1. Chapter One: General Introduction

1.1. Background

Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) are not only of significant economic value but, more importantly, an iconic symbol of the river systems in the United Kingdom (UK). The epic journey of the migratory Atlantic salmon and sea trout to the very location which they themselves were born is a reminder of the incredible strength and determination of these species. However, overfishing in the marine environment and the degradation of their freshwater habitat has resulted in a dramatic decline (Crozier and Kennedy, 1994; Parrish *et al*., 1998) in those returning to the rivers. Reductions in populations have instigated a growing concern and action has already been taken to protect these important species. However, numbers continue to decrease (Beaumont *et al*., 2010; Ellis and Sumner, 2011) and therefore, further information and research is essential if an attempt to conserve these incredible fish is to be a success.

There has been evidence of salmonids dating back to the upper palaeolithic period in the form of a cave relief (Figure 1.1), indicating their existence from possibly 10,000 to 40,000 years ago (WWF, 2001). Salmonids are therefore shown to be an enduring and successful species. However, Atantic salmon have suffered a decline of 35% in the past four decades in the UK (EA, 2005) and the number of sea trout caught each year has also shown a decrease in numbers since 2002 (EA, 2008). Such declines have been monitored using information from rod catch numbers and salmon counters. The Marine and Coastal Act (2009) contains many measures which will improve the protection of migratory, freshwater and inshore fisheries. The Salmon and Sea Trout byelaw is part of the Act and has banned the sale of rod caught salmon and trout as well as enforcing licensed netsmen to record and tag any catches. The Water Framework Directive will also help in the conservation of brown trout and salmon by improving the water quality of the freshwater habitat and demanding a good ecological status in European river basins by 2015.

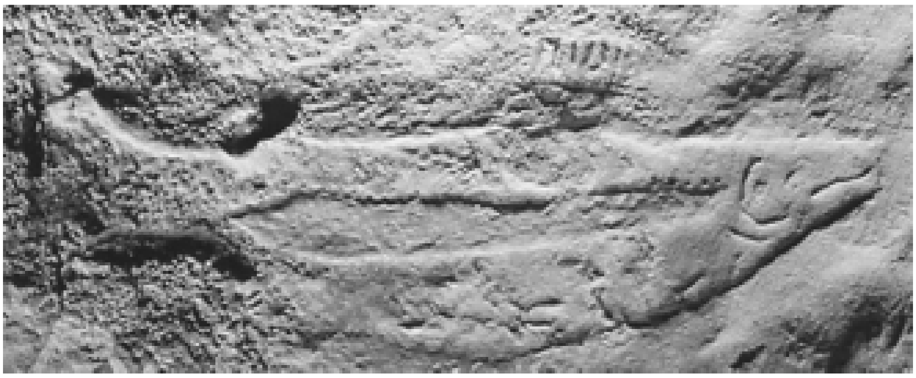

Figure 1.1. A 25,000 year old relief of a salmon from the cave Gorge d´Enfer in Les Eyzies de Tayac in France (sourced from WWF, 2001; Jacqueline Angot-Westin, Musée National de la Préhistoire).

It has been suggested that the most dramatic change in Atlantic salmon populations in England and Wales over the past 20 years has been the decline in two and three seawinter fish. This is a phenomenon which has appeared to be cyclical over at least 150 years. However, there is concern that exploitation and other anthropogenic actions may be excacerbating the decline, possibly compromising subsequent recovery (WWF, 2001; Erikson and Erikson, 1993; Johnson *et al*., 2009).

Although salmonids are exposed to potential risks and hazards in their seawater environment, there is evidence to suggest that it is the quality of the freshwater environment which may be contributing to declining stocks (Chapman 1988, Wheeldon, 2003; Julien and Bergeron, 2006; Dudgeon *et al*., 2006). The condition of rivers, which support the early life stages of salmonids have deteriorated, some even to the extent where extinction of Atlantic salmon has occurred (Figure 1.2).

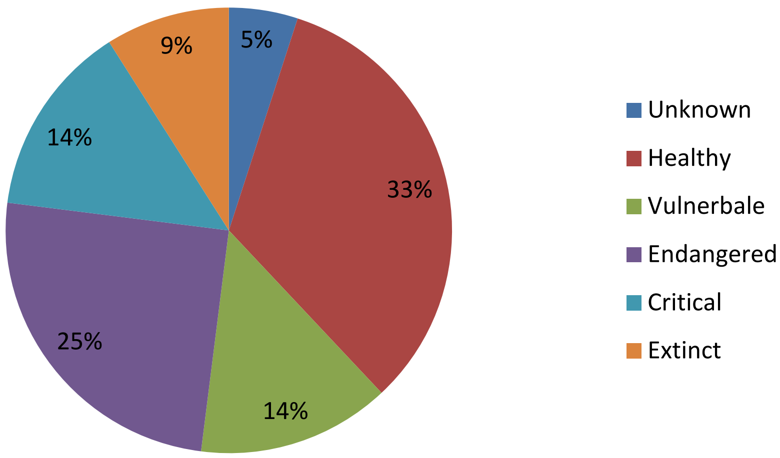

Figure 1.2. Categorization of salmon-bearing rivers in England and Wales (Adapted from WWF, 2001).

The early stages of development are considered the most vulnerable in many aquatic organisms (Weis and Weis, 1989; Peterson and Kristensen, 1998; Hamm and Hinton, 2000; Finn, 2007). As salmonid reproduction occurs in the freshwater environment, and the juveniles can spend up to five years in the river before they migrate to sea to feed, grow and mature (Hutchings and Jones, 1998), this could possibly be the most vulnerable stage of the life cycle. Water quality is one of the most important contributors to fish health (Malcolm *et al*., 2003b). Most fishes are dependent on water for the exchange of gases and ions across gills, and as a diluting agent for metabolic waste (Portz *et al*., 2006). Moreover, there is also evidence to suggest that the environmental conditions experienced by the juveniles in freshwater may have a significant effect on survival once the fish migrate to sea (Fairchild *et al*., 1999; Arsenault *et al*., 2004).

Survival of the intragravel life stages of salmonid fish, Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*), and the consequent success of development is imperative for long-term success of the population. Any factors that can affect the intragravel survival and production of juvenile salmonids, may affect sustainability of stocks and the fisheries dependant upon them. It is therefore important that there is an understanding of the potential risks associated with salmonid spawning gravels and the possible deleterious effects on the embryos and alevins.

1.2. Life Cycle of Salmonids

The Atlantic salmon are distributed in many countries where the watercourses empty into the Atlantic Ocean. In Europe, Atlantic salmon are found in most of the large rivers from Portugal to Northwest Russia. Salmon populations are also found in the UK, Iceland and Greenland. Greenland has only one spawning river however, the sea around Southwest Greenland is an important wintering and feeding area for salmon from other areas (Pyefinch, 1972; Friedland *et al.*, 1993; WWF, 2001).

The majority of salmonids start life in the freshwater riverine environment and migrate to sea for feeding during their adult stages, returning to their natal stream to spawn. Although some populations of Atlantic salmon and brown trout do not migrate and complete their entire life cycle in freshwater (land-locked populations of Atlantic salmon and non-migratory brown trout), the majority of Atlantic salmon have an anadromous life history (Figure 1.3).

