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Subtle sabotage: endocrine disruption in wild populations

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Abstract: How important is endocrine disruption as a threat to wildlife populations? This review applies causal criteria to existing studies of wild populations of fish, amphibians, reptiles, birds, and mammals to answer three questions: (1) Have endocrine-mediated effects of contaminant exposure been documented? (2) Have individual adverse effects that could lead to population effects been documented? (3) Have population level effects been documented? In fish, the possibility of population level effects is inferred from impaired individual fertility. Substantial evidence directly links fertility impairment to endocrine disruption. In amphibians, population declines are occurring worldwide and causes may vary among regions, with contaminant-induced sexual disruption being one of many insults that stymie population growth. In reptiles, local populations of alligators have been dramatically reduced by direct toxicity and recovery has probably been slowed by sexual disruption, particularly feminization induced by chronic contaminant exposure. In contrast, heavily contaminated snapping turtles display feminized secondary sex characteristics, evidence of disrupted sexual development, but populations are densest at the most heavily contaminated site. In birds, population decline and recovery have been directly linked to p,p'-DDE-mediated eggshell thinning and subsequent banning of the parent compound DDT in the US and Europe. Regional populations of predatory birds still suffer chick mortality related to PCB and DDT burdens, but no causal link with endocrine-mediated processes has been demonstrated. In mammals, contaminant-induced hormonal derangements in seals and polar bears have been documented, but no clear evidence links endocrine effects to population declines. Although endocrine disruption will rarely be the sole cause of failing recruitment or decreased abundance, it may subtly sabotage sexual development, sex ratio, and metabolic compensation for environmental stress. Endocrine disruption is unlikely to extirpate a species over its entire range, but in combination with other stressors such as habitat loss, over-harvesting, and global climate change, may contribute to local extinctions. Rev. Biol. Trop. 54(Suppl. 1): 1-19. Epub 2006 Sept. 30.

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AN ECOEPIDEMIOLOGY APPROACH TO EVALUATING THE IMPACT OF ENDOCRINE DISRUPTION

Since the 1980s, biologists around the world have accumulated evidence of hormonal derangements in wild populations of fish, amphibians, reptiles, birds, and mammals. Some cases clearly meet the most stringent definition of endocrine disruption-altered hormone status in a chemically contaminated population leads to adverse effects including developmental abnormalities and reproductive dysfunction (Daston *et al.* 2003). Some wildlife studies demonstrate altered hormone status in contaminated populations, but do not measure adverse effects, while others clearly delineate adverse developmental or reproductive effects without measuring hormone status.

How important is endocrine disruption as a threat to wildlife populations?

Is it linked to measured decreases in fertility, offspring survival, and overall population health? Answering this question requires an ecoepidemiology approach as outlined by Fox (1991). The fundamental goal of this approach is to evaluate whether environmental exposures cause a particular health effect, then devise ways to mitigate or prevent the problem. Causal criteria are used to evaluate evidence gathered in observational and experimental studies. Although no single standardized list of criteria for causal inference exists, several criteria are commonly used by epidemiologists: (1) strength of association, (2) consistency of association, (3) existence of a dose-response relationship, (4) biological plausibility, and (5) temporality (Fox 1991, Weed 2002). In the context of endocrine disruption, these criteria can be defined as follows:

1. Strength of association-Do chemical exposure and hormonal derangements or reproductive dysfunctions occur in the same place or within the same individual? Is the effect noticeable or statistically significant? Is the effect more prevalent in the exposed population relative to an unexposed one?

2. Consistency of association-Has the association between a chemical exposure and endocrine-mediated effects been observed by different investigators, in different species, in different habitats, or at different times?

3. Dose-response relationship-Does the effect become more pronounced as the level of chemical contamination increases?

4. Biological plausibility-Is there a plausible or proven mechanism by which the chemical could interfere with the endocrine system and produce the observed effect?

