment such as calculation of percentage organic matter, fluorescence analysis and particle size distributions (Phillips et al. 2000). These types of sampler were found to be approximately 70% efficient in laboratory tests relating collected sediment to a known input load (Phillips et al. 2000). The use of these samplers allowed bulk suspended sediment samples to be collected over a specified period of time and avoided issues surrounding the representativeness of suspended sediment sampling associated with ‘spot’ samples (Walling et al. 2006).

Two continuously monitored analite turbidity probes (specifications in Table 4.1) were installed at the super sites monitoring turbidity over the incubation period in 2008 and 2009. These records were used to create ratings curves to relate turbidity to
suspended sediment concentrations and periodic hand-held measurements were made of suspended sediment using a US DH-48 sampler. Filtration of samples using a vacuum pump and glass fibre filter papers (Whatman, <8μm) were used to accurately weigh the sediment collected. The time and weight of the sediment samples were noted to compare with turbidity readings taken from the continuous monitoring probes and relationships between the two were investigated.

Difficulties arose when attempting to construct ratings curves based on the relationship between spot suspended sediment samples and turbidity probe measurements over the incubation period. Despite regular weekly cleaning of the probe head, the continuous record was very noisy and required a lot of data cleaning at the analysis stage. No well-defined ratings curve could be derived between the suspended sediment concentration and turbidity, nor with suspended sediment concentration and discharge at all sites. Previous studies have also found that it is difficult to create ratings curves in this manner with small streams (Lewis 2002) and particularly chalk based rivers (Walling and Amos 1999; Greig 2004; Heywood et al. 2007). This is thought to be due to probe fouling by calcium carbonate deposits.

Figure 4.4 gives an example of the ratings curve created for the Arle site.

Figure 4.4. Ratings curve between suspended sediment spot measurements and turbidity at the Arle site in the 2009 field season.
Fifteen minute data and daily averaged data were supplied by the Environment Agency for discharge records at super sites (Arle and Bishopstoke 2009 and Arle and Gaters Mill 2008).

### 4.3.4 Survival experiments

The 2008 survival experiments used eyed eggs from the Environment Agency hatchery in Northumberland (Kielder) as due to population pressures on Itchen salmon, local eggs were unavailable. Approximately 1000 eyed eggs were sent via courier on ice to the Sparsholt hatchery where eggs were separated into batches for deployment to separate sites and a control batch was set up in an incubator. They were stored in tanks with continuously monitored recirculating freshwater until deployment at the field sites and the water temperature was recorded to estimate hatch (Crisp, 1992). Unfortunately due to the large increase in temperature from the northern birth river to the southern River Itchen temperature, the eggs hatched within a week of deployment to the field so survival experiments were abandoned.

Following the unsuccessful attempt at survival experiments in 2008, the 2009 survival experiments were modified to use River Itchen salmon eggs. By using green River Itchen salmon eggs the problem of population adaptation would be negated by using native fish and a more realistic view of catchment survival estimates could be made. Low returning numbers of salmon on the River Itchen meant it was only possible to acquire three hundred eggs from the Environment Agency, causing survival experiments to be limited to two sites. Control batches of eggs were monitored in an in-stream incubator on the Candover stream, an upper tributary of the River Itchen. Fifty eggs were placed in three separate wire mesh egg baskets (diameter 60mm, height 210mm, mesh 2mm). The mesh size of the basket allowed for fine sediment infilling and ensured recovery of hatchlings. Egg baskets were placed at a depth of 0.3m (Crisp and Carling 1986) within the redd of excavated redds at the Arle and Bishopstoke sites. Hatching was estimated from water temperature records in the field using the degree day method (Crisp, 1992). When hatch was deemed to have taken place, the egg baskets were removed and eggs/alevins were counted on the bankside using trays to retain the fine material for further analysis. Survival rates were recorded and a small sub-sample of alevins were retained in formaldehyde to measure length and visually compare upper and lower sites. Estimates made of embryo hatch rates were good as no healthy live eggs were recorded.
4.3.5  Groundwater influence

Based on the 2008 intra-gravel dissolved oxygen data there was considerable evidence that there may be groundwater influencing the artificial redds created at the Arle site in particular (Malcolm et al. 2003). A number of factors are thought to indicate the presence of upwelling groundwater as groundwater has been found to exhibit different characteristics to surface water in a range of ways (Boulton et al. 1998; Soulsby et al. 2001; Malcolm et al. 2003, 2004, 2008). The factors previously investigated included conductivity (Soulsby et al. 2001), temperature (Webb and Walling 1993; Grieg et al. 2005a), dissolved oxygen levels (Boulton et al. 1998; Malcolm et al. 2003; Soulsby et al. 2009) and a positive hydraulic head through river gravels (Malcolm et al. 2003, 2006). To further investigate the theory that upwelling groundwater might be influencing some sites, piezometers were installed to egg burial depth and surface sediment depth were fitted with pressure sensors to measure the hydraulic head in the 2009 field season (Malcolm et al. 2008; Malcolm et al. 2009). The difference between the pressures measured in the gravels to the surface gravels was then used to indicate if there was any further evidence of upwelling groundwater. Intra-gravel conductivity was also measured within standpipes to provide evidence for groundwater upwelling, as a difference in conductivity between the surface water and interstitial water would indicate that the source of the water was different (Soulsby et al. 2001). Intra-gravel temperature was monitored to see if there was any difference between the thermal regime exhibited in the surface water compared to the sub-surface water. Diurnal variation in temperature is characteristic of surface water however little diurnal variation registers in groundwater (Webb and Walling 1993).

4.3.6  Limitations of sampling methodology

Artificial redds can give an indication of natural spawning habitat but it should be noted that they are likely to be not exactly comparable to natural redds due to multiple factors including the cleansing of fine material (Kondolf 1993). However, previous studies have informed researchers about the morphology and size of redds which were emulated in the field seasons to allow the collection of representative data (Crisp and Carling 1989; Crisp 2000; Armstrong 2003).

Survival experiments were set up with salmon eggs sourced from the Environment Agency hatchery at Kielder in Northumberland. Due to the fragile nature of green eggs in transport, eyed eggs were thought to be the safest option for delivery. Controls were set up at Sparsholt college hatchery and mortality rate was less than 5% in the first few days after delivery. The eggs were deployed in Harriss baskets contained within
sediment baskets at all nine sites and using temperatures measured in the river and monitoring the control eggs, degree days were used to calculate hatch times (Crisp, 1992). Unfortunately the River Itchen river temperature was five times as high as the temperature the eggs had been incubating in and consequently they hatched after a week of being deployed in the egg baskets. Survival measured was estimated to be >50% although it should be noted that not all of the eggs/alevins were accounted for in every basket.

Absence of time-integrated suspended sediment concentrations are a major limitation within this project. Turbidity probes and suspended sediment ratings curves could not be computed due to the large degree of noise displayed in the turbidity reading. This problem has been documented by other studies (Walling 1983; Walling and Amos 1999; Old et al. 2006). The time-integrated method to measure suspended sediment load had an estimated efficiency rate of 70% and samplers were thought to decrease in efficiency during high flows (Phillips et al. 2000). A problem encountered on the River Itchen was the large amount of floating Ranunculus spp. impeding entry of river flow into the sampler. Underestimations of suspended sediment load are likely to have been made due to blocked inlet nozzles as samplers could only be checked and cleared on a weekly basis. An improvement to the method for this river could be to install some form of weed trap upstream, although careful thought is needed to ensure that this does not affect the sediment load in suspension or the passage of water into the sampler.

Freeze core samples were used to investigate the grain size distributions of uncut river gravels based on one sample per site due to small amounts of liquid nitrogen available. Therefore the resultant representativeness of these samples was intrinsically limited (Bunte et al. 2001). The freeze core method itself has limitations with a bias towards coarser particles and this could also have led to an underestimation in matrix sediment at sites (Evenson 2001)
4.4 Results

The results section is sub-divided into spawning habitat characteristics in terms of sediment dynamics, hydrological and hyporheic variables, survival data, groundwater influence and the relationships between habitat quality variables. Data was collected over two field seasons, 2008 and 2009 periods respectively.

4.4.1 Sediment Dynamics

4.4.1.1 Uncut particle size distributions

Analysis of freeze core data provided a base line reference for the particle size distribution of typical river bed sediments on the River Itchen. Figure 4.5 shows the cumulative per cent weight finer than grainsize distribution of uncut gravels at all nine sites on the River Itchen. In general, River Itchen uncut gravels can be described as polymodal, coarse to very coarse, poorly sorted, very fine skewed and meso-leptokurtic. The sites displaying the highest proportion of fine material were Martyr Worthy and Winchester which are located near to the centre of the catchment. Shawford House exhibits the greatest proportion of larger particles.

Figure 4.5. Cumulative % weight finer than particle size distribution of uncut gravels at all nine sites on the Itchen
River gravels can often be said to display two distinct populations, matrix and framework gravels (Carling and Reader 1982). The basic interlocking clasts are the framework gravels (Church et al. 1987) and the finer material (approximately <2mm) that fits between the pore space of the framework gravels is known as the matrix gravel (Carling and Reader 1982). The Itchen gravels displayed great variation in this respect by exhibiting bi-modal to polymodal grainsize distributions. Some other studies have found that bi-modality was not necessarily the general trend for grain size distribution in relation to salmonid spawning gravels, with only 10% of spawning rivers displaying bimodality (Kondolf and Wolman 1993; Burke, 2011). Table 4.3 describes the uncut gravel descriptors for sites.
<table>
<thead>
<tr>
<th>Site name</th>
<th>Geometric mean ($D_g$) mm</th>
<th>$D_{50}$ mm</th>
<th>Sorting coefficient ($S_o$)</th>
<th>Skewness ($SK_g$)</th>
<th>Kurtosis (K)</th>
<th>Fredle Index (Fi)</th>
<th>%&lt;2mm</th>
<th>%&lt;1mm</th>
<th>%&lt;63µm</th>
</tr>
</thead>
<tbody>
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<td>1.3</td>
<td>23.7</td>
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<td>1</td>
<td>27.1</td>
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<td>2.1</td>
</tr>
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<td>-1.9</td>
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<td>2.3</td>
<td>20.4</td>
<td>15.5</td>
<td>3.9</td>
</tr>
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<td>2.7</td>
<td>-1.6</td>
<td>0.27</td>
<td>1.8</td>
<td>20.8</td>
<td>18.0</td>
<td>7.1</td>
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<td>9.1</td>
<td>4.2</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Bishopstoke</td>
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<td>1.7</td>
<td>-2.2</td>
<td>0.3</td>
<td>5.8</td>
<td>11.0</td>
<td>7.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Gators Mill</td>
<td>7.2</td>
<td>13.8</td>
<td>2.2</td>
<td>-1.7</td>
<td>0.33</td>
<td>3.3</td>
<td>15.5</td>
<td>9.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 4.3. Uncut gravel particle size descriptors at all sites using methods described in table 2.
There is variation in gravel sizes within the catchment with the median grain size ranging from 5.2mm to 18.9mm between sites. With the exception of the upstream Abbotstone site, there is a trend towards larger median and geometric mean gravels occurring at the downstream sites. Previous studies on chalk streams have found that the median grain size was 6.33mm on the River Test (Grieg et al. 2004) and ranges from 13.6mm – 16.7mm on the River Itchen over different years (Riley et al. 1999). The results from this study show some sites fitting within the range of previous River Itchen work; however the upper sites generally report much lower median grain sizes, closer to the River Test average. Kondolf et al. (1993) reviewed over one hundred different salmonid spawning gravel sites and reported the median grain size range to be between 5.4mm and 78mm, with 50% falling between 14.5mm and 35mm. Whilst all but one of the River Itchen sites sit within the wider range reported, just two of the downstream sites fit within the 50th percentile.

Variation exists between the amounts of fine sediment present throughout the catchment (Table 4.3). Grieg et al. (2004) reported mean values of 25.4% and 9.3% for <1mm and <63µm fine sediment respectively for the River Test, whilst a range of 16 to 21.9% for <1mm fines was found on the Itchen in a previous study (Riley et al. 1999). Sear et al. (2008) describe summary statistics from freeze cores collected in 78 permeable catchments with 23.2% <1mm fine sediment and 28.2% <2mm fine sediment present. The River Itchen sites lie just below these averages. The upstream results from this study fit within the range of River Itchen % fine sediment, however the downstream sites display particularly low levels of fine sediment which could possibly be attributed to the under-representation of fine material by the freeze cores and smaller cores collected at these sites (Lisle and Eads 1991). Generally, good quality salmonid spawning gravels are reported to characteristically contain <20% fine sediment (<1mm) which all but one of the Itchen sites shows (Crisp and Carling 1989; Moir 1998).

4.4.1.2 Comparison of cut and uncut gravels

Cut gravels give an estimate of the redd environment at the beginning of the field season, immediately after eggs have been laid within the redd. The grain size distribution of cut gravels at all nine sites is displayed in Figure 4.6. Similarly with the uncut gravels, the cut gravels can be generally described as coarse to fine gravel, poorly sorted, very fine skewed and mesokurtic to leptokurtic. In contrast to the uncut gravels however, the site displaying the greatest proportion of fine material is Ovington and the least proportion of fine material is Abbotstone.
The inter-site variation is greater in the uncut gravels than the cut gravels. The uncut gravels generally display a much higher proportion of fine sediment compared with the cut gravels. This was expected based on previous evidence of cut and uncut gravels (Everest et al. 1980; Milan et al. 2001; Greig et al. 2005a). The elongated fine sediment tail diverges at different particle sizes for cut and uncut gravels, describing the inter-site variation in finer material occurs at 100µm and 10µm respectively (Figure 4.5 and Figure 4.6).

For uncut gravels, low $D_g$ suggests low permeability of gravels; reducing intra-gravel flow and alevins movement and high sorting coefficients indicate less permeable gravels as smaller particles fill the pore spaces (Lotspeich and Everest 1981). Cut gravel Fredle Index are similar to those calculated for uncut gravels (range: 1.2-9.4). The higher indexes are displayed by most upper sites indicating that greater survival of salmonid embryos would occur at these sites than the lower sites. In general this outcome is incongruous with current perception on the river as higher quality spawning habitat is thought to reside in the upper parts of the catchment (Environment Agency, Adrian Fewings, personal communication 2008).

A similar pattern is displayed in Table 4.3 and Table 4.4 with the upper River Itchen sites having a lower $D_{50}$ than more downstream sites. There is little variation between the cut and uncut gravels $D_{50}$ for the upper sites, suggesting that smaller particles make up a larger proportion of the bed sediments at these sites. The discrepancy in $D_g$ calculated for cut and uncut gravels, where cut gravels display a smaller $D_g$ than uncut gravels, could be partially due to the bias of the freeze-core method to attach larger material and miss finer material which in turn leads to an overestimation of the geometric mean ($D_g$) (Evenson, 2001).
Figure 4.6. Cut gravel cumulative particle size distributions for all sites on the Itchen
<table>
<thead>
<tr>
<th>Site name</th>
<th>Geometric mean ($D_g$ mm)</th>
<th>$D_{50}$ mm</th>
<th>Sorting coefficient ($S_o$)</th>
<th>Skewness (SK$_g$)</th>
<th>Kurtosis (K$_g$)</th>
<th>Fredle Index (Fi)</th>
<th>%&gt;2mm</th>
<th>%&gt;1mm</th>
<th>%&gt;63µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>9.4</td>
<td>12.5</td>
<td>2.0</td>
<td>-1.7</td>
<td>0.3</td>
<td>4.8</td>
<td>8.3</td>
<td>4.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Abbotstone</td>
<td>16.0</td>
<td>17.0</td>
<td>1.7</td>
<td>-1.2</td>
<td>0.3</td>
<td>9.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Cheriton</td>
<td>4.4</td>
<td>6.0</td>
<td>1.9</td>
<td>-1.2</td>
<td>0.3</td>
<td>2.3</td>
<td>16.5</td>
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</tr>
<tr>
<td>Ovington</td>
<td>2.9</td>
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<td>2.4</td>
<td>-1.2</td>
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<td>Martyr Worthy</td>
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<td>2.5</td>
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<tr>
<td>Bishopstoke</td>
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<td>Gators Mill</td>
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<td>-1.3</td>
<td>0.3</td>
<td>3.5</td>
<td>11.9</td>
<td>5.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 4.4. Cut gravel particle size descriptors for all sites.
Comparing the percentage fine material (<1mm) between the cut and uncut gravels, found that material <1mm is always higher in the uncut gravels than the cut; however cut gravels appear to have marginally more sediment in the <2mm class than the uncut samples (Figure 4.7). No difference was found to be displayed in the larger particle size, (+8mm) however three groups including the <63µm fine sediment were found to be statistically different. The finer material would be expected to be significantly less in the cut gravels than the uncut gravels because of the action of redd cutting. On the River Itchen this appears to be only the case for the very finest material in the clay-silt size proportions, rather than the fine sand proportion (63µm-2mm).

![Figure 4.7. Mean percentage weight in each particle size class in uncut and cut reds. Standard deviation is represented by bars and stars (•) denote particle size classes which are significantly different to one another (paired t-test results, where p = 0.05)](image-url)
Figure 4.8. Amount and comparison of cut and uncut fine sediment (%<1mm) particles present in samples at all sites. Dashed line denotes the level of fine sediment generally found in salmonid spawning gravels (Crisp and Carling 1989; Moir 1998). Amount and comparison of cut and uncut fine sediment (%<1mm) particles present in samples at all sites. Dashed line denotes the level of fine sediment generally found in salmonid spawning gravels (Crisp and Carling 1989; Moir 1998).

Figure 4.8 indicates that the River Itchen contains high levels of fine sediment (<1mm) within uncut gravels and occasionally in cut gravels. Earlier research has suggested that good quality salmonid spawning gravels in all types of rivers contain <20% fine material (<1mm) (Crisp and Carling 1989; Moir 1998). Interestingly the cut gravels at two sites (Ovington and Shawford House) still exhibit greater fines (<1mm) than the uncut gravels which indicate that the artificial redd building process does not winnow out all of the fine material as there is still a large proportion present in the cut gravels. It also lends weight to the argument that freeze coring river bed samples can often under-estimate the amount of fine material in a sample due to the smaller surface area of finer particles being selected against (Lisle and Eads 1991). There appears to be greater amounts of fine sediment in the upper catchment compared with the lower catchment, with the exception of the Abbotstone site. The action of redd cutting may also impact differently at different sites depending on the flow regime and amount of fine sediment within the river gravels, allowing for the preferential removal of fine sediment (<1mm) at sites where there is greater flow and less fine sediment originally.
4.4.1.3 Accumulation of fine sediment within redds

The amount of fine sediment (<4mm, <2mm and <63µm) was calculated for each field site at the end of the 2008 field season and at the end of the 2009 field season (Table 4.5). Figure 4.9 describes the percentage mass of fine sediment (<63µm) accumulated within redds over the 2008 field season.

![Bar chart showing accumulation of fine sediment](chart.png)

Figure 4.9. Mass of fine sediment in the clay and silt size range (<63µm) calculated as a percentage of the total sediment within each basket to allow easy comparison between sites.

Downstream sites appear for the most part to display the largest accumulation of silt and clay sized particles, except for the Winchester site which is situated on a side channel of the main river. Abbotstone and Winchester display the lowest accumulation of sediment within the catchment. These two sites displayed the lowest percentage of <63µm in the cut gravels at the beginning of the incubation also, suggesting winnowing of fines during redd construction was successful at these sites and also less sediment infiltration suggests lower levels of suspended sediment which will be
explored later in the chapter. Figure 4.10 displays a catchment map describing the spatial accumulation of percentage fine sediment (<63µm) at sites in 2008.

![Catchment map](image)

**Figure 4.10.** Spatial variation within the catchment of percentage silt and clay (<63µm) sediment during the 2008 incubation period.

### 4.4.1.4 Comparison of accumulated sediments with baseline gravels

The grain size characteristics of the sediment retrieved from the sediment baskets in the 2008 and 2009 field season are displayed in Table 4.5 and Figure 4.11. A box and whisker plot was used to show the range of variation within the cumulative frequency distribution of gravels at all sites compared with uncut base line gravels (Kondolf et al. 1993; Burke 2011). The particle size classes are plotted on a logarithmic axis to better encompass the wide range of data and the mid line on the box represents the median grain size.

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Similar to the uncut and cut gravel baselines, the redd sediment baskets display inter-site variation and also intra-site variation as two parallel reds were monitored at each site. The range of the median grain size class ($D_{50}$) in the 2008 sediments is 5.4–15.3mm and in 2009 sediments 4.2–15mm. The 2008 gravels sit within the range of salmonid spawning gravels reported in the literature of 5.4–78mm (Kondolf et al. 1993); however the 2009 gravels at Ovington are slightly below this range at 4.2mm. In comparison to the uncut gravels, there appears to be little difference between the $D_{50}$ of the distribution of gravels in the 2008 or 2009 period (Table 4.5). There proved to be no significant difference between the uncut gravels $D_{50}$ and the 2008 sediment baskets or 2009 sediment baskets when applying a paired t-test to the data ($p = 0.05$). Longer whiskers ($D_{10}$) in the uncut gravels point towards there being greater fine sediment (<4mm) in the uncut gravels than the sediment baskets, except for the longer tail present at the Ovington site in the 2009 field season (Figure 4.11).

In general, it can be seen that there is less fine sediment found in the redd sites in the 2008 and 2009 period in comparison to the uncut freeze cores which indicates that there is not a return to a baseline state over the salmonid incubation period.

<table>
<thead>
<tr>
<th>2008 Site</th>
<th>$D_{g}$</th>
<th>$D_{50}$</th>
<th>sorting</th>
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<th>% &lt;4mm</th>
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<td>3.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Winchester</td>
<td>9.8</td>
<td>11.5</td>
<td>1.8</td>
<td>0.3</td>
<td>0.6</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Shawford House</td>
<td>8.5</td>
<td>11.7</td>
<td>1.7</td>
<td>0.3</td>
<td>6.3</td>
<td>4.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>13.6</td>
<td>20.9</td>
<td>1.8</td>
<td>0.3</td>
<td>5.7</td>
<td>4.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Gaters mill</td>
<td>8.8</td>
<td>12.9</td>
<td>1.7</td>
<td>0.3</td>
<td>4.4</td>
<td>4.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2009 Site</th>
<th>$D_{g}$</th>
<th>$D_{50}$</th>
<th>sorting</th>
<th>kurtosis</th>
<th>% &lt;4mm</th>
<th>% &lt;2mm</th>
<th>% &lt;63µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>11.1</td>
<td>13.9</td>
<td>1.7</td>
<td>0.3</td>
<td>4.7</td>
<td>3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Ovington</td>
<td>4.2</td>
<td>6.7</td>
<td>1.6</td>
<td>0.2</td>
<td>12.7</td>
<td>8.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Winchester</td>
<td>7.5</td>
<td>10.6</td>
<td>2.0</td>
<td>0.3</td>
<td>9.4</td>
<td>6.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>15.0</td>
<td>21.2</td>
<td>1.9</td>
<td>0.3</td>
<td>4.4</td>
<td>2.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 4.5. Mean grain size distribution and per cent fine material for sediment baskets over the 2008 and 2009 field season.
The difference between the fine sediment content of redd gravels at the end of the incubation period and uncut gravels was compared to observe the amount of infilling occurred and to ascertain whether the artificial redds had returned to their original state. The mean values from uncut freeze cores and accumulation baskets for three classes of percentage fine sediment (<4mm, <2mm and <63µm) can be found Table 4.6.

<table>
<thead>
<tr>
<th>Fine sediment (%)</th>
<th>Redd sediment 08</th>
<th>Redd sediment 09</th>
<th>Freeze-core uncut</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4mm</td>
<td>5.0 (1.9)</td>
<td>7.1 (3.8)</td>
<td>23.2 (9.2)</td>
</tr>
<tr>
<td>&lt;2mm</td>
<td>3.4 (1.0)</td>
<td>4.9 (2.7)</td>
<td>18.0 (7.9)</td>
</tr>
<tr>
<td>&lt;63µm</td>
<td>0.9 (0.3)</td>
<td>0.6 (0.3)</td>
<td>3.2 (2.4)</td>
</tr>
</tbody>
</table>

Table 4.6. Mean fine sediment comparison between redd sediment at end of the incubation period and uncut gravels. Standard deviation is in brackets.

The uncut gravels contain 4.6 times, 5.2 times and 3.5 times the amount of sediment for the <4mm, <2mm and <63µm fine sediment categories respectively at the end of the 2008 field season. This suggests that there was little infilling of redds over the incubation period. In the 2009 field season the end of the incubation season saw 3.2, 3.6 and 5.3 times the amount of fine sediment in the uncut gravels, <4mm, <2mm and <63µm respectively, suggesting similar results to the 2008 season. There is an inversion from the larger to the smaller particle size fine sediment from the 2008 to the 2009 field season implying that larger particles were deposited over smaller particles in the 2008 field season and the opposite occurred in the 2009 field season. This could be due to slightly higher flows experienced in the 2008 field season compared to the 2009 field campaign, where coarser particles are transported by higher flows (Walling et al. 2000) and are more readily available to infill cut redds.

Discharge records can be found in section 4.4.4. Precipitation data from Met Office records also showed higher levels in the 2008 field season compared with the 2009 field season, with a mean rainfall of 114.8mm for the beginning of the 2008 field season in comparison with a low mean of 45mm for the 2009 field season. By the end of the incubation period (April) the means had dropped to 80.9mm for 2008 and 68.2mm for the 2009 period. This rainfall in the 2008 period could have positively contributed to fine sediment loads in the catchment as catchment sediment sources would be more readily connected with the river via run-off from field drains and ditches. The source of fine sediment within in the Itchen is discussed in greater detail in Chapter 5.
Figure 4.11. Box and whisker plot (Turkey 1977; Kondolf et al. 1993) of the cumulative grain size distributions of sediment baskets in the 2008 (08) and 2009 (09) field season with the uncut gravels of all sites as a baseline (uc).
4.4.1.5 Rate of accumulation of fine sediment

Using the mass of fine sediment accumulated within the sediment baskets over the incubation period, the rate of accumulating fine sediment (<2mm) could be calculated. This enabled comparison with other UK salmonid catchments. Table 4.7 describes the range of Itchen infiltration rates in comparison with other similar catchments for 2008 and 2009.

<table>
<thead>
<tr>
<th>River</th>
<th>Infiltration rate (kg m² day⁻¹)</th>
<th>Study (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Arle</td>
<td>0.00024 - 0.00063</td>
<td>This study</td>
</tr>
<tr>
<td>Candover stream</td>
<td>0.00007</td>
<td>This study</td>
</tr>
<tr>
<td>Cheriton stream</td>
<td>0.00023</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Ovington</td>
<td>0.00024 - 0.00149</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Martyr Worthy</td>
<td>0.00027</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Winchester</td>
<td>0.00019 - 0.00089</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Shawford House</td>
<td>0.00019</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Bishopstoke</td>
<td>0.00029 - 0.00050</td>
<td>This study</td>
</tr>
<tr>
<td>River Itchen, Gaters Mill</td>
<td>0.00024</td>
<td>This study</td>
</tr>
<tr>
<td>River Test (Bossington)</td>
<td>0.014 - 1</td>
<td>Acornley and Sear 1999</td>
</tr>
<tr>
<td>River Test (Horsebridge)</td>
<td>0.01 - 0.037</td>
<td>S.M. Grieg and D.A. Sear, unpublished data</td>
</tr>
<tr>
<td>Wallop Brook</td>
<td>0.04 - 0.4</td>
<td>Acornley and Sear 1999</td>
</tr>
</tbody>
</table>

Table 4.7. Infiltration rates of fine sediment (<2mm) within salmonid spawning gravels and example rates from previous studies in similar catchments (after Sear et al. 2008)

Comparisons between studies should be undertaken with caution. Differences occur in sampling strategy and methods of measurement of fine sediment (Zimmerman and Lapointe 2005) and it can be seen from the table that accumulation rates within the Itchen and its tributaries are at least two orders of magnitude lower than documented rates in similar catchments.

The rate of accumulation of fine sediment was measured temporally in the 2009 field season at different incubation stages. Figure 4.12 describes the accumulation rate over time at four sites on the River Itchen. It is clear to see that after an initial increase in accumulation from zero, which represents the initial cutting of the redd and placement of clean gravels within the redd, there is a marked decline in accumulation towards the end of the field season at emergence time for the two mid-catchment sites. Grieg et al. (2005)
noticed an initial high sediment accumulation rate just after redds were built, followed by a gradual decline in accumulation. This has been attributed to the filling up of pore spaces from the bottom of the redd to the top. The steep decrease in accumulation rate at sites does not follow with a gradual decrease; however the lack of data may create the illusion of a rapid decline. The Arle and Bishopstoke sites display the lowest accumulation rates and only show a negative trend in accumulation at the final removal of baskets at emergence. These two sites follow the pattern more of Burke (2011) who described an initial high accumulation rate, followed by a more stable period of steady infilling, before declining towards the end of the period.

Accumulation of fine sediment (<2mm) is low compared with other chalk rivers in different studies (e.g. River Test: 10% (Grieg et al. 2005), 24.5% (Arcornley and Sear 1999) River Piddle: 22.6% (Walling and Amos 1999)) and very low in comparison to different river typologies across the UK, which often record accumulation of fines as an order of magnitude higher than that recorded on the Itchen (Frostick et al. 1984; Soulsby and Malcolm 2001; Greig et al. 2005). The delivery of fine sediment to artificial redds over the incubation period does not appear to be an issue on the River Itchen, based on low accumulation rates and percentage fine sediment; however it should be noted that due to the high levels of fine sediment already found within the river bed, small increases in fine sediment could have a marked effect on survival. Sear et al. (2008) comments on the higher sensitivity of permeable catchments to fine sediment accumulation in comparison with other rivers which

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**Figure 4.12.** Accumulation rate over time at all sites in during the 2009 field season.
is based on the low mobility of framework gravels. Deposited sediment does not experience flushing flows within permeable catchments because of stable flow regimes, coupled with low-gradients and anthropogenically modified reaches (Sear et al. 1999). Low fine sediment accumulation could indicate limited fine material available on the Itchen. This factor will be discussed in more detail in a later chapter that addresses the issue of the source of fine sediment within the catchment.

4.4.2 Suspended sediment load

Estimates were made of the estimated suspended sediment load passing the redds at sites over the incubation period. Time integrated samplers were used to give estimates of the suspended sediment passing a site and also to provide information about the particle size distribution of suspended sediment. Limitations surrounding this method from the outset indicate that the samplers are approximately 70% efficient at trapping sediment load (Phillips et al. 2000) and the specific problems which arose during the field seasons, including blocking of the inlet nozzle by *Ranunculus spp.* and subsequent loss of suspended sediment capture, limit the data to broad estimates of both particle size distribution and total load. Total suspended sediment loads have been calculated by multiplying the sediment load passing the nozzle width of the sampler, proportional to the depth over the redd with respect to the width of a sediment basket (Burke 2011).

Figure 4.13 displays the total suspended sediment captured by the time integrated samplers at all sites in the 2008 field season over the salmonid spawning incubation season. The magnitude of the 2008 data in comparison with the 2009 data is different due to the shorter field season undertaken in 2008. Therefore it is not possible to compare the two years’ data in any detail. The lower sites appear to have the greatest suspended sediment loads, except Shawford House which was located on a distributary off the main stem of the river. Martyr Worthy and Bishopstoke display the highest suspended sediment loads overall. These two sites are situated on the main stem of the River Itchen, similar to the Ovington site which has the highest sediment load of the upper sites. The headwater tributaries display the lowest load of suspended sediment which is consistent with previous research suggesting suspended sediment yield increases further downstream in catchments (Schumm 1977).
Figure 4.13. Total suspended sediment load captured over the field season 2008. * denotes sites where major weed impacts impeded suspended sediment capture.

Figure 4.14 displays the total suspended sediment captured by the time integrated samplers at each site at different stages during the incubation period in the 2009 field period. The upper sites appear to have greater suspended sediment loads than the downstream sites. This could be due to underestimations of suspended sediment load due to weed damage but also these two downstream sites were situated off the main stem of the river in tributaries, so suspended sediment load may well be lower than the main river stem sites (Ovington). The amount of sediment collected at sites is variable and there appears to be no pattern in the results. Ovington displays the greatest amount of suspended sediment collected over the period which is where the greatest sediment accumulation was measured within the sediment baskets in 2009 (Figure 4.14).
Figure 4.14. Total suspended sediment trapped by sampler estimated at all sites 2009 field period. * denotes sites where major weed impacts could have affected sampler.

The suspended sediment trapped by the sampler calculated to a range of approximately 0.05 – 3.1 kg day⁻¹, based on the site and incubation stage when the load was collected (Figure 4.14). Compared with other studies this range of suspended sediment load is particularly low for different stream types (Russell et al. 2001; Walling 2005; Burke 2011) and also low for other chalk rivers (Acornley and Sear 1999; Greig 2004). Acornley and Sear (1999) reported that greater than 96% of the annual suspended sediment load was transported between November and April in the River Test, exactly the same time period that incubating salmonid eggs are residing in redds in river gravels.

Average particle size distribution of suspended sediment loads (sand, silt and clay) for the 2008 period are displayed in Figure 4.15 and for 2009 in Figure 4.16. In general sand comprises the greatest proportion of fine sediment in the upper and lower catchment in both years, with a much more even share between silt and sand in the lower catchment corresponding with the greater load. Clay fractions seem to be higher in the furthest downstream sites compared with the upstream sites. The sand proportion appears to be greater in the upper catchment in comparison with the lower catchment.
Figure 4.15. Percentage fine material sand (>63µm), silt (>2µm) and clay (<2µm) at all sites in suspended sediment load over the incubation period 2008.

The upper Arle and Ovington sites show similar patterns of silt, sand and clay proportions during the two field seasons (Figure 4.15 and Figure 4.16). The Bishopstoke site was in a different location in the 2008 and 2009 periods with the 2009 site being sited in a side channel due to land owner permission. This explains the difference in suspended sediment load at these sites between the two field seasons. Winchester shows much greater silt proportions than sand in the 2009 field season compared with the 2008. This is likely to be due to the sampling efficiency of 70% (Phillips et al. 2000) where coarser particles are likely to have slightly increased rates of transport in higher flow years (Walling et al. 2000). The 2009 field season was characterised by slightly lower precipitation and also slightly lower flows, particularly at downstream sites at the beginning of the incubation season (see section 4.4.4). However this inversion of particle size is not present in all samples so solid conclusions cannot be reached based on these data. Whilst these graphs give an indication of the relative proportions of sand, silt and clay present in suspended sediment loads, it should be noted that due to method error and environmental factors (weed interference) these estimates of particle size distribution are very general and should be treated as broad estimates.
Other field studies have shown that the accumulation of sediment within redd gravels is often related to the overlying suspended sediment loads within catchments (Acornley and Sear 1999; Grieg et al. 2005). Figure 4.17 shows the amount of suspended sediment passing a basket compared with the amount accumulated at hatch incubation stage for the 2008 data. There appears to be a slight increasing trend in both suspended sediment and accumulated sediment for the downstream sites, however there seems to be little similarity between calculated loads of fine suspended sediment and accumulated redd sediment. In order to see if there is any correlation between accumulated sediment and suspended sediment, a scatter graph with corresponding linear regression was plotted (Figure 4.18). When looking at the relationship between suspended sediment passing the baskets and the amount accumulating within the redd at sites, a weak correlation was found with an $R^2 = 0.2$, $p = 0.05$. Abbotstone appeared to be an outlier in the graph so was highlighted. It exhibited an extremely low accumulation rate within the redd (0.00048 kg m$^{-2}$ day$^{-1}$).
Figure 4.17. Total suspended sediment (<2mm) passing a sediment basket (kg) with total accumulated redd fine sediment (<2mm) at hatch 2008.

Figure 4.18. Relationship between suspended sediment and accumulated sediment at the 2008 sites. Circle highlights Abbotstone which exhibited a very low redd sediment accumulation rate.
4.4.3 Organic sediment

Loss on ignition experiments provided an estimate of the amount of organic material found at each site and at two sizes of fine sediment (<2mm and <63µm). Greig (2004) found that chalk streams contained a large proportion of organic material within spawning gravels (~20%) in comparison to other freshet and non-permeable lowland rivers (5-7%). Organic content of fine sediment recovered at the end of the incubation period in 2008 was in the same magnitude and often exceeded that found in Greig (2004) and ranged from 12-30% (<63µm) and 0.5-20% (<2mm) in two redds at all field sites (Figure 4.19).

![Figure 4.19. Mean organic content (based on two redds) of <63µm and <2mm fine sediment in the 2008 field season. Sites are ordered upstream to downstream.](image)

Similarly the organic material found in the 2009 season ranged from 0.1-6% (<4mm), 3-11% (<2mm) and 13-18% (<63µm) (Figure 4.20). These values are slightly lower than those exhibited in the 2008 field season which could be due to the lower number of sites studied in the 2009 field period. As in the 2008 results the 2009 results show that the smaller particle size material contains the largest amount of organic material. These results indicate that there is a lot of organic material available within spawning gravels on the River Itchen. Chalk streams have a high density of in-stream vegetation which seasonally have growth spurts and die back (Welton 1980). These macrophyte beds are often managed by river keepers to enable sport fishing and keep gravels clear. Management strategies and natural
cycles of weed dying will add large amounts of readily available organic matter to the stream system and coupled with the stable flow regimes and low occurrences of gravel entrainments (Sear et al. 1999), it is likely that organic material will deposit readily on the river bed and experience long residence times (Greig 2004).

![Figure 4.20](image)

Figure 4.20. Mean organic content (based on two redds) of <63µm, <2mm and <4mm fine sediment in redds at the end of the 2009 field season.

4.4.4 Hydrological and hyporheic characteristics of the River Itchen

This section introduces the discharge, temperature profiles and intra-gravel flow velocity measurements of study sites. Discharge data was supplied by the Environment Agency from nearby gauging stations.

4.4.4.1 Discharge

Discharge data were made available from Environment Agency gauging stations near to the study sites. Characteristically chalk rivers, like the Itchen, display relatively low and stable discharges in long term hydrographs (Acornley and Sear 1999; Greig 2004). One such long term discharge record is available for the River Itchen at Gaters Mill, the most downstream site measured in the 2008 field season. Figure 4.24 describes the long term discharge record for the gauging station at Gaters Mill that was closed in 2006. This long term record
allowed this study’s discharge data to be put into the context of longer term trends of higher and lower flow years. Discharge is displayed for the super sites, Arle, Bishopstoke and Gaters Mill in Figure 4.21, Figure 4.22 and Figure 4.23 respectively. The 2009 Bishopstoke site discharge record was calculated from the Highbridge gauging station in proportion to the width of the site.

![Discharge over incubation period](image)

**Figure 4.21.** Daily mean discharge for Arle site from the Environment Agency Drove Lane gauging station over the incubation period in 2008 and 2009 field season.

The discharge record from the Arle gauging station, which is situated approximately five metres upstream of the redd site, displays low magnitude flows over the incubation period (Figure 4.22). There is a slight increasing trend found in the discharge record to the end of February in the 2008 and 2009 field seasons. From February onwards, a decreasing trend in the discharge record is displayed to the end of the field season (April). The flow at this site is artificially regulated as Old Arlesford Pond is upstream of the gauging station and sluices control the outflow from this man-made lake into the river.
The downstream sites display greater magnitude discharges than the upper site as expected. The 2008 flow record for Bishopstoke exhibits higher flow for the January period compared with the 2009 field period, which corresponds with precipitation records (Jan 08, 114mm compared with Jan 09, 45mm). Figure 4.23 displays the flow regime experienced at the furthest downstream site, Gaters Mill in the 2008 field season.

Figure 4.22. Daily mean discharge estimates calculated from Highbridge gauging station over the incubation period at the Bishopstoke site in 2009.

