Endocrine disruptors: an overview and discussion on issues surrounding their impact on marine animals

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Abstract

Endocrine disrupting compounds (EDCs) are a growing concern because they are seriously harmful to wildlife both by direct damage to animals and through more subtle effects on growth, development and reproduction. In the past decades, there have been many studies about toxicological impact of EDCs on species. The NCBI Data-Base has amassed hundreds of research papers regarding the effects or mechanism of EDCs in marine animals. However, researchers still face new scientific challenges in some critical issues related to the understanding of EDCs and their effect on animals. Specifically, issues surrounding the selection of an appropriate model organism to document their effects, reconsidering biomarkers, and using "omics" technique to study the precise mechanism of EDCs warrant discussion. In addition, EDCs are now taken into account to partly explain the dwindling biodiversity of marine ecosystem. Yet, whether there exists a causal association between EDCs exposure and variety deterioration of species is still unknown. This review provides an in depth commentary on the issues mentioned above. [JMATE. 2009; 2(2): 7-17]

Keywords: Endocrine disrupting compounds; ecotoxicology; marine animals; discussion

1. The possible mechanisms of action of EDCs on marine animals

Environmental pollutants in the various ecosystems are a major concern worldwide. Many toxic, mutagenic, and carcinogenic aromatic pollutants are known to pose serious threats to the health of marine animals. Among contaminants, endocrine disrupting compounds (EDCs) are the most typical ones. They are a heterogeneous class of chemicals both man-made and naturally existing, which are present in the environment and have the potential of disturbing the endocrine system of organisms (45). Many EDCs are classified as "xenoestrogen" because their action mimics that of oestrogen hormones. EDCs act as inadvertent exogenous signals and therefore have the potential to disrupt hormone-controlled physiological processes such as growth, development, immunity, sexual differentiation, or reproduction. In the past two decades, many populations of marine animals have declined in a number of geographical locations worldwide, especially in coastal regions; the reasons are probably over-exploitation and habitat loss. However, in recent years, more and more evidence obtained from laboratory or field studies are reporting that EDCs are also potentially key factors contributing to bioresource decrease and biodiversity decline. EDCs have been found in estuarine, coastal and ocean fields, representing a potential hazard for marine animals. The adverse effects of EDCs on aquatic animals have increasingly been attracting peoples' attention. BBC News (2006) reported that the breeding biomass in the Pacific population of marine fish had declined more than 65% from historic levels, and some populations had decline more than 80%. Across the world, EDC pollution is extremely critical and it's effect on marine organisms deserves our concern. By summarizing previously published material, the possible mechanisms of action of EDCs on various marine

JMATE

species are clarified in this review.

Firstly, EDCs have been shown to affect the normal function of the hormone system at several different levels, including mimicking by binding its receptors, blocking receptor binding, as well as altering the synthesis, metabolism and activity of native hormones (17). In teleost fish and most vertebrates, the thyroid gland appears to be a rather well-defined target of polychlorinated biphenyls (PCBs) exposure. Altered levels of circulating thyroid hormones and morphological changes in the thyroid gland have been demonstrated in sea bass exposed to organic pollutants (42). Trout exposed in sea water contaminated by 3', 4, 4', 5-pentachloro biphenyl was observed to decrease concentrations of plasma E_2 and thyroid hormones when compared to control group (6). Histological lesions were also reported in thyroid glands of harbor seals found in waters polluted with EDC's (46). The adrenal gland is another target organ affected by organochlorines. Keymer et al reported that adrenal hyperplasia in otters in the Baltic Sea, were associated with high loads of organochlorine pollutants (28).

More recently, abnormally high concentrations of organ chlorines were also demonstrated in sea birds and fish, and metabolites were found to covalently bind adrenal cortex cells where their toxicity was expressed (4). Gerald reviewed that EDCs disrupted the steroidal hormones in crustaceans and impacted their growth and development (16). In our laboratory, we found the abalone, an important indigenous species in China, phthalates impacted its steroidal hormones process (Fig. 1, unpublished data).

