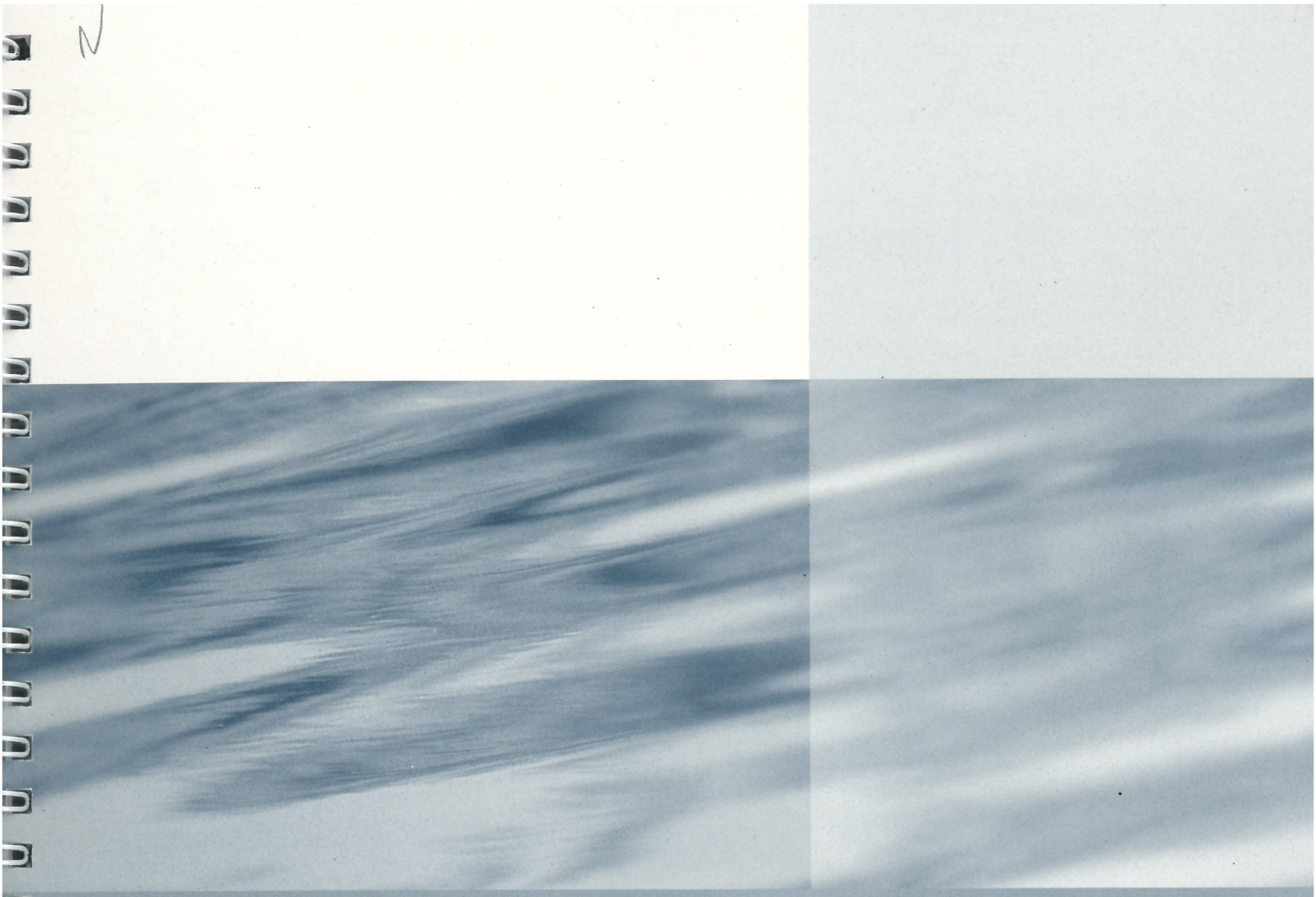

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The Effect of Atrazine Exposure on the Timing of Salmon (*Salmo salar* L.) Smolt Emigration

R&D Technical Report W2-052/TR

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The Centre for Environment, Fisheries & Aquaculture Science

ENVIRONMENT AGENCY

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Statement of Use
This technical report contains the results of a study into the effects upon the time of migration of salmon smolts treated with the herbicide atrazine. The information in this document is for use by Agency staff and others involved in the management of salmonid populations and the regulation of surface water quality.

