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The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.).

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Abstract

The synthetic pyrethroid pesticide cypermethrin, a known contaminant of tributaries supporting spawning salmonid fish, had a significant sublethal impact upon the pheromonal mediated endocrine system in mature male Atlantic salmon (*Salmo salar* L.) parr. Previous studies have demonstrated that ovulated female salmon release a priming pheromone in their urine (considered to be the F-type prostaglandin, $PGF_{2\alpha}$) which is subsequently detected by the olfactory system of mature male salmon parr and results in increased levels of plasma sex steroids and expressible milt. Exposure of mature male parr for a 5 day period to a water concentration of < 0.004 μg l^{-1} cypermethrin significantly reduced or inhibited the olfactory response to $PGF_{2\alpha}$. In addition, exposure of male parr to cypermethrin significantly reduced their ability to respond to the priming effect of the pheromone. The priming effect on milt and plasma 17,20β-dihydroxy-4-pregnen-3-one levels were abolished at water concentrations of < 0.004 and 0.028 μg l^{-1}cypermethrin, respectively. The effect of cypermethrin on the priming response did not appear to be due to a direct effect on the testes, since the ability of testes to respond to pituitary extract stimulation in vitro was not impaired in males exposed to cypermethrin. In addition, exposure of salmon milt and eggs to a concentration of 0.1 μg l^{-1} cypermethrin during fertilisation subsequently reduced the number of fertilised eggs. The results of the study suggest that low levels of cypermethrin in the aquatic environment may have a significant effect on Atlantic salmon populations through disruption of reproductive functions.

Keywords: Atlantic salmon; Olfaction; Reproduction; Pyrethroid pesticide; Cypermethrin

1. Introduction

The low toxicity to mammals of synthetic pyrethroid insecticides has encouraged their use in intensive agriculture. In aquaculture they have been used as replacements for more toxic pesticides such as organophosphates. However, non-target organisms such as aquatic invertebrates and fish are extremely sensitive to the neurotoxic effects of these insecticides when they enter surface water-courses (Reddy et al., 1991; Eshleman

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and Murray, 1991; Mian and Mulla, 1992; Philip et al., 1995). Pyrethroids have been shown to be up to 1000 times more toxic to fish than to mammals and birds at comparable concentrations (Edwards et al., 1986; Bradbury and Coates, 1989; Eells et al., 1993). The hypersensitivity of fish to pyrethroid intoxication is due partly to species specific differences in pyrethroid metabolism, but principally to the increased sensitivity of the piscine nervous system to these pesticides. One such type II pyrethroid, cypermethrin (*RS*-α-cyano-3-phenoxybenzyl, IRS, *cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate), is being increasingly used in the UK as the active ingredient in many dips which are used to prevent and treat ticks, lice and scab on sheep, and as a treatment against infestation by the parasitic sea louse *Lepeophtheirus salmonis* in intensive salmonid aquaculture. In 1995 there was a significant increase in the number of surface freshwater sites in England and Wales where cypermethrin exceeded the maximum allowable concentration (MAC) environmental quality standard (EQSs) of 1 ng l^{-1} (Environment Agency, 1997). More recently during an Environment Agency monitoring programme levels of cypermethrin ranging between 0.078 and 0.101 μg l^{-1} were measured in streams supporting spawning salmonids (Environment Agency, 1999). The source of contamination of river courses occurs as a result of the direct use of pyrthroid based dips, and also from the processing of sheep skins in the wool industry and knitwear manufacture. Although the environmental concentrations of cypermethrin are often below those that are lethal to many freshwater teleosts (McLeese et al., 1980; Stephenson, 1982; Ansari and Kumar, 1988; Philip et al., 1995) there is very little data on the potential sublethal effects of the pesticide on reproduction, and the long-term viability of fish populations.

Previously, a number of pesticides which commonly occur within the aquatic environment have been shown to have sublethal effects upon pheromone mediated endocrine function and reproduction in the Atlantic salmon (Moore and Waring, 1996a; Waring and Moore, 1997; Moore and Waring, 1998). For instance, exposure of mature male salmon parr to environmental levels of the pesticides atrazine, carbofuran and diazinon, inhibited the olfactory detection of the female reproductive priming pheromone which is considered to be involved with the synchronisation of spawning between the sexes (Moore and Waring, 1996b). Atrazine was also shown to have a direct impact upon the testes, modifying the release of androgens and suggesting an additional toxic mechanism to male salmon reproduction.

