6. Chapter Six: General Discussion

The major aim of this thesis was to investigate whether contaminants within the freshwater environment are impacting the survival and success of salmonids in the wild. Salmonid populations are in decline and identification of the exact cause of this reduction has proven to be a major challenge. It has been suggested that the early life stages of salmonids may be vulnerable to the contamination of rivers (Parrish *et al*., 1998). Anthropogenic activities can have profound, and usually negative, effects on freshwater fishes from the smallest streams to the largest rivers (Karr, 1981). The physico-chemical properties of water can be heavily influenced by a range of factors and one of these factors, contaminants (Karr, 1981) can enter water bodies through a number of sources (Carter, 2000). Indeed, any compound in water, which brings about changes in water quality, can impair the development and the survival of fish (Sipaúba-Tavares *et al*., 2007). Water quality is known to be one of the most crucial factors associated with the health of fish species (Malcolm *et al*., 2003b) and the input of contaminants into water bodies can vastly affect the early life stages of fish, potentially resulting in poor survival, which in turn leads to poor recruitment and a potential decline in population (Karr, 1981). Each fish species has characteristic tolerances for water quality (Karr, 1981). The trials conducted within this research suggest that contaminants found within the freshwater environment may indeed have implications for the success of the early life stages and survival of salmonid populations. Any factors which are found to have a potential role in affecting the early life stages of salmonids should be further investigated and used to direct any conservation efforts.

Field trials allowed for a realistic picture of all the factors acting upon and impacting on wild salmonid populations. The River Avon and its tributaries, the River Wylye and River Nadder, were selected for the field trials due to their historical recognition for supporting salmonid populations and the presence of natural spawning grounds. The River Avon system is predominantly made up of chalk streams, therefore the physico-chemical parameters should generally remain stable, especially the flow. Despite the ideal conditions provided by chalk streams, populations of *Salmo salar* and *Salmo trutta* have declined across the UK, including those in the River Avon (Giles, 1989; Jarvie *et al*., 2005;

Summers *et al*., 2005; Heywood and Walling, 2007). Declines may be due to water quality degradation, habitat degradation, or a combination of the two (e.g. high suspended solid loadings and resulting siltation) (Karr, 1981). Chalk streams across the UK have been in decline over the past few decades and this decline is brought about largely by increased anthropogenic activity (Casey and Smith, 1994). In Southern England most chalk streams are heavily utilised, with abstraction for urbanisation, industrialisation and farming increasing year on year as population growth increases. The discharges associated with these activities could also account for the declines in wild fish stocks that are being widely reported (Waring *et al*., 2011). All of the field sites selected for this research varied in respect of riparian zones, physico-chemical properties, as well as water chemistry composition. Some of these differences may have resulted in different survival rates and development traits of the eggs and alevins buried in the rivers. A greater flow rate at the River Avon site may have affected the survival of the eggs and alevins, although an increase in river flow has been shown to improve the conditions for incubating eggs, rather than promote mortality (Bjornn and Reiser, 1991; Geist and Dauble, 1998; Jensen and Johnsen, 1999). It is not clear why the survival rates in the River Avon were so poor, but considering the evidence that modest flow rates can be detrimental to egg survival it appears that other factors are potentially impacting on the survival of the eggs and alevins at this site. It was noted that the sediment and nutrient loading were generally greater into the River Avon compared to the other sites. These may account for the lack of survival in the first trial and final year of the trials. The site was adjacent to a grazed field, used for dairy herds, which may have also been the cause of greater sediment loading into the stretch of the river which was analysed. This increased sediment loading and increase in eutrophication could also help to explain the lower survival rates at the Avon site, despite an increase in flow rate. Studies have shown that the intensification of agriculture on sites adjacent to rivers increases the diffuse input of phosphorus and nitrate which can lead to eutrophication (Casey *et al*., 1993). Eutrophication has been found to be detrimental to fish populations, due to the bloom, and subsequent crash, of algae, causing hypoxia, or the release of toxins from certain species of algae. The toxins from such algal blooms have been found to influence entire ecosystems and cause large fish kills (Rosenberg and Lindahl, 1988). Eutrophication was observed to have deleterious effects on fish communities in low-order streams in Ohio when total inorganic nitrogen (TIN) and dissolved inorganic phosphorus (SRP) concentrations exceeded 610 and 60 mg m^{-3}

