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Environmental Toxicity and Oxidative Stress on Gonads of Fishes

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Abstract

The knowledge of oxidative stress in fish and other animals has a great importance in the environmental and aquatic toxicological studies. Oxidative stress is evoked by many chemicals, including some pesticides, metals and other organic pollutants. Antioxidant defense systems in fish and other animals can be used to assess a specific area of toxicity. The present understanding of the role played by numerous chemical and environmental toxins in the onset of oxidative stress in the gonads of fishes is summarized in this article. These toxins play a significant role in the development of reactive oxygen species by inducing oxidative stress in both aquatic and terrestrial organisms. Reactive oxygen species are produced in excess, which causes oxidative damage such as lipid peroxidation, protein and DNA oxidation, and enzyme inactivation. This review paper reveals how different natural and artificial toxins affect the reproductive capability and capacity of fishes, by showing how these toxins initiate oxidative stress and how in turn affect the structure, anatomy, physiology and functioning of gonads as well as reproductive cells. Antioxidant defense mechanisms are employed as biochemical indicators of oxidative stress. The study of these biomarkers and their effects can be used for biomonitoring the level of environmental toxicity in different organisms.

Keywords: Oxidative stress; Fishes; Antioxidants; Pollutants; Toxicity; Gonads

Introduction

The degree of harm that a material or chemical may do to an organism, organ, or cell is known as its toxicity. Due to the fact that different people react differently to the same amount of a poisonous chemical, toxicity is dose-specific and may be assessed by its effects on an organism, organ, tissue, or cell. Since water is the most frequent source of toxicity, aquatic creatures, particularly fish, are more susceptible to water toxins owing to gill respiration. Toxicants can be of different types like physical, chemical and biological. Physical toxicants include coal, asbestos; chemical toxicants include inorganic and organic substances like lead, mercury, benzene, methyl alcohol; and biological toxicants include harmful or venomous secretions of plants or animals (Auten and Davis, 2009). There is another category of toxicants that are formed automatically inside the animal body which leads to physiological stress in which some free radical species like reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chloride species (RCS) are formed in the cell. The presence of these species may lead to the death of the cell or organism. Oxidative stress is caused due to ROS which is oxygen-derived oxidants formed in the body due to continuous exposure to toxic chemicals and mainly include anionic radicals like superoxide radicals (O^{2-}), hydrogen peroxide radical (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), peroxyl radicals ($ROO^{\cdot}$), and hypochlorous acid (HOCl). They are continuously formed in the body regularly.

Under normal circumstances, the body is capable of detoxification, but oxidative stress occurs when there is an increased rate of ROS formation and less rate of activity of the biological system's ability to detoxify them (Pizzino et al., 2017). The main component of the biological system which detoxifies these ROS are the antioxidative enzyme system of the body. These antioxidative enzymes act by scavenging ROS, inhibiting their formation, blocking the activation of phagocytes, binding the transition metal ions, preventing the formation of OH radicals or decomposing lipid hydroperoxides. These antioxidative enzymes work together with certain enzymes either to prevent these reactive species from being formed or to eliminate them out of the body. Antioxidative enzymes mainly include glutathione peroxidase (GPx), superoxidase dismutase (SOD), and catalase.

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SOD catalyzes the breakdown of two molecules of superoxide anion to oxygen and hydrogen peroxide (H_2O_2), to convert them to less hazardous substances. Catalase works with iron (Fe) or manganese (Mn) as cofactor and catalyze the conversion of hydrogen peroxide to water and oxygen, thus completing the process of detoxification started by SOD. CAT activity occurs by completing two steps in which the first step includes the oxidation of heme to an oxyferyl species by H_2O_2.

The generation of porphyrin cation radical by removal of the equivalent of oxidation product from Fe and another removal of oxidation equivalent from the porphyrin ring to produce water and oxygen molecule (Ighodaro and Akinloye, 2019). Glutathione is a selenocysteine containing peptide. It has a thiol group in its cysteine moiety which is a reducing agent which can be reversibly oxidized or reduced. (MatÉs et al., 1999). The work of GPx is to breakdown hydrogen peroxide produced by CAT into water and peroxides of lipid into their respective alcohol (Ighodaro and Akinloye, 2019). The mechanism involves the oxidation of selenol (RSeH) group of selenocysteine by hydrogen peroxide which generates a selenenic acid (RseOH) group, it gets converted again into selenol (RSeH) by involving two-step process in which GSH (glutathione) form the GS-SeR and H_2O. In the second step, reduction of GS-SeR intermediate back to selenol occurs by using a second molecule of GSH, thereby giving GS-SG (Glutathione disulfide/oxidized glutathione) as a by-product and then the oxidized glutathione (GSSH) is reduced by glutathione reductase enzyme by using NADPH (Lubos et al., 2011). These three antioxidants thereby undergoing certain biotransformation pathways eliminate or neutralize chemicals or intoxicants from the body. There are two phases or pathways, which are phase-1 and phase-2 reactions. Phase-1 reaction primarily processes chemicals for the reactions of phase-2. Phase-1 converts toxins via oxidative, hydrolytic and reductive reactions. The most common are oxidation reactions. Phase-2 reactions involve several pathways in which molecules undergo conjugation. The substances being metabolized are attracted to endogenous molecules such as acetate, glutamine, and glutathione to form water-soluble metabolites which are then excreted out in the urine or in the bile (Beiras, 2018). Thus, the biotransformation pathway eliminates unwanted hydrophobic substances by transforming them into more hydrophilic substances. The objective of this paper is to study the way in which different environmental toxins; generated by different natural activities, anthropogenic activities, industrial activities or addition of pollutants; affect the spawning activity, structure of gonads (testes and ovary) and the structure and activity of germ cells and gametes (sperm and ovum) of different fishes found either in fresh water or marine water.

