THE IMPACT OF ENVIRONMENTAL LEVELS OF PERSISTENT AQUATIC CONTAMINANTS ON ATLANTIC SALMON

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Introduction

During the last decade, DEFRA has funded research within the Salmon and Freshwater Fisheries Team at the CEFAS Lowestoft Laboratory in order to investigate the effects of environmental levels of persistent aquatic contaminants on Atlantic salmon and sea trout.

The research has focused primarily upon the impact of agricultural pesticides on sensitive stages in the life history of salmonids, in particular reproduction, embryo survival and development, the parr-smolt transformation and marine survival. Initial studies at Lowestoft identified a pheromone that is involved in priming the reproductive system of the male fish just prior to spawning. The pheromone (an F-series prostaglandin) is released by the female in her urine and, when detected by the olfactory system of the male fish, results in an increase in plasma reproductive steroids (17,20βP; testosterone; 11-ketotestosterone; GtH II) and the levels of expressible milt. Both the olfactory and endocrine responses to the pheromone have been used as sensitive bioassays to assess the impact of four generic pesticides on olfactory mediated reproduction in the salmon. More recently, the studies have been expanded to examine the impact of mixtures of pesticides on these sensitive stages in the life cycle and also on the migratory behaviour of emigrating smolts

The four common pesticides studied were diazinon, carbofuran, atrazine and cypermethrin, which are all known to be contaminants of both ground and surface waters in the UK. Each pesticide was selected because routine monitoring of the aquatic environment had shown that they occurred in a number of rivers and tributaries supporting spawning salmon at critical periods during the freshwater life history. In addition, the concentrations that the fish were exposed to during the experiments were chosen to reflect environmental levels within the ranges measured during routine monitoring by the Environment Agency.

1. Diazinon (Organophosphate pesticide).

Diazinon (*O.O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothiate: IUPAC) is an organophosphate (OP) pesticide and one of the active ingredients used in dips to prevent and treat ticks, lice and scab on sheep. Diazinon has a proposed Average Annual Environmental Quality Standard (EQS) of 0.01μg l^{-1} and a Maximum Admissible Concentration (MAC) of 0.1 μg l^{-1}in the aquatic environment. However, levels of diazinon within the range 18.5 - 35 μg l^{-1} have been measured in spawning tributaries.

- ***Reproduction***

Diazinon was demonstrated to have a sub-lethal effect on the olfactory system of the salmon, reducing the ability of the male fish to detect and respond to the priming pheromone that is important in synchronising reproductive physiology and behaviour in salmon. The olfactory system of the male was significantly affected by diazinon after exposure to concentrations as low as 0.4 $\mu g\ l^{-1}$. The endocrine response of the male salmon to the pheromone was also reduced after exposure to significantly lower concentrations of diazinon (0.06 $\mu g\ l^{-1}$).

- ***Embryo development***

Exposure of salmon embryos to environmental levels of diazinon resulted in significant mortalities at concentrations between 5 and 10 $\mu g\ l^{-1}$. Sub-lethal effects were evident in the surviving embryos and these included significantly increased levels of steroids (e.g. testosterone and 17,20βP), increased levels of cortisol and reduced levels of the thyroid hormones T_3 and T_4. The results suggest a significant effect on the growth and development of the embryos and may have implications when assessing effective egg deposition and determining whether spawning targets have been met in rivers.

2. Carbofuran (carbamate pesticide)

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) is a water-soluble systemic insecticide used on winter crops. Carbofuran has not been designated an EQS for the aquatic environment, but has been measured at concentrations up to 26 $\mu g\ l^{-1}$during autumn/winter.

- ***Reproduction***

The carbamate pesticide also had a similar deleterious sub-lethal effect as diazinon on pheromonal mediated endocrine function in mature male Atlantic salmon parr at concentrations as low as 2.7$\mu g\ l^{-1}$. Carbofuran directly effected the olfactory system of the male salmon reducing the ability of the male fish to detect and respond to the female priming pheromone Prostaglandin $F_{2\alpha}$. Relevant plasma reproductive steroids (17,20βP; testosterone; 11-ketotestosterone) were not elevated in the males and there was a significant reduction in the production of sperm.

