

CEC Final Report 2005: Interferon induced protection of post-smolts against IPN.

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Introduction:

The main purpose of the project was to investigate the possibility of preventing mortalities of post-smolt Atlantic salmon from IPN by stimulating the innate antiviral response. This is mainly governed by interferon which stimulates production of the antiviral protein, Mx . In mammals interferon can be induced by injection of a synthetic viral RNA (poly I:C) as well as some bacterial and yeast products. The project aimed to identify potent interferon inducing agents in salmon and then go on to test their ability to induce protection against IPN virus challenge in susceptible post-smolts.

The aims of the first study were i) to identify various Mx stimulatory compounds in Atlantic salmon (*Salmo salar* L.) and to characterise the kinetics and intensity of the stimulated responses and ii) to investigate the effect of temperature on such responses by semi-quantitative RT-PCR. Mx transcripts were measured from Atlantic salmon parr kept at 14°C and injected with either *E.coli* LPS, the synthetic double stranded polyribonucleotide poly I: C, *Vibrio anguillarum* serotypes I and II-*ordalii* bacterin, β -glucan, whole yeast cells or yeast RNA. Sampling periods lasted until transcripts were undetectable or up to three weeks after treatment. The effect of temperature on poly I: C-induced Mx response was studied by injecting parr kept at 6°C. Newly hatched salmon fry were immersed once, twice or three times in the *Vibrio* bacterin diluted five or ten times and sampled for three weeks.

None of the yeast compounds induced Mx expression in Atlantic salmon parr. *E.coli* LPS induced a very low Mx response 2 and 3 days after injection. The *Vibrio* bacterin administered by injection in parr (but not by immersion in fry) resulted in strong Mx induction on days 2 and 3, disappearing by day 6. Poly I: C induced Mx responses that were more intense and longer lasting than those induced by the bacterin, peaking on day 3 and lasting over 6 days, disappearing by day 9 at 14°C. Lower temperature caused a longer lasting Mx response to poly I:C (at least 21 days), which peaked on days 7-14, with a similar intensity and no delayed onset as compared with the response at 14 °C. However, some toxicity of the poly I:C was indicated in treatments at 6°C.

A further study on the use of DNA vaccines as interferon inducers was performed as this was known to occur in rainbow trout:

The kinetics of the Mx response were compared in rainbow trout and Atlantic salmon parr kept at 10C and injected with the DNA vaccine or the synthetic double-stranded RNA, poly I:C. In both species there was a rapid response to poly I:C detectable from day 1, reaching maximum from days 3 to 9 and decreasing to background level by day 12. The peak level and return to background was reached slightly later in salmon. In both species the response to the VHS/DNA vaccine was slower to begin, not being detectable on days 1 and 3, but elevated levels were found on day 6. However, in the salmon parr the response was more transient than in trout, the peak level being on day 6 and the signal disappeared by day 12, while in the rainbow trout, the response peaked at day 12 and lasted until day 21.

Aims of 2nd study: As poly I:C appeared to be the most potent inducer of interferon/Mx in parr, the experiments were now repeated in post-smolts.

Intraperitoneal injection of 500 mg poly I:C/fish into Atlantic salmon parr in freshwater and post-smolts and growers in seawater (all at 11C) induced enhanced expression of Mx mRNA in liver tissue 24 h post-injection. The level of Mx transcripts peaked at day 3 (Mx:b-actin ratio of about 0.8) and the response disappeared by day 7. In post-smolts, mortalities occurred up to day 14 post-injection, which was dose-dependent. Histological examination of tissues revealed severe pathological changes in the liver of poly I:C injected post-smolts resulting from apoptosis and necrosis of hepatocytes. All other organs appeared histologically normal. Levels of Mx mRNA expression on day 3 post-injection were similar for fish with normal and pathological livers.

In untreated or control fish injected with PBS, low levels of Mx transcripts (Mx:b-actin ratio about 0.1) were sometimes detectable in parr but not in growers. Constitutive Mx expression was variable in post-smolts. Some populations had no detectable transcripts while in others moderate ratios (about 0.3) were detectable over a 3-week period of sampling.