5. Temporality-Did the effect appear only after the chemical was present in the environment?

Admittedly, the process of causal inference is subjective, depending upon the experience and judgment of the person performing the analysis. However, the goal is not to prove irrefutably that a particular factor is solely responsible for an effect, but to evaluate available evidence that a problem exists, so that decisions can be made regarding methods of preventing or remediating the problem.

This review applies causal criteria to existing studies of wild populations of fish, amphibians, reptiles, birds, and mammals to answer three questions: (1) Have endocrine-mediated effects of contaminant exposure been documented? Answers to this question will address all five causal criteria, but particularly that of biological plausibility. To conclude that endocrine disruption is likely to cause measured or predicted population effects, interference with hormone homeostasis must be demonstrated. (2) Have individual adverse effects that could lead to population effects been documented? Answers to this question will also address all five criteria, but particularly that of biological plausibility. Not only must hormone homeostasis be altered, but alterations in individual fertility (at minimum) must occur for population parameters such as recruitment and abundance to be affected. (3) Have population level effects been documented? Answers to this question will address three criteria-strength and consistency of association and temporality.

CASE STUDIES

Fish

Biologists around the world have accumulated substantial evidence of reproductive endocrine disruption in wild fish, particularly in waters receiving sewage treatment plant or pulp mill effluents (Mills and Chichester 2005). Three different kinds of endocrine disruption have been described in fish: feminization-males develop female-like characteristics, masculinization-females develop male-like characteristics, and suppression-both sexes

have sub-normal reproductive capacity. Causal criteria are applied to studies investigating each of these three types of endocrine disruption to evaluate the consequences of endocrine disruption for populations.

Feminization

Endocrine mediated and fertility effects

A strong association exists between sewage effluent exposure and feminization of male roach (*Rutilus rutilus*), a cyprinid fish. Male roach living near municipal sewage outlets in England have a high incidence of intersex gonads-part testis and part ovary (9-100% of males) compared to males in reference lakes (4% of males) (Jobling *et al.* 2002). Males near sewage treatment outfalls also produce vitellogenin (VTG), an egg-yolk protein normally found at high concentrations in females and extremely low to non-existent concentrations in males (Jobling *et al.* 1998). Males with the most severe gonadal abnormalities also had very low sperm counts and poor fertility (Jobling *et al*. 2002).

The association between sewage effluent and fish feminization is consistent across investigators, geographical regions, species, and habitats. Males of fresh and saltwater species suffer from varying degrees of feminization, including inappropriate vitellogenin production and intersex gonads in common carp (*Cyprinus carpio*) in Spain (Lavado *et al.* 2004), flounder (*Platichthys flesus*) from UK estuaries (Kirby *et al.* 2004), and marbled sole (*Pleuronectes yokohamae*) in Japan (Hashimoto *et al.* 2000), intersex gonads in male white perch (*Morone americana*) from the lower Great Lakes (Kavanagh *et al.* 2004) and reduced male secondary sex characteristics in sand goby (*Pomatoschistus minutus* and *P. lozanoi* Kirby *et al.* 2003).

In laboratory studies with roach, the degree of feminization, measured as plasma vitellogenin concentration or percentage of males with a female-like ovarian cavity, intensified with increasing concentration of sewage effluent (Rodgers-Gray *et al.* 2001). Two groups of chemicals, the alkylphenols (breakdown products of chemicals found in detergents and plastics) and steroidal estrogens, including estradiol-17beta, estrone, and 17alpha-ethinylestradiol have been identified as the most likely culprits (Jobling and Sumpter 1993, Desbrow *et al.* 1998, Routledge *et al.* 1998). Dose-response relationships between doses of steroidal estrogens (E2 or EE2) or alkylphenols (nonylphenol and octylphenol) and vitellogenin concentration or percentage of intersex individuals have been demonstrated in numerous laboratory studies of fish (e.g. Jobling and Sumpter 1993, Purdom *et al.* 1994, Ackermann *et al.* 2002, Seki *et al.* 2003). In addition, steroidal estrogens and alkylphenols can bind to and activate transcription from fish estrogen receptors in cultured liver cells (e.g. Flouriot *et al.* 1995, Jobling *et al.* 1995), a proven mechanism by which VTG synthesis is induced and a likely mechanism by which sexual development of exposed males is re-directed along a female pathway.

