Current status of environmental endocrine disruption in selected aquatic invertebrates

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Abstract Endocrine disruption by environmental contaminants has recently emerged as a new subdiscipline of environmental toxicology. Aquatic environments are increasingly contaminated with various anthropogenic chemicals, many of which are capable of disrupting endocrine functions of vertebrates. Where aquatic invertebrates are concerned, most of the recent reports are concerned with the impact of organic xenobiotics on endocrine functions in cnidarians, rotifers, mollusks, crustaceans and echinoderms. This review provides an overview of the current status of this research on endocrine disruption in these invertebrates. Suggestions for future research directions in the field of invertebrate endocrine disruption are also presented. Because of the disparities between the endocrine systems of invertebrates and vertebrates, care must be exercised in extrapolating to invertebrates, particularly the non-deuterostome invertebrates, the results of endocrine disruption studies with vertebrates. It is also inappropriate to suggest that an endocrine disrupting effect in a non-deuterostome invertebrate can be used as a marker to monitor environmental endocrine disruption in vertebrates [Acta Zoológica Sinica 49 (5): 551-565, 2003].

Key words Endocrine disruption, Environmental hormones, Cnidarians, Rotifers, Mollusks, Crustaceans, Echinoderms.

1 Introduction

Environmental endocrine disruption, a new subdiscipline of environmental toxicology, has recently emerged as a result of numerous reports on the adverse effects of various environmental chemicals, including some pesticides, pharmaceuticals and industrial chemicals, on hormonally mediated functions in various groups of vertebrates. These include feminization of male fish (Gmeno et al., 1996; Jobling et al., 1996), demasculinization of male frogs (Hayes et al., 2002), sexual abnormalities of male alligators (Guillette et al., 1994, 1996), reproductive impairment in panthers (Facemire et al., 1995), and alleged low sperm count in men (Sharpe et al., 1993).

Disruption of endocrine functions regulated by estrogen and androgen in vertebrates by environmental
contaminants is the original focus of this new field. Several mechanisms for disruption of estrogen and androgen responses have been reported thus far. Some chemicals such as bisphenol A (Nagel et al., 1997), diethylstilbestrol (DES) (Teng, 1995), and nonylphenole (Tollefsen et al., 2002; Kwack et al., 2002) produce their estrogenic effects by binding to, thereby activating the estrogen receptor (ER). Other chemicals can activate the ER without directly interacting with the ER. Hatakeyama et al. (2002) reported that β-hexachlorocyclohexane (β-HCH), an organochlorine with no affinity to the ER, is capable of activating ER via a c-ErbB2 mediated, ligand-independent pathway. A further study has shown that several organochlorinated compounds appear to exert their estrogenic effects mainly through such a mechanism in MCF7 human breast cancer cells (Zou et al., 2002). Hayes et al. (2002) found that atrazine, a widely used herbicide, can feminize the male frogs by, presumably, inducing aromatase. These aforementioned chemicals whose estrogenic effects are ultimately mediated by the ER in vertebrates have been called environmental estrogens. Environmental antiandrogens have also been reported. The fungicide vinclozolin and the DDT metabolite p,p′-DDE (Kelce et al., 1995; Vinggaard et al., 1999) can bind to but block the androgen receptor in vertebrates. Interestingly, no synthetic organic contaminants are found to act as the agonists of the androgen receptor.

Disruption of thyroid functions by environmental chemicals is also an important area of environmental endocrine disruption. Hypothyroidism, an abnormality of thyroid function, arises from the deficiency in thyroid hormone. Environmental chemicals such as polychlorinated biphenyls (PCBs), TCDD and methoxychlor have been linked to the occurrence of hypothyroidism (Crisp et al., 1998). PCBs can also alter the expression of thyroid hormone-responsive genes in the developing brain (Zoeller et al., 2002). Thyroid disruption appears to be mediated by mechanisms other than the direct interactions between chemical agents and thyroid receptors (TRs). Cheek et al. (1999) found that alterations in thyroid hormones can be caused by disruption of thyroid hormone transport. No environmental chemicals were found to be able to bind to the TR with an appreciable affinity, but hydroxylated polychlorinated biphenyls (PCBs) have a strong affinity to the serum transport protein transthyretin, capable of displacing the natural thyroid hormone from the transthyretin (Cheek et al., 1999). There is also evidence that PCBs (van Birgelen et al., 1995) and alachlor (Wilson et al., 1996) can decrease thyroid hormone concentrations in the serum by promoting thyroid hormone metabolism.

Invertebrates constitute over 95% of known animal species. Because of the ecological importance of these animals in ecosystems and the commercial value of many invertebrate species, attention is increasingly being paid to the disrupting effects of environmental chemicals on endocrine functions, such as growth, sexual development and reproduction, of various groups of invertebrates, particularly the aquatic invertebrates. This review presents the current status of research on environmental endocrine disruption in selected aquatic invertebrates, including cnidarians, rotifers, mollusks, crustaceans and echinoderms. Perspectives for future directions of research on invertebrate endocrine disruption are also given.

2 Cnidarians

2.1 Endocrinology

Cnidarians represent the earliest invertebrates that possess a nervous system. The cells that make up such a primitive nervous system have combined properties of sensory, motor and interneurons (Leshr-Laurie, 1988). Very little is known about cnidarian endocrinology. Although cnidarians lack a defined endocrine gland, certain cells in the nervous system are known to produce neuropeptides, which are implicated in the regulation of metamorphosis (Leitz et al., 1994) and peristaltic contractions (Anctil, 2000). Cnidarians often have a life cycle consisting of planula larva, polyp and medusa stages. A neuropeptide, pGlu-Gln-Pro-Gly-Leu-Trp-NH₂, was found to induce metamorphosis of the marine hydroid (Hydractinia echinata) from a hydroid planula larva to a polyp (Leitz et al., 1994). Gonadotropin-releasing hormone (GnRH)-like peptides have recently been detected in endodermal neurons of the sea pansy (Renilla koellikeri), and the sea anemone (Nematostella vectensis) (Anctil, 2000). The GnRH-like factors were found to inhibit the amplitude and frequencies of peristaltic contractions in Renilla koellikeri, suggesting that these neuropeptides may play a role in the modulation of neuromuscular transmission in the sea pansy.