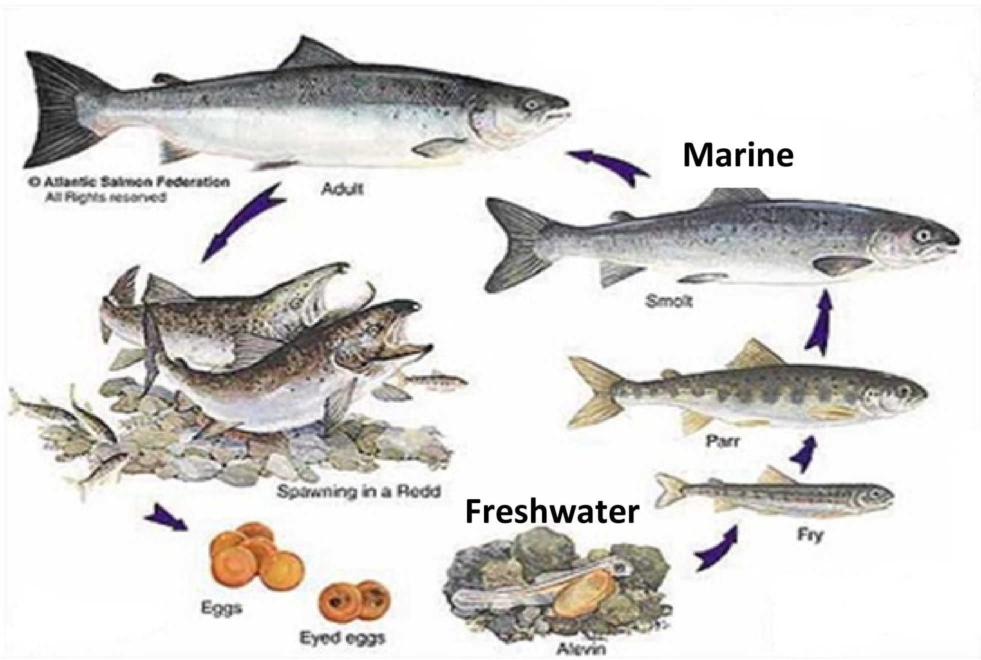

Figure 1.3. The life cycle of the Atlantic salmon (adapted from J.O. Pennanen; Atlantic Salmon Federation).

Anadromous organisms have a life cycle that includes both freshwater and marine phases (McDowall, 2001). Adult salmon can spend one to four years in the sea before returning to the natal river to spawn (Hutchings and Jones, 1998; Mills, 1989). It has been shown that differences in the length of marine occupation however does not reflect the fecundity or egg size at a given parent size (Thorpe *et al.,* 1984). The importance of the freshwater environment within salmon life history should not be underestimated, as reproductive success and survival of the early stages of development (Table 1.1) is dependent on the freshwater habitat. These early life cycle stages in the freshwater environment therefore represent potentially the most critical and sensitive stages, and could have significant implications for wild populations.

Table 1.1. Terminology of Salmonid Early Life Stages.

Term	Definition
Anadromous	Spend most of the adult life stage in the marine environment but return to the natal stream to reproduce.
Redd	Depressions made in the gravels by the female salmonid in which her eggs are laid and fertilised. The eggs are covered and remain within these nests for the first stages of development.
Alevin	Newly hatched salmonids which remain in the gravel and which are nutritionally supported by a yolk sac.
Emergence	Newly hatched salmonids swimming up out of the gravel to feed.
Fry	The stage at which the yolk sac has been completely absorbed and now ready for exogenous feeding.

Salmonid nests, known as redds, are built by females in locations of accelerating flow and increasing depth (Hobbs, 1937), usually covering an area of few square metres (Bardonnet and Bagliniere, 2000). The female salmonid constructs a series of excavations in the gravel substrate using tail movements (Jones, 1959; Tautz and Groot, 1975; Beall, 1994). Redds begin as a pocket, from which the female has removed fines and small gravels, located at a depth of 15-25cm beneath the river bed surface (Chapman, 1988). Crisp and Carling (1989) noted that *Salmo salar* excavate redds up to a depth of 0.3m. Grost *et al*., (1991) examined the redds of brown trout and found the eggs were buried between 2 and 23 cm below the substrate surface, but were most frequently 9-12 cm deep. The bottom of California golden trout (*Oncorhynchus mykiss aguabonita*) egg pockets were found to average a depth of 5.4 cm (Knappe and Vredenburg, 1996). The average depth for the egg burial for bull trout (*Salvelinus confluentus*) eggs was approximately 15 cm (Weeber, 2006). The female removes a large proportion of the fine sediment to increase both gravel porosity and permeability (McNeil and Ahnell, 1964) and to favour the flow of oxygenated water within the redd to the eggs (Guerrin and Dumas, 2001a). However, research has suggested that the fine sediment removed during redd preparation essentially returns to pre-spawning conditions by subsequent sediment transport (Peterson and Quinn, 1996a). Field evidence has shown that an abundance of fine sediment surrounding coho salmon eggs is detrimental (Meyer, 2003). Elevated levels of fine sediment have been shown to reduce the survival of *Salmo salar* eggs and alevins, as a result of low intragravel oxygen and reduced gravel permeability (Chapman, 1988; Acornley and Sear, 1999; Malcolm *et al.*, 2003, Heywood and Walling, 2007; Kemp *et al.*, 2011). Slight changes in fine sediment concentration in the redds produce large changes in embryo mortality (Peterson and Quinn, 1996b).

The female salmonid then covers the fertilised eggs with gravel substrate, usually by the excavated materials from the construction of a new redd upstream, into which further eggs are shed (Hendry and Cragg-Hine, 2000). Males spawn with a number of females each season and the fertilisation success by the dominant male is high (Maitland and Campbell, 1992). In addition, the proportion of salmon returning as previous spawners is between three and six percent (Mills, 1989).

The fertilised eggs develop in redds over the winter and hatch in the spring as alevins (Guerrin and Dumas, 2001a). The hatching period is dependent on water temperature. For example at a water temperature of 3ºC, the incubation period is approximately 145 days (Drummond Sedgwick, 1982). Once the alevins hatch they remain in the redd absorbing the yolk-sac until they emerge from redds as fry and begin independent feeding on invertebrates (Hendry and Cragg-Hine, 2003). *Salmo trutta* and *Salmo salar* both exist as anadromous and non-anadromous freshwater resident forms. Atlantic salmon are indigenous along the West and East coast of the North Atlantic Ocean. Brown trout are native to Europe, North Africa and western Asia (MacCrimmon *et al.*, 1970). Killeen *et al.* (1999) described the early life stages of *Salmo trutta* as a series of 40 developmental steps. Additionally, Gorodilov (1996) provides descriptions of a method for quantifying the degree of successive developmental states in *Salmo salar*. However it has been found that the overall rate of development is identical between brown trout and Atlantic salmon (Gorodilov, 1989). Temperature was also found to have no effect on the relative timing of formation of anatomical structure in salmonid species (Gorodilov, 1989). Although in terms of habitat, *Salmo trutta* have been shown to exhibit a much greater range of habitat use than *Salmo salar* (Riley *et al*., 2006).

1.3. Salmonid Spawning Gravel Habitat

Chalk waters rise from Cretaceous chalk aquifer through springs and boreholes and are rich in calcium carbonate (>200mg l^{-1}) and are historically known to have operated as water meadows (Mann *et al*., 1989). It has been noted that the decline in the water meadows has been a benefit to salmonid fish (Solomon, 1990). Chalk streams are renowned for high nutrient quality (Casey and Smith, 1994) although antropogenic influences have led to reduced water quality and degradation of fish habitats (Hendry *et al*., 2003). Chalk streams are groundwater fed so have little fluctuations in flow and temperature, as well as being generally low in suspended sediment concentration (Heywood and Walling, 2003). Many of the chalk stream headwaters are now used to grow watercress (*Rorippa nasturtium-aquaticum*) using water sourced directly from springs and boreholes (Crisp, 1970). There are water quality implications of watercress beds which are represented by an increase of diffuse input of phosphorus which could result in nutrient enrichment and reduced dissolved oxygen levels (Casey and Smith, 1994). Although sewage effluent can also be a major source of phosphorus in many lowland rivers (Jarvie *et al*., 1998). Watercress beds in lowland chalk streams can also lead to an increase in the quantity of suspended solids of which a high proportion contains fine organic sediments derived from the watercress farms (Casey and Smith, 1994). Catches of salmon were noticeably reduced in the late 1980s and early 1990s in southern chalk streams (EA, 2007) and this is potentially due to the degradation of habitat, pollution and increased silt accumulation, in these southern chalk streams which are known to support native populations of salmonids.

