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野生生物への影響

Effects on Wildlife

Endocrine Disrupting Contaminants: Lessons from Wildlife

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Studies over the last decade have clearly demonstrated that numerous wildlife populations have been affected by the endocrine disruptive actions of various environmental contaminants. Some pollutants have been shown to induce non-lethal, developmental effects by acting as endocrine disrupting agents. A well documented case is that of the alligators living in contaminated lakes in Florida, U.S.A. We have previously reported that the alligators from pesticide contaminated lakes exhibit reproductive disorders including reduced hatchability of eggs, reduced viability of offspring, impaired hormone secretion and modified reproductive anatomy. Recent field work has extended many earlier studies. For example, we have observed that animals living at contaminated locations on Florida's largest lake, Lake Okeechobee, have depressed thyroxine concentrations. Many avian species exposed to organochlorine pesticides, such as those found in the animals living on lake Apopka, FL exhibit egg shell thinning. However, we observed the opposite, with alligator eggs from contaminated lakes having significantly thicker egg shell. These thicker shells are correlated with reduced hatch rates. Recent studies with the Mosquito fish on Lake Apopka have also observed that males have gonopodia 25% smaller than males of similar size on reference lakes. These and additional recent data extend our observations indicating that an anti-androgenic contaminant is common in Lake Apopka and is influencing the development of male genitalia. We have also performed experimental studies to clarify the relationships between reproductive and endocrine abnormalities and pesticide exposure. For example, egg obtained from lakes Woodruff and Apopka have been incubated at a temperature (31.5℃) that produces animals of both sex. Eggs from Lake Woodruff produced 56% females at this temperature whereas eggs from Lake Apopka gave 75% females. Further, both male and female neonates from Lake Apopka had significant greater plasma estradiol concentrations when compared to neonatal alligators from Lake Woodruff. Previously, we have reported altered circulating steroids in juvenile alligators. The mechanism of this alteration has been unknown. A recent study using an FSH-challenge has demonstrated that animals held in captivity under identical conditions for three years and given an i.v. treatment with FSH showed dramatic increases in plasma testosterone that were similar, irrespective of their lake of origin. These data support earlier studies with 6 mo old animals suggesting that gonadal responsiveness to gonadotropin is not the major mechanism for altered circulating steroid concentrations. We have also examined hepatic biotransformation of androgens and observed sexual dimorphism in reference alligators but no such phenomenon in animals from Lake Apopka or contaminated areas of Lake Okeechobee. Finally, new studies indicate that exposure to contaminants at ppt concentrations alter developing alligators. Together, these data from wildlife, combined with studies of traditional laboratory species, suggest that pesticides are capable of disrupting the developing embryo at ecologically relevant levels. Finally, although species specific differences are observed among developmental and endocrine pathways in vertebrates, many common features exist demonstrating that wildlife models are important indicators of potential environmental health issues for humans.

Endocrine Disruption in Mollusks: Case Studies on the Rock Shell, the Ivory Shell and the Giant Abalone, Linking with Organotin Contamination in Korea and Japan

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1. Introduction

Imposex, formation of male genital organs (penis and vas deferens) on female gastropods, occurs cause specific and at low concentrations of certain organotins such as tributyltin (TBT) and triphenyltin (TPT). Spawning disturbance is known at severely affected stages. Since the first report from Plymouth, England in 1969, imposex have been reported from all over the world involving more than 140 species. Among them, the main cause of population declines in at least seven species is considered to be spawning disturbance related to imposex. In the symposium, we will report the present status of imposex of gastropods and organotin pollution in Korea and Japan, together with endocrine disruption found in abalones.

2. Present status of organotin pollution and imposex in rock shells along the Korean coast

Organotins (TBT and TPT) are artificial compounds that have been used worldwide as major components of anti-fouling paints on ship hulls and have widely polluted coastal areas. Because they are very toxic and exert a great influence on aquatic organisms including imposex in rock shells and other gastropods, the production and usage of organotins have been subjected to various regulations mainly in developed countries. They are recently drawing attention as endocrine disruptors.

As part of a project to study organotin pollution and effects on marine organisms along the Korean coast, we sampled water from sea surface, bivalves (common blue mussels, *Mytilus ediulis*, and Pacific oysters, *Crassostrea gigas*), and gastropods (rock shells, *Thais clavigera*) at 31 locations between October 1995 and August 1997. We investigated organotin concentrations in seawater and organisms as well as the occurrence of imposex in rock shells.

