

**PARALYTIC SHELLFISH TOXINS AND X
ENOBIOTIC METABOLISING ENZYMES
IN ARTIFICIALLY INTOXICATED
ATLANTIC SALMON (*SALMO SALAR*)**

Matt J. Gubbins¹

¹ FRS Marine Laboratory, PO Box 101,
Victoria Road, Aberdeen, AB11 9DB, UK.
tel: 01224 295522; fax: 01224 295511;
e-mail: gubbinsm@marlab.ac.uk

F. Brian Eddy², Susan Gallacher¹ and Ron M. Stagg¹

² Department of Biological Sciences,
University of Dundee, Dundee, UK.

EXTENDED ABSTRACT ONLY – DO NOT CITE

Paralytic shellfish toxins (PSTs) are a group of potent neurotoxins produced by toxic strains of dinoflagellates. Exposure of fish to such toxins can result in fish kills (White, 1977) and other deleterious effects in marine food webs (Geraci et al. 1989). A greater understanding of the precise fate of PSTs in marine organisms is therefore required. Studies in this field to date have focussed on the dynamics of PST depuration from bivalve molluscs (Bricelj and Shumway 1998), yet little is known of the mechanisms responsible for eliminating these toxins from fish. It has been suggested that xenobiotic metabolising enzymes (XMEs) may be involved in the metabolism of algal toxins (Washburn et al. 1996). Intra-peritoneal (ip) exposure of salmon (*Salmo salar*) to saxitoxin (STX) has been shown to effect the induction of cytochrome P-4501A, a phase I XME, as measured by 7-ethoxyresorufin *O*-deethylase activity (Stagg et al. 1998).

Investigated here is the potential role of the phase II XME glutathione *S*-transferase (GST) in PST metabolism. GSTs catalyse the conjugation of reduced glutathione (GSH) to electrophilic centres on substrates. This activity is inducible on exposure of the organism to the substrate.

The objective of this study was to determine the induction response of GST activity in Atlantic salmon exposed to PSTs by injection. This was achieved by three injections of post-smolts with PSTs (STX [2 :g/kg] or an extract from a cultured toxic dinoflagellate [*Alexandrium fundyense* CCMP 1719, 2.26 :g/kg] in physiological saline over 21 days. For control purposes fish were exposed to physiological saline or an extract from a non-toxic dinoflagellate (*Scrippsiella trochoidea* NEPCC 15).

Hepatic GST activities were found to differ significantly between treatment groups ($P < 0.001$). Fish exposed to both saxitoxin and a toxic dinoflagellate extract containing a number of PST analogues demonstrate nearly two-fold induction of activity over controls (Fig. 1).

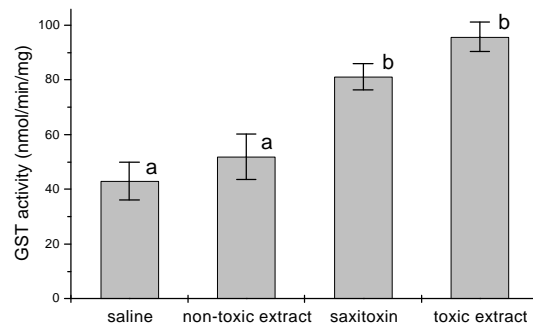


Fig. 1. Hepatic glutathione *S*-transferase activity of Atlantic salmon exposed over 21 days to multiple ip injections of: physiological saline, saxitoxin and extracts of toxic (*Alexandrium fundyense*, CCMP 1719) and non-toxic (*Scrippsiella trochoidea*, NEPCC 15) cultured dinoflagellates. Data are means \pm SE, $n = 10$. ab Groups with different notation are significantly different ($P < 0.05$).

The major GST isoform in salmon livers has been shown to be homologous to mammalian B class GST (Dominey et al. 1991). Antibodies raised against rat B class GST (obtained from John Hayes, Dundee University) detect a single protein band in protein fractions from both rat (positive control) and salmon

livers (Fig. 2a). Rat B class GST was estimated at 26.8 kDa in size, while the 'B class GST-like' protein detected in salmon samples was 27.6 kDa.

