

## Sex difference in tissue distributions of tetrodotoxin in grass puffer *Takifugu niphobles*

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Pufferfish is known to possess fatal toxin, tetrodotoxin (TTX). Although the toxicity is different among species and tissues [1], little is known about tissue distributions of TTX. In this study, detailed localization of TTX in male and female grass puffers *Takifugu niphobles* was investigated by immunohistochemical techniques with a commercial anti-TTX monoclonal antibody. The pufferfish *T. niphobles* were captured at the coastal waters of Enoshima Island, Kanagawa, Japan, July 2009. Tissues of skin/muscle, liver, gonad and intestinal tract were fixed with 4% paraformaldehyde, and embedded in paraffin followed by sectioning. Sections were reacted with anti-TTX monoclonal antibody based on the fluorescent immunohistochemical techniques. Immuno-positive reaction was observed in skin and liver in male, whereas it was detected in skin and ovary in female. In the skin, TTX was localized at epidermis, basal cell layer, mucous cells and sacciform cells, especially with intense immunoreaction at flat epithelial cell layer in the epidermis and sacciform cells. Signals on liver cells were stronger in male than those in female. Furthermore, the germinal vesicles of oocytes at perinucleolus and yolk granule stages exhibited intense immunoreactivity for TTX. On the other hand, no specific reaction was observed in muscle, testis and intestine. The present observations demonstrated that tissue distributions of TTX in the pufferfish *T. niphobles* were sex-dependent.

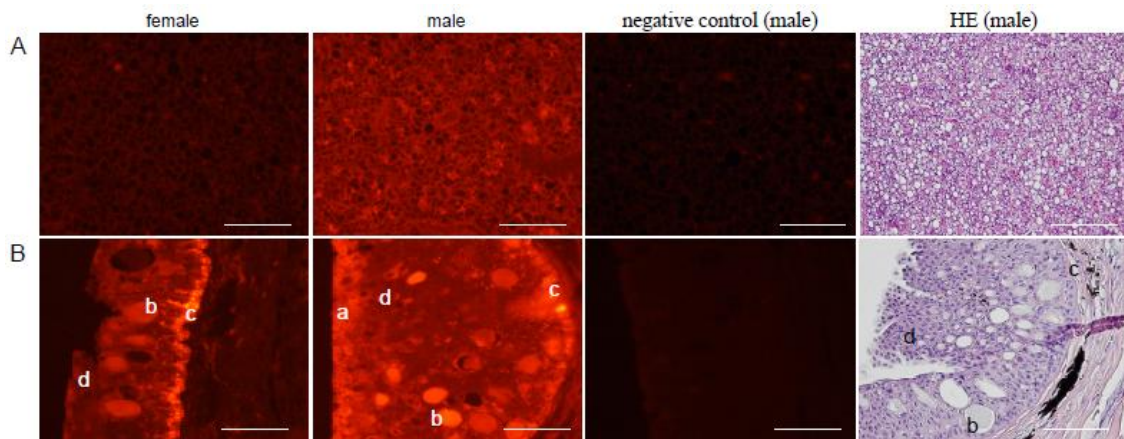


Fig. 1. Sex difference in the immunoreactivity for TTX in skin (A) and liver (B) of grass puffer. Exposure time was 400 and 125 ms in skin and liver, respectively. Positive signals to TTX-antibody are observed as red color. The negative control was reacted with mouse IgG and tissue structure was observed by HE staining. a, flat epithelial cell layer; b, sacciform cell; c, basal cell layer; d, mucous cell. Scale bars: 5  $\mu$ m.

### References:

- [1] Noguchi, T., Arakawa, O. and Takatani, T. 2006. TTX accumulation in pufferfish. *Comp. Biochem. Physiol. D* 1: 145–152.