respectively (Miltner and Rankin, 1998). It was also discovered that the biotic integrity of aquatic ecosystems was negatively correlated with increased nutrient loading, with a loss of pollution sensitive fish and top predators (Miltner and Rankin, 1998). Such studies indicate that the poor survival at the River Avon site could be due to high grazing activity adjacent to the river, which in turn affect the early life stages of salmon. Such findings could suggest that a greater buffer zone should be introduced by land owners and managers to livestock fields which are in close proximity to rivers. It is possible that the sedimentation from the grazing animals is having a negative effect on the survival of the early life stages of salmonids. A well planned and managed riparian zone can also create a stable bank environment, which again can reduce the erosive qualities of unplanted banks. In areas where there is no riparian zone, storm run-off has been witnessed to erode large tracts of land as it finds its way in to the water body. In well planted areas the buffer strip can also act to trap sediment, preventing some of the soil and related contaminants from entering the water.

Some of the sites were located in close proximity to carparks and roads which may have contributed to the greater observed levels of sediment-bound PAHs. Road runoff has been found to increase both sediment loading and PAH levels in river systems (Krein and Schroder, 2000; Brown and Peake, 2005; Mangani *et al.*, 2005). Both water and sediment samples were taken periodically and it is possible that this method of monitoring salmonid habitat quality (including parameters such as nutrients, dissolved oxygen, temperature, pesticides, and other toxics) could have missed short-term events that may be critical to assessment of biotic impacts (Karr, 1981). Studies have shown that some activities, such as crop spraying, and storm events can increase the availability of contaminants into the freshwater system (Schulz, 2001). Therefore, sampling just after such an event or even missing an event may drastically alter the recorded levels of contaminants in the water, and possibly even the sediment (Schulz, 2001). The actual concentration of the sediment-bound contaminants present in the substrate is unlikely to alter dramatically following a storm event. However, sediment from the banks which already contains contaminants may be washed into the water course. This could also significantly increase the sediment loading into the rivers, resulting in a greater proportion of fines, which could potentially reduce survival of incubating eggs and alevins. Drainage channels which run parallel to waterways may prevent the road run off from entering the water system. However, not every situation is suitable for this approach and the questions still remain as to what happens to the water and associated contaminants that enter these ditches. However, such a system would still decrease the total amount of inputs of contaminants into small stretches of river where the impacts could be cumulative. It is apparent that there was a degree of variability of contamination between sites. Location of spawning grounds close to areas of high agricultural use or areas of dense urbanisation can also mean that various populations of salmonids can be affected at different rates by contaminants entering river water.

The salmonid redd is both a dynamic and complex environment which is affected by a number of factors, such as flow, temperature and gravel size. These can operate independently or collectively to influence survival success and potentially alter the development of salmonids during the early life stages. Complex interactions between hyporheic dissolved oxygen concentrations, physico-chemical parameters, substrate composition, water-borne contaminants and sediment-bound contaminants may exist and these processes may all affect the success and development of the early life stages of *Salmo* species. In order to ascertain the level of impact that pesticides may have on spawning gravels, laboratory trials were carried out. These exposed salmonid eggs and larvae to environmental levels of contaminants, both in the water and sediment, in a controlled environment. This was carried out in order to eliminate the effects of external influences, such as flow rates, disturbance and decreased levels of dissolved oxygen that may be experienced in redds within the natural environment.

The measured levels of water-borne contaminants were replicated in the laboratory environment so that direct effects of these pollutants could be monitored. The water exposure trials examined individual compounds, as well as groups of compounds (which would better reflect the conditions in the natural environment). Some treatment dependent morphological and biochemical effects were observed during the trials, which could suggest that water-borne contaminants at environmental levels could potentially affect the development of salmonids during the early life stages. However, when individual compounds were used in isolation (i.e. naphthalene, atrazine and phenanthrene) no significant effects were observed on exposed eggs or larvae when the control groups and the treatment groups were compared. This may suggest that some pollutants function synergistically with other pollutants. Hayes *et al.* (2006) suggested that individual pesticides which produce no effects alone may act as 'enhancers' that worsen the effects of pesticides that act as 'effectors' when combined with other pesticides.