Figure 4.23. Daily mean discharge for Gaters Mill site from the Environment Agency, Riverside Park gauging station over the incubation period in the 2008 field season.
Gaters Mill discharge record from the 2008 field season stays relatively stable around 7-8 m$^3$ s$^{-1}$ until two higher peaks are observed (10 m$^3$ s$^{-1}$) mid-March suggesting flows in the upper range of the average longer term record (Figure 4.24). Mean precipitation for the incubation periods (Jan-April) was 80.9mm in 2008 and 68.2mm for the 2009 period which is respectively slightly higher and lower respectively than the 30 year average years (64-75mm). This may explain the slightly higher magnitude flows exhibited in the downstream catchment between 2008 and 2009. Apart from a slight inversion of the larger particulate fine sediment being deposited during the 2008 field season which experienced slightly higher flows, there is little difference in infilling of sediment at sites in the 2008 or 2009 field seasons which may suggest that the amount of infilling sediment to redds is not related closely to discharge on the River Itchen. Previous research on chalk streams have found that suspended sediment load in chalk streams is often related to the supply of sediment to the system and therefore precipitation rather than discharge (Walling and Amos 1999). Sediment is preferentially stored in upper and middle reaches of other chalk streams in winter months and then transmitted very slowly downstream (Walling and Amos 1999).

4.4.4.2 Intra-gravel dissolved oxygen

In the 2008 field season dissolved oxygen was measured at weekly intervals from eyeing stage to hatch in standpipes located in the centre of artificial redds. Figure 4.25 displays the oxygen profiles measured at each site. The upstream sites, Arle and Abbotstone recorded
stable, high levels of DO throughout the duration of the incubation period and also one downstream site, Bishopstoke, recorded stable and high levels of dissolved oxygen too. Cheriton and Winchester, one upstream and one downstream site respectively displayed the lowest recorded DO measurements with the final measurement dipping to approximately 5 mg l$^{-1}$, which has been described as a threshold level for incubating salmonids (Davis 1975). Gaters Mill, being the furthest downstream sites, recorded one of the lowest DO readings at the final reading also. Generally oxygen concentrations measured within standpipes varied between the range 8mg l$^{-1}$ – 12.5mg l$^{-1}$ and upstream sites (excepting Cheriton) consistently recorded higher levels of dissolved oxygen duringeyeing to hatch in 2008.

![Graph showing spatial variation of intra-gravel dissolved oxygen averaged between two redds over the incubation period at all sites on the River Itchen in 2008. Sites are in order of furthest upstream to furthest downstream.](image)

Concentrations of dissolved oxygen measured within the water column at the same time as standpipe measurements ranged from 10-12mg l$^{-1}$ and there was little inter-site variation with all sites displaying similar standard deviation describing spread around the mean (Figure 4.25). However a general spatial trend in average dissolved oxygen measured within the water column over the incubation period showed that downstream sites displayed lower dissolved oxygen levels than upper sites (Figure 4.26).
Figure 4.26. Average oxygen concentration within the water column over the incubation period. Error bars display standard deviation which show approximately the same amount of variation displayed at all sites.

In the 2009 field season dissolved oxygen levels in the standpipes stayed well above the critical threshold level of 5mg l\(^{-1}\) reported (Davis 1975) as can be seen in Figure 4.27. Greig (2004) noted that there was a likelihood of <50% survival if the dissolved oxygen level within the redd dropped below 8mg l\(^{-1}\) which is only reached after hatch at one site (Arle). In comparison with the 2008 measurements, there is slightly more variation displayed between sites, although it should be noted that the entire incubation season (green eggs – emergence) was measured in 2009 so there are a greater number of measurements taken.
4.4.3 Temperature

Very little inter-site variation is displayed in the thermal regime in the 2008 field season (Figure 4.28) or the 2009 field season. The temperature stayed relatively stable over the period and varied over the range 8-10°C, with a slight increase towards the end of the incubation period when spring got into full swing. Air temperature records from Met Office records explain this increase with mean maximum temperature increasing from 10.1-13.3°C (2008) and 7.1-14.2°C (2009). These results are in agreement with previous studies which found that chalk streams naturally contain little variability in temperature (Shepard et al. 1986; Acornley 1999). The relatively warm temperature leads to faster development rates of salmonid embryos than other rivers in the UK which could be the reason that survival remains relatively high despite the high levels of fine sediment within river gravels to start with.
4.4.4.4 Intra-gravel flow measurements

Intra-gravel flow velocity was measured using a conductiometric standpipe (Carling and Boole 1986; Greig et al. 2005b). The method records the dilution rate of a known volume of solution (in this case saline) through the gravel bed environment via insertion of a conductivity probe into a standpipe (Greig 2004). The resulting decay rates are then compared with previously calibrated curves to give estimates of intra-gravel flow velocity. This study used the exponent decay calibration curves for intra-gravel flow velocity created by Grieg et al. (2005b). The raw data measured on site was normalised to the peak conductivity measured during a single measurement and an exponential curve was fitted to the data. The exponent from the curve relationship was then transferred into one of two calibration equations based on a threshold level of 0.28 which relates to low or high permeability within gravels;

An exponent of >0.28 would be entered as x into equation 1: \[ y = 2460.2x - 214.97 \]
An exponent of <0.28 would be entered as x into equation 2: \[ y = 881.23x - 43.27 \]
Equation 1 relates to gravel permeabilities >6260 cm h\(^{-1}\) and equation 2 relates to gravel permeabilities that are <6260 cm h\(^{-1}\) (Greig et al. 2005b). The use of these calibration curves to estimate intra-gravel flow (IGF) is an improvement from previously reported calibration procedures (Greig et al. 2005b). However for very low velocities and very high velocities the probe could still be under or over estimating flow velocity. Velocity estimates need to be compared with other IGF values to be more certain of the effective measuring of velocities as in the field, hydraulic gradient and water column velocity can influence the relationship between intra-gravel flow and gravel permeability (Greig, 2004). A limitation of estimating intra-gravel flow within this exponent intra-gravel flow to velocity relationship is that occasionally the intra-gravel flow recorded for some reds did not display a strong exponential relationship e.g. R\(^2\) ≤0.7. A cut-off of measurements giving an R\(^2\) ≥0.5 was used to standardise measurements meaning that some measurements were disregarded as erroneous. Intra-gravel flow rate was measured at weekly intervals at all sites and exhibited high intra-site and inter-site variability. Figure 4.29 displays 2008 data and Figure 4.30 displays data from the 2009 field season.

![Intra-gravel flow velocity for all sites in the 2008 field season.](image)

Figure 4.29. Mean Intra-gravel flow velocity for all sites in the 2008 field season. A logarithmic scale was used for intra-gravel flow velocity to display the range of data from all sites.
The 2008 intra-gravel flow results do not show a clear negative trend over time. There is some evidence that intra-gravel flow declines over time within redds at certain sites (Cheriton, Shawford House and Abbotstone) but this is not a general trend in the 2008 data as some sites show an increase towards the end of the period (Winchester). As Figure 4.30 is plotted on a logarithmic scale, the mean intra-gravel flow along with the variation displayed at sites (standard deviation) and the range of values measured over the period are reported in Table 4.8 for clarity.

![Figure 4.30. Intra-gravel flow velocity displayed at four sites during the 2009 field season.](image)

Final measurements end just before hatch, due to probe malfunction on the last few readings.

There is a general trend in the 2009 sites of declining intra-gravel flow over time, similar to other studies using the same method, which display negative trends of intra-gravel flow within the redd environment over time for other study rivers (Greig 2004; Burke 2011). Table 4.8 displays the range of values measured over the period.
<table>
<thead>
<tr>
<th>Site</th>
<th>Mean velocity (cm$^1$ hr$^{-1}$)</th>
<th>Standard Deviation</th>
<th>Minimum (cm$^1$ hr$^{-1}$)</th>
<th>Maximum (cm$^1$ hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>600</td>
<td>132</td>
<td>475</td>
<td>782</td>
</tr>
<tr>
<td>Abbotstone</td>
<td>680</td>
<td>463</td>
<td>271</td>
<td>1373</td>
</tr>
<tr>
<td>Cheriton</td>
<td>225</td>
<td>173</td>
<td>14</td>
<td>357</td>
</tr>
<tr>
<td>Ovington</td>
<td>454</td>
<td>126</td>
<td>359</td>
<td>668</td>
</tr>
<tr>
<td>Martyr Worthy</td>
<td>146</td>
<td>35</td>
<td>101</td>
<td>188</td>
</tr>
<tr>
<td>Winchester</td>
<td>160</td>
<td>227</td>
<td>23</td>
<td>563</td>
</tr>
<tr>
<td>Shawford House</td>
<td>997</td>
<td>1209</td>
<td>140</td>
<td>2728</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>812</td>
<td>495</td>
<td>148</td>
<td>1413</td>
</tr>
<tr>
<td>Gaters Mill</td>
<td>352</td>
<td>297</td>
<td>55</td>
<td>765</td>
</tr>
<tr>
<td><strong>2009 Sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arle</td>
<td>815</td>
<td>538</td>
<td>29</td>
<td>1688</td>
</tr>
<tr>
<td>Ovington</td>
<td>275</td>
<td>429</td>
<td>0</td>
<td>1048</td>
</tr>
<tr>
<td>Winchester</td>
<td>441</td>
<td>636</td>
<td>10</td>
<td>1887</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>536</td>
<td>405</td>
<td>4</td>
<td>1273</td>
</tr>
</tbody>
</table>

Table 4.8. Descriptive statistics for intra-gravel flow velocities (cm$^1$ hr$^{-1}$) reported in the 2008 and 2009 field season.

Inter- and intra-site variability is very large in the 2009 field season following similar patterns with the 2008 field season as described in the standard deviation. As mentioned previously other studies have noted the steady decline of intra-gravel flow when measuring over weekly intervals (Greig 2004; Greig et al. 2005a; Burke 2011). Potential reasons for the non-uniform declines found in both years’ data could be due to differing dispersal mechanisms displayed within chalk stream river gravels. The river bed sediment structure is composed of greater matrix material than other river types and contains a much greater proportion of fine sediment (section 4.4.1; Acornley and Sear 1999). This could imply that a longer measurement time than ten minutes (Greig et al. 2005b) could be needed to fully show the decline curve relating intra-gravel flow velocity to dilution rate. The calibration curves may not be suitable for use on the Itchen, although they were successful in estimating intra-gravel flows in a neighbouring chalk river in a previous study (Greig et al. 2005b). The cumulative grain size distributions are similar to the Test for the majority of sites on the Itchen, which suggests it is reasonable to use the existing calibrations.
4.4.5 Embryonic survival 2009

As previously mentioned in section 4.3.4, survival experiments were only possible in the 2009 field season and then only with a small number of native River Itchen eggs (300). Control eggs monitored within in-stream incubators located in the Candover stream recorded 74% survival from green egg to emergent alevin. The control measured survival rate was comparable with expected hatch rates which indicated that the survival measured within the artificial redds should not be adversely affected by high rates of natural mortality. Due to the low number of eggs available for survival experiments, it was not possible to place replicate control boxes at each site to ensure that survival was not affected by natural mortality effects and could be attributed to sediment accumulation and oxygen availability within the redd environment. Each basket displayed variation in survival rates are shown in Table 4.9. Maximum survival recorded was 84% at Arle site and minimum survival recorded was 28% at Bishopstoke. Recovery rates varied from 100-44% and was affected by the inability to accurately count dead and decomposing alevins and eggs.

<table>
<thead>
<tr>
<th>Site</th>
<th>Basket location within redd</th>
<th>Total % survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>EB 1 (front)</td>
<td>84</td>
</tr>
<tr>
<td>Arle</td>
<td>EB 2 (left rear)</td>
<td>76</td>
</tr>
<tr>
<td>Arle</td>
<td>EB 3 (right rear)</td>
<td>62</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>EB 1 (front)</td>
<td>28</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>EB 2 (left rear)</td>
<td>66</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>EB 3 (right rear)</td>
<td>64</td>
</tr>
</tbody>
</table>

Table 4.9. Rates of survival recorded within artificial redds at sites

It is clear to see from Table 4.9 that the upstream River Itchen exhibited higher rates of survival than the lower Itchen. Personal communications from Environment Agency staff predicted that spawning habitat appears to be of better quality in the upper reaches of the catchment and consequently survival should be higher which is supported with the results displayed in Table 4.9.

Decay of eggs was limited at the Arle site which allowed for easy identification. This could suggest that the expired eggs had died reasonably recently. Dead alevins at the Arle site were also not in a state of decay so mortality is estimated to have occurred when pulling out the egg baskets or just before. In contrast the dead eggs and alevins
found at the Bishopstoke site were at a more advanced state of decay leading to difficulty with identification. This indicates that these eggs had been dead for a longer time period than the Arle eggs, suggesting that catchment pressures on incubating eggs is greater the further downstream in the catchment.

Alevin appearance was examined from the upper and lower River Itchen to see if there were any noticeable differences (Figure 4.31). The Arle alevins (mean = 2.3mm, st.dev = 0.098) were in general larger than the Bishopstoke alevins (mean = 2.1mm, st.dev = 0.091) with darker colouring, suggesting healthier development rates. The alevins from the Arle site were slightly heavier than the Bishopstoke alevins also with respective mean weights of 0.199g and 0.189g. There was little difference in temperature between the sites so this was unlikely to affect development rates and the development stage seemed uniform among the samples (10°C based on Gorodilov scale (Gorodilov 1996)). The yolk sac morphology was the most noticeable difference between sites. The Bishopstoke alevins displayed elongated yolk sacs, in comparison to the Arle alevins.

One hypothesis for the size difference in yolk sac could be that the Bishopstoke alevins display slight oedema (swelling induced by increased fluid in tissues), which can cause the slight elongation of the yolk sac (Gorodilov 1996). Another explanation could be that Bishopstoke exhibits less favourable spawning habitat quality than the Arle as the larger yolk sac could be indicative of slower rates of development. Malcolm et al. (2003) observed that alevins developing under low dissolved oxygen conditions had a larger percentage mass of yolk sac remaining compared with alevins developing in more favourable conditions. This highlights the slight delay in development of alevins in lower dissolved oxygen environments.
4.4.6 Temporal datasets 2008 and 2009

In this section continuously monitored data for intra-gravel and surface water oxygen concentrations, intra-gravel and surface water temperature and piezometer pressure data are reviewed. Evidence for upwelling groundwater patches at sites is described.

4.4.6.1 Intra-gravel dissolved oxygen

Dissolved oxygen was recorded continuously over the incubation period in the water column and within the redd (Figure 4.32) in 2008. At the upstream Arle site, dissolved oxygen stays fairly constant in the water column, with a slight increase towards the end of the period and intra-gravel oxygen generally declines with a number of sharp troughs over the incubation period. For short periods of time the dissolved oxygen levels drop below 5mg l⁻¹ which is thought to be the threshold level for incubating salmonid embryos (Davis 1975). A possible explanation for the trend displayed in intra-gravel dissolved oxygen levels could relate to the presence of low oxygen concentration upwelling groundwater.

Figure 4.31. Photograph of a recovered Arle and Bishopstoke alevin. Top specimen is from the Arle and the bottom is from Bishopstoke sites.
Gaters Mill, the lower River Itchen site, in 2008 in contrast to the Arle site displays steadily declining dissolved oxygen in the redd from the beginning of the incubation period to the end. There is a large difference between DO measured in the water and the redd environment from the beginning suggesting that surface water at this site does not penetrate far into the gravels or that there is longer residence water flowing through the redd. Field observations from the site during construction and insertion of the probes noted high levels of settled fine sediment within the river bed and this site contained one of the higher levels of fine sediment at the end of the incubation period (Table 4.6).

The 2009 data (Figure 4.33) shows a similar pattern for intra-gravel oxygen to that for the 2008 field season at the upper Arle site. The lower sites display different profiles due to the change in location of the monitoring probes. The downstream Bishopstoke site intra-gravel oxygen profile follows a similar pattern to the upper site on the Arle. Oxygen levels do not decrease far below 8 mg l⁻¹ at the lower site, in contrast to the upper Arle site however, which suggests shorter residence flow and possibly less groundwater influence.
Figure 4.33. Intra-gravel and surface water and interstitial dissolved oxygen profiles at the Arle and Bishopstoke sites over the incubation period 2009 field season. Note probe malfunction led to a gap in the Bishopstoke data-set from 12-27 February. Hatch is denoted by the dotted vertical line.

Non-parametric Mann Whitney-U tests were employed to see if there was any significant difference between the upstream and downstream sites in terms of dissolved oxygen levels. Intra-gravel dissolved oxygen measurements were found to be significantly different between the upper and lower sites in 2008 (p = <0.001) and again between the upper and lower sites in 2009 (p=0.002).

A two-way analysis of variance on ranks was performed to test the null hypothesis that there was no difference between dissolved oxygen levels measured within the water column and the redd environment between sites and over different years; the results showed that the furthest downstream site displayed a significant difference between oxygen levels in the water column and redd, whereas the Bishopstoke and Arle (upper) sites did not display significantly different dissolved oxygen levels within the water and redd environment. Gaters Mill was also found to be significantly different to the other sites in all variables (except water column dissolved oxygen) which highlights the different regime that this site exhibits to the other sites in terms of higher flows.
(Figure 4.23) and sediment accumulation which occurred at the beginning of the incubation season at the same time as the higher flows were exhibited.

### 4.4.6.2 Groundwater influence

In addition to the factors measured in 2008, groundwater influence was also investigated in the 2009 field season. Based on the dissolved oxygen records observed at super sites over the incubation period, there was evidence of upwelling groundwater affecting the upper site as there were numerous troughs in the long term record (Figure 4.32 and Figure 4.33). Other studies have highlighted the influence of groundwater which is often characterised by having low dissolved oxygen levels (Malcolm et al. 2003; Malcolm et al. 2005; This was in contrast to the downstream site, which displayed a consistent, smooth decline in dissolved oxygen over time. Piezometers were installed to measure the hydraulic head of interstitial water. Figure 4.34 displays the hydraulic head measured within a redd at the upstream Arle site, along with intra-gravel dissolved oxygen measures and discharge for the same time period. A relationship between dissolved oxygen and the presence of upwelling groundwater is apparent.
It is clear to see that periods of prolonged groundwater upwelling, shown from the beginning of April to the end of the incubation period, coincided with a decline in the amount of dissolved oxygen within the redd environment. Discharge does seem to correlate with hydraulic gradients as there is a peak in discharge with corresponding peaks in hydraulic head. The final section of the graph where hydraulic head appears to increase linearly does not correspond with a similar increase in discharge in surface water; however it does correspond with a continued and prolonged decline in dissolved oxygen levels. This could indicate a prolonged period of ground water upwelling in this particular patch of river bed. Hatch occurred at the beginning of April, so it does seem that the developing embryos would have experienced some short periods of low dissolved oxygen below the 5mg l\(^{-1}\) threshold before removal from the redd. Survival data for this site was particularly high ranging from 62-84% which may suggest that prolonged periods of low dissolved oxygen impact on survival more than short bursts.

It can be seen from the graph that had the embryos been left to emerge naturally they would have experienced a prolonged period of low dissolved oxygen around hatch time which could possibly have detrimentally affected survival or led to early emergence of alevins (Malcolm et al. 2008). Early emergence from the gravels could lead to increased predation and decreased fitness of salmonid young which still contain yolk sacs, leading to reduced recruitment (Phillips et al. 1975). The relationship between upwelling groundwater and declining dissolved oxygen within the redd is further investigated in Figure 4.35. There is a significant relationship (p = 0.001) displayed between upwelling groundwater and low dissolved oxygen levels.
Figure 4.35. Linear regression describing the relationship between upwelling groundwater and intra-gravel dissolved oxygen within the redd environment at the Arle site. Where $y = -0.717x + 11.282$ with an R2 of 0.7, $p = <0.001$.

In contrast with the upper Arle site, the Bishopstoke site displays no evidence of upwelling groundwater and there was no relationship between hydraulic head and intra-gravel dissolved oxygen (Figure 4.36). Ground water influence using hydraulic head could not be measured for the Gaters Mill site in the 2008 field season due to time constraints so there is no evidence to suggest the low dissolved oxygen levels found at this site during the incubation period.
Conductivity measurements can often show the presence of upwelling groundwater as higher conductivity is more often found in groundwater than surface water (Soulsby et al. 2001; Malcolm et al. 2004). Conductivity was measured within the standpipes of reds and within the water column at weekly intervals at sites during the field season to assess if there was any difference in measurements that might suggest groundwater influence. There was little evidence of any difference in the range of conductivity measured in the surface water or the water column over the two field seasons (Table 4.10). However this lack of difference could be due to the low temporal resolution of the weekly monitoring regime which is likely to miss fine scale differences in redd and water environments as the continuously monitored hydraulic head measurement and dissolved oxygen readings show evidence of groundwater upwelling at the Arle site.

Figure 4.36. Hydraulic head measured at Bishopstoke site 2009 with discharge and intra-gravel dissolved oxygen within the redd environment for the same period. Note the hydraulic head are all negative numbers, indicating surface water down-welling. Missing DO data is displayed by the gap in the blue line. Hatch is denoted by the vertical dashed line.
Table 4.10. Conductivity ranges (min and max) in redd and water column over the 2008 and 2009 incubation period.

<table>
<thead>
<tr>
<th>2008 sites</th>
<th>Redd conductivity (x $10^4$ siemens)</th>
<th>Water column (x $10^4$ siemens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>4 - 4.4</td>
<td>4 - 4.35</td>
</tr>
<tr>
<td>Abbotstone</td>
<td>4 - 4.3</td>
<td>4.2 - 4.4</td>
</tr>
<tr>
<td>Cheriton</td>
<td>4.1 - 4.5</td>
<td>4.1 - 4.4</td>
</tr>
<tr>
<td>Ovington</td>
<td>3.85 - 4.3</td>
<td>3.9 - 4.3</td>
</tr>
<tr>
<td>Martyr Worthy</td>
<td>4.2 - 4.35</td>
<td>4.1 - 4.35</td>
</tr>
<tr>
<td>Winchester</td>
<td>4.15 - 4.45</td>
<td>4.2 - 4.45</td>
</tr>
<tr>
<td>Shawford House</td>
<td>4.3 - 4.45</td>
<td>4.3 - 4.45</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>4.3 - 4.6</td>
<td>4.3 - 4.6</td>
</tr>
<tr>
<td>Gaters Mill</td>
<td>4.2 - 4.6</td>
<td>4.3 - 4.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2009 Sites</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>3.5 - 4.9</td>
<td>3.6 - 4.8</td>
</tr>
<tr>
<td>Ovington</td>
<td>3.7 - 4.8</td>
<td>3.7 - 4.8</td>
</tr>
<tr>
<td>Winchester</td>
<td>3.7 - 5.1</td>
<td>3.7 - 4.8</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>3.6 - 4.8</td>
<td>3.5 - 4.8</td>
</tr>
</tbody>
</table>

4.4.6.3 Intra-gravel temperature

Temperature data are useful in delineating the presence of upwelling groundwater (Evans et al. 1995; Malcolm et al. 2004). A more stable thermal profile than surface water is characteristic of groundwater as diurnal and seasonal temperature fluctuations do not affect it (Sear et al. 1999). The temperature profiles for the upstream and downstream super sites for 2008 and 2009 period are shown in Figure 4.37 and Figure 4.38 respectively. In both figures the upper and lower sites display very similar profiles with cyclical, steadily increasing temperature (diurnal variation) from the beginning to the end of the period. Generally the upper sites recorded slightly lower temperatures than the lower sites. This is likely to be due to its higher altitude and distance from the coast. There was also very little variation between the intra-gravel environment and the water column which could give an indication that surface river water is the major source for the water found in the river gravels, rather than groundwater. This suggests that the surface water is affected by upwelling groundwater exhibiting stable temperatures despite fluctuations in air temperature.
Figure 4.37. Temperature profiles in the river and interstitial environment over the incubation period at the Arle and Gaters Mill sites in the 2008 field season. Typical chalk stream temperature shown in blue box (Crisp et al. 1992).
Figure 4.38. Temperature profiles in the river and interstitial environment at Arle and Bishopstoke sites over the incubation period 2009 field season. Note probe malfunction led to a gap in the Bishopstoke data-set from 12-27 February for redd data. Typical chalk stream temperature ranges are shown in the blue band (Crisp et al. 1992)

Non-parametric Mann Whitney U tests were carried out on the continuous datasets to see if there were any statistically significant differences between river and intra-gravel temperature. There was no significant difference found between the river and water temperatures measured at the upper and lower sites or between years and the similarity in diurnal variation between intra-gravel temperature and water column temperature indicates that the two waters were of the same origin. There was little difference in amplitude of temperature fluctuations in the redd compared to the water column suggesting little evidence of groundwater and the redd temperature was not warmer than the surface water again suggesting no groundwater impact.

### 4.4.7 Relationships between variables affecting survival

Due to the lack of survival data for salmonid embryos it was not possible to assess the relationship between habitat variables and survival of eggs as other studies have demonstrated (Greig et al. 2005a; Burke 2011). However it was possible to assess
whether there were any relationships between the driving factor of fine sediment and other habitat variables. Greig (2004) suggested linear regression models for sediment accumulation (<4mm) and intra-gravel flow velocity in agreement with recent studies (Burke 2011). Scatter plots were drawn to explore whether there was any relationship between the amount of fine sediment (<63µm) and intra-gravel flow rate and intra-gravel dissolved oxygen levels measured at hatch in the stand pipes over both field seasons.

Little or no correlations were found between <4mm and <2mm percentage fine sediment and intra-gravel dissolved oxygen levels, however there was a very weak correlation found between <63µm sediment and dissolved oxygen (Figure 4.39). Burke (2011) found a weak linear relationship ($p = <0.01$) between dissolved oxygen measured in spawning redds and percentage fine sediment accumulation, similar to that seen in Figure 4.39.

![Figure 4.39](image)

**Figure 4.39.** Relationship between % fine sediment (<63µm) and intra-gravel dissolved oxygen in the standpipes on the Itchen at sites in two redds for the 2008 and 2009 field seasons.

A weak negative correlation ($R^2 = 0.4$, $p = <0.01$) between intra-gravel flow velocity and fine sediment (<63µm) was found when looking at redd 1 at all sites in 2008 and two sites in 2009 (Figure 4.40). Limited intra-gravel flow velocity data was available for the hatch period due to probe malfunction so data was limited to exploring the one redd. Similar relationships were observed in intra-gravel flow velocity and % fine sediment at hatch where a linear relationship gave an $R^2$ of 0.35 (Greig 2004; Burke 2011). A weak
A positive correlation ($R^2 = 0.3$, $p < 0.01$) was found between intra-gravel flow velocity and dissolved oxygen measurements taken from redd one in the 2008 field sites (Figure 4.41). These results suggest that there is a relationship between fine sediment and intra-gravel flow velocity and intra-gravel flow velocity and dissolved oxygen. There must be other factors affecting these variables other than inorganic fine sediment.

**Figure 4.40.** The relationship between fine sediment (% <63µm) and intra-gravel flow rate from redd one data at sites near to the hatch incubation stage (8, 2008 sites and 2, 2009 sites).
Greig (2004) forwarded the hypothesis that the high levels of organic material found in spawning gravels on a chalk stream, induced high oxygen demands of these deposited material which consequently lowered the amount of dissolved oxygen available to incubating eggs. If the oxygen demand of organic material within redds decreases intra-gravel oxygen concentration, then there should be a relationship between the amount of organic material found in redds and the subsequent intra-gravel oxygen in the redd. To test this hypothesis a scatter plot was drawn to elucidate if there was any relationship between these two variables (Figure 4.42). Interestingly no relationship was found between oxygen measured within the standpipes and the amount of organic material (<63µm) found at the end of the incubation period in the 2008 data. It appears from the graph that there is a slight increase in intra-gravel dissolved oxygen and increasing organic content of sediments which is contrary to theories proposed about the importance of organic material within salmonid redds (Alonso et al. 1996; Greig et al. 2005a; Heywood et al. 2007; Sear et al. 2008).
Figure 4.42. Organic material and intra-gravel dissolved oxygen scatterplot of sites at the end of the 2008 incubation period.
4.5 Discussion and implications

The spawning habitat of the River Itchen is characterised by high intra-gravel dissolved oxygen levels prior to hatch, low fine sediment accumulation at hatch (<4mm, <2mm and <63µm) and highly variable intra-gravel flow rates over the incubation period. Inter- and intra-site variation existed within all variables measured, highlighting the importance of catchment-wide studies (Greig et al. 2005a; Sear et al. 2008; Burke 2011). The baseline uncut gravels investigated for River Itchen spawning sites suggest that they contain a high proportion of fine sediment (<2mm) often exceeding 20%. This is in agreement with other studies conducted in similar rivers (Milan et al. 2000; Greig et al. 2005a; Heywood et al. 2007). Sear et al. (2008) states that most permeable catchments contain large amounts of matrix (<2mm) sediment within their gravel frameworks in comparison to other river typologies and often display low suspended sediment yields and infiltration rates. This incongruity is based on the stable flow regime, low gradient and lack of sediment transport in terms of bed mobility found within these catchments (Sear et al. 1999; Sear et al. 2008).

This study shows that there is relatively low sediment accumulation over the salmonid incubation period on the Itchen. Survival of salmon embryos was greater than 50% in most baskets and exceptionally high in the upper catchment with 85%+ which indicates that there are high quality spawning habitat patches on the Itchen and salmonid embryos in artificial redds can exhibit high survival. Sediment accumulation results are low in comparison to other studies and seem to agree with other studies’ conclusions of low accumulation rates leading to high salmonid survival, however significant relationships between these two variables could not be found due to limited survival data (Greig, 2004; Greig et al. 2005, 2007; Heywood et al. 2007; Burke, 2011).

Despite accumulation levels being low during the incubation period, fine sediment content of uncut and cut river gravels was high compared to other types of rivers in other studies (Greig 2004; Burke 2011) and contained similar amounts too other chalk rivers (Riley et al. 1999; Grieg 2004). This indicates that any small amount of additional fine sediment accumulated over the incubation period could potentially cause much larger impacts on survival of embryos than would be the case in a freshet river system for example. Heywood et al. (2007) commented that the relationship between survival and fine sediment accumulation was highly sensitive in chalk rivers and indicated that a small increase in fine sediment could produce a large decrease in survival. Figure 4.43 displays the relationship of <1% fines and % survival of salmonid embryos in this study and others looking at chalk rivers and other types of rivers. The
River Itchen data fits well with other chalk rivers investigated (Grieg 2004; Heywood and Walling 2007). The steep, negative gradient of the linear relationship supports the statement that small increases in accumulation could lead to a relatively large decrease in survival. Heywood et al. (2007) reported a 50% drop in salmonid embryo survival when fine sediment (<1mm) reached >8% within spawning redds in a chalk catchment. Within the context of the survival experiments in this study, fine sediment (<1mm) ranged from 3.4 - 1.9 % at hatch so did not reach the threshold set by this recent study. However survival ranged from 28 - 86% which was variable for such small changes in fine sediment lending weight to the argument that small increases in fine sediment accumulation over the incubation period may have larger affects in permeable catchments (Sear et al. 2008).

Figure 4.43. Meta data for studies looking at the relationship between fine sediment (<1mm) and salmonid survival (%). Itchen data is characterised by the red points, other chalk rivers are black points and other river types are white points. The blue line shows the linear regression with equation for chalk rivers % egg survival in relation to % <1mm sediment. Chalk river data from Greig (2004) and Heywood and Walling (2007). Run-off dominated river data from Greig (2004), Julien and Bergeron (2006) and O’connor and Andrew (1998).

In view of the fact that there was not enough survival data to explore meaningful relationships with spawning habitat variables over space and time, relationships
between each of the variables were explored. In contrast with other recent studies (Greig 2004; Heywood et al. 2007; Burke 2011), little or no relationships were found to exist between <4mm fine sediment and intra-gravel flow velocity or dissolved oxygen levels in the redd. However the finest clays and silts (<63µm) showed weak correlations with intra-gravel flow and dissolved oxygen. This indicates that the finer fraction of fine sediment influences habitat variables that affect the spawning success of salmonids more than larger fine sediment fractions in chalk rivers, unlike other types of river where the bulk fine sediment <4mm is often shown to be of similar importance to the finer fractions (Grieg et al. 2005a; Burke 2011). Lapointe et al. (2004) found in laboratory studies that the clay and silt fraction (<63µm) content of infiltrated sediment was the most sensitive to survival of eggs, with changes of one or two per cent causing large declines in survival, particularly in gravels containing >10% sand. Redefining ‘fine sediment’ within the context of the River Itchen may well indicate that particles with a smaller diameter in the silt/clay region (<63µm) are the most problematic to salmonid survival.

The weak relationships described between fine sediment, intra-gravel flow and intra-gravel dissolved oxygen concentrations suggest there are other factors influencing spawning habitat quality on the Itchen. One such factor has previously been thought to be organic inputs. Results from this study show that despite the high levels of organic material found in redds (>20%) there is no relationship between dissolved oxygen levels at the end of the incubation period. This could be explained by the low temporal resolution of the weekly dissolved oxygen measurements and that the total amount of organic material does not fully describe or give an estimate of the most labile fractions of organic sediments which are likely to exhibit greater oxygen demands within redds. This result indicates that total organic material is not an indicator of oxygen consumption by organic sediments. Chapters 5 and 7 explore the role of organic sediments within redd sediments in greater detail.

Other possible factors influencing spawning habitat on the River Itchen could be groundwater interactions, hydrologic regime and longer residence pollutants residing in river bed sediments. Groundwater influence was found to have a marked effect on intra-gravel dissolved oxygen at the upper Arle site during the 2009 field season. It was not found to influence the lower Bishopstoke site. Catchment-wide investigation at all sites may confirm the importance of upwelling groundwater along the river and it may be hypothesised from these results that the upper catchment may experience a higher occurrence of groundwater interactions than the lower catchment. Particularly with intra-gravel dissolved oxygen measurements this study shows the importance of the difference between spatial and fine-scale temporal resolution. The weekly
standpipe measurements at the Arle site did not pick up the drop in dissolved oxygen measured by the continuously monitored probe; however the weekly measurements allowed large scale monitoring programmes to be devised with many sites which highlights the importance of using both types of method when collecting data from natural systems.

The implications of these results for salmonid spawning habitat on the River Itchen are:

- <63µm fine sediment is likely to be the most important size fraction in terms of sediment accumulation within redds over the incubation period
- Fine sediment (<63µm) affects intra-gravel flow velocity and to a much lesser extent dissolved oxygen levels within redds
- Groundwater upwelling decreases dissolved oxygen levels in the hyporheic zone on the upper catchment
- Large amounts of organic material found in redds has implications for the usefulness of remediation method for fine inorganic sediment; organic loads need to be identified and reduced
- Higher survival of embryos was recorded in the upper catchment in comparison to the lower catchment

There is a real risk on the River Itchen that if accumulation rates of fine sediment increase on the river there may well be a large negative impact on salmonid embryo survival. Climate change, changing land use and existing land management practices could well impact on the sediment load entering the river, increasing the amount available for redd infiltration and as previously stated small changes in sediment infiltration into chalk stream redds can have large impacts on survival (Heywood et al. 2007). Ormerod (2009) discusses the implications of climate change on rivers and their conservation and particularly notes that rivers with current issues are likely to be exacerbated by climate change. Despite the uncertainty surrounding climate change predictions (Quiggan 2008), the wealth of science surrounding the likely scenarios can provide information on the kind of future issues likely to arise on the River Itchen. Sediment delivery to the River Itchen could be increased by wetter winters and abstraction pressures in the summer from a growing population could have large impacts on flow which could exacerbate sediment concretion issues already present on the Itchen.

Spawning site distribution on the river may well be confined to areas that have previously been spawned on or have been gravel cleaned in other years and hen salmon overcutting redds could also be reducing recruitment to the population. A
previous report indicated that over-cutting of redds in chalk rivers could be a major problem, compounded by the lack of suitable gravels (Cragg-Hine 2002). Evidence to back up this statement was observed in a study conducted on the Piddle and Avon which found that there were fewer redds than salmon observed and 22% of redds were being utilised by two or more hen salmon (Cragg-Hine 2002). This highlights the continued need for gravel cleaning on the catchment to ensure there are enough suitable habitat patches for fish to utilise. Survival experiments looking into the impact of cleaned and un-cleaned gravels suggest that salmonid eggs have greater success in cleaned gravels, 40-66% survival and 2-34% survival in un-cleaned gravels, respectively (Riley et al. 1999; Cragg-Hine 2002).
Chapter 5. Quantifying Sediment Oxygen Consumption Rate and Total Sediment Oxygen Demand within salmonid redds

5.1 Chapter synopsis and information

This chapter introduces the concept of sediment oxygen demand (SOD) and sediment oxygen consumption rate (SOC) and describes it in relation to rivers and spawning gravels, with a view to quantifying its potential importance for determining spawning habitat quality and successful egg incubation. Firstly the key theories and previous literature describing SOD/SOC and its measurement are reviewed. Secondly an experimental laboratory method and results are presented to quantify and estimate the SOD and consumption exhibited by infiltrated sediments within salmonid redds. Analysis will explore the spatial and temporal variation in SOC/SOD over the incubation period as well as different particle size SOC/SOD in an attempt to understand the role played by sediment in the consumption of available oxygen to salmonid embryos. Organic inputs to the redd environment should be considered in salmonid survival to emergence metrics as a dissolved oxygen sink (Chevalier and Carson 1985). SOD/SOC which is created by organic material is often overlooked in many models. Recent studies have highlighted oxygen as a major limiting factor in relation to salmonid embryo success (Greig et al. 2005a, 2007b) and a lack of knowledge surrounding sediment oxygen demand of infiltrated sediment has been noted. SOD/SOC is often mentioned as a source utilising dissolved oxygen within the redd environment in these studies but insufficient quantitative data supports the SOD/SOC values quoted by models and studies (Chevalier and Carson 1985; Greig 2004; Greig et al. 2005; Sear et al. 2008). This study attempts to address the knowledge gap surrounding sediment oxygen demand present in salmonid redds.

It is important to note that SOD and SOC are often used interchangeably in much of the previous literature to define the rate of oxygen lost from the overlying water column to underlying sediments. However in this research, definitions are set out at the beginning to avoid confusion.
- Sediment Oxygen Consumption (SOC) is defined as the rate of oxygen consumed from overlying water by sediments measured in mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$
- Sediment Oxygen Demand (SOD) is defined as the total oxygen demanded by sediments over a certain defined period of time e.g. the SOC rate multiplied by the number of days exhibiting that rate. SOD units are defined as mg O$_2$ g$^{-1}$ dry sediment.

In the interest of coherency, wherever a rate is supplied in the literature, whether it is called SOD or SOC it will be written in this study as SOC.
5.2 Introduction

5.2.1 Dissolved oxygen transport to underlying sediments

As previously stated within the literature review adequate dissolved oxygen availability is required for the successful development of salmonid embryos within the interstitial environment (Grieg et al. 2005a; Sear et al. 2008). Understanding the pressures on oxygen availability within the interstitial environment is integral to the further development of this research area. Therefore it is first relevant to understand the main mechanisms by which oxygen is transported to bed sediments before looking at the consumption of oxygen by bed sediments. The transfer of oxygen between gravel bed streams and the water column above is an extremely complex process that involves many different factors (Chevalier and Carson, 1985). Previous research has described the transport mechanisms and flux of oxygen between sediment and the overlying water and has elucidated a diffusive boundary layer with distinct zones where oxygen is transported by different processes and can limit the concentration of oxygen reaching the underlying sediments (Revsbech et al. 1980; Jorgensen and Revsbech 1985).

Within the water column where turbulent flows exist, eddy diffusion drives the transport of oxygen through mixing with deeper levels of water where laminar flow regimes dominate (Jorgensen and Revsbech 1985). Here oxygen is transferred between parallel layers by molecular diffusion (Jorgensen and Revsbech 1985). In rivers complex and dynamic hydraulic conditions exist where upstream factors have a marked impact on the flow regime experienced downstream.
A – defines the outer limit or start of the boundary layer
B – defines the effective diffusive boundary layer
C – defines the true (viscose) boundary layer where oxygen concentrations decline prior to reaching the bed causing a potential limit to oxygen supply to sediment

Figure 5.1. A schematic of the boundary layer at the sediment water interface to show the oxygen concentration change with depth within the water column (Jorgensen and Revsbech 1985)

The oxygen profiles and transport to and from the bed sediment described in Figure 5.1 were observed in controlled laboratory experiments using microelectrodes in marine and lake sediments (Jorgensen and Revsbech 1985). House (2003) when describing river sediments and dissolved oxygen profiles within gravel beds in similar laboratory conditions found no viscose boundary layer with coarser river sediments but observed a boundary layer where finer river sediments were found with high organic inputs. This suggests that the transport of dissolved oxygen in coarse river gravels is not limited by diffusion through a boundary layer but is limited by oxidation reactions in the sediment (House 2003) and the direct transport of oxygenated water to the interstitial environment by intra-gravel flow.