For organotin compounds in seawater, TBT concentrations ranged from an undetectable level (ND) to 35.2 ng Sn/L and TPT concentrations from ND to 7.8 ng Sn/L were determined. This suggested that organotin pollution was widely spread. TBT generally showed greater concentrations than TPT. High concentrations were found in areas with heavy vessel traffic and areas close to shipyards. High organotin concentrations in organisms were also found in areas closely associated with vessels. TPT concentrations up to 1000 ng Sn/g (dry weight) were found in Pacific oysters. These results indicate that high levels of organotins are concentrated and accumulated in marine organisms along the Korean coast.

Organotins (TBT and TPT) are known to cause imposex in rock shells. Imposex occurrence rates in rock shell populations were 100% in most of the locations surveyed along the Korean coast. An imposex occurrence rate of 0%, i.e. all the females were normal, was found only in Deukryang Bay situated in the southwestern part of the Namhae while the rates collected from locations close to this bay were in the range of 67%-88%. The occurrence rates of severe imposex (i.e. sterilized because of the oviduct blockage by vas deferens formation involved by progression of imposex) were higher (60% or more) in the eastern part than in the western part of the Namhae. No sterilized females were found in the open-sea-side areas and some other areas studied.

The above findings indicate that the Korean coast is widely polluted with organotins and imposex in rock

shells is in a serious state. Also, potentially wide influence of organotin pollution on marine organisms in Korean coast is suggested.

3. Imposex in rock shells and organotin pollution in Japan

Among rock shell samples collected between September 1996 and January 1999 from 93 locations along the Japanese coast, imposex were found in 86 locations whereas no or rare cases were found in the remaining seven locations. The occurrence rates of imposex at 73 locations were as high as or close to 100%. It is expected that spawning obstruction occurs in more than half the population of females when the Relative Penis Length (RPL) index exceeds 40, based on the relationships of RPL index, the Vas Deferens Sequence (VDS) index and the occurrence rate of oviduct (vulva) blockage. Among the 93 locations, RPL index values exceeded 40 were found at 40 locations. High values of RPL and VDS indices were found in Shizugawa, the southern part of the Miura Peninsula, Shimoda, Hamana Lake, Mikawa Bay, the head of Ise Bay, Osaka Port, the Seto Inland Sea, the vicinities of Kanmon-kaikyo, Sasebo, Nagasaki Port, Uchinoura, and the vicinities of Miho Bay. Compared to the survey conducted last time, the index values slightly decreased or remained almost unchanged in some locations. TPT concentrations determined in organisms showed decreases with time but varied distinctively with locations, and relatively high pollution remained in some locations was detected. Decreases in TBT concentrations were not so distinctive and decrease degrees were low compared to TPT. Changes with time were not observed in some locations.

4. Imposex and ovarian dysmaturity in ivory shells and their decreased catches

Occurrence of imposex, decreases in catches, and in the amount of spawning from parent snails at seed producing facilities have been recognized in ivory shells. In contrast to rock shells, however, oviduct blockage by vas deferens formation is not observed in ivory shells, so physical suppression of spawning is unlikely. It was suspected that decreases in catches were caused by ovarian insufficiency associated with imposex. Gonad tissue preparations were obtained by a usual method from a total of 135 individuals of ivory shells sampled every month between December 1988 and November 1989 in Prefecture A. The results of microscopy showed a distinctive reproductive cycle (peak maturity in summer) in males (43 specimens) but not in females (92 specimens). Females did not show prominent maturity and reproductive cycle like males even in summer. In addition to the suppressed ovarian maturity, spermatogenesis in ovaries was found in six female specimens (five imposex and one normal). It can be inferred that suppressed ovarian maturity included spermatogenesis in some cases appeared as dysfuncion of ovaries and this might cause the reduction of spawning volume.

5. Occurrence of imposex in other marine gastropods in Japan

Imposex was found in Alabaster False Tun, Galeoocorys leucodoma, (Order Mesogastropoda, Family Oocorythidae) trawled last year from 200-250 m in depth off the Atsumi Peninsula. Among the 69 species of Japanese marine gastropods studied including the rock shell and the ivory shell, a total of 39 species (7 from Mesogastropoda and 32 from Neogastropoda) were found imposex. Although imposex has been found mostly in shallow-water species in the previous surveys, we need to conduct detailed studies on species living at depths of 200 m or more because of our latest finding of imposex in Alabaster False Tun.

6. Endocrine disruption in abalones

The catches of abalones in Japan have been decreasing since 1970s. Various studies have been conducted to pursue the cause of the decrease but still unresolved. With a hypothesis that organotins (TBT and TPT) cause

reproductive insufficiency in abalones, we have studied this subject continuously since 1994. Our findings up to now are outlined below.

