Xenobiotics as Stressful Factors in Aquatic System (in fish)

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Abstract: A round 1500 substance have been listed as pollutants in freshwater ecosystems, xenobiotics represent the most important one which affect on most organs particularly the reproduction system. The toxicity of any xenobiotic is related to the bioaccumulated chemical residue in the organism. The evaluation of the xenobiotic-derived hazard has traditionally been related to the chemical concentration in the ambient media. The different pollutants put forth different problem to different freshwater ecosystem. Mostly, expressed in the amount of oxygen that is available for fish and other species. This sometimes results in habitat destruction and extinction of local populations.
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Introduction:

Food helps human beings maintain good health by providing all essential nutrients. Consuming a variety of foods in balanced proportions will prevent deficiency diseases and chronic diet- related disorders.

Fish is known to be a source of protein rich in essential amino acids cystine, threonine and tryptophan). The essential amino acids are lysine, methionine, threonine, tryptophan, isoleucine, leucine, phenylalanine and valine. Failure to obtain enough of even one of the essential amino acids results in the degradation of the muscle proteins in the body. Recent evidence shows that some amino acids and their metabolites are important regulators of key metabolic pathways that are necessary for maintenance, growth, feed intake, nutrient utilization, immunity, behavior, larval metamorphosis, reproduction, as well as resistance to environmental stressors and pathogenic organisms in various fishes.

Fish meal has been the most important feedstuff used as a source of protein in aquaculture feed because of its essential amino acid composition and palatability. Demand for protein ingredients in aquaculture is expected to exceed supply in the next decade. The growth of the aquaculture industry will also raise the price of feedstuffs.

Heterotrophic organisms:

Heterotrophic organisms consume autotrophic organisms and use the organic compounds in their bodies as energy sources and as raw materials to create their own biomass (Manahan, 2005). Euryhaline organisms are salt tolerant and can survive in marine ecosystem, while stenohaline or salt intolerant species can only live in freshwater environment (USEPA, 2006).

Freshwater Ecosystems and Pollution

The dissolved O_2 concentration highly depends also on the amount of pollutants, because most water pollutants cause low oxygen levels in freshwater. These pollutants make it difficult for species to live, and many aquatic organisms, especially fish, die when dissolved oxygen levels fall below 4 or 5 ppm. There a few natural sources of pollutants present in aquatic ecosystems. But mostly, freshwater ecosystems may become unbalanced by factors due to human activities. Human activities affect the bioavailability of chemicals to organisms, cause temperature fluctuations, and modify rainfall, pH salinity.

Water plays a key role in diluting pollutants and because of that superiority as a solvent, it also means that water- soluble wastes pollute water easily. For instance, runoff from nearby land provides freshwater life zones with an almost constant input of organic material, inorganic nutrients, and other pollutants. Some 1500 substances have been listed as pollutants in freshwater ecosystems.

Freshwater Biota

The types of species that could become affected by water pollution in freshwater ecosystems are: Insects, Crustaceans, Fish, Amphibians, Arthropods, Aquatic plants, Fungi, Bacteria, Algae, Viruses, etc.

Very few invertebrates are able to inhabit the cold, dark, and oxygen poor profundal zone. Those that can are often red in colour due to the presence of large amounts of hemoglobin, which greatly increases the amount of oxygen carried to cells (Brown, 1987). Because the concentration of oxygen within this zone is low, most species construct tunnels or borrows in which they can hide and make the minimum movements necessary to circulate water through, drawing oxygen to them without expending much energy.

Xenobiotics and toxicity:

A wide range of man-made chemicals used for several industrial and household activities have been shown to disturb normal physiology and endocrinology in living organisms.

The effects of xenobiotics on the whole organism are considered under three main headings, namely neuro-physiological, reproductive and behavioural effects. These effects can often be inter-related: neurological changes can affect behaviour; changes in behavior can affect reproduction and so on. A compound doesn't always put forth an effect on a target organism or a community. It always depends on the concentration of that compound and the time of exposure to it. These effects eventually can be either acute or chronic. Acute toxicity occurs rapidly, are clearly defined, often fatal and rarely reversible. Chronic effects develop after long exposure to low doses or long after exposure and may ultimately cause death.

A xenobiotics is lethal when it causes death, or sufficient to cause it, by direct action. And it is sub lethal when the poison is below we level that directly causes death. Then it results in the regression of the physiological or behavioural processes of the organism, and its overall fitness is reduced. Only in the case of radioactive pollution, it is likely that it will cause irreversible effects at the ecosystem (www.lenntech.com).

The effect of pollution on freshwater species are registered in the loss of some species, with maybe some profits for some of them. There normally is a reduction in diversity but not necessarily numbers of individual species, and a change in the balance of such processes as predation, competition and materials cycling. Because of the complexity of pollution, the effects of take-up in the aquatic life are also depended on the pollutants characteristic feature. It two or more poisons are present together in an effluent they may exert a combined effect to an organism, which can be additive, antagonistic or synergistic.

An example of an additive interaction is the combined toxicity of zinc and cadmium to fish. Calcium in antagonistic to lead, zinc and aluminium. Copper is more than additive with chlorine, zinc cadmium and mercury, while its decreases the toxicity of cyanide. The toxicity to the mayfly Baetis rhodani of phenol and ammonia at low concentrations is additive, but at higher concentrations the effect is more than additive.

Stress:

Another generally accepted paradigm is that stress, especially chronic stress, will suppress immune response and lower the disease resistance.

Numerous experiments have been conducted on different fish species to examine the effects of stress on the immune system. In these experiments stress hormones like cortisol and adrenaline or other stress related proteins like heat shock proteins and plasma glucose levels have been monitored, as well as innate and adaptive immune parameters and the effects on disease resistance.

Confinement, high density, handling and transport are stress inducers which are highly relevant to aquaculture and have received considerable attention. Long term exposure to these stressors has generally suppressive effects on the immune system and disease resistance of fish. Some examples of initial stimulation have been reported, for example, following short exposure to handling stress of Atlantic salmon and rainbow trout to basal levels is also commonly seen during chronic stress induction. Similarly, fish in aquaculture appears to adapt to confinement and show lower stress response than the wild type.