House (2003) described the oxygen profiles measured in different riverine sediment mixes and at different flow rates to emulate lotic systems. They described the
transport of dissolved oxygen via diffusion into the sediment with the use of differential equations as is in Equation 5.1:

\[
dc(x)/dt = D_s \left( d^2c(x)/dx^2 \right) - g
\]

Equation 5.1.

where;
\( c \) = the concentration of dissolved oxygen in the pore water (mg/l)
\( D_s \) = diffusion coefficient of oxygen in the sediment
\( x \) = distance from the water sediment interface (cm)
\( g \) = the net reaction of dissolved oxygen within the pore water

Biochemical reactions, such as the oxidation of organic matter and chemical redox reactions, are both combined within the function \( g \), which describes the oxygen consumption involved within sediments (House 2003). The diffusion coefficient was created using the porosity of sediments proportional to temperature dependant molecular oxygen diffusion. Essentially Equation 5.1 explains that diffusion rates in riverine gravels are controlled by the concentration of dissolved oxygen in pore waters in relation to its distance from the sediment-water interface and its diffusion coefficient (temperature dependant) minus the net reactions of dissolved oxygen in the pore water. The velocity of the fluvarium was low so as not to disturb the sediment surface and oxygen penetration depths, defined as the distance from the interface to the depth where oxygen reached zero, were measured and ranged from <1mm on day one to <3mm by day 38 (House, 2003). In the natural environment and over a range of flows and substrates, the oxygen penetration of sediments will vary greatly in rivers so this experiment gave very broad estimations of oxygen penetration into river gravels.

When considering incubating salmon eggs in river gravels, it is not only important for oxygen to be transported and transferred effectively into the gravel bed so it is available for use by eggs, but also oxygen transport directly to the eggs that needs consideration. At the egg micro-scale the transport of oxygen occurs by a combination of diffusion and direct transport of dissolved oxygen to the egg surface from the environment surrounding the egg (Daykin 1965). Dissolved oxygen is contained within a solute layer surrounding the egg which always contains a lower concentration than the concentration of dissolved oxygen within the macro-environment (Wickett 1975). This creates a concentration gradient for the diffusion of oxygen across the egg membrane. This gradient exists and is dependent on egg respiration and the supply of the water directly surrounding the egg (Daykin 1965). The dissolved oxygen
concentration of the water must contain higher levels of dissolved oxygen than the eggs themselves to enable this concentration gradient to exist.

Mass transfer theory is a quantitative method which can be applied to the problem of the transport of oxygen from the macro-environment to the micro-scale egg environment (Daykin 1965; Wickett 1975; Greig 2004). There is a need to expand on this theory as all oxygen sinks are not included within the model process equations. For example, the consumption of oxygen by other biological entities such as aerobic bacteria within gravels is not accounted for. Gravel permeability and pore space affect the direct movement and pathways of flow through river beds and these factors will affect the supply of water containing dissolved oxygen to embryos.

5.2.2 Sediment oxygen demand and consumption

Sediment oxygen consumption (SOC) describes the rate at which dissolved oxygen is removed from the overlying water column by biological and chemical reactions in the stream bed sediments (Hatcher 1980; Miller-Way et al. 1994; Mackenthun and Stefan 1998; Moodley et al. 1998; Nakamura 2003; He and Liu 2011). Benthic communities within the sediment in natural systems consume dissolved oxygen via respiration consuming organic matter. Combined with chemical reactions known as redox reactions, these are the major origins of sediment oxygen demand (Rauch et al. 2008). Figure 5.2 describes the major processes occurring in freshwater environments regarding sediment oxygen consumption.
Organic matter is the collective term used to describe complex organic molecules and biological compounds found in the water environment. Within the freshwater environment this could mean any plant or animal matter that is derived from in-stream sources (autochthonous), such as macrophyte or invertebrate matter or riparian zone plant and animal matter (allochthonous) (Wipfli et al. 2007). When organic matter settles down into the river bed sediment, it can either be permanently buried or can provoke a sequence of redox reactions that remove carbon from the sediment system by oxidizing it to CO$_2$ (Rauch et al., 2008). These redox reactions are mostly facilitated by microbial organisms as their enzymes act as catalysts (Libes 2009). Table 5.1 describes some of the main chemical reactions that occur in sediments and the general order of the oxidation of organic matter in terms of Gibbs free energy (Libes 2009). The biological mineralisation and oxidation-reduction reactions are driven by microbial density and activity within the sediment and as such are temperature dependant (Cerco et al. 1992; DiTorro 2001; Libes 2009).
<table>
<thead>
<tr>
<th>Process or pathway name</th>
<th>Oxidation formula</th>
<th>Gibbs free energy change (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Respiration</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \frac{1}{4} \text{O}_2 = \frac{1}{4} \text{CO}_2 + \frac{1}{4} \text{H}_2\text{O} )</td>
<td>-29.9</td>
</tr>
<tr>
<td>Denitrification</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \frac{1}{5} \text{NO}_2^- + \frac{1}{5} \text{H}^+ = \frac{1}{4} \text{CO}_2 (g) + \frac{1}{10} \text{N}_2(g) + \frac{7}{20} \text{H}_2\text{O} )</td>
<td>-28.4</td>
</tr>
<tr>
<td>Manganese respiration</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \frac{1}{5} \text{Mn}O_2(s) + \text{H}^+ = \frac{1}{4} \text{CO}_2 + \frac{1}{2} \text{Mn}^{2+} + \frac{3}{4} \text{H}_2\text{O} )</td>
<td>-24.6</td>
</tr>
<tr>
<td>Iron respiration</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \text{Fe(OH)}_2(s) + 2\text{H}^+ = \frac{1}{4} \text{CO}_2 (g) + \text{Fe}^{2+} + \frac{11}{4} \text{H}_2\text{O} )</td>
<td>-12.6</td>
</tr>
<tr>
<td>Sulphate reduction</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \frac{1}{8} \text{SO}_4^{2-} + \frac{1}{8} \text{H}^+ = \frac{1}{4} \text{CO}_2 (g) + \frac{1}{8} \text{HS}^- + \frac{1}{4} \text{H}_2\text{O} )</td>
<td>-6.1</td>
</tr>
<tr>
<td>Methane fermentation</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} = \frac{1}{8} \text{CO}_2(g) + \frac{1}{8} \text{CH}_4 (g) )</td>
<td>-5.6</td>
</tr>
<tr>
<td>Hydrogen fermentation</td>
<td>( \frac{1}{4} \text{CH}_2\text{O} + \frac{1}{4} \text{H}_2\text{O} = \frac{1}{4} \text{H}_2(g) )</td>
<td>-1.6</td>
</tr>
</tbody>
</table>

Table 5.1. Pathways of organic matter decomposition and oxidation of organic compounds (represented as 'CH₂O') in the order of decreasing energy yield – the more negative the free energy value the more favoured the reaction. Adapted from Libes (2009).

Benthic organisms such as algae, bacteria, protozoans and fungi convert dead plant and animal molecules into smaller molecular species and soluble end products by extra-cellular enzymatic hydrolysis (Sierra and Gomez 2007) which leads to the excretion of extra-cellular polymeric substances (EPS). EPS are sticky, high weight compounds mostly made up of polysaccharides, secreted by microbes during lysis and hydrolysis of macromolecules (Sheng et al 2010). They are capable of fusing particulate matter together within the stream bed environment, creating molecules
with large surface areas and initiating the creation of biofilms (Petticrew and Arocena 2003; Lundkvist et al. 2007).

Biofilms can potentially increase the SOC and SOD within river ecosystems. Different structural biofilms (web or film-like) can be observed when excessive organic inputs enter a system; for example large scale pacific salmon deaths post spawning (Petticrew and Arocena 2003) or raw sewage inputs to a river. Biofilms created by excessive organic matter inputs can increase the sediment oxygen consumption of bed sediments, especially in anthropogenic disturbed systems where sewage outfall inputs and land-use run-off increase organic matter loading, due to the increase in microbial density and increased respiration rates (Sierra and Gomez 2007).

The majority of previous research has focused on measuring SOD in estuarine and coastal mud flats (Revsbech et al. 1980; Jorgensen and Revsbech 1985; Beringer and Huettel 1997), marine sediments (Engelsen et al. 2008; Rauch and Denis 2008) and lake bottom sediments (Mackenthun and Stefan 1998; Josiam and Stefan 1999) although some recent studies have experimented with river sediments in field and laboratory studies (House 2003; Utley et al. 2008; Liu et al. 2008; He and Liu 2011). River beds that contain small pore spaces, fine sediments and large surface areas in these lentic ecosystems create the potential for sediment oxygen consumption rates to substantially affect dissolved oxygen concentrations in the overlying water body. In the case of salmonids and other benthic dwelling organisms, it is the accumulation and flow of organic matter that are important to the overall oxygen budget in the gravels.

The area of bed sediment which contains oxygen is known as the oxic zone and is shallower in lakes and marine sediments than river sediments due to less mixing potential at the sediment-water interface and finer sediments (Mackenthun et al. 1998). This can lead to depleted levels of dissolved oxygen due to the imbalance of sedimentary oxygen consumption rate and advection of oxygen into sediments (Mackenthun et al. 1998). In open sea bed sediments the first few decimetres of the bed surface contains oxygen and penetration is limited from mm – 10cm in continental shelf regions (Cai and Sayles 1996). From intra-gravel dissolved oxygen and hydraulic flow rates measured in the Itchen catchment it is observed that the oxic zone can reach further than this in rivers, to at least 30-40cm within the river bed (see section 4.3.5). Measuring the oxygen consumption of bed sediments in lentic environments is therefore arguably more straightforward than measuring lotic ecosystems. There is likely to be much greater mixing of anoxic pore water with oxygen rich surface inputs when turbulent flows are experienced in events in lotic ecosystems. Specifically in chalk based rivers, groundwater inputs could also contribute to the mixing of pore
waters within the oxic and anoxic zones in bed sediments. Groundwater infiltration can increase nutrient loads directly via the water itself and also by remobilising trapped organic material within bed sediments (Douglas et al. 1997). This can lead to greater oxygen demand by sediments (Douglas et al. 1997). Many SOD methodologies are not suited to measuring lotic systems as flow influences dissolved oxygen levels and therefore determines sediment oxygen demand and penetration of oxygen in to the sediments (House, 2003).

Sediment oxygen consumption can be the largest consumer of dissolved oxygen especially within shallow sediments or stagnant systems at certain times of the year, e.g. summer months where eutrophication can occur in some lakes and also ice covered lakes in winter where there is little transfer of inputs (Mackenthun and Stefan 1998; Madenjian, 1990). This is problematic for biota inhabiting these regions as oxygen is vital to their development, therefore it is imperative to understand the capacity for SOC and potential for disrupting the balance of water ecosystems. Lotic ecosystems with organisms dependant on a good supply of dissolved oxygen and or high levels of organic inputs i.e. pollution or dense macrophyte populations, could also exhibit high SOC rates and are overlooked within the research area, particularly in relation to the success of incubating salmonid progeny within river gravels. SOC has been highlighted as a key research area in many publications concerned with salmonid embryo survival in the redd environment (Grieg, 2004; Grieg et al. 2007; Sear and DeVries 2008). Chalk rivers with shallow, slow flowing sections could then potentially be at greater risk of exhibiting high SOC rates than other native salmonid rivers in the UK.

There are varying rates of sediment oxygen consumption found throughout the literature relating mostly to marine, coastal and estuarine and lake sediments. Previous literature report typical measured SOD rates that range from 0.2g O₂ m⁻² d⁻¹ for sandy sediments to 10g O₂ m⁻² d⁻¹ for finer sediments with a high percentage of organic material (Thomann and Mueller 1987 cited in Mackenthun and Stefan 1998). Table 5.2 lists the varying SOD rates found throughout the literature for rivers.
<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Type of sediment</th>
<th>SOD rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayes and MacAulay 1959</td>
<td>Laboratory controlled bottles</td>
<td>Lake</td>
<td>1.49 - 10.63 mg O₂ cm⁻² day⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– 1063 g O₂ m⁻² day⁻¹</td>
</tr>
<tr>
<td>Edwards and Rolley 1965</td>
<td>Laboratory controlled experiments – undisturbed control core</td>
<td>River mud</td>
<td>0.13 - 0.15g O₂ m⁻² hour⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.005 - 0.006 g O₂ m⁻² day⁻¹</td>
</tr>
<tr>
<td>Alonso et al. 1996</td>
<td>Freeze core laboratory experiments from artificial reds</td>
<td>River/redd</td>
<td>- Mean 5 day = 0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Mean 20 day = 7.11 mg g⁻¹</td>
</tr>
<tr>
<td>Doyle and Rounds 2003</td>
<td>In situ SOD chambers</td>
<td>River</td>
<td>1.32 - 4.59g O₂ m⁻² day⁻¹</td>
</tr>
<tr>
<td>Greig 2004</td>
<td>Laboratory BOD method</td>
<td>River</td>
<td>Test – 3.21 Blackwater – 4.86 Ithon – 7.36 Aran – 7.98 mg O₂ g (organic material)¹ hour⁻¹</td>
</tr>
<tr>
<td>Liu et al. 2007</td>
<td>In situ SOD chambers</td>
<td>Tidal River</td>
<td>2.54 - 3.66g O₂ m⁻² day⁻¹</td>
</tr>
<tr>
<td>Bakan and Cuce 2007</td>
<td>Laboratory respirometer</td>
<td>River</td>
<td>0.37 - 1.04g O₂ m⁻² day⁻¹</td>
</tr>
<tr>
<td>Utley et al. 2008</td>
<td>In situ SOD chambers</td>
<td>River</td>
<td>0.1 - 2.3g O₂ m⁻² day⁻¹</td>
</tr>
</tbody>
</table>

Table 55.2. SOD rates found in the literature (primarily relating to rivers). *Published rates converted below for ease of comparison with other studies. **SOD here is independent of time and is a measure of the silt and clay fraction settling in the control volume within SIDO UK model.***Temperature corrected to 20°C

Notably the values stated by Grieg (2004) for river sediments on the River Test, a southern chalk stream, were the lowest recorded out of the different river types sampled in this study. Although the sediments contained a high quantity of organic material, it was mostly thought to be composed of older in-stream organic detritus and therefore refractory carbon which is thought to have a lower oxygen consumption in contrast to labile carbon catchment surface inputs (Grieg 2004). Grieg (2004) noted that it was of importance to record the time after an event when removing sediment samples, as fresh organic inputs often consume greater oxygen within sediments than older more decomposed material.
Calculating the SOC and SOD of natural systems is a complex task. A range of factors can affect the flux of oxygen to the interstitial environment and hence the SOC rate, such as: density of biological micro-organisms, composition and amount of organic material, interstitial flow rates and depths reached, sediment pore space, oxygen concentration of surrounding water, sediment re-suspension which increases the surface area enhancing oxygen uptake, photosynthesis/respiration and macro-faunal activity which can cause re-suspension and can exhibit local oxygen consumption (Cerco et al. 1992), depth of sediment and organic matter content of sediment (Truax et al. 1995). Previous research however, suggests that the most significant factors affecting SOC are temperature at the sediment water interface, water velocity of overlying sediments and organic content of the sediment (Truax et al. 1995; Utley et al. 2008).

Biological and chemical reactions are temperature dependant which has obvious implications for SOC rates. Biological processes are shown to double with a 10°C increase in temperature (within reaction range) as described by Van't Hoff’s rule (McDonnel and Hall 1969) which means that SOC will be temperature dependant due to increased microbial reaction rates (Doyle and Rounds 2003). Dissolved oxygen concentrations within the overlying water column are negatively correlated with increasing temperature due to out-gassing (Lin, 2007). The combination of these two relationships indicates the possible significance of SOC in high temperature-low dissolved oxygen environments. Previous laboratory research into the effect of temperature on the consumption of oxygen by river muds found that oxygen consumption increased with temperature (Figure 5.3) (Edwards and Rolley 1965).
Figure 5.3. Average oxygen consumption (g m$^{-2}$ h$^{-1}$) rates of river sediments from (a) R. Ivel and (b) R. Hiz over a 14 month period in relation to temperature (°C) and oxygen concentration of the overlying water (ppm) (Edwards and Rolley 1965).

The hydrological regime in rivers can affect the flux rate of oxygen from water to pore space and can either aid or impede oxygen transfer over the interface, where the oxygen uptake depends entirely on the utilization of oxygen by the viable microorganisms decomposing organic matter (Higashimo et al. 2008) and the disturbance of the bed by hydrological events. Decomposition is limited by the dissolved oxygen and biodegradable organic matter available. Higashimo et al. (2008) modelled the transfer of oxygen from the water column to the sediment layer using the diffusive boundary layer concept and found that oxygen concentration varies not only vertically through the sediment layer but also in the direction of bulk flow, horizontally across a stream's cross-section and downstream with long profile. Microbial uptake of oxygen and turbulent diffusion were incorporated into the model to give a more comprehensive view of water-sediment interface dynamics. The flux of oxygen between the interstitial environment and the overlying water column is dependent on the residence time of the overlying water and influences the dissolved oxygen concentration within the water (House, 2003). For example in turbulent mixing flows sediment can be disturbed and the mixing of sediment and pore water can consume oxygen within the bulk water column (Jubb et al. 2001).

Mackenthun and Stefan (1998) showed with laboratory experiments and models on lake sediments and sawdust how SOC rate increases linearly with increasing flows. When flows exceed approx. 3 cm s$^{-1}$ the upper boundary for velocity related increased SOC had been reached (Mackenthun and Stefan 1998). Model validation confirmed that observed real SOC rates from sawdust and lake sediments compared well with modelled SOC rates and were velocity dependant only at low velocities (Josiam and Stefan 1999). Conversely oxygen profiles measured in intertidal sediments in the
laboratory showed that with increased velocity, oxygen concentration through the sediment was reduced due to a decrease in the diffusive boundary layer (Beringer and Huettel 1997). This suggests that higher flow velocities could limit the amount of dissolved oxygen reaching the sediment-water interface and hence limit SOC rate due to an insufficient supply of oxygen. A similar experiment, this time with river sediments in the laboratory, showed how velocity (low velocity and high velocity respectively, 10 – 20 cm s\(^{-1}\)) had no significant effect on oxygen penetration depth; however sediment surface area and organic content were the greater controls on SOC (House, 2003). There are conflicting results describing the relationship of flow velocity and SOC rate. This may partly be due to different methods and types of sediments used within the experiments described above (Table 5.2).

### 5.2.4 Review of different methodologies

In general SOC and SOD methods usually consist of taking a known volume of sediment contained within a known volume of water, sealing the mixture off from outside influence and measuring the amount of oxygen consumed within the water over time (Truax et al. 1995). The linear portion of the oxygen decline curve is used to estimate SOD (Utley at al. 2008). There are two general categories of methods that have been developed to measure SOC and SOD: \textit{in-situ} experiments carried out within the field and \textit{ex-situ} experiments where sediment is transported back to the laboratory for use in controlled experiments (Bakan and Cuce 2007; Liu 2009). Specific methods used within these categories include the use of micro-electrodes measuring oxygen consumption at micro scales within sediment beds (Revsbech et al. 1980; Jorgensen and Revsbech 1985; House 2003; Glud et al. 2005), \textit{in situ} SOC chambers (Doyle and Rounds 2003; Utley et al. 2008; Liu et al. 2009), sediment cores \textit{in ex-situ} chambers in controlled laboratory conditions (Hayes and MacAulay 1959; Bakan and Cuce 2007; Rauch and Denis 2008), flume sediment controlled laboratory experiments (Mackenthun and Stefan 1998; Higashino et al. 2008), chamberless methods which use general water quality and laboratory sediment methods to determine SOC rates at certain temporal scales using a spreadsheet of data (Charbonnet et al. 2006; Osborn et al. 2008) and more recently planar optode data which provides 2D images of oxygen distributions within bed sediments (Glud et al. 2005).

Essentially there are advantages and disadvantages to both categories. Laboratory experiments have the advantage that parameters can be controlled increasing the accuracy of results obtained, but are then unable to reproduce field conditions correctly and can only provide estimates of natural measurements (Doyle and Rounds...
2003; He and Liu 2011). In situ field experiments have the advantage of measuring real environments but not all data can be collected for example, interstitial oxygen concentrations or accurate flow measurements (Glud et al. 2005).

Micro-electrode studies have been instrumental in exploring the diffusive boundary layer and mechanisms of oxygen transport from the overlying water column to marine and lake sediments (Figure 5.1) in laboratory and field studies (Revsbech et al. 1980; Jorgensen and Revsbech 1985; Glud et al. 2005). House (2003) used river sediments and micro-electrodes to determine SOC rates in controlled laboratory experiments. River sediments displayed different behaviour to lake and marine sediments as regards SOC rates, as stated previously. It is important to note that different sediments with different characteristics i.e. particle size, pore space, and source inputs, will display different SOC rates. A limitation of using micro-electrodes is that they only measure at very small spatial scales within sediments and it is difficult to extrapolate data to give SOC rates for a larger area such as a reach or tributary (Glud et al 2005). Planar optode data is extremely useful to measure in situ sediments as it allows for oxygen concentrations and profiles to be measured as well as giving a detailed view of sediment composition and macro-faunal activity (Glud et al. 2005). However there are still issues with temperature compensation and image treatment. It is still one of the better methods for obtaining information on oxygen concentrations and flux at the sediment water interface in real systems without causing disturbance to them (SenseNet 2009).

Trade-offs are made when deciding which methods to use in SOC/SOD experiments. The literature suggests that in situ measurement of SOC is preferable to laboratory methods as the sediment is not disturbed and ‘real’ rates can be measured in natural, undisturbed conditions (Truax et al. 2995; Doyle and Rounds 2003; He and Liu 2011). Most previous river studies focus on fine sediment systems rather than mixed gravel bed systems that experience flows >0.3 m/s. With respect to the present study however, river sediment contains larger pore spaces and is created from a larger matrix framework than lake and estuarine sediments. To date oxygen profiles in relation to depth within river sediments and salmonid redd sediments in particular are little known. The fine fraction of sediment is reported to block pore spaces within the redd which reduces the efficiency of dissolved oxygen passage through the redd environment, leading to inhibited salmonid embryo development (Grieg 2004; Grieg et al. 2007; Heywood et al. 2007). In order to explore if there were any relationships between organic content, SOC and particle size of the redd sediment on the Itchen the removed sediment cores were separated into different particle size fractions, which created the need to develop a new method for measuring SOC rates. The finer particles
(<2mm) were particularly of interest because of the previous work suggesting that this size fraction causes the most damage to salmonid embryos (Greig 2004). Due to the varied methods existing for measuring SOC, it is often difficult to compare rates created by different methods which may lead to difficulties when discussing the importance of results.
5.3 SOD method

The aims of the experiment were:

- To develop a new method which would be able to measure SOC rates from artificial salmonid redd sediments with various particle sizes and use the method successfully to measure SOC and calculate SOD in redd sediments from the River Itchen.
- To explore the temporal and spatial variability of SOC/SOD within the catchment and at different development stages using field samples collected from various sites along the River Itchen.
- To create a unique set of SOC rates and SOD to be used within survival models for salmonid species.

An adequate supply of dissolved oxygen is one of the main factors highlighted within the literature review that affects survival of salmonid embryos during the incubation period, so understanding the oxygen requirements for the sediment surrounding the eggs becomes important when determining available oxygen to incubating salmonid progeny.

5.3.1 Experimental methodology

Artificial redds were created at four locations on the River Itchen, Hampshire, (Figure 5.4) to represent the different spawning habitat utilised by salmonids following the method of Grieg et al. (2005a, 2007b). Sediment baskets containing sediment truncated to 4mm were deployed within the pot section of the redd, surrounded by polyethylene bags which sit at the base of the basket and are used to ensure no infiltrated fine material is lost on removal (Greig et al 2005a). Redd sediment was collected from sediment baskets at different salmonid embryo development stages of the incubation period, namely the eyeing stage, hatch and emergence. The sediment baskets were placed in sealed dark containers on ice to minimise biodegradation and oxygen utilisation prior to the commencement of the laboratory experiment.
Figure 5.4. River Itchen site location map. Arle and Ovington are the upstream sites and Winchester and Bishopstoke were the downstream sites.

Sediment was collected over two field periods, 2009 and 2010. The 2009 sediment was used to trial the method and investigated the spatial variation in SOC rates along the river Itchen along with the difference that particle size made on SOC rate. Variables that remained constant during the experiments, such as temperature, agitation and light exposure were explored in this set. Redd sediment was separated into different size fractions on return to the laboratory by wet sieving to <4mm and <63µm using the wet sieving method described in the general methods chapter. Separate SOC rates could be determined for the different sized infiltrated material, which is important as <63µm sediment is thought to be the most damaging particle size to the development of salmonid embryos due to a number of factors including, blocking pore space and impeding passage of oxygenated surface water to the embryos (Sear et al. 2008).

Based on data retrieved in the 2009 experiments, only the finer material (<63µm) was used in the 2010 experiments. Spatial variation along the Itchen was also investigated in the 2010 experiments. Sediment was processed immediately on return to the
laboratory to ensure that oxygen consumption measurements were started as soon as possible to accurately capture decomposition of organic matter. Whilst previous laboratory studies have kept sediment cores intact to measure the depth of oxygen penetration and mimic field conditions better (Truax et al. 1995; Rauch and Denis 2008), it was considered necessary to disturb the sediment for the accurate measurement of SOC rates of different particle size. The intact removal of river sediments using the bag and basket method is not possible and with the likely mixing of materials throughout the basket due to their disturbed nature (i.e. redd building and subsequent removal from river) and river flow it was thought that representative sediment sub-samples of the sieved basket material would allow for sediment oxygen demand to be measured.

For the 2009 experiments a sub sample of sediment (<4mm and <63 μm) from each site was placed into 250ml of de-ionised water and transferred into the 500ml BlueSens shake flasks (Germany). It is important to note that an adequate amount of overlying water was used in the experiments to allow for accurate measurable oxygen decline and also ensured that the dissolved oxygen concentration in the headspace and water would not become a limiting factor for SOD (Truax et al. 1995). Volumes for individual flasks were noted down (approx. 250ml) to use in equations to determine SOD rates later. Slight variations on the above method for the 2010 experiments were a larger sub-sample of sieved <63μm material taken from each site was split into two flasks with 300ml water, allowing for replicate measurements to be taken of each site.

The flasks were filled with sediment slurries created from the sub-sample of sediment taken from the Itchen. Lids were screwed on to the flasks which had been fitted with a previously calibrated In-Q-OX MediceL oxygen probe and sealed with araldite, forming a closed system. They were securely fastened into the GallenKamp orbital incubator by the use of clip fasteners. The incubator allowed for the control of temperature, agitation and light interference. Experiments were carried out in the dark mimicking the environment that the sediment would be in if they were still buried within the river bed in salmon redds. Throughout the separate experiments, during the incubation period (i.e. at eyeing, hatch and emergence) the temperature was set to the average temperature observed at the field locations from the day of removal from the redd environment. The agitation plate inside the incubator was set to 100 revs min⁻¹ which ensured the adequate mixing of material so that oxygen gradients did not form in the sediment or in the water column, which could give a false impression of the rate of oxygen decline and be a limiting factor in actual SOD rates (Revsbech et al. 1980; Jorgensen and Revsbech 1985). The oxygen probes were wired up to a Delta-T DL2-e
logger which continuously measured and recorded % oxygen saturation within the gas head space every 15 minutes.

5.3.2 SOD calculation and method theory

In biological oxygen demand experiments, there is an assumption that there is a proportional relationship between oxygen consumption by aquatic organisms and the amount of organic matter present. The hypothesis of these sediment oxygen consumption experiments is that, similar to the concept of biological oxygen demand (BOD) experiments that there should be some relationship between the amount of organic material present and SOC. The SOC rate measured in these closed experiments is inferred from the loss of oxygen from the gas headspace based on the principles of mass balance; that matter can neither be created nor destroyed. Closed systems allow for equilibrium to occur between states, in this case gaseous oxygen and aqueous dissolved oxygen. Gases dissolve in liquids to form solutions in an equilibrium process e.g. the equilibrium between oxygen gas and dissolved oxygen in water is defined as:

\[ O_2(aq) \rightleftharpoons O_2(g). \]

The concentration of a solute in one state is always bigger than the other, by a fixed proportion that is temperature dependant (Lewis and Evans 2006). Equilibrium is disrupted when microbes within the sediment consume oxygen. A steady state is attempted to be regained. The amount of oxygen consumed in the sediment mixture therefore decreases the partial pressure of oxygen in the gas head space according to Henry's law (Equation 5.2).

\[ HK = \frac{p(O_2)}{c(O_2)} \]

Equation 5.2.

Where:

\[ HK = \text{Henry's law constant (atm mol}^{-1}) \]
\[ p = \text{partial pressure (atm)} \]
\[ c = \text{molar concentration (mol l}^{-1}) \]

The change in concentration of dissolved oxygen (calculated using Henry's law) in the closed flasks was found to be very small (<0.004) for all runs in comparison to the change in partial pressure in the gas head space. This is due to the constant in the equation above being such a large number (HK for oxygen = 769.2 l\(^1\) atm/mol).
Because the partial pressure of gas in the head space and concentration in the water are proportional to each other it is acceptable to use one as a proxy for the other.

### 5.3.3 Calculation of SOC rates and SOD

Within the literature, previous studies using *in-situ* sediment chambers and undisturbed sediment cores in the laboratory have calculated SOC rates using a formula based on the volume of water and surface area of the sediment bed being measured (Truax et al. 1995). Equation 5.3 is the model used by Liu (2009) to calculate SOC rates within river sediment cores in the laboratory:

\[
SOC_t = \frac{SV_s}{A_s}
\]

Equation 5.3.

Where;
- \(SOC_t\) = sediment oxygen consumption at temperature °C (mg O₂ g⁻¹ day⁻¹)
- \(S\) = linear portion of oxygen curve slope
- \(V_s\) = volume of sampler (m³)
- \(A_s\) = surface area bottom of sampler (m²)

However the calculation of the SOC rates from this experimental methodology demands a different calculation due to the fact that the sediment was not kept in undisturbed cores so therefore the surface area of the sediment becomes irrelevant. An explanation of the calculation of SOC rates calculated in this study follows below (Equation 5.4).

Manufacturer specifications for the ln-Q-OX MediceL probes described repeatability as <1% of signal (City Technology, specification). A stabilisation time of 15 minutes was allowed for when measurements were taken as per manufacturer advice. The oxygen probes measured millivolts and were calibrated using a two point calibration in ambient air oxygen levels (21%) and 0% oxygen conditions (nitrogen saturated conditions) and were set to record % oxygen saturation within the gas head space of the flasks.

\[
\frac{(c_1(t^3) \times g) - (c_2(t^2) \times g)}{m^{\gamma_p}} \times A \times 1000 = O_2(H)
\]
Where:

\( C_1 \) = \( O_2 \) concentration at \((t_1)\) time 1 (% sat converted to fraction)
\( C_2 \) = \( O_2 \) concentration at \((t_2)\) time 2 (% sat converted to fraction)

\( G \) = gas head space volume inside flask (ml)

\( T \) = temperature (°C)

\( p \) = pressure kPa (dependant on temperature)

\( A \) = atomic weight of oxygen

\( m \) = number of moles present at \( T \) and \( p \) (ideal gas law)

\( O_2 \) (H) = oxygen lost in the headspace (mg) over time \((t_1-t_2)\)

Equation 5.4 calculated mg of oxygen lost in the headspace over time. An example calculation is displayed in Equation 5.5. Values for the equation variables are presented first and then substituted into Equation 5.5 to fully explain the process followed. Data is from 2010 eyeing experiments, Arle site (<63µm).

\[
\frac{\left(0.249 \times 358 - 0.1436 \times 358\right)}{23220} \times 32 \times 1000 = 30.27 mg O_2
\]

The mg oxygen was divided by the dry weight of sediment and by the number of days incubated to give sediment oxygen consumption rate in mg O\(^2\) g\(^{-1}\) dry sediment day\(^{-1}\). Total SOD was calculated by multiplying the calculated SOC rate by the number of days exhibiting this rate. Sediment slurries were filtered through 550°C burnt Whatman <8µm glass fibre filters and then oven dried for 2 hours at 100°C before weighing when cool to supply the dry weight of sediment. A representative example of an original oxygen decline curve from an SOC/SOD experiment is shown in Figure 5.5. It shows that oxygen declines over time in the chamber. Similar oxygen decline curves to that in Figure 5.5 were produced for all sites. Slight variations between the gradient of the slope produced differing sediment oxygen demand rates.
There is a fast decline in oxygen at the beginning of the experiment in section A and then a steady more linear oxygen decline in section B (Figure 5.5) which corresponds with results shown in previous oxygen consumption experiments (Butts, T 1974; Jubb et al. 2001; Utley et al, 2008). Two distinct periods, A and B were observed in all the oxygen decline graphs that informed the theory behind calculating the sediment oxygen consumption. Standard BOD methods in water quality studies suggests that five days is a standard time period to observe the oxygen demand of organic effluents and sewage wastes on water.

The 25-day time period is considered by convention to be an adequate time for the complete bio-chemical oxidation of organic material within water samples, sometimes known as the total biological oxygen demand (Delzie and Mckenzie 2003). It was considered that the longer term 5-day and 25-day periods were of significant importance to incubating salmonid embryos as these rates give a more accurate account of oxygen consumed by sediment within the redd over the incubation period. It is important to understand the long term sediment oxygen consumption of redd sediments due to the retention time of sediments and also to provide a more accurate indication of the demand imparted by deposited organic material (Greig 2004). Rates were calculated from 0-5 days and 5-25 days based on the two distinct areas A and B.

Figure 5.5. A representative example of dissolved oxygen decline in the gas head space of a SOC chamber.
described in the oxygen decline curve (Figure 5.5) and the theory that longer term rates were hypothesised to be more important to incubating salmonid embryos due to the residence time within the gravels. The 0-5 day rate is written as ‘SOC<sub>5</sub>’ and the 5-25 day rate is called ‘SOC<sub>25</sub>’. Total oxygen demand for the entire period (SOD<sub>0-25</sub>) was calculated based on the SOC rates multiplied by the number of days exhibiting those rates, e.g. (SOC<sub>5</sub> x 5) + (SOD<sub>25</sub> x 20).
5.4 Results

5.4.1 2009 data

Calculated SOC rates for the 2009 experiments are displayed in Table 5.3 and Table 5.4 for hatch and emergence development stages respectively. The pilot experiment, carried out on eyeing stage sediment, to trial the experimental method highlighted an issue with leaks in the sealed system. This led to slight modifications with probe insertion and cable attachments for the preceding runs.

<table>
<thead>
<tr>
<th>Site</th>
<th>Particle size</th>
<th>$SOD_{5}$ mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
<th>$SOD_{25}$ mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
<th>$SOC_{0.25}$ mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle egg basket 2</td>
<td>&lt;4mm</td>
<td>0.32</td>
<td>0.03</td>
<td>2.17</td>
</tr>
<tr>
<td>Arle egg basket 2</td>
<td>$&lt;63\mu$m</td>
<td>5.81</td>
<td>1.84</td>
<td>65.78</td>
</tr>
<tr>
<td>Arle egg basket 1</td>
<td>&lt;4mm</td>
<td>1.48</td>
<td>0.52</td>
<td>17.90</td>
</tr>
<tr>
<td>Arle egg basket 1</td>
<td>$&lt;63\mu$m</td>
<td>8.27</td>
<td>2.83</td>
<td>97.97</td>
</tr>
<tr>
<td>Bishopstoke egg basket 2</td>
<td>&lt;4mm</td>
<td>3.09</td>
<td>0.90</td>
<td>33.44</td>
</tr>
<tr>
<td>Bishopstoke egg basket 2</td>
<td>$&lt;63\mu$m</td>
<td>29.25</td>
<td>3.75</td>
<td>221.21</td>
</tr>
<tr>
<td>Bishopstoke egg basket 3</td>
<td>&lt;4mm</td>
<td>1.58</td>
<td>0.53</td>
<td>18.49</td>
</tr>
<tr>
<td>Bishopstoke egg basket 3</td>
<td>$&lt;63\mu$m</td>
<td>5.17</td>
<td>0.65</td>
<td>38.89</td>
</tr>
<tr>
<td>Ovington redd 1</td>
<td>&lt;4mm</td>
<td>0.52</td>
<td>0.15</td>
<td>5.59</td>
</tr>
<tr>
<td>Ovington redd 2</td>
<td>$&lt;63\mu$m</td>
<td>7.16</td>
<td>1.81</td>
<td>71.97</td>
</tr>
<tr>
<td>Winchester R1</td>
<td>&lt;4mm</td>
<td>0.57</td>
<td>0.12</td>
<td>5.22</td>
</tr>
<tr>
<td>Winchester R1</td>
<td>$&lt;63\mu$m</td>
<td>6.71</td>
<td>1.86</td>
<td>70.78</td>
</tr>
</tbody>
</table>

Table 5.3. Hatch SOC rates and SOD for different sites, particle size and time periods.

It is clear to see from Table 5.3 that the smaller particle size material exerts a larger oxygen demand than the larger material. The $<63\mu$m material at all sites along the Itchen displays a larger sediment oxygen consumption at all time periods. This observation is mirrored in Table 5.4. The egg baskets at the upper Arle and lower Bishopstoke sites display slightly higher SOC rates in Table 5.3 for the SOC$_5$ and SOC$_{25}$. This could potentially be due to the retention of organic material from decomposing salmon eggs as this formed part of the fine sediment within the sediment baskets at hatch.
Table 5.4. Emergence SOC rates and SOD for different sites, particle size and time periods.

Table 5.4 mirrors the higher values shown in Table 5.3 for the upper Arle site, although the other three sites display very similar SOC rates for SOC₅ and SOC₂₅. The greater SOC₅ rate can be attributed to the larger proportion of readily available carbon over the first few days of the experiment. Other studies have observed similar patterns and generally discount the first two hours of the experiment (Bowman and Delfino 1980; House 2003). Table 5.4 also shows that the finer sediment exerts a greater SOC rate than the larger particles. This is hypothesized to be due to finer material having a greater surface area making biodegradable organic carbon more available promoting oxidation reactions between molecules. It could also be partly due to the finer material containing greater organic matter content than the coarser material. This assumption agrees with the % organic content of the sediment. Loss on ignition experiments estimates the total % organic material of the sediment, where sub-samples were burnt at 550 C for 2 hours and then weighed when cool to determine the weight lost which equates to the organic material present within the sediment (Lamb, 2004). A further burn at 950 C removes the inorganic carbonate content from the sample (Lamb, 2004). Figure 5.6, Figure 5.7, and Figure 5.8 display the difference in % organic content and particle size at all sites. These figures clearly display that the smaller particle size, the larger the % organic content of the sediment.
Figure 5.6. Eyeing stage different particle size and organic content of accumulated redd sediment 2009.

Figure 5.7. Hatch stage different particle size and organic content of accumulated redd sediment 2009.
The assumption made from the SOC rates that higher rates are experienced within the <63μm chamber as there is more organic material available for consumption is valid according to Figures 5.6, 5.7, 5.8 and 5.9. On this assumption the <63μm SOC rates were then used for further analysis.

The % organic content contained within <63μm infiltrated sediments were found to range from approximately 12% - 17% over the incubation period (Figure 5.9).
The eyeing development stage displays the lowest organic content suggesting that organic material accumulated over time within the redd environment. However hatch and emergence organic content were reasonably similar, with hatch organic content surpassing that of emergence at the two sites (Arle and Bishopstoke). Potential causes of increased organic material could be due to the accumulation of drift material deposited by flow over time or due to the increased amount of organic material found within the river resulting from increased macrophyte and riparian vegetation growth attributed to warmer temperatures (Cotton et al. 2006). One possible theory for the increased organic content of sediments at hatch exhibited at the two super sites could be the remnants of decomposing fish eggs present within the fine sediments.