The vertebrate steroids, estrone and 17β-estradiol (E₂), were detected in the tissue of the scleractinian coral (Montipora verrucosa) (Tarrant et al., 1999). Tissue concentrations of estrone and E₂ in Montipora verrucosa were found to vary during the year, with peaks for estrone occurring in April, a time of rapid gamete growth, and in early July, prior to spawning, while E₂ peaks preceded peaks for estrone. Based on this pattern of changes in estrogen levels, Tarrant et al. (1999) proposed that estrogens may play a role in regulation of coral gametogenesis and spawning. Whether these steroids are synthesized
in the coral, derived from dietary sources or sequestered from the overlying water is unclear. There is evidence that Montipora verrucosa is capable of absorbing estrone from the water column rather efficiently (Tarrant et al., 2001).

2.2 Disruption of sexual development, regeneration and symbiosis

The information on the impact of environmental contaminants on endocrine functions of cnidarians is scarce. Pascoe et al. (2002) found that exposure of the freshwater cnidian (Hydra vulgaris) to the synthetic estrogen 17β-ethinylestradiol at 0.5 mg/L for 6 weeks resulted in a significant reduction in both the number of testes and the time for which the sperm remained active. 17β-ethinylestradiol at 0.5 mg/L was found to reduce the number of oocytes and the time the oocyte was attached to the polyp. Besides, these investigators found, in a 72-hr assay, that 17β-ethinylestradiol at 0.15 - 2.6 mg/L and bisphenol A at 1.0 - 4.6 mg/L resulted in tentacle damage and inhibition of regeneration of Hydra vulgaris. The structure and physiology of polyps were also adversely affected at concentrations greater than 58 μg/L. 17β-ethinylestradiol and 42 μg/L bisphenol A following a 72-hr exposure.

Mercier et al. (1997) found that exposure of the sea anemone (Aiptasia pallida) to the antifoul agent tributyltin (TBT) can not only disrupt the symbiosis through expulsion of the symbiosis partner zooxanthellae but also produce other effects, such as increased mucus secretion, thickening of the pedal disc ectoderm and a decrease in the number of undischarged nematocysts. It is not known whether this disruption of symbiosis in Aiptasia pallida by TBT is due to the interference with the signaling between the host and its symbiotic partner.

3 Rotifers

3.1 Endocrinology

Rotifers are a group of free-living, planktonic pseudocoelomates characterized by possessing a wheel of cilia called a corona at the anterior end. There is an alternation of parthenogenic and sexual reproduction in the life cycle of rotifers. At the beginning of the growing season, diploid parthenogenic females hatch out from the resting eggs of the previous season. Parthenogenic females reproduce unisexually by laying diploid eggs, which develop into females. Upon receiving appropriate environmental cues, rotifers can switch the mode of reproduction from parthenogenic reproduction to sexual reproduction, in which diploid sexual females are produced. Diploid sexual females then produce haploid eggs through meiosis, which develop into haploid males or resting eggs if fertilized by males (Preston et al., 2000).

Information on the endocrinology of rotifer reproduction is scarce. Gallardo et al. (1997) found that exposure of the marine rotifer (Brachionus plicatilis) to several vertebrate and invertebrate hormones can result in changes in reproduction. Gamma-aminobutyric acid (GABA) at 50 mg/L, growth hormone (GH) at 0.0025 and 0.025 IU/mL, human chorionic gonadotropin (HCG) at 0.25 and 2.5 IU/mL, and 5-hydroxytryptamine (5-HT) at 5 mg/L significantly increased population growth, whereas E2 at 50 mg/L caused a decrease in population growth. An increase or decrease in population growth of the rotifer (Brachionus plicatilis) treated with an exogenous hormone is obviously mediated by stimulatory or inhibitory actions of such an agent on parthenogenic reproduction. JH at 0.05 and 0.5 mg/L, 5-HT at 0.05 and 5 mg/L, GH at 0.0025 and 0.025 IU/mL, E2 at 50 mg/L, GABA at 0.5, 5 and 50 mg/L, and 20-hydroxyecdysone at 0.05 mg/L were also found to significantly stimulate the production of sexual females, whereas T3 and HCG had no effect on the production of sexual females. A further study by Gallardo et al. (2000a) has shown that treatment of maternal rotifers with JH at 5 and 50 mg/L significantly enhanced production of sexual females in the second and third generations, but had no effect on the production of sexual females in the first generation. The effect of 5-HT on sexual female production depends on food concentrations. A significant stimulatory effect of 5-HT on sexual female production was only manifested at a low food concentration of 7 × 10^5 Nannochloropsis oculata cells/mL. Of these hormonal agents, GABA and 5-HT have been detected in rotifers (Gallardo et al., 2000b), suggesting these two factors may be involved in the regulation of cyclic reproduction of rotifers.

A sex pheromone has also been identified in the marine rotifer (Brachionus plicatilis) (Snell et al., 1995). A glycoprotein, 29 kDa in molecular weight, bound to the body surface of the female rotifer, acts as a contact-mating pheromone. Recognition of this glycoprotein by the chemosensory receptors in the male corona can initiate mating behavior.

3.2 Disruption of reproduction

Several investigations have been undertaken to investigate the impacts of environmental contaminants on the reproduction of rotifers. Snell et al. (1995) found that the sexual reproduction of the rotifer (Brachionus calyciflorus), is more sensitive to pollutants than the parthenogenic reproduction. Pentachlorophenate (PCP) at 0.2 mg/L and chlorpyrifos at 0.3 mg/L did not affect parthenogenic reproduction of Brachionus calyciflorus, but significantly re-
duced sexual reproduction. This reduction in sexual reproduction was due to the inhibition of sexual female production. Chlorpyrifos also inhibited the production of males by sexual females. It is not known whether these effects arise from the disruption of hormonally regulated processes.