Based on the results of a preliminary survey started in 1994, the gonadal maturity of giant abalones, *Haliotis madaka* was histologically studied, with area B selected as a control area, which was considered to be normal in view of catches and other characteristics, and area C selected as an affected area, where decreases in catches were prominent and the ratio of artificially raised and released individuals among the total catch was very high (i.e. reproducibility was very low in the natural abalone population). The results showed that both sexes of giant abalones in area B became sexually mature between the late fall and the early winter whereas those in area C did not became sexually mature at the same time. In area C, the males showed several peaks of sexual maturity in a year but the sexual maturity of females varied widely among individuals and the average level of maturity in the female population was suppressed. While few hermaphrodites (evidenced by ovarian spermatogenesis) were found in area B, 11 (20.4%) hermaphrodites out of 54 specimens were found in area C.

Because abalones fertilize externally by releasing sperms and eggs in seawater, it is very important for both sexes to become sexually mature at the same time in order to heighten fertility. Their fertility may drop if there are many immature individuals in either sex or if the peaks of sexual maturity do not coincide between both sexes. Because abalones are known to be dioecious, hermaphrodites (masculinazation of females) of about 20% found in area C is abnormal. This is considered to be a masculinization phenomenon similar to the imposex found in rock shells.

Because imposex in gastropods have been known to be specifically caused by certain organotins such as TBT and TPT, organotins are also suspected as a cause of masculinization found in giant abalones in area C. Determined values of organotin concentrations in giant abalones from both areas revealed significantly higher concentrations in area C than area B.

An *in situ* exposure test was conducted on an abalone species, *Haliotis gigantea*, transplanted from area B to a location close to a shipyard in area C (giant abalones could not be used for the test because of difficulty in obtaining a sufficient number of individuals). The test was carried out for seven months from June 1998 and we collected about 40 subjects in January 1999. Gonad tissue preparations of individuals were observed under a microscope, and masculinization, such as ovarian spermatogenesis, was found in 15 female specimens (88.2%) out of 17. Also, significant accumulation of organotins during the test period was observed. No ovarian spermatogenesis was observed in the control specimen from area B.

The microscopic features of the observed ovarian spermatogenesis are the same as those found in ivory shells suffering from imposex. Ovarian spermatogenesis and testicular development associated with imposex have been found also in rock shells and other Neogastropoda species. The findings in our study indicate that masculinization (ovarian spermatogenesis) essentially equal to imposex occurs in abalones as an endocrine disruption phenomenon, without development of external genital organs such as a penis. We infer that some environmental factor, most probably organotins, caused ovarian spermatogenesis in abalones around the shipyard in area C.

The Occurrence of Intersex in a Japanese Freshwater Crab

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The intersex phenomenon has been found to occur in a common Japanese freshwater crab, *Geothelphusa dehaani*, which inhabits mountain streams in Japan. In addition, the intersex of *G. dehaani* occurred not only in females but in males (dual-gender intersex): either female genital openings (gonopores) or a single ovary occurred in male crabs.

During a one-year survey, beginning in September 1990, six abnormal crabs were found out of 263 individuals collected in the River in Japan. This crab species populates Japan's mainland, excepting Hokkaido. The possibility of environmental endocrine disrupters (EEDs) affecting animals in the environment, we reinvestigated Geothelphusa population. While the definitive causal factors and mechanics relating to the occurrence of intersex are still unclear, it was found that there is a positive relationship between the concentration of some chemicals in the river water and the frequency of abnormality in each sex.

Sexual Disruption of Wild Fish in U.K. Rivers.....What Does It All Mean?

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It has been well established that there are a wide variety of endocrine-disrupting chemicals (EDCs) in the aquatic environment. Furthermore, there is a growing body of evidence that suggests that exposure to these hormonally-active chemicals may result in detrimental health and reproductive effects to wildlife and humans. Laboratory exposure data supporting this hypothesis is significant, although field data is very limited and thus the consequences of long-term exposure of wildlife to EDCs are largely undetermined.

In freshwater and estuarine fish, intersex (the simultaneous presence of both male and female gonadal characteristics) has been reported in gonochoristic species throughout the U.K. and other parts of Europe, the USA and Japan. It is generally believed that intersex in fish results from exposure to EDCs, as statistical associations between the incidence and severity of intersex and exposure to contaminants exist. In our own studies on a UK freshwater fish, the roach (*Rutilus rutilus*), both our experimental results and field observations suggest that intersex occurs as a result of exposure to treated sewage effluents containing oestrogenic chemicals. The link between specific chemicals in the effluents and intersexuality is, however, much weaker and the critical windows in the life cycle of roach during which partial sex reversal can occur have not been fully characterised. Notwithstanding all of this, our data suggest that intersex fish, in U.K. rivers, result from a feminisation of males.