Keywords
Atrazine, salmon, migration, diffuse pollution, smolt, smoltification.

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Technical summary

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The Effect of Atrazine Exposure on the Timing of Salmon (*Salmo salar L.*) Smolt Emigration

R&D Technical Summary W2-052/TS

Recent research has shown that sub-lethal levels of pesticides could have significant effects on Atlantic salmon (*Salmo salar* L.) reproductive and migratory physiology. One such pesticide is atrazine (2-chloro-4-ethylamino-6-isopropoylamino-s-triazine), which has been shown to have a significant effect on pheromonal mediated reproductive function in adult salmon (Moore & Waring, 1998) and to reduce the seawater tolerance in smolts (Waring & Moore, 1996).

The results of the work suggest that exposure of juvenile salmon to the pesticide may inhibit the smoltification process and result in the fish not being adapted to the marine environment. Another possible consequence is that the pesticide may effect the motivation to migrate and either delay or inhibit the movement of the smolts into the marine environment. Previous work has indicated that the timing of smolt migration into the sea is important to their survival and successful return as spawning adults (Hansen & Jonsson, 1989).

In 1992 and 1993, atrazine was one of the five pesticides most frequently present in both ground and surface water at levels in excess of the Maximum Admissible Concentration (MAC) 0.1 µg l-1 imposed by the Water Act 1991 (National Rivers Authority, 1995).

This study examined the impact on run-timing in groups of hatchery reared Atlantic salmon smolts exposed to different concentrations of pesticide prior to release in the River Test, southern England. Fish were identified using passive integrated transponding tags and exposed to the pesticide for 10 days prior to release.

Fish were recaptured using a smolt trap, which was operated continuously during the trapping period. As only one of the treated fish were recaptured no conclusions could be drawn regarding the effects of pesticide exposure. Analysis of the gill Na^+ K^+ ATPase activity indicated that both treated groups of fish had significantly ($p<0.05$) lower Na^+ K^+ ATPase activity than the control group. It was therefore suggested that exposure to even 5µg l^{-1} Atrazine may have reduced the ability of the fish to hypoosmoregulate and therefore survive the transition from fresh to saltwater.

This study was not able to provide further evidence to support or contradict the hypothesis that exposure to atrazine modified the migratory behaviour of salmon parr of smolt age and size.

Recommendations were made regarding the testing of smolts prior to inclusion in studies of this nature to ensure their suitability for study. The information in this document is intended for use by Agency staff and others involved in the management of salmonid populations and the regulation of surface water quality.

This R&D Technical Summary relates to information from R&D Project W2-052 reported in detail in the following output:-

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The effect of Atrazine exposure on the timing of salmon (*Salmo salar* L.) smolt emigration
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ENVIRONMENT AGENCY

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EXECUTIVE SUMMARY

Exposure of Atlantic salmon (*Salmo salar* L.) to sub-lethal concentrations of pesticides has been shown to have significant effects on their reproductive and migratory physiology. One such pesticide is atrazine (2-chloro-4-ethylamino-6-isopropoylamino-s-triazine), that is commonly used as a herbicide for the control of annual and perennial grass and broad-leaved weeds. In 1992 and 1993 this pesticide was found to be one of the most commonly occuring herbicides in ground and surface waters.

Salmon smolts exposed in freshwater to sub-lethal doses of atrazine and subsequently transferred to seawater experienced mortality of ~30% (Waring & Moore, 1996). It was suggested that the pesticide inhibited the smoltification process and the ability of the fish to adapt to the marine environment. This present study further examined the impact of atrazine on smoltification and in particular the potential effects on the run-timing of hatchery reared smolts. Groups of smolts were individually tagged using passive integrated transponding (PIT) tags and exposed to different concentrations (5µg l^{-1} and 20µg l^{-1}) of the pesticide, for 10 days prior to release in the River Test, southern England.