Direct administration of stress hormones or neuropeptides in vivo in vitro studies has similarly demonstrated varied but generally the suppressive effects on immune parameters of fish. For example, in a study of Atlantic salmon, Ig positive lymphocytes were down regulated following cortisol injection and mitogenic stimulation of lymphoctyes was suppressed when incubated with cortisol. On the other hand, β-endrophin was shown to stimulate in vitro phagoctyic activity of leukocytes (macrophages) isolated from kidneys from both rainbow trout and carp.

In aquaculture, selective breeding of low cortisol responders has been considered and modulating stress response through feeding regimes has also been suggested. As well as affecting the immune system and disease resistance of fish stress also affects other factors like growth, sexual maturation, gamete quality and larval health. Because of these wide reaching effects, husbandry practises in aquaculture aim at avoiding stress by maintaining steady environmental conditions, rearing at optimum density, controlling sexual maturation and avoiding excessive handling.

One important class of these compounds (the xenoestrogens) is able to mimic the natural hormone estrogen. Among these are synthetic steroids such as those used in the contraceptive pill (Pelissero *et al.,* 1993), some organochlorine pesticides such as dichlorodiphenyltrichloroethane, DDTs, hexachlorocyclohexanes, HCHs (Wester & Canton, 1986; Palmer & Palmer, 1995; Donhoe & Curtis, 1996), surfactants and detergents as: alkylphenol polyethoxylates, APEs (Soto *et al.,* 1991; Jobling & Sumpter, 1993; White *et al.,* 1994; Jobling et al., 1996; Arukwe *et al.,* 1997a & b), plasticizers (phthalates), polychlorinated biphenyls, PCBs (Mclachlan, 1985), and some natural chemicals such as phytoestrogens and mycoestrogens (Pelissero *et al.,* 1991a & b). In many cases, the estrogenic activity of these chemicals have been discovered accidentally (Soto *et al.,* 1991). Estrogenic responses in fish, like the induction of zona radiata proteins (Zrp) and vitellogenin (Vtg) by these compounds have received great attention (Sumpter & Jobling, 1995; Arukwe *et al.,* 1997b).

Alkylphenol polyethoxylates (APEs) represent an important class of non-ionic surfactants that are widely used as detergents, emulsifiers, wetting and dispersing agents, and also in plastic products for industrial, agricultural and domestic use (Ahel *et al.,* 1994b). Alkylphenols (APs) are formed by microbial degradation of APEs. Some studies have identified APs as the most critical metabolites of APEs because of their enhanced resistance toward biodegradation, toxicity, estrogenic effects, and ability to bioaccumulate in aquatic organisms (Ahel *et al.,* 1994a). A complex microbial degradation pattern, characterized by the formation of several metabolic products that are more toxic than the parent compound, has been established for APEs (Ekelund *et al.,* 1990; Naylor *et al.,* 1992).

Effects of Xenoestrogens

In classical pharmacology, an agonist is defined as a ligand that can bind to a receptor and 'turn it on' and the potency of an agonist depends on its ability to turn on the receptor. For the ER, E2 is the natural agonist. On the other hand, an antagonist is a ligand that blocks responses elicited by agonists (Nimrod & Benson, 1996). An antagonist can be competitive (i.e. competes for the same binding site as the agonist) or functional (i.e. through a non-receptor mediated mechanism). Xenobiotics (foreign compounds) with the ability to mimic natural estrogens have generally been referred as xenoestrogens (Mclachlan, 1985; Colborn & Clement, 1992).

The general mechanisms by which xenoestrogenic compounds mediate their effects is not well understood, but it is known that they can bind with high affinity to the ER (agonists) and initiate the action on target tissues, which is typical of natural estrogens. Some compounds also have the ability to bind to the receptor, but not eliciting estrogenic activities (antiestrogens or antagonists), thereby blocking the binding site of natural estrogens (Safe, 1995; Safe & Krishnan, 1995; Ahlborg *et al.,* 1995).

During ovarian recrudescence, incorporation of vitellogenin accounts for the major growth of the developing oocytes. A probable indirect measure of altered hepatic vitellogenin synthesis in fish exposed to xenobiotic is reduced or increased gonadosomatic index (GSI). A more direct quantification of these alterations can be obtained from plasma, hepatic and ovarian vitellogenin concentrations (Kime, 1995).

A-Effect of Xenobiotics on Fish Reproduction

There are several reports of xenobiotic induced reproductive disturbances in aquatic organisms, including fish living in polluted environments (Mclachlan, 1980; 1985;Colborn & Clement, 1992; Guillette *et al.,* 1995a).

Several methods and parameters have been used for assessing reproductive success of feral fish species. These includes reduced viable hatch in the Baltic flounder (*Platichthys flesus*) and Baltic herring (*Clupea harengus*) in correlation with elevated PCB concentrations in the eggs (von Westernhagen *et al.,* 1981; Hansen *et al.,* 1985), high egg mortality of Lake Geneva charr (*Salvelinus alpinus*) in correlation with elevated PCB and DDT in charr eggs (Monod, 1985), reduced fertilization success and viable hatch in female starry flounder (*Platichthys stellatus*) from contaminated areas of San Francisco Bay (Spies and Rice, 1988).

In studies on developmental effects in eggs of dab (*Limanda limanda*), whiting (*Merlangius merlangus*), cod (*Gadus morhua*), flounder and plaice (*Pleuronectes platessa*) in the southern North Sea, highest incidences of embryo malformations were observed near coastal waters known to receive high pollution loads (Dethlefsen *et al.,* 1996; Cameron *et al.,* 1996; Cameron and von Westernhagen, 1997). Common defects recorded by these authors include blister proliferation in early and late embryos, failure to close the blastopore and deformation of the notochord. However, significant correlations were only found for malformations of dab and concentrations of p,p'-DDE residues. Further, Cameron *et al.* (1988) have reported chromosomal and embryo malformations in fish caught in the North Sea. These authors found a positive correlation between anaphase aberrations and the levels of organochlorines such as PCBs, DDT and DDE in gonads and livers of whiting with highest malformation rates from stations near the coast of The Netherlands and off the Rhine River Estuary.