Almost all rivers in the U.K. receive effluents from sewage treatment works and hence the widespread nature of intersex in roach throughout the U.K. is not surprising. It has also been observed in other native fish species, including the gudgeon (*Gobio gobio*), although the full extent of the problem in other fish and their relative sensitivities to sexual disruption compared with roach have not been established. Major research efforts are currently being directed at determining the impact of widespread intersexuality in wild roach populations, particularlywith respect to the reproductive competence of affected individuals. Any effect of intersex on gamete production and/or quality to the individual may impair the contribution of that fish to the population and hence an assessment of the reproductive capabilities of intersex roach is of paramount importance.

Examinations of the reproductive potential of intersex roach clearly demonstrate that their ability to produce gametes is highly variable, and it is dependent on the degree of disruption in the reproductive ducts and/or altered germ cell development. Small numbers of wild roach have been found that cannot produce any gametes at all, due to the presence of severely disrupted gonadal ducts. In addition, at spawning time, some intersex fish produce both male and female gametes. These observations are, however, unusual, as the majority of 'intersex' fish produce male gametes that, although viable, appear to be of poorer quality than those from normal males obtained from environments that do not receive treated sewage effluent. Furthermore, our most recent studies suggest that poor fertility in roach may be a direct consequence of intersexuality, as feminised fish have a poorer fertilisation success compared with both less severely feminised and normal males. As the long term survival of a fish population is determined by the size of the spawning stock and the annual number of offspring (recruits) produced, together with the subsequent survival of these recruits, our results suggest that populations of roach, in at least some areas, are adversely affected. The effect on the population from this impact will need to be assessed in comparison with other pressures on the fish populations, to determine its significance.

From a broader perspective, extensive samplings of roach from some U.K. rivers between 1995-2000 has

indicated that the sex ratio is skewed in favour of females in some rivers, and suggests that, in these areas, full sex-reversal of male to female fish may be occurring. A skewed sex ratio will obviously influence the population dynamics of any fish species. Furthermore, the reproductive output of fully sex-reversed male fish may differ, when compared with normal female fish. Fully sex-reversed fish are, however, phenotypically indistinguishable from normal genotypic females as sex-specific genetic probes for roach are unavailable, presently. At this stage, therefore, we are, thus, unable to assess whether the skewed sex ratio seen is the result of sampling bias in favour of predominantly female populations, or whether they are, indeed, a result of widespread sex reversal. Complicating this issue is the knowledge that androgen-active substances can be detected in some U.K. effluents and in some US rivers, intersex fish have been shown to arise through masculinisation of genotypic females. It is theoretically possible, therefore, that intersex fish, in some UK rivers, have resulted from androgenisation of females, rather than (or as well as) by feminisation of genotypic male fish.

In summary, although intersexuality is widespread in fish in U.K. rivers, our understanding of the consequences of these effects is limited. Our most recent results do, however, suggest that widespread intersexuality as a result of endocrine disruption, may well have a deleterious impact on reproduction in wild fish. Further studies are, however, needed in order to fully understand the consequences to fish populations of widespread contamination of U.K. rivers by EDCs.

Detection of Thyroid Hormone Disrupting Effects Using Frogs and a Molecular Toolbox

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Recent evidence demonstrates that a growing number of substances released into the environment that are not deemed toxic *per se* function as disruptors of critical, normal endocrine mechanisms in a wide range of vertebrates. Malformations have featured prominently both in the popular and scientific literature dealing with the global problem of declines and disappearances of amphibian populations suggesting exposure to retinoid and thyroid hormone (TH) endocrine disruptors (EDs). Estrogenic EDs are implicated in many reports of feminization in fish, reptiles and amphibian males. These EDs, as well as those acting as androgens or anti-androgens, are implicated in the significant increases in the incidences of hormone-dependent diseases including breast, prostate and testicular cancers. Most research on EDs has focused on reproductive hormone disruption, but it is becoming increasingly clear that disruption of thyroid hormone-regulated pathways can have significant influence on these and other pathways.