A trap was operated continuously during the smolt run in order to monitor the movements of the different groups of smolts. However only a single fish subsequently migrated downstream and was captured in the trap. Analysis of the gill Na^+ K^+ ATPase activity from fish sampled prior to release indicated that both treated groups of fish had significantly ($p<0.05$) lower Na^+ K^+ ATPase activity than the control group. This suggests that had the salmon migrated, exposure to atrazine may have reduced their ability to adapt to the marine environment.

CONTENTS

1 INTRODUCTION

Recent work at the Centre for Environment Fisheries and Aquaculture Science (CEFAS), Lowestoft Laboratory has shown that sub-lethal levels of pesticides, which regularly occur in the aquatic environment could have significant effects on salmon reproductive and migratory physiology. One such pesticide is atrazine, which has been shown to have a significant effect on pheromonal mediated reproductive function in adult salmon (Moore & Waring, 1998) and to reduce the seawater tolerance in smolts (Waring & Moore, 1996). Atrazine (2-chloro-4-ethylamino-6-isopropoylamino-s-triazine) is a 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 five 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 (National Rivers Authority, 1995). In addition, some analyses of United Kingdom (UK) surface waters demonstrated levels approaching, and in a few cases exceeding, the proposed Environmental Quality Standard (EQS) of 2.0 μgl^{-1} based on the annual combined average of atrazine and simazine[1]. Since 1993 there has been a general decline in the detection of atrazine in UK surface waters, as a result of a ban on use on non-cropped land. However, its main use is now in the production of maize particularly in the south west of England where it is still detected in surface waters exceeding the 0.1$\mu g\ l^{-1}$ MAC (Environment Agency, 1997). Concentrations of atrazine up to 275 $\mu g\ l^{-1}$ have been detected in run-off water from agricultural land (Southwick *et al,* 1990).

In laboratory studies, salmon smolts exposed in freshwater to low levels of atrazine and then transferred to seawater, showed significant increases in plasma ion and cortisol concentrations and plasma osmolarity which resulted in a 30% mortality (Waring & Moore, 1996). The results of the work suggest that exposure of juvenile salmon to the pesticide may inhibit the smoltification process and result in the fish not being adapted to the marine environment. Another possible consequence is that the pesticide may effect the motivation to migrate and either delay or inhibit the movement of the smolts into the marine environment. Previous work has indicated that the timing of smolt migration into the sea is important to their survival and successful return as spawning adults (Hansen & Jonsson, 1989).

The present study therefore examined the impact of atrazine on run-timing in different groups of hatchery smolts exposed to the pesticide prior to release in the River Test, southern England.

[1] Simazine is an organic white solid, used as a pre-emergence herbicide used for control of broad-leaved and grassy weeds on a variety of deep-rooted crops. It is a closely related chemical to atrazine.

2 METHODOLOGY

2.1 Treatments

During 1-3 March 2000, 500 Atlantic salmon smolts (138-234mm fork length) were PIT (passive integrated transponder) tagged at the Lower Brook Hatchery on the River Test, Hampshire. The fish were then divided into three groups and transferred to large circular holding tanks. On 17 March 2000, two of the tanks were dosed with the pesticide to give final concentrations of 5 and 20 $\mu g\ l^{-1}$ atrazine. The third tank acted as a control and was dosed with an ethanol carrier. The water inflow to each tank was closed and oxygen was pumped into the water. The smolts were exposed for a period of 10 days to the pesticide. At the end of the exposure period, samples of 10 fish from each group was sacrificed, and gill tissue taken in order to measure gill Na^+ K^+ ATPase activity (McCormick, 1993), which is a physiological indicator of saltwater adaptation. The remaining fish were then released into the River Test at Romsey.