Damaging the immune system of aquatic species may be another mechanism whereby EDCs exert their toxicological effects. Ample evidence indicates that organohalogens have detrimental effects on the immune system of fish or shellfish over the past decade. These compounds alter the function of both arms of the immune system, cellular and humoral immunity. Bisphenol-A (BPA) has been found to cause lymphoid depletion in lynx (30), increase natural cell toxicity in mussels (9), decrease the number of T cells as well as reduce T cell mediated cytotoxic activity in jurkat cell lines
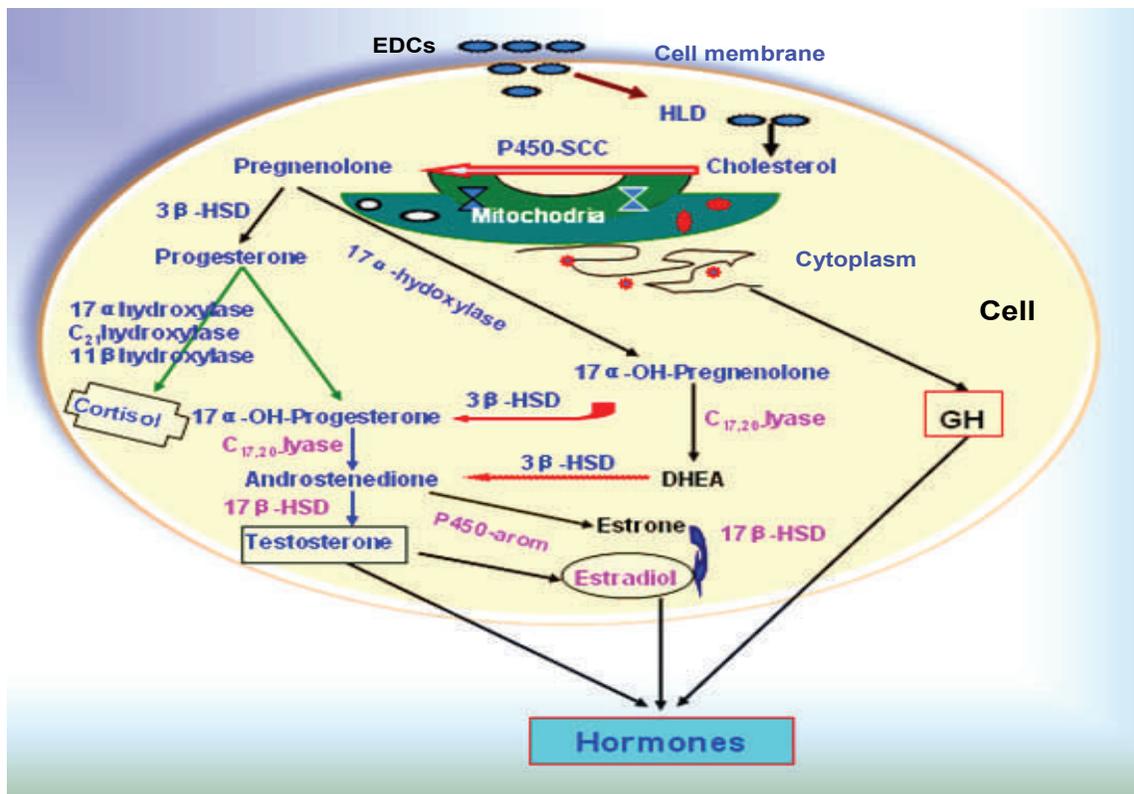

Figure 1. A hypothetical molecular mechanisms of EDCs substance (e.g. phthalate esters) effect the steroidal hormones process of abalone (*Haliotis diversicolor supertexta*).