In at least one case, identification of the causes of fish feminization has allowed partial remediation of the problem. For instance, the VTG-inducing capacity of a combined domestic and industrial sewage effluent released to the Aire River, England was significantly reduced, but not completely removed by additional effluent processing before final release (Sheahan *et al.* 2002). This remediation effort demonstrated that feminization is temporally associated with the presence of effluent.

Population effects

At present, studies demonstrating morphological, biochemical, and fertility effects of endocrine disruption in roach are the most comprehensive examination of a wild fish population. Still, no effects on recruitment or abundance of roach have been documented. However, Hurley *et al.* (2004) modeled the impact of constant exposure of fish to a feminizing agent over 30 generations. The model predictions were surprising: if "supermales"-YY males produced by mating between normal males and genetic males with female phenotypes-could

survive, the proportion of genotypic males in the population increased above 0.50. If "supermales" were inviable, the proportion of genotypic males hovered between 0.50 and 0.58. In both cases, the proportion of phenotypic males was approximately 0.20 and genotypic females did not die out. Hurley and colleagues (2004) concluded that even strong, constant feminization pressure would not lead to population extinction and that feminized populations would be capable of full recovery to sex ratios of 1:1 if the feminizing pressure were removed. These predictions have not yet been tested experimentally.

Masculinization

Endocrine-mediated and fertility effects

A strong association exists between the occurrence of masculinized females and pulp and paper mill effluent exposure. In one case, all female mosquitofish (*Gambusia affinis*) collected downstream from a bleached kraft pulp mill in northwest Florida, USA had a gonopodium-an elongated portion of the anal fin normally found only on males. No females collected upstream of the mill had a gonopodium. Males use the gonopodium as an intromittent organ to deliver sperm to females. After internal fertilization, females bear live young. Pregnant female mosquitofish with gonopodia displayed male-like courtship behavior toward other females and successfully delivered live young (Howell *et al.* 1980). Interestingly, male mosquitofish living in the same effluent-receiving stream showed apparent precocious sexual development, growing a gonopodium at a smaller body size than males in an unexposed population (Howell *et al.* 1980).

Masculinization is consistent across investigators, species, location, and time, although masculinization has not been demonstrated in as many localities as feminization. Masculinized female mosquitofish, least killifish (*Heterandria formosa*), and sailfin mollies (*Poecilia latipinna*) were collected downstream from a bleached kraft mill discharging into a second Florida stream, the Fenholloway River (Bortone *et al.* 1989). Approximately 10 years later, female mosquitofish in the Fenholloway River still had elongated, male-like anal fins (Parks *et al.* 2001), but masculinized females had the same number of follicles in the ovary as females collected from a reference site (Orlando *et al.* 2002). Together with Howell *et al.* (1980) observation that masculinized females successfully delivered live young, these data suggest that fertility is not altered.

The kraft mill effluent in the Fenholloway River contains constituents that bind and activate transcription from the androgen receptor in a dose-dependent manner (Jenkins 2001, Parks *et al.* 2001). The suspected masculinizing agent is a wood-derived compound that has not been conclusively identified, although several steroids, including progesterone, androstenedione, and androstadienedione have been identified in river water and sediments downstream from the mill (Jenkins *et al.* 2003, Jenkins *et al.* 2004). A plausible hypothesis is that bacteria in the pulp mill treatment ponds or receiving streams transform wood sterols into sterols or steroids that can bind the fish androgen receptor (Denton *et al.* 1985) and masculinize females.

A different form of masculinization, induction of a male-skewed sex ratio, occurs in a species of live-bearing marine fish, the eelpout (*Zoarces viviparus*). This form of masculinization is strongly associated with the effluent plume from an elemental chlorine-free bleached pulp mill: females captured in the plume had broods that were 55-65% male, while females collected from six reference sites had broods that were 50% male (Larsson *et al.* 2000, Larsson and Forlin 2002). The investigators found the same effect during three of four breeding seasons. In the season when normal sex ratios were recorded, a mill shutdown had coincidentally occurred during early eelpout gestation, indicating a strong temporal association of masculinization with effluent presence (Larsson and Forlin 2002). No data are available on dose-response relationships between effluent constituents and sex ratio effects, nor were fertility data presented.