The inorganic carbonate content found in sediments at sites was found to exhibit similar relationships to the particle size graphs found for % organic content. The <63\(\mu\)m size fraction of sediment exhibits the greatest % carbonate content. These graphs for the different incubation stages can be found in Appendix 3. Figure 5.10 displays the % carbonate content of <63\(\mu\)m sediments over the incubation period. There is little difference between the amount of inorganic carbon present at different incubation stages and, with the exception of Winchester which consistently displays higher carbonate content than the other sites, inorganic carbon content appears to be similar at all sites on the River Itchen. Winchester was a particularly concreted site which indicates a high density of calcium carbonate deposits. Increased calcium concentrations have a tendency to flocculate fine particles and could be one of the reasons for the elevated levels of carbonate found at this site (Figure 5.10).

![Figure 5.10](image.png)

Figure 5.10. Carbonate content of <63\(\mu\)m particles accumulated within redd sediments at different incubation stages at all sites.
The higher SOC rates found on the Arle could be as a result of discharges containing higher organic matter content than normal river discharges from fish farm outlets just upstream of the site location. Organic sediment infiltration into the artificial redds on the Arle seems to be high, suggesting greater organic inputs from sources in the upper catchment than sites further downstream. However SOC rates were generally higher at emergence time than at hatch which suggests greater organic material had accumulated in redd sediments at this stage. An explanation of this increase in organic content is the seasonal macrophyte growth during the spring and the potential for more autochthonous decaying plant parts in the river at this time is greater. Other studies have found that macrophyte distribution and growth varies throughout the year (Flynn et al. 2002).

In order to display the relationship between SOC rate and % organic matter content, scatter plots containing best-fit regression lines were drawn in Minitab to explore whether any correlation between the two variables existed. The % organic matter content of samples were normalised to a scale range of 1-10. Figure 5.11 and Figure 5.12 show the relationship between SOC$_5$ and SOC$_{25}$ and % organic matter content. Positive correlations are observed between increasing SOC rate and % organic matter. This can be seen in both the SOC$_5$ and SOC$_{25}$ results.

Figure 5.11 displays a linear regression fit with associated 95% confidence intervals between SOC$_5$ and normalised organic matter with an $R^2$ value of 0.72 suggesting a correlation between the two variables. The analysis of variance results (for full table see Appendix 4) show the amount of variation in SOC$_5$ explained by normalised organic matter and the resulting P-value of 0 suggests that the association between the two variables is significant where the significance level is set at $p = 0.05$. It should be noted here that one sample was removed from this analysis (Bishopstoke egg basket SOC$_5$ = 29.25) as it appeared to be an outlier. Figure 5.12 displays a linear regression fit with 95% confidence intervals, between SOC$_{25}$ and normalised organic matter. An $R^2$ value of 0.57 indicates a weaker correlation between SOC$_{25}$ and organic content than the SOC$_5$. Regardless of the lower $R^2$ value, the ANOVA results indicate a significant association between SOC$_{25}$ and organic matter with a P-value of 0 at $p = 0.05$ (Appendix 4).

Looking at other similar results, House (2003) found that SOC rate constants showed a linear increase with organic matter content ($R^2 = 0.8$) and oxygen penetration increased with decreased organic content. It should be noted that SOC rate was calculated on a shorter time period in the House (2003) study than the longer term period used in this study. However the results shown in Figure 5.11 and Figure 5.12 seem to suggest that
larger organic matter content of samples will lead to a higher SOC rate over 5 and 25 day periods.

Figure 5.11. Linear regression fit between normalised organic matter content and SOC\textsubscript{5} of fine redd sediments 2009.

Figure 5.12. Polynomial relationship between normalised organic matter content and SOC\textsubscript{25} of accumulated fine sediment within redds 2009.
All sediment sizes (2mm and <63µm) were used in the drawing of these graphs which explains the clustering of groups. The smaller particle size, exhibits the larger % organic material with greater SOC rates and the greater particle size displays the reverse. The relatively small number of data points presented here could potentially influence the relationships.

The relationships between carbonate content and SOC rate show some interesting results. There is a negative correlation between both SOC$_5$ and SOC$_25$ and normalised carbonate content. In order to perform regression analysis on the % carbonate content the values were normalised to a scale of 1-10 to remove the % issue. Figure 5.13 displays a linear regression with confidence intervals of SOC$_5$ and carbonate content. The $R^2$ value is 0.27 suggesting a very weak correlation between the two variables. Significance of the regression line was tested using ANOVA which generated with a P-value = 0 indicating a significant association between the two variables set at $p = 0.05$.

$$y = -4.881 + 12.75x - 2.882x^2 + 0.1747x^3$$

$$R^2 = 0.27$$

Figure 5.13. Polynomial regression and 95% confidence intervals of SOC$_5$ and carbonate content of accumulated fine sediment in redds 2009.

Similarly in Figure 5.14, the relationship between SOC$_{25}$ and carbonate content gives a linear regression with an $R^2$ value of 0.64 only slightly less than Figure 5.11. ANOVA results give a P-value of 0 demonstrating the significance of the relationship between the two variables at $p = 0.05$. These results suggest that the larger carbonate content of the sediment the lower the oxygen demand rate imparted by that sediment.
5.4.2 2010 data

The 2010 field season allowed further sediment oxygen demand experiments to take place. Following the laboratory method described above a series of experiments were set up similar to the 2009 set of experiments. However replicates included this year were intended to test the replication of the method and give a more accurate picture of the rates exhibited across the catchment at different sites. Only the oxygen consumption associated with fine particulate matter (material <63µm) was observed in these experiments at four sites along the Itchen Arle, Ovington, Winchester and Bishopstoke. Alonso (1996) discussed the important contributory portion of SOC coming from the silt and clay fractions in the SIDO manual, which verifies the smaller fraction of sediment used in these further experiments.

<table>
<thead>
<tr>
<th>Site</th>
<th>Particle size (µm)</th>
<th>SOC_{25} mg O_2 g(^{-1}) dry sediment day(^{-1})</th>
<th>SOC_{25} mg O_2 g(^{-1}) dry sediment day(^{-1})</th>
<th>Total SOD_{25} mg O_2 g(^{-1}) dry sediment day(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle Redd 1</td>
<td>&lt;63</td>
<td>13.27</td>
<td>0.007</td>
<td>66.48</td>
</tr>
<tr>
<td>Arle Redd 2</td>
<td>&lt;63</td>
<td>19.48</td>
<td>1.77</td>
<td>132.90</td>
</tr>
<tr>
<td>Ovington Redd 1</td>
<td>&lt;63</td>
<td>10.39</td>
<td>1.35</td>
<td>78.91</td>
</tr>
<tr>
<td>Winchester Redd 1</td>
<td>&lt;63</td>
<td>22.70</td>
<td>2.20</td>
<td>157.49</td>
</tr>
</tbody>
</table>

Figure 5.14. Linear regression and 95% confidence intervals of the relationship between SOC\(_{25}\) and carbonate.
<table>
<thead>
<tr>
<th>Site</th>
<th>Particle size (µm)</th>
<th>SOC$_1$, mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
<th>SOC$_2$, mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
<th>Total SOD, mg O$_2$ g$^{-1}$ dry sediment day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bishopstoke R1</td>
<td>&lt;63</td>
<td>16.91</td>
<td>0.27</td>
<td>89.87</td>
</tr>
<tr>
<td>Arle rep 1</td>
<td>&lt;63</td>
<td>5.32</td>
<td>3.10</td>
<td>88.65</td>
</tr>
<tr>
<td>Arle rep 2</td>
<td>&lt;63</td>
<td>44.05</td>
<td>-2.28</td>
<td>174.71</td>
</tr>
<tr>
<td>Ovington rep 1</td>
<td>&lt;63</td>
<td>5.25</td>
<td>2.98</td>
<td>85.89</td>
</tr>
<tr>
<td>Ovington rep 2</td>
<td>&lt;63</td>
<td>8.13</td>
<td>3.80</td>
<td>116.72</td>
</tr>
<tr>
<td>Winchester rep 1</td>
<td>&lt;63</td>
<td>4.22</td>
<td>2.08</td>
<td>62.70</td>
</tr>
<tr>
<td>Winchester rep 2</td>
<td>&lt;63</td>
<td>2.42</td>
<td>1.53</td>
<td>42.66</td>
</tr>
<tr>
<td>Bishopstoke rep 1</td>
<td>&lt;63</td>
<td>3.64</td>
<td>1.18</td>
<td>41.86</td>
</tr>
<tr>
<td>Bishopstoke rep 2</td>
<td>&lt;63</td>
<td>3.27</td>
<td>0.98</td>
<td>35.99</td>
</tr>
</tbody>
</table>

Table 5.5. Eyeing SOC rates and SOD for fine sediment accumulated within redds over different time periods. NB: not all replicate results are presented here due to anomalous data.

Table 5.6. Hatch SOC rates and SOD for fine sediment accumulated within redds over different periods. All replicate samples displayed.
<table>
<thead>
<tr>
<th>Site</th>
<th>Particle size (µm)</th>
<th>Hatch SOC $\text{mg O}_2 \text{ g}^{-1}$ dry sediment day$^{-1}$</th>
<th>Emergence SOC $\text{mg O}_2 \text{ g}^{-1}$ dry sediment day$^{-1}$</th>
<th>Total SOD $\text{mg O}_2 \text{ g}^{-1}$ dry sediment day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle rep1</td>
<td>&lt;63</td>
<td>4.72</td>
<td>2.48</td>
<td>73.27</td>
</tr>
<tr>
<td>Arle rep2</td>
<td>&lt;63</td>
<td>13.04</td>
<td>2.90</td>
<td>123.14</td>
</tr>
<tr>
<td>Ovington rep1</td>
<td>&lt;63</td>
<td>5.38</td>
<td>2.85</td>
<td>84.00</td>
</tr>
<tr>
<td>Ovington rep2</td>
<td>&lt;63</td>
<td>5.67</td>
<td>4.49</td>
<td>118.17</td>
</tr>
<tr>
<td>Winchester rep 1</td>
<td>&lt;63</td>
<td>3.02</td>
<td>1.79</td>
<td>50.88</td>
</tr>
<tr>
<td>Winchester rep 2</td>
<td>&lt;63</td>
<td>2.42</td>
<td>1.37</td>
<td>39.42</td>
</tr>
<tr>
<td>Bishopstoke rep 1</td>
<td>&lt;63</td>
<td>4.39</td>
<td>1.97</td>
<td>61.27</td>
</tr>
<tr>
<td>Bishopstoke rep2</td>
<td>&lt;63</td>
<td>3.69</td>
<td>1.23</td>
<td>43.15</td>
</tr>
</tbody>
</table>

Table 5.7. Emergence SOC rates and SOD for fine sediment accumulated within redds over different time periods with replicate samples displayed.

Hatch and emergence SOC rates reported in Table 5.6 and Table 5.7 respectively display larger SOC rates than at the eyeing stage. Hatch and emergence rates display similar values to each other which emulate the earlier results from the 2009 experiments. The lower eyeing values could potentially be due to lower amounts of organic material being present. As in the 2009 experiments it is of use to explore the organic content of the finer material.

Total SOD appears to be greater at more upstream sites which is surprising as the further downstream you are the greater the number of inputs there that could potentially increase SOD. The 2009 results show similar patterns, although at hatch the lower Bishopstoke site has the greatest SOD possibly due to decomposing fish egg material confined within sediments.

### 5.4.3 Organic content and SOC rate

Similar values to the % organic content found in the 2009 experiments were observed in the 2010 data (Figure 5.15) finer portion of the organic content % of infiltrated sediment (<63µm) was investigated using loss on ignition experiments.
Figure 5.15. Displays the different organic matter concentrations found in redd sediments at developmental stage at all sites.

There appears to be little difference between sites in % organic content of infiltrated fine material. Ovington, an upstream site shows the highest values whilst the other three sites are approximately even. There is little change over the different developmental stages, unlike the 2009 data where the furthest downstream site exhibited the highest values of organic material at hatch and second highest at emergence. It seems less likely that the lower SOC rates for eyeing are due to the amount of organic material present in sediments. Figure 5.16 displays similar results to the 2009 carbonate content at sites, with Winchester displaying the largest amount of carbonate within accumulated sediments. The other sites show little spatial variation or temporal variation over the incubation period.
Figures 5.16 and 5.18 display the relationship of % organic content and SOC rate for all sites and development stages. There is a very weak correlation displayed in Figure 5.18 between SOC$_{5}$ and organic matter ($R^2 = 0.27$) with a cubic regression line. This suggests that there is a very weak relationship between the two variables. ANOVA results (appendix 4) give a P-value of 0.16 which suggests that the relationship is not significant at $p = 0.05$. This is in contrast to the 2009 data which display a significant correlation between SOC$_{5}$ and organic content. Figure 5.19 shows that there is a weak positive correlation displayed between SOC$_{25}$ and organic content ($R^2 = 0.35$), which is a lot lower than the 2009 results. The P-value obtained from ANOVA (Appendix 4) is 0.02 which means that the relationship is significant where $p = 0.05$. There is a large degree of scatter displayed in both Figure 5.17 and Figure 5.18. Better relationships might be displayed if there were a greater number of data points.
Figure 5.17. Relationship between normalised organic matter and SOC$_5$ of redd sediments with cubic polynomial regression and related 95% confidence intervals.

Figure 5.18. Quadratic polynomial regression of the relationship between SOC$_{25}$ and normalised.

Carbonate content of samples and the relationship with SOC rate were also explored. Figure 5.19 shows that there is no relationship between SOC$_5$ and carbonate content.
with the 2010 sediment samples. This is in stark contrast to the 2009 results which saw a significant relationship between SOC\textsubscript{5} and carbonate content.

![Graph showing relationship between SOC\textsubscript{5} and carbonate content](image)

**Figure 5.19.** Relationship (lack of) between SOC\textsubscript{5} and carbonate content of redd sediments 2010.

The relationship between SOC\textsubscript{25} and carbonate content (Figure 5.20) is similar to that of SOC\textsubscript{5} with 2010 experiments and sediments. A quadratic polynomial regression line of $R^2 0.17$ suggests that there is no relationship which is confirmed by the ANOVA results which returned a $P$-value of 0.2 at $p = 0.05$. 
It is notable that the two years describe very different relationships between organic matter, carbonate content and SOC rate. Organic content and carbonate content are similar in both years and little spatial variation is displayed in these two variables between sites. The strong significant relationships shown in the 2009 data set could be due to the different classes of particle sizes (<4mm and <63µm) that were used in the SOC experiments. The 2010 experiments only used the smaller size fraction. This is the major difference displayed between the two years results. When investigating this hypothesis the relationships between only the smaller (<63µm) size fraction and organic matter and carbonate content are a lot weaker ($R^2 = 0.4$), however the correlations were still greater in the 2009 results compared with the 2010 results. There are a lot fewer data points so the relationship cannot be statistically significant.

5.4.4 Egg oxygen consumption rates versus SOC

In relation to the oxygen requirements of salmonid embryos, Greig (2004) reported egg oxygen consumption estimates at different stages of development. At hatch average Atlantic salmon (*Salmo salar*) oxygen consumption rate is 0.0067 mg O$_2$ egg$^{-1}$ h$^{-1}$. A long term SOC$_{25}$ value ranging from 0.98 – 3.8 mg O$_2$ g$^{-1}$ day$^{-1}$ (Table 5.6), equating to 0.0408 – 0.158 mg O$_2$ g$^{-1}$ h$^{-1}$ is between an order of magnitude and two orders of magnitude higher than the average estimates of egg oxygen consumption. The SOC$_{25}$
values from the 2010 data-set at hatch (Table 5.6) were chosen to compare with oxygen requirements for salmonid embryos at hatch as they represent SOC rates at this incubation stage. The longer term rate would be indicative of the oxygen consumption occurring if there was little sediment infilling into the redd, which was the case on the Itchen over the incubation period. If this SOC rate were to be displayed by sediment directly surrounding an egg then the oxygen consumption exerted by the sediment would be greater in relation to the egg. SOC rates measured in this study are of an order of magnitude higher than egg oxygen consumption rates so the likelihood is that within the redd, the sediment surrounding the egg will have a far greater oxygen consumption than the eggs. The water transporting dissolved oxygen through the redd would therefore have to contain greater dissolved oxygen than is needed by the joint consumption of the developing eggs and the sediment to satisfy the oxygen demand of both.

It is difficult to say what effect that SOC rate would have on egg oxygen consumption as intra-gravel flow and oxygen concentrations need to be taken into consideration. This is where existing survival metrics can look at the data as a whole and attempt to devolve the many factors affecting survival to give an estimate of embryo survival.

5.4.5 Existing survival metrics and SOD

The reasoning behind this research was to enhance quantitative data about the rate oxygen was consumed by the surrounding sediment. Oxygen availability is shown to be one of the most important factors affecting survival of salmonid embryos in the interstitial environment (Chevalier and Carson 1985; Greig et al. 2007). Current survival metrics and models require many variables to provide accurate estimates of embryo survival to emergence and available oxygen is one if the major inputs. Any factors affecting the available oxygen within the redd environment need to be taken into account within parameter-based and process-based models. Sediment oxygen consumption rate and demand is often overlooked or estimated (Greig 2004). Three examples of existing models that look at predicting survival from variable inputs including intra-gravel oxygen measurements are: Chevalier and Carson's (1985) dissolved oxygen consumption model who apply their oxygen transfer model to the survival of salmonid embryos in the interstitial environment; the SIDO-UK a sediment intrusion and dissolved oxygen model developed by the United States Department of Agriculture (Alonso et al. 1996) and adapted to suit UK river habitats that predicts survival based on many variables and Wickett's (1975) process-based mass transport
model (further developed by Grieg (2004)) to quantify fish egg survival based on available oxygen and egg oxygen requirements.

5.4.5.1 SIDO-UK modelling

SIDO-UK which is based on SIDO – (sediment intrusion and dissolved oxygen) model, an American made stream habitat deterministic model, developed by the United States Department for Agriculture, that predicts salmonid survival based on accumulating sediment and interstitial dissolved oxygen and associated processes (Alonso 1996). It is a numerically based, physical process model that represents the movement of water, sediment and dissolved oxygen through the hyporheic zone of a redd (Alonso et al. 1988). SIDO-UK can be described as a conceptual, deterministic model which means that the model is based on certain given sets of input data that will always produce the same output, but the linkages and parameters in it are based on the actual physical processes that take place within the redd environment (Alonso et al. 1988).

Mathematical, physical process based models are a key element in predicting and describing dynamic, natural systems, particularly when there is more than one variable changing through time and relative to other variables (Haddon 2001; Lane 2003) which highlights the advantages of using models on these kind of systems. Models are useful in predicting future events and furthering understanding natural processes that can often be understood poorly.

The model uses a simulation of salmonid spawning habitat that includes constituents interacting with one another. Constituents e.g. water, sediment, temperature and dissolved oxygen move through the spawning habitat and this movement and interactions that occur with other constituents in the redd environment are known as processes. There are a number of processes used in SIDO-UK to represent occurrences in natural spawning habitats. These processes are simulated in two distinct domains, the stream and redd based domains. Processes that occur in the stream domain are linked to transport processes within the reach containing the redd and the output from this domain is then passed onto the redd domain where the movement of constituents through the redd exist (Alonso et al. 1988).

Within the stream domain, the channel is represented by a number of cross sections of equal length apart which afford all the data needed to inform the important topographical and hydraulic features found within the reach. Processes such as flow and suspended sediment transport and proportional silt, sand and clay, suspended sediment are represented within this domain as one day events. Infiltration of sediment to the redd is assumed to be directly correlated with the amount of
suspended sediment contained within the water column above it (Alonso et al. 1988). Dissolved oxygen saturated, suspended sediment laden stream water is the output modelled by the stream domain phase.

Transport processes within the redd domain are simplified to two dimensions, rather than three (interactions between bed shape of redd, intra-gravel flow circulation and gravel substrate) by using a vertical, two dimensional domain of the redd’s plane of symmetry (Alonso et al. 1988). A computational grip represents the redd zone in discrete cells which have values for porosity, material composition and hydraulic connectivity with other cells based on the infiltration of sediment from the stream domain. The settling of sediment within the redd grid is assumed to occur from the bottom up and settling occurs unimpeded. Fine sediment and dissolved oxygen transported over the stream-substrate interface in-fill sediment and decrease dissolved oxygen within the redd domain. Dissolved oxygen is modelled non-conservatively, meaning other processes including intra-gravel flow and biological sinks can consume it, removing it from use by the eggs in the redd (Alonso et al. 1988).

The SIDO model uses a de-oxygenation constant \( k \) of \( k = 0.0471 \text{ day}^{-1} \) to describe the effects of SOD on oxygen availability within the parameters of the model in Equation 5.6:

\[
\sum t_i \ln \left( 1 - \frac{SOC_i}{SOD} \right)
\]

\[
k = \frac{\text{SOD}}{\sum (t_i^2)}
\]

Equation 5.6.

Where;
- \( k \) = de-oxygenation constant (day\(^{-1}\))
- \( SOC_i \) = mean sediment oxygen consumption for \( t_i \) (mg/g)
- \( SOD \) = mean total sediment oxygen demand (mg/g)
- \( t_i \) = time at which was taken \( SOC_i \) (days)

Within the model this constant is used to explain the oxygen consuming capacity of the fine sediments infiltrating the redd environment. The model relates SOD to the silt and clay fraction of the sediment which is in agreement with the experimental results presented where the <63μm fraction of sediment exerted the greatest oxygen demand of the accumulated sediment. There is potential for site specific calibration of the SIDO
model in relation to SOD\textsubscript{0.25} calculated for the Itchen catchment. This could help in creating more accurate survival estimates of salmonids on the river.

One previously calibrated site, Bishopstoke was used to explore the effect of changing the total SOD on embryo survival within the SIDO-UK model. A description of the procedure followed for model calibration for the Bishopstoke site can be found in model calibration and set-up.

5.4.5.2 Model calibration and set-up

SIDO-UK contains model parameters and boundary conditions. The first being changeable in order to calibrate SIDO-UK for the Itchen, one site was chosen which had the data needed for all the model parameters, Bishopstoke. Input parameters included in the model, explanations and sources are found in Table 5.8.

<table>
<thead>
<tr>
<th>Input Parameter</th>
<th>Explanation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross sections</td>
<td>To represent the channel reach within the model</td>
<td>Total station measurements taken on site u/s, redd and d/s 5m</td>
</tr>
<tr>
<td>Daily Discharge</td>
<td>To provide the hydrologic conditions within the reach</td>
<td>Environment Agency gauged data (Highbridge gauging station)</td>
</tr>
<tr>
<td>Daily suspended sediment load</td>
<td>To give the input of different particle size % sand, silt and clay of suspended solids passing the redd site</td>
<td>See suspended sediment (section 7.4.5.2) for a full description of calculation</td>
</tr>
<tr>
<td>Uncut and cut gravel bed samples</td>
<td>To give original grain size distribution so model approaches the uncut distribution from the beginning state of cut</td>
<td>Freeze core and bed disturbance methods</td>
</tr>
<tr>
<td>Elevation</td>
<td>To provide an estimate of channel slope within the model</td>
<td>GPS unit</td>
</tr>
<tr>
<td>Temperature</td>
<td>To allow the model to calculated estimated hatch time of salmonid embryos</td>
<td>Aanderra probe – daily mean data</td>
</tr>
</tbody>
</table>

Table 5.8. Input parameters for SIDO-UK model
5.4.5.3   Suspended sediment

Section 4.3.3 highlighted the issues creating a continuous suspended sediment record from the turbidity probes and handheld spot suspended sediment samples taken. A solution to this problem was to model suspended sediment loads based on discharge related to each spot suspended sediment sample taken for the 2009 field period. The daily discharge measured at EA gauging stations was transformed into percentage discharge values from the total discharge and then each spot suspended sediment sample was divided by this percentage value to give the amount of that sediment that would be carried in 1% of the total discharge. Then this variable was multiplied by the actual mean discharge for that day. The range of modelled estimates for the super site Bishopstoke is shown in Figure 5.21.

Figure 5.21. Displays modelled suspended sediment for a range of spot suspended sediment samples taken to represent 1% of discharge and multiplying by the actual % at the Bishopstoke site. Model 1 was chosen to represent suspended sediment load in SIDO-UK.

In order to decide which model would give the best estimate for suspended sediment load a comparison of the modelled results to the actual measured suspended solids for that discharge allowed a summary of the errors associated with a particular model. Based on these errors, the models containing the lowest values were calibrated within SIDO-UK and the outputs of the different models were validated against observed data like infilling sediment within the redd and dissolved oxygen profiles within the redd.
The suspended sediment models that created SIDO-UK outputs the nearest to measured observed data, were model 1 for Bishopstoke (Figure 5.21).

![Figure 5.21](image)

Based on the SIDO-UK model description of ‘SOD’ which is defined not as a rate of oxygen demand but the total consumption of all accumulating organic matter within the redd, the values substituted into the source code for the model were the calculated total SOD\(_{0.25}\) values taken from Tables 5.3-5.7. Table 5.9 displays the information used in the model source code for separate runs and then model runs were compared to see if changing SOD made any difference to the outcome of the model. A linear regression relationship was found between previous SOD and k (de-oxygenation constant) which is co-dependant with the differing SOD values input, i.e. for every individual SOD value there is a corresponding k value. The linear model \(y = 0.006x – 0.1577; R^2 = 0.95\) was used to predict the k value for nine SOD.

<table>
<thead>
<tr>
<th>Sido run</th>
<th>SOD(_{0.25}) mg O(_2) g(^{-1})</th>
<th>k constant</th>
<th>Figure legend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sido_1</td>
<td>221.21</td>
<td>1.17</td>
<td>Model 1</td>
</tr>
<tr>
<td>Sido_2</td>
<td>38.89</td>
<td>38.89</td>
<td>Model 2</td>
</tr>
<tr>
<td>Sido_3</td>
<td>53.84</td>
<td>53.84</td>
<td>Model 3</td>
</tr>
<tr>
<td>Sido_4</td>
<td>89.87</td>
<td>89.87</td>
<td>Model 4</td>
</tr>
</tbody>
</table>
Model results based on field data from Bishopstoke (cross sections, intra-gravel flow data and fine sediment proportions within suspended sediment) describe high embryo survival coupled with little infilling of fine sediment to the interstitial environment. The modelled dissolved oxygen concentrations were compared with observed field data measured which validated the model as they display similar patterns (Figure 5.22) over the incubation period. The magnitude is slightly greater in the observed data of about 1mg l\(^{-1}\) larger than the modelled concentrations, but the shape of both data are very similar displaying the same patterns of increases and decreases. Comparing all nine model runs, each with a different SOD\(_{25}\), showed that there was no alteration of the model outputs in any of the variables measured.
SIDO-UK predicts 99.8% survival of salmonid embryos at Bishopstoke to emergence. This is not comparable with field estimates of salmonid survival (≈50%). The model appears to overestimate survival based on an underestimation of the infilling of fine sediment over the incubation period. It is likely that this is due to the insensitivity to low levels of infilling within the model which only registers an increase in sediment at 11.1% infilling of the redd. This insensitivity will lead to underestimations of salmonid survival rivers with infilling levels smaller than 11.1%, like the River Itchen.

Due to this insensitivity to relatively small inputs of fine sediment the model is likely to underestimate the impact of sediment oxygen demand. Modelled fine sediment accumulation within the redd domain over the incubation period equated to 0% for all models. This is unlike the observed field measurements, where <2mm fine sediment within redds increased from 0% at burial of eggs to 3.6% at hatch and 2% at emergence at the Bishopstoke site. Sediment accumulation has been found be low on the River Itchen over the incubation period in comparison to other rivers. For the Bishopstoke site it appears that changing the SOD does not alter the model outcome. However it should be noted that SIDO-UK is less sensitive to rivers where there are only small increases in sediment accumulation over the incubation period, which all the River Itchen sites displayed apart from the lowest site at Gaters Mill. This site was not used in this model testing due to the fact that no SOC/SOD experiments were carried out on sediments collected from this site.

**5.4.5.4 Other model applications**

Chevalier and Carson (1986) have included SOD as an input variable within their model to predict salmonid survival. They commented on the lack of research into oxygen consumption of gravel-bed rivers and the large variability displayed within SOC rates obtained from lakes, inhibited their estimates of background SOD were employed in their model (Chevalier and Carson 1986). Wickett (1975) does not take into account SOC rate or SOD within the mass transport model, although specifies that a correction factor could be added to the initial oxygen concentration variable to include substrate oxygen consumption rate. The total SOD calculated for the River Itchen could be applied simply within the mass transport model as a correction factor to the original oxygen concentration prior to running the model using different incubation stage SOC
rates and total organic content and potentially could show variation in egg survival rates.

The SOD_{0.25} values can also be input into the ‘SOD background estimates’ parameter used in the Chevalier and Carson (1986) dissolved oxygen consumption model to explore the impact of SOD on different intra-gravel flow velocities and oxygen concentrations. Greig (2004) created a modified version from the Chevalier and Carson model (1986) which was renamed the SOD model and can be found in Equation 5.7.

\[ C = C_0 - (SOD) \frac{s}{V} \]

Equation 5.7.

Where;
- \( C_0 \) = initial oxygen concentration (mg l\(^{-1}\))
- \( V \) = intra-gravel flow velocity (cm h\(^{-1}\))
- SOD = sediment oxygen demand imparted on one litre of interstitial water
- \( s \) = distance flow path length (m)

Field data from the 2009 period were input into Equation 5.7 to explore the impact of different SOD_{0.25} on oxygen concentrations. Initial oxygen concentrations were measured in the field. Average flow path length was not measured in this study but an estimate of 0.75m was used from previous research looking at another chalk river and assuming that down-welling laminar flow was the dominant form of intra-gravel flow (Greig, 2004). Total SOD_{0.25} data indicating the entire oxygen consumption over a 25-day period were input along with intra-gravel flow field data to give estimates of declining oxygen concentration into a field situation context (Figure 5.23).
Bishopstoke is the only site that it is possible to delineate any kind of regression line for. A second order polynomial regression of the equation, \( y = -4E^{-06}x^2 + 0.0072x + 9.0843 \) with an \( R^2 = 0.7 \) explains the relationship best, however a linear regression line with \( R^2 = 0.4 \) indicated that there is a correlation between modelled oxygen concentration and intra-gravel flow rate at this site. The other sites display little or no relationship between the two variables.

Figure 5.24 displays the observed intra-gravel oxygen concentration relationship with intra-gravel flow. Comparing the modelled oxygen concentrations with the observed intra-gravel oxygen concentrations, it appeared that measured values did not drop as low as modelled oxygen concentrations. Very weak linear regression lines describe the relationship between intra-gravel flow and field measured dissolved oxygen for the Winchester (\( R^2 = 0.28, p = <0.01 \)) and Bishopstoke (\( R^2 = 0.26, p = <0.01 \)). In Figure 5.23, Ovington displayed a marked decrease to nearly 6mg l\(^{-1}\) which is not displayed in the measured values seen in Figure 5.24. This suggests that SOD has less impact on oxygen concentrations in the natural environment than the model suggests.
Figure 5.24. Observed measurements of intra-gravel flow and oxygen concentrations from field sites. Logarithmic scale is used to show the intra-gravel flow data.

This simplified model allows potential impacts of total SOD on the interstitial environment to be measured and can be developed further to include egg oxygen consumption rates and boundary concentration gradients that need to be maintained for the healthy development of the eggs in different intra-gravel flow and oxygen availability scenarios. It should be noted that variable rates of flow, sedimentary structure and diffuse processes also influence oxygen concentration and could potentially affect demand (Greig 2004).

Similar to SIDO-UK, this model does not express sediment oxygen consumption as a rate, but as ‘the finite demand by organic matter that can be placed on surrounding dissolved oxygen within the substrate’ and uses the units mg O\(_2\) per litre of substrate (Chevalier and Carson 1986). Once the organic material has accumulated within the interstices of the redd environment, there is only a finite amount of oxygen that can be consumed by a specific kind of organic matter regardless of the time (Chevalier and Carson 1986). Whilst this may be a valid assumption about the filling of pore space within the redd, it may well also be true that the time factor of oxygen consumption for different organic sources may be crucial to embryo development and success. Acute short term oxygen consumption may not be as damaging as chronic, longer term oxygen consumption in the case of salmonid embryo development if oxygen is reduced over a longer time period. However it could also be true that the timing of infilling organic sediments i.e. just before hatch or at eyeing stage when eggs exhibit a greater oxygen demand, may well play a very important role in the survival and development success of the incubating eggs. Organic sediments that exhibit a fast
oxygen demand rate will have a more damaging impact on salmonid eggs at this point than slower oxygen demand rate by organic sediments.

For the purposes of exploring the field data within the context of sediment oxygen consumption values calculated, SIDO-UK shows that SOC rate and total SOD is possibly not as important a factor for incubating salmonid embryos on the Itchen as the modelled relationship appears to show. However the sensitivity of the model is too coarse for the low rates of sediment accumulation data experienced in the River Itchen, which will impact on the total oxygen consumption by sediments. There is a need perhaps for these models to be developed further to increase their sensitivity to low sediment accumulation rates, particularly for the benefit of lowland, chalk rivers. The timing issue of the rate of oxygen consumption at different egg development stages and also the specific timing of loading of fine sediment could prove to be crucial factors in whether or not SOD/SOC acts a major sink of dissolved oxygen within the redd interstitial environment, damaging survival success of salmonid progeny. Models need to incorporate these factors to improve their ability to predict egg survival.
Limitations of method

The experimental method has been tested and oxygen consumption and demand can be measured within the chambers. However replicate experiments will help to ensure the rates calculated are reasonable representations of the oxygen demand imparted by redd sediment. Due to the lack of standard SOD methodologies, the various calculations of rates and the lack of data on riverine and redd sediments in particular, it is difficult to compare these results with others (Ziadat, 2004; Miskewitz et al. 2010). SOC rate and SOD was inferred by measuring the rate of oxygen decline in the head-space above the water-sediment mixes and employing the ideal gas law and mass balance laws to use this rate of change as a proxy for oxygen consumption within the sediment slurry. This was a valid assumption because the experiment was run as a closed system. An experimental set up where oxygen probes measure the dissolved oxygen concentration within the water mixes could be an improvement on this method.

A major factor limiting the accuracy and representativeness of the calculated SOC rates is the ex-situ nature of the experiment. Many studies advise that in-situ sediment oxygen demand chambers give a better indication of real rates of oxygen consumption from sediments as they are undisturbed and exist in natural systems (Liu, 2009; He and Yun 2011). It should be noted that laboratory experiments of this nature are capable of giving an approximate indication of SOC rates not an exact equivalent of rates that would be observed in field conditions.

Controlled laboratory conditions are different to the dynamic environment encountered in the field. Factors such as intra-gravel flow rate, discharge, temperature, diurnal effects, up-welling groundwater and supply and transport/settling rates of organic and inorganic materials are all likely to have an influence on the SOC rates (Truax et al. 1995). Furthermore the supply rate of organic matter is dependent on many factors like hydraulic action, settling rates, abundance of invertebrate communities, presence of riparian vegetation, density of in stream macrophytes and the concentration of micro-organisms within the bed sediments that break down the material once it has settled in the bed (Petticrew et al. 2003). It is highly likely that due to hydraulic action and dynamic conditions within channels, SOC could be smaller or larger than estimates presented here.
Field experiments using *in-situ* SOC chambers could be deployed to allow for comparisons between controlled SOC rates observed in the laboratory with this method and real life trends observed in natural systems, however they still fail to recognise the impact of accumulated material or flow within the redd sediment as the chambers are sealed from the main flow of the river.

Closed system experiments used to measure oxygen consumption could lead to oxygen becoming a limiting factor in the experiments (Truax et al. 1995). Truax et al. (1995) suggests that if a sufficient water column exists above the sediment base then oxygen should not become a limiting factor. Allowing for a large gas headspace within the chamber alleviated this problem. Sufficient volumes of water are not available within the literature so an estimated volume was used within the chambers to allow for steady state conditions to return and so that oxygen declines can be clearly measured within the chambers. Different amounts of sediment should also be tested with this set up as small amounts of sediment were used in these experiments (0.3-5g) and there is some concern over whether the oxygen consumed was purely the sediment and not the water and sediment combined. Other controlled factors include temperature, light penetration and agitation.

Temperature was set in the incubator to the average temperature observed in the field over all sites for the entire duration of the experiment. This has obvious implications for SOC rates as increasing temperature increases biological and chemical reactions and dissolved oxygen is less readily available in the water column with increasing temperature (Stoker 1939). The amount of light entering the incubator was controlled to imitate the environment of the gravel bed, however it does not fully replicate light penetration in shallow stream sections.

Redd sediments are less compacted and contain greater pore spaces than other areas of the river bed which could potentially mean that water can penetrate deeper into the sediments at these points. The topography of redds has been thought to induce flow down into the gravels (Crisp and Carling 1989; Sear et al. 2008) as stated previously, which suggests that the oxygen demand exerted through the sediment at different depths in a field situation could be far different to the SOC rates measured in the laboratory study. Surface water velocity above the gravels in a field situation would also influence SOC rates (Parkhill and Gulliver 1997) which could not be replicated in the laboratory experiments. To improve the laboratory method a circulatory pump with split tubes to all chambers could create flow within the chambers and could be controlled. SOC rates could then be measured in terms of flow and experimental SOC rates could be measured when under different flow regimes.
Immediate problems were mostly with the seal of the probes to the flask lids. Araldite was used as an impervious seal to block out the atmospheric oxygen seeping into the flask and disrupting the desired degradation curve. However by the third experimental batch the seal appeared to be weakening so seals need to be checked at regular intervals during experiment runs.

The indirect effect of the infiltrating sediment consuming available oxygen within the redd needs to be compared with the direct effect of in-filled sediment smothering the eggs and blocking off diffusion pathways in the chorion. This direct effect of the sediment intrusion could have a greater detrimental effect than the demand imparted on the interstitial water for dissolved oxygen. It is noted here that when talking about egg oxygen consumption and sediment oxygen consumption there is a lack of consensus as to the definition of both. Comparing SOC rates and total oxygen consumption by eggs and sediment is the only way to accurately compare the two factors and currently there is a disparity in measurement of the two factors. Egg oxygen consumption rates are generally measured in the region of hourly time periods (Hayes et al. 1951; Hamor and Garside 1979) over short experimental studies and there seems to be little recent evidence of experiments carried out on individual river salmonid populations. The local adaptation displayed within populations of different rivers could affect tolerance levels to oxygen concentrations and possibly oxygen consumption of the eggs themselves could vary. SOC rate and SOD should be measured synonymously with egg oxygen consumption rates to give a meaningful measure of the affect one has on the other.
5.6 Discussion and implication

The experimental method allows for the calculation of SOC rates and SOD from different spatial locations along a catchment. There are variations in SOC rates and SOD found within the River Itchen catchment, however no significant variation can be proven between sites or salmonid embryo development stage due to the small data range. SOC rate is weakly correlated with % organic matter in sediments at different incubation stages.

Generally riverine sediments exhibit a lower SOC (1.58 – 1.84 mg O$_2$ g$^{-1}$ m$^2$ day$^{-1}$ (Doyle and Rounds 2003)) than lake sediments (1200-2200 mg O$_2$ m$^2$ day$^{-1}$ (Lucas and Thomas 1972)). This research reports results nearer to the reported riverine results than those reported for other systems (0.2-4.5 mg O$_2$ g$^{-1}$ day$^{-1}$). It is not clear whether the SOC rates and SOD calculated here are likely to detrimentally impact on the available oxygen to salmonid embryos. It is certainly shown that SOC rates are of an order of magnitude larger than egg oxygen consumption rates displayed within the literature but the number of factors that influence oxygen availability within the redd such as intra-gravel flow, temperature and dissolved oxygen concentration need to be accounted for in association with SOC/SOD before conclusive results can be obtained. Existing survival metrics and models appear to show that total SOD makes little or no difference to the model outcomes as regards egg survival, however this could be partially due to oversimplifying the SOD inputs within these metrics. Simplifying SOC rates to one single value is not representative of the oxygen consumption of sediment which degrades at different rates over time and thus incubation stages, which is why total SOD was also calculated in this study.