Furthermore, the grounding of the tanker Exxon Valdez in 1989 that spilled huge amounts of crude oil into the Prince William Sound in Alaska, has resulted in severe effects on the reproductive success of pink salmon (*Oncorhynchus gorbuscha*, Wertheimer & Celewycz, 1996) and Pacific herring (*Clupea pallasi*, Hose *et al.,* 1996; Kocan *et al.,* 1996; Norcross *et al.,* 1996). Parameters used in evaluating Pacific herring reproductive success include egg and larval mortality, morphological deformities, cytogenetic abnormalities and premature hatch. Significant correlations were found between these effects and crude oil exposure. Nevertheless, there have also been reports of no clear effects on fish reproductive activity, of exposure to contaminants from petroleum production sites in the Gulf of Mexico (Stott *et al.,* 1980, 1981). In 1994, Purdom and coworkers (Purdom *et al.,* 1994) showed that sewage effluent contain estrogenic substances that induce Vtg synthesis in male trout and this response was attributed to alkylphenolic compounds.

Several studies have been performed in the United Kingdom (UK) freshwater and marine environments with the aim of identifying rivers and estuaries where these compounds occur (Sumpter, 1995; Harries *et al.,*1996, 1997; Jobling &Sumpter 1993; White *et al.,* 1994; Lye *et al.,*1997). In Norway, STW effluents have been shown to be estrogenic to rainbow trout (Elsrud Schou *et al.,* 1996) in a similar manner. In addition, Arukwe *et al.,* 1997b shown that effluents from an oil refinery treatment plant (ORTP) was estrogenic (using Zrp and Vtg as markers) to juvenile salmon in a dose-dependent manner, again with Zrp as the more sensitive biomarker. Johnson *et al.*(1988) and Casillas *et al.* (1991) have reported the effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington (USA). One significant finding of these authors was that female English sole from sites heavily contaminated with polychlorinated biphenyls (PCBs) and PAHs had lower plasma estradiol levels and were significantly less likely to undergo gonadal recrudescence than females from the less contaminated sites.

Collier *et al.*(1998) have also reported precocious juvenile sexual maturation and inhibited gonadal development in female flatfish from the Hylebos Waterway, in central Puget Sound known to be severely contaminated by variety of organic and inorganic contaminants. In another study, epizootics of thyroid hyperplasia and hypertrophy that affected 100 % of the pink (*Oncorhynchus gorbuscha*), coho (*O. kisutch*) and chinook (*O. tshawytscha)* salmon populations taken from the Great Lakes (North America), in addition to reduced fertility, high prevalence of embryo mortality and low plasma steroid hormone levels, when compared to Pacific Northwest populations, were reported by Drongowski *et al.* (1975) and Leatherland (1992). It is evident that there are effects on the reproductive abilities of the feral fish of many species.

Masculinization responses involving the development of male secondary sex morphological characters (such as modified anal fin into a gonopodium-like structure) was observed in female mosquitofish (*Gambusia affinis*, Poecilliidae) sampled from streams receiving kraft mill effluent (Howell *et al.,*1980; Rosa -Molinar & Williams, 1984; Bortone *et al.,*1989;Kme *et al.,*1992;). These effects were irreversible and often concomitant with male fish behavior, including mating attempts, and have been associated with androgenic properties of compounds in the KME. Another important aspect of reproduction disturbances in marine organisms is the occurrence of the imposex phenomenon, which is the development of male sexual characteristics (such as penis and vas deferens) in female neogastropod molluscs. The imposex phenomenon, which was first described for dogwhelk *Nucella lapillus* L., an estuarine snail (Babler, 1970), is caused by pollution of the marine environment by organotin compounds, such as tributyltin (TBT), which have been used in antifouling paints for ships, boats and fishing nets (Horiguchi *et al.,* 1994; Bryan *et al.*, 1986; 1987; Gibbs& Bryan, 1986; Din & Ahamad, 1995). Imposex was reported for the first time in the open ocean in female whelks (*Buccinum undatum* L.) from the North Sea by Ten Hallers-Tjabbes *et al.* (1994). However, the use of TBT as antifouling paint have been banned for small boats and fishing nets in most countries.

Furthermore, in Lake Apopka (Florida), Guillette and coworkers (Guillette *et al.,* 1994; 1995b) have reported impaired gonadal steroidogenesis (E2 and testosterone; T) and abnormal gonadal morphology in juvenile alligators inhabiting the lake. When compared with alligators from control sites, Lake Apopka male and female alligators synthesized significantly higher testis and ovarian E2 levels, respectively, while normal T levels were displayed. They attributed the effects to the contaminants and nutrients in the lake that are derived from the extensive agricultural activities around the lake, a sewage treatment facility associated with the Winter City Garden, Florida, and a major pesticide (dicofol; kelthane) spill from the Tower Chemical Company in 1980 (Clark, 1990).

B. Estrogenic potencies

All the chemicals with estrogen activity discovered to date are comparatively weak estrogens. Their potencies vary, with many orders of magnitude (3-4) less than estradiol-17β. However, it could be argued that their relative potencies depend on the assay system used to assess them, but it is interesting to note that they appear to possess the full activity and interact with the ER in exactly the same manner as the natural estrogens. It should be emphasized that more than one estrogenic assay should be employed in determining organismal response to xenoestrogens (Korach & Mclachlan, 1995). This is because the sequence of estrogenic responses do not necessarily have the same time curve or the same strength of response (Arukwe *et al.,* 1997a& b). The utilization of in vivo studies is critical when compounds have the potential of enhanced estrogenicity only after metabolic activation.

Concentrations of estrogenic chemicals in fish will depend on a number of factors, such as bioavailability, bioconcentration/bioaccumulation, and biotransformation. Most of the known estrogenic chemicals are lipophilic and hydrophobic and therefore have a strong potential to accumulate in aquatic biota.

Therefore, determining environmental exposures is very difficult and might not be particularly meaningful.

Few attempts have been made to measure the concentrations of alkylphenolic compounds in organisms. Ekelund *et al.*(1990) have reported bioconcentration factors (BCFs) between 13 and 3400 in fish. One consequence of bioaccumulation is that chemicals that are not estrogenic or weakly estrogenic in vitro might be active in vivo at considerably lower concentrations, given sufficient exposure time. In addition, they may be physiologically inactive while stored in fat (adipose) tissues, but when this fat is mobilized such as during sexual maturation, the compounds may be released to give toxicity elsewhere or metabolized into other compounds that might be more toxic than the parent compound. Furthermore, alkylphenolic compounds with long ethoxylate chains are more water soluble, and will not be expected to bioconcentrate to any significant level. For example, Ahel *et al.* (1994b) have reported concentrations of 0.1-0.2 mg NP/kg in tissue samples taken from animals in a river with low NP contamination (3.9 µg/l).