(an immortalized line of T-lymphocyte cells) (33). The immunotoxicity of other EDCs has also been demonstrated in frog, fish, shrimp, and sea turtle (11, 23). It is not surprising that EDCs induced immunosuppression of experimental animals resulted in their experiencing a higher susceptibility to a wide variety of infectious agents, such as bacteria, protozoa and viruses. Recently, several studies confirmed this xenobiotic-related immunosuppression of humoral and cellular responses as well as the decrease in natural resistance to viral and bacterial infections (26). In our laboratory, a study to correlate immunosuppression of abalones with levels of contamination in polluted sites versus pristine sites is under the way.

The third effect of EDCs on species is the dysfunction of reproduction, which deserves close attention. EDCs can increase reproductive and developmental abnormalities in many aquatic organisms. The common effects which were most often reported include an alteration in androgenic steroid metabolism. This alteration could result in imposex development in the animal (especially in gastropod mollusks) and consequently interfering in their sexual differentiations or affecting their secondary sexual characters (19). Imposex is a descriptive term applied to some sea snails, marine gastropod mollusks which, under the toxic effects of pollutants, develop sex organs that are in contrast to their actual sex. It is a pathological condition where male sex characteristics, such as the development of male sex organs, (for example the penis and the vas deferens) form in female gastropods. In some marine species, reproductive failure, induced perhaps by high pollution, has been suspected as one of potential reasons for the declining populations of fish inhabiting highly polluted ecosystems (12, 20). Tributyltin (TBT), the most typical of the EDCs, has been reported to induce reproductive dysfunction in most marine animals, such as gastropods and mussels (22). Similar to TBT, Phthalate esters (PAEs) could cause premature births, reduced reproductive rate, decreased survival of juveniles as well as depressed sperm quality (count, viability, motility and percent of abnormalities) (55). In

marine mammals, the number of pregnant beluga females appeared dramatically low in polluted areas, and study of serial sections of ovaries demonstrated little ongoing ovarian activity compared to what was reported in belugas from pristine sites (8).

2. How to select an ideal model organism?

A model organism to study EDCs should offer technical and practical advantages for studying principal biological processes, effects and mechanisms. In addition, it needs to have traits that can be generalized, i.e. the model organism has to be representative for large group of organisms. Carvan reviewed the long known phenomenon that model species is necessary in ecotoxicology. In his view, if a species to be considered a model or bioindicator, it must be meet some criteria (10). Species that are appropriate for study are:

·low cost and easily managed

·not subject to legal protection or a
 conservation issue

·easily sampled

·abundant and easily manipulated

·an important component of their ecosystem/
 habitat

·able to accumulate pollutions

·known to exhibit well defined responses
 after exposure

According to this, there are a minimum of a dozen species models for toxicological studies (zebrafish, medaka, trout, fathead minnow, Atlantic killfish, pufferfish, flounder, trout, eel, mussel, seastar and crab). The model species mentioned above mostly consist of vertebrate fish. Hahn summarized the advantages of using fish as a model organism in marine ecotoxicology. Fish are vertebrates and therefore have a close evolutionary relationship to mammals. Thus, mechanisms discovered in fish are more likely to be relevant to humans (18). It