Population effects

No data have been published regarding offspring survival or abundance in populations with masculinized females. Because masculinized female mosquitofish did not have compromised fecundity (Howell *et al.* 1980, Orlando *et al.* 2002), population level declines seem unlikely. Male-skewed sex ratio in eelpouts may have more serious consequences at the population level. Hanson *et al.* (2005) used population data from eelpouts collected in an uncontaminated area over a 10 year period to build an age-structured matrix population model. The model predicted that population growth would be slower if the proportion of females were 38.7% (observed in the effluent plume) vs. 50% (observed at uncontaminated sites). The probability of extinction after 50 years rose from 5% to 28%, suggesting that local extinction of populations with male-skewed sex ratios could occur. Hurley *et al.* (2004) generalized fish population model predicted that exposure to a weakly masculinizing agent would result in approximately 50% phenotypic males, but only 10-40% genotypic males. A strongly masculinizing agent would lead to population extinction because although phenotypic males (genetic females with a male phenotype) would constitute 60-80% of the population, the proportion of genotypic males would decline to 0. When the masculinizing pressure was removed from the simulation, the population died out after one generation (Hurley *et al.* 2004).

Reproductive Suppression

Endocrine-mediated and fertility effects

Reproductive suppression in fish is strongly associated with kraft mill effluent and is consistent across species, investigators, and habitats. Suppression includes effects such as delayed sexual maturity, reduced gonad size, suppressed steroid hormone and VTG levels, and impaired pituitary hormone release (Karels *et al.* 1998, Munkittrick *et al.* 1998, Karels *et al.* 2001, Sepulveda *et al.* 2001, Fentress *et al.* 2006). The fertility effects of kraft mill effluent are less consistent across species. In some species, hormonal effects were accompanied by a limited ability to spawn (lake whitefish, *Coregonus clupeaformis* and perch, *Perca fluviatilis*) or by reduced fry number and size (largemouth bass, *Micropterus salmoides*), whereas other species were able to spawn normally (longnose sucker, *Catostomus catostomus*; white sucker, *Catostomus commersoni*; and roach, *R. rutilus*) (Karels *et al.* 2001, Lister and Van Der Kraak 2001, Sepulveda *et al.* 2003).

Mesocosm studies exposing wild fish to varying kraft mill effluent concentrations show that increasing effluent doses cause decreasing steroid hormone and VTG concentrations and decreasing gonad investment in largemouth bass (Sepulveda *et al.* 2003) and decreased steroid hormone concentrations in mummichog (Dube and MacLatchy 2000). Interannual variation of kraft mill effluent concentration in the Pearl River, Louisiana, USA created a natural dose-response experiment. When effluent concentration was higher, steroid hormone and VTG concentrations were decreased in longear sunfish (Fentress *et al.* 2006). The specific compound responsible for these reproductive effects is unknown, but the most likely culprit is a lignin degradation product (Hewitt *et al.* 2002). How these wood-derived compounds interact with the reproductive endocrine system is not clear, but exposure to kraft mill effluent stimulates white sucker livers to produce ligands for the aryl hydrocarbon receptor (AhR), rainbow trout estrogen receptor (ER), goldfish androgen receptor (AR), and goldfish sex steroid binding protein, indicating interaction with multiple components of the reproductive axis (Hewitt *et al.* 2000).

Reproductive suppression is tightly time-coupled to kraft mill effluent presence. During a short kraft mill shutdown, male white sucker 11-ketotestosterone levels returned to normal (Munkittrick *et al.* 1992). Likewise, livers from white sucker captured from a kraft mill effluent plume, then caged at a reference site for four days had very low concentrations of AhR, ER, AR and sex steroid binding protein

ligands. A reciprocal experiment gave the same results: white sucker captured from a reference site and caged in the effluent plume for four days had 10-20 fold higher concentrations of receptor and binding protein ligands (Hewitt *et al.* 2000).