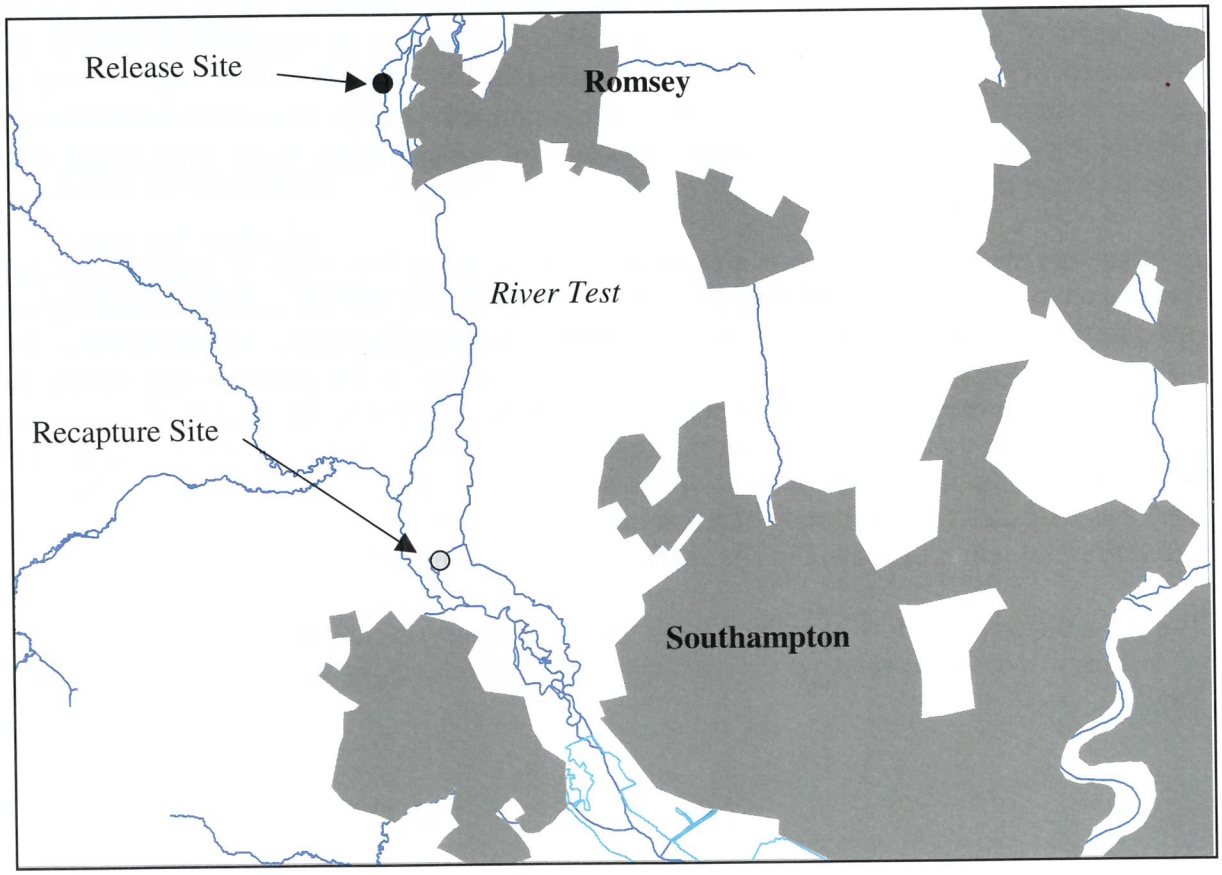

Figure 2.1 Map of lower River Test

During April and the beginning of May the Nursling smolt trap was operated continually in order to sample smolts and to determine the migration patterns of the three groups of fish.

2.2 Smolt Trapping

The recapture of PIT tagged fish was achieved using the main River Test smolt trap at Nursling. Calibration of that traps' efficiency was realised by the release of marked smolts from an upstream trap at Romsey or from the upstream release of fish caught and marked at Nursling. Since a likely consequence of atrazine exposure to the smolts was a disruption in the normal timing of smolt emigration, the downstream trapping operations were run 24hrs a day. Four main groups of smolts could be differentiated at the Nursling site.

Table 2.1 Identification of smolt groups

Smolt Group	Identification Method
Wild smolt	No marks
Hatchery smolt	Adipose clipped
Hatchery smolt grown at Mawddach	Adipose clipped and coded wire tagged
Atrazine treated hatchery smolt	Adipose clipped and PIT tagged

In addition, fish caught by the upstream trap were marked by removal of part of the right pelvic fin. Fish caught at Nursling and released upstream were marked by the partial removal of the anal fin. Details of the number and location of hatchery origin fish introduced in 1999/2000 are described in Appendix A.