As very few significant effects were observed for the water exposure trials it is difficult to ascertain to what extent water-borne contaminants affect survival and development of salmonids during the early life stages. It was believed after the initial trials, the lack of effects may be a result of the exposures only occurring once the eggs had already water hardened and the eye stage had been reached. Therefore, the final trial used eggs which were fertilised using contaminated water so the eggs water hardened in the pollutants of interest. Again, the results showed that there was a limited impact of exposure to these contaminants on both the egg and alevin stage. Similarly, exposure of alevins (without the protection of the chorion) to these contaminants showed few effects. In order to relate the results to environmental conditions it was imperative that levels of contaminants mimicked those measured in salmonid rivers. Further research, exposing the eggs to lethal doses of environmental contaminants, may help to develop an understanding of how the pollutants affect the salmonid eggs and alevins. Also, it is possible that the contaminants may have a genotoxic effect rather than acute effects. Allowing the trials to continue until at least the emergence stage and running genotoxicity tests may have uncovered the potential of the contaminants to impact the early life stages of salmonids. Although the findings in this study have proved largely inconclusive in terms of specific impacts of contaminants on the early developmental stages of both brown trout and Atlantic salmon, the fact remains that the inputs by farming and other sources of contamination are potentially having a deleterious effect on wild fish populations.

Farming activities are on the increase and the inputs from farms are able to easily enter the waterways. Many fisheries across the UK, and indeed globally, are located adjacent to farmland, which in turn increases the likelihood of agriculturally related contaminants directly entering the streams and rivers. The threat to fisheries in terms of agricultural inputs in these areas is therefore increased and strategies are required to be implemented in order to restrict the inputs of pesticides and other contaminants into water bodies. This puts fishery managers and other environmentally focused organisations under increasing pressure to act in order to prevent the potential decline of valuable fish stocks. However, there is often conflict between land owners and fishery managers in deciding how best to utilise the available resources. It is evident that conflict of interest often arises as a result of differing needs of both parties and these should be resolved prior to any strategic developments and conservation efforts are put in place. Ideally, the source of the contaminant input should be investigated and if possible stopped, but with increasing global pressure for food production will be a challenge.

The Environment Agency are responsible for some of the water quality monitoring in the UK, however the exact levels of contaminants which the salmonids are actually exposed to is still not fully understood. The behaviour of compounds in the environment is dependent on many variables such as the K_{ow}, K_{oc} and water solubility, as well as the composition of the substrate and quantity of humic substances and dissolved organic matter. The development of passive samplers and solid phase micro-extraction (SPME) techniques in the field will potentially lead to a more accurate method of monitoring contaminants in the environment (Vrana *et al.*, 2007).

It was important that any measured levels of sediment-bound contaminants in the environment were tested under controlled laboratory conditions in order to ascertain any effects on the early life stages of salmonid fish. These exposure trials eliminated any other influencing factors which may also affect the survival and development of *Salmo* species, such as physico-chemical parameters, nutrient loading and predation, which would be apparent in the natural environment. The sediment exposure trials were designed so that any monitored effects could be attributed to the sediment-bound contaminants. There were several effects which were noted during the trials which could indicate that sediment-bound contaminants may indeed be having a negative effect of the success of the salmonids in the natural environment. However, overall survival of the contaminant exposed salmonids did not differ to those of the controls, so at a population level there is little evidence of any impact.