Poly I:C administered to parr by bath or orally did not induce upregulation of Mx expression.

Aims of 3rd study: As poly I:C appeared toxic in post-smolts, attention was turned to the potential of *Vibrio* products and optimising the Mx response in parr.

A commercially available vibrio bacterin, intended for immersion vaccination, was shown to be a potent inducer of Mx gene expression in Atlantic salmon parr following intraperitoneal injection. The response was dose and temperature dependent. At 10C and 10 times concentration the bacterin induced Mx response kinetics similar to that induced by poly I:C.

At 10C, enhanced Mx responses were detected from days 1 to 9 with both 1 times (1x) and 10 times (10x) concentrated bacterin, with a tendency for a higher response to the concentrated bacterin on days 1 and 3. Basal levels of Mx mRNA were detected on day 12 after injection to both concentrations. The response induced by poly I:C was higher on day 1 and it was still present at day 12, with basal levels being reached on day 18. At 6C, there was a more definitive dose effect of the vibrio bacterin and the Mx response was delayed in comparison to that at 10C. Increased Mx expression did not appear until day 6 and with the 1x dose it had disappeared by day 9. However, the 10x dose continued to induce Mx at day 12, disappearing by day 18.

The Mx response to the purified *Vibrio anguillarum* lipopolysaccharide (LPS) and DNA in fish held at 10C showed some differences in the rate of onset. The response to DNA was faster, beginning on day 1 compared with day 3 for the LPS. The response to DNA peaked on day 3 while for LPS the peak was on day 9. However, the response to both components had disappeared by day 12. The response kinetics to the *V. anguillarum* DNA was essentially similar to the 10x dose of the vibrio bacterin and to poly I:C at 10C.

Aims of 4th study:

As the previous work in Atlantic salmon parr showed that a *Vibrio anguillarum* bacterin ip injected at 10x the normal concentration was a potent inducer of interferon (IFN), in this study using post-smolts, 4 weeks after transfer to seawater, we sought to determine the optimum concentration of *V.anguillarum* vaccine, with and without an oil adjuvant, that elicits the greatest IFN response as determined by Mx mRNA expression. We also investigated if treatment of post-smolts with *Vibrio anguillarum* vaccine provided any protection against challenge with Infectious Pancreatic Necrosis Virus (IPNV) by cohabitation one week after treatment .

None of the vibrio preparations induced an Mx response in the post-smolts. There was about 35% mortality in treated fish by day 9, but no mortalities occurred in the PBS injected group. This indicated a toxic effect of the treatments.

In the challenge experiment, very low mortality occurred in the untreated fish injected with IPNV and the PBS injected cohabitants, indicating that the challenge had failed. This was also indicated by the low titres of IPNV/g kidney found in the few fish which did die. However, considerable mortality (about 40%) occurred in the vibrio bacterin injected fish which again indicated toxicity of the treatment.

It is concluded that treatment of post-smolts 3 weeks after seawater transfer by injection of a vibrio bacterin causes considerable mortality and is therefore not a feasible means of controlling mortalities from IPN.

Conclusions:

It would appear that the induction of interferon/Mx responses by the methods used above, while being successful in parr, are lethal in post-smolts when treated 3-4 weeks after seawater transfer. The reasons for this are not known.

Nevertheless, the vibrio vaccine has been shown to be a potent and safe inducer of the Mx response in parr in freshwater. With the tools presently available we have been able to measure the expression of Mx mRNA only, not the Mx protein which is the anti-viral factor. This would persist much longer than the mRNA but the actual duration is still not known. The duration of the Mx protein production and the expected duration of innate protection against IPN is, therefore, still not clear but it is possible that injection vaccination of pre-smolts with the vibrio vaccine may stimulate this response long enough to provide post-smolts with protection over the IPN-susceptibility window following seawater transfer. However, this would require further research to confirm.

Communication of results:

All of the results from this work have now been published in scientific journals and also have been communicated to Marine Harvest who kindly provided most of the fish used in the experiments. MH are currently investigating the possibility of using vibrio vaccines in pre-smolts to protect against IPN in the post-smolt period.

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