Basic knowledge of the physiology of vitellogenesis as a prerequisite for the interpretation of this biomarker

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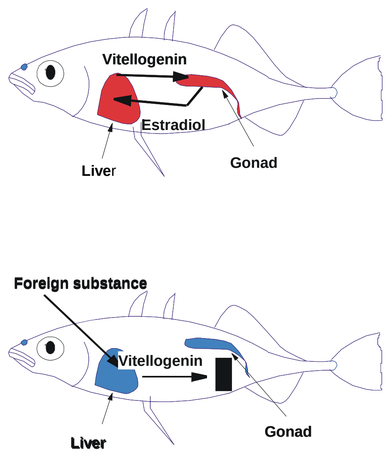

Fig. 1

At the beginning of egg maturation, follicle cells of the ovary secrete 17ß-estradiol (E2), which induces the synthesis of the yolk protein vitellogenin (VTG) in the liver (Fig.1 above, Fig.3c). VTG is transported via blood to the ovary and deposited in the maturing oocyte.
Estrogenic xenobiotics can also trigger the synthesis of VTG, regardless of age and the sex of the fish. In male fish, VTG accumulates in the blood (Fig.1 below) and is detectable as an unphysiological protein in serum (Fig.2). Juvenile and male fish react differently on exogenous estrogens depending on the species (Fig.2).

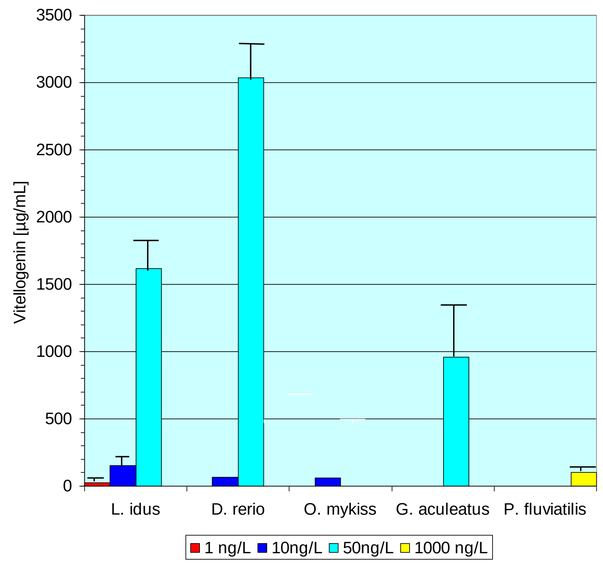

Fig. 2: Comparison of the sensitivity of different fish species after 7 days of ethinyl estradiol (EE2) exposure in different concentrations. L. idus and D. rerio clearly show a dependence of concentration. In P. fluviatilis only low VTG levels are detectable in blood even at exposure to high concentrations of EE2.

Vitellogenesis in roach (*Rutilus rutilus*)

Rutilus is dominant in many european river basins and is used as an indicator species for the assessment of exposure to estrogenic compounds.

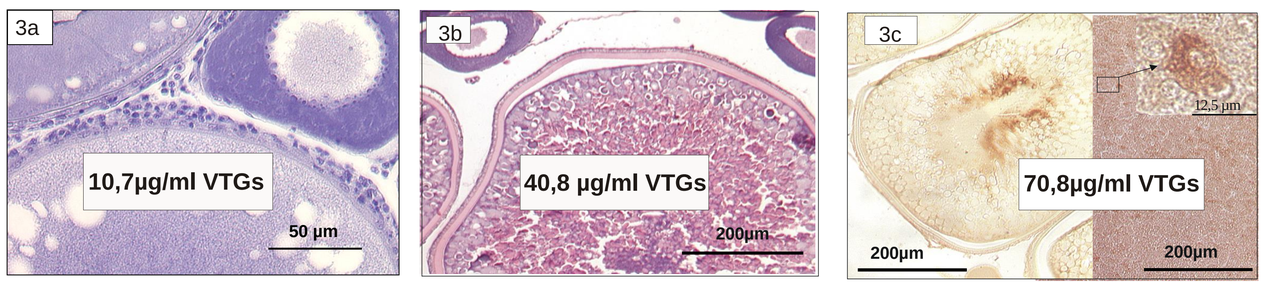

Fig. 3: a) and b) Cross sections of roach ovar in different stages of egg maturation (HE staining); a) in the first ripening stage, formation of a two layered (i.e. E2-producing) follicle epithelium leads to serum VTG content (VTGs) of 10,7µg/ml; b) in late ripening stage, 40,8µg/ml VG detectable in blood; c) immunohistochemical localization of VTG in ovary (left) and liver (right); distinct immunoreactive regions in the centre of the ovum (brown coloration) and in liver tissue.

In roach VTG can be detected immediately after formation of a functional follicular layer in early stages of egg maturity in serum (VTGs). The serum VTG levels rises concomitant with egg maturation. VTG immunological detectable in liver as well as in the egg cell.