Effect of metals and non-metals on gonads of fishes

Different metals and non-metals whether in free or compound states find their way to reach water bodies either directly or indirectly, thereby entering the food chain and harming the aquatic organisms. The effects of some of the metals and non-metals are discussed in different fishes.

Mercury

The effects of heavy metals on the reproduction, reproductive processes, and gonads (ovary and testis) of several fish species, including marine and freshwater fish, have been well researched. The major fresh water fishes used for studying are *Channa punctatus*, *Danio rerio*, *Gambusia affinis*, *Gymnotus carapo*, *Notopterus notopterus*, *Heteropnuestus fossilis* and the major marine water fish species include- *Acanthurus japonica*, *Balistes carolinensis* as these are easy to approach and to study. Different results have been obtained; some fish species showed deformity in gonad structure, some showed a reduced or irregular increase in gamete number, others showed problems in fertility and reproduction while some showed increased oxidative stress. The fresh water fish, *Notopterus notopterus* exposed to sublethal concentrations (0.088 & 0.44mg/l) of mercury for 30 days showed a decrease in lipid content and vitamin A & D content in the ovary (Verma and Tonk, 1983). Damaged follicular lining, deshaped oocytes, severe damage of oocytes and decrease in number and activity of previtellogenic, vitellogenic oocytes and clamping of primary oocytes with necrosis was observed in some of the regions of ovary in the fish, *Clarias*

gariepinus exposed to a sublethal concentration of 0.08mg/l of mercury (Masarat et al., 2014). When the same fish was exposed to the heavy metal (mercury) for 3 weeks followed by normal water for 1 week [15 & 25mg Copper-nicotinate complex/100g wet feed]; increased ROS production was observed that affected fertility of the fish. Addition of Cu-N complex reversed the mercuric-chloride induced oxidative stress by showing recovery of ovary (Al-Salahy, 2011). Hedayati and Katuli (2016) observed an increase number of pre-vitellogenic oocytes, adhesion in ovaries of females, oocyte atresia at higher mercury concentrations for the fish *Acanthopagrus latus*. Autometallography technique used to trace mercury uptake and accumulation in ovary of crucian carp, *Carassius auratus gibelio* showed mercury accumulation in early and late previtellogenic oocytes in the ooplasmic region, with positive immunohistochemistry reaction for caspase-3 in nuclei of early pre-vitellogenic oocytes (Zarnescu, 2009). Mercury given in combination with different compounds (Elsan, Ammonia) to the fish, *Channa punctatus* resulted in a continuous significant decrease in ovary weight, number and diameter of mature oocytes at stage 2 & 3, changes in occurrence of stages of oocytes thus interfering the breeding process (Dey and Bhattacharya, 1988). Total disappearance of mature yolky oocytes, reduced and destructed follicular cells and atretic follicles, reduced ovary size, destruction of germ cells was observed by Abhay (2014) in fresh water teleost A. *mola* when exposed to Arsenic trioxide, cadmium chloride along with mercuric chloride. Gautam and Chaube (2018) evaluated the effect of different heavy metals (Pb, Cd, Co, Hg) in post-vitellogenic oocytes of the fish, *Heteropnuestus fossilis* and observed lead (Pb) & mercury (Hg) showing significant increase ($P<0.05$) in germinal vesicle breakdown (GVBD) and ovulation at all concentrations during 4 & 8 hours of incubation, decline in GVBD and ovulation with cadmium (Cd) & cobalt (Co) at 16 & 24 hour of incubation and Hg & Pb showed stimulatory effect on ovulation at 16 & 24 hour of oocyte maturation.

Testes and sperm of male tropical fish, *Gymnotus carapo* exposed to mercuric chloride (1µM-30µM) for 24 & 96 hour showed complete disorganization of seminiferous tubules, reduction of germ cells, congested blood vessels, interstitial tissue proliferation, sperm aggregation, decrease in number of sperms at 20µM and change in morphology of sperm after 24 & 96 h (Vergilio et al., 2013). Reduced gonad size, destruction in germ cells, atrophy was also observed in testes of fresh water teleost, A. *mola* exposed to mercury chloride, arsenic trioxide and cadmium chloride (0.04583ppm, 0.2911ppm, 0.6279ppm) for 21 days (Abhay, 2014). Loss of lobular structure of testes, vacuolization and destruction of mature stages of spermatocytes and arrest of spermatogenesis was also observed.