The present study examined the impact of low levels of cypermethrin on a number of aspects of reproduction in mature male salmon parr. First, the effect of the pesticide on olfactory mediated reproductive endocrine function was studied and the ability of male fish exposed to cypermethrin to detect and respond to the salmon priming pheromone $PGF_{2\alpha}$. It has been shown in previous studies that pheromones released by reproductively mature female salmonids are detected by the olfactory systems of the males and that this results in an increase in the levels of plasma reproductive steroids and expressible milt (Liley et al., 1993; Olsén and Liley, 1993; Moore and Waring, 1996b). Second, the possible direct effect of the pesticide on the ability of the testes to respond to pituitary stimulation was investigated. In addition, free and glucuronidated steroid levels in the bile were measured to ascertain whether cypermethrin altered aspects of the metabolism and excretion of steroids in mature male salmon parr. Third, in order to investigate whether the waterborne pesticide could inhibit fertilisation, salmon milt and ova were mixed in the presence of low concentrations of cypermethrin and the subsequent development of the embryos was monitored.

2. Materials and methods

In November 1997 mature male Atlantic salmon parr were obtained from the Environment Agency, Cynrig Hatchery, Wales, and transported to the CEFAS, Lowestoft Laboratory. The fish were kept in 1000 l tanks, under natural light conditions, with a constant flow of aerated dechlorinated water (flow rate of 85 l min^{-1}).

Water temperature ranged from 7.1–9.8°C and the physico-chemical characteristics of the water have been reported previously (Moore and Waring, 1996a). The fish were fed to satiation twice a day with commercial salmon pellets.

2.1. Effect of cypermethrin on the olfactory detection of $PGF_{2\alpha}$

The effect of cypermethrin on the detection of the priming pheromone $PGF_{2\alpha}$ by the olfactory epithelium of male Atlantic salmon parr used the same electrophysiological technique (electro-olfactogram: EOG) that has been used previously in other pesticide studies on mature male salmon parr (Moore and Waring, 1996a; Waring and Moore, 1997; Moore and Waring, 1998). EOG recording measures trans-epithelial voltage gradients from the surface of the olfactory epithelium and is considered to reflect multi-unit cell activity (Evans and Hara, 1985). A full description of the technique is given by Moore and Waring (1996a).

Two groups of spermiating male salmon parr (length 126 ± 1.1 mm; weight 24.2 ± 0.7 g; GSI $7.1 \pm 0.29\%$; $n = 10$), were transferred to 63 l glass tanks and left to recover for 96 h without feeding. Each tank had a constant flow of dechlorinated tap water ($1\ l\ min^{-1}$) with no re-circulation. A natural photoperiod was followed and the water temperature was 10.9 ± 1.1°C.

Cypermethrin (Greyhound Chromatography and Allied Chemicals) was prepared from a stock solution of 200 mg l^{-1} absolute ethanol, and stored in amber glass bottles. The pesticide was added to one of the tanks for a period of 5 days via a multichannel peristaltic pump (Watson-Marlow) and silicon tubing (Altec) and vigorously mixed by aeration. Stock solutions were prepared in order to give a nominal tank concentration of 0.01 μg l^{-1} cypermethrin. The stock solutions were renewed every 12 h. The second tank of fish was exposed for 5 days to an ethanol carrier control. At the end of the 5 day exposure period, the responses to a 10^{-9} M concentration of $PGF_{2\alpha}$ (Sigma) was recorded from the olfactory epithelium of five individual fish from each of the two treatment tanks using the EOG technique. The $PGF_{2\alpha}$ was prepared from a stock solution consisting of 500 μg ml^{-1} absolute ethanol. The $PGF_{2\alpha}$ was prepared fresh before each experiment with water taken from the inlet pipe of the salmon tank and allowed to stand at room temperature until required (room temperature 10.5°C). Control dilutions of ethanol were also prepared and tested. A stock solution of 10^{-5} M L-serine in dechlorinated water was also prepared and tested at the beginning and end of each recording. The solution was prepared fresh prior to each experiment. Subsequently, the responses of the olfactory epithelium of the remaining ten fish to a 10^{-9} M concentration of $PGF_{2\alpha}$ were recorded by EOG over a 5 day period to study the recovery time of the olfactory epithelium after exposure to cypermethrin.

The amplitude of each EOG response was measured from the baseline to the peak of each phasic displacement and expressed in millivolts (mV). All recordings in response to the dechlorinated water controls were subtracted from the EOG responses. The EOG responses recorded from each group of salmon were compared using a paired two sample for means t-test.