enhanced our understanding of human environmental health and toxicology. However, this is not to say that all vertebrate species are useful or usable for models in marine ecotoxicology. When using the fish as a model organism for EDCs research and toxicological assessment, we should not overlook their limitations. For example, vertebrates (fish) make only a limited contribution of the overall biodiversity in the natural ecosystems and many of the species studied do not represent the lower trophic levels. Furthermore, their relatively long generation times make it difficult to conduct transgenerational or life-long exposure studies and these limitations may be compounded by issues of ethics and the cost of maintaining large numbers of experimental animals. Besides, we also need to be aware of the specialization that makes these species differ from each other. For example, in most fish species, the egg yolk protein vitellogenin (VTG) and the eggshell protein, zone radiata (ZR), zone pellucida (ZP), or vitelline envelope protein, are synthesized in the liver under estrogen signal in female fish. This is secreted to the circulation from the hepatocytes and incorporated into the growing oocytes (18). However, in zebrafish and other cyprinids, the eggshell proteins are synthesized only in the maturing oocyte, so, whereas ZR or ZP proteins are useful biomarkers for xenoestrogens in the trout and the medaka, they are not detectable in the circulation of estrogenized zebrafish or fathead minnow. The reason behind of this is not known, and surely some interesting issues for evolutionary studies are raised. Similar differences can be anticipated for other aspects, such as nuclear receptor ligand affinities and enzyme substrate specificities. Of course, we cannot go into the details of all marine species, but we may need to map some fundamental differences between major branches and groups.

Among the species mentioned so far, only the mussel, sea-star, oyster and crab are totally invertebrate models (39, 49). Compared to the vertebrate, concern and scientific studies of EDCs impacts using invertebrate have lagged behind. Conceptually, invertebrates should be another excellent model for marine ecotoxicology. First,

invertebrate animals are essential elements of the coastal ecosystems, necessitating protection from the harmful effects of contaminants (e.g. EDCs). Second, invertebrate EDCs assays may be useful in predicting or indicating potential EDCs responses in vertebrates, in part because some invertebrates can be more readily manipulated than vertebrates. In this capacity, invertebrate assays may serve either as sentinels of potential effects from exposure to conditions or chemicals, or as actual predictors of effects that have a counterpart in other species. In addition, advantages of selecting invertebrate systems include short generation times, easy to culture, and fewer legal/regulatory issues. Mussels as a sentinel organism has been well reported, and the "Mussel Watch Project" has also been conducted (37). Besides, to some extent, invertebrates can act as predictors for vertebrate systems. Luciferase and its gene comprise a notable example of invertebrate material that has been used with great success in various molecular and cellular assays. In addition, some invertebrates may contain genes or other biological components that are sufficiently similar to vertebrates to offer predictive power. Peter demonstrated that invertebrates also contain the DNA sequences (and thus, the putative genes) for vertebrate hormonal systems, even when the invertebrate gene is a homolog or an analog (37). Recently, we found *H. diversicolor supertexta* (Fig. 2) have good sensitivity to toxic chemicals. In addition, since EDCs can lead to chronic alterations of development and reproduction, the relatively short generation time of small laboratory animal such as abalone represents an important advantage of this species as experimental organisms. It could serve as a candidate for model species.

3. Biomarkers re-explored

Bio-monitoring is a very important method in environmental assessments. It has inherent advantages over simple chemical measuring the presence or concentration of a particular stressor or toxicant, due to the fact that organisms represent an integrated response to factors in their environment over time. Biomarkers are often regarded as a signal

Figure 2. The *Haliotis diversicolor supertexta* (abalone), a important indigenous marine species in China.

to respond to adverse toxicant effects. However, several authors imply that this definition has certain limitations, because adverse effects may be influenced by a number of factors, including habitat utilization in the environment, feeding behavior, metabolism, and physiology. Larsson indicates that when we find effects on the molecular level in marine organisms exposed to chemicals, or in organisms sampled in contaminated environment, we should interpret this as a warning flag, not as evidence of an adverse effect (29). Furthermore, Thomas also points out biomarkers should act as 'signposts' not 'traffic lights' (47). In our opinion, for general bio-monitoring, one may want to assess exposure and interpret positive results as a warning flag or signpost. However, if one wants to try to assess whether cleanup efforts within a given ecosystem were effective, one might want "biomarkers of exposure" and "biomarkers of effects. "Biomarkers of effects" are more robust than "biomarkers of exposure", especially with exposure to complex mixtures. Of course, we have to wait and see if 'markers of effect' or 'markers of exposure' derived from different systems biology approaches are more robust when we move out in the field. Maybe, there would be use for both.