Preston et al. (2000), in their attempt to use the freshwater rotifer (Brachionus calyciflorus) as a screen for endocrine disruptors, found that flutamide (androgen antagonist) at 1 μg/L, testosterone at 10 μg/L and nonylphenol at 50 μg/L significantly inhibited fertilization of sexual females by decreasing the percentage of fertilized sexual females. Besides, testosterone at 10 μg/L was found to reduce the proportion of unfertilized sexual females, while no other chemicals tested, including cadmium, methoprene (JH agonist), precocene (JH antagonist), flutamide, and nonylphenol, affected the percentage of unfertilized females. Additionally, none of the test chemicals was found to affect the proportion of parthenogenetic females. Since fertilization and resting egg production in rotifers are likely to be regulated by endocrine mechanisms, these investigators proposed that rotifer reproduction assays may be an effective assay for the detection of endocrine disrupting activities in aquatic invertebrates.

In an attempt to obtain suitable endpoints for the screening of environmental endocrine disruptors, Radix et al. (2002) investigated the effects of endocrine disruptors and steroid hormones on reproduction and the ratio of sexual/parthenogenetic females of the rotifer (Brachionus calyciflorus). Nonylphenol at concentrations higher than 0.59 μmol/L, ethinylestradiol at concentrations higher than 1.72 μmol/L and testosterone at concentrations higher than 8.88 μmol/L were found to significantly decrease the intrinsic rate of population increase of Brachionus calyciflorus, which was used as an indicator of reproduction, in a dose-response manner. Ethinylestradiol treatment also led to a decrease in the ratio of sexual/parthenogenetic females, while nonylphenol enhanced the ratio of sexual/parthenogenetic females. Because of a clear dose-response relationship, Radix et al. (2002) concluded that the intrinsic rate of population increase is a suitable endpoint for the screening of endocrine disruptors. It is not clarified, however, by these investigators that whether this endpoint is specifically for the screening of endocrine disruption in rotifers or for the detection of endocrine disrupting activities in aquatic invertebrates in general.

4 Mollusks

4.1 Endocrinology

Mollusca consists of diverse groups of animals, including gastropods, bivalves, cephalopods, and oly- placophores. Because of the differences in morphologies and life history of various groups of mollusks, it is not unexpected that great variations in endocrine systems exist in these discrete mollusk groups. Since gastropods have received most attention in studies of environmental endocrine disruption, only a concise review of gastropod endocrinology is presented here.

Neuropeptides play an important role in the regulation of growth and reproduction in gastropods. The egg-laying hormone (ELH), a neuropeptide secreted from a bag cell cluster in the abdominal ganglion, regulates gonad maturation and ovulation in Aplysia, while the caudodorsal cell hormone (CDCH), produced from the cerebral ganglion, controls the development of female accessory sex organs, gonad maturation and ovulation in Lymanea (Geraerts et al., 1988). The penis morphogenic factor (PMF), produced from the pedal ganglion, induces differentiation of the penis while a lysis neurohormone from the pedal ganglion controls the regression of the penis after the mating season is over (Joosse, 1988). The chemical nature of the PMF has yet to be determined. APGWamide, a neuropeptide produced in the pedal ganglion, appears to be a good candidate of the PMF. APGWamide is known to regulate male sexual activities in Lymanea stagnalis (Li et al., 1992) and treatment with APGWamide can induce the development of the penis in the mud snail (Illyanassa obsoleta) (Oberdörster et al., 2000).

Although gastropods possess enzymatic systems for metabolism of the vertebrate steroid hormone testosterone (Oberdörster et al., 1998b; Goding et al., 2001), hormonal roles of steroids in these animals have yet to be established. Administration of the exogenous testosterone has been found to induce development of male sex organs in gastropods (Spooner et al., 1991; Bettin et al., 1996), but steroid receptors, which mediate the actions of steroid hormones, have not been discovered in mollusks.

4.2 Disruption of sexual development and reproduction

The best example of environmental endocrine disruption in invertebrates is the imposex of neogastropods. Neogastropods, a group of prosobranchs, are normally gonochoristic. Imposex is a type of sexual abnormality where male sex organs, such as the penis and vas deferens, develop on the female neogastropods as a result of exposure to the low levels (parts per trillion) of the antifouling agent tributyltin (TBT) (Matthiessen et al., 1998). Since the male organs are superimposed upon the female, this kind of sexual anomaly has been termed the imposex. Imposex phenomenon was first reported by Blaber (1970) in the snail (Nucella lapillus). Now, this form of sexual anomaly has been described in over
100 neogastropod species from locations worldwide (Kiran et al., 1999; Barroso et al., 2000; Shim et al., 2000; Takeda, 2000; Evans et al., 2001a; Evans et al., 2001b; Ramôa et al., 2001; Hung et al., 2001; Bech, 2002).

In some species, such as Ilyanassa obsoleta and Nassarius reticulatus, the development of male sex organs does not appear to cause reproductive problems to the affected female, whereas in other species, such as Nassarius lapillus and Ocenebra erinacea, imposition of male sex organs can cause sterility of the female snail (Matthiessen et al., 1998). The sterility of the affected females is caused by the blockage of the genital pore by convolutions of the vas deferens (Evans et al., 2001a). Occurrences of imposex have been linked to extinction of gastropods in some areas of high shipping and boating activities (Bryan et al., 1986; Evans et al., 1996).

The masculinization of female snails after exposure to TBT appears to be mediated by an elevation in testosterone titer in the snail. Exposure of the female Nuclella lapillus to TBT at an environmentally realistic concentration 98 ng/L resulted in the occurrence of a penis and an elevation in testosterone levels (Spoonier et al., 1991). Bettin et al. (1996) found that treatment of female Nuclella lapillus and Hinia reticulata with TBT at 5, 50 and 100 ng tin/L or testosterone at 500 ng/L induced imposex development and that a marked increase in endogenous testosterone level was exhibited by the females of both species treated with TBT. Moreover, both cyproterone acetate, an antiandrogen, and estrogen were found to be capable of suppressing the imposex-inducing effects of TBT in both snail species. It has been suggested that an increase in testosterone level in the female snail after exposure to TBT may be caused by inhibition of cytochrome P450-dependent aromatase-mediated metabolism of testosterone to E2 (Bettin et al., 1996). Oberdörster et al. (2002) reported the inhibition of aromatase activity in a gastropod by TBT in a gastropod. The inhibitory effects of TBT on aromatase activity have also been observed in human placental tissues (Heidrich et al., 2001) as well as in the human granulose-like tumor cell line KGN (Saitoh et al., 2001). The possibility that an elevation in testosterone level after TBT exposure may result from inhibition of testosterone excretion has also been suggested. Ronis et al. (1996) found that exposure of the periwinkle (Littorina littorea) injected with [14C]testosterone to 54 mol/L TBT can significantly decrease the concentrations of sulfur-conjugated testosterone in the exposure media, suggesting that inhibition of testosterone excretion through suppressing sulfur conjugation may be the alternative mechanism for TBT-induced elevation of testosterone in gastropods.