2.2. Effect of cypermethrin on the priming response of males to $PGF_{2\alpha}$

In November and December 1997 spermiating male parr (length 130 ± 1.3 mm; weight 27.2 ± 0.8 g; GSI $7.9 \pm 0.34\%$; $n = 63$), were transferred to 63 l glass tanks. Each tank had a constant flow of dechlorinated tap water ($1\ l\ min^{-1}$) with no recirculation. A natural photoperiod was followed and the water temperature was 11 ± 1°C. Males were gently stripped of milt and groups of seven were placed into each tank and left to recover for 96 h without feeding.

Solutions of cypermethrin were again added to the tanks via a multichannel peristaltic pump (Watson-Marlow) and silicon tubing (Altec), and vigorously mixed by aeration. Stock solutions were prepared in order to give nominal tank concentrations of cypermethrin of 0 (control), 0.0001, 0.001, 0.01, 0.05, 0.1 and 0.5 μg l^{-1} for 5 days. Stock solutions were renewed every 12 h. At the end of this period groups of males were then either given a 5 h exposure to $PGF_{2\alpha}$ or to the

ethanol carrier. The $PGF_{2\alpha}$ was further diluted from the stock and added to tank water to give a final dilution of 10^{-8} M.

At the end of the $PGF_{2\alpha}$ exposure period, males were anaesthetised in 0.4 ml l^{-1} 2-phenoxyethanol and milt and blood were sampled (Moore and Waring, 1996a). The gall bladders were removed from each fish and the contents were left to drain for 10 min into pre-weighed 1.5 ml micro-centrifuge tubes. Bile was then diluted 1:10 (w/v) with distilled water and stored at −20°C.

Testes were removed from some of the males in the control group and the group exposed to 0.5 μg l^{-1} cypermethrin, but which was not subsequently primed with $PGF_{2\alpha}$. They were then washed in the incubation medium, chopped into 50 mg fragments and incubated in vitro (Nagler et al., 1996). Acetone-dried chum salmon pituitary powder was obtained from Syndel Laboratories (Vancouver, BC, Canada). The powder was ground in 30 vol. (w/v) of incubation medium, centrifuged and the pellet was discarded. The supernatant (PE) was then used immediately for the experiment at a dosage of 0.025 mg of original powder per well. Testes fragments (50 mg per well), were incubated in the presence or absence of PE at 11°C in a humidified atmosphere on a shaking platform. After 18 h the media were removed, centrifuged, and the supernatants were stored at −20°C until analysis.

Free steroids were extracted from plasma (50 μl), bile (50 μl), and testes incubation medium (100 μl) with 3 ml diethyl ether. Plasma levels of testosterone (T), 11-ketotestosterone (11-KT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) were measured using the radioimmunoassays described previously (Scott et al., 1982, 1984). Levels of glucuronidated and sulphated T, 11-KT and 17,20βP were also measured in bile and incubated media using the methods described previously (Scott and Liley, 1994; Waring et al., 1996; Waring and Moore, 1997). The data have been expressed as ng of free steroid equivalents and have not been adjusted to take into account the mass of the conjugated moiety.

The milt, bile and plasma steroid data were analysed using a one-way ANOVA followed, if significance was indicated, by Student–Neuman–Keuls (SNK) tests as the multiple range test. The testes incubation data were analysed using a two-way ANOVA with the presence or absence of PE and pesticide treatment as factors. When significance was indicated, SNK was used as the multiple range test.

2.3. Effect of cypermethrin on egg fertilisation

On 10 December 1997 unfertilised eggs and milt from adult salmon were collected from the Environment Agency, Kielder Hatchery, and transported on ice to the Lowestoft laboratory. Six groups of 500 eggs were placed in 200 ml glass containers. The milt from five male salmon was mixed in a 20 ml glass container and then 1 ml of the mixture was added to each of the groups of eggs. Five seconds later the milt was further activated with 50 ml of water containing different concentrations of cypermethrin (control (no cypermethrin), 0.001, 0.01, 0.05, 0.1 and 0.5 μg l^{-1}), and the milt and eggs were gently mixed. After 30 s the eggs were washed in tank water to remove the milt and then placed in egg trays within plastic troughs with a constant flow of aerated water (flow rate 10 l min^{-1}). The eggs were then monitored daily within the hatchery until embryonic development was evident. At this stage the number of fertilised eggs within each group were calculated.