Southern England chalk streams are characterised by the consistent flow and mineral-rich nutrient water (Mann *et al*., 1989). The conditions are ideal for the successful spawning of salmonids and their consequent development and recruitment. The chalk streams have been affected by intensive agricultural practices in the last century and data from the Environment Agency has demonstrated the increased agricultural practices around chalk rivers. It is estimated that almost 50% of land in chalk stream catchments is in fact arable (EA, 2005) and pesticides associated with this may affect wild salmonids (Moore and Waring, 1996; Moore and Waring, 2001).

The River Avon is one of the chalk river systems which has witnessed a decrease in salmonid populations. Wheeldon (2003) suggested that a poor recruitment rate, and thus declining populations, is possibly linked to a reduction in spawning and the success of embryo survival. The fresh water conditions during early life stages of salmonids can have subsequent effects on the adult population and the survival in the marine environment, consequently affecting the spawning success on the return to fresh water (Waring and Moore, 2004; Finn, 2007).

Laboratory and field studies have suggested that infiltration of large amounts of fine sediment into salmonid redds can reduce gravel permeability (McNeil and Ahnell, 1964). Additionally, a study on high suspended sediment concentrations suggest a significant impact on the fertilisation and reproductive success of sockeye salmon (*Oncorhynchus nerka*) leading to detrimental effects on adults and egg incubation habitat due to sediment exposure (Galbraith *et al.*, 2006). There have been many studies which have investigated the effects of fine sediments on salmonids (Chapman, 1988; Lisle and Lewis, 1992; Acornley and Sear, 1999; Soulsby *et al.*, 2001; Greig *et al.*, 2005; Julien and Bergeron, 2006). An increase in fine sediment content in spawning gravels can reduce permeability, limiting the amount of dissolved oxygen reaching embryos and the rate at which waste products are removed, thus increasing embryo mortality (Adams and Beschta, 1980). A similar relationship was observed by Olsson and Persson (1986) where gravel with a high proportion of fine sediment and a consequent low permeability tended to have low dissolved oxygen levels. However, high levels of suspended solids have been shown to induce gill damage, which may have caused anoxia and stress, despite the oxygen levels nearing saturation (Lake and Hinch, 1999).

It has been shown that 76% of all sediment loading into rivers of England and Wales is accountable by agricultural sources (Collins *et al*., 2009). Figure 1.4 describes the contribution of sediment loading by identifying the source. Sediment mobilisation, transport and delivery through river channel systems is also a key vector governing the transfer and fate of contaminants (Warren *et al*., 2003; Cave *et al*., 2005).

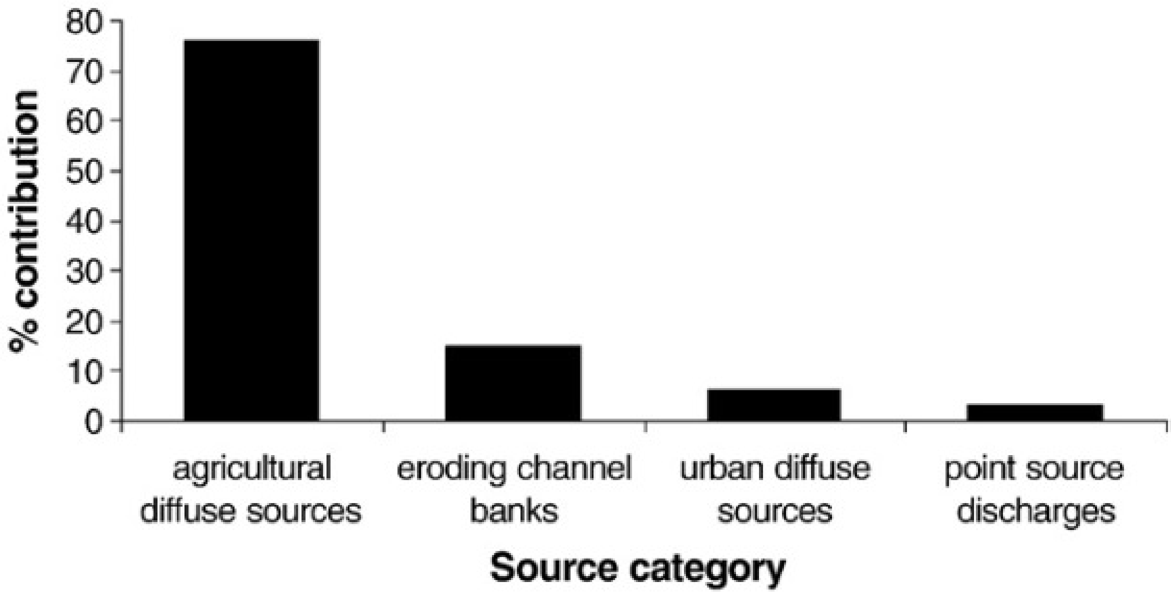

Figure 1.4. National scale sediment source apportionment for England and Wales, under current environmental conditions (year 2000). (Collins *et al*., 2009).

Early emergence is believed to possibly be induced by a stress response in relation to the confinement effects of the small interstices in fine substrata (Olsson and Persson, 1986). Additionally, Fudge *et al.* (2008) noted early emergence of rainbow trout (*Oncorhynchus mykiss*) fry, as a result of increased sediment loading, and observed greater residual yolk sac remaining in these early emerged fry. Moreover, it has been observed that fry from coarser gravels were larger and emerged at a later time (Witzel and MacCrimmon, 1981). Consequently they were better developed and retained less yolk sac material than those emerging from finer gravels. Additionally, high rates of delivery of fine sediment to the redds can prevent fry emergence altogether (Reaney *et al.*, 2010).

Research on steelhead trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) observed that the major controlling factor influencing egg mortality is dissolved oxygen (Reiser and White, 1988). Heywood and Walling (2007) noted that salmonid embryo survival was reduced in areas of lower interstitial oxygen levels, which correlated with the finer substrate composition of the redds. Oxygen deficits have been found to be the result of a reduction of intra gravel velocities which are a consequence of large amounts of sediment (Reiser and White, 1988). Rubin and Glumsäter (1996) showed that the egg-to-fry (ETF) survival was correlated generally with the interstitial oxygen concentration. Generally when the permeability of the substratum was low, the interstitial water supply was reduced. Therefore the interstitial oxygen concentration decreased and the waste concentration increased (Rubin and Glumsäter, 1996). Research carried out by Sowden and Power (1985) showed low rainbow trout embryo survival in their field trials, averaging only 7.6±2.7% and ranged from 0 to 43.5%. No survival occurred in redds where mean dissolved oxygen was less than 4.3mg/l and survival was negligible (<1.0%) until mean oxygen concentration exceeded 5.2mg/l. Survival was also significantly related to the velocity of groundwater in redds. However, velocity of groundwater only had a direct effect on egg survival when the levels of dissolved oxygen were higher (>5.3mg/l). The study concluded that water velocity through sediments can limit oxygen delivery in natural streams, and therefore survival, when dissolved oxygen is less than 5mg/l.