Population effects

The potential for population effects of kraft mill effluent varies among fish species. Although many species experience suppressed steroid hormone levels, some, including white sucker, longnose sucker, and roach have normal fecundity (Karels *et al.* 2001, Lister and Van Der Kraak 2001). Others, including lake whitefish, perch, and largemouth bass suffer reduced fecundity (egg number) or fry number and survival (Karels *et al.* 2001, Lister and Van Der Kraak 2001, Sepulveda *et al.* 2003). Impaired fecundity and fry survival are directly related to population growth, but no data indicate decreased abundance in effluent-receiving areas. Kraft mill effluent does delay sexual maturity, altering age-related fecundity and potentially reducing population-wide fry production (Munkittrick *et al.* 1998).

Amphibians

Endocrine-mediated and fertility effects

Wild frogs in the USA are suffering sexual disruption. Nearly 3% of cricket frogs (*Acris crepitans*) collected in Illinois during 1993-95 had intersex gonads-either testes with large oocytes or even a complete testis and a complete ovary (instead of two complete testes or two complete ovaries)(Reeder *et al.* 1998). Juvenile cricket frogs captured from PCB (polychlorinated biphenyl) and PCDF (polychlorinated dibenzofuran)-contaminated sites had a heavily male-skewed sex ratio (58-75% male) relative to the expected female-skewed sex ratio (20-40% males) of juveniles caught at reference sites (Reeder *et al.* 1998).

This study stimulated an extensive survey of preserved museum specimens (Reeder *et al.* 2005), a geographically broad-scale field survey (Hayes *et al.* 2003), and laboratory dose-response experiments (Hayes *et al.* 2003). The subsequent studies indicate that intersex is not part of normal frog sexuality, but is probably due to organic pollutants.

Upon examining more than 800 museum specimens, Reeder *et al.* (2005) found that 5% of cricket frogs collected in Illinois over the past 150 years had intersex gonads. The proportion of intersex cricket frogs was strongly associated with geographic region, 10.9% in northeastern Illinois, 4.9% in central Illinois, and 2.6% in southern Illinois. Northeastern Illinois is the most heavily industrialized and urbanized part of the state and is the most heavily contaminated with organochlorine chemicals, including dioxins, polycyclic aromatic hydrocarbons (PAHs), and PCBs.

This study also revealed a strong temporal association between organochlorine use and intersex frogs. Statewide, only 1% of frogs collected between 1852 and 1929-before the use of organochlorine chemicals in industry and agriculture - were intersex. Between 1946 and 1959, the period of heaviest organochlorine use, 17% of frogs were intersex. Coincident with decreased usage and banning of DDT and PCBs, the frequency of intersex frogs declined to 9% between 1980 and 1996. The spatial and temporal associations between decreased exposure and decreased intersexuality suggest a dose-response relationship.

Consistent with observations of intersexuality in cricket frogs, 10 - 90% of male Northern leopard frogs (*Rana pipiens*) collected in 2001 in the upper western and midwestern USA (Utah-Iowa) had eggs in the testis (Hayes *et al.* 2003). The suspected culprit in this species is the herbicide atrazine. Collection sites with 0.2-6.7 ppb atrazine in the water had 10-90% intersex males, while the only site with 100% normal males had less than 0.2 ppb atrazine. Confusingly, the site with 90% intersex frogs had only 0.2 ppb atrazine, while the site with 6.7 ppb atrazine had only 15% intersex frogs. The absence of a clear dose-response relationship in wild-caught frogs may be explained

by the short half-life of atrazine and rapid temporal variation in atrazine levels due to evaporation/irrigation cycles. The concentration of atrazine measured at the time of frog collection is unlikely to be the same as the concentration experienced by the developing tadpole. Leopard frog tadpoles exposed to atrazine (0.1 or 25 ppb) in the laboratory developed eggs in the testes, but the proportion of intersex individuals was greater at the lower dose (Hayes *et al.* 2003).