2.4. Water cypermethrin concentrations

At the end of each experiment water samples were taken from the tanks and stored in the dark at 4°C in amber glass bottles before analysis of actual cypermethrin contents. Water cypermethrin concentrations were measured at the CEFAS Burnham on-Crouch Laboratory using high-resolution gas chromatography/mass spectrometry. The limit of detection for cypermethrin was determined to be 0.004 μg l^{-1}.

The measured cypermethrin concentrations in the water sampled from the tanks ranged between 38 and 66% of the nominal concentrations aimed for with one anomalous reading of 150%. The measured cypermethrin concentration from the

tank of parr used in the olfactory studies was < 0.004 μg l^{-1}. The measured cypermethrin concentration from the tanks of parr used in the endocrine studies were between < 0.004 and 0.33 μg l^{-1}. The measured water concentrations are referred to in the rest of the text and figures. However, these values were terminal measurements and we have no data regarding water concentrations earlier in the exposure period.

3. Results

3.1. Effect of cypermethrin on the olfactory detection of $PGF_{2\alpha}$

Exposure of the male parr for 5 days to a concentration of < 0.004 μg l^{-1} cypermethrin either abolished or significantly reduced the EOG response to $PGF_{2\alpha}$ (Fig. 1). Five days after exposure to the cypermethrin the mean EOG response of the dosed group (0.15 ± 0.11 mV) was only 11% of the control group (1.31 ± 0.18 mV). The response to the amino acid L-serine was also significantly reduced ($P < 0.01$) in the dosed group (0.11 ± 0.08 mV) compared to the control group (0.68 ± 0.09 mV). Over the subsequent 5 days there was a small but significant recovery in the olfactory response to the priming pheromone. The mean EOG response to $PGF_{2\alpha}$ in the group exposed to cypermethrin (0.22 ± 0.07 mV) was 18% of the control group (1.21 ± 0.13 mV). Cypermethrin itself did not produce a typical EOG response from the olfactory epithelium of the mature male parr.

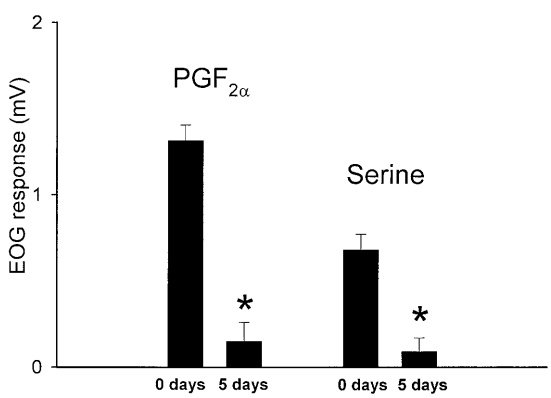

Fig. 1. EOG responses of two groups of mature male salmon parr to $PGF_{2\alpha}$ (10^{-8} M) and L-serine (10^{-5} M) before and after a 5 day exposure to a concentration of < 0.004 μg l^{-1} cypermethrin. The amplitude of each EOG response was measured from the baseline to the peak of each phasic displacement and expressed as mV. Data represents the mean ± S.E.M. of five fish per group. Asterisks designate significant differences ($P < 0.001$) between EOG responses before and after exposure to cypermethrin.

3.2. Effect of cypermethrin on the priming response of males to of $PGF_{2\alpha}$

Exposure to $PGF_{2\alpha}$ for 5 h significantly increased levels of expressible milt and plasma 17,20βP, T and 11-KT concentrations (Fig. 2) in male parr compared to the control group. However, when male parr were exposed to cypermethrin at concentrations of 0.028, 0.038 and 0.33 μg l^{-1}, the increase in plasma levels of 17,20βP was abolished. Similarly, the increase in the level of expressible milt was also abolished but at concentrations of cypermethrin of < 0.004, 0.015, 0.028, 0.038 and 0.33 μg l^{-1}. Exposure of male parr to cypermethrin in the water also impacted on the plasma androgen response which was abolished at water concentrations of 0.028, 0.038 and 0.33 μg l^{-1} (T) and 0.038 and 0.33 μg l^{-1} (11-KT).