In stream ecosystems, surface and ground waters interact through a hydrologic continuum and mix within the hyporheic zone (Battin *et al*., 2003). The transition between groundwater and streams was first recognised as a distinct zone by Orghidan (1959). The hyporheic zone (Figure 1.5) is a mixing zone in which there are gradients in the concentration of dissolved gases, pH and temperature (Runkel *et al*., 2003).

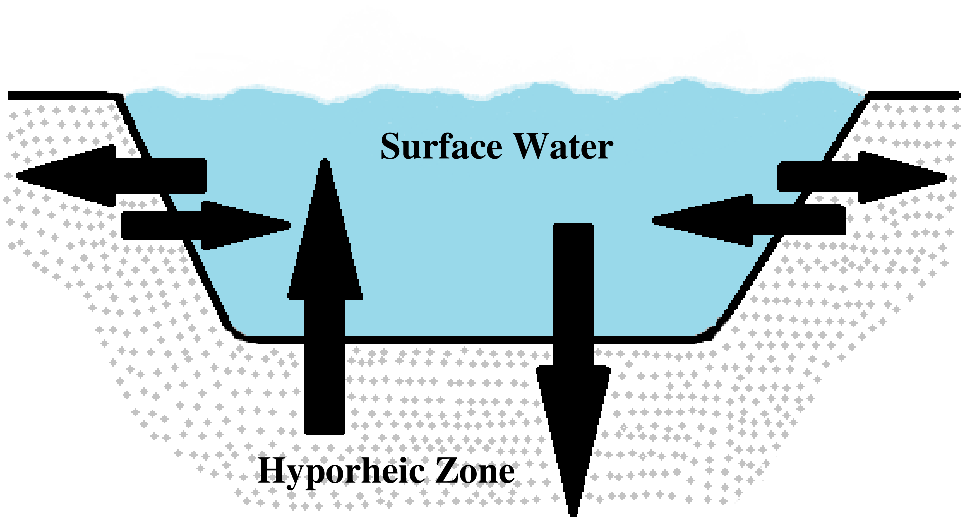

Figure 1.5. A schematic of the hyporheic zone. The arrows indicate the movement of water between the surface water and hyporheic zone. (Drawn by author).

The hyporheic zone offers its inhabitants protection against high discharge, desiccation and extreme temperatures and provides stable conditions for the development of fish embryos (Brunke and Gonser, 1997). The upwelling hyporheic flow is usually associated with the spawning locations of salmonids (Sowden and Power, 1985). Water temperature has a direct affect on the survival of salmonid eggs (Crisp, 1996) as it influences both the solubility of oxygen and oxygen demand of developing fish embryos (Acornley, 1999). Water quality in this hyporheic zone affects salmonid egg survival and may limit juvenile recruitment. Research has shown the hyporheic water quality in a degraded agricultural stream had a significant affect on salmonid eggs (Malcolm *et al*., 2003). It has been noted that a better understanding of the hydraulic and geomorphic processes occurring in the hyporheic zone would help predict the availability of salmonid spawning habitats (Geist and Dauble, 1998).

Toxicity tests performed on freshwater fish have demonstrated the acute sensitivity of the early life stages of fish (Gorge & Nagel 1990) and it is generally well agreed that factors influencing the first 60 days of embryonic life will ultimately affect the abundance of recruiting adults, as demonstrated in experiments on striped bass (*Morone saxatilis*) stocks (Westin & Rodgers 1978). Embryos have been considered to be the most sensitive developmental stages in the lifecycle of fish. This is due to the high number of critical developmental events, such as rapid proliferation, differentiation and growth of tissues, which occur over a short period of time (Petersen and Kristensen 1998; Honkanen 2004) and because of an underdeveloped capability to metabolise the chemicals that readily accumulate within the yolk tissue (Petersen & Kristensen 1998). Because of this, natural embryonic and larval mortality are already high without the added pressures of mortality due to the teratogenic effects of pollutants (Weis & Weis 1989). Heintz *et al.* (2000) reported delayed effects on the growth and a 15% reduced marine survival rate of pink salmon (*Oncorhynchus gorbuscha*) which were exposed to oil as embryos, compared to non-treated embryos. Additionally Fairchild *et al.* (1999) demonstrated a decline in the numbers of returning spawning adults to rivers in Canada, when smolts were exposed to an insecticide containing 4-nonylphenol. Such studies corroborate with the suggestion that the early life stages of salmonids in terms of recruitment and population in the marine environment are possibly the most important.

1.4 Freshwater Pollution

Pollution of freshwater is perhaps the single most significant factor in the decline of Atlantic salmon populations (Maitland *et al*., 1994). A large amount of published work has been carried out on the effects of agriculture on water quality and spawning habitats of salmonids and other fish species (Chapman, 1988; Rubin and Glumsäter, 1996; Acornley and Sear, 1999; Walling and Amos, 1999; Armstrong *et al*., 2003; Greig *et al*., 2005). Many salmonid streams, or potential salmonid streams, have been affected by channel modification for agriculture and other purposes (Malcolm *et al*., 2003). It is noted that although the physical characteristics to the changes in geomorphology are slowly being recognised, it has largely been neglected (Soulsby *et al*., 2001a). There is a high level of degradation in lowland rivers across Scotland which has been associated with agricultural intensification. This has resulted in the widening and deepening of streams, which increases the sediment loading of the rivers and streams through soil erosion (Soulsby *et al*., 2001b).

There are several ways in which pollution can enter the water course (Figure 1.6), however they are mainly classified as point source or non-point source pollution.