Fluctuating asymmetry (FA) was investigated to determine if there were any significant differences in the meristic traits of the fry. FA has been proposed as a tool to monitor stressors in the environment (Allenbach *et al.*, 1999). FA has also been used in various studies as a potential indicator of genetic stresses, such as inbreeding (Wagner, 1996), captivity stress (Almeida, 2008) and the overall condition of the larvae (Somarakis *et al.*, 1997). Many studies have examined FA as an indicator of pollution. In this study FA was used for brown trout fry in order to establish whether paired morphological features are affected by sediment bound organic pollutants. The fin rays of brown trout fry were shown to display fluctuating asymmetry, with more fin rays being present in the right fins than the left. However, these findings did not show a treatment dependent effect. Allenbach *et al.* (1999) found pelvic fin rays to show low asymmetry when exposed to pesticides. Similar finding have been recorded in the perch (Østbye *et al.*, 1997). The findings suggest that the contaminants used in this study, at environmentally relevant concentrations, do not have an effect on the fluctuating asymmetry of *Salmo trutta*. However whether such traits would be observed at a later stage of development still remains to be seen.

The most interesting effects noted for the sediment-bound contaminant trials were the potential genotoxicity of the compounds. The comet assay revealed that some sediment-bound contaminants (OCPs and PAHs) have the potential to cause DNA damage to salmonids. This DNA damage was observed at the fry stage of development which suggests that the exposure to the contaminants from fertilisation did in fact cause potential harm at a later stage of development. It is only whilst the eggs and alevins remain in the gravel that the sediment-bound contaminants could directly impact the salmonids. However if damage at a molecular level is being caused over such a short period of time then there is potential that this damage could also cause problems during later stages of development. Experiments with prolonged exposure to contaminants and allowing the development of the *Salmo trutta* to continue through to adult life stages would allow for a better understanding of the genotoxic effects of certain compounds found in the natural environment.

Although some significant effects of sediment-bound contaminants were noted (in particular the genotoxic effects) there was no significant difference in the survival rate or conclusive evidence for dramatic morphological or biochemical effects. This may have been a result of the bioavailability of the compounds to the incubating salmonids. In the laboratory environment there were no invertebrates, which are known to release compounds from the sediment surface through bioturbation (Goedkoop and Peterson, 2003). The same process may be ocurring in the natural environment as sediment interactions are dynamic and complex. It could be that sediment-bound contaminants, such as pesticides, may not be available to sediment-dwelling organisms because they are bound to sediment particles. However, sediment pore water plays an important role in sediment-water sorption chemistry and chemical bioavailability. The amount of pore water available for desorption of sediment-bound organic chemicals depends on sediment porosity. This in turn is determined by the particle size distribution and the degree of compaction.

As commercially important species, Atlantic salmon and brown trout are well studied and there are other species of fish which also have an intragravel period of their life cycle that should be considered. For instance the larval lamprey may spend a number of years within gravel before developing and migrating downstream (Sawyer, 1959; Potter, 1980). The juvenile eel also spends a period partially buried in the substrate (Jellyman and Chisnall, 1999; Edeline, *et al*., 2006). It is likely that these species may be impacted by sediment derived contaminants. It is recommended that research is also undertaken on these species. Therefore, there is a requirement to fully understand the potential impact of the interactions of diffuse contaminants on the intragravel stages of salmonids within the context of providing advice to protect the freshwater environment. The impact of the waterborne contaminants may also affect other course fish which remain largely unstudied. It appears from an ecologically viewpoint the importance of lesser studied species needs to be noted and the impacts of pesticides and other contaminants needs to be considered. An effect on recruitment of course fish, due to exposure to agricultural contaminants could lead to a loss of population of predatory salmonids.

Although the trials conducted in this thesis did not reveal conclusive results regarding the effects of contaminants on salmonids, it is still suggested that whilst further investigation is undertaken that precautionary measures are taken to protect this important species. The research in this thesis has indicated that contaminants could have sub-lethal effects on the early life stages of salmonids and how this may later impact future stages of development is still unclear. The results do suggest that the conditions of the spawning grounds are important and measures to protect them should be enforced. Improving agricultural practices, coordinating the timings of pesticide applications around the spawning season and introducing buffer zones or barriers to agricultural land may all help to improve the habitat quality of salmonids.