3. Atrazine (triazine herbicide)

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a water-soluble pre- and post-emergence herbicide for the control of annual and perennial grass and annual broad-leaved weeds. Atrazine is known to have high mobility through soil and is a known contaminant of aquatic ecosystems in England and Wales. In 1992 and 1993, atrazine was one of the 5 pesticides most frequently present in both ground and surface water at levels in excess of the Maximum Admissible Concentration (MAC) 0.1 $\mu g\ l^{-1}$ imposed by the Water Act 1991. In addition, analyses of UK surface waters demonstrated levels exceeding the proposed Environmental Quality Standard (EQS) of 2.0 $\mu g\ l^{-1}$ based on the annual combined average of atrazine and simazine. Since 1993, the use of atrazine has been banned on non-cropped land and as a result there has been a decline in its detection in UK surface waters. However, its main use now is in the production of maize, particularly in SW England. Concentrations up to 275 $\mu g\ l^{-1}$ have been detected in run-off water from agricultural land, but at these concentrations it is considered not to be a risk to aquatic life. Concern regarding the toxic properties of atrazine is now prevalent in North America where it is considered to have caused developmental abnormalities in amphibians.

- ***Reproduction***

Exposure to environmental levels of atrazine (0.04 - 14.0 $\mu g\ l^{-1}$) resulted in a sub-lethal effect on the olfactory system of the salmon, reducing the ability of the male fish to detect and respond to the male priming pheromone, Prostaglandin $F_{2\alpha}$. Both plasma 17,20βP levels and sperm production were significantly reduced at concentrations of 0.04 µg l^{-1} and above. Atrazine also had a further direct impact upon the testes of the male salmon modifying the release of androgens and suggesting an additional toxic mechanism affecting reproduction. Previous studies on endocrine disrupting chemicals in UK rivers and streams have indicated that many are oestrogen mimics and act as xenoestrogens. However, it would appear that atrazine also modifies the production and metabolism of the androgens in the male which are involved in reproduction.

It is not evident what the long-term impact of exposure of Atlantic salmon to atrazine is in relation to reproductive function and population viability. However, the rapid accumulation of atrazine by salmonids, the direct effect of the pesticide on steroid synthesis of the testes and inhibition of the priming response suggest that male fish exposed to atrazine may be at risk. Of particular concern is the spring run component of Atlantic salmon populations, which are resident within freshwater, and may therefore be exposed, for longer periods. Exposure to atrazine may either enhance the males' reproductive status too early or delay it, and this may subsequently have adverse effects on the motivational mechanisms controlling the run-timing of the fish and subsequent reproductive success.

It is not known what effect atrazine may have on the reproductive biology of mature female salmon. Atrazine, is known to have a direct effect on kidney structure and function in freshwater salmonids. Disturbance to renal function may also inhibit the release of pheromones within the urine of the female even if there had been no initial effect on reproductive status.

- ***Smoltification and marine survival***

The research on atrazine was further extended to examine its impact upon smolt physiology and the adaptation of juvenile salmon to saltwater. Smolts that were exposed to environmental levels of atrazine in freshwater were physiologically stressed at concentrations of 6.5 µg l^{-1}, as evidenced by increased plasma cortsiol, osmolarity and monovalent ion concentrations. Subsequent exposure of the smolts to seawater for 24 hours after prior exposure to sub-lethal levels of atrazine in freshwater, resulted in 14-28% mortality. The transfer of the smolts to saltwater after exposure to atrazine elevated plasma thyroxine and reduced the gill Na^+K^+ ATPase activity in the smolts, which is an indication of the seawater adaptability and osmoregulatory capabilities of fish. Exposure to atrazine in freshwater may therefore reduce the subsequent marine survival of salmon smolts and post-smolts. The main implication from this work is that the freshwater and marine phases of the Atlantic salmon and sea trout cannot be separated. The freshwater history of the juvenile salmonid (e.g. growth rates, exposure to water quality) plays a significant role in modifying the survival and behaviour in the marine environment.