Another issue worthy of note is whether there exist 'universal biomarkers'? Anders deems cytochrome P450 (CYP) response proves itself universal, in the sense that it shows up in almost all fish species studied, and responds well in field situations with AHR-type agonists present (1). Also in applying the new toxicogenomics and proteomics technologies, CYP is generally found as a high responding gene or protein. Similarly, vitellogenin (VTG) shows up in estrogen-type exposures in all fish species that have been studied; it responds well in the field, and it comes out on top of genomics and proteomics scores. With regard to this issue, one problem that can occur is with cross-species comparisons. If one thinks that a universal marker must respond to all types of environmental stress, it is our feeling that there really are none. For example, not all animals respond similarly with regard to CYP induction, and there are also notable differences in phase II enzyme induction across species (3, 56).

Accordingly, it is crucial to conduct validation studies for the end-point of interest across species. One fish might be highly inducible with regard to CYP or VTG expression but another may not be, and the need for careful validation studies across species. We believe that there are exposure situations, such as mixed exposures, where the best biomarkers are not yet to be found. Fortunately, the

"–omics" technologies are the tools that will help us to find them.

4. "–Omics" technique in ecotoxicology

We hear a lot about the advancement of biomedical sciences as they relate to new and emerging "–omics" technologies. Is this really going to be valuable for research targeted to the 'brook-side' rather than the human version 'bedside'? How is the development of "-omics" technologies going to advance our understanding of marine ecotoxicology? Benninghoff has reviewed the "–omics" technologies and indicated that "–omics" disciplines apply high-throughput methodologies in which change in expression of hundreds to thousands of genes, proteins, or metabolites are assessed simultaneously (3). We have an opportunity to ask open questions on what the effects of a given chemical may be, thus, the chances of discovering unexpected effects are greatly improved. The possibilities of this field, often referred to as ecotoxicogenomics / ecotoxicoproteomics, are great for the identification of specific and sensitive markers of exposure and adverse effects, understanding toxic mechanisms, directing testing to certain outcomes, identifying sensitive species, environmental monitoring, and more (2,39). The potential of genomics or proteomics techniques in marine environmental science and especially in environmental risk assessment is recognized by many researchers and official organizations. Compared with genomics or proteomics, application of metabolomics in marine ecotoxicology is particularly underutilized today. Metabolomics encompasses large-scale analyses of a variety of small endogenous molecules such as amino acids, sugars, energy carriers, and lipids. The best advantage of it is that metabolic changes reflect real changes in cell or organ function. Analyzed metabolites are well conserved between organisms, thus allowing fast identification regardless of species. For these reasons we expect metabolomics will receive more interest in the near future from ecotoxicologists, particularly those working with less explored fish species. In addition, to our knowledge, there are received relatively less literature within the field of ecotoxicology that have integrated the three areas of transcriptomics, proteomics, and metabolomics. These approaches supply complementary information, but the information is mechanistically related. In combination, the three may reduce noise by validating each other, which would increase the possibilities of identifying affected pathways and understanding the modes of action of chemicals. More important, integration of techniques could be used to answer some open questions such as whether there is a correlative relationship between degeneration of variety and exposure to EDCs.

5. Is there a causal association between EDCs exposure and variety deterioration of species?

EDCs are reactive chemicals that exhibit multiple mechanisms of toxicity acting at different sites within the body, including endocrine dysfunction, reproductive failure, developmental abnormalities, metabolic disturbances and immunosuppression. Figure 3 showed a hypotheses for explaining the multiple mechanisms of toxicity induced by EDCs. Endocrine system dysfunction, genetic material damage and immunity decrease may each contribute toward the overall toxic impact for population decline. They exhibited some portentous characteristics of species degeneration, including growth retardation, sexual precocity, individual sizes smaller as well as increasing the susceptibility of infections. Hence, perhaps we can say these phenotypic characteristics of aquatic species reflect genotype-environment interaction at a specified time or place. There exists a link between endocrine disruptors exposure and deterioration of species.