Further work to develop this method could include:

- Testing the method with different amounts of sediment – a greater mass of sediment used could significantly alter SOD values obtained
- Comparing the laboratory results obtained with data collected in-situ on the river using SOD chambers
- Investigate the effect of flow using a split tube pump system to simulate river conditions
- Infilling experiments that explore the change in SOD rate with inputs of fine sediment over a different time periods. This would be extremely useful to divulge the variable SOD values that salmonid embryos could experience in rivers where sediment intrusion is large.
Chapter 6. Sediment Fingerprinting Study

6.1 Introduction

The River Itchen is a catchment particularly vulnerable to degradation from fine sediment due to its highly modified state, low gradient and stable flow regime (Agency 2004b; Section 3.3). As fine sediment within spawning gravels has been identified as a major limiting factor affecting spawning success on the River Itchen (Itchen Sustainability Study 2004; Environment Agency 2004a), a study to assess the source of the fine sediment within spawning gravels was thought appropriate. This chapter details the method and experiments carried out to attempt to identify the source of fine sediment throughout the catchment. Firstly a review of current literature on sediment sourcing studies and the latest methodologies used is carried out. A sediment fingerprinting method is then presented and the results obtained are elucidated and discussed. The results highlight the importance of catchment specific studies to investigate the sources of interstitial sediment to inform water policy and practical land management, when considering the improvement of spawning habitat quality by reducing fine sediment inputs to rivers. To date there is no comprehensive, in-depth catchment study of the sources of fine sediment within salmonid redds on the River Itchen. This chapter aims to address this issue using sediment fingerprinting technology to quantify the provenance of sediment sources on the Itchen.

6.1.1 Sediment sourcing studies and methods

Fine particulate sediment can exert significant influence on hydrological and geomorphological regimes within rivers and can significantly affect the biological functioning of species by altering and maintaining certain habitats (Owens et al. 2005; Armitage and Keating 1997). Whilst sediment within river systems is part of a natural succession of geo-morphological processes that occur within catchments, artificially heavy fine sediment loads are often seen as diffuse pollution in certain catchments (Agency 2004b). Diffuse, non-point source pollution causes particular problems within river networks and catchments directly and indirectly. Direct effects of high loads of sediment include clogging of river bed sediment pores leading to low flow and low dissolved oxygen levels reaching sub-surface organisms, reducing the amount of light
available to primary producers affecting the entire riverine food chains and altering niche habitats. Fine sediment deposits can indirectly provide transport pathways and can be sinks for other labile and mobile pollutants such as pesticides, phosphates and nitrates, heavy metals and PCBs (Armitage and Keating 1997; Walling 2005; Owens et al. 2001). These kinds of persistent organic pollutants can have chronic effects on aquatic life long after the initial exposure. (Rees et al. 1999; Haddox and Cutright 2003; Moore and Langner 2012)

Research into tracing sediment movement through ecosystems has improved dramatically over the last forty years (Foster 2000) with the development of new tools, models and the quantification of sediment movement. The previous main research focus was to estimate movement of sediment through a catchment and ultimately the magnitude of the sediment load and yield from a particular system (Peart and Walling 1988). Traditional techniques of measuring sediment magnitude and the associated problems caused in water courses include: estimating sediment budgets, soil loss equations and sediment delivery ratios, cross-profiles of gullies, erosion pins, sediment baskets and photographic surveys (Peart and Walling 1988; Collins and Walling 2004). Many of these methods used on their own do not adequately address the spatial and temporal variability in sediment delivery to river systems and involve intensive fieldwork campaigns (Collins et al. 1997). Studies have evolved over time from measuring qualitative erosion patterns and mass sediment accumulation to more quantitative methodologies that take into account the sources of sediment as an important factor in managing suspended sediment pollution (Collins et al. 1997).

The ability to trace diffuse pollutants from end point back to the original source has been identified as an effective way to be able to target control measures for diffuse pollution, such as excessive fine sediment loading (Collins and Walling 2004; Walling 2005; David and Fox 2009). There has been a shift therefore from investigating only sediment flux and sediment budgets in rivers to a more preventative scenario where targeting the root source of the sediment problem leads researchers to find the origins of the fine sediment (Collins and Walling 2007). Many government initiatives and policies aim to reduce diffuse pollution problems in vulnerable catchments and target the reduction of fine sediment through programmes of action to such as Catchment Sensitive Farming (CSF) and the Nitrates (NO₃) Directive (Agency 2009). More recently the Catchment Restoration Fund has been set up to deliver Water Framework Directive objectives to reduce diffuse pollution and achieve good ecological status by 2015 (EA 2012).
Over the last three decades the sediment fingerprinting method has been developed by a number of researchers to improve understanding of the movement of sediment through river systems (Collins et al. 1998; Collins et al. 2001; Motha et al. 2003). The major concepts which define the sediment fingerprinting methodology are the ability to identify sources of sediment from discrete natural or anthropogenic tracers ($^{137}$Cs), the capability to statistically define those tracers best at discriminating between sources and finally utilising sediment mixing model technology to model the contributions of each source to fine sediment loadings in river systems (Davis and Fox 2009; Collins et al. 1998).

Early fingerprinting methods have conventionally used only one tracer to relate river sediment back to sources; however using only one tracer property has been proved to be unrepresentative and unreliable in matching sources to sediment (Collins et al. 1997). This is because spurious source ascription can occur when one property is used as the only tracer (Collins et al. 1997; Walling and Woodward 1995). It is highly unlikely therefore that there is any one property that can effectively differentiate between sediment sources in natural river systems. Using a combination of different properties such as mineral magnetic properties (Jenkins et al. 2002), geochemical (Russell et al. 2001; Collins and Walling 2007a), organic compounds, physical properties like particle size and colour (Krein et al. 2003) and radio-nuclides (Walling and Woodward 1992) of source and river sediment afford greater discriminatory power between sources, can alleviate the risks of spurious source to sediment matches and allow for tracers that can experience physical and chemical changes in the riverine environment (Collins et al. 1997). In allowing for transformations from one environment to another, it is important to note that the range of values exhibited by a particular fingerprint property for a source is an advantage when using composite signatures (Collins and Walling 2007a).

A consideration and appreciation of primary and secondary sediment sources is needed when apportioning sources to sediment using the fingerprinting approach (Adrian Collins, personal communication, ADAS). Primary sources can be defined within different studies as the original geological parent material, soil type or land-use that sediment is derived from e.g. chalk, peat or arable sources (Collins et al. 1997; Collins and Walling 2007; Davis and Fox 2009). A secondary source could be described as a mix of these primary sources, for example road verge material which is often a combination of other sources. Road drainage systems are particularly good at transporting sediment, often facilitated by precipitation and generally the sediment will not originate directly from the road. One way to establish whether a source is secondary or not is to apply quantitative statistical tests to see if tracers' properties
can distinguish the primary sources (land-use, soil type or source derived from different geological parent material) of the material, so the source is effectively used as a sediment sample (Collins et al. 1997). If the source is found to be statistically different then it can be included as a primary sediment source. Some studies have used roads as a primary source and have found that the fingerprint properties are statistically different to indicate a separate source from other primary potential sources identified (Motha et al. 2004).

Previous studies have investigated the sources of fine suspended sediment loads within rivers. However, recent studies have been directed towards the sources of settled river bed sediment (Jenkins et al. 2002; Collins and Walling 2007a; Collins and Walling 2007). One particular study, which examined the amount of fine sediment found in salmonid redds across the country, was the first study to specifically investigate the sources of infiltrated fine sediment to the redd environment (Walling et al. 2002; Walling et al. 2003). Artificial redds and sediment baskets facilitated the retrieval of sediment from the redd environment and sources varied in different catchments around the UK. South West catchments displayed greater source apportionment to channel bank and sub-surface sources compared with the lowland river systems found in Southern England where 89-97% of sediments within redds were derived from land surface sources (Walling et al. 2002). The results of this study demonstrate the great variation between catchments and regions in the origin of fine sediment settling in the redd environment.
6.2 Methods

The methodology used to reveal the provenance of fine sediment within salmonid spawning gravels is described below. Each section details the different stages in the context of the sediment fingerprinting method. Firstly an assessment of the likely sediment sources in the catchment was made followed by the collection of potential catchment source samples and fine sediment samples from artificial redds. Secondly, a number of different laboratory methods were adopted to analyse the sediment and soil samples. Range tests and statistical procedures were then used to define the number of properties needed to create a composite fingerprint suite that could discriminate between source categories. Finally a sediment mixing model (Collins et al. 1997) was used, employing a Monte Carlo approach to identify the provenance of sources within redd sediments.

6.2.1 Assessment and collection of catchment sediment sources

In order to identify potential sediment sources within salmonid redds in the River Itchen catchment, two primary objectives were considered:

- all major land-use practices in the catchment were included
- pathways of these land use practices that might enable sediment to enter the river system

Numerous river walkovers were conducted to visually assess the potential sources of fine sediment in the catchment (Collins and Walling 2004). Field drains from rough and improved pasture, a lack of riparian vegetation, fencing off of farm animals and silted river margins were identified as major potential sources throughout the catchment. Pockets of woodland were noted on some steep banks adjacent to river channels (Ovington) and cultivated arable fields, often situated where slight gradients heading down to the river were observed in the upper catchment. Evidence of recent transport of sediment via roads from arable fields towards the river was found, particularly near to the Cheriton and Candover streams.

A literature search of results from similar catchments to the River Itchen helped to identify the likely sediment sources (Walling et al. 2006; Collins and Walling 2007a).
Collins and Walling (2006) identified that bed sediment remobilisation significantly contributed to the overall sediment budget in two lowland catchments similar to the River Itchen. In-stream, marginal sediment sources were therefore thought to be a primary source of fine sediment in salmonid redds. Catchment maps played a role in identifying potential problem areas, such as soil and land-use maps. Three main source categories were identified using different land-use types as the defining, distinguishing characteristic for the categories. The geology of the Itchen catchment is dominated by chalk deposits (80+%) laid down in the cretaceous period for the majority of its length, with a small amount of tertiary deposits from Bishopstoke down to its tidal limit. The soils are therefore fairly uniform due to its uniform geology, leading to the conclusion that categorising sources in terms of land-use would be the more reliable option. The three categories identified were:

i. Rough and improved pasture
ii. Mixed arable
iii. In-stream channel margin sediment

As observed in the walkovers, the upper catchment does contain small amounts of woodland. Connectivity of sediment from wooded areas to the river was not observed in catchment walkovers although the potential for sediment delivery to the river based on gradient and proximity to a number of upland stretches of the River Itchen was thought to be possible. Previous literature has suggested that unless large scale forestry operations are operating in river basins, it is unlikely that stable, undisturbed woodland soils are likely to contribute to fine sediment problems in small catchments (Motha et al. 2003). Therefore it was not thought necessary to include woodland as a source due to the lack of large scale forestry within the catchment. Woodland sources were however monitored to allow for analysis of samples within the framework of the sediment fingerprinting method. The results are not presented here.

Pasture and arable were the major land-use types that appeared to be dominant in the catchment, observed from maps, walkovers and other literature sources (Figure 6.1). It was clear to see from catchment walkovers and land cover maps that pasture was often the dominant riparian land use type. In-stream sources were included as a potential primary source due to the large, visible patches of marginal river bed sediment present on the River Itchen. Long residence times of stored sediment, low mobility and subsequent altering of characteristics of the sediment in the aquatic environment led to the decision to include it as a potential primary sediment source (Davis and Fox 2009).
Two further commercial categories were identified as being potential sources on the Itchen; water cress beds and to a lesser extent fish farms. The Itchen supports these two industries and both produce waste materials that could potentially add to the fine sediment load and have the potential to pollute water courses. Mazzola et al. (2000) found that there was a large increase in organic sediments deposited on the sea bed underneath coastal fish farm systems, reducing the oxygen available within sediments. A potential hypothesis for river systems might be that organic sediment deposition downstream of fish farms is greater than in other areas of the river. Sampling of these commercial ventures as sources and including artificial redds immediately downstream...
of these two types of industry could not be undertaken, however, due to land access permission issues.

The size of the catchment also played a role in deciding source categories and thinking about source contributions to a system. One particular land-use could play a more important role in contributing to source apportionment in smaller catchments compared with larger catchments which generally have more developed channel bank erosion regimes (Walling 2005).

In order for the samples within the source categories to be representative of the entire catchment, 10 sub samples (approx. 1kg) of each source category were collected from 10 different potential source sites around the catchment. In order to allow for unbiased and representative sampling within the sub samples, the method followed consisted of collecting multiple samples from a particular source area, e.g. four of five scrapings/shovels from different spatial areas within that one source area which were then combined to create the 1kg of sample for a sub sample. Samples were collected from the top few centimetres of surface soils (Collins and Walling 2007a) which were readily eroded by climate and/or anthropogenic activities. All samples were collected from sites which exhibited either proximal or distal (e.g. pathways linking slope material to river) fine sediment connectivity to the river (Walling et al. 2003; Collins and Walling 2004). Approximately 1kg of each sub-sample was collected to ensure the completion of all laboratory analyses.

Figure 6.2 displays the geo-referenced source collection undertaken in 2009 of possible sediment sources potentially feeding into the River Itchen and its tributaries. Sources were collected upstream from the furthest downstream artificial redd site and a larger proportion was collected upstream of Winchester to account for the fact that sediment travels at varying rates along rivers. Sediment which has entered the system further upstream is potentially more likely to have a longer residence time in the main stem of the river for a proportionally longer time than material deposited at lower points within the river network. Sediment entering the river nearer to the mouth has a greater chance of being removed from the system faster.
6.2.2 Collection of sediment samples

In order to study the sources of fine sediment infiltrating into salmonid spawning gravels on the River Itchen, artificial salmonid redds were constructed so as to avoid damage to existing salmon redds and to ensure that redds were as standard as possible in a semi-controlled environment. Redds were constructed at the beginning of the incubation period for salmonids on the River Itchen (Dec/Jan) so as to investigate sediment infiltrating during the correct time period when embryos and alevins will be buried in the gravel nests. Sites were chosen based on the following criteria:

- Established spawning activity of salmonids occurred within reach or historic spawning known to have occurred in the past
- Depth <50cm
- Flow and substrate were characteristic of spawning habitat (i.e. gravels not concreted and not slack areas of water)
- To be representative of the whole catchment
- Ease of access and landowner agreement

Nine sites were chosen in 2008 field season to incorporate good spatial variation in spawning habitat quality, reducing to four in 2009 field season. Figure 3.7 displays maps with site locations for the 2008 and 2009 field seasons. Following the method of Grieg (2004), hollows were dug into the river bed approx. 0.3m deep, an average depth for natural salmon redds from previous estimates of between 0.5 and 0.1m (Crisp and Carling, 1989). Sediment accumulation baskets were filled with 4mm truncated on-site gravels which were encased in retracted polypropylene bags and placed in the pot area of the redd. Using a kicking action similar to that of a hen salmon, the pot area containing sediment baskets and bags were in-filled with u/s river gravels. For full details and diagrams of this method see section 3.5. Baskets were excavated from redds at periodic intervals during the incubation season to measure fine sediment accumulation and sub samples were taken from this infiltrated material for further laboratory elemental analysis and source fingerprinting investigations (Walling et al. 2003).

Samples were removed from the redds with minimal loss of fine infiltrated sediment through using the polypropylene bags which were pulled up from the base of the basket prior to removal from the redd. Samples were transported in 1 litre dark, clean plastic buckets and were processed within 24 hours of arrival in the laboratory.

Isokinetic samplers were installed approximately 10cm from the river bed facing the direction of main flow at each site (see section 4.3.3, Figure 4.3). Following the method of Phillips et al. (2000) samplers were created to estimate suspended sediment loads spatially and temporally across the catchment.
6.2.3 Laboratory processing of sediment and source material

A number of different laboratory procedures were carried out to characterise the sediment and source samples. Sediment and source samples were investigated to obtain as much information about the physical, elemental composition and organic content as possible in order to inform either the fingerprint properties themselves or provide detailed information about correction factors that were applied within the mixing model to account for transformations within the river systems. The different laboratory methods used for exploring different facets of the samples are explained below.

6.2.3.1 Wet sieving sediment samples and source soil samples

In order to facilitate direct comparisons with redd sediment and source samples all samples were wet sieved to <63μm (Walling et al. 2003) using a series of standard stainless steel sieves to separate out the finer material from the rest of the material in samples. Sediment was truncated through sieves 4mm, 2mm and finally 63μm using minimal distilled water to ensure the inclusion of all the fine material. The <63μm fraction was then dried at 40 C in convection ovens until all moisture was evaporated. Dried sediment and source samples were stored in sealed plastic bags until further analysis could be undertaken.
6.2.3.2 Particle size analysis

Particle size has been used in previous studies as a tracer parameter to relate source sediment to river sediment (Santiago et al. 1992). It is also an important correction factor to account for in composite sediment fingerprinting studies as it is known that particle size can alter geochemical element concentrations (Horowitz and Elrick 1987) and fluvial sediments, particularly in chalk streams which are often enriched with fine sediments in comparison with catchment soil samples (Collins et al. 1997). Thus it is useful to compare fingerprint properties using a particle size correction factor (Walling et al. 2002; Collins et al. 1997; Collins and Walling 2007a) for accurate comparison of soil and sediment samples. Although wet sieving to <63µm for all samples already limits the influence particle size might have on element concentration, it is necessary to further explore the particle size difference in this fraction between source samples and river sediment samples. It is hard to standardise the wet sieving method to the certainty level required to accurately estimate particle size correction factors as there are errors associated with sediment retained on the pan. To address this issue laser diffraction of particles helps to further breakdown the particle size of samples to inform the correction factor.

The mean specific surface area of <63µm sub-samples of all source and sediment samples were established using a Coulter Counter LS 130. Liquid samples (50ml) were treated with calgon to ensure particles were disaggregated and thorough mixing was achieved by stirring for two minutes before placing a 15ml sub-sample into the sample cell. A mechanical stirrer ensures entire mixing of sample whilst particle distribution is being measured via laser diffraction within the range 0.1µm – 900µm. Dilution of samples with distilled water occurred only if the obscuration rate measured by the Coulter Counter reached levels greater than 12%. The Coulter Counter assumes that the particles in the samples are non-porous and spherical in shape to calculate mean surface area in m².

6.2.4 ICP-MS elemental analysis and AAS

The dry <63µm material for all samples was analysed by different methods to obtain information about the individual properties of the samples. The fingerprint properties chosen to differentiate between sources were a mixture of heavy and trace metals and cations. The list of properties used were: Li, Na, Mg, Al, K, Sc, T, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Y, Zr, Mo, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb,
Dy, Ho, Yb, Hf, Tl, Pb and Bi (Collins and Walling 2007b). Most element concentrations were obtained using inductively coupled plasma mass spectrometry (ICP-MS), although Fe, Zn and Mn were re-analysed using atomic absorption spectroscopy (AAS) due to particularly high concentrations of these elements observed in the ICP-MS data.

Samples were acid digested with concentrated nitric acid (HNO₃) prior to ICP-MS and AAS analysis using a microwave to extract the elements from sediment and soil samples. The standard method followed for this procedure can be found with the Environment Protection Agency methods sheets (Agency 1994). Samples were entered automatically into the ICP-MS and all samples had previously been spiked with Beryllium (Be), Indium (In) and Rhenium (Re) to act as internal standards. All elements were measured relative to these standards. Four repetitions were measured and the mean standard deviation and % relative standard deviation were calculated for each element in a sample.

6.2.5 Pre-processing data and statistical tests

A number of pre-processing techniques were applied to element concentration data obtained from ICP-MS and AAS. Firstly it should be noted the data without internal standards were used due to large variation in standard concentrations of Be, In and Re. This could have been due to inaccurate pipettes or sample mixing problems with the auto sampler. The data without internal standards was thought to give a more accurate measure of the actual concentration of elements in a sample. The second data pre-processing carried out was to account for instrument drift. Each fingerprint property was corrected by measuring the drift experienced in the concentration of the standards measured in between sample runs and incrementally increasing or decreasing the element concentration depending on the direction of the drift. Replicate samples also helped to inform the direction of machine drift. The mean average for each element concentration was corrected in this way. Similarly the AAS results for iron (Fe) and manganese (Mn) were corrected for instrument drift using standards run in between samples. The ICP-MS data were used in the case of zinc (Zn) data were used because the AAS data were inconclusive.

It is important at this stage (Figure 6.3) to define whether the redd sediment samples and suspended sediment samples fit within the range of the source materials collected. In other words the potential source categories identified at the beginning of the sediment fingerprinting process are tested here to ensure no sources are missed out. The range of element concentrations in the source categories should incorporate the
range of concentrations displayed by redd sediment and suspended sediment samples. Using correction factors and applying them to element concentrations takes into account some possible transformations of sediment.

Figure 6.3. The sediment fingerprinting procedure simplified in a flow diagram describing the major steps taken from identification of sediment potential sources through to attributing sediment back to its original source.

Figure 6.4 describes this simple concept in diagrammatic form. Most fingerprint properties of redd and suspended sediment exhibit similar characteristics to its source material. It is clear to see that river sediment samples that fall outside of the range of the potential sources are likely to be due to some form of transformation which has led to an inability to link river sediment back to its origins. Completely different elemental characteristics in sediment samples could also suggest that the source material is different to those identified as potential sources; however this is unlikely to be the case if sources have been robustly identified. In order to satisfy the condition that all redd sediment samples were comprised of materials found in at least some of the
source samples, range tests were applied. The range of all combined source fingerprint properties were presented and the range of all redd sediment and suspended samples’ element concentrations were checked against these ranges for each element to ensure that they occurred within the source ranges. Appendix 2 describes the processes involved with this. The blue Y or N indicate ‘yes’ or ‘no’ according to whether that sediment sample fits within the source ranges for a particular fingerprint property. Particle size correction factors are applied to all properties in the range test due to its later inclusion in the sediment mixing model. Any source trace element concentrations that do not incorporate the full range of element concentrations exhibited in the sediment samples are removed from further analysis.

**Sediment mixing model space** - source samples represented by coloured circles and redd/suspended sediment samples represented by pentagons. If redd/suspended sediment mean concentrations of a property lie outside the space between the different source samples mean property concentrations then it is discounted from further analysis.
Discriminant function analysis was then carried out using SPSS statistical software to define the optimum number and order of fingerprint properties that discriminate between sources (Collins and Walling 2007a). The method used was the minimisation of Wilks lambda combined with forced entry for elements. Properties that improved the discrimination of sources were included in the final composite fingerprint suite (Collins and Walling 2007a). All properties that exhibited values of Wilks lambda near to zero or at zero were included in the suite of composite fingerprints (Collins et al. 1997). The outcome of this robust statistical procedure was the creation of a composite fingerprint suite of elements that discriminated between catchment sources. The next stage of the fingerprinting procedure was to input the composite suite into a mixing model and test the redd sediment samples against it to determine which source contributes the most to fine sediment accumulating in salmon redds.

6.2.6 Sediment Mixing Model

Collins et al. (1997) described the sediment mixing model which was used as the basis for this study. The aim of the model was to approximate the individual source categories distribution and their percentage contribution within sediment samples using the composite suite of fingerprint properties defined by the previous statistical tests. Using the solver optimisation tool in MS Excel, constraints were applied to the
model to force the calculations to reflect realistic properties (Collins et al. 1997). These are described in Equation 6.1 and Equation 6.2;

- The individual source category contributions must be non-negative
  \[ 0 \leq S_p \leq 1 \]  
  Equation 6.1.

- The total percentage contribution of all source categories must sum to unity
  \[ \sum_{j=1}^{n} S_p = 1 \]  
  Equation 6.2.

Objective functions in the form of linear equations for each fingerprint property and corresponding source category were created that minimised the sum of squares of the combined weighted relative errors (Collins and Walling 2007a) and were able to give an estimate of a source contribution, relative to a given redd sediment sample. The objective functions are shown in Equation 6.3;

\[
\sum_{i=1}^{n} \left( \frac{C_i - \left( \sum_{j=1}^{m} S_p F_{ij} Z_j \right) / C_i}{W_i} \right)^2 
\]  
Equation 6.3.

where;
- \( C_i \) = fingerprint property \( (i) \) concentration in sediment sample
- \( S_p \) = percentage contribution from source category
- \( F_{si} \) = concentration of fingerprint property in source category \( (s) \)
- \( Z_s \) = particle size correction factor for source category \( (s) \)
- \( W_i \) = tracer specific weighting
- \( n \) = number of fingerprint properties contained in composite suite
- \( m \) = number of source categories

Correction factors applied within the mixing model were the particle size factor and the tracer specific weighting. The particle size correction factor was calculated using ratios from the mean of the mean specific surface area of a set of sediment samples with the mean of the mean specific surface area of a particular source category. Table 6.1 displays the particle size correction factors used in the model for redd and suspended sediment.
### Table 6.1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pasture</th>
<th>Arable</th>
<th>Instream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redd sediment</td>
<td>1.43</td>
<td>2.36</td>
<td>1.20</td>
</tr>
<tr>
<td>Suspended sediment</td>
<td>1.80</td>
<td>2.97</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Table 6.1. Particle size correction factors for redd and suspended sediment applied within the mixing model for each source.

The tracer specific weighting takes into account the different errors associated with the individual fingerprint properties and explains the variation in precision of laboratory methods used to measure them (Collins and Walling 2007a). The tracer specific weighting was specific to the mean % relative standard deviation of the individual measured element concentrations. It was calculated by taking the inverse of the square root of the % relative standard deviation (RSD) for each element (Collins et al. 1997). Table 6.2 presents the tracer specific weightings used in the model for all elements used in composite fingerprint suite for both sets of sediment samples.

### Table 6.2

<table>
<thead>
<tr>
<th>Fingerprint property</th>
<th>Weighting</th>
<th>Fingerprint property</th>
<th>Weighting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>0.97</td>
<td>As</td>
<td>0.57</td>
</tr>
<tr>
<td>Mg</td>
<td>1.08</td>
<td>Rb</td>
<td>1.14</td>
</tr>
<tr>
<td>Al</td>
<td>1.21</td>
<td>Y</td>
<td>1.22</td>
</tr>
<tr>
<td>V</td>
<td>1.11</td>
<td>Cd</td>
<td>0.56</td>
</tr>
<tr>
<td>Cr</td>
<td>1.12</td>
<td>Cs</td>
<td>1.06</td>
</tr>
<tr>
<td>Mn</td>
<td>1.29</td>
<td>Ba</td>
<td>1.21</td>
</tr>
<tr>
<td>Co</td>
<td>1.07</td>
<td>La</td>
<td>1.27</td>
</tr>
<tr>
<td>Ni</td>
<td>0.98</td>
<td>As</td>
<td>0.57</td>
</tr>
<tr>
<td>Ga</td>
<td>0.97</td>
<td>Rb</td>
<td>1.14</td>
</tr>
<tr>
<td>Ce</td>
<td>1.29</td>
<td>Pr</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Table 6.2. Tracer specific weightings for each fingerprint property applied within the sediment mixing model.

Uncertainty within the model regarding the source contributions predicted for sediment samples was quantified by following a Monte Carlo approach (Motha et al. 2003; Collins and Walling 2007a). Monte Carlo simulations involve carrying out multiple simulations of the model by using random numbers defined by pre-defined probability distributions (Motha et al. 2003; Collins and Walling 2007b). Random
numbers were calculated to represent each source fingerprint property within the composite suite, based on the mean and standard deviation of that property to repeatedly test the model (3000 repetitions). Random numbers were generated using the random number generation tool in Excel. Confidence limits were calculated by finding the standard error of the mean associated with the repeat iterations in order to estimate the relative contribution of each source type to each redd and suspended sediment sample.
6.3 Results

Outlined below are the results from the statistical verification of fingerprint properties after range testing river sediment samples with source samples. Twenty eight different elements passed the range test for redd sediment samples and fourteen elements passed the range test for the isokinetic, suspended sediment samples. The mean sediment mixing model outputs and different model scenarios tested are also described.

5.4.1 Kruskal-Wallis H test results

Table 6.3 describes the fingerprint element properties that could discriminate between the three source categories. H-values are calculated from the statistical procedure and here the H-values have been transformed into p-values. H-values can be compared with a Chi-squared distribution table to determine whether or not the result for each fingerprint property is significant to 95% significance level (p=0.05). Significance denotes that this property can statistically differentiate successfully between source categories and the null hypothesis that there is no significant difference between element concentrations in different sources was rejected.

<table>
<thead>
<tr>
<th>Potential fingerprint property (ppb)</th>
<th>H- Value</th>
<th>p - Value</th>
<th>Statistical significance (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 7 Li</td>
<td>17.58947368</td>
<td>0.000534</td>
<td>*</td>
</tr>
<tr>
<td>2 24 Mg</td>
<td>21.15</td>
<td>0.000098</td>
<td>*</td>
</tr>
<tr>
<td>3 27 Al</td>
<td>21.60182186</td>
<td>0.000079</td>
<td>*</td>
</tr>
<tr>
<td>4 48 Ti</td>
<td>3.515991903</td>
<td>0.318694</td>
<td></td>
</tr>
<tr>
<td>5 51 V</td>
<td>21.83967611</td>
<td>0.000070</td>
<td>*</td>
</tr>
<tr>
<td>6 52 Cr</td>
<td>10.02267206</td>
<td>0.018374</td>
<td>*</td>
</tr>
<tr>
<td>7 55 Mn</td>
<td>22.77591093</td>
<td>0.000045</td>
<td>*</td>
</tr>
<tr>
<td>8 59 Co</td>
<td>21.60506073</td>
<td>0.000079</td>
<td>*</td>
</tr>
<tr>
<td>9 60 Ni</td>
<td>14.07125506</td>
<td>0.002810</td>
<td>*</td>
</tr>
<tr>
<td>10 69 Ga</td>
<td>18.74190283</td>
<td>0.000309</td>
<td>*</td>
</tr>
<tr>
<td>11 75 As</td>
<td>21.48846154</td>
<td>0.000083</td>
<td>*</td>
</tr>
<tr>
<td>12 85 Rb</td>
<td>19.53927126</td>
<td>0.000211</td>
<td>*</td>
</tr>
<tr>
<td>Potential fingerprint property (ppb)</td>
<td>H- Value</td>
<td>p - Value</td>
<td>Statistical significance (*)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------</td>
<td>------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>89 Y</td>
<td>23.69898785</td>
<td>0.000029</td>
<td>*</td>
</tr>
<tr>
<td>111 Cd</td>
<td>22.68846154</td>
<td>0.000047</td>
<td>*</td>
</tr>
<tr>
<td>133 Cs</td>
<td>19.26174089</td>
<td>0.000241</td>
<td>*</td>
</tr>
<tr>
<td>137 La</td>
<td>14.87530364</td>
<td>0.001926</td>
<td>*</td>
</tr>
<tr>
<td>139 La</td>
<td>25.66659919</td>
<td>0.000011</td>
<td>*</td>
</tr>
<tr>
<td>140 Ce</td>
<td>20.79048583</td>
<td>0.000116</td>
<td>*</td>
</tr>
<tr>
<td>141 Pr</td>
<td>22.47307692</td>
<td>0.000052</td>
<td>*</td>
</tr>
<tr>
<td>144 Nd</td>
<td>22.94919028</td>
<td>0.000041</td>
<td>*</td>
</tr>
<tr>
<td>146 Nd</td>
<td>23.70769231</td>
<td>0.000029</td>
<td>*</td>
</tr>
<tr>
<td>152 Sm</td>
<td>21.84210526</td>
<td>0.000070</td>
<td>*</td>
</tr>
<tr>
<td>157 Gd</td>
<td>23.31356275</td>
<td>0.000035</td>
<td>*</td>
</tr>
<tr>
<td>159 Tb</td>
<td>20.62044534</td>
<td>0.000126</td>
<td>*</td>
</tr>
<tr>
<td>163 Dy</td>
<td>23.58724696</td>
<td>0.000030</td>
<td>*</td>
</tr>
<tr>
<td>174 Yb</td>
<td>21.82206478</td>
<td>0.000071</td>
<td>*</td>
</tr>
<tr>
<td>209 Bi</td>
<td>2.732793522</td>
<td>0.434683</td>
<td></td>
</tr>
<tr>
<td>Fe ppb</td>
<td>17.24392713</td>
<td>0.000630</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 6.3. Redd samples elements that pass the Kruskal-Wallis H test.

Table 6.3 shows that 26 out of the 28 elements put through the KW test can significantly distinguish between sources at three degrees of freedom and p = 0.05. The elements; Li, Mg, Al, V, Cr, Mn, Co, Ni, Ga, As, Rb, Y, Cd, Cs, Ba, La, Ce, Pr, 144Nd, 146Nd, Sm, Gd, Tb, Dy, Yb and Fe all went onto the next stage of statistical verification.

Table 6.4 displays the elements that pass the Kruskal-Wallis H test for suspended sediment samples collected by the isokinetic samplers. Thirteen elements out of the fourteen tested could discriminate between all source categories for suspended sediment samples. Significance was tested at 95% significance level and three degrees of freedom where p = 0.05. The elements put forward for further analyses were; Li, Mg, Al, V, Mn, Co, Ni, Ga, As, Rb, Cd, Cs and Ba.
<table>
<thead>
<tr>
<th>Potential fingerprint property (ppb)</th>
<th>H- Value</th>
<th>p - Value</th>
<th>Statistical significance (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 7 Li</td>
<td>17.58947368</td>
<td>0.000534</td>
<td>*</td>
</tr>
<tr>
<td>2 24 Mg</td>
<td>21.15</td>
<td>0.000098</td>
<td>*</td>
</tr>
<tr>
<td>3 27 Al</td>
<td>21.60182186</td>
<td>0.000079</td>
<td>*</td>
</tr>
<tr>
<td>4 51 V</td>
<td>21.83967611</td>
<td>0.000070</td>
<td>*</td>
</tr>
<tr>
<td>5 55 Mn</td>
<td>22.77591093</td>
<td>0.000045</td>
<td>*</td>
</tr>
<tr>
<td>6 59 Co</td>
<td>21.60506073</td>
<td>0.000079</td>
<td>*</td>
</tr>
<tr>
<td>7 60 Ni</td>
<td>14.07125506</td>
<td>0.002810</td>
<td>*</td>
</tr>
<tr>
<td>8 69 Ga</td>
<td>18.74190283</td>
<td>0.000309</td>
<td>*</td>
</tr>
<tr>
<td>9 75 As</td>
<td>21.48846154</td>
<td>0.000083</td>
<td>*</td>
</tr>
<tr>
<td>10 85 Rb</td>
<td>19.53927126</td>
<td>0.000211</td>
<td>*</td>
</tr>
<tr>
<td>11 111 Cd</td>
<td>22.68846154</td>
<td>0.000047</td>
<td>*</td>
</tr>
<tr>
<td>12 133 Cs</td>
<td>19.26174089</td>
<td>0.000241</td>
<td>*</td>
</tr>
<tr>
<td>13 137 Ba</td>
<td>14.87530364</td>
<td>0.001926</td>
<td>*</td>
</tr>
<tr>
<td>14 209 Bi</td>
<td>2.732793522</td>
<td>0.434683</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.4. Isokinetic suspended samples elements that pass the Kruskal-Wallis H test.

### 6.3.1 Discriminant function analysis results

Discriminant function analysis (DFA) was carried out in SPSS statistical software, using the classify tool. The purpose of this statistical test was to investigate the optimum number of fingerprint properties that were capable of distinguishing between the different source categories (Collins and Walling 2007a). The minimisation of Wilks lambda algorithm was the method chosen to test the boundaries between source categories, created using Fisher linear functions, for each fingerprint property (Collins et al. 1997; Russell et al. 2001). Cross-validation of the accuracy of these functions is an important part of the procedure as it tests how well samples are classified into certain source groups. SPSS automatically runs cross-validation by removing one sample at a time and then attempting to classify that sample using the remaining samples as discriminant functions (Fowler et al. 1998). Separate statistical tests were run for redd sediment elements and suspended sediment elements.
6.3.1.1 Redd samples discriminant function analysis results

From the Kruskal-Wallis H test 26 fingerprint properties were used in further discriminant function analysis (DFA). The first step was to enter all elements using the stepwise selection algorithm (Collins et al. 1997). From these results only two properties, Mn and V, were able to discriminate between the four source categories with 93.3% of original cases classified correctly and a lower cross-validation result of 86.7% of cases classified correctly (Figure 6.5) shows that there is some overlap between samples in source categories, explaining why there is low classifying power for samples since the boundaries defined by the linear functions are not clear.

![Figure 6.5. Stepwise selection algorithm results with Mn and V correctly discriminating between source categories.](image)

Due to the low classification results, all properties were entered again, although independently this time, with the result that 19 elements correctly classify the differences between all source categories. Entering independents together for the DFA created the output below in which 100% of original classes and 56.7% of cross-validated cases are correctly classified. The separation displayed between the sources shows that the three sources were statistically different from each other. The larger number of fingerprint properties capable of discriminating between sources leads to
more clearly defined group boundaries. Comparison of Figure 6.5 and Figure 6.6 show that classification of groups has been improved by entering properties independently.
Figure 6.6. Independently entered properties where 19 elements (Li, Mg, Al, V, Cr, Mn, Co, Ni, Ga, As, Rb, Y, Cd, Cs, Ba, La, Ce, Pr and Tb) discriminate effectively between source categories.

However the object of the discriminant function analysis is to find the smallest number of elements that give the best classification between sources. With a smaller number of elements the mixing model is less complex and error sources are reduced. There is a trade-off between the most effective discriminators between sources and reducing the number of error sources within the mixing model. In order to find the optimum number of fingerprint properties to enter into the mixing model, forced entry of properties, based on the original stepwise classification results (Mn, V) and the independent analysis results will inform those capable of discriminating between source categories (Table 6.5). This process also highlights the importance of using different properties to define source categories (Collins and Walling 2007a).
Further forced entry of fingerprint properties did not improve the original classification of sources from independently entering all nineteen fingerprint properties (Table 6.5). However although the original classification using all 19 elements (Li, Mg, Al, V, Cr, Mn, Co, Ni, Ga, As, Rb, Y, Cd, Cs, Ba, La, Ce, Pr and Tb) gives the best result (100%), the cross validation classification of 56.7% is a lot lower than some of the forced entry results. Therefore the suite of fingerprint properties chosen to enter into the mixing model were Mn, V, Li, Mg, Al and La (emboldened in Table 6.5). Comparing the 19 elements proved to distinguish between the three sources in this study to other fingerprinting studies demonstrated that there are similarities between the properties chosen for composite suites to discriminate between sources (Collins et al. 1998; Collins and Walling 2002; Minella et al. 2008). For example, Walling et al. (2008) in a similar chalk catchment found that Mn, Cd, Al, and Cr were among the suite of fingerprint properties used within their mixing model and Collins and Walling (2006) used Ba, Al and Ga in their composite fingerprints to source the provenance of sediment sources in two lowland chalk streams in Dorset.

### Table 6.5. Example of forced entry and improvement/no improvement on the classification of samples within the correct source group.

<table>
<thead>
<tr>
<th>Element combination</th>
<th>Original Classification</th>
<th>Cross-validation Classification</th>
<th>Improvement or not</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn, V, Li</td>
<td>90%</td>
<td>86.7%</td>
<td>N</td>
</tr>
<tr>
<td>Mn, V, Li, Mg</td>
<td>93.3%</td>
<td>86.7%</td>
<td>the same</td>
</tr>
<tr>
<td>Mn, V, Li, Mg, Al</td>
<td>93.3%</td>
<td>86.7%</td>
<td>the same</td>
</tr>
<tr>
<td>Mn, V, Li, Mg, Al, Cr</td>
<td>93.3%</td>
<td>83.3%</td>
<td>N</td>
</tr>
<tr>
<td>Mn, V, Li, Mg, Al, Co</td>
<td>90%</td>
<td>86.7%</td>
<td>N</td>
</tr>
<tr>
<td>Mn, V, Li, Mg, Al, Ce</td>
<td>93.3%</td>
<td>83.3%</td>
<td>N</td>
</tr>
<tr>
<td>Mn, V, Li, Mg, Al, La, Ce</td>
<td>93.3%</td>
<td>86.7%</td>
<td>the same</td>
</tr>
</tbody>
</table>

6.3.1.2 Isokinetic samples discriminant function analysis results

Thirteen elements passed the Kruskal-Wallis H test to be put forward for discriminant function analysis for the suspended sediment samples. These were Li, Mg, Al, V, Mn, Co, Ni, Ga, As, Rb, Cd, Cs and Ba. The same process to the one outlined above in section 6.3.1.1 was employed to distinguish the optimum composite fingerprint suite for use in the sediment mixing model. Firstly stepwise algorithm results found that V
and Mn were the only two elements capable of distinguishing between sources and classifying samples within a group correctly (original cases 93.3% and cross-validation cases 86.7%). These results are shown in Figure 6.7.