Cadmium

The effects of heavy metal cadmium exposure on gonads, reproduction, and reproductive systems were evaluated in several fish species collected from various water sources. No mature oocytes, higher number of atretic follicles observed in *Labeo bata* (Annabi et al., 2013). The effects of sublethal concentrations (6mg/l & 9mg/l) of cadmium chloride was observed for different time intervals (15, 30, 45 days) on the air breathing fish, *Heteropnuestes fossilis*. Results observed were enlarged oocytes, thin and ruptured ovary wall after 15 days of exposure to 6mg/l & 9 mg/l concentrations respectively. Degeneration of egg envelope (at 6mg/l) and enlarged interfollicular spaces (at 9mg/l) after 30 days, atretic follicles (at 6mg/l) and degenerated egg envelope (at 9mg/l) after 45 days were observed (Sharma et al., 2011).

The gonads of male fish species exposed to various quantities of cadmium and its compounds were examined for a variety of effects. The tropical fish, *Gymnotus carapo* exposed to increasing concentration of cadmium chloride ($CdCl_2$) (5-40µM) (for 24 & 96h) showed reduction and absence of germ cells in testes, sperm aggregation, variation in cyst size, necrosis, vacuolization in cytoplasm of spermatogonia, spermatocytes and spermatids, infiltration of inflammatory cells; alterations in sperm number and morphology at 20µM concentration (at 24 & 96h) (Vergilio et al., 2015). Reduced gonadosomatic index with absence of spermatids and spermatozoa in testes were observed in the fish, *Labeo bata* obtained from different water sources (Annabi et al., 2013).

Chromium

Mishra and Mohanty (2008) examined the acute and chronic effect of heavy metal chromium (Cr VI) on the ovary of *Channa punctatus*. High percentage of atretic oocytes in acute exposure, decrease in percentage of vitellogenic oocytes during chronic exposure; vitellogenic impairment were observed.

Zinc

Channa punctatus, a freshwater teleost, was subjected to zinc concentrations of 8 mg/l, 10 mg/l, and 15 mg/l to study the effects of zinc. Reduced gonadosomatic index, shrinkage of oocytes, large interfollicular spaces, ootoxic conditions, decrease in number of immature oocytes and increase in number of atretic follicles observed. Oocytes showed distorted appearance and accumulation of heavy metal till the end of experiment. A linear relation observed between damage, dose and duration (Verma and Srivastava, 2008).

Effects of commercially produced chemicals (pesticides & insecticides) on gonads of fishes

Pesticides and insecticides are widely employed in agricultural areas across the world to reduce pest and bug population, however these chemicals also severely affect both humans and animals in addition to damaging crops. From the agricultural land, these chemicals enter into the water resources like ponds, rivers, lakes, seas and oceans and affect the aquatic ecosystem and aquatic flora and fauna in diverse ways. A lot of these chemicals like organophosphates and organochlorines such as Cythion, Hexadrin, Aldrin, Parathion; insecticides like Malathion, Endosulfan, Pyrethroids, Dichlorovos; pesticides like Chlorodecone, Carbaryl pesticide, Carbamate, Deltamethrin, DDT have their impact on reproductive structures, reproductive mechanisms and gonads of fishes. Decreased ovary protein content and significantly reduced total RNA, significantly reduced DNA content in ovary from 30 to 60 days were observed in the fish, *Channa punctatus* exposed to organophosphate (cythion) (Ram and Sathyanesan, 1985). The freshwater catfish's (*Heteropnuestus fossilis*) ovarian activity decreased during the whole yearly reproductive cycle, and there was a gradual decline in ovarian 32 P uptake and Pituitary gonadotrophin levels from the preparation phase to the spawning phase after 4 weeks of exposure to sublethal or safe concentrations of two pesticides (Cythion & Hexadrin) (Singh and Thakur, 1980). The same fish when exposed to same chemicals, no change in ovary lipid concentration, increase in ovarian cholesterol level during pre-spawning, spawning phases observed (Singh and Singh, 1980).