C. Ecological consequences

Reproductive development is a continuous process throughout ontogeny. Consequently, it is susceptible to the effects of xenoestrogens and/or xenobiotics at all stages of the life-cycle, including fertilization, embryonic development, sex differentiation, oogenesis or spermatogenesis, final maturation, ovulation or spermiation and spawning. Thus, the sensitivity to a particular compound will vary depending on the stage of reproductive development (Donaldson,1990).

Effects at higher hierarchical levels are always preceded by changes in 'earlier' biological processes, allowing the development of early warning biomarker signals. It is however difficult to interpret the early biological responses with regard to their significance at the population and ecosystem levels. The difficulty is attributed to, among others, that the changes in fish population and ecosystem diversity may be caused by myriads of other factors than xenobiotics, like seasonal fluctuations in temperature, salinity, food availability, fishing intensity etc. Understanding the general principles by which chemical substances or foreign compounds (xenobiotics) interfere with fish reproduction is particularly important for meeting the larger objectives in aquatic reproductive toxicology, as it is impossible to empirically determine the biological specificity or how every compound affect the reproductive life-history strategy of every species.

Current risk assessment of the reproductive effects of xenobiotics on aquatic organisms rests explicitly or implicitly on in vitro and in vivo laboratory studies. However, ecological consequences of xenobiotic-induced Zrp and Vtg synthesis are not known. Presently, there is ample evidence that aquatic organisms living in and bioaccumulating xenobiotic chemicals are adversely affected.

In these respects, there is an absolute need for concern, given the several roles played by endogenous estrogens in normal physiology such as during adult sexual maturation and sex differentiation at the early life (egg and embryo) stages (Hunter & Donaldson, 1983; Piferrer & Donaldson, 1989). Furthermore, there are myriads of other factors than can modulate the effects of xenobiotics, and these might be difficult, if not impossible, to quantify. Given the energetic cost of reproduction and the long decision time, it seems most likely that xenobiotically induced hepatic Zrp and Vtg synthesis may cause an imbalance in the reproductive strategy of a given fish population. Thorpe (1994) suggested that during maturation, the internal responses that are synchronized by external signals depend upon some genetically determined performance threshold, and that maturation processes will continue if this performance exceeds a set point at this critical time. Furthermore, maturation has developmental priority over somatic growth, and in salmonids survival after spawning implies a chance dependent balance between stored energy and that spent on reproduction (Poplicansky, 1983).

Therefore, xenoestrogen-induced Vtg synthesis outside normal maturation period may result in wasteful use of stored energy resources. The ecological implication of this might be failure in the reproduction of affected individual fish, and in the long-term affecting recruitment in the entire population.

Effects of environmental estrogens have mostly focused on males and juveniles, because of the very low cellular levels of estrogens (Jobling *et al.,* 1996; Arukwe *et al.,* 1997a). One possible deleterious effect is that high Vtg and/or Zrp concentrations might cause kidney failure and increased mortality rates as a result of metabolic stress (Herman & Kincaid, 1988). Furthermore, although not yet demonstrated, there is a possibility that the reduced testicular growth could reduce fertility (Jobling *et al.,* 1996). Continued synthesis of Vtg diverts available energy resources (lipids, proteins), thereby reducing chances of juvenile survival before start feeding. Loss of calcium from bones and also from the scales during active Vtg synthesis (Carragher & Sumpter, 1991) may increase the susceptibility of fish to disease.

Xenoestrogen-induced changes in Zrp synthesis appear to have a higher potential for ecologically adverse effects than Vtg induction, because critical population parameters such as offspring survival and recruitment may be more directly affected. The argument for this, is that whereas subtle changes in Vtg content would not be of great significance to the survival of the offspring, small changes in Zrp synthesis might cause the thickness and mechanical strength of the eggshell to be altered, thus causing a loss in its ability to prevent polyspermy during fertilization and to protect the embryo during development (Arukwe *et al.,* 1997b).

The antiestrogenic and estrogen potentiating effects of typical CYP1A-inducers reported recently by Villalobos *et al.* (1996) and Anderson *et al.* (1996a & b) were dependent upon the concentration of estradiol-17β and the CYP1A inducing agent. These responses were mediated through the ER and Ah-receptor, respectively. With regard to other reproductively important ER containing organs in fish, these modulatory effects will have some added significance. For example, ER mRNA has been detected in the hypothalamus and pituitary of rainbow trout (Pakdel *et al.,* 1990). Anglade *et al.* (1994) have also reported immunolocalization of ER in three brain regions (ventral telencephalon, anterior ventral preoptic region and mediobasal hypothalamus) of rainbow trout. Further, using cultured pituitary cells from rainbow trout, Xiong *et al.* (1994) have reported the identification of ERE in the proximal and distal regions of the gonadotropin II (GtH II) gene promotor. Since extrahepatic targets for ER modulating compounds exist, such compounds may have significant and deleterious consequences on neuroendocrine and endocrine regulation of fish reproduction. Such effects might include, but not be limited to, altered gonadotropin secretion, gonadal synthesis of estradiol-17β, and thereby interfering with Zrp and Vtg synthesis and gonadal maturation.

In several studies, a relationship between elevated P450 activities and disturbed physiological endocrine functions, essential for successful reproduction have also been found (Sivarajah *et al.,* 1978; Spies *et al.,* 1985; Thomas, 1990).

Some authors reported that a xenoestrogen (4-nonylphenol) that induces Zrp and Vtg at higher concentrations, also elevated (at lower doses) and inhibited (at higher doses) different hepatic P450 isoforms that metabolize xenobiotics and steroid hormones (Arukwe *et al.,* 1997a). It is a known phenomenon that E2 inhibits the expression and activity of CYP1A (Anderson, 1989; Pajor *et al.,* 1990; Arukwe Goksoyr, 1997). Inhibition of P450 activities by environmental estrogens might result in reduced ability of the individual fish to metabolize and excrete xenoestrogens. On the other hand, elevated P450 levels might result in increased metabolism and excretion of steroid hormones necessary for active Zrp and Vtg synthesis. Although no links between the induction of P450 and impaired reproductive functions have yet been established, it is nevertheless important that the mechanism by which potential P450 inducers may affect sexual development and fertility is elucidated.