Figure 6.7. Suspended sediment samples stepwise DFA results. V and Mn elements capable of 93.3% original cases classified correctly and 86.7% cross-validation cases classified correctly.

To improve the classification of sources, all 13 elements were entered independently (Figure 6.8). It is clear to see when comparing Figure 6.7 and Figure 6.8 that group separation and distinction is marginally better with independently entered properties.
Figure 6.8. Suspended sediment entered independently into DFA. 100% original cases classified correctly and 86.7% cross-validation classification.

Working on similar principles to the redd data, forced entry of fingerprint properties was used to discover the optimum number and combination of fingerprint properties able to discriminate effectively between sources. After trying numerous combinations based on stepwise and independent results there was no improvement on the original 13 element independent classification percentages. Therefore these 13 elements (Li, Mg, Al, V, Mn, Co, Ni, Ga, As, Rb, Cd, Cs and Ba) were put forward as the composite suite of fingerprint properties best capable of discriminating sources.

### 6.4 Sediment mixing model results

The mean mixing model outputs displayed in this section show the percentage source apportionment of redd sediment samples in different years and at different spatial scales. Model runs were separate depending on upstream and downstream sites, whole catchment and different years to observe any variation in source apportionment. 3000 iterations within a run allowed for the calculation of descriptive statistics to be calculated, such as the mean, standard deviation, minimum and maximum range and 95% confidence intervals.
6.4.1 Redd sediment source apportionment

Figure 6.9 displays the mean contribution of each type of source at all sites in the 2008 field season. Table 6.6 gives the actual percentage with the associated confidence intervals for each site in the 2008 field season. Pasture was found to contribute the most to fine sediments accumulating within salmonid redds and arable sources proved to be important at all sites. The upper catchment had a greater percentage of the fine sediment from pasture than the downstream sites. In-stream sources were not found to contribute to the fine sediment accumulating in redds in either year.

2008 Percentage source apportionment

Figure 6.9. Fine sediment source apportionment for the three potential categories (pasture, arable and in-stream) in redds over the 2008 field season.
<table>
<thead>
<tr>
<th>Source %</th>
<th>Pasture</th>
<th>Arable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arle</td>
<td>56 ± 1.0</td>
<td>44 ± 1.0</td>
</tr>
<tr>
<td>Abbotstone</td>
<td>55 ± 0.9</td>
<td>45 ± 0.9</td>
</tr>
<tr>
<td>Cheriton</td>
<td>68 ± 1.4</td>
<td>32 ± 1.4</td>
</tr>
<tr>
<td>Martyr Worthy</td>
<td>54 ± 0.8</td>
<td>46 ± 0.8</td>
</tr>
<tr>
<td>Winchester</td>
<td>59 ± 1.2</td>
<td>41 ± 1.2</td>
</tr>
<tr>
<td>Shawford House</td>
<td>51 ± 0.6</td>
<td>49 ± 0.6</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td>53 ± 0.7</td>
<td>47 ± 0.7</td>
</tr>
<tr>
<td>Gaters Mill</td>
<td>56 ± 0.9</td>
<td>44 ± 0.9</td>
</tr>
</tbody>
</table>

Table 6.6. Percentage source proportions of fine sediment within salmonid reds over the 2008 field season and associated 95% confidence limits.
Figure 6.10. Fine sediment source apportionment for the three potential categories (pasture, arable and in-stream) in redds over the 2009 field season.

Table 6.7. Percentage sediment source proportions found within salmonid redds over the 2009 field season (emergence) with associated 95% confidence intervals.
Pasture sources are slightly more prominent than arable sources at all redd sites in the 2008 and 2009 field seasons with often just over half of the total sources of fine sediment being attributed to pasture sources. These results are similar to other recent studies conducted in chalk-based catchments, where the most prominent source of fine sediment was often found to be pasture or cultivated arable land (Walling et al. 2006; Collins and Walling 2007). Arable land has become an increasingly important source of fine sediment in catchments where cereal crops are Autumn sown as opposed to Spring sown, leaving the strength resistance of the soil low and susceptible to transport in the winter rains (Collins and Walling 2004).

The proximity of rough and improved pasture to the river may well explain its dominance at sites as it was the major land use recorded at most sites (Figure 6.1). However, cultivated soils are often thought to be more erodible than soil under pasture regimes which may explain why the remaining apportionment of sediment is to arable sources (Walling 1990; Wood and Armitage 1999). Recent risk mapping of diffuse sediment effects on salmonid fry abundance have shown that pasture sources may well have a greater impact than previously thought and have suggested that the erodibility factor within the model should be raised from 0.2 to ~0.75 (Reany et al. 2011). This is based on the reasoning that while pasture sediment may not be as erodible as arable sources, there could prove to be more damaging components in pasture than arable sources, e.g. animal faeces (Reany et al. 2011).

In-stream sources, collected from marginal sediments, do not contribute to the fine sediment accumulating within redd sediments over the incubation period. It has been reported in previous studies on chalk rivers that river margins and river bank sediment sources are of limited importance due to their stable flow regimes which result in a general lack of sediment mobilising flows and consequently bank erosion does not occur on large scales (Walling et al. 2006). However bed sediment remobilisation was found to be a particularly important source in lowland, groundwater-fed rivers in other studies (Collins and Walling 2006) which is incongruous with the findings of this study.

The 2008 results display very slight variations in source apportionment between pasture and arable sources, with the Cheriton stream exhibiting the greatest variation; 68% of fine sediment attributed to pasture and only 32% attributed to arable sources. This site was one of two sites, during the field season, where evidence of cattle ‘poaching’ had been noted. The lack of riparian vegetation and shallow banks allowed livestock to enter the reach of the Cheriton stream containing the field site which could increase the likelihood of pasture sediment entering the river.
Figure 6.11 describes the mean source apportionment found in the 2008 and 2009 field season within salmonid redds. As already noted from the separate site maps, pasture sediment sources are slightly more dominant in the catchment than arable sediment sources and in-stream marginal sediment sources contribute nothing in both years (Figure 6.9 and Figure 6.10). There is a marginal difference between fine sediment source apportionment in 2009 from 2008, where pasture sources contribute 4% more to fine sediment accumulating in salmonid redds. This difference could be due to slight variations in river flow rate but is more likely attributed to greater precipitation and snowfall experienced over the incubation period in the 2009 field season. It is likely that transport and connectivity of fine sediment to the River Itchen over the 2009 field season at times was slightly higher due to snow melt in January and February. Walling et al. (2002) found that 97% of the fine sediment found in salmonid spawning gravels could be attributed to surface sediments which agrees with
the results from this study. Despite the majority of arable land-use occurring in the headwaters of the catchment, this is not reflected in the source apportionment of fine sediment within redds at sites. There appears to be very little difference between upstream and downstream sites (Figure 6.12).

![Figure 6.12. Comparison of the difference in mean fine sediment source origin between upstream and downstream sites in the 2008 and 2009 field season](image)

<table>
<thead>
<tr>
<th></th>
<th>2008 u/s</th>
<th>2008 d/s</th>
<th>2009 u/s</th>
<th>2009 d/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>pasture</td>
<td>57.15</td>
<td>54.83</td>
<td>55.59</td>
<td>59.45</td>
</tr>
<tr>
<td>arable</td>
<td>42.85</td>
<td>45.17</td>
<td>44.41</td>
<td>40.55</td>
</tr>
</tbody>
</table>

6.4.1.1 Temporal variation in sediment source apportionment

Temporal variation over the incubation period was monitored in the 2009 field season to see whether there was any difference in fine sediment sources found within redds at different stages over the incubation period. At eyeing, hatch and emergence, sediment baskets were removed from redds. Figure 6.13 shows the results of the mixing model outputs for different incubation stages at all four sites.
Figure 6.13. Temporal variation in source apportionment during the 2009 field season at different incubation stages. 1 = eyeing (18/02/09), 2 = hatch (3/04/09) and 3 = emergence (29/04/09).

The hatch incubation stage describes a slightly greater percentage of fine sediment originating from pasture sources than either eyeing or emergence, which could indicate that there is greater connectivity between pasture sediment and the river at this time. Recent research into the impact of cattle on chalk rivers found that there was a positive correlation between time spent by cattle in a chalk river and air temperature (Bond et al. 2012). It was also noted that cattle defecate five times more in the river and riparian zone than on land (Bond et al. 2012). Although air temperature was not monitored over the incubation zone, Met Office data from the region suggests that 14.2 °C was the maximum temperature experienced in April which is higher than March
(12.3°C) and February (8.3°C) when eyeing occurred. Hatching of the eggs occurred on the 2nd of April and emergence on 29th April so it is possible that there were higher temperatures around hatch, encouraging more cattle into the river and connecting pasture sediments with the river. At all incubation times, the furthest downstream site (Bishopstoke) had the greatest proportion of sediment attributed to pasture sources and the least attributed to arable, as would be expected considering the proximity of this site to the majority of arable inputs which are largely situated in the headwaters of the catchment. Stable hydrological regimes within the river limit the sediment transport capacity of the river (Sear et al. 1999) indicating that settled sediment within the river bed stays near to where it has settled.

### 6.4.2 Suspended sediment source apportionment

The sources of fine suspended sediment were also explored in order to see whether there was any difference between the sediment entering the river and being carried in suspension and that being deposited within salmonid spawning gravels. Figure 6.14 displays the mean percentage origin of suspended fine sediment in the 2008 and 2009 field season.

![Figure 6.14](image1.png)

Figure 6.14. Mean source apportionment of suspended sediments for 1) 2008 and 2) 2009 field seasons on the Itchen.
There is no difference between the average source apportionment of suspended fine sediment on the River Itchen over the 2008 and 2009 field seasons. Upstream sites in both years exhibit a mean source apportionment of: pasture - 57%, arable - 43% and in-stream sources - 0% and the downstream sites displayed: pasture - 55%, arable - 45% and in-stream sources - 0%. Small variations were present between sites but not as much as was found within redd sediments. This could be explained by the longer residence time of sediments in river beds and accumulation over time compared with the mobile nature of suspended sediments.

6.4.2.1 Temporal variation in suspended sediment sources

Very little difference is displayed in the sources of fine suspended sediment over different incubation periods and sites in the 2009 field season. Bishopstoke is the only site that displays noticeable variation in the apportionment of sources, with hatch time exhibiting the greatest source being attributed to pasture sources, similar to the fine sediment found within redds (Figure 6.15).

Figure 6.15. Temporal differences in suspended sediment source apportionment over the salmonid embryo incubation period at all sites during the 2009 field season. A = Arle, B = Ovington, C = Winchester and D = Bishopstoke.
The similarity displayed between the proportions of sediment sources within suspended sediment and redd sediments suggests there is a strong connection between them. The fine sediment accumulating within salmonid redds is most likely to be from suspended sediment settling out of suspension. This finding is in contrast to work carried out in the Torridge catchment where it was observed that there was variation between the source provenance of suspended sediments and river bed sediments (Nicholls 2000). The hypothesis here was that sediment deposition occurred in periods of stable flow and not during the greatest suspended flux period (autumn/winter months) leading to the conclusion that different sources contributed to the fine sediment found in bed sediments and suspended sediments. Possibly due to the stable regime exhibited by the River Itchen, deposition may occur at a more stable rate throughout the year which would account for the similarities found between suspended and redd sediments.

6.4.3 In-stream sediment sources

A question alluded to in previous research and posed by this study, due to the lack of source contribution to fine sediment, was the role of in-stream sediment as a primary source in the potential source categories. Large amounts of fine sediment were observed adjacent to the river bank and within the main channel, particularly upstream of macrophyte colonies during catchment walkovers on the River Itchen. The lack of mobilising flows to flush the sediment through the reach, due to the stable hydrological regimes, suggests that the bank marginal sediments in particular could be stored for long periods of time. Based on the residence time of these sediment ‘sinks’ within the river, marginal in-stream sediment was thought to have been subject to change in the aquatic environment over time which could have altered the fingerprint properties to define it as a primary source in its own right. It is clear to see from the discriminant function analysis that the element concentrations of its fingerprint properties are significantly different to that of the other sources. Figures 6.6 and 6.8 show in-stream sediment can be defined as a separate source category from the other sources and this suggests that it has a different signal as a distinct and unique source. Some overlap between in-stream sources and other sources would be expected if similar elemental concentrations to the other sources were displayed.

To be sure that in-stream sediment was not being incorrectly classified as a primary potential source it was decided to carry out quantitative statistical verification of source discrimination on these samples to see if they could be attributed back to the other primary sources, namely pasture and arable sources. Three elements passed the
range test, indicating that the range of in-stream fingerprint property characteristics were different to those displayed by arable and pasture combined. Statistical testing with Kruskal-Wallis H test showed that Ti, Sn and Sb could all distinguish between arable and pasture as source categories. However further discriminant function analysis could not correctly classify pasture and arable as separate sources with only three elements. This indicates that in-stream source material is significantly different from the other source contributors identified. Degradation of organic material and the formation of inorganic-organic complexes would alter the composition of the type of sediment fingerprinting properties used in this study (e.g. heavy metals) in the riverine environment which argues the case for including in-stream sediment as a primary source category (Collins and Walling 2002).

The lack of representation of in-stream sediment within suspended and redd gravels perhaps illustrates there is a lack of connectivity between river margin sediment and the main river, although this does not apply to sediments accumulating within macrophyte colonies as they gather seasonally from suspended and particulate sediment deposits (Cotton et al. 2006). In-stream sediment has been found to be a significant source within previous studies in groundwater dominated catchments. Collins and Walling (2006) found that in three lowland, groundwater-fed catchments, re-mobilised bed sediment comprised a minimum of 7% and a maximum of 28% to suspended sediment loads. It should be noted that this study was comparing aquatic sediment sources (river bed re-mobilised sediment, headwater tributary suspended sediment and eroded channel bank material) of suspended sediment and not looking at the original source of sediments within the river. It is likely that the in-stream sediment was originally derived from catchment sediment sources. Recent studies have found that surface catchment sediments are the dominant source of riverine sediments found in lowland, groundwater dominated rivers (Walling et al. 2002; Walling et al. 2003; Collins and Walling 2007a).
6.5 Discussion and implications

The results of this study show that pasture sources (50-68%), closely followed by arable sources (32-50%) are the major sources of fine sediment accumulating within salmonid redds and within suspended sediment on the River Itchen. Walling et al. (2002) suggests that the major sediment sources on the River Itchen that can be identified within salmonid redds were mostly surface derived material (97%). The findings of this study agree with recent findings, with surface sources contributing 100% of the fine sediment found in redds. Walling et al. (2002) suggested that the majority of fine sediment in redd gravels on the River Itchen originated from arable fields; however this study only split sources into surface catchment and sub-surface sources. Interestingly, this study shows that pasture soils are a marginally more important source on the River Itchen than arable land, highlighting the importance of monitoring rough and improved pasture soils' connectivity with the river. The major riparian land use at sites studied in the upper catchment was rough and improved pasture, with many fields containing remnant water meadow systems, old field drains and evidence of cattle poaching. However arable land constitutes 49% of land use on the River Itchen catchment (Environment Agency 2004a), explaining the importance of this land management regime as a source of fine sediment in redds and suspended sediment.

Arable land contributes a significant source of fine sediment in the catchment which is not surprising due to the fact that cultivation of land for crops physically alters soil structure and decreases the potential for roots to stabilise soil so more material is available for erosion and transport. Arable cultivation has long been thought to contribute the majority of fine sediment to rivers, particularly in catchments with high arable land use (Boardman 1990; Walling and Amos 1999). There are some sloping arable fields in the upper catchment, particularly on the Candover and Cheriton streams, that have direct connectivity with the river via farm tracks and drainage ditches. There was observational evidence of sediment trails from fields and along roads into the river. Other studies of small lowland catchments in different parts of the country have shown variable source provenance. (Russell et al. 2001) reports that field drains are the most important source (55%) with surface sediment next important (33% - mostly arable) and channel banks providing only 12%. However within different catchments the sediment load was found to be split; field drains (30%), surface soils – mostly pasture sources (64%) and channel banks (6%). The catchment with greater surface sources originating from pasture soils had a greater number of livestock utilising riparian areas in winter (Russell et al. 2001). The River Itchen catchment does
have local incidences of cattle poaching along its banks. The upstream sites on the Cheriton and Candover tributaries suffered particularly from a lack of riparian vegetation and evidence was noted of cattle entering the river within these reaches. It is therefore possible that cattle entering the river could form part of the transport process by which pasture top soil is deposited within the River Itchen.

In-stream sources are negligible in all of the mixing model results. Identifying marginal sediment as a primary source was presumed to be a reasonable assumption from the statistical verification results which displayed clear distinction between in-stream sediment and other sources. The secondary source testing of in-stream sediment to see whether it could be attributable to arable or pasture sources also concluded that in-stream sediment cannot be classified into either source group. Another study on a chalk stream suggests that surface sources are more important contributors to fine suspended sediment than remobilised sediment and channel banks (Walling and Amos 1999). However this study did not look at bed accumulated sediments, therefore an argument to support in-stream material as a major source of redd sediment could be that groundwater hydrological regimes might be more important than surface water infiltration and move fine sediment into bed sediments from further upstream having already deposited as matrix sediment in gravels upstream. Surface flows in chalk streams can be low due to natural and anthropogenic issues such as the chalk aquifer regulating surface water and over-abstraction (Walling and Amos 1999). Stored marginal sediments are potentially therefore not very mobile as would be expected due to the stable nature of chalk stream flow regimes (Sear et al. 1999).

A limitation of the study were that watercress beds, fish farms and urban waste water and sewage treatment works could not be included as possible sources of fine sediment within sediments. The two former are large industries operating in and next to the River Itchen and the latter would have indicated whether there is an urban impact from diffuse sediment pollution. The scope of this study could not include these categories as potential sources although they were identified as potential sources due to permissions not granted to sample these areas. These sources should be included in future work.

Had a greater number of source samples (>10 per source) and river sediment samples been collected, the accuracy and precision of the results obtained would have been better. The mixing model could be improved with a greater number of iterations and more sources of error included. In some previous studies an organic matter correction has been used alongside the particle size correction factor which has increased source ascription ability of the model (Collins et al. 1997; Collins and Walling 2007a; Davis
and Fox 2009). Modelling uncertainty was minimised by averaging many iterations for the mean source proportion results, however models are inherently uncertain as they cannot reproduce real world situations and thus the results should be seen as an estimation of source proportions of fine sediment in the catchment.

This study highlights for the first time the importance of both pasture and arable sources contributing to fine sediment inputs on the Itchen, which is an advance on the previous reconnaissance study that suspected arable as the major source of fine sediment in the River Itchen. Catchment management strategies, including Catchment Sensitive Farming (CSF), Environmentally Sensitive Areas (ESAs) and the ‘set aside’ scheme (where farmers are compensated for taking land out of agricultural production) aimed at promoting awareness of land and river connectivity (Hendry et al. 2003) should include recommendations for arable and pasture land. One of the major catchment management strategies that could greatly decrease diffuse sediment pollution on the Itchen is the encouragement and management of riparian buffer zone vegetation which would exclude these areas from livestock and mechanical disturbance altogether, thereby reducing connectivity between surface sources (pasture and arable) and the river. Fencing off of the riverbanks would have similar results in excluding livestock from the river.
Chapter 7. The composition of organic material in salmonid redds

7.1 Chapter synopsis and background

The lack of data and information pertaining to the composition, source and concentration of organic matter accumulation within salmonid spawning redds could be reducing the effectiveness of present fisheries management policies. Whilst the delivery of fine sediment to rivers has been recognised as detrimental to salmonid population recruitment and policies have been implemented to reduce the effect of this diffuse pollutant on salmonid spawning gravels (Catchment Sensitive Farming, riparian buffer zones and river restoration projects focusing on reinstating natural river morphology and creating niche habitats), there has been little attention focused on the fraction of sediment that has the potential to affect salmonid embryo survival due to its oxygen consuming capacity (House 2003), toxic metal binding potential (Sader et al. 2011) and excessive bacteria growth (Vallino et al. 1996). A number of researchers have highlighted the need to investigate the complex nature of particulate and dissolved organic matter (POM and DOM) and consider the impact of such substances on the early life stages of salmonids in UK rivers (Chevalier et al. 1984; Greig et al. 2005a; Sear and DeVries 2008). Salmonids have themselves been identified as a source of organic material within catchments of the Pacific North West of America and researchers there have looked at nutrient transfer from mass post-spawning mortalities and the fate of decomposing salmonids organic by-products in the production of bio-films in rivers (Jonsson and Jonsson 2003; Petticrew and Acrocena 2003; McConnachie and Petticrew 2006). Whilst the presence of organic material within spawning gravels has been highlighted as a potential oxygen sink (Chevalier et al. 1984; Greig et al. 2005a; Sear and DeVries 2008), there is still very little evidence about the source or composition of infiltrated organic sediments to assess the likely impacts on incubating salmonid embryos in Atlantic salmon spawning rivers.

In order to deal with this research gap, the main aim of this chapter is to identify and describe accumulated organic matter within the redd environment. This chapter synthesises previous literature about organic material in natural systems and critically reviews the methods employed to investigate sources, composition and concentration of organic matter originating within natural systems. An integrated method to characterise the organic material obtained from salmonid redds is presented. The method uses spectrophotometric
techniques to determine organic matter composition and potential sources, complementary techniques were used to support the results of macro-flora and macro-fauna percentage cover counts and percentage total organic material and carbon.
7.2 Introduction

7.2.1 Organic Matter definition and description

The term organic matter describes any organic compound or molecule found in natural systems that is biodegradable, based on its source being of biological origin. It is often termed natural organic matter (NOM) within the literature. Specifically in freshwater systems, organic matter can be described as a heterogeneous mix of aliphatic and aromatic polymers with varying composition depending on decay processes and proximity to source (Stedmon et al. 2003). Organic matter present in freshwater systems can originate from a wide range of different sources (Hudson et al. 2007), occurring naturally or from anthropogenic origins such as riparian or in-stream vegetation, microbial and bacterial colonies, waste products from animals and sewage treatment discharges. Some researchers suggest that one of the major sources of DOM in freshwater systems is that derived from terrestrial plant matter from the surrounding catchment and dissolved and transported through the system (Stedmon et al. 2003). Another likely major source, particularly in groundwater fed chalk streams, is the exudation and biodegradation of aquatic plants, known as macrophytes and bryophytes present in abundance in these systems (Jaffe et al. 2008; Lapworth et al. 2009).

Organic matter can be found in different forms within freshwater environments, including particulate (POM), colloidal and dissolved (DOM) and regularly dissolved organic carbon (DOC) is the measure used to represent dissolved organic material in research (Komada et al. 2002). Dissolved organic matter (DOM) is the most studied fraction of organic material in freshwater and marine environments due to the role it plays in the global carbon cycle (Stedmon at al. 2003; Jaffe et al. 2008). Approximately 97% of the organic carbon found in the ocean is found in a dissolved state (Benner 2002) and similarly in freshwater systems, DOM represents the largest pool of organic carbon (Jaffe et al. 2008). Despite the large amount of literature on the composition, concentration and interactive processes of dissolved organic matter, it is still a very complex topic to study due to its inherent changeable structure, reactivity and state (Hudson et al. 2007).

The variation in organic matter composition and dynamics in natural systems is vast and its importance in many processes differs depending on the interaction between sources, hydrological regime, land management practices and land cover (Jaffe et al. 2008). Organic matter in freshwater systems is known to be highly labile and reactive leading to its
involvement in processes such as forming colloids and biofilms with inorganic sediments (Petticrew and Arocena 2003; Droppo, 2001), controlling microbial food chains and nutrient availability within the system (Jaffe et al. 2008) and binding and transporting trace metals and other pollutants (Berault et al. 1996). Figure 7.1 describes some of the processes involving dissolved organic matter in freshwater ecosystems.

Figure 7.1. Schematic highlighting different processes involving DOM in freshwater ecosystems. Note the interaction between processes and that all processes are first connected with biodegradation of organic matter.
7.2.2 Salmonid spawning habitat and organic matter

Salmonids are benthic spawners and the successful incubation of salmonid embryos depends on optimal environmental factors surrounding the embryos which include an adequate supply of dissolved oxygen, removal of metabolic wastes and appropriate temperature ranges (Chapter 2, section 2.3). An adequate supply of dissolved oxygen to embryos has been found to be one of the most important factors in successful hatching of salmonid eggs (Malcolm et al. 2003; Grieg et al. 2007) and therefore identifying the factors that limit oxygen availability to incubating embryos is of great importance. Whilst previous research has focused on the inorganic fraction of fine sediments, physically blocking sediment pores and egg membranes and thus indirectly and directly blocking the passage of oxygenated water through redds (Reiser 1998; Grieg 2005c; Heywood et al. 2007; Sear et al. 2008), the organic fraction of accumulated sediment has been largely overlooked. However, the literature has increasingly highlighted the importance of the organic fraction of infiltrated fine sediments in the redd environment being an important dissolved oxygen sink (Chevalier et al. 1984; Grieg et al. 2007; Sear et al. 2008).

Research conducted in the Pacific North West region of America describes the composition and structure of organic matter present in Pacific salmon streams, pre-, mid- and post-spawning (Petticrew and Arocena 2003). The experiments found that two types of organic structure formed; a less dense web-like structure and more dense film-like complexes overlying the gravel bed. Notably during spawning periods the web-like structures dominated the river bed and post-spawning film-like structures dominated (Petticrew and Arocena 2003). The disturbance of gravels by salmonids during redd building interrupts the flocculation of organic matter and inorganic sediment, however postspawning when eggs are incubating within the gravels, organic/inorganic flocs create a film-like coating covering the gravel bed which is described as expansive and dense (Petticrew and Arocena 2003). The production of biofilms within salmonid spawning redds could pose a threat to pre-emergent survival by directly smothering egg surfaces or by indirectly blocking the passage of oxygenated river water into the egg pockets.

It should be noted that Atlantic salmon (Salmo salar) are semi-iteroparous as opposed to their Pacific semelparous cousins which therefore have greater organic inputs post spawning. There is evidence to suggest that redd building can promote the accumulation of fine sediment as its morphology preferentially forces river water containing dissolved organic matter, into the redd (Crisp and Carling 1989; Sear et al. 2008).
The storage of organic material within river beds can cause a number of issues concerning the production of biofilms in redds; reduced intra-gravel flow through sediment pore spaces, leading to decreased oxygen availability to embryos and slower removal of metabolic products and indirectly increasing sediment oxygen demand (SOD) reducing available oxygen in the hyporheic zone (Greig et al. 2005; Sear et al. 2008).

A study quantifying the organic budget of a reach on a small, southern chalk stream in the UK found that the concentration of organic matter transported through the reach increased during winter months from 1-2 mg l\(^{-1}\) dry wt\(^{-1}\) organic matter to 10-12 mg l\(^{-1}\) dry wt\(^{-1}\) organic matter (Dawson 1981). This study highlighted the potential for organic material to have an impact on salmon embryo incubation in southern chalk streams as increasing organic material is present during the incubation period of salmonids. Lapworth et al. (2009) found that a significant proportion of fluorescent organic material can reside within the hyporheic zone of chalk catchments and fulvic-like fluorescence has longer residence times than protein-like fluorescence which is preferentially attenuated in shallower regions (0-0.5m). It was also noted that in deeper regions (~4-20m), groundwater inputs dampened the surface water organic matter fluorescent signal indicating reduced organic matter concentration because of dilution (Lapworth et al. 2009).

7.2.3 Fluorescence spectroscopy

Recent research into organic material in natural systems has been dominated by the use of fluorescence spectroscopy to identify the organic composition of samples in freshwater and marine environments (Coble et al. 1996; Coble et al. 2003; McKnight et al 2001; Baker 2002; Stedmon et al. 2003; Hudson et al. 2008; Henderson et al 2009). Recent studies measured total organic carbon (TOC) to give a measure of aquatic organic matter (Tipping et al. 1997 in Baker 2002); however limitations with this method included the limited delineation of carbon species found in different organic compounds (Baker 2002). Fluorescence measurements have the power to distinguish between different carbon complexes which are useful to estimate the effects of discrete fractions on ecosystem function and are able to trace organic matter sources through ecosystems (Newson et al. 2001; Baker 2002).
7.2.3.1 Principles of fluorescence spectroscopy analysis

Certain molecules absorb and emit light energy; typically these are aromatic molecules (Lakowicz 2006). Figure 7.2

Figure 7.2 displays a group of fluorescent molecules. These light emitting substances are known as fluorophores.

Fluorescein

Quinine

Rhodamine B

Figure 7.2. Describes three structural examples of typically fluorescent substances. Fluorescein (tracer used in natural waters and found in anti-freeze - green fluorescence), quinine (found in tonic water – blue fluorescence) and rhodamine B (often used as a dye tracer - orange fluorescence) (Lakowicz 2006).

When electrons in a fluorophore are excited they absorb light and emit energy, sometimes in the form of light on return to their original state. This emitted light is known as fluorescence and the difference between the excited and original states of the fluorophore determines the wavelength of the light absorbed and emitted (Stedmon et al. 2003). Fluorescence spectroscopy analysis is an analytical tool used by many scientists and industries to measure light energy emitted by fluorophores. Samples are delivered into the fluorimeter at their ground state where they are excited by a high energy light source, usually a xenon lamp (Sharma and Schulman 1999). After excitation, relaxation occurs to a state known as the ‘lowest excited singlet state’ which is the point where fluorescent compounds emit light which is then measured by the fluorimeter (Sharma and Schulman 1999).
Fluorescence excitation-emission matrices (EEM) are commonly used as a method to display complex fluorescence data. EEMs display the number, abundance and type of fluorescent material present in samples (Stedmon et al. 2003; Teymouri 2007). They consist of 3-dimensional arrays that merge a range of excitation and emission wavelengths together (Stedmon et al. 2003) to give fluorescence emission intensity measurements over a range of excitation wavelengths that correspond to the concentration of fluorophores in a sample (Teymouri 2007). Generally these EEMs are displayed as contour maps which describe the specific areas where peak intensity locations are found (Figure 7.3). The location of an intensity peak describes the type of fluorescent material that is contained within a sample (Coble et al. 1996; Baker 2002; Hudson et al. 2007) whilst the actual intensity indicates the relative concentration of that fluorophore within the sample (Teymouri 2007).

Figure 7.3. Displays a raw EEM and a scatter corrected EEM, with excitation and emission on the x and y axis respectively and the colour of the contours represents the fluorescence intensity of the sample (Zepp et al. 2004).

A number of corrections need to be applied to EEM fluorescence data to ensure the validity and comparability of results. As seen in Figure 7.3 uncorrected EEMs display linear features known as Rayleigh-Tyndall lines which occur due to the reflection of the excitation light on water molecules. These lines need removing as they are not directly related to the concentration of fluorophores in a sample (Zepp et al. 2004). Inner filter effects are one of the biggest issues to overcome. Primary and secondary inner filter effects relate to the absorption of the excitation beams by the sample prior to
measurement by the fluorimeter and absorption of emitted light from samples respectively. The effects are increased with a high concentration of fluorophores present in samples (Mobed et al. 1996; Ohno 2002). In order to avoid direct absorbance by the sample during fluorescence measurements some samples need to be diluted. Ohno (2002) reported that diluting samples to an absorbance value lower than 0.3 at absorbance wavelength of 254 (Abs$_{254}$) negated the need to correct data for inner filter effects. Raman scatter, also related to the reflection of excitation light by water molecules, but to a lesser extent than Rayleigh-Tyndall scatter should be accounted for in fluorescence results (Zepp et al. 2004). Raman water spectra of Milli-Q water samples is often monitored throughout the period of sample measurement to enable identification of instrument drift and EEMs can then be normalised to the Raman measured peak (spectra are divided by the average Raman peak) to convert measurements to Raman Units (R.U.) allowing comparison of results with other studies (Stedmon et al. 2003).

### 7.2.3.2 Fluorescence and freshwater ecosystems

Originally EEM fluorescence spectroscopy was used in marine research to study dissolved organic carbon and characterise dissolved organic matter in terms of its source; terrestrial or marine (Coble and Mayer 1990; Coble et al. 1993; De Souza Sierra et al. 1994). Fluorescence spectroscopy is now being employed in a large range of industries to quantify organic compounds and specifically in the water industry for drinking water treatment plants (Hua et al. 2007), recycled water systems (Foley et al. 2007; Henderson et al. 2009), groundwater flow determination (Lamont-Black et al. 2005) and also applications in measuring water quality and organic pollution monitoring in rivers (Baker 2001; Baker and Inverarity 2004; Hudson et al. 2007; Hudson et al. 2008).

Fluorescence spectroscopy is of particular use in investigating dissolved organic matter (DOM) in freshwater ecosystems as it is non-destructive and samples are quick to analyse, reducing the effects of biodegradation (Henderson et al. 2009). The expansion of fluorescence excitation-emission matrices in many sectors has led to a need to identify peak locations of fluorophores to allow comparison and quantification of samples (Teymouri 2007).

A number of different fluorescent constituents have been identified in EEMs which constitute the main constituents of fluorescent dissolved organic material (FDOM). Fluorophores which have been identified and characterised in NDOM from terrestrial
and aquatic systems by numerous researchers, include tyrosine and tryptophan protein structures and humic and fulvic acids (Coble et al. 1996; Mayar et al. 1999; Baker, 2002; Stedmon et al. 2003; Hudson et al. 2007; Liu et al. 2007). Humic and fulvic acids comprise the main composition of humic substances and are respectively defined as larger molecular, acid insoluble and smaller molecular, acid soluble groups of mixed compounds (Alberts and Takács 2004; Hudson et al. 2007). Humic substances are defined as the macro-molecular complex products of the chemical and biological degradation of plant residues including lignin, carbohydrates and proteins (Uygunar and Bekbolet 2005; Hudson et al. 2007; IHSS 2008).

Fluorophores are referred to as humic-‘like’ or protein-‘like’ due to the uncertainty that the peak location is specifically due to fluorescence from any one particular species, however based on consistency within the literature of identified peaks for certain groups of compounds and substance specific laboratory standard tests, it is acceptable to term fluorescence measurements as such (Teymouri 2007; Reynolds 2003). Figure 7.4 displays an example EEM of the location of fluorophores that are commonly found in freshwater ecosystems.

![Figure 7.4](image)

Figure 7.4. An example EEM describing peaks commonly found in freshwater (Hudson et al. 2008). Peak A and C indicate the presence of humic/fulvic-like acids, peak B represents protein, Tyrosine-like fluorescence and peaks T₁ and T₂ indicate protein, Tryptophan-like fluorescence.

Previous research has reported the peak locations of fluorescent substances and their likely composition. Table 7.1 describes the main peaks commonly found in aquatic ecosystems.
<table>
<thead>
<tr>
<th>Type of fluorescence</th>
<th>Peak name</th>
<th>Excitation wavelength (nm)</th>
<th>Emission wavelength (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humic/fulvic-like acids</td>
<td>A</td>
<td>230-260</td>
<td>400-500</td>
<td>Coble et al. 1996; Mayar et al. 1999</td>
</tr>
<tr>
<td>Humic/fulvic-like acids</td>
<td>C</td>
<td>300-370</td>
<td>400-500</td>
<td>Coble et al. 1996; Mayar et al. 1999; Baker 2001</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>275</td>
<td>340, 350</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>225-237</td>
<td>340-381</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.1. Commonly identified peak locations and types of fluorophores found in natural water ecosystems that form part of dissolved organic matter.

Protein-like compounds are reported to be derived from the breakdown of protein and peptide chains (Hudson et al. 2007). Tryptophan and tyrosine are two essential amino acids which display fluorescence and have been identified by previous researchers (De Souza Sierra et al. 1994; Coble et al 1996; Baker 2001). The origin of protein-like fluorescence is still under debate as to whether it occurs from free amino acids in the DOM pool or as to whether it is partially from amino acids still bound within cell walls and proteins (Hudson et al. 2007). Hudson et al. (2007) stated that there was clear evidence that tryptophan originated from bacterial sources, based on previous research relating tryptophan-like fluorophores to activity within freshwater bacterial communities and the products that came from those communities (Elliot et al. 2006).

Baker (2001) found that anthropogenic organic pollutants, found in some rivers such as farm wastes, sewage treatment outfalls and sewerage overflows, all contain high intensity tryptophan and tyrosine protein-like fluorescence. Organic pollutants would increase bacterial activity within the water as colonies multiply with larger food sources. Additionally correlations between BOD measurements taken in rivers and protein-like fluorescence (namely peak $T_2^-$ see Figure 7.4 and Table 7.1) indicate that protein-like fluorescence is related to the labile fraction of dissolved organic matter sources which consume greater amounts of dissolved oxygen than refractory DOM (Baker 2004; Hudson et al. 2008).
Previous studies have noted that freshwater samples tend to contain high levels of dissolved organic carbon (DOC) and humic/aromatic fulvic acids (McKnight et al., 2001; Coble et al., 1996; De Souza Sierra et al., 1994; Newson et al., 2001) which has been reported to be mainly derived from the humification of terrestrial plant material in soils (Senesi et al., 1991 reported in Newson et al., 2001).

7.2.4 PARAFAC Analysis

EEMs contain a large amount of data about the chemical composition of each sample in a dataset and it can often be hard to extract all the useful data by using traditional ‘peak picking’ techniques. This involves a researcher identifying peak locations by eye from every sample (Coble et al., 1996). In light of the fact that the amount of data in one EEM consists of the number of excitation wavelengths multiplied by the number of emission wavelengths, a typical EEM can have >10,000 data points (Teymouri, 2007) so this method warrants a certain degree of subjectivity and can be time consuming (Stedmon et al., 2003).

Multivariate data analysis methods have therefore been developed specifically to alleviate the issues surrounding traditional peak picking methods and help to decompose complex organic mixtures into separate organic components (Bro, 1997; Stedmon et al., 2003). Two-way Principle Component Analysis (PCA) and three-way Parallel Factor Analysis (PARAFAC) are two types of multivariate methods which have been used to statistically derive the organic composition of a range of samples in common datasets (Thoss et al., 2000; Stedmon et al., 2003; Stedmon and Bro, 2008). PARAFAC is an extension to the methods used in PCA and other two-way methods by using a three-way analysis (Harshman and Lundy, 1994). Arguably PARAFAC is better suited to analysing data obtained from EEMs due to the three-way matrix nature of EEM data (sample by excitation by emission) which otherwise would need to be transformed into a two dimensional array to create a more complex PCA model (Bro, 1997; Stedmon et al., 2003). PARAFAC enables the identification of specific fluorophores whereas it is not possible with PCA due to the intrinsic rotational freedom of the method (Stedmon et al., 2003; Teymouri, 2007). PARAFAC analysis displays uniqueness in mathematical terms because the model cannot be rotated without loss of fit to the observed data (Stedmon et al., 2003).