It appears that there is much more work needed in order to fully understand the problems associated with agricultural contaminants and there impacts on fish. It was not within the scope of this study to explore the full range of pesticides and contaminants which enter the rivers and streams. It is felt that much more work is required on a wider range of compounds and that new technologies such as passive samplers, are used to get a better understanding of the range and concentrations of contaminants that exist in the environment. One area of research that needs to be considered is the interaction of these compounds with other contaminants that enter the waterways, such as pharmaceuticals. It seems that much of the literature is focused on one element of pollution and often doesn't consider the interactions of a range of pollutants on any aquatic organisms. There is currently no literature which reports on the measurement of sediment-bound contaminants in natural spawning gravels of salmonids. This study exposed early life stages of two recreationally and commercially important salmonid species to environmentally relevant levels of contaminants. This was carried out in order to investigate the effects of these contaminants (OCPs, PAHs, Acid Herbicides and Triazines) on salmonid early life stages. Some literature exists on the observed genotoxic effects of environmental levels of sediment-bound contaminants on the early life stages of some aquatic organisms (Marvin *et al.*, 1995; Harvey *et al.*, 1997; Incardona *et al.*, 2004; Barbee *et al.*, 2008). However, as far as the author is aware there is no literature to date on these effects in the early life stages of any salmonid species. Few studies have measured *in-situ* levels of water-borne contaminants and mimicked these levels in a laboratory environment in order to test the toxicity of those concentrations to both eyed and newly fertilised salmonids. The results from such exposure trials could potentially be used to model the survival and development success of wild salmonid populations during their early life stages. These observations will hopefully provide a better insight to the problems associated with environmental stressors in the aquatic environment, such as contaminants. Long term monitoring of pesticide and hydrocarbon levels present in rivers, which support salmonid populations, is essential to understand the trends of concentrations of these contaminants. Passive samplers, such as the chemcatcher® (as discussed in Chapter 3) would give a more accurate assessment of pollutant levels over a known time. This would eliminate the problems associated with spot sampling, such as missed events (run-off and agricultural activity) (as discussed in Chap. 3). The genotoxicity results obtained from the sediment-bound contaminant trial have offered an evaluative insight into the possibilities of predicting future effects of pollutants on salmonids. The comet assay has proved to be a useful tool in analysing these potential effects and could be further used in future.

This research also focused on the early life stages of salmonids. This needs further developing to monitor the long-term effects of these compounds on the development and survival of later life stages. It is possible that exposure to contaminants, such as atrazine and simazine, could cause poor condition factors in adults or lead to production of poorly developed gametes. It has been noted that exposure to certain triazines can reduce the efficiency of immune defence mechanisms in goldfish (Fatima *et al*., 2007). This could suggest that other biomarkers are important in determining the toxicity of compounds to fish. This in turn could cause population declines within areas where agricultural inputs are high. There is also the impact of stages such as smoltification that needs consideration. Waring *et al.* (2011) found that exposure to trout farm effluents affected the ability of Atlantic salmon to survive at sea. It is possible therefore that exposure to other river contaminants will have similar effects, potentially creating high mortality rates during the migration process which will lower annual recruitment.

The recent climate change scenarios produced for the United Kingdom predict that there will be a general warming of many rivers, particularly in the south, with a rise in temperature of 2-3 ºC. In addition, predicted rainfall will decrease in the south of England reducing river flow and run-off. The modifications to the river characteristics, including flow, will be further exacerbated by the increased requirement for water for domestic, industrial, aquaculture and agricultural with pressure on local government and the Environment Agency for increases to abstraction licences. Under these changes to the freshwater environment there is a serious concern that diffuse pollution will have a further more significant impact on the intragravel stages of salmonids. Higher water temperature could also increase the toxicological effects of contaminants on fish whilst reduced flows will reduce the levels of oxygen within the spawning gravels, and further concentrate the levels of contaminants.

Continued research is required to combat the declining populations of salmonids in UK and worldwide river systems. There is still a great deal that is not fully understood about the life cycle of the salmonids. The early life stage component of the salmonid life cycle has been shown to be important for the recruitment and survival of the species. However, many questions still remain over the impact that the marine phase has on the future of the species. The pressure during this phase could affect the reproductive cycle. With fewer migratory salmonids returning to UK rivers annually there is further diminishment of the gene pool. Allied to the effects of contaminants, described in this study, it is clear that much more focus and research is required across the entire life cycle in order to fully mitigate the decline of these incredible species.