Whether an increase in testosterone titers relative to estrogen is the direct cause for the development of imposex in female gastropods is still controversial. Oberdörster et al. (2000) argued that increased testosterone titers were not the cause of imposex induction because of the fact that TBT and testosterone induced the penis and vas deferens in as little as two weeks, with maximum induction occurring after one month, and that testosterone titers did not elevate until two months after TBT exposure according to the results of Bettin et al. (1996). Oberdörster et al. (2000) found that injection of the neuropeptide APG-Wamide at less than 10^{-11} moles into the female mud snail (Ilyanassa obsoleta) induced imposex development. These investigators proposed that APG-Wamide, a likely candidate for the PMF, mediates the development of the penis and vas deferens in the female snail, therefore a more profound factor for imposex development. It is hypothesized that TBT can cause abnormal release of APGWamide from neurosecretory cells, triggering the development of the male sex organs, which then release androgens, possibly testosterone, to maintain the male sex organs and spermatogenesis (Oberdörster et al., 2000). Whether the actions of testosterone in gastropods are mediated by the receptor, as in the case with vertebrate systems, is still unknown. To my knowledge, no such a receptor has been discovered yet in mollusks.

Neogastropods are not the only mollusks adversely impacted by TBT. Horiguchi et al. (2000) reported the female giant abalone (Haliotis mida) inhabiting TBT-contaminated environment were masculinized with an ovotestis. The ovaries of the affected females were found to have a small amount of testicular tissue, where spermatogenesis or seminiferous tubule-like structure was observed.

Environmental contaminants other than TBT have also been found to cause sexual abnormalities in prosobranch snails. Oehlmann et al. (2000) found that treatment of the freshwater snail (Marisa cornearietis) with environmental estrogens bisphenol A and octylphenol can induce a type of sexual abnormality called the superfemales, where the affected females are characterized by the formation of additional female organs, e.g., a second vagina with vaginal opening to the mantle cavity and/or an enlargement of the pallial accessory sex glands. Oviduct malformation and an enhancement of spawning mass production were also observed in the superfemales. Similar sexual abnormality was also observed in the marine snail (Nuclella lapillus) treated with bisphenol A or octylphenol, where enlargement of accessory pallial sex glands and increased oocyte production occurred,
but no oviduct malformation was found. It is not known whether it is the estrogenicity of these xenobiotics that causes the superfemale phenomenon. An experiment utilizing E2 and other estrogenic agents as test chemicals may provide an answer to this question.

5 5 Crustaceans
5.1 5 Endocrinology

Crustaceans are one of the larger animal groups. Since most studies on crustacean endocrine disruption deal with sexual development, reproduction and molting, only the endocrinology for these processes is presented in this review.

5.1.1 Sexual development and reproduction 5 Much of the knowledge on endocrine control of sexual development and reproduction has been obtained from malacostracan crustaceans because of their large body sizes. There is evidence suggesting the existence of a genetic sex-determining mechanism in malacostracans, with the male possessing a heteromorphic sex chromosome pair and the female only having homomorphic chromosomes (Vainola, 1998; Barzotti et al., 2000). In malacostracan crustaceans, development of male secondary sexual characteristics is solely controlled by the androgenic gland hormone (Charniaux-Cotton, 1954), which is a protein produced in the androgenic gland (Hasegawa et al., 1993). The activity of the androgenic gland is regulated by the gonad-inhibiting hormone (GIH) produced in the X-organ-sinus gland complex and the gonad-stimulating hormone (GSH) produced in the brain and the thoracic ganglia, both of which are peptides (Fingerman, 1997). Therudiments develop further (Charniaux-Cotton et al., 1985). The development of female secondary sexual characteristics, such as the ovigerous setae and the brood pouch, is controlled by the ovarian hormone produced by the ovary (Charniaux-Cotton, 1952; Nagamine et al., 1987). The chemical nature of this hormone is still unknown.

Both GIH and GSH regulate the gonadal maturation. In females GIH and GSH act directly on the ovary, which then secrete the ovarian hormone, while in males GIH and GSH act on the androgenic gland (Fingerman, 1997). As mentioned above, the androgenic gland hormone not only directly controls the secondary characteristics of males but controls development and maintenance of the male reproductive system as well (Charniaux-Cotton, 1954). Additionally, there is evidence that methyl farnesoate, the unepoxidated form of insect juvenile hormone III (JH III) produced by the mandibular organ, enhances ovarian maturation (Lauber et al., 2001).

5.1.2 Molting 5 For growth to occur, crustaceans must periodically molt their rigid, confining exoskeleton. Molting in crustaceans is regulated by a multi-hormonal system, but is under immediate control of the steroid hormones called ecdysteroids (Chang et al., 1993). In decapods, ecdysteroids are produced in the Y-organs whose activity is held in abeyance during the intermolt stage by the molt-inhibiting hormone (MIH), which is a peptide, from the X-organ-sinus gland complex. When the animal enters premolt stage, this inhibition of Yorgan activity by the MIH is lifted and ecdysteroidogenesis in the Y-organs intensifies. The ecdysteroid titer in the hemolymph is therefore elevated. Ecdysteroids regulate gene activities at the transcriptional level by interacting with the EcR, which then heterodimerize with crustacean retinoid X receptor (RXR) (Durica et al., 1996; Chung et al., 1998). This EcR/crustacean RXR dimer binds to the DNA response elements of target genes.

5.2 Endocrine disruption
5.2.1 Disruption of sexual development and reproduction 5 Endocrine disruption of crustacean sexual development and reproduction in various crustacean groups has been extensively reviewed by Zou et al. (2003). Concerns were expressed over the possibility that the same chemicals capable of disturbing male development of vertebrates can also interfere with male differentiation of cladocerans. The proportions of male Daphnia in Lake Mendota, Wisconsin, have declined rather drastically over several decades (Dodson et al., 1995). These investigators hypothesized that the decrease was caused by some of the same estrogenic agents capable of disturbing male development of vertebrates. Such historical data and the hypothesis have generated several investigations to examine whether estrogenic agents can interfere with male differentiation in Daphnia. So far, the results are inconsistent, possibly because of differences in Daphnia species and experimental schemes used by different investigators.