D. Anaesthetic agents as stress factor

It is widely accepted that the stress response as a whole is characterised by physiological changes. These changes tend to be similar for stressors and could be as varied as anesthesia, flight, forced swimming, disease treatments, handling, scale loss, or rapid temperature change (Wedenmeyer and McLeay, 1981). A study by Wendelaar Bonga (1997) showed that stressor increases the permeability of the surface epithelia, including the gills to water and ions and thus induces hydromineral disturbances. Therefore, it is good indicator of toxicity in fish than in mammals, as the fish are exposed to aquatic pollutants by extensive and delicate respiratory surface of the gills.

E. Antibiotics as a stress factor

One of the main problems in fish farms is the occurrence of parasites and bacteria, (Cruz *et al.,* 2004). Parasite and bacterial diseases stand out as important limiting factors to productivity. These diseases may delay growth and cause high rates of mortality in fish (Ranzani-Pavia *et al.,* 1997). The outbreak of parasite and bacterial diseases is controlled by the use of antibiotics in the water. Therefore, when antibiotics are used in fish farms there is an environmental intoxication risk that should be assessed.

Regarding substances used to control diseases in aquatic organisms, most are classified as moderately or highly toxic (Lutzhft *et al.,* 1999; Straus, 2004). On the other hand, the uncontrolled use of antibiotics develops bacteria resistance (Lin, 1989).

The ecotoxicological assays and environmental risk assessment are helpful methods used to correlate toxicity data obtained in laboratory conditions and the predicted environmental concentration (PEC), in such way to predict what would be the adequate concentration that could be used without harming the environment (Zagatto and Bertoletti, 2008). It is concluded that xenobiotics have a deleterious effects on aquatic system and affecting all stages of reproduction of fish.

References:

1. Ahel, M., W. Giger & C. Schaffner 1994b. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - II. Occurrence and biotransformation in rivers. – Water Research 28:1143-1152.
2. Ahel, M., W. Giger & M. Koch 1994a. Behaviour of alkylphenolpolyethoxylate surfactants in the aquatic environment -I. Occurrence and transformation in sewage treatment.– Water Research 28:1131-1142.
3. Ahlborg, U.G., L. Lipworth, L. Titus-Ernstoff, C.-C. Hsieh, A. Hanberg, J. Baron, D. Trichopoulos & H.-O. Adami 1995. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: An assessment of the biological and epidemiological evidence. – Critical Review of Toxicology 25(6):463-531.

4. Alexander David E. (1999). Encyclopedia of Environmental Science. Springer. ISBN 0-412-74050-8.
5. Anderson, M.J., H. Olsen, F. Matsumura & D.E. Hinton 1996b. In vivo modulation of 17β-estradiol-induced vitellogenin synthesis and estrogen receptor in rainbow trout (Oncorhynchus mykiss) liver cells by b-naphthoflavone. – Toxicology and Applied Pharmacology 137:210-218.
6. Anderson, M.J., M.R. Miller & D.E. Hinton 1996a. In vitro modulation of 17-β-estradiol-induced vitellogenin synthesis: Effects of cytochrome P4501A1 inducing compounds on rainbow trout (Oncorhychus mykiss) liver cells. – Aquatic Toxicology 34:327-350.
7. Andersson, T. 1989. Sex differences in cytochrome P-450-dependent xenobiotic and steroid metabolism in the mature rainbow trout kidney. – Journal of Endocrinology 126:9-16.
8. Anglade, I., F. Pakdel, T. Baihache, F. Petit, G. Salbert, P. Jego, Y. Volataire & O. Kah 1994. Distribution of estrogen receptor-immunoreactive cells in the brain of the rainbow trout (Oncorhynchus mykiss). Journal of Neuroendocrinology 6:573-583.
9. Arukwe, A. & A. Goksøyr 1997. Changes in three hepatic cytochrome P450 subfamilies during a reproductive cycle in Turbot (Scophthalmus maximus L.). – Journal of Experimental Zoology 277:313-325.
10. Arukwe, A., F.R. Knudsen & A. Goksøyr 1997b. Fish zona radiata (Eggshell) protein: A sensitive biomarker for environmental estrogens. – Environmental Health Perspectives 105:418-422.
11. Arukwe, A., L. Förlin & A. Goksøyr 1997a. Xenobiotic and steroid biotransformation enzymes in Atlantic salmon (Salmo salar) liver treated with an estrogenic compound, 4-nonylphenol. – Environmental Toxicology and Chemistry 12:2576-2583.
12. Babler, S.J.M. 1970. The occurrence of a penis-like outgrowth behind the right tentacle in spent female of Nucella lapillus (L.). – Proceedings of the Malacological Society of London 39:231-233.
13. Blaylock MJ, Huang JW. Phytoextraction of metals. In: Raskin I, Ensley BD, editors. Phytoremediation of toxic metals: using plants to clean-up the environment. New York: Wiley; 2000. p. 53–70.
14. Bortone, S.A., W.B. Davis and C.M. Bundrick 1989. Morphological and behavioral characters in mosquito fish as potential bioindication of exposure to kraft mill effluent. – Bulletin of Environmental Contamination and Toxicology 43:370-377.
15. Brown, A.L. (1987). Freshwater Ecology. Heinimann Education Books. London. P. 163.
16. Bryan, G.W., P.E. Gibbs, G.R. Hurt & L.G. Hummerstone 1987. The effects of tributyltin (TBT) accumulation on adult dog-whelks, Nucella lapillus: long-term field and laboratory experiments. – Journal of the Marine Biological Association of the United Kingdom 66:767-777.
17. Bryan, G.W., P.E. Gibbs, L.G. Hummerstone & G.R. Hurt 1986. The decline of the gastropod Nucella lapillus around south-west England: Evidence for the effects of tributyltin from antifouling paints. – Journal of the Marine Biological Association of the United Kingdom 66:611-640.
18. Cameron, P. & H. von Westernhagen 1997. Malformation rates in embryos of North Sea fishes in 1991 and 1992. –Marine Pollution Bulletin 34(2):129-134
19. Cameron, P., J. Berg & H. von Westernhagen 1996. Biological effects monitoring of the North Sea employing fish embryological data. – Environmental Monitoring and Assessment 40(2):107-124.
20. Cameron, P., V. Dethlefsen, H. von Westernhagen & D. Janssen 1988. Chromosomal and morphological investigations on whiting (Merlangius merlangus) embryos from the North Sea in relation to organochlorine contamination. – Aquatic Toxicology 11:428-429.
21. Carragher, J.F. & J.P. Sumpter 1991. The mobilization of calcium from calcified tissues of rainbow trout (Oncorhynchus mykiss) induced to synthesize vitellogenin. – Comparative Biochemistry and Physiology A 99:169-172.
22. Casillas, E., D. Misitano, L.L. Johnson, L.D. Rhodes, T.K. Collier, J.E. Stein, B.B. McCain & U. Varanasi 1991.Inducibility of spawning and reproductive success of female English sole (Parophrys vetulus) from urban and nonurban areas of Puget sound, Washington. – Marine Environmental Research 31:99-122.
23. Celander, M., D.R. Buhler, L. Förlin, A. Goksøyr, C.L. Miranda, B.R. Woodin & J.J. Stegeman 1996. Immunochemical relationships of cytochrome P4503A-like proteins in teleost fish. – Fish Physiology and Biochemistry 15(4):323-332.
24. Clark, D.R. 1990. Dicofol (Kelthane) as an environmental contaminant. – U.S. Fisheries and Wildlife Technical Report 29:1-37.
25. Colborn T. & C. Clement (eds) 1992. Chemically-induced alterations in sexual and functional development: The wildlife/human connection. – Advances in modern environmental toxicology Vol. XXI. Princeton Scientific Publishing, Princeton, NJ.
26. Collier, T.K., L.L. Johnson, C.M. Stehr, M.S. Myers & J.E. Stein 1998. A comprehensive assessment of the impacts of contaminants on fish from an urban waterway. –Marine Environmental Research 45 (in press).
27. Cruz, C.; Machado-Neto, J.G.; Menezes, M.L. 2004 Toxicidade aguda do inseticida paration metílico e do biopesticida azadiractina de folhas de neem (Azadirachta indica) para alevino e juvenil de pacu (Piaractus mesopotamicus). Pesticidas: Revista de Ecotoxicologia e Meio Ambiente, Paraná, 14:93-102.
28. Daily, Gretchen C. (1997). Nature's Services. Island Press. ISBN 1-559-63476-6.
29. Dethlefsen, V., H. von Westernhagen & P. Cameron 1996. Malformations in North Sea pelagic fish embryos during the period 1984-1995. – ICES Journal of Marine Science 53(6):1024-1035
30. Dhankher OP, Li YJ, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and g-glutamylcysteine synthase expression. Nat Biotechnol 2002;20:1140–5.