Whether intersex cricket and leopard frogs are feminized males or masculinized females is unknown since the genetic sex of the intersexes has not been analyzed. Some evidence suggests that intersex cricket frogs may be masculinized females because where intersexes were found, the proportion of females dropped, but the proportion of males remained at the expected 50% (Reeder *et al.* 2005). The ratio of males, females, and intersexes has not been reported for wild leopard frogs.

Because gonad development is a hormonally mediated process, intersex gonads are considered an endocrine-mediated effect. How the suspected causal agents, PCBs, PCDFs or atrazine could interfere with sexual development is unclear. None of the compounds strongly bind or activate estrogen or androgen receptors (Tennant *et al.* 1994, Safe and Krishnan 1995), but they do interfere with enzymes responsible for steroid synthesis. PCBs and PCDFs bind the aryl hydrocarbon receptor (AhR) and activation of AhR by some PCBs prevents estrogen receptor-mediated gene expression. In addition, dioxin binding to AhR inhibits the enzyme that converts cholesterol to pregnenolone, the first step in sex steroid synthesis (Safe and Wormke 2003). The net effect could be reduced estrogen production and dampened estrogen signaling, potentially leading to female masculinization. Atrazine stimulates aromatase-the enzyme that converts testosterone to estrogen-in mammalian and fish cell cultures (Sanderson *et al.* 2001). The net effect could be enhanced estrogen production, potentially leading to oocytes in male testes (Hayes *et al.* 2003).

Population effects

Amphibian populations are declining worldwide (Wake 1991) and the search for causes continues to be controversial (Pechmann *et al.* 1991). Cricket frog populations have decreased markedly in portions of their range, particularly in northern Illinois where organochlorine contamination and intersex incidence are highest. Since 1960, almost no cricket frogs have been collected in this region, strongly suggesting that sexual disruption led to local extinction (Reeder *et al.* 2005). Although still common, northern leopard frog populations throughout the USA are listed as declining, particularly in areas of Colorado, Wyoming, and Montana (IUCN *et al.* 2004). In some regions, endocrine disruption may be one of many insults whose cumulative impact is decreased population growth and even local extinction.

Reptiles

Endocrine-mediated and fertility effects

Strong associations between organochlorine contaminant exposure, sex differentiation, and sex hormone levels have been demonstrated in wild alligators (*Alligator mississippiensis*). In 1980, Lake Apopka, Florida, USA and surrounding groundwater were contaminated by an extensive spill from a pesticide-manufacturing plant. The major pollutants were DDT and its metabolites, p,p'-DDE and p,p'-DDD, although a variety of other organic contaminants have been identified at the site (Environmental Protection Agency 2004). Alligator eggs from the lake contain high concentrations of organochlorine pesticides. Juvenile females had polyovular follicles and blood estradiol-17beta levels two times higher than females from a reference lake. Juvenile males had smaller than normal penises, premature spermatogenesis, higher plasma estradiol and lower plasma testosterone than juvenile males from a reference lake (Guillette *et al.* 1994).

The association between organochlorine contamination and feminized sexual

development is consistent across investigators, geographical regions, and species. Adult male snapping turtles captured in the Great Lakes region bear high body burdens of PCBs, DDE, and other organochlorines and have shorter, female-like precloacal lengths-the distance between the posterior tip of the plastron (the ventral shell) and the cloaca-compared to males with low body burdens of organochlorines. Normally, males have longer precloacal lengths than females. Hatchling males reared from wild-caught, contaminant-containing eggs displayed the same ambiguous precloacal length, indicating that developmental exposure is probably responsible (de Solla *et al.* 2002).

