Development of an enzyme-linked immunosorbent assay (ELISA) for assessing vitellogenin content in the skin mucus of Perciformes (Teleostei : Acanthopterygii)

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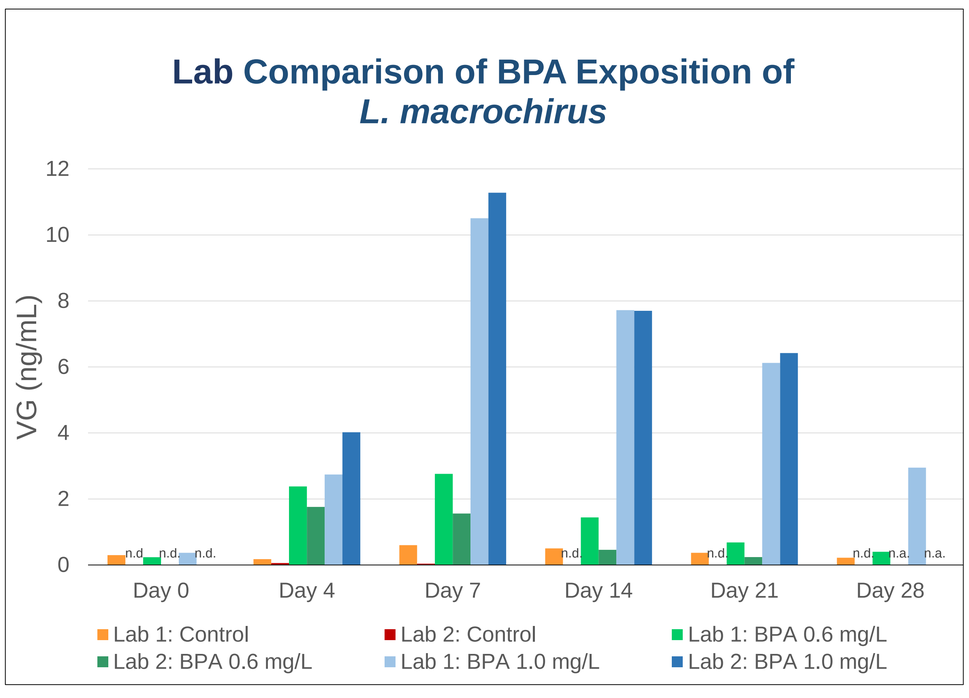
Background Perciform fish are the dominant vertebrate group in the aquatic environment. Thus environmental monitoring especially of estuarine and marine environments, as well as targeted chemical testing calls for perciformes related bioanalytical methods. A non-destructive ELISA-method to determine the egg yolk protein vitellogenin (VTG), a bioindicator for estrogenic endocrine disruption, in perciform fish is presented. Quantification of VTG induction is recommended by the OECD for assessing estrogenic potential of chemicals (EDCs) (OECD TG 229; OECD TG 230; OECD TG 234)

Method

Figure 1: (a,b) non destructive sampling of skin mucosa via FLOQ-Swabs.(c) application of the sandwich ELISA which quantifies the VTG with a reaction of tetramethylbenzidine (TMB) with streptavidin peroxidase. (d) shows the immune histology staining of VTG within the oocyte of *L. macrochirus* with the antibody that is used in the assay.

Lepomis macrochirus were exposed semi statically to sublethal Bisphenol-A (BPA) concentrations (600 µg/L and 1000 µg/L) and estradiol (data not shown) for 28 d in compliance with OECD 215 in order to check the suitability of the method in the frame of standard toxicity testing as an additional endpoint . In order to prove the option of non destructive application of the method in field studies perch (*Perca fluviatilis*) was exposed to 17β-estradiol (200 ng/L, 500 ng/L and 1000 ng/L)