- ***Smolt migration and run-timing***

Atrazine has previously been demonstrated to directly effect the parr-smolt transformation by modifying the physiological mechanisms associated with adaptation to the marine environment. A further effect on the smoltification process is a significant delay and in certain instances inhibition of downstream migratory behaviour during the Spring emigration. Recent studies in collaboration with the University of Stockholm have demonstrated that exposure of pre-smolts to low levels of atrazine inhibits migratory behaviour so that fish either do not migrate or there is a significant delay to the emigration. This has particular significance to the survival of smolts in the marine environment as previous telemetry studies at CEFAS have indicated that there is a very brief window of time for successful entry of smolts into the open sea. Smolts missing this brief window have reduced survival and return rates as adults.

4. Cypermethrin (synthetic pyrethroid pesticide).

Cypermethrin [(*R,S*)-α-cyano-3-phenoxybenzly (1 *R,S*)-*cis, trans*-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylate] is a synthetic pyrethroid (SP) insecticide, which is increasingly being used as the active ingredient in sheep dips to replace the organophosphates (OPs). Although less toxic to humans than OPs, SPs are significantly more toxic to aquatic invertebrates and fish. Cypermethrin has a proposed Average Annual Environmental Quality Standard (EQS) of 0.0001µg l^{-1} and a Maximum Admissible Concentration (MAC) of 0.001 µg l^{-1}in the aquatic environment. However, levels of cypermethrin in excess of 0.85 µg l^{-1} have been measured during routine monitoring of surface waters.

- *Reproduction*

The research on cypermethrin was expanded to investigate its effects on pheromonal mediated endocrine function in mature male salmon parr; sperm motility; fertilisation rates and embryo growth and survival. Low levels of cypermethrin (> 0.001µg l^{-1}) were demonstrated to have a sub-lethal effect on the olfactory system of the salmon reducing the ability of the male fish to detect and respond to the female priming pheromone. As a consequence, there was a significant reduction in the sperm produced by the spawning male salmon. Exposure to 0.5µg l^{-1} cypermethrin also reduced the motility and life of the sperm in water suggesting a secondary sub-lethal effect on salmon reproduction.

- *Embryo Development*

Exposure of eggs and milt to cypermethrin during fertilisation within a hatchery had a significant effect on the subsequent development of the eggs. At cypermethrin concentrations of 0.028µg l^{-1} and 0.33 µg l^{-1}, 62% and 74% of the eggs were not fertilised respectively. During exposure of the surviving eggs to the pesticide there was a significant decrease in egg weight and ionic content at all concentrations. A direct effect on fertilisation rates and embryo survival may have implications for salmon spawning targets and juvenile production in many rivers supporting salmonids.

- *Smoltification and marine survival*

Exposure of salmon smolts in freshwater to cypermethrin caused 50% mortality in the highest dose group (0.5µg l^{-1}). In all the other groups, the smolts were physiologically stressed at concentrations as low as 0.001µg l^{-1}, as evidenced by increased plasma osmolarity and monovalent ion ($Cl^{(-)}$) concentrations. However, there was no significant effect on the gill $Na^{+}K^{+}$ ATPase activity in the smolts, which is an indication of the seawater adaptability and osmoregulatory capabilities of fish. Indeed, when the smolts were subsequently transferred to full strength seawater there were no mortalities and gill $Na^{+}K^{+}$ ATPase activity was similar between cypermethrin treated and control groups. The study suggests that the main impact of cypermethrin on salmon is likely to occur in freshwater and not the marine environment.