TH is critical in mammals for infant brain development, maintenance of proper brain function and general metabolism. In frogs, it triggers the rapid metamorphosis of the tadpole into a juvenile. Virtually every tissue in the tadpole is a target of TH and the end result varies depending upon the target tissue. For example: the tail regresses, limbs grow, the brain remodels and liver cells change biochemically to synthesize urea cycle enzymes necessary for handling nitrogenous waste on land. Indeed, the frog tadpole represents a state in many ways analogous to the developing mammalian fetus with the distinct exception that premetamorphic tadpoles do not have any endogenous TH detectable in their serum, yet they are exquisitely sensitive to exogenous TH administration. Thus metamorphosis can be precociously induced by exposure to TH *in vivo* and in organ culture and can be enhanced or inhibited by various agents including retinoids, corticoids, and prolactin. TH can also modulate the expression of estrogen and retinoic acid receptors and there is some evidence that estrogen and estrogenic EDs may have significant effects on metamorphosis. Therefore, examination of the effects of suspected endocrine disruptors on tadpole metamorphosis promises to be an important tool in the identification of EDs that, not only identify substances that could directly contribute to frog species declines, but also could adversely affect other species.

The major mode of TH action is via binding to its nuclear receptors and triggering tissue-specific programs of gene activation and repression. These programs are clearly underway within 48 h of TH administration and thus represent a rapid way to detect ED activity and obtain leads on mechanism of action. Using two frog species, *Xenopus laevis* and *Rana catesbeiana*, we have examined the gene expression profiles of different tissues in response to treatment with known EDs such as the preemergent herbicide, acetochlor, and effluent samples containing possible EDs that affect TH action. We demonstrate that molecular analysis of gene expression is a rapid and sensitive method to determine exposure to EDs *in vivo*, *in situ* and in organ culture that correlates with overt morphological and behavioural consequences.

Use of Chemistry and Biology to Assess Endocrine Disruptors in the Environment

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The North American Great Lakes have become contaminated with a number of synthetic compounds. This is one of the locations where effects of synthetic organic compounds have been demonstrated to have demonstrative effects on wildlife. While this is not a new story, it is useful to review the story periodically to put into perspective current efforts to minimize the entry of these compounds into the environment. Synthetic organic chemicals have been associated with birth defects and embryo-lethality in fish, mammals and birds. The observed effects are similar among the different classes of organisms, though the threshold concentrations to cause effects vary greatly among classes. To understand the mechanisms of action and determine the causative agents required a combination of instrumental analyses, toxicology and ecology to identify the putative causative agents and to understand the interactions among the many compounds in these complex mixtures. Some of the compounds that have been released into the Great Lakes are classified as persistent, organic pollutants (POPs). Included in these are a number of classes of compounds. One of the most toxic classes are those that have the capacity to interact with the aromatic hydrocarbon receptor (AhR). These are the so called dioxin-like compounds including polychlorinated dibenzo-p-dioxins (PCDFs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated Napthalenes (PCNs). Here I present the history of exposures of these compounds and the effects they have had on lake trout, mink and bald eagles. The focus of the discussion is the deformities and embryo-lethality that is caused by dioxin and structurally similar compounds. The results of studies of effects at the molecular, individual and population levels of organization will be discussed. The results of studies conducted in both the laboratory and field will be presented in a weight of evidence approach. The relative importance of the toxic effects of the dioxin-like compounds, relative to effects of habitat, nutritional, genetic and habitat changes on the observed population-level effects of the dioxin-like compounds will be discussed. Furthermore, techniques for understanding complex mixtures of residues will be presented.

Review of Present Knowledge Concerning Endocrine Disrupters and Wildlife

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With the advent of the new millennium, an appropriate time has come to take stock of our knowledge and understanding of endocrine disruption in wildlife. The subject has been studied very widely since the early 1990s, and as well as being interesting in their own right, the findings provide useful pointers to potential parallel effects in human populations. Much of the literature on endocrine disruption concerns experimental laboratory-based studies, which although essential for understanding the mechanistic basis of these effects, do not address the questions of how widely and to what degree of severity impacts are occurring in wildlife. However, although many good field studies have now been undertaken, the majority of these have been confined to studies of a variety of biochemical, cellular or morphological biomarkers, with relatively few attempting to tackle the all-important question of whether populations and communities are at risk. The numbers of such studies are probably inversely proportional to their degree of difficulty, but nevertheless there are several which have made substantial progress. These show that given appropriate exposure and sensitivity to endocrine disrupters, certain wildlife populations have undoubtedly been placed at risk from these substances. The available evidence suggests that these adverse effects are generally localised in the vicinity of large domestic and industrial discharges, large scale run-off situations, or other specific sources. However, some of the persistent organohalogens and aromatic hydrocarbons are causing more global impacts, especially on top predators, although most sources of these materials are now known and under control in the developed world. This paper will briefly discuss some key case histories taken from the major wildlife groups to have been studies to date.