The levels of free and glucuronidated 17,20βP in the bile were significantly elevated in the pheromonally primed males compared to controls (Fig. 3). However, this response was abolished when water cypermethrin concentrations were $<$ 0.004, 0.015, 0.028, 0.038 and 0.33 μg l^{-1}. Similar increases in free and glucuronidated T concentrations in the bile were also evident in males exposed to $PGF_{2\alpha}$. These responses were not apparent in the bile when the pesticide was present at < 0.004, 0.015, 0.028, 0.038 and 0.33 μg l^{-1} (glucuronidated) and at < 0.004 μg l^{-1} and > 0.015 μg l^{-1} (free). The exposure to $PGF_{2\alpha}$ did not result in an increase in either free and glucuronidated levels of 11-KT in the bile. However, in the presence of cypermethrin at concentrations of 0.33 μg l^{-1} without the addition of $PGF_{2\alpha}$, the levels of free 11-KT in the bile were significantly reduced compared to the control.

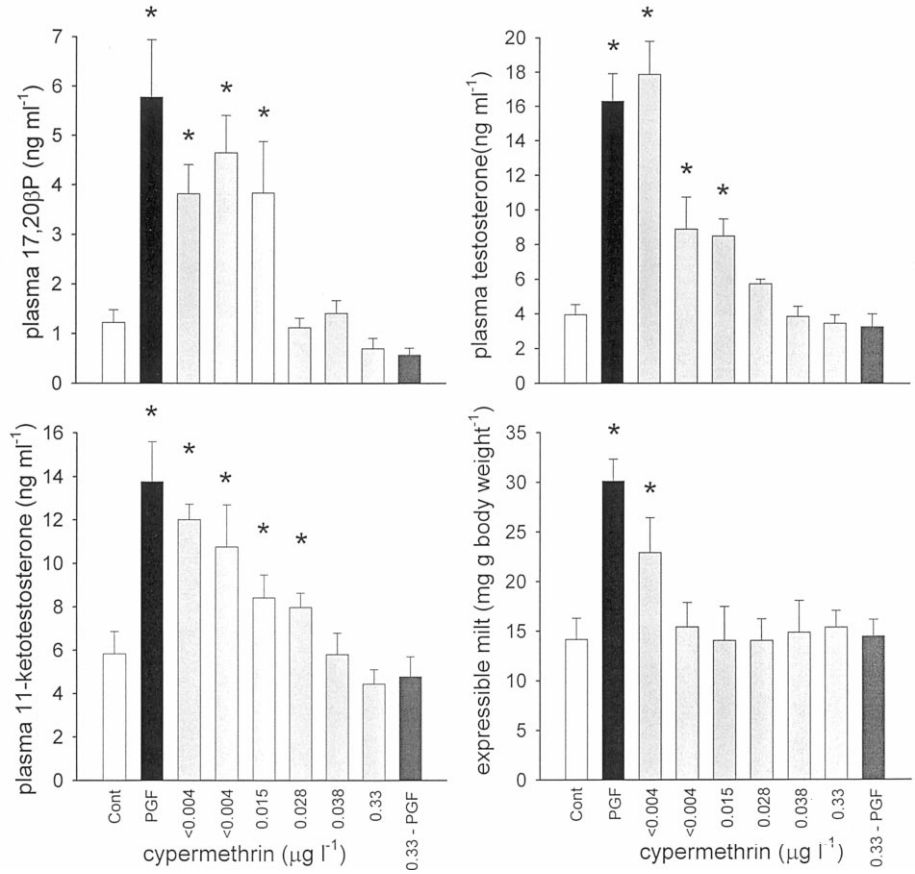

Fig. 2. The effect of cypermethrin on expressible milt, plasma 17,20βP, T and 11-KT concentrations in various groups of mature male salmon parr. Open column, control group not exposed to $PGF_2\alpha$; solid column, control group exposed to $PGF_2\alpha$; light shade column, group exposed to cypermethrin and $PGF_2\alpha$; dark shade column, group exposed to cypermethrin only. Data represents the mean $\pm$ S.E.M. of seven fish per group. Asterisks designate significant differences ($P < 0.05$) between control fish not exposed to $PGF_{2\alpha}$ and the groups exposed to $PGF_{2\alpha}$/cypermethrin.

When exposed to PE in vitro, with the exception of T-S and 11-KT the release of free and conjugated steroids from the testes was significantly higher than from the non-stimulated testes (Table 1). When the testes were exposed to PE in vitro and in the presence of 0.33 μg l^{-1} cypermethrin there was no difference between the release of the free and conjugated steroids when compared to the control groups. However, in non-stimulated testes the presence of cypermethrin significantly reduced the release of T-S, 11-KT and 11-KT-S (Table 1).