5. Mixtures of pesticides

The majority of research carried out on pesticides has examined the effects of individual chemicals. Yet Atlantic salmon may be exposed for periods to a complex mixture of contaminants within tributaries and rivers supporting spawning salmon. Where a mixture of pesticides occurs within the aquatic environment, the overall impact on the biota may be additive, antagonistic or synergistic.

- ***Atrazine and simazine (Triazine herbicides)***

Two such pesticides that regularly occur within water-courses are the s-triazine pesticides atrazine and simazine. Both atrazine (2-chloro-4-ethylamino-6-isopropylamino -s-triazine) and simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) are pre- and post-emergence herbicides used in the control of annual and perennial grass and annual broad-leaved weeds. The proposed Environmental Quality Standard (EQS) of 2.0 μgl^{-1} for atrazine and simazine is based on the annual combined average of these pesticides and is calculated using the best available environmental and ecotoxicological information (Environment Agency, 1999).
Short term exposure of the olfactory epithelium of mature male Atlantic salmon parr to either the pesticide simazine (concentrations 1.0 and 2.0$\mu g\ l^{-1}$), or the pesticide atrazine (concentration 1.0$\mu g\ l^{-1}$), significantly reduced the ability of the male salmon to detect the reproductive priming pheromone released by the spawning female salmon. Once again the levels of expressible milt were subsequently reduced in the male fish. When the olfactory epithelium was exposed to a mixture of simazine and atrazine, (concentrations of 0.5/0.5 and 1.0/1.0$\mu g\ l^{-1}$), there was no significant reduction in the olfactory response when compared to the single pesticides at equivalent concentrations. In addition, exposure to a mixture of simazine and atrazine had no synergistic effect on the priming response, and plasma levels of testosterone, 11-ketotestosterone and 17,20β-dihydroxy-4-pregnen-3-one were similar in the groups of male parr exposed to the individual pesticides. Although the levels of expressible milt were reduced in all groups, there were no significant differences between the different pesticide treatments. The results suggest that the two s-triazine pesticides have an additive and not a synergistic impact on olfactory mediated endocrine function in mature male salmon parr.

- ***Atrazine and 4-nonylphenol.***

Recently there has been a great deal of research examining a suite of chemicals that can significantly affect the endocrine systems of aquatic organisms. These so-called endocrine disrupting chemicals (EDCs) include natural and synthetic oestrogens (e.g. oestradiol and ethinyl oestradiol, respectively) man-made xenoestrogens (e.g.4-nonylphenol) and a number of pesticides. The majority of recent research has concentrated on the impact of xenoestrogens on the reproductive endocrine systems of fish where plasma vitellogenin has been used a biomarker for exposure of male fish to oestrogens. However, CEFAS has focused on the the impact of EDCs on other endocrine systems in fish such those controlling the parr-smolt transformation.

During smoltification, there is evidence that certain hormones positively regulate some aspects of the transformation. These include thyroid hormones, growth hormone (GH), cortisol and insulin-like growth factor-I. Other hormones such as prolactin negatively regulate smoltification by counteracting the SW-adaptive effect of GH and cortisol. The sex steroids testosterone and 17β-estradiol (E_2) have also been shown to have an antagonistic effect on smoltification. Generally, circulating levels of sex steroids are low in juvenile salmonids but where they are elevated such as in mature male parr this may subsequently inhibit osmoregulatory and other physiological aspects involved in smoltification. Exposure of wild juvenile salmonids to xenoestrogens and pesticides during the parr-smolt transformation could therefore have an antagonistic effect on smolt physiology and compromise the ability of the fish to osmoregulate and survive in the marine environment.