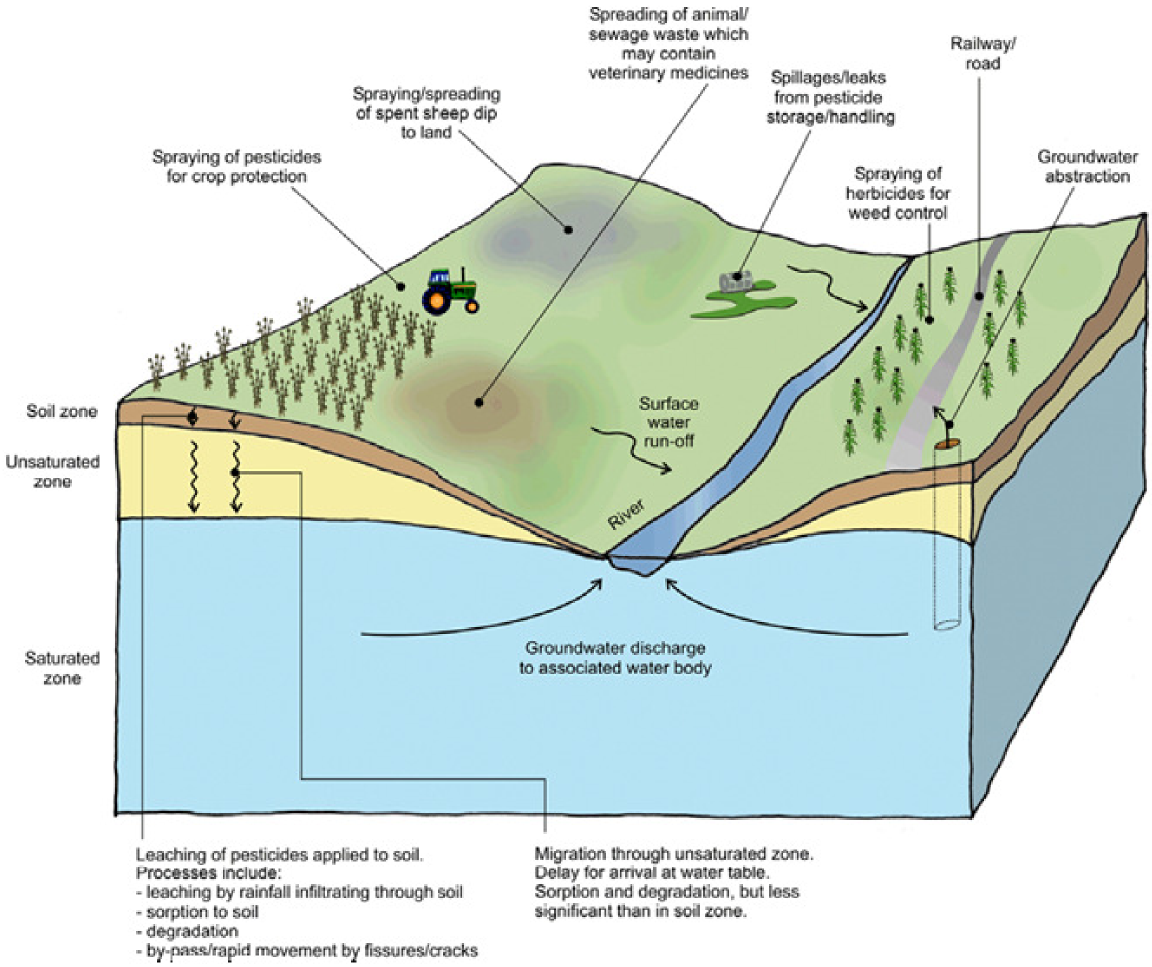

Figure 1.6. Routes of entry into the freshwater environment (Pesticide sources, pathways and receptors, courtesy of EA).

Point source pollution originates from a traceable location and involves a discharge from a single source or a small area, such as sewage effluent. Some environmental legislation has been enforced since the 1970s which has helped to control the point source pollution from industries and agriculture (Cave *et al.*, 2005). However, it is non-point source pollution (diffuse pollution) which is much harder to regulate. Diffuse pollution is problematic as it is difficult to quantify and manage due to its tendency to fluctuate with seasons and weather (Carpenter *et al.*, 1998). A high proportion of diffuse pollution originates from land use activities, including the use of herbicides, pesticides, organic, and inorganic fertilisers (Wheeldon, 2003). However, pollution as a term also encompasses the sedimentation of the salmonid spawning habitat as previously discussed.

As a result of their application, agricultural products are introduced into the environment and may enter into groundwater, surface water and sediment. Fertilisers, containing nutrients and chemicals, can enter the rivers and groundwater as a result of leaching. During heavy rainfall, less soluble components can be washed overland to rivers and large concentrations of pesticides and chemicals are known to be washed into surrounding waters (EA, 2007). However, pollution remains most problematic in small rivers close to densely populated areas (Iversen *et al*., 2000). Moreover, water temperature also affects the lethality of pollutants on fish. Fish exposed to pollutants typically have a decreased survival time by a factor of two or three with each increase of 10°C (Wakefield *et al.,* 2004).

1.4.1 Pesticides

In the UK, pesticides are mainly used in association with agriculture and horticulture and include herbicides, insecticides and fungicides, and the application of these chemicals has increased over the years (Figure 1.7). The use of chemicals for agricultural purposes is not a recent event, in fact evidence has shown the application of pesticides as early as 200BC (Wijbenga and Hutzinger, 1984).

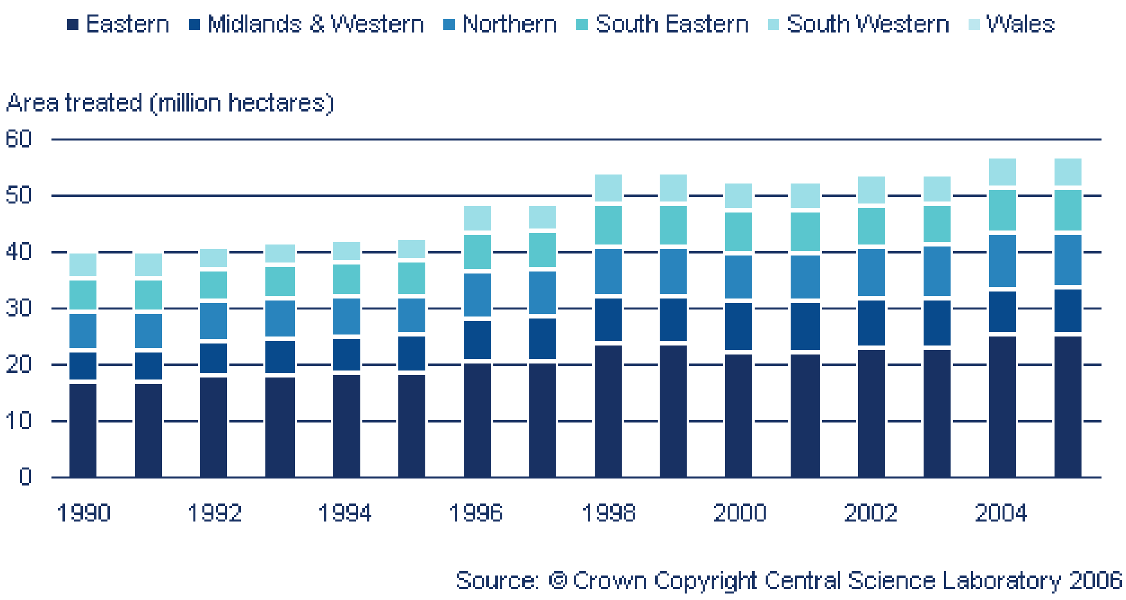

Figure 1.7. Area treated with pesticides in England and Wales (source EA, 2006).

Physical, chemical and biological processes transform most pesticides into one or more transformation products. Transformations can be susceptible to abiotic, or biotic factors, therefore surface water contaminants may be prone to abiotic transformation processes such as photochemical degradation (Farre *et al*., 2008). For instance, DDT is broken down by sunlight to DDE, while microorganisms further degrade it to DDD (Ssebugere *et al*., 2009). Also, atrazine is known to be transformed into at least seven compounds (Belfroid *et al*., 1998), which include desisopropylatrazine and desethylatrazine. Due to the application, these compounds are introduced into the environment and may distribute into groundwater, surface water and sediment (Hutson and Roberts, 1990). There has been a steady increase in the total area in which pesticides are applied over the years in the UK, which has already been shown in the previous figure (Figure 1.8).

The percentage of pesticides detected in England and Wales, which are found at levels greater than 0.1µg/l, does not exceed 8% (Figure 1.8). However, concentrations found to exceed 0.1ug/l are greater than the maximum permissible level (MPL) of the EU Drinking Water Directive 98/83/EC (European Community, 1998).