The fish, *Heteropnuestes fossilis* exposed to pesticides (Cythion, Paramour M50, Hexadrin and Aldrin) showed reduced gonadotrophic potency; no effect on ovarian 32 P uptake with Cythion and paramour M50 treatment and reduced ovarian 32P uptake with aldrin and Hexadrin treatment (Singh and Singh, 1982). Reduced uptake of ovarian 32P observed when the same fish was exposed to parathion and aldrin (SC or LC (1)50) for 4 weeks (Singh and Singh, 1981). The pre-spawning teleostan fish, *Colisa fasciatus* exposed to Endosulfan (Thiodon) EC35 (1ppm solution) for 30 days showed thick ovary wall, retarded ovarian activity, decreased stage 2 & 3 oocytes; increase in percentage of oogonia, stage 1 and atretic oocytes; stage 3 oocytes with clumped and damaged yolk (Pandey, 1988). The treatment of same chemical (0.001ppm) given to fish, *Oreochromis mossambicus* (Trewaves) showed decreased sugar level of ovary after 30 & 60 days, decrease in free amino acid level of ovary after 30 & 60 days, decreased protein content of ovary after 30 days and increased protein content after 60 days, degenerative changes in the cell (vacuolization, necrosis and histolysis), rupture of follicular epithelium and degeneration of immature oocytes (Saravanan et al., 2003). Clumping of cytoplasm, degeneration of follicular cells, increased number of nuclei, shrinkage in nuclear material, adhered and atretic oocytes were observed in *Labeo rohita* exposed to sublethal concentration (0.4mgl^{-1}) of Endosulfan (for 10, 20, 30 days) (Archana and Sitre, 2014). The insecticide Endosulfan (0.5, 0.1, 0.05, 0.01ppb) and Malathion (1000, 500,100, 50ppb) given in combination with different concentrations of LH (10µgm/l) to study the effect on germinal vesicles and oocytes of ovary of *Cyprinus carpio* revealed high germinal vesicle breakdown (GVBD) in LH- induced ovary and low GVBD with Malathion

(13.4±0.4, 14.0±1.0, 12.9±3.5 & 18.1±3.9% repectively); Endosulfan with LH (12.8± 1.6, 8.8±1.2, 20.9±2.1, 26.0±2.2% respectively); no ovulation on treatment with Endosulfan (Haider and Inbaraj, 1988). The sublethal and median lethal concentrations (0.06, 0.15, 0.30mg/L) of carbofuran given to fish, *Labeo rohita* for 96h resulted in significant decrease in total number of eggs, decrease in hatching percentage (Adhikari, et al., 2008).

Minimum ovarian damage in spawning phase, reduction in Gonado-somatic-index (GSI) prominently in spawning phase than in pre-spawning phase and post-spawning phase observed in same fish treated with sublethal dose of carbofuran (0.06, 0.15mg/l) & cypermethrin (0.16 & 0.40µl/L) for 4 weeks. Cypermethrin showed more impact than carbofuran in all doses (Sarkar, et al., 2013). Reduction in diameter of stage 1, 2, 3 oocytes (shrinkage), atretic follicles and large interfollicular spaces were observed after exposure of subacute concentration (0.024, 0.012mg/L) of chlorodecone (CD) (i.e. Kepone/ Deca-chloro-octahydro-1,3,4-metheno,2,H-cyclobuta-pentalen-2-one) in the ovary of fresh water catfish, *Heteropnuestus fossilis* (Srivastava and Srivastava, 1994). Reduced GSI of ovary, vacuolization and necrosis, arrested ovarian recrudescence, interfollicular odema in ovary were observed in the fish, *Clarias batrachus* exposed to sublethal concentration of carbaryl pesticide (Jyothi and Narayan, 1999).

Different concentration of Sumithion (2.0,5.0,5.5,10.0,15.0,15.5,18.5,20.0,20.5,25.5,100 ppm) were given to *Channa* for 7days. Degenerative changes like hypertrophy of cells and their nuclei, vacuolation in cytoplasm, necrosis, ruptured cell membrane, fragmentation of ova was observed (Bhuiyan et al., 2001). Reduced number of oocytes, increased atretic oocytes that too grow unhealthy, discontinued growth of developing follicles and follicle destruction were observed in the gonad (ovary) of zebrafish exposed to different concentrations of deltamethrin [group1 (0.5µg/L), group 2 (1µg/L), group3 (control group)] for 5 days (Koç et al., 2009). The fish samples of *Oreochromis niloticus* were divided into 3 groups- control group, vitamin-E treated group, deltamethrin-treated group, vitamin E + deltamethrin treated group. Results observed were atretic oocytes, oocytes without nuclei surrounded by macrophages, deformed oocytes, focal necrotic area in ovary of deltamethrin treated groups. Vitamin E showed protective effects against deltamethrin induced histopathological changes (Bayar et al., 2014). The same fish when treated with sublethal concentrations (22.5µg/L & 45.0µg/L) of dimethoate for 21 days showed alterations in histo-anatomy of ovary like disrupted follicular cells, vacuolization (Desai et al., 2011).Partial disruption of ovarian follicle with vacuolization in cytoplasm of germ cells (with lethal dose of 5.012ppm), cytomorphological changes in ovarian follicle, some degenerative atretic follicles were observed in the dimethoate treated fish, *Puntius ticto* (Ham) (Marutirao, 2013).

