

# **Estrogen Endocrine Disruption Monitoring**

A technical guide to field studies and cage experiments





The challenge of monitoring xenoestrogen-mediated endocrine disruption via recording vitellogenin induction is to combine bioanalysis of trace amounts of an unstable protein with nondestructive field sampling.

#### Please find our suggestions on

Sampling sites and period

Electrofishing

Sampling and sample conservatio

Habitat-related selection of monitor

Riverine upper reaches

Grayling zone

**Riverine lower reaches** 

Lentic waters

Caged and bypass experiments

Allocation of indicator species to E

Detailed information on TECO products for environmental monitoring at <a href="http://www.tecomedical.com/en/ecotoxicology">http://www.tecomedical.com/en/ecotoxicology</a> Services in environmental monitoring by Gobio GmbH http://www.gobio-gmbh.de/

	5
	7
on	11
oring species	13
	13
	15
	17
	21
	23
ELISA	26





Length-frequency diagram of the European perch, with the arrows indicating the total length variation from individual age groups (age determination by scale age analysis).

#### Sampling sites and period

Previous endocrine disruption monitoring studies have shown that destructive sampling comprising a sufficient number of individuals (n>= 40) incurs in a large impact on the fish population under investigation. Several measurement points are required to distinguish between exogenous impacts and the natural hormonal changes in the course of seasonal gonadal differentiation. These experiences led us to design a monitoring strategy based on non-invasive sampling of fish mucosa for VTG determination

For *in vivo* estrogen monitoring, sampling from late summer to late autumn is suggested.

Since fish fauna varies depending on the socio-geographical condition of aquatic habitats, data from EUWFD fish monitoring campaigns are useful planning tools.

Monitoring should focus on the condition of different riverine habitats and site-related species instead of migrating species (like salmonids).







#### Electrofishing

Is the method of choice when fish fauna should be monitored in strong relation to their habitats. A fishing team comprised of at least 3 persons is advisable to conduct a fishing. The operator of the electric catcher should be assisted in the water by a second person also dressed in appropriate protective gear, both for safety reasons and to bring the narcotized fish to the shore to be placed in a small tank for sampling, which should be performed by the third person.

For the benthonic species *Gobio gobio*, *Barbatula barbatula* and *Cottus gobio*, a representative sample (n=20) was caught over a 2h lasting fishing activity in a previous event. According to the EUWFD, a river stretch of 100 m should be monitored.







#### Electrofishing

For medium-sized rivers, usage of a boat may be required for the fishing event. Since the indicator species inhabiting these zones are less sensitive to the treatment, the fish can be stored in small tanks on the boat for later sampling.

Whereas the small-sized species proved to be resilient regarding the electric impulse, maturing trouts over 25 centimeters long seemed to be strongly impaired. Trouts often become spastic and need to be treated before normal swimming behaviour is restored.



9





## Sampling and sample conservation

For mucosa sampling, the fish should be transferred directly to small tanks at the shore or at an accompanying boat.

Soft nets should be used to place the fish at humid paper towel.

Smear samples should be taken according to the instructions of the TECO<sup>®</sup> Mucus Collection Set. After recording the species and length of the individuals, specimens should be checked regarding their vitality and returned to the river after responsiveness is completely restored.

For conservation and transport, the samples should be placed in Styrofoam boxes with ice for maintenance of low temperatures. We suggest the use of dry ice to avoid water condensation. Samples should be taken to the laboratory or shipped on ice to the contract facility immediately after sampling events.







### Habitat-related selection of monitoring species

Based on a pilot study with 4 different ecological sampling sites, we suggest distinct species for different riverine environments.

### Riverine upper reaches, salmonid zone

Apart from specific technical reasons, we do not suggest monitoring of the brown trout, since this species is often restocked for fishery purposes in these riverine zones. In this area, two species of interest for monitoring from the Cyprinidae family may be encountered: *Gobio gobio* and *Barbatula barbatula*.

Both cyprinid species feed on benthos and are therefore closely connected to the breeding grounds of the brown trout.

In both habitats, a significant number of individuals of different sizes and/or ages was caught in the frame of an EUWFD monitoring campaign. The fish were not compromised either by the applied electricity or by the sampling (i.e. swab smear) itself.

The only perciform species living in this area is *Cottus gobio*. In July 2015, we measured only trace amounts (0.1 ng/ml) of VTG in the epidermal mucosa using the TECO<sup>®</sup> Perch (Perciformes) ELISA.







#### Grayling zone

In a monitoring fishing addressed to measure body burden of priority compounds in April 2015, smear samples were taken from mature fish from the entire catch. Sex was determined for a restricted number of individuals based on gonad morphology or appearance of tubercles in male individuals of *Chondrostoma nasus*.

In this zone, significant numbers of immature cyprinids and European perches should be accessible (by electrofishing) in a size suitable for non-destructive sampling. These cohorts show low but measurable VTG values in the epidermal mucosa. Elevated VTG levels (above 4 ng/ml) are suspected to be due to exogenous estrogens.

Concomitantly, in older maturating individuals a clear distinction between male and female specimens was measurable. Reproductive toxicants may be reflected by elevated values in males and strongly lowered values in females, due to endocrine disruption of estrogen dependent gonadal ripening (anti-estrogenic effect).







#### **Riverine lower reaches**

What refers to habitat preference, roaches and perches show a certain degree of overlapping with other indicator species. Since both species are euryhaline (i.e. able to survive a wide range of salinity), they also inhabit estuarine and Baltic habitats.