Male sex reversal of wild-caught eggs exposed to chemical contaminants in the laboratory has been demonstrated in alligators (o,p'- and p,p'-DDE, Matter *et al.* 1998) and caiman (bisphenol A, Stoker *et al.* 2003), but did not occur in wild-caught green sea turtle (*Chelonia mydas*) hatchlings with p,p'-DDE burdens in egg yolk (Podreka *et al.* 1998). Laboratory experiments with farm-raised red-eared slider turtles (*Trachemys scripta*) showed that steroidal estrogens, hydroxylated PCBs, chlordane, p,p'-DDE, trans-nonachlor and Aroclor 1242 switched some, but not all males to females (Bergeron *et al.* 1994, Bergeron *et al.* 1999, Willingham *et al.* 2000). Steroidal estrogens, hydroxylated PCBs, trans-nonachlor, and p,p'-DDE can bind estrogen receptors in the alligator uterus (Vonier *et al.* 1996), suggesting a mechanism for contaminant-induced feminization. However, the non-steroidal compounds bind at concentrations higher than those found in the environment.

Changes in circulating steroid hormone levels are less consistent across species. Adult yellow-blotched map turtles (*Graptemys flavimaculata*) from southern Mississippi, USA have PCBs and DDTs in their liver, fat, and muscle (Kannan *et al.* 2000) and males captured from a contaminated site had lower circulating testosterone than males from a reference site. A small percentage of the exposed males had female-like levels of estrogen, but no changes in external sex characteristics were present (Shelby and Mendonca 2001). In the laboratory, hatchling male red-eared slider turtles that had been dosed with chlordane and Aroclor 1242 (a commercial PCB mixture) in ovo had lower blood levels of testosterone and females had lower progesterone and testosterone than vehicle-dosed controls. Estrogen was non-detectable in both the normal and chemical-exposed young turtles (Willingham *et al.* 2000). In contrast, even though external sex characteristics were feminized in PCB-exposed snapping turtles, circulating testosterone and estrogen concentrations were unaffected (de Solla *et al.* 1998).

Circulating steroid hormone levels are influenced by synthesis rate, disposal rate, and steroid binding protein concentration. Juvenile alligators exposed to different intensities of environmental contamination had different rates of testosterone metabolism, suggesting that contaminants may enhance testosterone hydroxylation (disposal), indirectly lowering circulating testosterone levels (Gunderson *et al.* 2001).

Population effects

Alligator population problems in Lake Apopka were clearly caused by pesticide contamination. Prior to the 1980 pesticide spill, alligator populations in the lake were flourishing, but declined by 90% during the four years following the spill and remain low. One year after the spill, juvenile abundance decreased severely. Clutch viability decreased four years after the spill and has remained low (Guillette and Crain 1995). USA Environmental Protection Agency analyses conducted in 2003 revealed that groundwater near the lake is still so contaminated that carbon filters have been installed on residential wells to protect humans (Environmental Protection Agency 2004). Because of alligator population declines, the pesticide concentrations in eggs and the endocrine and morphological status of juvenile alligators were investigated. Juvenile mortality immediately following the spill and continued poor clutch viability are probably due to direct toxicity (Guillette and Crain

1995). In addition, stalled population growth can reasonably be attributed to endocrine-mediated derangements in sexual development that impair breeding success of individuals chronically exposed to contaminants.

In a surprising contrast, snapping turtle population growth at a site heavily contaminated with PCBs, DDT metabolites, and other organochlorines appears to be unaffected. Even though eggs contain significant concentrations of contaminants and hatchling and adult males have feminized secondary sex characteristics, the population at this site is one of the densest recorded (de Solla *et al.* 2002). Apparently, endocrine disruption is mild-sexual development is slightly altered, but not enough to cause fertility problems.

Any link between endocrine disruption and population declines in yellow-blotched map turtles is tenuous. These turtles are listed as threatened under the USA Endangered Species Act, but the causes of recent rapid population declines are not known. No fertility or recruitment data are available to link mild endocrine disruption-slightly altered testosterone concentrations in males-to population declines. Other factors such as hatchling mortality due to flooding or nest predation have been suggested (Kannan *et al.* 2000).