Zou et al. (1997a) continuously exposed the neonate Daphnia magna (age < 12 hr) to the synthetic estrogen DES at up to 0.20 mg/L and the estrogenic pesticide endosulfan at up to 0.15 mg/L for 40 days under male-inducing conditions, i.e., short day length, crowding and food limitation. Neither of these estrogenic agents was found to significantly alter male production of Daphnia magna. Endosulfan was also found to have no effects on male production of Daphnia galeata (Dodson et al., 1999b). Baer et al. (1999) found that food concentration plays a role in a chemical's effect on male differentiation of
Daphnia magna. At a food concentration of 150 000 cells/ mL, none of the three endocrine disruptors tested, DDT, methoxychlor and 4-nonylphenol, significantly affected male differentiation of Daphnia magna. At low food concentration of 50 000 cells/ mL, E₂, TBT, DDT and methoxychlor had no significant effects on male differentiation, but 4-nonylphenol significantly promoted male production. In a 21-day reproduction test. Caspers (1998) found that the environmental estrogen bisphenol A at 3.16 mg/L did not significantly affect the reproduction rate (number of offspring per parent) of Daphnia magna.

The absence of consistency concerning the effects of various estrogenic chemicals on sex differentiation of Daphnia, especially the lack of response to the natural estrogen E₂ (Baer et al., 1999) and the potent synthetic estrogen DES (Zou et al., 1997a), strongly suggests that the strength of a chemical’s estrogenicity is not a significant determining factor for the shift of sex determination in Daphnia. The decline in proportions of male Daphnia in Lake Mendota, Wisconsin, over several decades (Dodson et al., 1995) does not appear to result from the estrogenicity of environmental chemicals in the lake. More research attention should be directed towards elucidation of the endocrinology behind sex determination in cladocerans.

Abnormal sexual characteristics of copepods have also attracted much attention. Harpacticoid copepods normally have separate sexes (Du, 1987). However, intersexuality, an occurrence of an individual carrying sexual characteristics of both sexes, has been recorded in benthic harpacticoids in the vicinity of a large sewage discharge near Edinburgh in the United Kingdom (Moore et al., 1991). In order to examine whether there exists a link between the occurrence of intersexuality and pollution, Moore et al. (1994) surveyed the seabed off the Edinburgh coast to examine the distribution of harpacticoid intersexuality in relation to sewage discharge. These investigators found no evidence for a correlation between intersex frequency and proximity to the sewage outfall, intersexuality in copepods being not restricted to polluted areas. This ecological finding was supported by the results of two independent laboratory studies, Hutchinson et al. (1999) continuously exposed nauplii of the copepod (Tisbe battaglaii) (age < 24 hr) to E₂, estrone, and 17α-ethynylestradiol at 0.1 - 100 μg/L and 20α-hydroxyecdysone at 8.7 - 269 μg/L for 21 days and found that none of these chemicals significantly changed the female/male sex ratio. Exposure to 20α-hydroxyecdysone significantly reduced the fecundity (total number of nauplii per female) of Tisbe battaglaii, but E₂, estrone, and 17α-ethynylestradiol had not effects on copepod fecundity, suggesting the reproduction of Tisbe battaglaii is only responsive to the ecdysteroid (Hutchinson et al., 1999). Using the estrogenic compound nonylphenol as the test chemical, Bechmann (1999) found no significant differences in the percentages of female Tisbe battaglaii in either the P or the F1 generation between the 31 μg/L nonylphenol exposure and the controls nor significant effects on any life table parameter for Tisbe battaglaii at 31μg/L nonylphenol. Breitholtz et al. (2001) found that exposure to DES at 0.3 - 30μg/mL, 17α-ethynylestradiol at 0.5 - 50 μg/mL, and 17β-estradiol at 0.5 - 50 μg/mL for 18 days had no significant effect on the fecundity (nauplii/female) or the sex ratio of the copepod (Nitocra spinipes). The absence of any alterations of the copepod sex ratio and reproduction to high doses of estrogenic chemicals strongly suggests that the endocrine control for sexual development in copepods is different from that for vertebrates.

Malacostracans are mostly gonochoristic, but intersexuality is common among certain parasitic isopods (Du, 1987), caridean shrimps (Yaldwyn, 1966; Bauer, 1986), and anomurans such as Emerita analoga (Barnes et al., 1968) and Emerita emerita (Subramaniam, 1981). Among these malacostracan crustaceans, two types of hermaphroditism have been recognized. One is sequential protandrous hermaphroditism in which individuals first mature as sperm-producing males, and then with increasing size, switch sex to become breeding females (Bauer, 1986) and the other is called simultaneous hermaphroditism where an intersex individual can function as either a male or a female at any given time (Bauer et al., 1998).

Neither of these two types of hermaphroditism has been found in brachyurans. However, several forms of sexual abnormalities have been reported for brachyuran decapods (Hartnoll, 1960; Farmer, 1972; Johnson et al., 1981; Manning et al., 1981). The fiddler crab (Uca pugilator) exhibits striking sexual dimorphism, with males characterized by a pair of asymmetrical chelipeds and a narrow abdomen and females having two small, identical claws and a broad abdomen that covers the entire sternum. A sexually abnormal Uca pugilator with a female-type abdomen and a pair of asymmetrical male-type chelipeds was reported by Deecaraman et al. (1992). More recently, Zou et al. (2000) found a sexually aberrant Uca pugilator out of 1 000 individuals. This specimen exhibited reduced asymmetry in its chelipeds. Its abdomen was intermediate between those of a typical male and a typical female and carried the appendages of both sexes. It is still unknown whether these sexual abnormalities are related to en
environmental pollution.