The seasonal variation in epidermal mucosa VTG content of roaches is comparable to those found in upstream cyprinid indicator species. Measurements of subadult individuals in autumn reflect the strong increasing levels in females during ovarian ripening. No clear increase in approximately 50 % of subadults may indicate failure in gonadal ripening of females, possibly due to anti-estrogenic endocrine disruption. Increasing values in juveniles imply estrogenic impact. Regarding roaches, it is strongly recommended not to conduct surveys in the summer since the species is highly susceptible to any kind of exogenous impacts, and harmful effects may result from sampling.







#### **Riverine lower reaches**

For perches, evidence of seasonal variation in females is not conclusive, but differences in VTG contents of juveniles and sensitivity to estrogens and xenestrogens suggest that the species is suitable for monitoring purposes.

The neozooan genus *Neogobius* caught in river Rhine showed low but reliable VTG contents in epidermal mucosa with the TECO<sup>®</sup> Perch (Perciformes) ELISA.







#### Lentic waters

In lentic waters the common Rudd (*Scardinius erythropthalmus*) is suggested as a cyprinid monitoring species, showing comparable reproductive physiology as the riverine indicator species. Regarding the exposure situation, a lower impact of sediment borne contaminants due to herbivore nutrition must be considered.

Apart from the European perch, the stickleback (*Gasterosteus aculeatus*) represents a widespread perciform species in riverine as well as in lentic environments. The epidermal mucosa VTG values differ between males and females in reliable concentration ranges when measured with the TECO<sup>®</sup> Perch (Perciformes) ELISA.









#### Caged and bypass experiments

Caged and bypass experiments are the method of choice to monitor spot contaminations and habitats missing indigenous fish fauna.

Since repeated sampling is possible, the pre-exposure status can be recorded and only a restricted number of controls is required to determine the species-specific effects of the caged exposure situation. Stock fish suited for cage and bypass experiments are the common carp (*Cyprinus carpio*) and the goldfish (*Carassius auratus*). Both species proved to be sensitive against xenestrogens in laboratory experiments.

Carps up to 10 months of age are available from July to February in suitable sizes for mucosa monitoring experiments. During this period, carps show low variation of epidermal mucosa VTG levels between males and in females. They are sensitive to exogenous estrogens, which cause increased values of mucosa VTG that can be reliably measured with the TECO<sup>®</sup> Cyprinid Vitellogenin ELISA.







### Caged and bypass experiments

Amongst perciforms, juvenile bluegills are suggested for caged experiments. Regarding the usage of perciform species, an adaption of 3 days to the exposure group is recommended to even VTG values possibly altered by the social interactions in the pre-exposure situation.

Another suggestion for caged monitoring trials is the exposure of all-male groups of guppies (*Poecilia reticulata*). The low but reliable mucosa VTG values obtained in the TECO<sup>®</sup> Perch (Perciformes) ELISA are clearly distinguishable from the VTG contents in female mucosa.



## Monitoring in marine habitats

For the monitoring of fish in the marine environment the Teco Marine VTG Assay was developed.





gill smear as an alternative with a less potential of cross contamination.



The gender of flatfishes is easily distinguishable when hold against light. Therefore, low or high VTG values may be more significant.



Possible contamination of mucosa VTG due to contact of the fish during holding. Sampling of











Sample dilution per species in the TECO® Cyprinid Vitellogenin ELISA

Common	Species	Family	Dilution
name			range
Carp	Cyprinus carpio	Cyprinidae	undiluted to 1:100
Goldfish	Carassius gibelio auratus	Cyprinidae	undiluted to 1:100
Zebrafish	Danio rerio	Cyprinidae	undiluted to 1:10
Fathead minnow	Pimephales promelas	Cyprinidae	undiluted to 1:10
Japanese rice fish	Oryzias latipes	Adrianichthyidae	undiluted
Common bream	Abramis brama	Cyprinidae	1:10 to 1:100
Roach	Rutilus rutilus	Cyprinidae	undiluted to 1:100
Common rudd	Scardinius erythrophthalmus	Cyprinidae	undiluted to 1:10
Chub	Squalius cephalus	Cyprinidae	undiluted to 1:100
Common nase	Chondrostoma nasus	Cyprinidae	undiluted to 1:100
Bleak	Alburnus alburnus	Cyprinidae	undiluted to 1:10
Neon Tetra	Paracheirodon innesi	Characidae	undiluted
Gudgeon	Gobio gobio	Cyprinidae	undiluted to 1:10
Common dace	Leuciscus leuciscus	Cyprinidae	undiluted to 1:10
Stone loach	Barbatula barbatula	Nemacheilidae	undiluted to 1:10

Sample dilution per species in the TECO<sup>®</sup> Perch (Perciformes) ELISA

Common name	Species	Family	Dilution range
Tilapia	Oreochromis niloticus	Cichlidae	undiluted
Bluegill	Lepomis macrochirus	Centrarchidae	undiluted
<b>European perch</b>	Perca fluviatilis	Percidae	undiluted
Ruffe	Gymnocephalus cernua	Percidae	undiluted
Goby		Gobiidae	undiluted
Three-spined stickleback	Gasterosteus aculeatus	Gasterosteidae	undiluted
Guppy	Poecilia reticulata	Poeciliidae	undiluted









#### **TECO**medical AG

Headquarters **TECOmedical GROUP** Gewerbestrasse 10 4450 Sissach Switzerland Phone +41 (0) 61 985 81 00 Fax +41 (0) 61 985 81 09 Mail info@tecomedical.com Web www.tecomedical.com GOBIO-GmbH Scheidertalstraße 69a 65326 Aarbergen/Kettenbach Germany Phone: +49 6120 926434 Fax: +49 6120 903817 Mail: stahlschmidt@gobio-gmbh.de www.gobio-gmbh.de

Partnership with:

© 09/2015 TECOmedical Group, Switzerland