Recent work on mixtures of contaminants investigated the effects of environmental concentrations of two EDCs on some aspects of smoltification in the Atlantic salmon. Firstly, smolts were exposed in freshwater to low levels of 4-nonylphenol (4-NP) and the effects on gill Na^+K^+ATPase activity, plasma vitellogenin (VTG), ions and sea water survival measured. 4-NP is an alkylphenol, which is a degradation product of alkylphenol polyethoxylates used widely as non-ionic surfactants. Secondly, smolts were again exposed in freshwater to combinations of 4-nonylphenol and environmental levels of the pesticide atrazine, which has been demonstrated to effect smolt physiology and hypoosmoregulatory performance.

Exposure of Atlantic salmon (*Salmo salar* L.) smolts in freshwater to environmental levels of the oestrogenic chemical 4-nonylphenol (4-NP) during the peak migration period had no significant effect on gill Na^+K^+ATPase activity, plasma vitellogenin (VTG) levels or hypo-osmoregulatory performance as indicated by survival in sea water. However, where smolts were exposed to mixtures of 4-NP and the pesticide atrazine at concentrations of respectively, there were significant differences in the gill Na^+K^+ATPase activity and plasma Cl^- and Na^+ and increased mortalities when transferred to sea water. The results indicate that where certain chemicals are present together in the aquatic environment they may act together syergistically to increase the toxic effects on the fish.

- ***Diazinon and cypermethrin***

Mixtures of environmental levels of the two sheep dip insecticides diazinon and cypermethrin commonly found together in tributaries during the salmon spawning season also disrupted pheromonal mediated endocrine function in male Atlantic salmon. The impact of the mixtures was synergistic together the pesticides had a greater impact on salmon reproduction that the individual compounds at the same concentrations.

A similar synergistic effect was seen on salmon embryos after a brief 2 minute exposure of eggs and milt to mixtures of the pesticides during fertilisation. The subsequent survival of the embryos was significantly reduced in the groups exposed to the two pesticides when compared to the individual compounds. In addition, the timing of emergence of the surviving alevins was delayed in embryos that had been exposed to the mixtures. These results may have implications for the recruitment of juvenile salmonids to the population and subsequent effects on smolt production.

Modelling the impacts of contaminants on salmonid populations.

The potential effects of these contaminants at the population level were investigated by exploring models of the Atlantic salmon life-cycle. Several available models were examined, including one used by the Environment Agency to assess the effectiveness of stocking procedures and the Canadian ASRAM model which had been developed to examine the long-term effects of acidification on stocks. However, these models were not directly applicable to the present research. Therefore CEFAS developed a separate model, which permitted the study of the effects of contaminants, impacting at particular life stages, on the population dynamics of different types of stock.

The model divides the salmon life cycle into five periods and makes the following assumptions about the survival of individuals:

1. Spawner to eggs: density independent (eggs produced per adult)
2. Eggs to fry: density independent (intra-gravel survival data)
3. Fry to Parr (~1 month): density dependent (Ricker) - $\text{Parr} = \alpha * \text{Fry} * e^{-ß*\text{Fry}}$
4. Parr-smolt: density dependent (Beverton & Holt) - $\text{Smolt} = \alpha * \text{Parr} / (1+ ß * \text{Parr})$
5. Smolt to adult: density independent (smolt return data)

While it is widely accepted that the survival of juvenile salmonids in freshwater is density dependent, there has been considerable debate about the nature of this relationship (Elliot, 2000). Different authors have proposed dome-shaped (e.g.

Kennedy & Crozier, 1993) and asymptotic (e.g. Buck & Haye, 1984) relationships for egg to smolt survival . In this study the juvenile phase was divided into two stages and assumed that in the first stage the survival is dome-shaped, as proposed by Elliot (1994), and for the latter juvenile period it is asymptotic; these were described with Ricker and Beverton & Holt relationships respectively. (In an alternative formulation of the model a Shepherd relationship was employed for both life stages, but using a different value for the third, lambda, parameter. However this had no advantages over the simpler approach above.)