Alterations in structural, physiological and morphological patterns of ovary, shrinkage of oocyte with interfollicular odema, leaky yolk with ruptured oocyte membrane and stromal hemorrhage was observed in *Channa striatus* exposed to sublethal concentration of cypermethrin for 96h (Tantarpale and Rathod, 2014). Lethal and sublethal concentrations of cypermethrin in combination with Deltamethrin was given to same fish species for 12h, 24h, 72h & 96h; results observed were disruption of mature follicles, broken oocytes, ooplasm disrupt, atretic follicles, irregular shaped oocytes in the ovary (Mondal et al., 2015). The fish, *Channa punctatus* was treated with chlorpyrifos (a pesticide, insecticide, miticide, acaricide) for its sublethal concentrations of 1.106µl/L (1/3rd of LC_{50}) & 0.332µl/L (1/10th of LC_{50}) for 3 & 7 days respectively. Changes in oocytes and follicles, elongated and degenerative ovarian follicle, fragmented and irregular ova, necrosis and larger intracellular spaces, vacuolization and exclusion of karyoplasm in ovarian follicle were observed (Pandey et al., 2014). The same chemical in combination with cypermethrin (50% EC) having sublethal concentration (8.4 & 4.2µg/L) was given to zebrafish (*Danio rerio*) for 7 days. Minimal to mild follicular atresia observed in the ovary (Rajini et al., 2015). Oocyte damage, cytoplasmic retraction of oocytes, destruction of follicles, broken ovarian wall, extrusion of karyoplasm was observed in the fish, *Heteropneustes fossilis* exposed to sublethal concentrations of 0.2ppm of Malathion (Deka and Mahanta, 2012). Reduced size of mature oocytes, disruption and vacuolization of cytoplasm, elongated ovarian follicle, abnormal ovary configuration,

necrosis, fragmented ova with abnormal shape during chronic exposure were observed in the fish *Channa punctatus* exposed to Malathion for acute (4 days) & chronic (15 days) (Magar and Bias, 2013).

Significant decrease in GSI (after 15 days) & maximally at higher concentration of 2ml/L, decreased vitellogenic activity and oocyte atresia, vacuolization and tissue necrosis were observed for the fish *Channa punctatus* exposed to two sublethal concentrations (1ml/L & 2ml/L) of Monocrotophos for 15 & 45 days (Maqbool and Ahmed, 2013). Reduced size of mature oocytes, damaged follicles, deformity in ovarian follicle, fragmented ova with atretic oocytes, broken ovary wall with necrosis were observed in the ovary of fresh water, air breathing fish, *Channa gachua* exposed to lethal (0.3ppm) & sublethal (0.06ppm) concentrations of profenofos for 4 & 15 days (Anamika et al., 2015). The eggs of trout, *Salmo gairdneri* collected from Lake Rerewhakaaitu showed least viability after assessing the possible effects of DDT received from drainage of agricultural land (Hopkins et al.,2010).

In the fish *Labeo rohita*, changes were seen in the form, composition, and structure of the spermatogonia, spermatocytes, sertoli cells, and interstitial cells. Additionally, the walls of the primary spermatocytes were broken, causing them to become detached from the seminiferous tubules when exposed to sublethal concentration (0.4mgl^{-1}) of endosulfan for 10, 20, 30 days (Archana and Sitre, 2014). The fish, *Cyprinus carpio* exposed to carbamate pesticide (16ppm) was observed for testicular recrudescence and recovery response after long term exposure of 45, 75, 105 days; insignificant change after 75 days, increased GSI in recovery groups, HIS reduced in exposed groups but increased in recovery groups; decreased water and lipid content in exposed groups, increased water and lipid content in recovery group; impaired spermatogenesis observed (Chandra et al., 2003). Reduced GSI of testes, vacuolization and necrosis, cessation of spermatogenesis, thickening of basement membrane were observed in the testes of fish, *Clarias batrachus* for the sublethal concentration of carbaryl pesticide (Jyothi and Narayan, 1999). The fish, *Heteropneustes fossilis* exposed to acute (0.048mg/L), subacute (0.024, 0.012mg/L) and sublethal concentration (0.008mg/L) of chlorodecone (CD) resulted to show ceased sperm maturation and reproductive function, reduced and flattened seminiferous tubules, degenerating germinal epithelium and their inter-tubular connective tissue, atrophy and vacuolization in leydig cells for acute and sub-acute concentration (Srivastava and Srivastava, 1994). Nuclear pyknosis, cell necrosis, increase in number of macrophages, degeneration of spermatogonial cells, decrease of spermatogonia within lumen of seminiferous tubules, decrease in number of spermatocyte cells were observed in the testes of Deltamethrin exposed fish, *Oreochromis niloticus* divided into 4 groups (control, vitamin E treated, Deltamethrin treated & vitamin E +Deltamethrin treated group) (Bayar et al., 2014). Presence of large number of inflammatory cells, necrosis, inter-tubular vacuoles formation, swelling of seminiferous tubules observed in same fish exposed to Dimethoate for sublethal concentrations of 22.5µg/L & 45.0µg/L for 21 days (Desai et al., 2011). Increase in CAT activity, levels of MDA and GSH in spermatozoa, decrease in GSH-Px and SOD activity and motility and survival of sperm was observed for the cypermethrin treated fish *Salmo coruhensis* (Kutluyer et al., 2018).