2.3 The Trapping Sites

The two smolt traps operated since 1992 were reinstated in March 2000 at Romsey Trout Farm (National Grid Reference SU 348 216) and Nursling Mill (NGR SU 352 158) as shown in Figure 2.1. Russell (1992) details the construction of these traps with subsequent minor modifications in 1993 (Russell, 1993). Prior to the 1999 smolt trapping the iron bar risers of the Nursling trap were replaced with wedge wire panels. This modification was introduced in an attempt to minimise any damage to smolts and ease the task of weed removal. In addition the trap at Romsey was modified with the replacement of the hardwood slats, which form the de-watering surface, by polypropylene ones. This modification was also introduced to minimise any damage to smolts and to reduce the need for maintenance due to damaged de-watering slats.

2.4 Trap Operation

The Romsey trap was installed on 30 March 2000 and operated between the dates of 30 March to 12 May 2000. During this time routine debris clearance was carried out as required by the staff of the Romsey Fish Farm. Each day at approximately 1700hrs the holding tank was sampled and any smolts marked with the removal of approximately one quarter of the outer corner of the right pelvic fin. During this process, captured smolts were anaesthetised (using 2-Phenoxyethanol), examined for the presence an adipose fin, pelvic fin clipped, allowed to recover and released downstream of the smolt trap.

The Nursling trap was installed on 27 March 2000 and operated between the dates of 27 March to 15 May 2000 continuously. Smolts were removed from the holding tank and anaesthetised as above. Each fish was then weighed, the fork length measured and checked for the presence of an adipose fin, pelvic clip, coded wire tag and PIT tag. Fish released as part of this study were indicated by the presence of a PIT tag and the absence of an adipose fin. The data for each fish caught was recorded in a log with the date and time of capture as

given in Appendix A. On detection of fish with a PIT tag the tag number was recorded, the fish anaesthetised and the spinal chord severed. Within 20 minutes, the fourth gill raker was removed and immediately placed in SEI buffer (150mM sucrose, 10mM EDTA, 50mM imidazole, pH 7.3). Samples were then placed in liquid nitrogen storage at a nominal -80°C. The assay of Na^+K^+ ATPase activity was then carried out at CEFAS, Lowestoft using the method described by McCormick (1993).

3 RESULTS

3.1 Migration

Only a single PIT tagged smolt from the experiment was recorded in the Nursling Trap. The smolt was recorded on 26 April 2000 and was from the control group. No other fish were detected migrating downstream in the river and the remaining smolts were considered to have either died or to have remained in freshwater during the study period.

3.2 Gill Physiology

The gill Na^+K^+ ATPase activity of the three groups of smolts released into the River Test are shown in Table 3.1. The gill activity in the groups of parr exposed to atrazine was significantly reduced ($p<0.05$) when compared to the control group (no atrazine). This suggests that atrazine exposure may have reduced the ability of the fish to hypo-osmoregulate in seawater.

Table 3.1 Gill Na+K+ ATPase activity from smolts exposed to the pesticide atrazine Means ± S.E.M. N=10

Treatment Group	Gill Na+ K+ ATPase activity (µmol Pi/mg protein/h)
Control Group	12.85 ± 2.64
5 µg l^{-1} Atrazine	6.58 ± 1.17 *
20 µg l^{-1} Atrazine	5.77 ± 1.29 *
Control Group (Lowestoft)	23.46 ± 4.76

* $p<0.05$ compared to the controls

In addition, the gill activity of the control group was significantly lower ($p<0.01$)[2] than a group of smolts from the Agency's Cynrig Hatchery which had been maintained at the Lowestoft Laboratory and which was sampled at the end of April. This suggests that physiologically the smolts from the Lower Brook Hatchery may not have been ready to enter seawater and that the required smoltification processes had not been completed.