3.3. Effect of cypermethrin on egg fertilisation

The exposure of salmon eggs and milt to cypermethrin during mixing reduced fertilisation when compared to the control. At nominal concentrations of 0.1 and 0.5 μg l^{-1} cypermethrin, the fertilisation rates were 47 and 39% of the control group, respectively. However, the remaining fertilised eggs subsequently appeared to develop normally and there were no differences in the percent hatching rates at the time when the salmon alevin emerged.

Table 1
In vitro release of free, glucuronidated and sulphated 17,20βP, testosterone and 11-ketotestosterone from testes of mature male parr exposed in water to 0.33 μg l^{-1} of cypermethrin[a]

Control		Cypermethrin (0.5 μg l^{-1})		Control		Cypermethrin (0.5 μg l^{-1})		Control		Cypermethrin (0.5 μg l^{-1})	
No-Pe	PE	No-Pe	PE	No-Pe	PE	No-Pe	PE	No-Pe	PE	No-Pe	PE
17,20βP				Testosterone				11-Ketotesterone			
7.19 ± 1.21	123.3 ± 18.99**	5.63 ± 0.83	1.0.2 ± 11.0**	11.23 ± 1.11	36.04 ± 5.08**	12.05 ± 1.60	22.71 ± 3.90*,**	6.45 ± 1.15	65.41 ± 18.03**	2.82 ± 0.48*	32.26 ± 7.38**
17,20βP-G				Testosterone-G				11-Ketotesterone-G			
3.42 ± 0.45	14.29 ± 6.11**	3.39 ± 0.66	5.43 ± 0.61	15.15 ± 1.46	23.59 ± 2.19**	18.07 ± 1.83	20.58 ± 2.26	3.88 ± 1.34	6.79 ± 1.63	5.07 ± 1.39	5.38 ± 1.24
17,20βP-S				Testosterone-S				11-Ketotesterone-S			
6.38 ± 0.61	81.51 ± 11.27**	4.63 ± 0.65	63.47 ± 7.4**	21.77 ± 5.43	16.27 ± 2.86	9.1 ± 1.43*	11.07 ± 0.93	8.29 ± 0.95	15.1 ± 3.06**	6.186 ± 0.34*	10.558 ± 1.26**

[a] Data represents S.E.M. of $n = 7$ per group.
* $P < 0.05$ compared to the relevant control.
** $P < 0.05$ compared to the relevant no pituitary extract.

4. Discussion

Exposure of mature male salmon parr to low levels of water-borne cypermethrin inhibited their ability to detect and respond to the female salmon priming pheromone $PGF_{2\alpha}$. The increase in expressible milt and the levels of plasma sex hormones were significantly reduced in the presence of the pyrethroid as the result of impaired olfactory detection of the priming pheromone. Previous studies have also indicated that a number of generic pesticides have a similar toxic effect on