Effects of variations in environmental conditions on gonads of fishes

When environmental pollutants such as those found in soil, water, and the air enter the bodies of living things, they begin at the lower trophic level and build up until they reach the higher trophic level, a process known as biomagnification. As a result, the organism's physiology undergoes a number of changes, including impairments to its normal metabolic rate, reproductive rate, and impacts on gonads and gametes. Like humans, fish also face the same effect of contaminants on its body. To examine these effects, various fish species has been studied. The ovary of English sole, *Parophrys vetulus* was examined for the effect of contaminants on its developmental pattern by collecting the fish from 4 different sites in Puget sound, Washington. Fishes from two highly contaminated sites showed increased gonadal recrudescence (Johnsan et al., 1988). The fish rock sole again collected from 4 different places (Eagle Harbor, Sinclair Inlet, Yukon Harbor, Pilot point)

of same site in Washington and investigated for the effect of aromatic hydrocarbons (AH) and polychlorinated biphenyls (PCBs) contaminated on ovarian development and spawning activity. Sinclair Inlet showed highest concentrations of PCBs and produced eggs with significantly reduced weight (Johnsan et al., 1998).

Changes in gonadal tissue observed in fish, *Barbus peloponnesius* due to polluted water of Vardar River (Iseni et al., 2015). Increased MDA levels in ovary was observed in the van fish having abnormal ovary and exposed to contaminated water of a lake (Ozok et al., 2017). The effects of temporal variations were studied on the spawning activity and reproductive strategy of European Perch, *Percha fluviatilis*. Results showed a long vitellogenic process and a short spawning season, ovary development in one clutch of oocyte having 700-900µm oocyte diameter but no maturation of further clutch in spawning season; low average fecundity in late vitellogenic stage than potential fecundity; atretic oocytes in early vitellogenic stages (Saemi-Komsari et al., 2014). Delayed development of gonads (immature stage 2), ovaries with post spawning oocytes (in April), longest phase of gonad development with oocytes in advanced vitellogenesis during stage 4 were observed in the same fish collected from natural sites (Oder River, Lake Dabie) and drainage canal carrying post- cooling (warm water, 8˚C high than normal water) from the Dolna Odra power plant to analyze the annual development cycle of gonads of fish (Kirozuk et al., 2015). The post stripping of *Carassius auratus* at 20˚C was done and eggs were incubated in vitro for 18 hours to study the involvement of oxidative stress in oocyte ageing by using real-time PCR. No alterations in any relative mRNA abundance of examined genes through oocyte ageing observed (Samarin et al., 2019).

Samples of male fish, *Puntius javanicus* collected from Mas river of Indonesia on 19 July, 2012 were analyzed for the effect of pollution on gonads. Samples of male fish got feminized and some showed the presence of inter-sex individuals (Shobikhuliatul et al., 2013). The male fish, Nile Tilapia, *Oreochromis niloticus* was analyzed for the effect of different photoperiods with different conditions of light i.e. oh (no light), 12h (natural light), 24h (lamp light) on the development of gonads for 60 days. Results showed increased tubular lumen and germinal epithelium in fish provided with 0 and 24h photoperiod (Navarro et al., 2015). The effect of chronic hypoxia (DO<0.8mg/L) on the testes of *Cyprinus carpio* showed decreased ATPase activity, high LDH activity (sign of forced entry into anaerobic phase of metabolism); significant increase in CAT, SOD and significant loss in GSI in male (Bera et al., 2020).

Effects of anthropogenic contaminants on gonads of fishes

Many industrial businesses today discharge their wastes into water bodies, where they infiltrate the aquatic environment and interfere with the physiological functions of aquatic creatures like fish. These articles like paint, food additives, various flavouring substances, artificial colors may enter their bodies and undergo certain bio-transformations that could convert these substances into other toxic compounds. The effect of two antifouling paints [Berger TBT-free (A/F 783(H)-reddish brown color and silka marine lead based paint- pale orange color] was observed on catfish, *Clarias gariepinus* by exposing the fish to 96h acute toxicity and then to chronic toxicity [using $1/10^{th}$& $1/100^{th}$ 96h median lethal concentration (LC_{50})] for 28 days. Significantly higher levels (P<0.05) of Malondialdehyde in silka-exposed catfish; significantly higher enzymatic activity of SOD, CAT, reduced GSH, GST in Berger paint exposed catfish; with follicular degeneration of gonads were observed (George, et al., 2017). Decreased weight, decreased egg production and decrease in percentage of viable eggs observed when the fish *Clarias gariepinus* was exposed to industrial effluents (food and beverage industry) for 30 days to study the change in reproduction and growth patterns (Olubukola and Victor, 2012). The juvenile zebrafish fed with Biodiet starter and TCDD (0, 0.1, 1, 10, 100ppb) after 0, 7, 14, 28, 42 d of exposure was studied for the effect of dietary TCDD on histopathological alterations associated with global gene expression. Results showed multiple lesions in ovary, diminution in some ovarian follicles having vitellogenic oocytes, endocrine disruption based on alternatively spliced vasa transcripts (biomarker for ovarian development and disruption) (Liu et al., 2014). The fish, *Oreochromis niloticus* fed to a basal diet