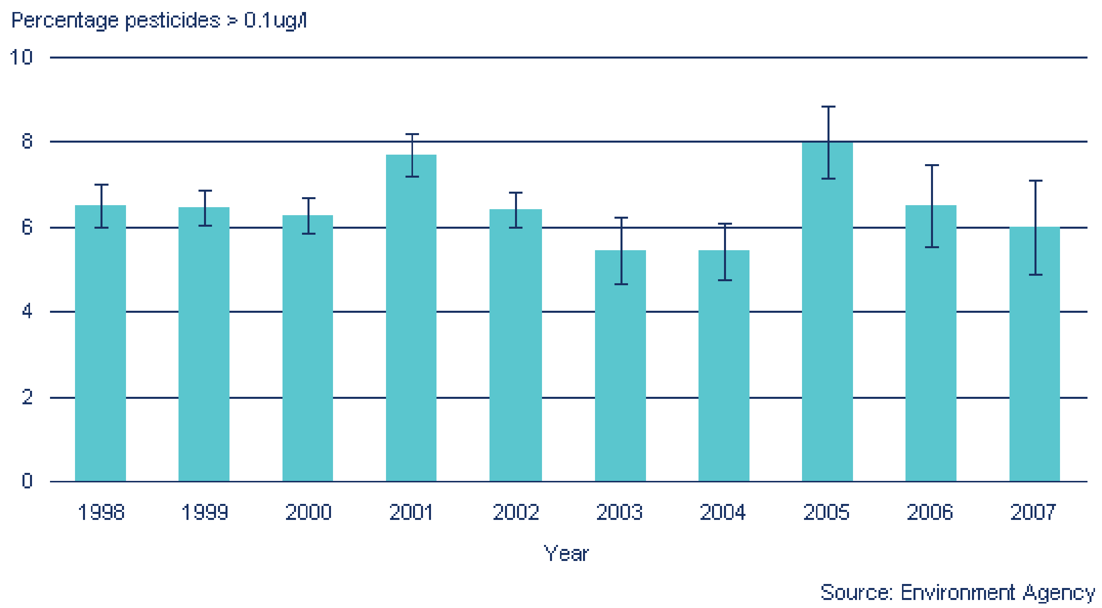

Figure 1.8. Pesticides in surface waters in England and Wales, 1998 to 2007 (EA, 2007).

Various types of pesticides have been detected in ground water monitoring sites (Figure 1.9), including herbicides, insectides and fungicides. Levels of detection in groundwater have also been shown to exceed the MPL (European Community, 1998).

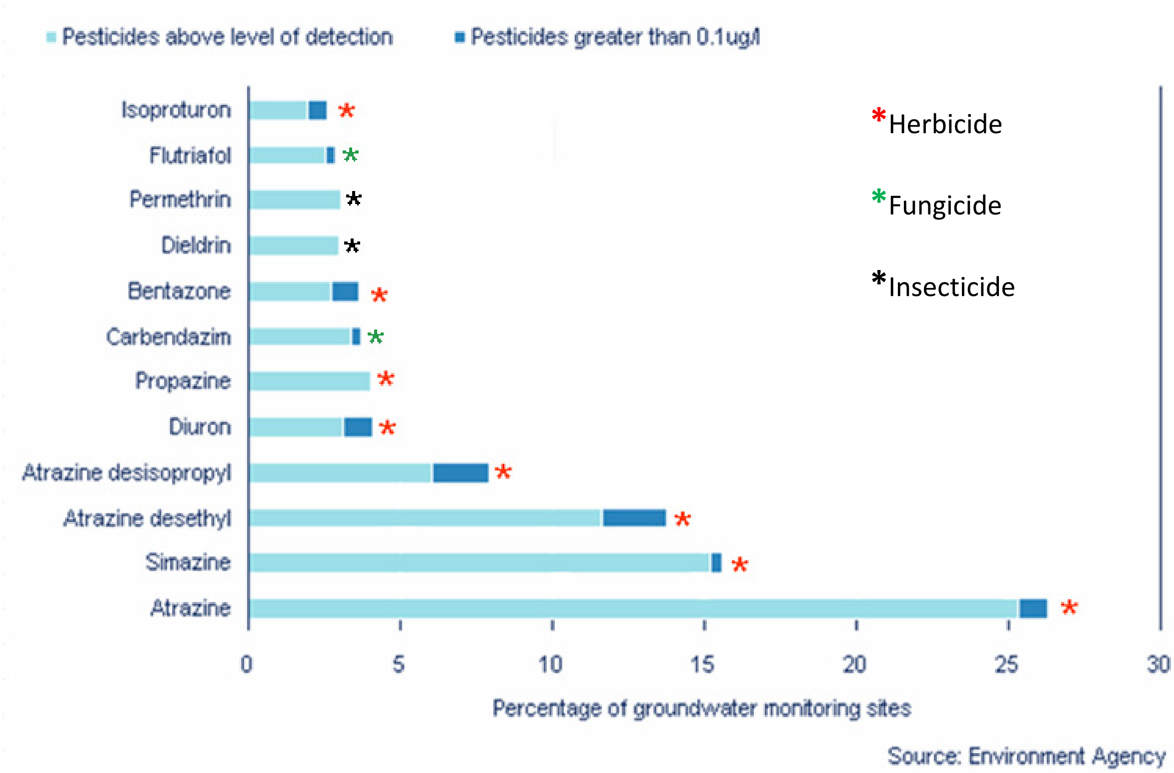

Figure 1.9. The percentage of groundwater monitoring sites which have detected pesticides (EA, 2007).

Organochlorine pesticides are manmade chemicals (Scorecard, 2005) and the first synthetic compound produced was dichlorodiphenyltrichloroethane (DDT) in Switzerland in 1939. Now a banned agricultural substance in the UK since 1984 (Pesticide Action Network UK), DDT was originally designed to combat human head and body lice, until it was developed for agricultural purposes. However, traces of DDT are still found in the aquatic environment and have been shown to accumulate in aquatic organisms (MacGregor *et al.*, 2001). DDT residues accumulate in fatty tissues of organisms (Cox, 2002). Additionally, the organochlorine pesticide, pentachlorophenol, has been found to produce yolk sac oedemas (accumulation of fluid in the yolk sac) in freshwater fish during the early stages of development (Chapman, 1969; Helder, 1981). Few recent studies have examined waterborne concentrations of DDT in UK rivers, as it is no longer routinely monitored. However a more recent study has looked at the atmospheric concentrations of DDT and found detectable levels in a large UK city (Harrad and Mao, 2004). This may suggest that despite the ban there is still some continued use of the contaminant. DDT has found to be practically insoluble in water and has a reported half-life of 28 days in river water. Residues are lost by adsorption on particulate matter and a reported half-life of 2-15 years (Cox, 2002). Dieldrin and lindane are both organochlorine compounds which can be metabolised in animals and accumulation tends to occur in the fatty tissue. OCPs, including the metabolites, have been found to accumulate in liver samples of many marine fish species including dab, plaice, bib and whiting, with highest concentrations of 2.1 and 8.0 ng g^{-1} (Voorspoels *et al*. (2004). Water solubilities are 0.19mg l^{-1} and 7.3mg l^{-1} respectively. However dieldrin is persistent in soils whereas lindane shows low soil affinity (Cox, 2002). Even compounds within the same class of pesticides behave very differently when exposed to the environment. It has been suggested that the bioavailability of organochlorine compounds, such as DDT, to fish could change according to seasonal variations in surrounding water and sediment (Linkov *et al*., 2002). In turn, this may modify contaminant bioavailability and change the uptake of contaminants from water by aquatic organisms and change the nature of the exposure (Sethajintanin and Anderson, 2006). Endosulfan has also been shown to be toxic to freshwater organisms and a concentration of 1.75µg l^{-1} (96 hour exposure) killed 50% of the exposed rainbow trout Capkin *et al.* (2006). The toxicity of the organochlorine pesticide, endosulfan, is suggested to be dependent on the on the size of fish, water temperature as well as water quality and a level greater then 1.3µg l^{-1} is considered toxic to adult rainbow trout once mixed with water. Therefore the early stages of fish development may be more susceptible to the toxicity of endosulfan as well as other contaminants.