Results and Discussion



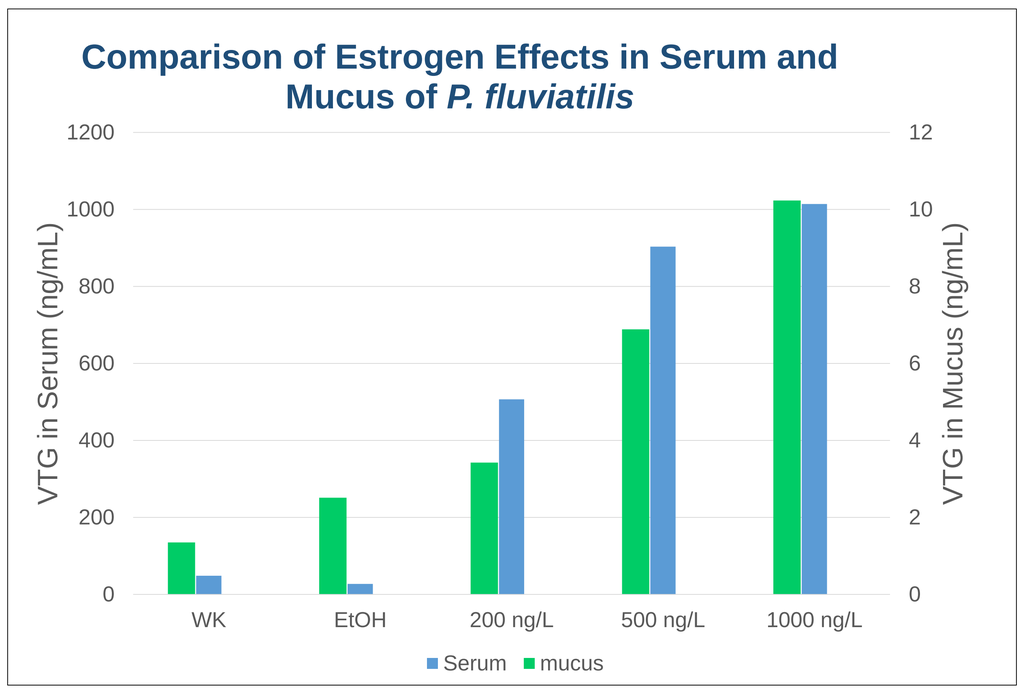


Figure 2: (a) shows the dose-dependent induction of VTG in distinct parallel (i.e. taken from different sides of each specimen) mucus samples of *L. macrochirus* monitored individually, as quantified by two distinct laboratories (Gobio GmbH, TECOdevelopment) using the TECO Perch ELISA.
(b) shows the dose-dependent induction of VTG in serum and mucosa by exposures of *P. fluviatilis* to different concentration to 17-β estradiol after six days.

VTG values in the epidermal mucosa of *L. macrochirus* samples increased from 0.2 to 11 ng/mL, with highest values obtained in day 7 samples for both concentrations, pointing to the acclimatization (i.e. of the estrogen receptors in epidermal cells) to waterborne BPA. Estradiol induced VG concentrations in Perch epidermal mucosa are about one order of magnitude lower than in blood, but they are comparable in terms of dose dependency and time course.

Conclusion

The assay enables reliable detection of estrogen effects in perciform fish species. Epidermal mucosa is a well-defined matrix without lymphatic fluid contamination to measure VTG induction non-destructive. Pre exposure testing and repeated sampling in course of long term assays facilitates to record the kinetic of VTG induction.
Due to the high sensitivity TECO Perch ELISA mucosa VTG determinations are directly comparable to homogenate or serum testing. The method has a high potential to reduce the expense of endocrine disruptor environmental monitoring and ED related risk assessment in chemical testing in compliance with OECD test guide lines.

All procedures described herein were performed in compliance with the German and European laws, with permission number RTK / Anz. 1000. Specimen were obtained from certified rearing facilities and maintained and exposed at the Gobio GmbH laboratory.

Allner B., Gönna von der S., Griebeler E.M., Nikutowski N., Schaat A., Stahlschmidt-Allner P. (2010) Reproductive functions of wild fish as bioindicators of reproductive toxicants in the aquatic environment. ESPR Environ Sci. Pollut. Res. (2010) 17: 505-518
Moncaut, N., Lo Nostro, F., Maggese M. C. (2003) Vitellogenin detection in surface mucus of the South American cichlid fish Cichlasoma dimerus (Heckel, 1840) induced by estradiol-17b. Effects on liver and gonads. Aquatic Toxicology 63, 127-137.