A field study showed that the amphipod (*Gammarus pulex*) collected from an unpolluted reference site had a significantly higher male/female ratio than did those living below sewage treatment works (STW) (*Gross et al.*, 2001). The male/female size differential was significantly reduced in *Gammarus pulex* collected from one of the polluted sites. Additionally, the allometric relationships of the male secondary sexual characteristics, gnathopod and genital papillae, to body size for *Gammarus pulex* living below STWs were different from those from the reference site. Moreover, abnormal oocytes were observed in *Gammarus pulex* collected from one of the polluted sites, while no significant difference was found in the gonadal structure of males from the two polluted sites. While the significant departure from the 1:1 sex ratio seen at the reference site was deemed biologically insignificant, *Gross et al.* (2001) interpreted the changes in male reproductive morphology as being caused by interference with androgenic hormone signaling by substances in the sewage effluents. These investigators reasoned that since ecdysone is required for full vitellogenesis, the low number and aberrant distribution of yolk bodies in the abnormal oocytes were likely induced by steroid mimics in the effluents capable of interacting with the ecdysteroid receptor.

A laboratory investigation (*Brown et al.*, 1999) demonstrated that continuous exposure of the amphipod (*Corophium volutator*) (age < 5 days) to nonylphenol at 10 - 200μg/L for 120 days did not significantly affect the sex ratio, but nonylphenol at 10 - 100μg/L significantly induced elongation of the second antenna, which is a male characteristic. Also, fertility, i.e., number of juveniles per female, was significantly enhanced by nonylphenol at 10 - 50μg/L. *Brown et al.* (1999) proposed that the stimulating effect of nonylphenol on the second antenna is likely due to nonylphenol triggering hypersecretion of the androgenic gland hormone that controls the development of male secondary sexual characteristics. The large second antenna would be a selective disadvantage to the males because the large antennae make the males more vulnerable to predation in the field, which could lead to a skewed sex ratio and the subsequent population decline.

The possibility that sexual and reproductive aberrations can be induced in malacostracans, particularly among decapods, by exposure to environmental pollution needs to be extensively investigated, considering the economic importance of many of the malacostracan species. Both field and laboratory studies should be done. The laboratory component would examine the alterations in physiology of hormonal cascades that regulate sexual development and reproduction.

**5.2.2** Disruption of molting Disruption of crustacean molting by environmental xenobiotics has also been reviewed by *Zou et al.* (2003). Adverse effects of environmental chemicals on growth and molting of cladocerans have recently been reported. Using the time needed for completing four molts as a parameter, *Zou et al.* (1997a) found that the estrogenic agents endosulfan at 0.10 and 0.15 mg/L and DES at 0.1 and 0.2 mg/L can significantly delay the molting of neonate *Daphnia magna*. A further study by these investigators (*Zou et al.*, 1997b) demonstrated that other vertebrate endocrine disruptors, such as Aroclor 1242, 2,4,5-trichlorobiphenyl (PCB29) and diethyl phthalate, can also significantly slow the molting of daphnids. Later, *Baer et al.* (1999) found that the insecticide methoxychlor at 1.0μg/L significantly inhibited the molting of *Daphnia magna*.

Without recording molt data, *Olmstead et al.* (2000) found in a 6-day experiment using newborn *Daphnia magna* (age < 24 hr) that the juvenile hormone mimic methoprene at 0.16 and 0.32μmol/L significantly inhibited growth of both male and female daphnids, while DES at 3μmol/L significantly reduced the body length of female daphnids but had no effect on the growth of male daphnids. Perhaps, the inhibitory effect of DES on daphnid growth is sex related.

Growth inhibition by xenobiotics can also occur in a molt-independent manner. *LeBlanc et al.* (1999) observed in a 21-day assay utilizing young *Daphnia magna* (age < 24 hr) that continuous exposure to the vertebrate antiandrogen cyproterone at concentrations ranging from 0.3 - 2.5μmol/L had no significant effect on molt frequency and cyproterone at 5.0μmol/L significantly decreased molt frequency. However, the body length of *Daphnia magna* was significantly reduced by cyproterone at concentrations as low as 1.2μmol/L. These results strongly suggest that inhibition of *Daphnia* growth can occur without molting being affected.

Not all vertebrate endocrine-active compounds are capable of affecting the molting of cladocerans at sublethal concentrations. *Zou et al.* (1997b) found that neither 4-octylphenol at 0.01 - 0.04 mg/L nor lindane at 0.05 - 0.2 mg/L significantly affected the molting of *Daphnia magna*. *Caspers* (1998) observed that bisphenol A at 0.316 and 3.16 mg/L did not significantly impact the molting of *Daphnia magna*. Furthermore, nonylphenol at 25μg/L, E2 at 270μg/L, TBT at 1.0μg/L or DDT at 1.0μg/L had no effect on the molting of *Daphnia magna* (*Baer et al.*, 1999).

In copepods, the development from a nauplius
larva to a copepodite includes several molts, with the last molt leading to metamorphosis (Du, 1987). These processes are regulated by ecdysteroids and, presumably, also by methyl farnesoate, the crustacean analog of insect juvenile hormone (Lauffer et al., 2001). Using the larval development of the copepod (Acartia tonsa) as an endpoint, Andersen et al. (2001) found that proctophylenol, tamoxifen, 1α-ethinylestradiol, flutamide, and juvenile hormone III are potent inhibitors of the nauplius development of Acartia tonsa while other endocrine-active chemicals, such as E2, estrone, bisphenol A, testosterone and 20-hydroxyecdysone, had little or no potency. The absence of an effect of 20-hydroxyecdysone may have been due to low permeability through the exoskeleton.

Adverse effects on growth and molting of malacostracan crustaceans by xenobiotics have also been reported. Fingerman et al. (1977) earlier found that the polychlorinated biphenyl formulation Aroclor 1242 can drastically inhibit the frequency of molting of the fiddler crab (Uca pugilator). Brown et al. (1999) reported that continuous exposure of the amphipod (Corophium volutator) to nonylphenol at concentrations from 10 - 200 μg/L for 100 days significantly reduced the body length relative to the untreated controls. However, this inhibitory effect on amphipod growth was not detectible with only 30 days of exposure. Since molting data were not collected, it is unknown whether this nonylphenol-induced growth inhibition was due to molt-inhibition or other mechanisms. Had these investigators terminated the experiment on the 30th day of exposure, they would not have seen growth inhibition in Corophium volutator by nonylphenol.