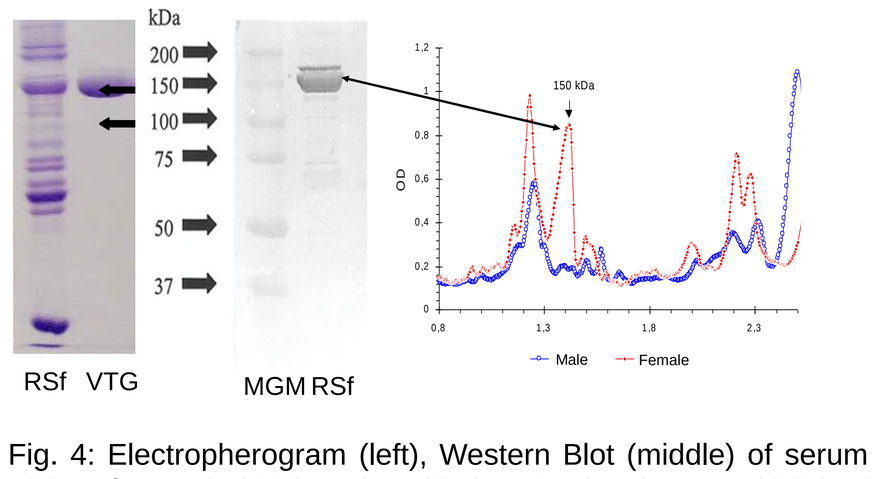

Fig. 4: Electropherogram (left), Western Blot (middle) of serum of female roach (RSf) and purified VTG; densitogram (right) of serum of a male and a female roach; the arrow connects the immunological detected VTG and the corresponding protein peak in the densitogram.

Vitellogenesis in perch (*Perca fluviatilis*)

Perch as a dominant member of perciform species is also used as an indicator species in studies of population biology.

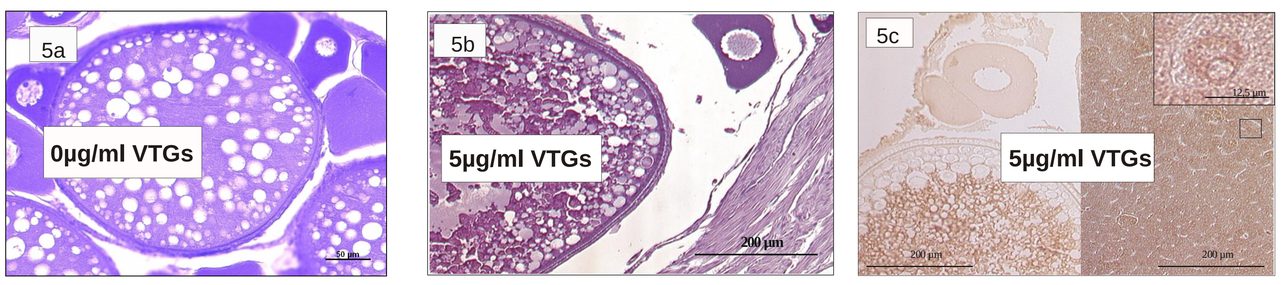

Fig. 5: a) and b) Cross sections of perch ovar in different stages of egg (HE-staining); a) in the first ripening stage, Formation of a two layered follicle, no VTG detectable in blood; b) in late ripening stage, only 5µg/ml VTG detectable in blood; c) immunohistochemical localization of VTG in ovary (left) and liver (right); distinct immunoreactive regions in the centre of the ovum (brown coloration), detection in liver questionable.

In perch VTG is only detectable in late stages of egg ripening in very low concentrations in blood (VGs), independent from the determination method (Abb.5). An explicit immunological detection of VTG formation in liver is only possible after exposure to unphysiological high concentrations of ethinyl estradiol. In contrast, the detection in the egg cell is explicit.

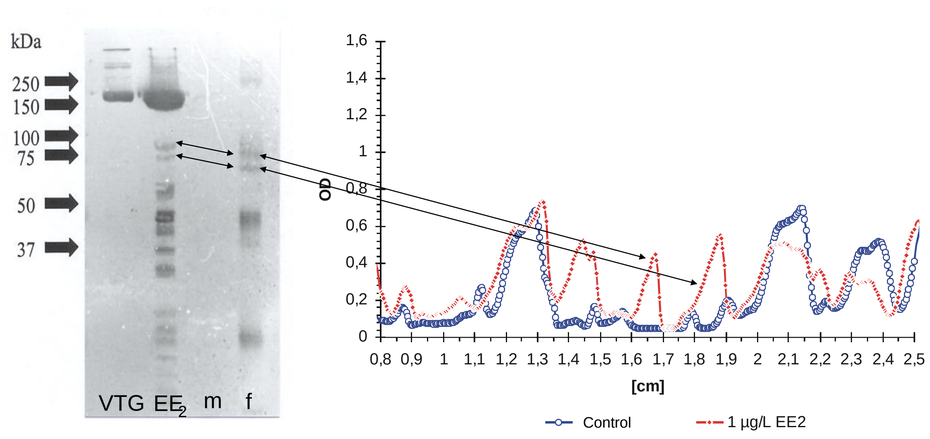

Fig. 6: Western Blot (left) of purified VTG and serums of a female, a male and a juvenile with EE2 induced perch; Densitometrical presentation of a electropherogram (SDS-PAGE) of serum of a male control fish and a EE2 induced juvenile perch (right); the arrows connect immunological detected, relatively stable VTG decomposition products and the corresponding protein peaks in the densitogram. Both bands also appear regularly in serum of maturing female perches and are therefore suited for the qualitative detection of VTG.

In perch, a quantitative VTG detection is not safely applicable (Fig.6).

Conclusion

Perch and Roach differ fundamentally in terms of physiology of yolk formation, even though the cytology of oocytes shows no differences. The ideas, which were obtained by investigating cyprinids and salmonids, do not apply for perch. It is not acceptable to compare serum vitellogenin contents of different fish species as an indicator of estrogen pollutant burden. In order to estimate the estrogenic potential underlying exogenously induced VTG synthesis, it is necessary to asses the sensitivity of the species to estrogens in laboratory experiments . Due to the instability of VTG a comparison of quantity is only possible if the same method of detection was used.