Dissertation abstract

Larsson, D. G. Joakim, 2000: **Endocrine disruption in fish: sex ratios, secondary sex characters and estrogen-induced proteins.** Department of Zoology/Zoophysiology, Göteborg University, Box 463, SE-405 30 Göteborg, Sweden

Anthropogenic substances in the environment are known to cause reproductive disturbances in vertebrates by interfering with their endocrine systems. Disruptions of androgen or estrogen function in fish may have effects on a number of events, including the gonadal differentiation, the development of secondary sexual characters and the induction of sex-specific proteins. The major aims of this thesis were to develop new or adapt existing *in vivo* biomarkers in fish to be able to identify endocrine disturbances.

Immunological assays (ELISA and Western blotting) were developed against vitellogenin, an egg yolk protein precursor, and vitelline envelope (eggshell) proteins in several fish species. These proteins are induced by estrogen and an induction in males is thought to reflect an exposure to exogenous estrogen or estrogen-like chemicals. Vitelline envelope proteins were induced by estradiol in 10 teleost species; thus the induction of these proteins may be used as a biomarker for estrogenic contamination in a wide spectrum of species.

The viviparous eelpout was given special attention due to its suitability for biomonitoring purposes. To better understand its reproductive physiology the gonad differentiation of the embryos and the seasonal variations of sex-steroids and estrogen induced proteins in the adults were followed. The levels of estradiol-17β were similar in both sexes, but vitellogenin was present only in females, suggesting a mechanism for supressing estrogen-induced vitellogenin production in males. In contrast to vitellogenin, vitelline envelope proteins were present in plasma of both sexes and displayed clear seasonal variations.

Gonadal differentiation in eelpout is completed before birth, which enables sexing of embryos. Male biased embryonic sex ratios were found near a large Swedish pulp mill. Together with data on masculinised fish near pulp and paper mills in North America, this observation suggested the presence of masculinising compounds in the effluent. This was further investigated by exposing female guppies to diluted effluent from the mill. Both guppies exposed to effluent or to methyltestosterone developed bright colours, confirming the suspected androgenicity of the effluent. However, morphological changes of the anal fin, characteristic of androgen-treated fish, were not induced in guppies exposed to effluent and the sex ratios of their fry were normal. Therefore, more research is needed to confirm if the effluent indeed contains masculinising substances.

The findings of estrogenic effects in fish downstream sewage treatment works in the United Kingdom led us to investigate if estrogenic substances are released from Swedish sewage treatment works in concentrations sufficient to affect fish. Experiment with exposed rainbow trout showed a strong induction of vitellogenin as far downstream as two kilometres from the sewage treatment works, demonstrating the presence of relatively stable estrogenic compounds in the effluent. Chemical analyses of the effluent revealed highly potent concentrations of the synthetic estrogen ethinylestradiol used in contraceptives, and other estrogens in less potent amounts. The estrogens were concentrated up to one million times in the bile of the fish. Thus, a widely used drug, intended for human use, is released into the environment and affects aquatic organisms.

The adaptation and development of novel biomarkers in this thesis have extended our battery for identifing endocrine disruption in fish. The generation of new knowledge about the reproductive endcrinology of the eelpout facilitates future interpretations of endocrine responses in this species. Finally, physiological biomarkers together with analytical chemistry proved to be a useful blend for identifying the release of endocrine disrupters to the aquatic environment.