A stimulatory effect on molting by xenobiotics has been reported also. Clare et al. (1992) demonstrated that the nonsteroidal edysone mimic RH 5849 at 0.1 and 1.0 mg/L can accelerate larval molting of the crab (Rhithropanopeus harrissii) and that 10 mg/L RH 5849 promoted attachment and metamorphosis of the barnacle cyprids. Accelerated molting of crab zoae and increased metamorphosis of barnacle cyprids are consistent with the known stimulatory actions of the molting hormone on these processes.

As is mentioned above, molting in crustacean is regulated by a multi-hormonal system, but is under immediate control of the steroid hormones called ecdysteroids (Chang et al., 1993). An elevation in ecdysteroid titer in the premolt stage triggers secretion of the molting fluid by the epidermis. This molting fluid contains hydrolytic enzymes necessary for degradation of the chitinous exoskeleton that will be shed. In theory, any step in the cascade of endocrine events (e.g., ecdysteroidogenesis, metabolism of ecdysteroids, and EcR binding) could be the target of xenobiotics. Should a chemical agent be capable of assaulting this hormonal system, the effect would be manifested at the terminal step, the activity of the exoskeleton-degrading enzymes.

Chitobiase, also known as N-acetylglucosaminidase, is one of the two chitinolytic enzymes found in the molting fluid, which are required for complete digestion of exoskeletal chitin. Chitobiase activity was found to correlate well with the profile of the hemolymph ecdysteroid titer during the molting cycle (Zou et al., 1999a). Injection of exogenous 20-hydroxyecdysone resulted in a significant increase chitobiase activity in the epidermis of Uca pugilator (Zou et al., 1999b). These two lines of evidence strongly suggest that this enzyme is a product of the gene regulated by the molting hormone. In their investigation the mechanisms of molt-inhibition by xenobiotics, Zou et al. (1999c,d) examined alterations of chitobiase activity in the epidermis after Uca pugilator was exposed to molt-inhibiting xenobiotics. Seven-day exposure to the molt-inhibiting agents endosulfan, DES, Aroclor 1242, PCB29, and diethyl phthalate did indeed result in decreased chitobiase activity. Inhibition of epidermal chitobiase activity is obviously a general response of Uca pugilator to exposure to molt-inhibiting agents. This inhibition of epidermal chitobiase activity can at least partly account for the slowing of molting caused by the molt-inhibiting xenobiotics because chitobiase is indispensable for degradation of exoskeletal chitin. Since chitobiase is a marker for the actions of the molting hormone, inhibition of this enzymatic activity by these molt-inhibiting chemicals strongly suggests that exposure to these xenobiotics disturbs the Yorgan-EcR axis (Zou et al., 1998, 1999d).

It is possible that the disturbance of the Yorgan-EcR axis arises from direct action of xenobiotics on the ecdysteroid receptor, where xenobiotics act as antagonists of this EcR (Zou et al., 1997b, 1999c,d). Using an ecdysteroid-responsive cell line from Droso phila melanogaster as the assay system combined with an EcR binding experiment, Dinan et al. (2001) screened about 80 potential environmental contaminants and found that the vast majority of these xenobiotics and vertebrate steroids showed neither agonist nor antagonist activity. The few that had affinity to the EcR were found to be EcR antagonists. Two chemicals, diethyl phthalate and nonylphenol, which are capable of inhibiting molting (Zou et al., 1997b) and growth (Brown et al., 1999) of crustaceans, respectively, were shown to produce antagonistic actions according to the assay of Dinan et al. (2001). Therefore, the antagonistic actions on EcR can at least partly explain the inhibition of molting in...
Daphnia magna (Zou et al., 1997b) and chitobiase activity in Uca pugilator (Zou et al., 1999d) by di-ethyl phthalate and growth retardation in Corophium volutator by nonylphenol (Brown et al., 1999). DES was found to have neither agonist nor antagonist effect on the EcR (Dinan et al., 2001), suggesting the inhibitory effects of DES on molting in Daphnia magna (Zou et al., 1997a) and chitibiase activity in Uca pugilator (Zou et al., 1999c) are not mediated by the interaction between DES and EcR. More screenings are needed to examine whether other molt-inhibiting agents can also act as ecdy steroid antagonists.

The disturbance to the Y-orgarcrecdy steroid receptor axis may also result from inhibition of ec dys teroidogenesis in the Yorgans and/or elevated excretion of ecdysteroids. There is still no report on im- pacts of xenobiotics on ec dys teroid synthesis. But attempts to look into the impacts of xenobiotics on the enzymatic system responsible for ec dys teroid metabolism have been made. Exposure of specimens of Daphnia magna to nonylphenol and nonylphenol polyethoxylate while they are also exposed to testos terone can inhibit the metabolic elimination of glucose- and sulfate-conjugated testosterone, thereby result- ing in increased accumulation of testosterone (Baldwin et al., 1997; Baldwin et al., 1998). Sublethal exposure to tributyltin does not affect molting of Daphnia magna but enhances the production of hydroxylated, reduced/dehydrogenated, and glu- cose-conjugated metabolites of exogenous testosterone (Oberdörster et al., 1998a). However, there is still no evidence that the testosterone-metabolizing en- zymes are also involved in ec dys teroid metabolism in crustaceans. Snyder (1998) has identified a new P450 member, CYP45, in the hepatopancreas of the lobster (Homarus americanus) whose expression level coincides with modulations of hemolymph ec dys teroid titer during the molting cycle. Injection of the molting hormone 20-hydroxyecdysone significantly increases CYP45 expression in the hepatopancreas of Homarus americanus, suggesting the possible in- volvement of CYP45 in either ec dys teroid metabolism or other molting-related events.

Unlike the endocrine disrupting effects on sexual development, which are readily visible, the disrupting effects on molting of crustaceans are not easily seen and may have been going on unnoticed in the wild in view of the fact that aquatic environments have been increasingly contaminated with xenobi-otics. More environmental contaminants should be as- sayed for their abilities to interfere with crustacean growth and molting. Mechanistic studies, especially EcR binding and alterations of ec dys teroidogenesis and ec dys teroid metabolism, are also urgently needed for elucidation of the action routes for molt-interference by xenobiotics.