Parallel factor analysis is defined as a multi-way decomposition method that analyses fluorescence data from multiple samples in a 3-way matrix array to uncover specific
organic components/fluorophores, common to that dataset (Anderson and Bro 2003; Stedmon and Bro 2008; Teymouri 2007). Equation 7.1 describes the PARAFAC model used as reported in Stedmon et al. (2003) and Stedmon and Bro (2008).

\[ x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk} \]

, \( i = 1, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K; \)

Equation 7.1. (Stedmon et al. 2003)

Where:

- \( x_{ijk} \) = fluorescence intensity of \((i)th\) sample at emission \((j)\) and excitation \((k)\)
- \( a_{if} \) = proportional to the concentration of the \(f\)th analyte in sample \(i\)
- \( b_{jf} \) = estimated emission of the \(f\)th analyte at emission \(j\)
- \( c_{kf} \) = estimated excitation of the \(f\)th analyte at excitation \(k\)
- \( \varepsilon_{ijk} \) = residuals not accounted for in the model
- \( F \) = number of components
- \( I \) = intensity
- \( J \) = emission wavelength
- \( K \) = excitation wavelength

\( x_{ijk} \) represents one sample which is one element of the three-way matrix array that has dimensions \( I, J \) and \( K \) and parameters \( a, b \) and \( c \) represent the model outcome; the underlying fluorophores (Stedmon and Bro 2008). The solution to the model is found by minimising the sum of squared errors of the residuals (Stedmon et al. 2003). Assuming that the fluorophores in a sample adhere to Beer-Lamberts Law (linear relationship between absorption and concentration) and there are no interactions between fluorophores, equation 1 states that the concentration of \( F \) fluorophores in a sample is the sum of \( F \) fluorophores (Stedmon and Bro 2008). The location of each fluorophore does not change, only the concentration within each respective sample. PARAFAC allows for more adequate, robust and more easily interpretable results than other statistical methods (Anderson and Bro 2003).
7.3 Methods

7.3.1 Fluorescence analysis

7.3.1.1 Sediment sample collection

In order to explore the organic fraction of fine sediment available on the River Itchen to infiltrate into salmon redds, three different types of samples were collected:

- Redd samples – collected from sediment baskets in artificial redds constructed in the River Itchen (see Chapter 3, section 3.5 for detailed methodology; Grieg, 2004) to provide representative samples of the type of organic material infiltrating the redd environment over the incubation period.
- Suspended sediment samples – collected using the material accumulated in the isokinetic samplers (see Chapter 4, section 4.3.3 for detailed methodology; Phillips et al. 2000) which collected suspended sediment over the salmonid embryo incubation period.
- Source samples – collected from top soil from various sites around the catchment that display connectivity with the river and thus the potential to be sources of fine sediment deposited in the River Itchen and hence salmonid redds. Source categories include in-stream material, pasture, arable and woodland (see Chapter 6, Figure 6.2 for detailed map of source distribution and source choice).

Samples were collected during the 2008 and 2009 field seasons. Site maps and details for both 2008 and 2009 field seasons can be found in Figure 3.7. Once collected, samples were brought back to the laboratory in a controlled environment in dark and cool containers. Samples were processed within 24 hours after collection from the field to ensure that organic material in the samples did not alter significantly. Every sample was wet sieved to <63µm to obtain the fraction most detrimental to salmon embryo survival and to standardise samples (Grieg et al 2005; section 4.2). After sieving, representative 50ml sub-samples were taken of the <63µm suspension for further laboratory analysis.
Chapter 7.3.1.2 Sample pre-processing

Two different sample preparation methods were used prior to collecting the fluorescence data as measuring organic components within redd and suspended sediments had not been attempted before to the author’s knowledge. The first method used on the 2008 samples involved measuring suspensions of field samples after wet sieving (<63µm) on the day they were obtained (method 1), whilst the second method used on soil source and river and redd sediments from the 2009 field season involved extracting organic material from the sediment using chemical reagents (method 2). Both methods are described below.

On return from the field, sediment samples were wet sieved (section 4.2) to <63µm and a 50ml sub-sample was removed after mixing the remaining bulk of suspended sample to ensure it was representative. The raw supernatant fluid overlying settled sediment samples after shaking for 30 minutes and settling for 2 hours was analysed using fluorescence spectroscopy (method 1). A potassium chloride (KCl) solution was used to strip the organic sediment from the inorganic sediment. Approximately 1g of dried sediment was added to 5ml of reagent (2M KCl) with approximately 1:5 weight/volume ratio and left on a mixing plate for 48 hours to allow adequate extraction of organic carbon (Jones and Willett 2006). After centrifugation (15 minutes at 1400 rpm), the supernatant liquid was removed from the sediment sample and allowed to reach room temperature before analysis by fluorescence spectroscopy (method 2).

The extraction method was used to ensure that no fluorophore signals were missed within fluorescence measurements of original field samples in 2008, as dilution with laboratory water occurred during the wet sieving process which may have diluted the natural fluorescence intensity of material and some signals might not have been picked up by the fluorimeter. Jones and Willett (2006) suggest that soil and sediment extracts can provide a comparative estimate of DOC and DON concentrations, although there is little difference between amino acids (tryptophan and tyrosine fluorophores) determined fluorometrically from soil solutions and using extracted material with reagents such as deionised water and potassium chloride (KCl).

Extraction methods are known to overestimate actual concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) compared with original field soil solutions. Drying and storing samples and extraction methods have the potential to alter the concentration of DOC and DON (Jones and Willet 2006). A 3-10 fold increase in extraction of DOC/DON has been observed in previous studies when
soil samples were air dried, compared with original field states (Jones and Willet 2006). However, as all samples were being dealt with in a methodical and standard way the extraction results proved a useful tool to compare organic components measured at different sites.

It is therefore worth noting that the fluorescence intensities and hence the actual concentrations reported of organic materials (fluorophores) derived using all methods should not be taken to be the actual concentration of organic material found at sites, but rather an indication of the composition of organic components and relative concentrations present between samples handled in the same manner. Other studies which used fluorescence to measure solid samples such as soil organic carbon and compost extracts use extraction methods (Ahmed et al. 2005; Jones and Willet 2006). The removal of inorganic solid material allows the organic components signal to be measured without interference from inorganic/organic complexes masking fluorophore intensity and hence actual concentrations (Cox et al. 2000; Ahmed et al. 2005).

### 7.3.1.3 Method comparison

Data collected using method 1 for the pre-processing of samples displayed low intensity fluorescence for fluorophores identified as shown in Table 6.4 in comparison with published data on freshwater DOM intensities for humic-like and protein-like fluorescence (Newson et al. 2001; Baker 2002; Hudson et al. 2008). This was thought to be due to inadvertent dilution of samples by the wet sieving procedure used to separate the fine sediment from larger grain size particles. In order to verify the peak locations identified in the diluted samples from 2008 and 2009 samples an alternative extraction method using KCl to strip organic constituents from inorganic material of dried samples was employed to ensure that no signals were missed.

A visual comparison of the EEMs of extracted samples and diluted samples appeared to display the same locations (Ex/Em (nm)) for the five fluorophores originally identified in the diluted samples with no additional signals found (A, C, B, T₁, and T₂). A comparison of samples showed that the extraction method produced greater magnitude in intensity values (in Raman units) than the diluted samples. On average the sum of fluorescence intensities were 32 times greater in the extracted samples than in the diluted samples. The variance described by the standard deviation was 11.91 and the minimum magnitude was 21.5 and maximum magnitude of 63.4. The variation in magnitude could be explained by the presence of different formations of
inorganic/organic complexes within samples which could lead to quenching of fluorophores.

In order to assess whether the proportion of specific fluorophores in samples differed between the two methods, samples were normalised by dividing a specific intensity value for one fluorophore by the sum of fluorophore intensities within that sample. Each sample’s sum of intensities was therefore now equal to 1. The difference between the two methods was examined by calculating the square root of the sum of squared differences in measured intensities in a sample. The mean difference in proportions equated to 0.04 with standard deviation equal to 0.01 and a minimum difference 0.03 ranging to a maximum difference of 0.07. These results indicate that there is a very small difference in the proportions of fluorophores found in the two methods.

7.3.2 Absorbance measurements

Prior to fluorescence analysis, absorbance was measured in order to check that samples were not too concentrated to negate primary and secondary inner filter effects (Ohno 2002). Most of the catchment source soil samples needed to be diluted and all of the samples from the extracted organics methods were diluted to remove any inner filter effects as they contained a high concentration of organic material and therefore decreased the clarity of the sample. Any samples with an absorbance reading over 0.3 at Abs(254) were diluted with Ultrapure milliQ water until the absorbance level was at Abs(254) was lower than 0.3 (Ohno 2002).

Absorbance measurements were taken using a Shimadzu UV1800 absorbance spectrophotometer. The range of absorbance measurements took into account the unknown quantity of organic material within the samples so the range measured was every 0.5 nm from 200 – 700 nm. Table 7.2 provides details of the instrument settings for all sample runs.
In Table 7.2, the Shimadzu UV1800 absorbance spectrophotometer instrument settings and chosen parameters are detailed.

### 7.3.3 Fluorescence measurements

Fluorescence measurements were made on a Varian Cary Eclipse fluorescence spectrophotometer with a xenon lamp. The fluorescence intensity of the samples was measured from 200 – 600 nm excitation wavelengths and 280 – 700 nm emission wavelength at an interval of 5 nm (Table 7.3). A full range of excitation and emission wavelengths were measured to take account of the fact that the samples were of an unknown mixture of organic material. Due to the large number of wavelengths explored, a scan speed of 24000 nm was used to allow for fast analysis of samples.

<table>
<thead>
<tr>
<th>Instrument settings</th>
<th>Specific parameter set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Scan mode</td>
<td>Emission</td>
</tr>
<tr>
<td>Excitation (nm)</td>
<td>200 – 600</td>
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<tr>
<td>Emission (nm)</td>
<td>280 – 700</td>
</tr>
<tr>
<td>Excitation slit width (nm)</td>
<td>5</td>
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<tr>
<td>Emission slit width (nm)</td>
<td>5</td>
</tr>
<tr>
<td>Sample increment (nm)</td>
<td>5</td>
</tr>
</tbody>
</table>
### Correcting EEM data

As previously stated EEMs need substantial correcting in order to make full use of the data and allow comparisons with other studies. Inner filter effects were accounted for by ensuring adequate dilution of samples. Intensity values at all excitation and emission spectra were multiplied by a sample specific dilution factor before being divided by the Raman peak to Raman normalise measurements and remove the Raman signal (Jiang et al. 2008) and finally by having a blank solute EEM subtracted from them. Figure 7.5 denotes a flow chart explaining the corrections applied to the data in this study and the order in which they were carried out.

![Flow diagram showing the process of EEM correction](image)

**Figure 7.5.** Describes the procedure of EEM correction applied to samples and the order in which corrections were carried out.

---

<table>
<thead>
<tr>
<th><strong>Scan speed</strong></th>
<th>24000 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Path length (cuvette cm^3)</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

Table 7.3. Varian Cary Eclipse fluorescence spectrophotometer instrument settings and chosen parameters.
7.3.4 PARAFAC modelling

PARAFAC analysis of fluorescence data was carried out in MatLab (v.R2011a) using the N-way toolbox (vr.1.6) created specifically for this purpose (Anderson and Bro 2002). Non-negativity constraints were applied to the model to ensure that only chemically relevant results were modelled (Marius et al. 2011). In order to achieve more accurate modelling results, greater than 20 samples were used in each run of the model (Stedmon and Bro 2008). Between 2 and 7 components were computed for each separate model run as there was no improvement in model residuals after 7 components (Figure 7.6 step 2). The different component models were then validated using split-half analyses, where the data were split and both halves modelled separately and then compared (Figure 7.6 step 3). Random initialisation was also used for validation purposes where a series of models were fitted using random numbers for the initial estimates and compared to ensure the least squares result is correct (Stedmon and Bro 2008). Figure 7.6 displays the main steps carried out in the modelling process. For further details see Stedmon and Bro (2008).

Figure 7.6. Describes the process steps followed in PARAFAC modelling as described in Stedmon and Bro (2008).
7.3.5 Supplementary organic data methods

Whilst fluorescence analysis was the main method used to explore organic composition of sediments and soils, two other laboratory methods; loss on ignition experiments and microscopy analysis of the percentage cover of macroflora/fauna sources were used to provide supplementary information concerning the organic nature of the samples. The methods followed to obtain this data are outlined below.

7.3.5.1 Loss on ignition method

Loss on ignition experiments provided percentage organic carbon, carbonate and organic matter data for each sample and were performed using a standard method (Lamb 2004). Briefly, ~1 gram of sediment was weighed into a pre-washed and dried ceramic crucible that was burned for 2 hours at 550°C, allowed to cool and then weighed again. This gave a measure of the organic content of the sample in per cent. Multiplying by 0.4 gave an estimate of the amount of organic carbon present in the sample (Lamb 2004). The sample is then burned for a further 4 hours at 950°C, cooled and re-weighed to measure carbon dioxide loss to allow percentage CO$_2$ to be calculated. This number was then multiplied by 1.36 to give percentage carbonate content of the sample (Lamb 2004).

7.3.5.2 Macro fauna and flora identification

Microscopy analysis was used to provide data on the amount of identifiable plant and/or animal matter in each sample. Microscope analysis of 50ml sub-samples taken from the material retained on the 63µm sieves for redd and suspended sediment samples was used to visually identify the type of organic material present within samples. This method was modified from standard methods pertaining to the identification of plant and invertebrate macrofossils (Barber 1984; Smol et al. 2001). A petri dish containing a grid was used to enable the estimation of % cover of different categories; inorganic material, invertebrate material, plant material (including wood), white space and unidentified material. To reduce bias, a mean of 15 repetitions of counts per sample was calculated. The identification of source materials was limited to fresh sources, i.e. those that have not been biodegraded beyond recognition and rules were created to identify plant and invertebrate matter. Materials that fitted into the following descriptions were therefore classed as invertebrate or plant matter;
- Invertebrate and insect material: generally has a glossy texture that can sometimes be opaque and also iridescent. Brown and honey coloured materials were also indicative of invertebrate source material
- Plant and wood material: often single cells are visible in plant materials (leaves, stems). Green coloured material indicated plant matter and woody materials, bark like materials

A reference crib sheet for estimating % cover for each category was used to enable standard estimations to be made between samples. This can be found in Figure 7.7.

![Figure 7.7. Percentage cover estimations for sediment samples to allow standard estimation of % cover for categories in microscope analysis of samples.](image-url)
7.4 Results

Three dimensional EEMs were created for all samples in R. R is an open source, statistical software program written in S-Plus language (Lapworth and Kinniburgh 2009). Recent advances in EEM analysis have led to the creation of useful scripts, in software such as R, that draw EEMs of many samples in a dataset and show results for intensity peak locations of pre-specified fluorophore locations (Lapworth and Kinniburgh 2009). Rayleigh-Tyndall scatter lines from light reflecting off particles in suspension, water molecules and the light reflecting off the sides of the cuvette in the fluorimeter can be removed via the application of a data mask.

Initial peak picking data analysis was carried out to identify peaks in redd and suspended sediment samples collected in 2008 field season and to analyse the measured organic content by site and sample type. These samples were pre-processed using method 1, where samples were sieved and the supernatant liquid above the solid sample was analysed. Typically these EEMs displayed peak locations in some or all of the previously specified locations for named peaks, A, C, B, T1 and T2 (Figure 7.4) found in aquatic ecosystems. Examples of redd and suspended sediment EEMs created are displayed in Figure 7.8.
Figure 7.8. Fluorophores typically present within redd and suspended sediment samples collected in 2008. 1) Arle redd sediment: circled peaks A and C, 2) Martyr Worthy redd sediment: circled peak T₁, 3) Ovington suspended sediment: circled peak T₂, and 4) Bishopstoke redd sediment: circled peak B.

7.4.1 Spatial variability in organic composition

The mean fluorescence intensities for peaks found in the 2008 redd and suspended sediment samples are shown in Table 7.4 below. In general the suspended sediments displayed weaker intensity peaks which implies lower fluorophore concentrations in suspended sediments than redd sediments, indicative of lower organic material concentration.
<table>
<thead>
<tr>
<th>Site name</th>
<th>Peak A</th>
<th>Peak C</th>
<th>Peak B</th>
<th>Peak T1</th>
<th>Peak T2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ex 240</td>
<td>Ex 330</td>
<td>Ex 280</td>
<td>Ex 280E</td>
<td>Ex 230</td>
</tr>
<tr>
<td></td>
<td>Em 400-420</td>
<td>Em 410-440</td>
<td>Em 294-302</td>
<td>Em 340-350</td>
<td>Em 340-350</td>
</tr>
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<td>Arle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
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<td>2.00</td>
<td>3.26</td>
<td>2.61</td>
<td>4.11</td>
</tr>
<tr>
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<td>1.26</td>
<td>1.41</td>
<td>0.92</td>
<td>1.29</td>
</tr>
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<td>Abbotstone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>4.88</td>
<td>7.18</td>
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<td>1.37</td>
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<td>Cheriton</td>
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<td></td>
<td></td>
</tr>
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<td>3.68</td>
<td>3.05</td>
<td>4.35</td>
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<td>1.64</td>
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<td>1.60</td>
</tr>
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<td>Ovington</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>4.89</td>
<td>1.74</td>
<td>2.48</td>
<td>2.13</td>
<td>3.19</td>
</tr>
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<td>1.77</td>
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<td>1.91</td>
</tr>
<tr>
<td>Martyr Worthy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>5.00</td>
<td>2.30</td>
<td>6.12</td>
<td>5.75</td>
<td>7.76</td>
</tr>
<tr>
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<td>1.34</td>
</tr>
<tr>
<td>Winchester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>5.26</td>
<td>2.31</td>
<td>3.01</td>
<td>2.61</td>
<td>3.34</td>
</tr>
<tr>
<td>Suspended sediment</td>
<td>3.19</td>
<td>1.61</td>
<td>2.17</td>
<td>2.04</td>
<td>3.87</td>
</tr>
<tr>
<td>Shawford House</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>4.48</td>
<td>2.45</td>
<td>3.89</td>
<td>3.33</td>
<td>5.33</td>
</tr>
<tr>
<td>Suspended sediment</td>
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<td>1.52</td>
<td>1.47</td>
<td>1.40</td>
<td>2.18</td>
</tr>
<tr>
<td>Bishopstoke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>4.25</td>
<td>2.07</td>
<td>2.92</td>
<td>2.50</td>
<td>3.89</td>
</tr>
<tr>
<td>Suspended sediment</td>
<td>3.84</td>
<td>2.01</td>
<td>1.79</td>
<td>1.70</td>
<td>2.65</td>
</tr>
<tr>
<td>Gaters Mill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redd sediment</td>
<td>5.66</td>
<td>2.79</td>
<td>4.56</td>
<td>3.48</td>
<td>5.94</td>
</tr>
<tr>
<td>Suspended sediment</td>
<td>4.23</td>
<td>2.43</td>
<td>2.81</td>
<td>2.00</td>
<td>2.95</td>
</tr>
</tbody>
</table>

Table 7.4. Mean fluorescence intensities (in R.U.) calculated using R software for five pre-specified fluorophore locations in redd and suspended fine sediment collected from the 2008 field period.
7.4.2 Suspended sediment samples

Spatial variations in organic composition of redd and suspended sediment samples were assessed using 2008 data (method (1)) from the end of the incubation period (hatch). Figure 7.9 describes the spatial variation observed in suspended sediment between sites of the major freshwater aquatic peaks; A, C, B, T₁ and T₂. It is apparent that sites located further downstream (Winchester, Shawford House, Bishopstoke and Gaters Mill) contain the greatest fluorescence intensity of all five fluorophores, indicating a greater amount of organic material available in suspended sediments at downstream sites. Peak A, which is described as humic/fulvic-like fluorescence, exhibits the greatest fluorescence intensity at all sites, apart from Winchester, where the protein-like T₂ peak has the highest intensity. This site is surrounded by grazing pasture for animals and areas upstream of the site are exposed to cattle poaching and potentially animal waste depositing above the site could explain the high protein-like fluorescence peak.

The furthest downstream site, Gaters Mill, is situated just downstream of Chickenhall Sewage Treatment Works, where treated sewage is discharged into the River Itchen. Previous research has suggested that untreated and treated sewage is often characterised by high concentrations of tryptophan-like fluorescence (Baker 2002; Hudson et al. 2007) and also higher levels of fulvic acid-like fluorescence (Baker 2002). Results presented here compare favourably with previous work, suggesting that the highest levels of protein-like fluorescence can be found in suspended sediments downstream from the large sewage treatment works, however concentrations of fulvic acid-like fluorescence are the dominant type of signal at this site.

Higher amounts of humic/fulvic-like fluorescence in river suspended samples than protein-like fluorescence echoes results from previous studies in other chalk rivers (Lapworth et al. 2009a) and studies characterising DOM in riverine systems (McKnight et al. 2001; Teymouri 2007). Teymouri (2007) commented that the larger amounts of humic and fulvic-like material in the Missouri river samples could be attributed to the high levels of suspended sediment possibly derived from terrestrial sources. Suspended sediment levels are particularly low in the River Itchen (Chapter 4, section 4.4.2) so a correlation between these two factors is unlikely. Preferential attenuation of labile organic matter, including proteins, is possibly a more likely cause for the greater presence of fulvic and humic-like fluorescence in suspended sediment samples as hypothesised by Lapworth et al. (2009).
Variation in the fluorescence intensity of fluorophores identified in suspended sediment samples at hatch incubation stage in the 2008 field season. Sites are ordered upstream to downstream, left to right. A = humic/fulvic-like acid, C = humic/fulvic-like acid, B = tyrosine-like and T₁ and T₂ = tryptophan-like.

7.4.3 Redd sediment

The general fluorescence intensities measured in redd sediment (Figure 7.10) are greater than those observed in suspended sediment samples. This could be due to a higher concentration of organic material being deposited in riverbed sediments compared to that remaining in suspended sediments. There is less evidence of increasing fluorescence intensity within redd sediments further downstream, unlike the trend displayed within suspended sediment (Figure 7.9).

The upstream site Abbotstone and mid-stream site Martyr Worthy exhibit high intensity peaks of tyrosine-like and tryptophan-like fluorophores indicating the presence of protein-like organic material within redds at these sites. The location of these two sites could potentially explain the higher intensity protein-like fluorescence which ultimately could be due to anthropogenic causes. The Abbotstone site was located in a field with cattle where there was no bank-side protection or vegetation to deter animals from...
entering the site, therefore animal waste could contribute to the high protein-like fluorescence. The Martyr Worthy site is located ~2km downstream from a large fish farm located near Itchen Abbass. Waste from the fish farm could have travelled downstream in higher flows and caused the slightly elevated protein-like fluorescence intensity found at this site. Interestingly this is not mirrored in the suspended sediment results for these sites.

Humic-like and fulvic-like acid fluorescence intensity does increase slightly from upstream sites to downstream sites; however in comparison to the protein-like fluorescence it stays reasonably constant in redd material. Protein-like fluorescence, particularly peak T, often exhibits the greatest intensity at sites in the catchment which contrasts with suspended sediments where a humic-like fluorescence peak (peak A) dominates the organic composition at sites. Protein-like fluorescence is indicative of bacterial activity and signifies the more labile and easily biodegradable organic material in rivers (Hudson et al. 2007). These results suggest that the more labile and therefore more rapidly consuming oxygen material appears in greater quantities in redd sediments than suspended sediments, potentially indicating that there is a difference between the sources of the composition of fine sediment found in redd and suspended sediment.

Figure 7.10. Spatial variation in the average fluorescence intensity of fluorophores identified in redds sediments (mean of two redds) at sites at hatch incubation stage during the 2008 field season. Sites are ordered upstream to downstream, left to right. A = humic/fulvic-like acid, C = humic/fulvic-like acid, B = tyrosine-like and T₁ and T₂ = tryptophan-like.
7.4.4 Catchment source samples

Figure 7.11 depicts the difference in fluorescence intensity of fluorophores measured in catchment source samples. Potential sample sources used for some of the below analysis were: pasture, arable, in-stream and also woodland sources. The organic composition of woodland soils was thought to be of interest as it could possibly elucidate the previous issues experienced within the sediment mixing model when including woodland as a potential source (Chapter 6, section 6.2.1). Pasture soils exhibited the greatest intensity of all fluorophores with the humic/fulvic-like fluorescence, peak A being the most intense. The other two terrestrial soil sources, arable and woodland, also had peak A (humic/fulvic-like) as the dominant fluorescence signal. The in-stream sources, collected from marginal silt beds and older river bed sediment deposits in the river, however showed that peak T₂ was the dominant fluorescent signal. All redd sediments, except two, displayed a similar fluorophore compositional trend to that shown by the in-stream source samples, whereas the suspended sediments exhibited similar profiles to the catchment soil sources.

Total percentage organic matter measured by loss on ignition for arable, in-stream, pasture and wood sources was 9.21, 10.79, 24.15 and 27.36% respectively, which highlights that fluorescence intensity does not measure the total composition of organic material as the fluorescence intensity measured does not correspond with total organic material measured.

Figure 7.11. Catchment sources mean fluorescence intensity of identified fluorophore peaks, A = humic/fulvic-like acid, C = humic/fulvic-like acid, B = tyrosine-like and T1
and T2 = tryptophan-like. Samples pre-processed using method (2) KCl extraction of dried samples.

7.4.5 Temporal variability in organic composition

Temporal trends in fluorescence intensity and composition was observed in redd sediments measured at different incubation stages during the 2009 field season. Fluorescence intensity of the sum of all fluorophores was greatest at the emergence stage at all four sites (Figure 7.12). At the Arle and Winchester sites, protein-like fluorescence intensity (peaks B, T1 and T2) show a significant increase at emergence relative to the other incubation stages and a smaller increase was also observed in the humic-like peak A. This indicates that the amount of organic material contained within redd sediments is greatest at the end of embryo incubation when fry start to emerge from the hyporheic environment. A comparison of the loss on ignition (LOI) data obtained for each incubation stage showed that in only one site, Ovington, was the greatest percentage total organic matter found at emergence, whilst for the other sites, hatch was the period that exhibited the greatest amount of organic material (Figure 13).

Figure 7.12. Bar chart showing the proportion of fluorophores present within redd sediments over different incubation stages at different sites. Peaks A and C indicating humic/fulvic-like organic compounds and B, T1 and T2 representing protein-like material.
7.4.6 Fluorescence data and other organic parameters

Table 7.5 displays data on other organic parameters, including the amount of invertebrate and plant material present in samples. Suspended sediment samples exhibited a greater percentage total organic matter than redd samples which contrasts with fluorescence data where suspended samples displayed less fluorescence intensity than redd material, indicating lower organic constituents were present. Total organic matter is a measure of all organic compounds within samples, so it takes into account not just fluorescent organic material but every organic compound present within a sample. This would therefore explain the discrepancy between the two sets of results. Based on the premise that different fluorophores relate to different groups of compounds within organic matter, scatter plots were drawn to explore whether it was possible to relate any one particular peak to invertebrate material, plant material, % organic material and % organic carbon present in samples.
<table>
<thead>
<tr>
<th>Site</th>
<th>Type</th>
<th>%organic Matter</th>
<th>%organic Carbon</th>
<th>%invert</th>
<th>%plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arle</td>
<td>redd sediment</td>
<td>16.58</td>
<td>6.63</td>
<td>0.67</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>suspended sediment</td>
<td>27.91</td>
<td>11.16</td>
<td></td>
<td></td>
</tr>
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<td>Abbotstone</td>
<td>redd sediment</td>
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<td>10.45</td>
<td>1.13</td>
<td>1.27</td>
</tr>
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<td>suspended sediment</td>
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<td>16.57</td>
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<td>10.76</td>
<td>0.87</td>
<td>0.93</td>
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<td>suspended sediment</td>
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<td></td>
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<td>Martyr Worthy</td>
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<td>6.49</td>
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<td>*</td>
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<td>12.19</td>
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</tr>
<tr>
<td></td>
<td>suspended sediment</td>
<td>30.83</td>
<td>12.33</td>
<td></td>
<td></td>
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<tr>
<td>Shawford House</td>
<td>redd sediment</td>
<td>18.40</td>
<td>7.36</td>
<td>0.80</td>
<td>1.17</td>
</tr>
<tr>
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<td>suspended sediment</td>
<td>35.47</td>
<td>14.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bishoptoke</td>
<td>redd sediment</td>
<td>19.89</td>
<td>7.96</td>
<td>0.77</td>
<td>1.23</td>
</tr>
<tr>
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<td>10.35</td>
<td></td>
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<td>Gaters Mill</td>
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<td>6.99</td>
<td>0.40</td>
<td>1.60</td>
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<tr>
<td></td>
<td>suspended sediment</td>
<td>30.55</td>
<td>12.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.5. Supplementary organic material data for redd sediment and suspended sites in 2008. (* denotes missing data) Note the large percentage of plant material for the Ovington site was due to the presence of a large proportion of woody debris.

There was little relationship displayed between fluorescence intensity of fluorophores in redd and suspended samples and % organic matter and organic carbon. Figure 7.14 describes the lack of relationship between total percentage organic matter and all five fluorophores in suspended sediment. A slight decline in measured fluorescence intensity can be noted with increasing organic matter. This suggests that fluorescence data does not fully describe all of the organic material found in samples.
Figure 7.14. The lack of relationship between fluorophore intensity and percentage organic matter in suspended sediments in samples collected during the 2008 field season.

The relationship between the amount of plant and invertebrate material found in samples was investigated by means of scatter plots. Figure 7.15 presents the linear relationships found between humic-like (a) and protein-like (b) fluorescence and plant and invertebrate material. Humic/fulvic-like fluorescence displays a correlation ($R^2 = 0.45$, $p = 0.01$) with plant material, but no correlation with invertebrate material ($R^2 = 0.03$, $p = 0.01$). Protein-like fluorescence displays a weak correlation with invertebrate material ($R^2 = 0.21$, $p = 0.01$) and no relationship with plant material ($R^2 = 0.06$, $p = 0.01$). Despite the small number of points, the implication of these results is that fulvic/humic-like fluorescence is more indicative of plant origins on the River Itchen, rather than animal origins and that protein-like fluorescence denotes animal origins, specifically invertebrate material.
7.4.7 PARAFAC results

PARAFAC modelling provided a tool to statistically assess the underlying composition of organic material present in sediment and soil samples from the river and catchment, using the raw fluorescence data for each sample. This method allowed statistical verification of peak locations observed within samples, rather than visually peak picking or using pre-specified fluorophore location analysis methods like the former R-script. Samples were split into working groups; suspended sediment, redd sediment and source sediment to model the fluorescent organic components found within each
type of sample. The following results detail the validated model components arrived at for catchment source and redd sediment data.

The model to assess the underlying fluorophores exhibited in suspended sediment data was not validated, possibly due to the low number of samples available (~30) as PARAFAC has been reported to model data better with larger sample sizes (Stedmon and Bro 2008).

All modelled components resembled general fluorescence characteristics of organic fluorophores which have overlapping excitation and emission loadings and often multiple excitation peaks and single emission peaks (Stedmon and Markager 2005). The components modelled should be regarded as representative of groups of fluorophores not one specific fluorophore (e.g. rhodamine-B) as organic matter samples collected are very complex (Stedmon and Markager 2005). It should be noted that the number of components modelled may not describe all of the fluorescent compounds present in every sample, rather they represent the fluorophores that were present in the majority of samples and could be modelled and validated, explaining the majority of variation between samples (Stedmon et al. 2003)

7.4.7.1 Redd sediment PARAFAC results

All redd data was included in the model as no obvious outliers were identified in the first outlier inspection of the data. Between three and five components was thought to model the data best, as there was little improvement in the sum of squared errors with a greater number of components. The three component model could be split half validated in both sets of data, meaning that when the data was randomly split in half and modelled separately, the same components were modelled in both datasets (Stedmon and Bro 2008). This proved to be the only statistically validated model so this was accepted as the most robust result.

Component 1 is composed of a double excitation peak with one emission peak at the excitation/emission wavelength pair for the principle peak at 240/440nm and a secondary peak at 310/440nm (Figure 7.16). Component 2 similarly comprises a double excitation peak with one emission maxima; first peak at 225/410nm and the second peak at 280/410mn (Figure 7.17). Component 3 displays a peak at the excitation/emission wavelength pair of 275/295nm (Figure 7.18).
When comparing the three components with fluorophore peaks observed in previous literature (Table 7.1), the components display very similar locations to those found in aquatic ecosystems. Component 1 with its primary and secondary excitation maxima is similar to peaks A and C respectively which were observed by Coble et al. (1996) and Mayar et al. (1999) and are described as humic and fulvic like substances. The primary peak of component one is also similar to PARAFAC modelled data, component 4 (UVA humic) which is described in Stedmon et al. (2005) as terrestrially derived from autochthonous sources and primarily comprised of fulvic acids which are present in all environments.

Component 2 shows similarities with the location of peak A (humic/fulvic-like acids) and also with component 3 (UVC humic) found in Stedmon et al. (2005) which was described as originating from terrestrial sources and naturally occurring in dissolved organic matter as it is absent from wastewater dissolved organic matter. Component 3 could possibly be compared with peak B (Coble et al. 1996) which is described as tyrosine-like and protein based, although the emission wavelength (295nm) is slightly lower than that reported in the literature (309-321nm). Based on its proximity to peak B it can be hypothesised that its origin is likely to be derived from protein-like fluorescence. Stedmon et al. (2005) measured component 8 (UVB protein-like) with similar excitation/emission wavelengths as this studies component 3. They measured 275/304 nm and described the component tyrosine-like fluorescence, almost identical to free tyrosine proteins and as originating from autochthonous sources (Stedmon et al. 2005). It should be noted that there appears to be a reasonably high peak occurring just after the main peak in the excitation loadings for component 3 which possibly suggests that there is another component that hasn’t been modelled by PARAFAC (Figure 7.18). This is most likely due to low numbers of samples.
Figure 7.16. EEM of modelled component 1 found in accumulated redd sediments on the River Itchen.
Figure 7.17. Describes the EEM of modelled component 2 found in accumulated redd sediments on the River Itchen.
Figure 7.18. Describes the EEM of modelled component 3 found in accumulated redd sediments on the River Itchen.
A comparison of PARAFAC derived fluorescence intensity scores, which are relative to the amount of that particular component in a sample of the three components, revealed that there is little difference in organic components between samples from upstream and downstream sites as they both plot in similar places on the graphs (Figure 7.19).

Figure 7.19. Scatter plots of comparisons between component scores of upstream and downstream redd sediment samples on the River Itchen.

However due to the low number of samples, some slight differences can be seen which are described. Some of the emergence samples display higher scores for component 2 in the (Figure 7.19) upstream sites, similar to the temporal trends displayed in Figure 7.12 and a slightly anomalous point describes the Cheriton stream which appears to contain particularly high scores for components 1 and 2 (Figure 7.19, no. 1 and 2). The Bishopstoke site, at eyeing stage, contains higher levels of component 3 (Figure 7.19, no. 3).

7.4.7.2 Source soil PARAFAC model

All sources were modelled together to see if there were any similarities in underlying fluorophores in all source categories. In the initial explorative analysis and outlier
testing of the data, no samples were removed based on the loading and leverage plots drawn as no samples displayed erroneous spectra or measurement error so the final model was based on all source samples fluorescence data. Non-negative constraints were applied to the model. A three component model could be split half validated on both sets of results, giving a robust three component model for soil source materials found on the River Itchen.

Component 1 comprised a peak at the excitation/emission wavelength pair of 260/465 nm (Figure 7.20). Component 2 displayed a double excitation maxima with one emission peak, with the first principal peak at 230/415 nm and the second peak at 280/415 nm (Figure 7.21). Component 3 again described a double excitation maxima with a single emission peak; the first peak at 225/340 nm and the second peak at 280/340 nm (Figure 7.22).

In comparison with previous research, component 1 and 2 fit within the range of wavelengths measured for peak A, typifying humic/fulvic-like acids (Coble et al. 1996). The principle peak measured in component 2 is similar to the peak signal measured in component 3 (UVC humic) from Stedmon et al. (2005), describing humic-like fluorescence present in natural dissolved organic matter in aquatic systems, originating most likely from terrestrial sources. Component 3 displays similar peak locations as two published peaks, $T_1$ and $T_2$, which describe tryptophan-like or protein-like fluorescence (Coble et al. 1996; Baker 2001). The primary peak found in component three is in the similar region to peak $T_1$, and the secondary peak is in the exact location of peak $T_2$. 
Figure 7.20. Component 1 EEM and spectral loadings for soil source material on the River Itchen.
Component 2 EEM and spectral loadings for soil source material on the River Itchen.

Figure 7.21. Component 2 EEM and spectral loadings for soil source material on the River Itchen.
Figure 7.22. Component 3 EEM and spectral loadings for soil source material on the River Itchen.
Comparative analysis to see if there was any possibility of differentiating between the organic compositions of sources was used on the loading scores of the modelled PARAFAC components within each sample of the catchment source groups. Scatter plots were drawn to compare scores from each component to see whether the sources appear in different areas on the plot (Figure 7.23). It is clear that in-stream sources are quite different from the terrestrial soil sources, particularly in Figure 7.23 b) and c). Pasture and wood sources display very similar fluorescence so it may not be possible to differentiate between these two sources, but arable samples mostly occur in a different space to pasture and woodland sources.

Figure 7.23. Scatter plots comparing the scores of PARAFAC components from catchment source groups.

Most of the arable sources are clustered near to the origin with low scores, suggesting low levels of fluorescent materials being present in samples. Cultivated land is particularly vulnerable to leaching of nutrients and natural organic material due to mechanical disturbance and harvesting crops. The lack of differentiation between pasture and woodland organic components is possibly the cause of the difficulty the sediment mixing model had in accurately apportioning sources to redd sediment samples when woodland was included as a source within the model. The vast majority
of woodland in the catchment is found in small pockets, between areas of rough and improved pasture. Differentiating between in-stream, arable and pasture/woodland catchment sources by comparing PARAFAC modelled components in this manner is therefore practicable as shown by these results.

7.4.7.3 Comparison of redd and source PARAFAC results

The excitation-emission coordinates for the modelled components of redd and catchment source organic material is displayed in Table 7.6. It is clear that both types of sample contain humic/fulvic-like fluorescence and protein-like fluorescence. There are some differences displayed. Redd sediment samples exhibit shorter emission wavelength humic-like fluorescence as well as the lower wavelengths. The protein-like fluorescence also differs. Soil humic substances have been reported to exhibit longer wavelength emission maxima than river humic substances (Chen et al. 2003). This is due to the larger organic macromolecules present in soil complexes compared with lower weight molecules found in rivers (Chen et al. 2003). The two emission peak maxima for peaks A and C displayed by the catchment soil sources in table 6 is exactly the same as described for soil humic acids by Chen et al. (2003).

Catchment source samples exhibit tryptophan-like protein fluorescence and redd sediment exhibits tyrosine-like protein fluorescence. Mayer et al. (1999) describe some correlation between chlorophyll and tyrosine-like fluorescence in river water and disturbed bed sediments proved to be a source of protein-like fluorescence in their experiments and similar to the results displayed here. The tyrosine-like fluorescence displayed in redd sediments could therefore be originating from aquatic plants breaking down. This source of labile protein-like fluorescence would not be exhibited in catchment sources where there is less free chlorophyll compared with river systems like the River Itchen which have abundant macrophytes.
Table 7.6. Describes the locations of modelled PARAFAC components in redd and source material. Brackets denote secondary maxima. Suffix R = redd organic material and suffix S = catchment source organic material.

### 7.4.8 Organic sediment fingerprinting

Inorganic sediment fingerprinting methods and models were used to ascertain information pertaining to the source of fine material accumulating within salmonid redds (Chapter 6). In order to explore the provenance of the organic sediment within spawning gravels, the same method was applied using the catchment source categories: arable, pasture and in-stream and a number of the different organic parameters measured. Sediment fingerprinting technology allows fine sediments to be differentiated back to their original sources using discrete natural tracers such as radio-nucleides (Walling and Woodward 1992), geochemical (Russell et al. 2001) and mineral magnetic properties (Jenkins et al. 2002). These are then statistically derived to provide the best number of tracers able to distinguish between sources and modelled against fine sediment using mixing models (Collins et al. 1998).

Organic material is known for its changeable, ubiquitous nature in freshwater (Komada et al. 2002) which raises questions regarding the suitability of organic parameters in tracing fine sediment sources. Transformations and biodegradation which occur within terrestrial and aquatic environments suggest that these parameters are not discrete which could mean that direct source fingerprinting could prove difficult (Marius et al. 2011). However fluorescence data has been used in a number of previous studies to trace DOM in rivers, namely to trace microbial and terrestrially derived fulvic acids through alpine catchments in the USA (McKnight et al. 2001, 2003) and within an assay.
of parameters to trace dissolved organic matter in six catchments with contrasting land use in North Wales (Thoss et al. 2000).