along with food additives [Therigon (0.05, 1.0, 2.0g/kg diet), Nuvisol Hatch (0, 1, 2, 3g/kg diet), Gibberellic acid (0, 20, 40, 60mg/kg diet), carnitine (700, 900, 1100mg/kg diet)] for 19 days. Results showed that Gibberellic acid had most negative effect on oocytes at 60mg/kg diet; Therigon showed presence of lesions, necrosis, separation of follicular layers, atresia; ovary developed more with Nuvisol Hatch (1.0g/kg diet), later showed irregular walls (at 2.0g/kg diet), haemolysis at 3.0g/kg; liquefaction of yolk sphere with large vacuoles in ripe oocytes on treatment with Gibberellic acid at 60mg/kg (Abdelhamid et al., 2013). Reduction in GSI, weight of gonads, alterations in ovaries; significant decrease ($P<0.05$) in activities of antioxidant enzymes (SOD, CAT, glutathione reductase, glutathione peroxidase), significant increase in level of hydrogen peroxide (H_2O_2) and lipid peroxidation observed in ovaries of fresh water fish, *Anabas testudineus* exposed to sublethal concentrations [5mg/L for short- term duration and 10mg/L for long term durations (7, 15, 30, 60d)] with the median lethal concentration (96h-LC_{50}) of carbon nanoparticle, fullerene C60 (Sumi and Chitra, 2019).

The male fish, *Oreochromis niloticus* fed with a basal diet along with food additives for 19 days. Results observed were L-carnitine (700 & 900mg/Kg diet) showed better maturation of testes as compared to control but in meantime, degeneration and severe haemolysis observed in seminiferous tubules (Abdelhamid et al., 2013). Study of attenuation effect of antioxidant N-phenyl-4-aryl-polyhydroquinolines (6e-6g) was done on atrazine-induced histopathological changes in testicular tissues. Abnormalities induced by atrazine were significantly reduced when the atrazine-treated tissue was supplied with (6e-6g) compounds of antioxidants (Chandak et al., 2015). Significant decrease ($P<0.05$) in activities of antioxidant enzymes (SOD, CAT, glutathione reductase, glutathione peroxidase), significant increase in level of hydrogen peroxidase generation and lipid peroxidation, reduction in GSI, weight of gonads; alterations in testes were observed in the fish, *Anabas testudineus* exposed to sublethal concentrations [5mg/L for short term duration & 10 mg/L for long term durations (7,15, 30,60d)] with median lethal concentration (96h- LC_{50}) of carbon nanoparticle fullerene C_{60}(Sumi and Chitra, 2019). The male catfish, *Clarias gariepinus* exposed to pure (100mg/L) 4-nonylphenol to which quince (*Cydonia oblonga*) was added for 15 days. Results observed were decreased level of testosterone, FSH, increased 17 β-estradiol (E2), LH and cortisol; significantly increased ($P<0.05$) enzymes [SOD, CAT, Acetylchloinesterase (AchE), glutathione-S- transferase, total antioxidant capacity (TAC)], MDA, impaired histology of testes. Quince added balanced the hormonal level, repaired testicular histology, reduced AchE and MDA significantly (Sayed and Ismail, 2017).

Effects of naturally occurring plant chemical compounds on gonads of fishes

To research the impact on the reproductive systems, gonads, and reproductive structures of fish, various natural materials used in daily life are employed, such as food components, fruit seeds, aloe vera, numerous vitamins and oils. Low ovary weight and low occurrence of stage 3 i.e. yolky oocytes in ovaries of treated fish with significantly retarded ovary recrudescence and atresia were observed when the fresh water teleost, *Channa punctatus* was exposed to sublethal concentration (5%) of vegetable oil factory effluent for 120 days (Saxena and Bhatia, 1982). The fish, *Gadus morhua* was fed at 4 ration levels (starvation, maintenance, moderate, excess) prior and during spawning for 6-9 months. Results observed were maximum values of influx of proteins into ovary, egg dry weight subsequent decline of mean hydrated egg diameter at 10% proportion of eggs spawned; increase in mean vitellogenic oocyte diameter at PES=10%; production of more vitellogenic oocyte (in cod with high condition factors); specimen deprived of feed during spawning period have actual fecundity between 20% and 80%; increase (from 0% at PES= 0% to 33% at PES=80%) in atresia in moderate ration fish (Kjesbu et al., 1991). The English sole (*Parophrys vetulus*) from Puget Sound, WA exposed to xenoestrogen showed ovarian lesions, oocyte atresia but not related to xenoestrogen exposure; at Elliot Bay, spawning time in female with abnormal vitellogenin production observed that might be due to discharge of industries, surface run off or combined sewage conditions (Johnsan et al., 2008). The fish, *Glossogobius giuris* exposed to neem oil to examine its effect for different concentration (0.05ppm, 0.25ppm & 0.50ppm) up to different periods (24, 48, 72, 96h). Lower dose (0.05ppm) for 24h showed increase

in yolk vesicles, degeneration of few oocytes of stage 1 & 2, reduction in ovary weight, a higher dose for 72, 96h at 0.50ppm show more nuclei in stage 1 & 2 oocytes with more atretic follicles, liquefaction and swelling of follicular wall, necrosis along with ruptured follicles (Narayanaswamy and Ramachandra, 2010).