6 Echinoderms
6.1 Endocrinology

Echinoderms, including sea stars, sea urchins, and brittle stars, are deuterostomes. Evolutionarily, echinoderms and vertebrates belong to the same lineage. It is therefore, not surprising that vertebrate steroid hormones may be used as endogenous modulators of reproduction in echinoderms. Schoenmakers et al. (1980, 1981) demonstrated that the tissues of the sea star (Asrerias rubens) possess enzymatic systems necessary for conversion of cholesterol to C21 steroids, progesterone to C10 steroids, and androstenedione to other androgens. Conversion of androstenedione to testosterone by pyloric caecal and gonadal tissues of the sea star was also observed by Hines et al. (1992). Hines et al. (1992) found that sex steroid levels in the testes varied during the reproducti ve cycle, with progesterone level peaking during spermatogonial proliferation and prior to spawning, testosterone level reaching peak just prior to spawning, and estradiol being highest during spermatogonial proliferation. Similar patterns of variations in sex steroid levels were observed in the oocytes of Asrerias rubens. Ovarian progesterone levels did not change significantly throughout the annual gametogenic cy- cle, while testosterone and estradiol levels in the o- varies were highest at the onset of oogenesis. Reproductive functions of echinoderms appear to be responsive to exogenous steroids. Unuma et al. (1999) found that dietary administration of androstenedione or estrone can stimulate gonadal growth of the male sea urchin (Pseudocentrotus depressus). Dietary ad- ministration of E2 promoted ovarian growth but inhibited oocyte growth while testosterone stimulated oocyte growth in Lytechinus variegates but had no effect on ovarian growth (Wasson et al., 2000). These studies provide the strong evidence that verte- brate sex steroids are used by echinoderms to regulate reproduction.

A gonad-stimulating substance (GSS), secreted from the radial nerves and also called the radial nerve factor (RNF), is known to be involved in the control of maturation and spawning of oocytes in echino- derms. The GSS stimulates the follicular cells that envelope each primary oocyte to produce the maturatior-inducing substance 1-methyladenine (Kubota et al., 1975). 1-Methyladenine then acts on the prima- ry oocyte to stimulate its maturation and eventual spawning (Shirai et al., 1988). Schuetz (2000) re- cently reported an extrafollicular source of the maturatior-inducing substance that mediates the actions of the GSS in an echinoderm.
Echinoderms are well known for their striking regenerative abilities. Three classes of regulatory molecules have been proposed as possible candidates for growth-promoting factors in regeneration (Thornoyke et al., 2001). These molecules are monoamines, neuropeptides such as substance P and SALMFamides 1 and 2, and growth factor-like molecules such as nerve growth factor, transforming growth factor beta, and basic fibroblast growth factor.

6.2 Disruption of reproduction and regeneration

Information on endocrine disrupting effects of environmental chemicals on echinoderms is limited. Exposure of the sea star to Clophen A50, a PCB mixture, can reduce ovarian growth and impair the quality of offspring (den Besten et al., 1989; den Besten et al., 1990). A further study demonstrated that these adverse effects of the PCB mixture are related to a compromised ability of steroid synthesis in the pyloric caeca and gonads since treatment with Clophen A50 was found to inhibit steroid synthesis rates in the pyloric caeca and gonads of Asterias rubens (den Besten et al., 1991).

Laboratory evidence indicates that regeneration in echinoderms can be adversely affected by PCBs. Exposure of the crinoid (Antedon mediterranea) whose three arms were amputated at the level of the autonomy plane, to Aroclor 1260 at 14 ng/L for up to two weeks resulted in abnormal arm growth, characterized by an accelerated growth of the regenerate, massive cell migration/proliferation, hypertrophic development of the coelomic canals, and extensive rearrangement of differentiated tissues of the stump (Carnevali et al., 2001). However, an explanation was not provided by these investigators as to how Aroclor 1260 treatment induced hypertrophy of the regenerate. It is possible that the action of Aroclor 1260 may be mediated by the growth-promoting factors as suggested by Thornoyke et al. (2001).

7 Perspectives

Because of the importance of invertebrates in aquatic environments and the fact that aquatic environments are increasingly contaminated with various kinds of pollutants, more and more attention is being paid to the disrupting effects of environmental pollution on endocrine functions of invertebrates. The major hindrance to the assessment of endocrine disrupting effects of environmental chemicals on growth, development and reproduction of aquatic invertebrates is the general paucity of information on the endocrine mechanisms underlying these functions. Among all invertebrates, the understanding of insect endocrinology is most advanced. But most of insects are terrestrial and environmental endocrine disruption is not generally considered a problem for insects. In fact, the principles of endocrine disruption have been used to develop insecticides to control insect populations.

The hormonal control of molting and reproduction of malacostracan crustaceans is well understood, but little is known about the endocrine mechanisms for crustacean metamorphosis, a process that is possibly vulnerable to environmental pollution. The molecular aspects of the actions of crustacean hormones involved in the regulation of molting and reproduction still need to be elucidated. Information on the endocrinology of small-sized crustaceans, such as copepods and cladocerans, is scarce. Cladocerans may have their unique endocrine mechanisms for reproduction because these crustaceans undergo both parthenogenic and sexual reproduction. At present, the understanding of hormonal mechanisms in nonarthropod invertebrates is fragmental or nonexistent. For future investigations, more efforts should be directed toward the elucidation of basic endocrinology of various invertebrate groups. Without the endocrine mechanisms for a specific process being known, it would be difficult to conclude that an adverse effect on such a process by an environmental chemical is mediated through endocrine disruption.

Based on the available information on invertebrate endocrinology, it is obvious that the hormonal control of reproduction in echinoderms shares commonalities with that of vertebrates, echinoderm reproduction being responsive to vertebrate sex steroids. This is not unexpected since echinoderms and vertebrates belong to the same evolutionary lineage, the deuterostomes. However, profound differences exist between endocrinology of other invertebrates and vertebrates. Solid evidence is still lacking that vertebrate sex steroids play a hormonal role in invertebrates other than echinoderms. Therefore, caution must be exercised when extrapolating to non-deuterostome invertebrates the results of endocrine disruption studies with vertebrates. Conversely, it is to suggest that an endocrine disrupting effect in a non-deuterostome invertebrate can be used as a marker to monitor environmental endocrine disruption in vertebrates.

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