

**ENVIRONMENTAL EFFECTS ON THE ONTOGENY  
OF NON-SPECIFIC AND SPECIFIC DEFENCES  
IN TURBOT LARVAE**

C.A. Low  
Department of Zoology, University of Aberdeen  
Tillydrone Avenue AB24 2TZ  
Phone: 01224 272648 Fax: 01224 272396  
E-mail: [c.low@abdn.ac.uk](mailto:c.low@abdn.ac.uk)

M.F. Tatner, T.H. Birkbeck, I. Taylor  
Department of Infection and Immunity  
University of Glasgow

C.J. Secombes  
Department of Zoology, University of Aberdeen

**EXTENDED ABSTRACT ONLY – DO NOT CITE**

Flatfish are economically important in European fisheries and are becoming increasingly significant in aquaculture. Turbot have been identified as the most suitable species of flatfish for rearing in aquaculture in Northern Europe because of its ease of growth and high market value (Munro, 1995). The growth of aquaculture has clearly shown that infectious disease is an important limiting factor in the production of flatfish. Prior to metamorphosis larvae are extremely susceptible to a number of viral and bacterial diseases, particularly *Vibrio* and *Aeromonas* sp., which are frequently associated with turbot larvae (Bergh, 1997; Novoa and Figueras, 1996). One of the problems of intensive culture of turbot is the variation in the survival rate of the larvae during early rearing stages. This is characterised by high, though variable mortality rates which make final survival rates unpredictable (Padros, 1996; Munro 1995).

Increased knowledge of the turbot immune system, and the effects of environmental conditions such as temperature and nutritional effects, on the appearance of defence mechanisms during ontogeny will aid the introduction of new strategies to cope with the problem of high mortality. It may also have a major impact on the recruitment of turbot in the natural environment and in

fisheries. Despite recent advances in salmonid immunology there is relatively little information available on the immune system of flatfish at a molecular level. Since turbot larvae are very small, a molecular approach is ideal to monitor early gene expression of non-specific and specific defences.

In order, to determine the effects of environmental conditions on the ontogeny of non-specific and specific defences in turbot larvae, probes are being developed to genes of the turbot immune system, concentrating on genes already characterised in other fish species, where the immunological relevance is very apparent.

The main approach which has been taken, in order to develop probes and obtain partial sequences of the turbot immune genes is PCR based homology cloning. Known sequences are aligned to reveal sites of conservation for primer design. Following PCR with degenerate primers based on these regions, products of the correct predicted size are cloned and sequenced and analysed for homology. However, the cDNA used as a template is also an important consideration, and must be from tissues or cells likely to express the gene of interest, as gene expression can be tissue specific and many immune genes are not expressed constitutively. For example, transferrin and Rag-1 are expressed predominantly in the liver and expression of IL-1 $\beta$  requires induction. Stimulation can be easily achieved *in vivo* by bacterial challenge, as with an attenuated (aroA-) strain of the Gram negative bacterium *Aeromonas salmonicida*, or *in vitro* by incubation of head kidney leucocytes with lipopolysaccharide (LPS).

To date partial sequences have been obtained for turbot interleukin 1 beta (IL-1 $\beta$ ), transforming growth factor beta 1 (TGF $\beta$ 1), recombinase activating gene 1 (Rag-1) and Transferrin. The nucleotide identity of these sequences to other fish species is shown in Table 1.

Preliminary expression studies looking at immune gene development in turbot eggs and newly hatched larvae have been carried out using probes to IL-1 $\beta$ , TGF $\beta$ 1 and Rag-1. The results showed that TGF $\beta$ 1 was expressed in eggs (day 1 – day 4 post-fertilisation) but not in the early larval stages (day 5 at hatching – day 7). There was no IL-1 $\beta$  or Rag-1 expression detectable at any stage over this early period of development.

TABLE 1: Percent nucleotide identity of partial sequences of immune genes in turbot to those of other fish species.

Gene	Length Sequenced	% Nucleotide Identity
IL-1 $\beta$	197bp	80% Plaice
		72% Rainbow Trout
TGF $\beta$ 1	185bp	94% Plaice
		81% Rainbow Trout
		79% Goldfish
Rag-1	552bp	82% Rainbow Trout
		74% Goldfish
		74% Zebrafish
Transferrin	871bp	72% Japanese Flounder
		67% Rainbow Trout
		66% Atlantic salmon

In a second larval rearing study, larval turbot were reared at 10°C, 14°C and 18°C under (largely) bacterial free conditions. Larvae reared at 10°C were incubated in LPS 24 hours prior to sampling. Expression of IL-1 $\beta$  was detected from 1-4 days after hatching in larvae stimulated with LPS but not in unstimulated samples. This is consistent with our previous work which showed that turbot IL-1 $\beta$  required induction by *in vitro* stimulation of head kidney leucocytes with LPS or by challenge (intraperitoneal) with *Aeromonas salmonicida*. Expression of the Rag-1 gene was not detected at this stage of development and is presumably a later event.

Probe development is currently on-going for other turbot immune genes.

## References

- Bergh O., Hjeltnes B. and Skiftesvik A.B. 1997. Experimental infection of turbot *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* yolk sac larvae with *Aeromonas salmonicida* subsp. *salmonicida*. Diseases of Aquatic Organisms 29: 13-20
- Munro P. D., Barbour A. and Birkbeck T. H. 1995. Comparison of the growth and survival of larval turbot in the absence of culturable bacteria and those in the presence of *Vibrio anguillarum*, *Vibrio alginolyticus*, or a marine *Aeromonas* sp. Applied and Environmental Microbiology 61: 4425
- Novoa B. and Figueras A. 1996. Heterogeneity of marine birnaviruses isolated from turbot. Fish Pathology 31: 145-150
- Padros F. and Crespo S. 1996. Ontogeny of the lymphoid organs in the turbot *Scophthalmus maximus*: a light and electron microscope study. Aquaculture 144: 1-16