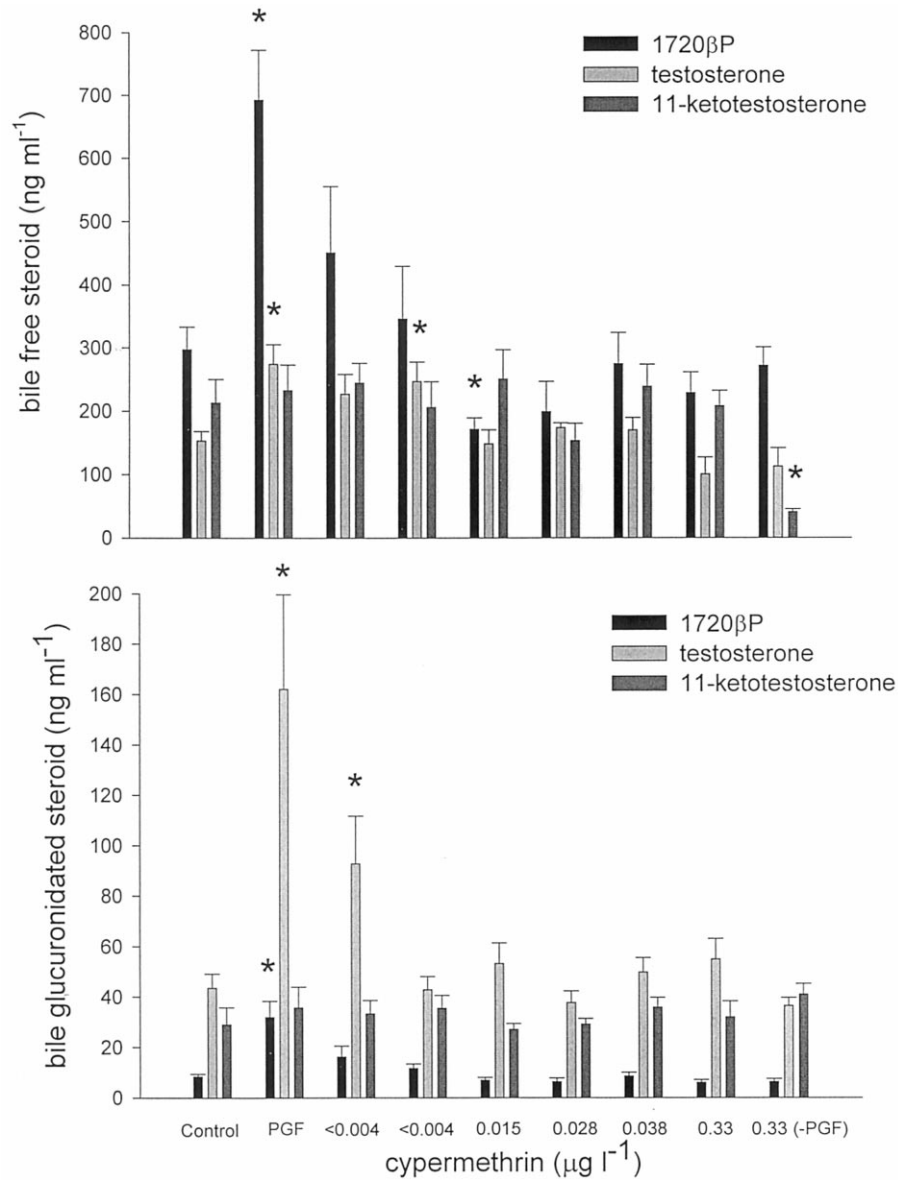

Fig. 3. Free and glucuronidated 17,20βP, T and 11-KT in the bile of male salmon parr exposed to various concentrations of cypermethrin. Data represents the mean ± S.E.M. of seven fish per group. Asterisks designate significant differences ($P < 0.05$) between control fish not exposed to $PGF_{2\alpha}$ and the groups exposed to $PGF_{2\alpha}$/cypermethrin.

pheromone mediated endocrine function in salmon (Moore and Waring, 1996a; Waring and Moore, 1997; Moore and Waring, 1998). However, unlike atrazine (Moore and Waring, 1998), there was no evidence that exposure to cypermethrin influenced either the metabolism of steroids or their accumulation within the bile. In addition, there appeared to be no direct impact of cypermethrin upon the testes and in this respect the toxic mode of action of cypermethrin on the reproductive endocrine function of the male salmon was similar to carbofuran (Waring and Moore, 1997). However, it is not clear to what extent the pesticide may effect the female reproductive system. Female salmon were not tested during the present study, but it is likely that the olfactory system of females would be similarly affected.

Exposure of salmon eggs and milt to cypermethrin also reduced the level of fertilisation, suggesting a further toxic impact of the pesticide on salmon reproduction. Therefore, even if the olfactory priming response was not significantly inhibited by cypermethrin the pesticide could have a deleterious impact on populations as a result of decreased fertilisation. However, it is not clear from the present study which of the gametes the cypermethrin was affecting and whether the pesticide was reducing the viability of the salmon egg or the sperm. Short-term exposure to the pyrethroid esfenvalerate also resulted in a reduction in fecundity of the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) and the failure of eggs to hatch (Barry et al., 1995a). Further studies on this species demonstrated that the fertilised eggs were not sensitive to the pyrethroid (Barry et al., 1995b), suggesting that the toxic effect again occurred prior to fertilisation. Reduced spawning and hatching of offspring has also been demonstrated to occur in bluegills (*Lepomis macrochirus*) exposed to esfenvalerate (Tanner and Knuth, 1996). A further effect of exposure to the pyrethroid was that spawning in the blue gill was delayed. The delay in spawning in this species may have been the result of disruption to the pheromone-based synchronisation of spawning between the sexes.