31. Din, Z.B. & A. Ahamad 1995. Changes in the scope for growth of blood cockles (Anadara granosa) exposed to industrial discharge. – Marine Pollution Bulletin 31(4-12):406-410.
32. Donaldson, E.M. 1990. Reproductive indices as measures of the effects of environmental stressors in fish. – American Fisheries Society 8:109-122.
33. Donohoe, R.M. & L.R. Curtis 1996. Estrogenic activity of chlordecone, o,p'-DDT and o,p'-DDE in juvenile rainbow trout: Induction of vitellogenesis and interaction with hepatic estrogen binding sites. – Aquatic Toxicology 36:31-52.
34. Drongowski, R.A., J.S. Wood & G.R. Bouck 1975. Thyroid activity in coho salmon from Oregon and Lake Michigan. – Transactions of the American Fisheries Society 2:349-352.
35. Ekelund, R., A. Bergman, A. Granmo & M. Berggren. 1990. Bioaccumulation of 4-nonylphenol in marine animals - A re-evaluation. – Environmental Pollution 64:107-120.
36. Elsrud Schou, A., E. Mona, M. Wiborg, K.-E. Tollefsen, J. Stenersen & F.R. Knudsen 1996. Increase in plasma vitellogenin in rainbow trout exposed to effluents from sewage - and oil refinery treatment works in Norway. Abstract VII International Symposium on Fish Physiology, 3-6 April 1996, Oslo Norway.
37. Gibbs, P.E. & G.W. Bryan 1986. Reproductive failure in populations of the dogwhelk, Nucella lapillus, caused by imposex induced by tributyltin from antifouling paints. – Journal of the Marine Biological Association of the United Kingdom 66:767-777.
38. Giller. S. and B. Malmqvist (1998). The Biology of Streams and Rivers. Oxford University Press, Oxford. P. 296.
39. Gliwicz, Z.M. (2004). Zooplankton (In: The lakes Handbook). P.E. O'sullivan and C.S Reynolds. pp.461-516
40. Guillette jr, J.L., D.A. Crain, A.A. Rooney & D.B. Pickford 1995a. Organization versus activation: The role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. – Environmental Health Perspectives 103 (Supplement 7):157-164.
41. Guillette jr, J.L., T.S. Gross, D.A. Gross, A.A. Rooney & H.F. Percival 1995b. Gonadal steroidogenesis in vitro from juvenile alligators obtained from contaminated or control lakes. – Environmental Health Perspectives 103 (Supplement 4):31-36.
42. Guillette jr, J.L., T.S. Gross, G.R. Mason, J.M. Matter, D.A. Gross, H.F. Percival & A.R. Woodward 1994. Developmental abnormalities of the gonad and abnormal sexhormone concentrations in juvenile alligators from contaminated and control lakes in Florida. – Environmental Health Perspectives 102:680-688.
43. Hansen, P.D., H. von Westernhagen & H. Rosenthal 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. – Marine Environmental Research 15:59-76.
44. Harries, J.E., D.A. Sheahan, S. Jobling, P. Matthiessen, P. Neall, E.J. Routledge, R. Rycroft, J.P. Sumpter & T. Taylor 1996. A survey of estrogenic activity in United Kingdom inland waters. – Environmental Toxicology and Chemistry 15(11):1993-2002.
45. Harries, J.E., D.A. Sheahan, S. Jobling, P. Matthiessen, P. Neall, J.P. Sumpter, T. Taylor & N. Zaman 1997. Estrogenic activity in five United Kingdom rivers detected by measurements of vitellogenesis in caged male trout. – Environmental Toxicology and Chemistry 16(3):534-542.
46. Herman R.L. & H.L. Kincaid 1988. Pathological effects of orally administered estradiol to rainbow trout. – Aquaculture 72:165-172.
47. Hillebrand, H.; A. I. Azovsky (2001). Body size Determines the Strength of the Latitudinal Diversity Gradient. Ecography, 24: 251-256. doi:10.1034/j.1600-587.2001.240302.x.
48. Horiguchi, T., H. Shiraishi, M. Shimizu & M. Morita 1994. Imposex and organotin compounds in Thai clavigera and T. bronni in Japan. – Journal of Marine Biological Association of the United Kingdom 74:651-669.
49. Hose, J.E., M.D. McGurk, G.D. Marty, D.E. Hinton, E.D. Brown & T.T. Baker 1996. Sublethal effects of the Exxon Valdez oil spill on herring embryos and larvae: morphological, cytogenetic and histopathological assessments, 1989-1991. – Canadian Journal of Fisheries and Aquatic Sciences 53:2355-2365.
50. Howell, W.M., D.A. Black & S.A. Bortone 1980. Abnormal expressions of secondary sex characters in a population of mosquito fish, Gambusia affinis holbrookii: Evidence for environmentally-induced masculinization. – Copeia 4:676-681.
51. Hunter, G.A. & E.M. Donaldson 1983. Hormonals sex control and its application to fish culture. – Pp. 223-303 in: Hoar, W.S. D.J. Randall & E.M. Donaldson (eds). Fish Physiology Vol. IX. Academic Press, New York.
52. Jobling S. & J.P. Sumpter 1993. Detergent components in sewage effluents are weakly oestrogenic to fish: An in vitro study using rainbow trout (Oncorhynchus mykiss) hepatocytes. – Aquatic Toxicology 27:361-372.
53. Jobling S., D. Sheahan, J.A. Osborne, P. Matthiessen & J.P. Sumpter 1996. Inhibition of testicular growth in rainbow trout (Oncorhynchus mykiss) exposed to estrogenic alkylphenolic chemicals. – Environmental Toxicology and Chemistry 15:194-202.
54. Johnson, L.L., E. Casillas, T. Collier, B.B. McCain & U. Varanasi 1988. Contaminant effects on ovarian development in English sole (Parophrys vetulus) from Puget Sound, Washington. – Canadian Journal of Fisheries nd Aquatic Sciences 45:2133-2146.
55. Kime, D.E. 1995. The effects of pollution on reproduction in fish. – Reviews in Fish Biology and Fisheries 5:52-96.
56. Kocan, R.M., J.E. Hose, E.D. Brown & T.T. Baker 1996. Pacific herring (Clupea pallasi) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. – Canadian Journal of Fisheries and Aquatic Sciences 53:2366-2375.