In general, when we talk about quality degeneration of one organism, the first thing that comes to mind is inbreeding. Breeding between close lineages is generally thought to be unfavorable from an evolutionary standpoint, because harmful mutations are easily propagated through populations in this way.
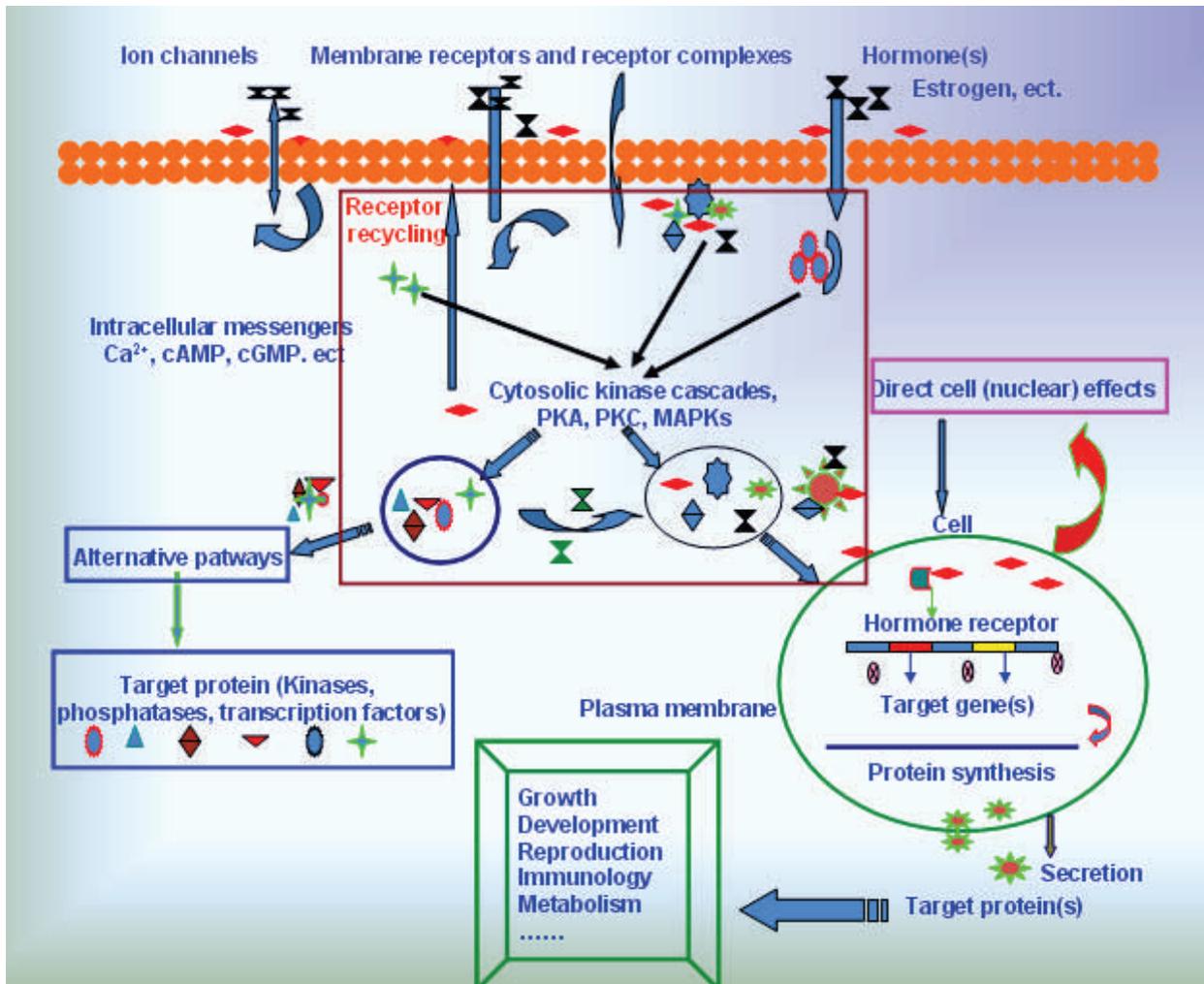
Figure 3. A hypotheses for explaining the multiple mechanisms of toxicity induced by EDCs.