Cypermethrin clearly reduced the ability of the olfactory system to detect the priming pheromone. Exposure to the pesticide for a period of 5 days either significantly reduced or abolished the olfactory response to the priming pheromone. Synthetic pyrthroid insecticides are potent neurotoxicants and their modes of action have been well described in many species of invertebrate and vertebrates (Bradbury and Coates, 1989; Narahashi et al., 1998). It is well established that pyrethroids exert potent actions on neuronal sodium channels (Narahashi, 1996; Song and Narahashi, 1996; Narahashi et al., 1998), keeping the sodium channel open for unusually long periods and causing repetitive after-discharges within the nerve fibres (Song and Narahashi, 1996; Narahashi et al., 1998). Repetitive activity of nerve fibres induced by pyrethroids has also been observed particularly in sense organs of invertebrates (van den Bercken et al., 1979). Vijverberg et al. (1982) also found that cypermethrin induced very long trains of nerve impulses in the lateral line sense organ of the clawed frog *Xenopus laevis*. During the present study it is suggested that exposure to the pesticide probably acted directly on the sodium channels, inhibiting nervous transmission within the olfactory system and resulting in the male salmons' inability to detect and respond to the pheromone. In addition, after exposure to the pesticide there appeared to be a slow recovery of the EOG response to the priming pheromone, which suggests that there could be a long-term impact of cypermethrin on the olfactory system.

The seasonal use of cypermethrin based sheep dips may enhance their toxic impact on the aquatic environment. In the UK many sheep are treated late in the year (October) and cypermethrin can occur in many spawning rivers and tributaries during November and December (Environment Agency, 1999). Pyrethroids have been shown to be more toxic at low temperature than at high temperatures in mammals and insects (Song and Narahashi, 1996). In addition, Kumaragura and Beamish (1981) reported that acute toxicity of synthetic pyrethroids to fish was negatively correlated to temperature. Vijverberg et al. (1982) demonstrated that the neurotoxic effect of cypermethrin in the lateral line organ of the clawed frog, *Xenopus laevis*, also increased dramatically at lower temperatures. Therefore, the

presence of pyrethroids in the aquatic environment when water temperatures are decreasing during the salmon spawning season may increase the toxic impact on reproduction.

The inability of the salmon parr to detect cypermethrin via the olfactory system would suggest that they have no mechanism by which to avoid contaminated areas which in turn may have a compounding effect on the fish (Waring and Moore 1997). Ishida et al. (1996) demonstrated that carp can detect and avoid pesticides mainly by the use of olfaction. However, the concentrations used in these studies on the carp were very high and the olfactory responses were relatively small and did not show a concentration dependency that is usually recognised in the olfactory response to other odorants.

Cypermethrin may also impact upon other critical life history stages of the Atlantic salmon particularly where the olfactory system has a major sensory role. The toxicological effect on the olfactory system was not only restricted to priming pheromones and reproduction. The pesticide also significantly reduced the ability of the olfactory epithelium to respond to the amino acid L-serine. Exposure of smolts to the pesticide during the freshwater stage may effect olfactory imprinting to the natal river (Hasler and Scolz, 1983). In addition, the presence of cypermethrin within the marine environment as a result of its use in intensive salmon aquaculture may reduce the homing abilities of returning adults and increase straying rates between river systems. Previous studies on pyrthroid pesticides have also demonstrated a range of significant sublethal effects on both the physiology and behaviour of other freshwater species. Responses of fish to cypermethrin toxicity include gill flailing, hyperactivity, loss of buoyancy and inability to remain upright in rainbow trout (Edwards et al., 1986); inhibition of AChE and ATPase activity in carp (Reddy and Philip, 1994).

Cypermethrin is also highly toxic to many of the freshwater invertebrates (Stephenson, 1982), which constitute a large proportion of the diet of juvenile salmonids (Keely and Grant, 1997). A reduction in the biomass and diversity of important prey items may have a significant impact on the survival of mature male salmon parr. In addition, toxic effects on salmon may occur as the result of the dietary uptake of cypermethrin through the food chain, in addition to the exposure to water-borne cypermethrin. Cypermethrin is also toxic to many marine invertebrates and fish (McLeese et al., 1980; Clark et al., 1987). A reduction in the marine prey items of the salmon and sea trout post-smolts during the early marine phase may also significantly effect survival. This would be most likely to occur in coastal zones where cypermethrin is used as a sea-lice control in intensive salmon aquaculture.

In conclusion, the synthetic pyrethroid inhibited the ability of male Atlantic salmon to detect and respond to the reproductive priming pheromone, $PGF_{2\alpha}$. As a result milt and plasma levels of 17,20β-dihydroxy-4-pregnen-3-one concentrations were not significantly elevated in the male parr suggesting a significant effect on reproductive function. In addition, low levels of the pesticide reduced fertilisation rates in salmon eggs. The results suggest that sub-lethal levels of cypermethrin in the aquatic environment may have a long-term impact on Atlantic salmon population by interfering with some aspects of reproduction.

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