Chlorophenoxy acids, or acid herbicides, are used as common weed killers (Ozcan Oruc *et al*., 2004) and also for the control of broad leaf weeds in crops such as wheat, oats and barley (Fletcher *et al*., 1996). Exposure to acid herbicides has been shown to cause considerable DNA damage in walking catfish (*Clarias batrachus*) and mussels (*Mytilus galloprovincialis*) (Micic *et al*., 2004; Ateeq *et al*., 2006) as well as affecting metabolism and histopathological parameters in fresh water fish species, silver catfish (*Rhamdia quelen*) and piava (*Leporinus obtusidens*), (Cattaneo *et al*., 2008; da Fonseca *et al*., 2008). Levels of acid herbicides have been detected in UK rivers, a river (Great Ouze) situated in agriculturally impacted lowland (Neal *et al*., 2000). Levels of Mecoprop (0.5µg l^{-1}), 2,4-D

(0.2μg l^{-1}) and MCPB (very low levels) were all detected in the river during the study (Neal *et al*., 2000). 2,4-D has a water solubility of 46mg l^{-1} at 25°C and is rapidly degraded in soils with an estimated half-life of less than seven days (Cox, 2002). 2,4-D has a low K_{oc} (20) so therefore is less likely to bind to particulates in the environment (Vogue *et al*., 1998). Sarikaya and Yilmaz (2003) found that exposure to 2,4-D induced behavioural abnormalities in the common carp (*Cyprinus carpio* L.) from a range of concentrations (48-96mg l^{-1}) and a calculated LC_{50} of 63.24mg l^{-1} for this acid herbicide.

The triazine group of contaminants includes a number of widely used herbicides. Triazines have a high solubility in water, but are also quite stable in soils (Lentza-Rizos, 1996). Atrazine and related triazine herbicides, such as simazine and cyanazine, are widely applied to corn, soybean, wheat and barley for controlling broadleaf weed and grass (Cai *et al*., 2004). The triazine herbicides have a relatively low absorption in soils so easily migrate through it into surface and ground water (Cai *et al*., 2004). Research has shown that exposure of mature male Atlantic salmon parr to sub-lethal levels of water-borne atrazine clearly inhibited their ability to detect and respond to female priming pheromones (Moore and Waring, 1998). Atrazine has been found to penetrate the chorion of zebrafish (*Danio rerio*) eggs and it has been suggested that atrazine could cause physiological effects in embryonic cells of zebrafish (Wiegand *et al.*, 2000; Mukhi, 2005) and the fathead minnow (*Pimephales promelas*) (Burton *et al*., 1994). Atrazine toxicity to some salmonids has been shown to be relatively low 4.5-8.8mg l^{-1} (Forrest and Caux, 1989).

Environmentally relevant concentrations of atrazine have been shown to have significant effects on fecundity in fathead minnows (Tillitt *et al*., 2010). Environmentally realistic levels of atrazine have been shown to not be directly lethal for red drum (*Sciaenops ocellatus*) larvae but effects on growth and behaviour were noted which could have an effect on survival during later development stages (del Alvarez and Fuiman, 2005). Additionally, atrazine exposure to *Daphnia magna* has shown to induce early development arrest, curved apical spine and underdeveloped antennae (Palma *et al*., 2009). Atrazine is an ubiquitous and persistent contaminant of ground water and has found to have more severe effects when combined with other pesticides. Hayes *et al*. (2006) noted that mortality of *Rana pipiens* and *Xenopus laevis* was more recognised in a nine-compound mix pesticide exposure (35%) compared to a atrazine as a single pesticide exposure (4%).

Although the roles of each individual pesticide is not able to be identified, it was suggested that that some single pesticides which have no effects alone may act as 'enhancers' to other pesticides acting as 'effectors' and magnify the effects as a combination (Hayes *et al*., 2006).

It is unclear how well triazine herbicides bind to sediment. However, it has been found that triazines are practically unaffected by microbial or hydrolytic degradation processes (Knuesli *et al*., 1969; Gamble *et al*., 1983) and in time the aqueous concentrations of triazines are likely to decrease with time as they will bind to bottom sediments due to the moderate K_{ow} (octanol-water partition coefficient) and K_{oc} (sorption coefficient) values (Tomlin, 2000). Atrazine has a water solubility of 33mg l^{-1} and a half-life of 35-50 days in soil (Cox, 2002). Atrazine also has a K_{oc} of 100 and has a high pesticide movement rating (Vogue *et al*., 1998) which indicates that the compound may be more bioavailable in the aqueous phase, however it still may have a tendency to bind to particulates. Other triazine compounds have slightly different characteristics which may suggest a different behaviour within the environment. Cyanazine, unlike atrazine, has a low pesticide movement rating and higher water solubility (170mg l^{-1}) and K_{oc} (190) (Vogue *et al*., 1998). However, propazine and simazine have more similar characteristics to atrazine with a high pesticide movement rating and comparable water solubility (8.6mg l^{-1} and 6.2mg l^{-1} respectively) and K_{oc} (154 and 130 respectively) (Vogue *et al*., 1998). A study on the Humber River found that commonly used agricultural herbicides, such as atrazine and propazine, which have a high water-solubility, were also detected within sediment (Long *et al*., 1998). However, the use of atrazine as a non-agricultural substance has been banned in the UK since 1993, with the enforcement of an EU ban since 2004 (Pesticide Action Network). Moore *et al.* (2000) found atrazine concentrations below the lower detection levels in all sediment samples collected from constructed wetland cells at the University of Mississippi Field Station. Additionally, it has been suggested that phytotoxicity is generally determined by the waterborne atrazine, rather than the sediment-bound (Forney and Davis, 1981). Little is known about the effects of river water and sediment on the toxicity and bioavailability of herbicides to vertebrates (Phyu *et al*., 2006). Research has also shown that the type of water and the presence of sediment will not significantly affect the bioavailability of atrazine (Akkanen *et al*., 2001; Phyu *et al*., 2005; Phyu *et al*., 2006).

There is little information on the acceptable levels of pesticides and other contaminants which are found in freshwater river systems. Table 1.2 shows the guidelines for drinking water quality (WHO, 2006) to give an indication of a benchmark value for environmental levels. However the toxicity of these compounds for aquatic organisms may still vary from the set values listed for drinking water quality.

Table 1.2 Guideline Values for Drinking Water Quality (adapted from WHO, 2006).

Compound	Guideline Value
Aldrin and Dieldrin	0.03 µg l^{-1}
Atrazine	2.00 µg l^{-1}
Cyanazine	0.60 µg l^{-1}
2,4–D	30.0 µg l^{-1}
DDT (and metabolites)	1.00 µg l^{-1}
Endosulfan	20.00 µg l^{-1}
Endrin	0.60 µg l^{-1}
Heptachlor	0.03 µg l^{-1}
Lindane	2.00 µg l^{-1}
MCPA	2.00 µg l^{-1}
Mecoprop	10.00 µg l^{-1}
PAH	0.70 µg l^{-1}
Simazine	2.00 µg l^{-1}

1.4.2 Polycyclic Aromatic Hydrocarbons

As well as diffuse pollution from agricultural sources, urban run-off is another entry pathway of pollutants into water courses. The development of buildings and roads can lead to increased run-off, thus an increased risk of pollution (Crisp, 1993). Moreover, emissions from outboard motors are also a route of entry and have been shown to delay fish embryonic development (Koehler and Hardy, 1999). Polycyclic aromatic hydrocarbons (PAHs) exist as a component of crude oil, and the by product from the chemical conversions of compounds such as steroids, coal, asphalt, creosote and roofing tar (Lyons, 1997). Neat crude oils generally contain fractions that consist of roughly 50-60% naphthalenes, 40-50% tricyclic compunds (fluorenes, dibenzothiophenes and phenanthrenes) and 1-3% chrysenes. Higher molecular weight PAHs, such as Benzo-[a]-Pyrene usually constitute <1% of the total PAHs in crude oil. PAHs are directly released into the environment from oil spills and the incomplete combustion of carbon-containing fuel such as diesel, wood, coal and tobacco (Lyons, 1997). Many PAHs are categorised as persistent organic pollutants (POPs) due to their environmental significance and potential toxicity to organisms (Chen *et al.*, 2006).

