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Fish reproduction disturbances induced by pollutants. Field assessment and search for biochemical targets and markers with special emphasis on cytochrome P450-dependent enzymes.

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Introduction

INRA (National Institute for Agronomic Research) has long been working on fish reproduction especially in the Department of Hydrobiology and Wildlife. Laboratories have developed studies on fish reproductive physiology and population dynamics, but little attention has been paid to (eco)toxicological aspects of fish reproduction. The present paper deals with some studies performed during the last decade on various salmonid species: (1) the relationship between survival of embryos and larvae of charr (*Salvelinus alpinus*) from lake Geneva and contamination of eggs by organochlorine compounds, (2) inhibition of oestrogen synthesis by pesticides in rainbow trout (*Oncorhynchus mykiss*), and (3) the development of xenobiotic metabolizing enzymes during fish ontogenesis.

Relationship between organochlorine compound concentration and embryolarval mortality in lake Geneva arctic charr (*Salvelinus alpinus*)

Lake Geneva (58240 ha, 145 m mean depth) is a French-Swiss lake where arctic charr is endogenous and classically spawns at important depths (30 to 120 m) especially along the French coast. This species supports active professional as well as recreational fisheries. By the end of the 1970s, the recruitment of charr had fallen to a low level. At the same time, some studies pointed out high levels of contamination of the Lake Geneva food web by organochlorine compounds,

especially PCBs. Therefore, a study was planned to examine the relationship between embryo-larval survival in charr and contamination by PCBs (Monod, 1985).

Wild charr were captured on spawning grounds. Batches of roe collected from 18 females were individually fertilized with milt of males captured at the same time. Subsequent egg incubation was performed in a local hatchery and mortality was monitored up to yolk sac resorption. Eggs sampled from each batch were analyzed for organochlorine pollutants (PCBs, Σ DDT).

The results showed a significant and positive correlation between PCB (and Σ DDT) levels in eggs and overall mortality when pollutant concentrations were expressed on a lipid-weight basis (suggesting a protective effect of fatty reserves against lipophilic micropollutants). When phases of embryo-larval development were considered, the results showed that this correlation was explained by the mortality occurring prior to hatching.

Analyses of PCBs demonstrated that the transfer of PCB congeners from female to eggs depended on chlorine content, with decreased transfer when chlorine content increased. These results suggested that maternal transfer of PCBs might result in the accumulation of highly toxic planar-PCBs in the eggs.

This work showed that mortality of arctic charr embryos could be at least partly explained by the contamination of Lake Geneva with organochlorine compounds. The occurrence of significant mortalities prior to hatching was the main difference compared with M74. During the 1980s, the arctic charr fishery has been rehabilitated due to an intensive stocking program with fingerlings produced from eggs of Lake Geneva's wild spawners (Champigneulle, personal communication).

Interaction of xenobiotics with oestrogen synthesis: inhibition of aromatase activity

In an attempt to identify specific targets for aquatic pollutants we examined aromatase activity, the enzymatic activity catalyzing the terminal step in the biosynthesis of oestrogens. Oestradiol-17 β has been demonstrated to govern the hepatic synthesis of vitellogenin, a lipoprotein corresponding to the major part of the embryonic trophic reserves. Thus, interaction of pollutants with aromatase activity might be of ecotoxicological significance. As Aromatase activity is catalyzed by the unique member of the cytochrome P450 19 family, we hypothesized that oestrogen synthesis might be disturbed by cytochrome P450 inhibitor xenobiotics.

We tested imidazole derivatives, which have antifungal activity, on ovarian microsomal aromatase activity *in vitro* and on oestradiol-17 β secretion by cultured ovarian follicles (Monod et al., 1993). A dose-dependent inhibition of rainbow trout aromatase activity and oestradiol secretion was observed. Although xenobiotic-metabolizing cytochrome P450s and steroid-metabolizing cytochrome P450s correspond to quite different members in the P450 super-family, we hypothesized that reproductive failure might originate from interaction between pollutants and cytochrome-dependent enzymes of steroid synthesis, particularly cytochrome P450 19. Further studies are necessary to check this hypothesis in environmental situations.

Ontogenesis of xenobiotic metabolizing enzymes and their induction by pollutants in salmonid species

Considering that xenobiotic metabolizing enzymes, particularly cytochrome P450, are major factors influencing the toxicity of pollutants (detoxication/toxication), and that fish embryo-larval stages are known to be highly sensitive to aquatic pollutants, we examined these enzymes during embryo-larval development in reared arctic charr (*Salvelinus alpinus*), whitefish (*Coregonus sp*) and grayling (*Thymallus thymallus*) (Monod et al., to be published).

Eggs were collected, fertilized and incubated in local hatcheries. Sampling was carried out from fertilization up to yolk sac resorption. At the time of sampling, whole eggs or larvae were suspended in the appropriate buffer and homogenized. Homogenates were centrifuged to obtain microsomal and cytosolic fractions, which were subsequently assayed for enzymatic activity measurements.

Results showed similar patterns regardless of fish species, but significant differences were observed depending on enzymatic activities. Each species showed a biphasic development with a transition from low to rapid increase of EROD and cytochrome P450 reductase activities at hatching, whereas glutathion S-transferase (GSH-t) activity exhibited such an increase well before hatching.

Further studies were conducted to investigate the effect of b-naphtho-flavone (bNF), an inducer of cytochrome P450 1A1 (as are dioxin and polycyclic aromatic hydrocarbons), on EROD activity in arctic charr eggs and yolk-sac larvae. Results showed that EROD was induced 5 to 10 times in eggs and larvae after 48 hours of exposure to 0.12 g bNF per ml incubation water, while cytochrome P450 reductase and GSH-t were not affected by this treatment.

Our results suggest that during the embryo-larval development of fish, metabolizing capabilities against pollutants are related to organogenesis but expression of different enzymatic systems (P450 1A1/GSH-t) seems to be

governed by distinct ontogenetic regulation. The toxicological significance of EROD induction during embryo-larval development will be the subject of further studies.

Our work on reproduction disturbances in fish in connection with exposure to pollutants has been conducted in different directions. At first, the assessment of embryo-larval survival performed on arctic charr from Lake Geneva suggests a relationship between pre-hatching mortality and the level of contamination by PCBs. This is a marked difference compared to M74, where mortality is mainly observed during yolk-sac resorption. Similarities seem to appear when symptoms of M74 are compared to symptoms reported in the literature after exposure of salmonids to dioxin. Unfortunately, dioxin and dioxin-equivalents were not investigated in eggs of arctic charr. On the other hand, preliminary studies on cytochrome P450-dependent enzymes have provided some insight into specific targets or biomarkers for toxicants interfering with fish reproduction.

References

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Report from the Uppsala workshop on Reproduction Disturbances in Fish

20-22 October 1993

Leif Norrgren (editor)

Swedish University of Agricultural Sciences, Uppsala, Sweden

Report from the Uppsala workshop on Reproduction Disturbances in Fish

20 - 22 October 1993

The objective of the workshop was to gain a clearer understanding of the causes of reproductive disorders in salmon and cod from the Baltic Sea, for comparison with salmonids in the north American Great Lakes and in Lake Geneva.

Participants from Sweden, Finland, Estonia, Latvia, Poland, France, The Netherlands, Canada and the USA attended the workshop.

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