Inbreeding degeneration has been observed in many higher plants and animals and its physical basis and its bearing on the genetic economy of these organisms have been widely discussed (15). Degeneration in species is not a single entity event but the result of complex interdependencies of genetic, physiological and environmental factors (21).

Presently, several references in the literatures reported that environmental contaminants are partly responsible for the variety deterioration except the inbreeding and genetic element (38, 51). In laboratory conditions, EDCs damage the gonadal tissues and germ cell quality. Veeramachaneni observed that exposure to certain chemicals could permanently alter sexual capacity in adult life, and proved the cause was environment factors rather than gene factors (51). EDCs stresses affect fish reproduction, gamete quality and progeny have also been reported by Schreck et al (43). The germ cell, gamete and embryo qualitative and quantitative characters were analyzed. In EDCs exposure condition, the fish has no more optimal growth to maturity, and produce no more gametes in optimal numbers, sizes and quality. Consequently, from an ecological as well as a genetic perspective, it will depress the genetic plasticity of the next generations. Additionally, behavior ecotoxicology has also been altered the result of EDCs exposure. Findings by Wong et al suggest that anthropogenic disturbance to the signaling environment can potentially reduce the evolutionary potential of sexual selection by diminishing the efficacy of visual displays and weakening socially-enforced signals of male quality (54). From an evolutionary

perspective, reducing the intensity of male-male competition is more difficult to transfer useful genes into descendants from parents than species in non-polluted area through competitive mating. In this case, it will threaten offspring quality (54). Thomas et al document that 17-ethinylestradiol reduces the competitive reproductive fitness of the male guppy fish by significantly decreasing sexual coloration, sperm amount and frequency of courtship behavior (48). Male competition for mating and sperm competition are important factors affecting next generation quality in many species, and the consequences of feminization or demasculinization may be more serious (13). If the competitive mating fitness decreases, the opportunity of excellent genes express will decline and the quality of offspring will be influenced. Besides laboratory or small area (scale) field experimental proofs, field studies have the potential to provide more direct evidence that aquatic organisms were impacted by EDCs in the marine environment. Reeder et al analyzed the proportion of intersex gonads in cricket frogs (*Acris crepitans*) using museum deposited samples. Their results showed that the percentage of intersex gonads increased during the period of industrial growth and initial uses of polychlorinated biphenyls (PCBs) (1930-1945). This was highest during the greatest manufacture and use of p,p-dichlorodiphenyltrichloroethane (DDT) and PCBs (1946-1959) and began declining with the increase in public concern and environmental regulations in the United States (1960-1979) (41). The research hinted a stronger linker between intersex and EDCs pollution. Similar field investigations were reported in heavily contamination area worldwide. In Japan, 15% of the wild male flounder (*Pleuronectes yokohamae*) contained scattered oocytes in Tokyo Bay which receives a large amount of industrial and domestic sewage effluent (35). Van et al reported intersex condition in the cyprinid gudgeon (*Gobio gobio*) from several British rivers and lakes (50). To our knowledge, adverse effects of this intersex on reproductive success have never been reported. Sole et al found the gonad malformation in wild carp collected from a section of the Anoia River (in

Spain), known to be polluted by estrogenic compounds, and high level vitellogenin (VTG) can be detected in male carp (44). In China, Fang confirmed that in East-Sea of China, dramatic population decline and germplasm resources collapse have occurred over the last 30 years because EDCs effects. Some of the precious fish germplasm are depleting or gradually declining day by day (14).