57. Korach, K.S. & J.A. McLachlan 1995. Techniques for detection of estrogenicity. – Environmental Health Perspectives 103(Supplement 7):5-8.
58. Leatherland, J.F. 1992. Endocrine and reproductive function in Great Lake salmon. – Pp. 129-145 in: Colborn T. & C. Clement (eds). Chemically-induced alterations in sexual and functional development: The wildlife/human connection. Vol. 21. Advances in modern environmental toxicology. Princeton Scientific Publishing, Princeton, NJ.
59. LIN, C.K. 1989 Shrimp culture in Taiwan: what went wrong? Aquaculture, Amsterdam, 20: 19-20.
60. Long XX, Yang XE, Ni WZ. Current status and perspective on phytoremediation of heavy metal polluted soils. J Appl Ecol 2002;13:757–62.
61. LUTZHOFT, H.C.H.; HALLING-SORENSEN, B.; JORGENSEN, S.E. 1999 Algal toxicity of antibacterial agents applied in Danish fish farming. Archives of Environmental Contamination and Toxicology, Paron, 36: 1-6.
62. Lye, C.M., C.L.J. Frid, M.E. Gill & D. McCormick 1997. Abnormalities in the reproductive health of flounder Platichthys flesus exposed to effluents from sewage treatment works. – Marine Pollution Bulletin 34(1):34- 1.
63. Manahan, Stanley E. (2005). Environmental Chemistry. CRC Press. ISBN 1-56670-633-5.
64. McLachlan J.A. (ed.) 1980. Estrogens in the environment. – Elsevier North Holland Inc., New York. 427 pp.
65. McLachlan J.A. (ed.) 1985. Estrogens in the environment II. Influences in development. – Elsevier Science, New York. 435 pp.
66. Mona. S. Zaki. Stress and pollution in fish. Discover Publishing House. PVT. LTD.
67. Monod, G. 1985. Egg mortality of Lake Geneva Charr (Salvelinus alpinus L.) contaminated by PCB and DDT derivatives. – Bulletin of Environmental Contamination and Toxicology 35:531-536.
68. Monod, G. 1985. Egg mortality of Lake Geneva Charr (Salvelinus alpinus L.) contaminated by PCB and DDT derivatives. – Bulletin of Environmental Contamination and Toxicology 35:531-536.
69. Nimrod, A.C. & W.H. Benson 1996. Environmental estrogenic effects of alkylphenol ethoxylates. – Critical Reviews in Toxicology 26(3):335-364.
70. Norcross, B.L., J.E. Hose, M. Frandsen & E.D. Brown 1996. Distribution, Abundance, morphological conditions, and cytogenetic abnormalities of larval herring in Prince William Sound, Alaska, following the Exxon Valdez oil spill. – Canadian Journal of Fisheries and Aquatic Sciences 53:2376-2387.
71. Pajor, A.M., J.J. Stegeman, P. Thomas & B.R. Woodin 1990. Feminization of the hepatic microsomal cytochrome P- 450 system in brook trout by oestradiol, testosterone and pituitary factors. – Journal of Experimental Zoology 253:51-60.
72. Pakdel, F., F. Le Gac, P. Le Goff & Y. Volataire 1990. Fulllength sequence and in vitro expression of rainbow trout estrogen receptor cDNA. – Molecular and Cellular Endocrinology 71:195.204.
73. Palmer, B.D. & S.K. Palmer 1995. Vitellogenin induction by xenobiotic estrogens in the red-eared turtle and African clawed frog. – Environmental Health Perspectives 103 (Supplement 4):19-25.
74. Pelissero, C., B. Bennetau, P. Babin, F. Le Menn & J. Dunogues 1991a. The estrogenic activity of certain phytoestrogens in the siberian sturgeon Acipenser baeri. – Journal of Steroid Biochemistry and Molecular Biology 38(3):293- 299.
75. Pelissero, C., F. Le Menn & S. Kaushick 1991b. Estrogenic effect of dietary soya bean meal on vitellogenesis incultured siberian sturgeon Acipenser baeri. – General and Comparative Endocrinology 83:447-457.
76. Pelissero, C., G. Flouriot, J.L. Foucher, B. Bennetau, J. Dunogues, F.L. Gac & J.P. Sumpter 1993. Vitellogeninsynthesis in cultured Hepatocytes; an in vitro test for the estrogenic potency of chemicals. – Journal of Steroid Biochemistry and Molecular Biology 44(3):263-272.
77. Piferrer, F. & E.M. Donaldson 1989. Gonadal ontogenesis in coho salmon, Oncorhynchus kisutch, after a single treatment with androgen or estrogen during ontogenesis. – Aquaculture 77:251-262.
78. Policansky, D. 1983. Size, age and demography of metamorphosis and sexual maturation in fishes. – American Zoologist 23:57-63.
79. Purdom, C.E., P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler & J.P. Sumpter 1994. Estrogenic effects of effluents from sewage treatment works. – Chemical Ecology 8:275-285.
80. RANZANI-PAIVA, M.J.T.; ISHIKAWA, C.M.; CAMPOS, B.E.S.; EIRAS, A.C. 1997 Hematological characteristics associated with parasitism in mullets, Mugil platanus, from the estuarine region of Cananéia, São Paulo, Brasil. Revista Brasileira de Zoologia, São Paulo, 14(2): 329-339.
81. Rosa-Molinar, E. & C.S. Williams 1984. Notes on fecundity of an arrhenoid population of mosquito fish Gambusia affinis holbrookii. – Northeast Gulf science 7:121-125.
82. Safe, S. & V. Krishnan 1995. Cellular and molecular biology of aryl hydrocarbon (Ah) receptor-mediated gene expression. – Archives of Toxicology (Supplement 17):99- 115.
83. Safe, S.H. 1995. Environmental and dietary estrogens and human health: Is there a problem? – Environmental Health Perspectives 103:346 - 315.
84. Sivarajah, K., C.S. Franklin & W.P. Williams 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. – Journal of Fish Biology 13:401-409.
85. Soto AM, Justicia H, Wray JW and Sonnenschein C 1991. p-nonylphenol: an estrogenic xenobiotic released from “modified” polystyrene. Environ Health Pers 92: 167-173.
86. Spies, R.B. & D.W. Rice 1988. Effects of organic contaminants on reproduction of the starry flounder Platichthys stellatus in San Francisco Bay. II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. – Marine Biology 98:191-200.