The fish Nile Tilapia, *Oreochromis niloticus* divided into 5 groups- 2 groups fed with basal diet, rest 3 were fed on basal diet along with 60, 90, 120g of pawpaw seeds respectively. Results showed decreased GSI and egg diameter, decreased fecundity in 3 groups after treatment and recovery months; permanent sterility of ovary at high dose after recovery months (Abdelhak et al., 2013). The same fish exposed to a plant nutrient, Librel TM to determine its effect on gonadosomatic (GSI) and hepatosomatic index (HIS) for sublethal concentration of 250mg/l for 15, 30,45d whose LC_{50} value was found to be 5000mg/l. Results observed were decrease in GSI, histopathological changes in ovary (Sadekarpawar and Parikh, 2013). The same fish exposed to Aloe vera latex (AL) (added to basal diet) at 5 different concentrations (0, 0.5, 1.0, 1.5, 2.0ml/kg) for 60 days showed decreased egg size, fecundity with each diet, less visible atretic follicles (at oml/kg), few lesions (at 1.0ml/kg), change in color of ovaries, ruptured follicles, necrosis, abnormal gonadal development, inflammation of granulosa mass in interstitial tissue of ovary (at 2.0ml/kg) (Kushwaha, 2013). Brain and gonad samples of wild coral reef (female blue headed wrasses) collected to study stimulus for sex change by subjecting the fishes (control small females & dominant TP males) to 6 successive stages base on behaviour at time of capture and gonadal histology. Transcriptomic and methylome study revealed repression of aromatase gene (encodes enzyme to convert androgens to estrogens) and exertion of environmental stimulus via stress-axis triggers a cascade collapse of feminizing gene expression and sex-specific gene neofunctionalization (Todd et al., 2019). Significantly lower level of SOD activity, vitamin E concentration and higher MDA content observed in healthy common carp (*Cyprinus carpio*) fed with basal diet with reduced vitamin E content of 0, 25, 50 $IUkg^{-1}$ (Wang et al., 2016).

Inbreeding depression in three spined sticklebacks, *Gasterosteus aculeatus* was studied by dividing them into 2 groups of outbred and inbred males. Result showed inbred males have higher body condition, brighter testes but low sperm quality and number as compared to outbred males; testes brightness gives a measure of oxidative stress inversely correlated with sperm number (Mehlis et al., 2012). Significant levels of vitellogenin in male fish from several urban sites with high number of fish affected in Elliot Bay; testicular lesions (not related to xenoestrogen exposure); altered spawning time with abnormal vitellogenin production observed (might be due to industrial discharge, surface run off or combined sewage conditions) in English sole (*Parophrys vetulus*) from Pujet Sound, WA (Johnsan et al., 2008). Significantly decreased levels of testosterone and estradiol after treatment and recovery months, changes in structure of testes of treated groups, permanent sterility at high dose after recovery months observed in fish, Nile Tilapia, fed with basal diet along with 60, 90, 120g Pawpaw seeds respectively (Abdelhak et al., 2013). Decrease in GSI, histopathological changes in testes observed in fish, *Oreochromis mossambicus* exposed for 15, 30, 45d whose LC_{50}value was found to be 5000mg/l (Sadekarpawar and Parikh, 2013). Aloe vera latex (AL) added to basal diet of fish, Nile Tilapia, *Oreochromis niloticus* at 5 different concentrations (0, 0.5, 1.0, 1.5, 2.0ml/kg) for 60 days. Results showed cystic seminiferous tubules at (0.5 & 1.5ml/kg), atrophy (at 1.0ml/kg, 1.5ml/kg), spermatid disintegration and necrosis (at 2.0ml/kg) in males (Kushwaha, 2013).

Conclusion

The gametes are chosen as a study material for toxicity and oxidative stress. These can be easily available during breeding seasons of animals without any surgical treatment. Gonads contain germ cells which represents the precursors or the hereditary information in the form of gametes so any alterations or change in gamete genetic material and cytoplasm can be easily studied to know the alterations in the body of future generation. Gametes obtained from the parents can be used for in-vitro fertilization and study of effects of different xenobiotics on the histological and physiological changes in the body of embryo or developing young one. Moreover, the further

studies of different types of chemicals whether natural or artificial with their effects on the gamete production and germ cells can be used in the evaluation of successive changes over the years which can help scientists to study about the origin and evolution of different and modified species and can also help in examining the presence of excess amount of certain type of chemical in the aquatic body under study by mere looking at the external features of the fish and by cellular changes happening inside a fish body. In addition to this, these studies can help scientists to study about the variations in the body features like skin color, spines shape, cellular changes due to exposure of fish body to different chemicals.

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Ethics approval
Not applicable.

Citation: Mahajan S and Randhawa JK (2023) Environmental Toxicity and Oxidative Stress on Gonads of Fishes. Environ Sci Arch 2(STI-2):3-17.