For the density independent relationships, normal (unimpacted) survival rates were based upon figures available from previous CEFAS studies (e.g. studies of intra-gravel embryo survival and smolt tagging studies) and from the literature. Specific parameter values (α and ß) were not available for the density-dependent relationships, and so a range of values were considered which gave survival rates during the life stages consistent with those quoted in the literature. The values of all parameters were adjusted to investigate different types of populations. The survival at each life-stage was then further modified in accordance with the results of various contaminant studies undertaken during this research programme. It is important to note, however, that the model was not designed to provide specific numerical estimates of the impacts of contaminants on populations but to explore the nature of the possible impacts on the overall dynamics of the population.

The results of the modelling show that the influence of contaminants at early and later life cycle stages has different population effects, as one might expect. Exposure to contaminants during the early stages (egg to fry and fry to parr) has little effect on the unexploited stock, due to compensatory effects, but significantly impacts the stock if it is reduced well below carry capacity. This may occur as the result of over-fishing (exploitation) or the impact of additional environmental stressors. For instance, exposure of eggs and milt to 0.05μgl^{-1} diazinon for two minutes during fertilisation results in a 27% reduction in survival rate from fertilisation to emergence. The model shows that this impact has little effect on recruitment (spawning escapement) if the stock is unexploited, but the effect increases up to 25% as the ‘equilibrium’ stock size is reduced (i.e. as exploitation increases). There is, however, a substantial reduction in yield at all stock sizes.

A different scenario is seen when later life stages are exposed to environmentally relevant contaminants. For example, since mortality after the smolt stage is independent of density, impacts at this stage will have the same proportional effect on recruitment at all stock levels. Thus, exposure of smolts to 22.7 μg l^{-1} atrazine for 24 hours prior to emigration reduces recruitment by about 30%. The equilibrium stock size is also reduced; the effect being about 30% on the unexploited stock and increasing as the level of exploitation increases. Potential yields are also very severely reduced (40-100%) at all stock sizes.

The model was also used to test the impacts of exposure to a combination of contaminants at different life cycle stages, as would occur in the wild. For instance, the results of the modelling show that exposure of gametes to 0.05μgl^{-1} cypermethrin and exposure of smolts from the same population to 13.9μgl^{-1} atrazine has a severe effect on the population. The stock becomes virtually non-self-sustaining, with the majority of the stock-recruitment curve falling below the replacement line and there being almost no surplus even at low population levels. This is a disturbing result as many populations in the wild are exposed to suites of contaminants throughout their lives. Results from experimental studies combined with these modelling scenarios show that exposure to environmentally relevant contaminants at sensitive periods (e.g. fertilisation, embryo development, and smoltification), may increase the pressure on the stock to theoretically dangerous levels, where recovery may not occur.

In conclusion, the incorporation of the laboratory based experimental data into the life cycle models demonstrates that low levels of environmental contaminants can have a serious impact on both individuals and populations of salmonids. As more data is gathered, both from laboratory and field-based research programmes the models and the predictions will become more robust.

Conclusions

The main conclusion from the research on the effects of contaminants on salmonids is that the present levels of certain pesticides in the aquatic environment may be too high and as such may impose a significant biological risk to migratory salmonid populations. The research has highlighted that the impacts of environmental levels of contaminants can operate throughout the freshwater life cycle particularly at sensitive stages such as embryo development, reproduction, smoltification and entry into the marine environment. Further, contaminants may operate in an additive or synergistic manner increasing their impact on sensitive stages in the life cycle of salmonids. It is also evident that the freshwater and marine phases of the salmon life cycle cannot be considered in isolation. The freshwater history of the juvenile salmonids in terms of exposure to aquatic contaminants is critical to their subsequent survival in the marine environment. Modelling the effects of the pesticides at the population levels has also indicated that exposure at the critical and sensitive stages in the life cycle may significantly reduce the number of returning adults and compromise the spawning biomass of many populations.

Related Publications

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15. Moore, A., Scott, A.P., Lower, N., Katsiadaki, I. & Greenwood, L. (2003). The effects of 4-nonylphenol and atrazine on Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* **222,** 253-263.

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