87. Spies, R.B., D.W. Rice, P.A. Montagna jr & R.R. Ireland 1985 Reproductive success, xenobiotic contaminants and hepatic mixed-function oxidase (MFO) activity in Platichthys stellatus populations from San Francisco Bay. – Marine Environmental Research 17:117-121. Stott, G.G., N.H. Mcarthur, R. Tarpley, V. Jacobs & R.F. Sis 1981. Histopathological survey of ovaries of fish from petroleum production and control sites in the Gulf of Mexico. – Journal of Fish Biology 18:261-269.
88. Stott, G.G., N.H. Mcarthur, R.F. Tarpley, R.F. Sis & V. Jacobs 1980. Histopathological survey of male gonads of fish from petroleum production and control sites in the Gulf of Mexico. – Journal of Fish Biology 17:593-602.
89. STRAUS, D.L. 2004 Comparison of the acute toxicity of potassium permanganate to hybrid striped bass in well water and diluted well water. Journal of the World Aquaculture Society, USA, 35: 55-60.
90. Sumpter, J.P. & S. Jobling 1995. Vitellogenesis as a biomarker for estrogenic contaminants of the aquatic environment. – Environmental Health Perspectives 103 (Supplement 7):173-178.
91. Sumpter, J.P. 1995. Feminized responses in fish to environmental estrogens. – Toxicology Letters 82(83):737-742.
92. Ten Hulscher TEM, Postma J, den Besten PJ, Stroomberg GJ, Belfroid A, Wegener JW, Faber JH, van der Pol JJC, Hendriks AJ and van Noort PCM 2003. Tenax extraction mimics benthic and terrestrial bioavailability of organic compounds. Environ Toxicol Chem 22: 2258-2265.
93. their degradation intermediates in marine alga. Fresenius J Anal Chem 371: 486-490.
94. Thomas, P. 1990. Teleost model for studying the effects of chemicals on the female reproductive endocrine function. – Journal of Experimental Zoology (Supplement 4):126-128.
95. Thorpe, J.E. 1989. Developmental variation in salmonid populations. – Journal of Fish Biology (Supplement A) 35:295-303.
96. USEPA (2006). Marine Ecosystems, United States Environmental Protection Agency, Retrieved 2006-08-25.
97. USEPA (2007). Whole Effluent Toxicity, an overview of the U.S. Federal Bio-monitoring Publication, U.S. Environmental Protection Agency.
98. Villalobos, S.A., M.J. Anderson, M.S. Denison, D.E. Hinton, K. Tullis, I.M. Kennedy, A.D. Jones, D.P.Y. Chang, G. Yang & P. Kelley 1996. Dioxinlike properties of a trichloroethylene combustion-generated aerosol. – Environmental Health Perspectives 104:734-743.
99. Wertheimer, A.C. & A.G. Celewycz 1996. Abundance and growth of juvenile pink salmon in oiled and non-oiled locations of western Prince William Sound after the Exxon Valdez oil spill. – American Fisheries society Symposium 18:518-532.
100. Wester, P.W. & J.H. Canton 1986. Histopathological study of Oryzias latipes (Medaka) after long-term β-hexachlorocyclohexane exposure. – Aquatic Toxicology 9:21-45.
101. Westernhagen, H. von, H. Rosenthalm, V. Dethlefsen, W. Ernst, U. Harms & P.-D. Hansen 1981. Bioaccumulating substances and reproductive success in Baltic flounder Platichthys flesus. – Aquatic Toxicology 1:85-99.
102. White R, Jobling S, Hoare SA, Sumpter JP and Parker MG 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135: 175-182.
103. Winfield, I.J. (2003). Fish Population Ecology (In: The Lakes Handbook). P.E. Sullivan and C.S. Reynolds, pp. 517-537.
104. Xiong, F., K. Suzuki & C.L. Hew 1994. Control of teleost gonadotropin gene expression. – Pp. 135-160 in: Farrell, A.P. & D.J. Randall (eds). Fish Physiology. Vol. 13. Academic Press, San Diego.

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