Fluorescence data has also been used to distinguish between organic material in seasonal run-off pathways in a catchment with two different soil types (Newson et al. 2001). A close relationship was displayed between fluorescence and organic loads entering the river, with peaty-gley soils displaying higher fulvic acid fluorescence (Newson et al. 2001). Therefore it was hypothesised that there may be the potential, with large enough sample sizes, to test whether it is possible to differentiate organic catchment sources of fine sediment found in redd sediment, using parameters such as fluorescence peaks, percentage organic material and organic carbon, within the context of the traditional inorganic sediment fingerprinting method. The first step was to determine whether it was possible to differentiate between catchment sources using organic parameters and based on this outcome, whether redd and suspended sediment samples could be modelled with catchment sources to find its origins.

7.4.9 Fingerprinting procedure

The fluorescence data used in this study was measured from the KCl extracted eluent (pre-processing method (2)) from soil and sediment samples and the peak intensities are those generated from analysis in R. The organic fingerprint properties chosen to test were: mean intensity fluorescence for peaks, A, B, C, T₁, and T₂, intensity ratios of peaks T₁:C and T₂:A, the fluorescence index (McKnight et al. 2001), total percentage organic matter, percentage organic carbon and percentage carbonate. As in section 5.2, definition that organic parameters for redd and suspended sediment samples lay within the range of catchment source parameters was essential to identify if any sources had been missed (Collins et al. 1998). Range tests compared the minimum and maximum values within source and sediment parameters and confirmed that the sediment samples fitted within the range of combined sources (section 6.2.5). The fluorescence index was the only category that did not fulfil the range test criteria so it was removed from further analysis.

The Kruskal-Wallis H test was used to statistically verify whether a specific organic fingerprint property could successfully differentiate between the three source types (pasture, arable and in-stream – see chapter 5 for source choice). Properties that could not discriminate between sources were removed from further analysis (Collins et al. 1997). Discriminant function analysis (DFA) was then employed to further test the classification power of each fingerprint property and to statistically determine the least
number of properties needed to successfully discriminate between sources (Collins et al. 1997, 1998; Collins and Walling 2007b). Finally data was input into the sediment mixing model (for full description see Ch.6, section 6.2.6) and using particle size correction factors that can be found in table 6.2 (Ch.6, section 6.2.5) and calculating tracer specific weightings (Collins et al. 1997), a Monte Carlo approach was used to quantify uncertainty within the model results for the apportionment of sources (Collins and Walling 2007a).

7.4.9.1 Results

The results of the Kruskal-Wallis H test found that all but three organic properties could discriminate between source categories. The three properties that could not distinguish between sources; $T_1$ : C, $T_2$ : A and % organic carbon, were removed and the remaining eight properties were put forward for discriminant function analysis. A first attempt using the stepwise method found that three fingerprint properties (C, B and $T_1$) could distinguish between sources (Figure 7.24) and 85.7% of cases were classified, with a cross validation classification of 75.0% (Table 7.7). Cross-validation classification removes one sample at a time and tries to re-classify that sample using the remaining samples’ functions (Fowler et al. 1998). Cross-validation tests the accuracy of sample classification using the functions generated so maximising this is important.

![Figure 7.24. Combined group classification of organic parameters of source material entered with a step-wise method.](image-url)
Table 7.7. Corresponding original and cross-validation classification results for Figure 7.24.

The sources appeared close to each other so there was not as good separation as was found with inorganic properties (Table 7.7) which was not found in the DFA carried out for inorganic sediment sources. The objective of the discriminant function analysis was to define the smallest number of parameters that could easily distinguish between the different sources. This is important to minimise error and create a less complex sediment mixing model. Different combinations and numbers of fingerprint properties were analysed to maximise the classification and cross-validation of source categories. There was no improvement by entering parameters independently for original classification and cross-validation classification so the three parameters taken forward to discriminate between sources of redd and suspended sediment in the sediment mixing model were peaks C, B and T₁.

The mixing model results are based on Monte Carlo simulations run using random numbers calculated from the mean and standard deviation for each fingerprint property within each source category (Collins and Walling 2007). The percentage of each source category found within averaged suspended and redd sediment samples are presented in Figure 7.25 and the descriptive statistics including 95% confidence intervals are displayed in Table 7.8.
Figure 7.25. Source contributions from the four source categories in a) average redd sediment at sites collected in 2008 and 2009 field seasons and b) average suspended sediment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Redd sediment</th>
<th>Suspended sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ (%)</td>
<td>$\sigma$</td>
</tr>
<tr>
<td>Arable</td>
<td>16</td>
<td>36.6</td>
</tr>
<tr>
<td>In-stream</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>Pasture</td>
<td>81</td>
<td>39.3</td>
</tr>
</tbody>
</table>

Table 7.8. Descriptive statistics of source contributions to suspended and redd sediment average samples. Percentage mean ($\bar{x}$ (%)), standard deviation ($\sigma$) and 95% confidence intervals ($\alpha$ (95%)).

The results from the mixing model display very similar findings to the source proportions of inorganic sediment in the River Itchen reported in Chapter 6, where pasture was found to be the greatest source contributing to fine sediment accumulated in redd and suspended sediments. Arable sources display less prominence than shown by the inorganic fingerprinting, which could be explained by the proximity of arable to pasture sources found in the DFA. In-stream sources were found to contribute the least to accumulated redd and suspended sediment. These results indicate that sources on the River Itchen exhibit discrete organic properties in addition to discrete inorganic properties. Woodland was not included as a source within the mixing model as it was not included in the inorganic results and PARAFAC analysis comparison of component
scores showed that woodland samples were not easily distinguishable from pasture samples.

These results display a first attempt at looking purely at organic parameters to attribute redd and suspended sediment back to their original sources which have returned similar results from inorganic parameters modelled on the same data. It tentatively suggests that despite the complex nature of organic material within natural systems, there is potential for using a composite suite of fingerprint properties which includes fluorescence intensity peaks to explore the provenance of fine sediment in aquatic systems.
7.5 Discussion and implications

The composition of infiltrated organic fine sediment into salmonid redds and the relation to suspended sediment and catchment source material has been explored using fluorescence data and other organic parameters. Humic/fulvic-like and protein-like fluorescence were the organic signatures observed in all types of samples. Variability existed between the fluorescence intensity displayed by fluorophore groups in accumulated fine sediments in redds, suspended sediment and catchment sources, within sample types, spatially throughout the catchment and temporally over the incubation period.

Suspended sediment samples collected from surface waters next to artificial redd sites showed that humic/fulvic-like fluorescence has the greatest intensity signal. Previous studies have stated that humic/fulvic-like fluorescence (peaks A and C) dominate river DOM (Katsuyama and Nobuhito 2002; Hudson et al 2007). The most common origin of these compounds in rivers has been reported to be from the breakdown of plant material in water, riparian zones and terrestrial soils by some researchers (Stedmon et al. 2003; Hudson et al. 2007) whilst others have speculated that the main source is from the breakdown of plant material in terrestrial soils (Senesi et al. 1991 reported in Newson et al. 2001) which has then been delivered to the river via processes such as run-off during rain events. Results from this study show that a correlation exists between peak A (fulvic-like) and to a lesser degree peak C (humic/fulvic-like) with plant material observed within samples. This suggests there is evidence for humic-like substances originating from the biodegradation of plants. Further research using larger sample sizes would allow greater exploration of this relationship and indeed more research on the differentiation between specific fluorescent signals exhibited by in-stream and catchment humic-like substances would lead to better discrimination of the sources of organic components within river DOM.

In contrast to the dominance of humic-like fluorescence in suspended sediment samples, redd sediment was mostly dominated by protein-like fluorescence. Conversely to the results presented here, Lapworth et al. (2009) concluded that tryptophan protein-like fluorescence in DOM exhibited lower intensity in river gravels than in river water and was being favourably removed by biodegradation processes in gravels 0-0.5m below the riverbed. The origin of protein-like fluorescence has been attributed by some as largely coming from bacterial communities within ecosystems (Elliot et al. 2006; Hudson et al. 2007), however others have postulated that protein-
like fluorescence can be derived from both floral and faunal organic material (Newson et al. 2001). Baker (2001), (2004) and Hudson et al. (2008) suggested that protein-like fluorescence correlates well with BOD (0.8 for peak $T_2$ and 0.9 for peak $T_1$) indicating that protein-like fluorescence is related to the labile and reactive compounds of organic material that readily consumes oxygen. Most literature suggests that protein-like fluorescence indicates the most labile and easily degradable organic material in natural systems (Coble 1996; Baker 2002, Hudson et al. 2007). High intensity tryptophan fluorescence has been attributed to diffuse pollutants such as farm waste material and sewage treatment effluent (Baker 2001). There is little evidence within this study that sewage treatment effluent creates greater tryptophan-like fluorescence, however greater fluorescence as a whole was observed at Gaters Mill, the furthest downstream site that is situated very near to Chickenhall sewage treatment works (STW). Further work, including monitoring sites downstream of other STW on the River Itchen, could allow for greater comparisons to be made with river reaches that do not contain discharges and STW-impacted reaches.

The presence of protein-like material in redd sediments could therefore point towards the presence of bacterial communities and or the presence of anthropogenic diffuse pollutants collecting in redd sediments over the spawning season. However further research is needed to understand fully the sources of fluorophores, as protein fluorescence occurs naturally in organic material, such as organism cell walls and free amino acids released from the breakdown of proteins (Hudson et al. 2007). The intensity of protein-like fluorescence in redd sediments was not as high as has been reported for heavily polluted rivers (Baker 2001; Hudson et al. 2008), which could partly be due to differences in pre-processing methods, but also could indicate that there was evidence of bacterial communities present at hatch time.

Lapworth et al. (2009) found that humic/fulvic-like fluorescence intensity decreased with depth (>0.5m) in the hyporheic zone in a chalk catchment and whilst the colloidal and particulate DOM fluorescence intensity of humic/fulvic-like and protein-like fluorescence decreased with depth in the hyporheic zone, dissolved organic matter fluorescence did not change with depth. These findings suggest that the larger size fraction organic matter is preferentially held in river gravels in or near to the bed surface, suggesting that in redds larger organic particles will accumulate in the top layers of redds.

This has implications for directly blocking pore spaces through the growth of biofilms (Petticrew and Arocena 2003) and decreasing oxygen available to salmonid embryos by the reducing intra-gravel flow. Transformations, such as adsorption to clay particles
and biodegradation by aerobic microbes (Lapworth et al. 2009), will consume dissolved oxygen from the overlying water in the upper layers of the redd. Redd morphology forces surface water into the egg pocket section of redds (pot) (Sear et al. 2008). This could potentially lead to organic material, most likely in the dissolved size fraction based on evidence above, collecting around incubating eggs and utilising available oxygen.

Fluorescence data, organic material and organic carbon data did not correlate well. This is similar to a previous study that found little or no correlation between fluorescence parameters and dissolved organic carbon (DOC) (Jaffe et al. 2008) which point to the fact that not all organic material fluoresces and there are unknown components of organic material in natural systems. The bulk of DOM in natural systems remains uncharacterised, with estimations that 50-70% is composed of humic-like substances (Baker et al. 2004). Research is needed to further the knowledge of organic material generally and within specific niches, such as accumulating in salmonid redds.

The 2009 temporal results show that over the incubation period the intensity of fluorescence signal increases from eyeing to emergence at all sites which is indicative of accumulating organic material over time. This corresponds with the accumulation of fine sediment (<63µm) over the period (Chapter 4, section 4.4.1.3), but is not backed up by % total organic material. The incongruousness of % organic material to relate to fluorescence results indicates that different methods characterise different types of organic material in natural systems.

The use of PARAFAC modelling to identify organic components within redd, suspended sediment and source samples proved successful. Those components identified fitted with the peaks identified in the R analysis. PARAFAC also proved a useful tool to decompose the fluorescence signal in salmonid spawning gravels. There is potential also for using PARAFAC component scores to differentiate between catchment source materials, particularly in-stream, arable and pasture sources. Marius et al. (2011) found that PARAFAC component combinations can be indicative of one particular source of organic material, however large datasets and further work is needed before success in discriminating between specific sources (e.g. land-use types and vegetation types) is achieved.

The sediment mixing model using fluorescence data and other organic parameters to source the provenance of catchment sources in suspended sediment and redd sediment gave similar answers to the sources of inorganic sediments (Chapter 6
Differentiation between pasture and woodland sources proved to be difficult from the PARAFAC component comparisons of source samples. Results should be reviewed conservatively as sample sizes were reasonably small.

Evidence suggests that increasing concentrations of DOC in rivers and lakes in the UK could be being caused by increasing temperatures and that other factors such as land-use change and eutrophication may be accentuating the increase (Worrall et al. 2004). There is potential then for organic compounds to increase in rivers based on the current temperature rise and climate forecast models could play a role in predicting the magnitude of the increase in organic material in rivers.
Chapter 8. Final discussion and summary

The results and discussions provided in the preceding chapters have provided an insight into the sources and impacts of inorganic and organic fine sediment accumulating in salmonid spawning habitat in the River Itchen specifically and provided a baseline for spawning habitat quality in chalk streams generally. Preliminary data collected from field studies allowed characterisation of the sedimentation of spawning habitats on the River Itchen which provided the basis for further laboratory and field studies pertaining to the source and composition of inorganic and organic sediments accumulating within the redd environment.

8.1 Summary of main findings

A summary of the main findings of this research is presented here, under the headings of the specific research objectives laid out at the beginning of the study (Chapter 1, section 1.3).

Objective 1. To characterise spawning habitat quality on the River Itchen over different spatial and temporal scales during the incubation period

In order to investigate the source and characteristic of accumulated sediment in spawning gravels, it was first pertinent to characterise the sedimentation problem on the Itchen in a whole catchment wide investigation of spawning habitat. Chapter four details the results of this study which are briefly summarised here;

- <63µm fine sediment is the most important size fraction in terms of redd accumulation over the incubation period, in comparison with larger fine sediment (<4mm,<2mm)
- Fine sediment (<63µm) affects intra-gravel flow velocity and to a lesser extent dissolved oxygen levels within redds
- Large amounts of organic material found in accumulating fine sediment (<63µm) often greater than 20%
- Groundwater upwelling decreases dissolved oxygen levels in the hyporheic zone in the upper catchment
• Higher survival of embryos was recorded in the upper catchment in comparison with the lower catchment
• Sediment accumulation rates were low (0.00007-0.00149 kg m\(^{-2}\) day\(^{-1}\)) during the incubation period, original levels of fines sediment in gravels were particularly high with an average of 15% for <1mm and 3.6% for <63µm.

These results agree with the few previous studies on chalk streams where spawning habitats exhibit these features; containing high amounts of organic material (>20%), <63µm fine sediment impeding intra-gravel flow and permeability of the redd and lower accumulation rates in comparison to other river types (Acornley and Sear 1999; Greig 2004; Heywood and Walling 2007). Due to the high level of fine sediment (<2mm) found in chalk stream river beds and the stable nature of flow regimes, the sensitivity of spawning gravels to additional deposition is far greater than that experienced in upland and freshet streams (Milan et al. 2000; Greig et al. 2005a; Sear et al. 2008) which has significant implications for the successful incubation of salmon in chalk streams. The high organic composition of the clay and silt fraction blocking pore spaces in salmonid redds increases the likelihood of low dissolved oxygen levels becoming a major limiting factor in spawning success. The results from this study indicate that fine sediment should be redefined as the clay-silt range (<63µm) of particle size for chalk streams and rivers as this size appears to affect intra-gravel flow and dissolved oxygen in redds more than larger fine sediment.

Due to limitations within the study a relationship between the survival of eggs and sediment accumulation could not be drawn. However combining the River Itchen data with other chalk stream data showed that the River Itchen catchment was within the range of other chalk streams and below the threshold proposed by Heywood and Walling (2007) of 8% (<1mm) fine sediment (Figure 8.1). Notice the gradient of the linear regression for chalk streams, indicating the sensitivity of the relationship.
Figure 8.1. Meta data for studies looking at the relationship between fine sediment (<1mm) and salmonid survival (%). Itchen data is characterised by the red points, other chalk rivers are black points and other river types are white points. Data source: O’Connor and Andrew (1998), Greig (2004), Julien and Bergeron (2006), Heywood and Walling (2007).

From the results of this study it is shown that fine sediment accumulates in spawning redds and although direct links between survival and accumulation were not attempted, groundwater influence and the hypothesis that high organic content could influence survival is still the most likely as accumulation rates were low and the finest fraction (<63µm) which is known to settle in redds from the bottom up (Frostick et al 1984) contains the highest content of organic matter. Therefore further investigation into the sediment composition, sediment oxygen demand and source of this organic material was further investigated in this research. Characterising spawning habitat showed the importance of catchment-wide investigations as spatial and temporal variation was present along the catchment and different factors had the potential to affect egg survival at different sites (Arle: groundwater upwelling; Bishopstoke: organic content of sediment). This study also highlighted the importance of measuring data at different resolutions, as large scale, low resolution data was collected from standpipe measurements, but fine scale resolution data provided in-depth data at a small number of sites, which highlighted groundwater upwelling as a factor on the river more effectively than the low resolution data could.
Objective 2. To assess the impact of sediment oxygen demand (SOD) of organic sediments on the availability of oxygen within the interstitial environment and the impact on oxygen consumption by incubating salmon embryos.

This objective was related to the oxygen consuming capacity of the organic sediments found within spawning gravels. The reduction of intra-gravel oxygen by labile organic material is hypothesised to be one of the major oxygen sinks influencing survival of salmonid embryos on chalk streams (Grieg et al. 2005a). A summary of the main findings include:

- Sediment oxygen consumption rates vary from 0.2-4.5 mg O₂ g⁻¹ day⁻¹
- <63µm fine sediment exerted the greatest oxygen consumption rate and subsequently sediment oxygen demand in comparison to other particle sizes
- SOC rate is weakly correlated with % organic matter in sediments at different incubation stages
- Total SOD makes little or no difference to the existing egg survival model outcomes
- SOC rates are of an order of magnitude larger than egg oxygen consumption rates displayed within the literature
- A number of factors that influence oxygen availability within the redd such as intra-gravel flow, temperature and dissolved oxygen concentration need to be accounted for in association with SOC/SOD before conclusive results can be obtained
- The SIDO-UK model is unsuited to catchments that experience low fine sediment accumulation rates. SOC will consequently have no impact to egg survival in the current modelling domain.

The experimental ex-situ method proved reliable in terms of repetitions and produced results of the consumption of oxygen by accumulated redd sediments similar to those found in laboratory study looking at riverine sediment oxygen consumption (House et al. 2003). It should be noted that it is particularly difficult to compare these results with other studies due to the lack of standard methodology in the literature regarding sediment oxygen consumption and sediment oxygen demand. The silt and clay range of sediment exhibited the greatest oxygen demand, which was expected based on the total organic matter content of the finer material; however there was only a weak correlation found between % organic matter and sediment oxygen consumption rate. This could be partly due to the small number of data points but may also point towards the
metric of total organic content not fully describing the oxygen demand and labile nature of accumulating material.

Survival metrics used in this study to observe the impact total sediment oxygen demand has on the survival of salmonid eggs found that little change occurred when altering the SOD value. This could be due to the oversimplification of the SOD inputs within these metrics. Simplifying SOC rates to one single value is not representative of the oxygen consumption of sediment which degrades at different rates over time and incubation stages. Total SOD was calculated in this study to create values capable of fitting into current metrics. Chevalier and Carson (1984) note that there is only a finite amount of oxygen that can be consumed by a specific kind of organic matter regardless of the time. This may be a valid assumption about the filling of pore space within the redd particularly in rivers where little flushing of fine sediment occurs like the River Itchen. It may also be true that the time factor of oxygen consumption for different sourced organic inputs may be crucial to embryo development and success. As demonstrated in chapter 7, more labile protein-like material will consume oxygen at a greater rate than humic-like substances so acute and chronic oxygen consumption by organic materials may have different impacts on incubating eggs. Further investigation into the timing of infilling sediments and the type of organic material they contain, be it acute or chronic oxygen consuming species, will potentially elucidate more information on the effect of organic material on salmonid egg survival.

Objective 3. To trace inorganic sediment sources throughout the catchment using sediment fingerprinting technology and the use of a composite fingerprint suite to determine where mitigation of sediment control policy might be effective on the Itchen and similar rivers.

Based on the results from objective one, it is apparent that although accumulation of fine sediment over the incubation period is low in the River Itchen redds, the sensitivity of chalk rivers to sediment inputs and the initial high levels of fine sediment within gravels warranted investigation into the sources of sediments. Chapter 6 contains the detailed results of the sediment fingerprinting study of inorganic sources of fine sediment accumulating in redds. This allowed areas defined as contributing to fine sediment inputs to redds to benefit from management solutions to control sediment delivery to the River Itchen and other like chalk rivers. A summary of the main findings were that;
Pasture sources contributing 50-68% of the fine sediment (<63µm) accumulating in redds and arable sources contributing a further 32-50% were found to be the major origins of fine sediment accumulating within salmonid redds and within suspended sediment on the River Itchen. Walling et al. (2002) suggested that the major sediment sources on the River Itchen that could be identified within salmonid redds were mostly catchment surface derived material (97%).

Sources of suspended sediment were very similar to those found in redds with mean values of pasture (56%) and arable (45%).

To date only one previous study has attempted to address the issue of the source of fine sediment within spawning gravels (Walling et al. 2002). This study focused broadly on whether sources originated from sub-surface (channel bank erosion) or surface soils (fields, ditches, road run-off) within a large number of catchments in the UK. The source of fine sediment in southern catchments, including the River Itchen, was found to be from catchment surfaces (97%). The results from this study improve the knowledge of sources of sediment and elucidate the specific types of land use which are contributing to sources of fine sediment in the river. Pasture soils are a marginally more important source than arable land, highlighting the importance of monitoring and managing rough and improved pasture soil connectivity with the river. A recent model assessing the risk of diffuse pollutants on the abundance of salmonid fry survival, found that intensively farmed pasture land was more important to the abundance of fry in rivers than arable land (Reany et al. 2011). This could be due to the type of sediment which originates from pasture land which contains higher quantities of organic matter than arable land livestock increase connectivity and low level pesticide spraying is common.

Based on previous studies there has been a lack of attention given to pasture soil connectivity to rivers and sediment delivery policies have largely been targeted at reducing inputs from arable land, which is reported to be of an order of magnitude more erodible than pasture soils (Walling 1995). Previous river sediment pollution studies have reported catchments with greater catchment surface sources originating from pasture soils had a greater number of livestock utilising riparian areas in winter (Russell et al. 2001). This may provide a greater understanding of the mechanism by which pasture soils is transferred to the river. Arable source connectivity to the river via roads and tractor trails may be more obvious than pasture sources which could include river crossings for animals, field drains, hedge ditches and compacted animal trails. In order to implement effective sediment management options on the River Itchen, equal weighting needs to be given to the two major land uses in the upper catchment.
Practical strategies that could be employed to mitigate sediment delivery from catchment surface sources to chalk river catchments could include riparian buffer strips along the banks of rivers (Deasey et al. 2010), reducing the amount of autumn and winter sown cereal crops (Collins and Walling 2007b) which leads to bare soils in the winter months (a particular problem in small, lowland catchments) or by replacing crops with fast growing nitrogen fixing plants to stabilise bare soil in arable fields. A recent study found that effective strategies in managing the sediment delivery to rivers from winter sown crops included minimum tillage, contour cultivation, in-field barriers and reducing compaction of tractor tracks by breaking the surface soils (Deasy et al. 2009). Existing strategies such as catchment sensitive farming (CSF), environmentally sensitive areas (ESAs) and the ‘set aside’ scheme (where farmers are compensated for taking land out of agricultural production) should continue to promote awareness of pasture and arable land and river connectivity (Hendry et al. 2003). Transport paths from pasture and arable land should be monitored and where possible blocked by the use of diversion channels or vegetation growth.

**Objective 4. To identify and describe organic matter sources within the redd environment and explore the potential for creating a composite fingerprint suite for sourcing organic matter throughout the catchment to investigate the impact on salmonid spawning habitat quality**

To the author’s knowledge to date, no study has attempted to describe and source the organic matter accumulating within salmonid spawning gravels. The high content of organic material highlighted in chapter four confirms the importance of organic material and its potential to impact on incubating salmonids on chalk rivers. New technology, known as fluorescence analysis, was employed to describe the composition of accumulated organic material within redds and organic catchment sources using the source categories from Chapter five were used to explore the potential of sourcing organic material accumulating in redds on the Itchen. The detailed results can be found in chapter 7. A summary of the main findings are that:

- Redd sediment was dominated by protein-like fluorescence.
- Suspended sediment samples collected from surface waters next to artificial redd sites showed that humic/fulvic-like fluorescence had the greatest intensity signal.
• The intensity of fluorescence signal increases from eyeing to emergence at all sites which is indicative of accumulating organic material over time. This corresponds with the accumulation of fine sediment (<63µm) over the period.
• Correlation exists between peak A (fulvic-like) and to a lesser degree peak C (humic/fulvic-like) with plant material observed within samples. Humic-like substances are therefore likely to have originated from the biodegradation of plants in the river.
• There is potential for using PARAFAC component scores to differentiate between catchment source categories, particularly in-stream, arable and pasture sources.
• Itchen catchment sources of organic material in suspended sediment and redd sediment were found to be attributed to 3% in-stream, 16% arable and 81% pasture sources.

Fluorescence measurements proved to be a rapid, cheap and useful method to describe the organic fraction in redd sediments. Redd sediment and suspended sediment on the River Itchen displayed fluorescent characteristics very similar to previous studies tracing dissolved organic matter in freshwater environments (Coble 1996; Baker 2002; Stedmon et al. 2003; Hudson et al. 2008). Interestingly redd sediment contained greater protein-like fluorescence than suspended sediment which contained high humic-like fluorescence, generally the most dominant form of fluorescence found in freshwaters (Coble 1996; Mcknight et al. 2001). This could indicate that sediment containing protein-like fluorescence is deposited rapidly to the riverbed or that river beds contain greater protein-like fluorescence which can be stored for longer within complexes formed with inorganic mineral. Researchers have attributed protein-like fluorescence to bacterial communities (Elliot et al. 2006; Hudson et al. 2007), diffuse pollutants such as farm waste material and sewage treatment effluent (Baker 2001) and correlations between BOD and tryptophan-like fluorescence (Baker 2001; Baker 2004; Hudson et al. 2008).

However the intensity of protein-like fluorescence in redd sediments was not as high as has been reported for heavily polluted rivers (Baker 2001; Hudson et al. 2008), so there could be another source of the protein-like material, possibly from benthic organisms and the breakdown of chlorophyll from in-stream macrophytes (Meyer et al. 1999; Newson et al. 2001). Lapworth et al. (2009) suggested protein-like substances were preferentially attenuated in river water due to their labile, reactive nature, which would explain the lower presence in suspended sediments. The implication of reactive, labile organic material present in salmonid redds is that short-term high consumption of dissolved oxygen could increase oxygen deficiencies to incubating eggs and result
in development retardation. In prolonged exposure to such conditions, mortality may result. Humic-like fluorescence is correlated with plant material identified in redd and suspended samples. However, more samples are needed to demonstrate this and to be able to differentiate between in-stream and riparian plants. The River Itchen contains large amounts of in-stream vegetation, characteristic of chalk streams (Berrie 1992; Cotton et al. 2006) and seasonal variations in growth describe die-back periods in January with increasing growth from March to April, where stands of macrophytes can dominate reaches (Cotton et al. 2006). Biodegrading macrophyte material is therefore likely to be readily available in river beds during the salmonid spawning season and stable flows are not likely to flush this material from the bed, allowing accumulation of organic material during this period. Further research using larger sample sizes would confirm this relationship and indeed more research on the differentiation between specific fluorescent signals exhibited by in-stream and catchment humic-like substances would lead to better discrimination of the sources of organic components within river DOM.

PARAFAC modelling of samples allowed fluorophores to be grouped together to create component fingerprints of the organic material found within samples. Initial analysis of the data suggests that source categories identified as contributing source material to the River Itchen can be discriminated using these components. Marius et al. (2011) found that PARAFAC component combinations can be indicative of one particular source of organic material but are not conclusive, however large datasets and further work is needed before success in discriminating between specific sources (e.g. land-use types and vegetation types) is reached. PARAFAC provided a useful and robust tool to rapidly analyse large numbers of samples and find common similarities between samples. PARAFAC modelling should be used in future research to define in more detail the organic sources of sediments found in spawning gravels to provide information about spawning habitat quality.

Employing the sediment mixing model to organic parameters, defined in chapter 6, of accumulated sediments provided more evidence of the importance of pasture sources accumulating within spawning gravels. The fluorescent signal of pasture sources was much greater in total than that from arable sources, suggesting greater organic material within pasture soils than cultivated soils. Arable soils are often depleted in organic material due to the intensively farmed nature of modern farming. This adds weight to the implication formed in Chapter 5 of the importance of monitoring pasture sources and connectivity on the Itchen as well as arable sources.
8.2 Research Recommendations

This study advances a number of proposals to better manage and ultimately improve spawning habitat quality for salmonids in chalk rivers. Two fundamental management considerations are highlighted by this project; the first being the requirement to undertake whole catchment studies as the basis to inform practical and policy recommendations for improving spawning habitat as variation exists between reaches even in relatively small catchments. The second relates to the necessity to consider the multiple interacting factors and processes which influence the quality of spawning habitat on chalk rivers. Management and policy strategies should therefore be based upon scientific studies which address these two considerations.

Low resolution, spatial data is of limited use to delineating the specific factors influencing spawning habitat quality in chalk rivers in comparison to high frequency, continuously monitored data. This is demonstrated in this study by the low frequency, spatial data collected to investigate groundwater upwelling at sites that indicated that this was not a factor affecting spawning sites on the Itchen; however the continuously monitored probes measured the influence of groundwater in spawning gravels at the Arle site. Monitoring programmes and continuing to fund high quality research into factors affecting spawning habitat quality is important to ensure that healthy salmonid populations are protected in UK rivers and numbers continue to increase in declining populations. Based on the findings of this study recommendations should be considered at policy, monitoring and management levels for chalk rivers and include;

Policy

Government and government agencies need to ensure that in-stream impacts in chalk streams (high fine sediment levels) are translated into land management practises that minimise fine sediment delivery to chalk rivers. Current policies like those under the banner of the Countryside Stewardship Scheme need to be updated to ensure that river managers and monitoring bodies looking after vulnerable chalk catchments mitigate the effect of fine sediment in spawning gravels, namely by reducing pasture and arable sources of inorganic and organic sediments to the riverbed. Chalk rivers should also be protected under laws and policies which restrict development and abstraction of water as these two factors increase the likelihood that the pressure of fine sediment on spawning gravels will be exacerbated.
**Monitoring**

Consider the use of modelling techniques which include SOD of fine sediments to predict survival of salmonids in chalk catchments. Monitor sources of organic sediments along with inorganic sediments found in spawning gravels. Deploy higher temporal resolution monitoring equipment in targeted monitoring programmes to assess spawning habitat quality in chalk rivers. High frequency groundwater monitoring programmes assessing intra-gravel oxygen concentrations should be considered of particular importance in chalk rivers and its effect on embryo survival.

**Practical management**

1. Both organic and inorganic in-stream sediment inputs and the processes they affect need to be understood by river managers.

2. Spawning gravel rehabilitation should be considered as a primary objective to improve salmonid numbers on chalk rivers as despite the fact that sediment accumulation is low over the incubation period, levels of fine sediment are very high (>20%) already in bed sediments which could reduce sites available to spawning salmonids. Increasing flows by decreasing abstraction levels and timing sluices at strategic times of the year could artificially flush sediment from river beds, for example November/December time prior to spawning activity occurring. Artificially cleaning gravels using jet washers should continue to be used on known spawning patches and consideration should be put into managing macrophyte growth as sediment is often naturally trapped around these plants in rivers. Natural silt traps might alleviate pressure of silted spawning gravels directly downstream of plant colonies.

3. Catchment surface inputs of fine sediment to rivers from pasture as well as arable sources need to be identified in chalk river catchments and targeted practical application to limit the delivery of sediment to rivers should be put in place. Riparian buffer zones should be encouraged along bare banks. Poached river areas should be built back up again and restored back to river banks to limit their transport capacity of climate driven sediment runoff. Initiatives that could be shared and carried out in partnership with farmers could include; fencing off of livestock from river edges, contour ploughing should be encouraged and ploughing in wet weather should be discouraged. Fields should not be kept bare for long periods of time over winter.
8.3 Limitations of the methodological approach

The wider inter-disciplinary and whole catchment approach taken during this research meant that many factors known to affect the survival of salmonid embryos during the incubation period could be monitored, however not every factor could be explored. The study investigated perceived gaps in the research that would enable the project to fit within time and budgetary constraints. A literature review formed the baseline for characterising spawning habitat quality on the River Itchen. Factors such as available dissolved oxygen, intra-gravel flow, sediment accumulation, groundwater upwelling and temperature were used based on the premise that previous researchers highlighted their impact on salmonid survival. Within the context of assessing spawning habitat quality on the River Itchen, it was advantageous to compare factors affecting survival with other studies. Based on previous research the work settled on organic sediments as one of the main factors affecting salmonid survival in chalk streams. Some of the factors not quantified in this study were; sub-lethal effects on emergent fry, the structure and nature of bio-films, all water quality parameters (BOD, nitrates etc.), flushing of metabolic wastes and the presence of toxic diffuse pollutants within river bed sediments (heavy metals, organo-phosphate pesticides etc.).

Survival experiments were limited due to the lack of available eggs for experiments. This made it difficult to create relationships between survival and potential factors affecting survival. Therefore factors previously highlighted in the literature as affecting salmonid survival in redds were assessed against each other. High resolution sites with continuously monitoring equipment and egg survival basket experiments were set up at just two sites. This was mainly due to budgetary constraints, which limited the amount of high resolution data available for analysis, but also because of permission and access issues on the River Itchen.

Innovative laboratory experiments were used as opposed to field experiments in some cases so only the fine sediment fraction accumulated in redds over the incubation period was measured. Future experiments should incorporate comparative field studies in the natural environment to ensure the rate of oxygen consumption is measured within redds.
8.4 Future research recommendations

A number of new research avenues were identified by the work in this thesis. Many relate to the advancement of the sediment oxygen consumption (SOC) and demand (SOD) method created and also develop further the novel approach taken to source organic sediments in spawning gravels. These include:

- Continued testing of the SOD method developed to explore variability on results e.g. the impact of using a greater mass of sediment and or overlying water column on the consumption rates measured.
- Laboratory studies to understand the relationship between egg oxygen consumption rates and sediment oxygen consumption rates in parallel.
- Field SOD studies on chalk rivers and other river types would produce more natural and realistic measurement of the oxygen consumption of spawning gravels using sealed chambers to measure oxygen consumption at the surface-water interface and also using fine-scale oxygen probes to measure oxygen consumption at the micro-scale within the egg pockets themselves. Comparison with the laboratory results obtained in this study to elucidate method differences.
- Related SOD of pure organic source materials present in catchments (riparian and in-stream vegetation, livestock waste, waste water treatment particulate matter) should be investigated to discover those materials with the greatest SOC/SOD and also the length of time it takes for material to biodegrade i.e. how long lasting are the effects if deposited within redds.
- Investigation into the effects of flow under laboratory conditions using a split tube pump system to simulate river conditions and in artificial redds in rivers to explore the effect natural flow has on sediment oxygen consumption.
- Experiments to explore the change in SOD rate with different loadings of fine sediment over different time periods. This would be extremely useful to find if variation exists of SOD values that salmonid embryos could experience in redds where sediment intrusion is large.
- Further fluorescence analysis of specific organic sources (in-stream macrophytes, algae, bacteria, invertebrates) to create robust PARAFAC models of groups of organisms that can be distinguished from each other. Sediment fingerprinting methods could then be employed to source organic sediments retrieved from redds. Marius et al. (2011) have already started this work, but
greater numbers of samples and a full suite of organic inputs within a catchment should be investigated

- The development of a database with major fluorescent signals of organic material found within spawning gravels in chalk streams and for all river typologies in the UK
- Laboratory studies into the effect different organic loadings and sources have on embryo survival – flume studies provide an excellent semi-controlled environment for this work where flow and organic loading can be controlled
- Develop SIDO-UK and other models to incorporate complex organic matter influence on salmonid survival; namely sediment oxygen consumption rates that change over time, rather than static values that do not change over time.
- Re-evaluate and develop SIDO-UK to represent river catchments that may experience low fine sediment accumulation rates.
Chapter 9. Appendices

9.1.1 Appendix 1 – Monitoring probe standardising

Standardising probes used to measure the same habitat variable. These include; continuously monitored temperature and dissolved oxygen (water and intra-gravel) and weekly spot samples within standpipes and the water column taken at the same site with a handheld probe.

![Graph showing DO difference across different probes](https://example.com/figure1.png)

Figure 1. Displays DO difference in measurement across different probes. Used to standardise dissolved oxygen readings across probes. Measured difference between probes:

- **Aandera 666 vs YSI**
  \[ y = 0.9768x + 0.0364 \]
  \[ R^2 = 0.9998 \]

- **Aandera 666 vs 667**
  \[ y = 1.7257x - 1.7457 \]
  \[ R^2 = 0.9119 \]
Figure 2. Displays the strong linear relationship between DO test for oxygen probes. Due to strong relationship between probes no dissolved oxygen correcting occurred.

Figure 3. Displays temperature difference between probes used in analysis. Used to standardise measurements from different probes measuring the same variable.
9.1.2 Appendix 2 – Example of range tests applied to fingerprint properties to determine sediment – source material relationship.

A snapshot from a larger spread sheet describing the range tests applied for redd sediment samples and source sediments

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<th>Type</th>
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<th>23Na</th>
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9.1.3 Appendix 3 - % Carbonate content of different particle sizes over different incubation stages at all sites 2009

Figure 1. Eyeing incubation stage carbonate content of different particle sizes at all sites 2009

Figure 2. Hatch incubation stage carbonate content of different particle sizes at all sites 2009
Figure 3. Emergence incubation stage carbonate content of different particle sizes at all sites 2009
9.1.4 Appendix 4 – ANOVA tables for regression analysis of the relationships between SOC, organic matter and carbonate.

Note: DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F-value, P = P-value.

**Figure 7.11. SOD and normalised organic matter 2009**

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
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<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>155.52</td>
<td>155.52</td>
<td>44.34</td>
<td>0.000</td>
</tr>
<tr>
<td>Residual Error</td>
<td>17</td>
<td>59.63</td>
<td>3.51</td>
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<tr>
<td>Total</td>
<td>18</td>
<td>215.16</td>
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</table>

**Figure 7.12. SOD and normalised organic matter 2009**

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<tbody>
<tr>
<td>Regression</td>
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<td>13.212</td>
<td>13.212</td>
<td>23.73</td>
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<td>10.022</td>
<td>0.557</td>
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<tr>
<td>Total</td>
<td>19</td>
<td>23.234</td>
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**Figure 7.13. SOD and normalised carbonate content 2009**

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<tbody>
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<td>145.673</td>
<td>40.93</td>
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<tr>
<td>Error</td>
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<td>56.939</td>
<td>3.559</td>
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<tr>
<td>Total</td>
<td>17</td>
<td>202.612</td>
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</table>

**Figure 7.14. SOD and normalised carbonate content 2009**

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<tbody>
<tr>
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<td>0.4694</td>
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**Figure 7.17. SOD and normalised organic matter 2010**

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</thead>
<tbody>
<tr>
<td>Regression</td>
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</table>
Total 19 697.747

**Figure 18. SOD$_{25}$ and normalised organic matter 2010**

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</tr>
</thead>
<tbody>
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<td>4.22092</td>
<td>4.66</td>
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<td>19</td>
<td>23.8501</td>
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</table>

**Figure 19. SOD$_{25}$ and normalised carbonate content 2010**

<table>
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<td>19</td>
<td>23.8501</td>
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