In the environment, lipophilic contaminants, such as Halogenated Aromatic Hydrocarbons (HAHs), Polychlorinated Biphenyls (PCBs) and PAHs readily bioaccumulate in fish and the bioaccumulation of these lipophilic chemicals by adult fish may have significant consequences on the development and survival of their offspring (Ostrander, 1988). HAHs and PAHs translocate from adult female body stores into eggs during oocyte maturation and early life stages of fish are often more sensitive (Ostrander, 1988). Comparison of bioaccumulation of low metabolisable compounds such as PCBs with that of PAHs may give a good indication whether PAHs have been accumulated and then metabolised (Petersen and Kristensen, 1998). Beyer et al. (1998) found that Atlantic cod accumulated PAHs from particulate matter in the seawater, however PAHs in the upper layer of sediment was not bioavailable to the fish.

Malformations, yolk sac oedema, premature hatching, jaw reductions and spine curvation have all been shown to be consequences of PAH exposure to fish embryos (Barron *et al.,* 2004; Carls *et al*., 1999; Heintz *et al*., 1999; Hose *et al*., 1996; Incardona *et al.*, 2004). Incardona *et al.* (2004) demonstrated that yolk sac oedemas formed in zebrafish larvae when exposed to phenanthrene. Additionally, PAH-exposed pink salmon embryos demonstrated delayed hatching and absorption of the yolk-sac (Carls and Thedinga, 2010). It has also been found that the genotoxicity of PAHs correlates with sediment-bound concentrations but not with levels detected in the water column (Barbee *et al.*, 2008). Research by Laetz *et al.* (2009) showed the importance of synergistic toxicity of pesticides on Pacific salmon by suggesting that single-chemical assessments could underestimate actual risks to aquatic organisms.

1.4.3 Sediment-Bound Contaminants

The partitioning of hydrophobic organic contaminants between particulate and dissolved phases controls their environmental fate and availability to aquatic organisms (Menon and

Menon, 1999). Moreover, bioturbation has been shown to significantly increase the release of organic contaminants to the water column, especially when the sediment is enriched with contaminants (Skei *et al*., 2000). Therefore, water samples alone could fail to address the impact of sediment-associated contaminants or the potential store of contaminants available for release, during sediment re-suspension.

Studies by Long *et al.* (1998), has shown the presence of a wide range of organic contaminants associated with bed-sediments in North-East England river catchments. PAHs were detected most frequently reflecting widespread atmospheric deposition and *in situ* production from industrial and domestic emissions, such as vehicle exhausts. Moreover, more water soluble agricultural herbicides, such as atrazine and propazine, were also regularly detected within sediment samples (Long *et al.*, 1998).

Contaminated sediments have been shown to induce avoidance or reduction in feeding intensity in spot (*Leiostomus xanthurus*) juveniles suggesting the potential for consequent detrimental biological effects (Hinkle-Conn *et al*., 1998). A study by Strmac *et al.* (2002) observed increased mortality in zebrafish exposed to polluted river sediments. Additionally, sublethal effects have been noted in brown trout and zebrafish embryos exposed to sediment-bound contaminants (Luckenbach *et al.*, 2001; Hollert *et al.,* 2003). Brown trout embryos that were exposed to contaminated sediments were found to exhibit retarded development, slower growth and increased mortality rates (Luckenbach *et al.*, 2001). In zebrafish, increased mortality rates, retarded development of the eyes and ears and the development of oedemas of the heart and yolk sac were commonly observed effects of exposure of embryos to contaminated sediments (Hollert *et al.,* 2003).

Schafer *et al.* (2008) surveyed a number of small streams in France and found a number of particle-associated polar and semi-polar pesticides. Polarity essentially determines the behaviour of the pesticide, and the tendency of the pesticides to bind to particulates in the environment. A polar pesticide will tend to be very water soluble and, generally, will not bind to particulates, whereas a non-polar compound will probably bind more strongly to sediments (Linde, 1994). So a semi-polar pesticide will be more likely be less soluble than a polar compound but may also bind to particulates. Schafer *et al.* (2008) concluded that particle-bound contaminants in aquatic environments require much more attention as they may represent major stressors for aquatic ecosystems. Additionally, Keiter *et al.* (2006) concluded that the bioavailability of particle-bound lipophilic substances in sediments was higher than generally assumed. As salmonid early life stages have a long period in the intra-gravel environment, any sediment-bound pesticides infiltrating salmonid spawning gravels potentially have a prolonged period to interact with eggs and alevins.

1.5 Summary of Aims

The major aim of this thesis was to investigate the potential impact that environmental contaminants may have on salmonid early life stages. One aim was to identify the levels of contaminants present in natural salmonid spawning gravels in the River Avon catchment and to monitor the effects on survival and development, as well as to determine the consequences of the contaminants at environmentally relevant levels. As discussed, it is clear that factors operating in the freshwater environment require further attention as factors that may be affecting wild salmon populations. The role of freshwater pollution has been linked to specific population declines, despite the fact that the effects of many contaminants remain unclear. The aim of these studies was therefore to measure contaminants found in the freshwater environment, so that there is a clear indication of the contaminants present in natural spawning gravel sites during the spawning season. Moreover, the focus of the studies was on early life cycle stages which could be considered to be the most vulnerable and to have the greatest potential impact on affecting recruitment. The objective was to describe the nature and extent of the impact of waterborne and sediment-bound pesticides derived from intensive agriculture and urban run off on intragravel survival and development of salmonid embryos. Survival and successful development of the intragravel life history stages of salmonids are fundamental to juvenile recruitment and the long term survival and success of salmonid stocks. Environmental chemists have researched some sediment-bound pesticides (Gomez-Lahoz and Ortega-Calvo, 2005; Chen e*t al*., 2006; Zhou *et al*., 2006), and biologists have assessed the quality of spawning grounds (Crisp, 1996; Heintz *et al*., 1999; Malcolm *et al*., 2003) from an organism aspect. However, no research has attempted to examine the impacts of sediment-bound pesticides in redds and the survival success of salmonids.

The results of the studies aim to demonstrate how survival, development, and emergence may be affected by specific environmentally-relevant contaminants, which could then be used to inform the direction of further monitoring and conservation measures in the wild.

1.6 Outline of Thesis

The major aim of the thesis was to investigate the role of environmentally-relevant pollutants on the survival and development in the early life cycle stages of salmonids. Chapter One provides the background of the project, including the decline in Atlantic salmon and brown trout populations, the life history of the species including the importance of researching the early life stages, a description of the habitat in which the salmonids development as well as an introduction to the potential freshwater pollutants found in spawning gravels. A more detailed introduction can be found at the start of each experiment chapter. A description of the materials and methods used in all experiments is given in Chapter Two. However, materials and methods, which were specific to each study, are presented in the relevant chapter. Chapter Three investigates the contaminant levels found in field sites, which are known to support wild populations of salmonids, as well as the construction of artificial redds to evaluate the *in situ* survival of both Atlantic salmon and brown trout eggs. Sediment samples and water samples from the salmon spawning habitat were collected and qualitatively and quantitatively analysed. Chapter Four describes three experiments looking at the impact of environmentally relevant water-borne contaminants on the survival and development of brown trout eggs. Exposure experiments were run with a range of pesticides and PAHs, which were detected in the field samples, to assess the effects on survival and development up until hatching. Chapter Five describes three experiments on the effects of sediment-bound pesticides and PAHs on the survival, development and emergence of brown trout. Exposure experiments were conducted using contaminants which were identified in the field samples. Individual and relevant discussions are included at the end of each Chapter. Finally, Chapter Six completes the thesis with a general discussion about the studies presented, their relevance to the decline of salmonids in the wild, and a summary of results and recommendations for future work.