In recent years, some new evidences at molecular level concerning the case has also been found. Researchers have found that EDCs could disturb gametogenesis of frog (7) and damage the DNA of germ cell of scallop (32). Josephine et al has confirmed that organotin induced the gene-toxicity and DNA rupture in dog-whelk (27). Jha et al has reported that TBTO (tributyltin oxide) exhibit cytotoxic and genotoxic effects (sister chromatid exchanges and chromosomal aberrations) to marine species, and this damage could have adverse effects on the exposed population and eventually to the ecosystem (24). Was & Wenne have also reported that environment deterioration can change the genetic structure and genetic polymorphism of trout populations by cytogenetic and allozymic analysis (53). Microsatellite DNA analysis confirmed that the number of alleles is lower in the hatchery populations in comparison with wild populations (52). It can affect the gene flow from parent to offspring and probably affect the progeny quality. In higher mammal, moreover, Matthew et al have reported that the endocrine disruptor altered the DNA methylation patterns in the germ line. They deem the environmental factor (for example, endocrine disruptor) to reprogram the germ line state has significant implications for evolutionary biology (31). Besides, there is evidence that certain EDCs interact with the placental function. In the mink, polychloriuated bipbenyls (PCBs) have been shown to cross the placental barrier and cause fetal death in a dose dependant manner. Exposure to PCB during gestation modified the uterine progesterone and estrogen-receptor content, altered the placental glycan expression, which is an early marker for pathological changes, affected maternal vasculature and produced degenerative changes in the

trophoblast and fetal vessels leading to impeded development or fetal death (25). These data showed that exposure to EDCs can affect reproductive function at molecular level, and to some extent, the genetic material and genotypic characterization.

Another important thing deserved noticing is using multigenerational protocol to trace EDCs effects, which can help us to understand better the cause-effect relationship between EDCs exposure and variety degeneration. The adverse effort of EDCs can be directly transferred from parents to their progeny or by gene and molecular propagated. For example, exposure to organochlorines such as DDT, caused embryo mortality, edema and deformities including egg shell thinning in water birds breeding in the Great Lakes region or North America (53). Effect of EDCs on these maternal/paternal behaviors could also affect the offspring's ability to cope with stress in future. Several reports have confirmed that EDCs have effects not only on F_1 generation, but also on F_n (n≥2) generation in medaka (36), perch (5) and zebrafish (34). Additionally, Raimondo et al demonstrate that continued exposure to E2 not only affect population growth rate of sheepshead minnow, but also alter their population structure and variability (40). It resulted in increasing the population risk of extinction and potentially decreasing its recovery time. These results indicated that EDCs exposure could results directly in gene-toxicity and probably, indirectly affects the genetic pool of the generations. However, linking EDCs and variety deterioration with multigeneration relevant impact on the organisms, with few expections, an open challenge. Multigeneration toxicity test for wildlife is still urgent if we want to elucidate the precise cause of this. In addition, presently, most of the understanding about variety deterioration remain at phenotypic level. In order to obtain more genotypic data and prove it in a real world, a thorough evaluation and a more extensive study are needed.

6. Outlook

In order to protect marine animals and biodiversity, the biggest challenge in the future will be in elucidating toxicological mechanisms and setting up screens, including high-throughput screens, testing environmental and endocrine disrupting chemicals. For mechanistic studies, we believe the combination between large-scale molecular screening methods and systems biology approach possibilities will make the invertebrate an increasingly important model. For detection methods, it is important to development new biomarkers when we move out to the complex field environment. So, we would like to highlight that molecular information and –omic tools are highly important to make a monitor attractive to environmental researchers, but the availability is not an absolute prerequisite. Another important issue is how to understand the relationship better between EDCs exposure and degeneration of species, as well as the eventually bioresource decrease.

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