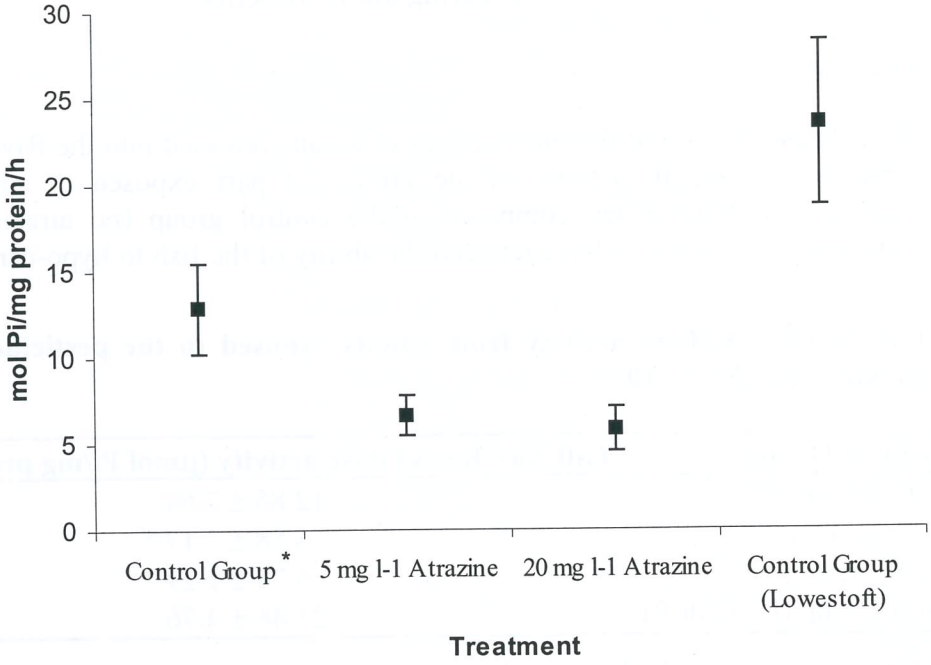

Figure 3.1 Gill Na+K+ ATPase activity from smolts exposed to the pesticide atrazine. Means ± S.E.M. N=10. * $p<0.05$ compared to the controls

[2] The data presented as mean ±S.E.M indicates that the S.E.M increased in proportion to mean i.e. That the relative S.E.M was approximately constant.

4 DISCUSSION

During the present study, exposure of the smolts to sub-lethal levels of atrazine in the hatchery clearly reduced the gill Na^+ K^+ ATPase activity and may therefore have reduced their ability to survive within the marine environment had they migrated to sea. The results are similar to previous work carried out at Lowestoft which also demonstrated that atrazine reduces the seawater adaptability of salmon smolts (Waring & Moore, 1994). A dramatic increase in gill Na^+ K^+ ATPase activity is one of the major physiological processes that occurs during smoltification and signifies an increase in the hypo-osmoregulatory ability of the fish which pre-adapts them to life in seawater.

Only a single smolt of the ~500 experimental smolts released into the River Test subsequently migrated downstream and was sampled at the Nursling smolt trap. The remaining fish are thought to have remained within the river close to the site of release. In addition, the majority of smolts from a larger group of 18 000 fish reared in the Lower Brook Hatchery and released into the River Test also did not migrate downstream past the Nursling site (Fewings A, personal communication). Although exposure to atrazine may explain why the majority of the experimental smolts did not migrate, it is not clear why more of the control group fish and the majority of the 18 000 smolts not exposed to atrazine were not subsequently sampled in the smolt trap. One possible explanation, is that although morphologically the smolts appeared to be migratory, physiologically they were not ready to enter saltwater and the smoltification process had not been completed. This is supported by the observation that the gill Na^+ K^+ ATPase activity of the control group sampled at the beginning of the smolt run was quite low when compared to other groups of smolts maintained at the Lowestoft Laboratory. Although, gill Na^+ K^+ ATPase activity is by no means a definite indicator of migratory behaviour in smolts, it does suggest that the hatchery fish may have required a further period of smoltification before they migrated to sea. Alternatively, as a result of the conditions within the hatchery the fish may have attained full smolt status earlier in the season and were subsequently losing the smolt characteristics. This can occur when fish are prevented from entering saltwater, and hatchery reared smolts in particular can lose their migratory urge, salinity tolerance and the underlying osmoregulatory changes (Duston *et al,* 1991; McCormick *et al,* 1999).

The gill Na^+ K^+ ATPase activity assay would be a useful hatchery management technique in determining the optimum time to release smolts to the wild. A small sample of fish taken at seven day periods from late February through to April would allow an indication of when the hatchery fish are physiologically adapted to migrate into saltwater. Release of the fish at this point would both increase the numbers of fish migrating and the survival and return of the fish as spawning adults.