Variation in salmon 'B class GST-like' protein was inferred from immuno-peroxidase stained dot blots by quantifying band intensities on scanned blots. This analysis showed that fish injected with a toxic dinoflagellate extract not only demonstrate the highest levels of hepatic GST activity (Fig. 1), but also contain significantly elevated levels of 'B class GST-like' protein (Fig. 2b). This suggests that elevated levels of the major GST protein in salmon livers may be partly responsible for inducing hepatic GST activity in salmon exposed to PSTs.

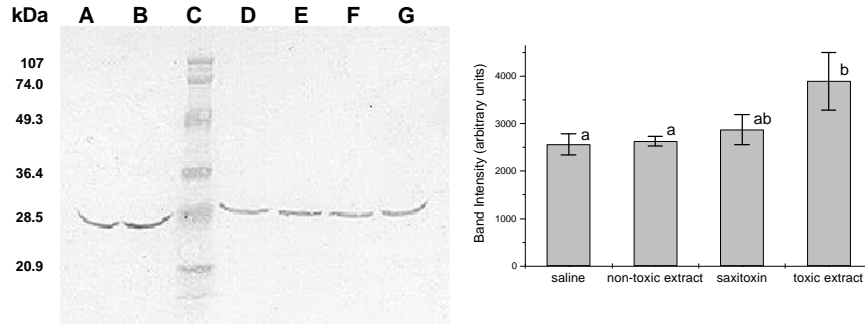


Fig. 2a. Western blot probed with anti-rat B class glutathione *S*-transferase antibodies and visualised by immuno-peroxidase staining. A-B) protein fractions from rat liver run as a positive control at 40 and 80 :g protein loadings respectively. C) pre-stained BioRad low range size marker. D-G) protein fractions from salmon livers (40 :g protein). **2b.** Band intensities from dot blots probed and visualised in the same manner. Samples (200 :g protein) are from salmon treated as per Fig. 1. Data are means \pm SE, n = 10. ab Groups with different notation are significantly different ($P < 0.05$).

In the fish model chosen, artificial intoxication with PSTs (saxitoxin or extracts from toxic dinoflagellates) results in induction of GST activity in the liver. This may be caused by increased protein expression. Such induction suggests that this enzyme system may play a role in the metabolism of this group of algal toxins.

Exposure of fish to PSTs from toxic dinoflagellate blooms may therefore result in a greater capacity of the liver to conjugate GSH to absorbed toxins.

References

- Bricej, V. M. and S. E. Shumway. 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics and biotransformation. *Rev. Fish. Sci.* 6:315-383.
- Dominey, R. J., I. A. Nimmo, A. D. Cronshaw and J. D. Hayes. 1991. The major glutathione *S*-transferase in salmonid fish livers is homologous to the mammalian pi-class GST. *Comp. Biochem. Physiol.* 100B:93-98.
- Geraci, J. R., D. M. Anderson, R. J. Timperi, D. J. St. Aubin, G. A. Early, J. H. Precott and C. A. Mayo. 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. *Can. J. Fish. Aquat. Sci.* 46:1895-1898.
- Stagg, R. M., S. Gallacher and P. Burgess. 1998. The toxicity of saxitoxin and effects on hepatic CYP1A activity in farmed Atlantic salmon (*Salmo salar*). Pages 607-608 *In* B. Reguera, J. Blanco, M^a. L. Fernández and T Wyatt, editors. Harmful Algae. Xunta de Galicia and International Oceanographic Commission of UNESCO, Santiago de Compostela.
- Washburn, B. S., C. A. Vine, D. G. Baden, D. E. Hinton and P. J. Walsh. 1996. Differential effects of brevetoxin and beta-naphthoflavone on xenobiotic metabolising enzymes in striped bass (*Morone saxatilis*). *Aquat. Toxicol.* 31:1-10.
- White, A. W. 1977. Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as apparent vector. *J. Fish. Res. Board Can.* 34:2421-2424.