5 CONCLUSIONS

This study was not able to provide further evidence to support or contradict the hypothesis that exposure to atrazine modified the migratory behaviour of salmon parr of smolt age and size.

Given the failure of the tagged smolts to migrate and therefore be sampled by the operation of the smolt trap few conclusions can be drawn from the experiment. It is likely from the observations of gill Na^+ K^+ ATPase activity assay that the hatchery smolts used as test subjects in this experiment were unsuitable due to their lack of physiological readiness for migration. Future studies of this nature should aim to reduce the risk of migratory failure through sourcing smolts more likely to migrate ie. pre-smolts or parr reared in near wild conditions prior to treatment and release.

If access to the pre-smolts or parr was possible then pre-treatment gill Na^+ K^+ ATPase activity assays should be carried out to ensure that normal maturation toward smoltification was underway.

Since gill Na^+ K^+ ATPase activity was depressed in both groups treated with atrazine (5 and 20 mg l^{-1}) compared with control groups ($p<0.05$) it was concluded that treatment with atrazine would have reduced the ability of emigrating smolts to osmoregulate. This reduction in osmoregulatory capacity may have prejudiced their survival on seawater challenge.

LIST OF FIGURES

LIST OF TABLES

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APPENDIX A Details of Hatchery Salmon Introductions 1999/2000

Date	No. Stocked	Mean wt. (g)	Stocked Location	Identification method
03/09/99	4180	5.3	Oakley stream/farm	AFC
06/09/99	4726	5.4	Houghton Club	AFC
09/09/99	4990	4.0	Bossington Estate	AFC
03/10/99	4376	4.2	Horsebridge	AFC
06/10/99	6196	3.3	Houghton Club	AFC
03/11/99	6188	5.0	Compton Estate	AFC
06/11/99	4527	4.8	Houghton Club	AFC
12/11/99	10290	17.0	Broadlands Estate	AFC
21/12/99	9850		Saddlers Mill (from Mawddach)	AFC + CWT
March-00	2400		Saddlers Mill	AFC
March-00	2300		Nursling Mill	AFC
28/03/00	500		Saddlers Mill Atrazine Treated	AFC + PITT
04/04/00	3000		Saddlers Mill	AFC
12/04/00	10000	80.0	Saddlers Mill	AFC
Total	**73523**			

Abbreviations:
AFC - Adipose Fin Clip
CWT - Coded Wire Tag
PITT - Passive Integrated Transponding Tag

APPENDIX B Daily Captures of Salmon Smolts at Nursling Trap

Date	Smolt Group			
	Atrazine	Hatchery	Mawddach	Wild
27-Mar-00			1	3
28-Mar-00		2	1	1
29-Mar-00		1	1	3
30-Mar-00		1		2
31-Mar-00		2		5
01-Apr-00		1		8
02-Apr-00		3	3	7
03-Apr-00		3	12	26
04-Apr-00		3		4
05-Apr-00		5		3
06-Apr-00		3		6
07-Apr-00		4	1	12
08-Apr-00		5	8	14
09-Apr-00		8	5	19
10-Apr-00		5	11	33
11-Apr-00		8	8	21
12-Apr-00		8	1	17
13-Apr-00		5	11	42
14-Apr-00		4	1	9
15-Apr-00		2		10
16-Apr-00		5		4
17-Apr-00		4		33
18-Apr-00		7	4	27
19-Apr-00		11	12	27
20-Apr-00		46	1	11
21-Apr-00		10		7
22-Apr-00		15		10
23-Apr-00		8		15
24-Apr-00		2	1	7
25-Apr-00		5		20
26-Apr-00	1	8		8
27-Apr-00		8		15
28-Apr-00		8	1	9
29-Apr-00		17		10
30-Apr-00		11		6
01-May-00		10		9
02-May-00		9	1	8
03-May-00				3
04-May-00		3		
05-May-00		5		4
06-May-00		7		9
07-May-00				3
08-May-00		7		5
09-May-00		3		3
10-May-00		3		2
11-May-00		1		
12-May-00		2		
Total	**1**	**288**	**84**	**500**