Birds

Since the 1950s, fish-eating and predatory bird populations have suffered a variety of health problems due to organochlorine pollutants, including poor reproductive success, growth retardation, and goiter (an over-sized, over-worked thyroid gland). Predatory and fish-eating birds are burdened with a stew of organochlorines, with p,p'DDE and PCBs being the most common. Reproductive failure in bald eagles (*Haliaeetus leucocephalus*), brown pelicans (Pelecanus occidentalis), gulls (Larus occidentalis, Larus argentatus), and other birds of prey during the 1970s through the early 1990s was mainly due to eggshell thinning caused by high body burdens of p,p'-DDE (Lundholm 1997). How p,p'-DDE caused thin eggshells has been studied and debated since the 1970s. One well-accepted explanation is that p,p'-DDE blocks the prostaglandin signaling that stimulates the eggshell gland to deposit calcium in the shell (Lundholm 1997, Bowerman *et al.* 2000, Dawson 2000). Since the ban on DDT in North America and Europe, p,p'-DDE concentrations in birds have declined, eggshell thickness has improved in most species, and populations are recovering.

Endocrine-mediated effects

Three types of endocrine disruption have been investigated in wild birds: reproductive disruption, thyroid disruption, and glucocorticoid disruption. Although organochlorines clearly cause serious reproductive problems in wild birds, there is little evidence that sexual development is permanently affected. In the laboratory, p,p'-DDE and other DDT metabolites stimulated primordial germ cells to migrate into a more female-like position in the testes of male Western gull (*L. occidentalis*) embryos. At hatching, some male embryos even had oviducts (Fry and Toone 1981). No adult gulls with feminized testes or oviducts were collected in wild populations. To test whether early signs of feminization might indicate continued abnormal sexual development, Hart and colleagues (2003) studied a common tern (*Sterna hirundo*) breeding colony in Buzzard's Bay, MA. Many of these birds feed near New Bedford Harbor, a site heavily contaminated with PCBs, and their eggs have very high PCB concentrations, similar to those found in bald eagles incapable of reproducing. Approximately half of the just-hatched male tern chicks had primordial germ cells arranged in a female-like pattern, but no oviducts. No 21 day old male chicks had female-like tissue in the testis, nor did any adult males have ovarian tissue in the testis. The researchers concluded that feminization of the gonad was temporary and unlikely to compromise reproduction in common terns (Hart, *et al.* 2003).

Some evidence links PCB, dioxin, and p,p'DDE contamination to thyroid hormone

disruption in wild birds, but results differ between species and life history stages. In the 1970s, herring gulls (*L. argentatus*) in the Great Lakes region had goiters - over-sized, over-worked thyroid glands. This abnormality was associated with heavy organochlorine contamination in the fish upon which the gulls were feeding and led to the hypothesis that contaminant exposure upset thyroid hormone homeostasis (McNabb and Fox 2003). Subsequent work found that thyroxine (T4) concentration declined as polyhalogenated hydrocarbon in the yolk sac increased in cormorant chicks (*Phalacrocorax carbo*), but thyroid hormone concentrations were unrelated to yolk sac contaminant levels in herring gull and common tern chicks. To help untangle these contradictory results, McNabb and Fox (2003) measured thyroid status in herring gulls at three life history stages: just-hatched chicks and prefledgling chicks over a three year period (1998-2000) and adults collected from reference, low, and high PCB sites in one year (2001). Just-hatched chicks with high PCB concentrations in the yolk sac had decreased stored T4 in the thyroid gland, the gland was enlarged, and plasma T4 was reduced in 42% of chicks. PCB-laden chicks seemed to be barely maintaining circulating T4 by increasing T4 production and secretion. Prefledgling chicks from contaminated sites also had less stored T4 in the thyroid gland and the gland was hypertrophied, but plasma T4 was reduced in only 28% of chicks. Adult herring gulls also had enlarged glands and less stored T4, but all gulls from the contaminated sites had plasma T4 concentrations similar to those of birds from reference sites. Overall, herring gulls with high PCB body burdens seem to suffer hypothyroidism as chicks, but not as adults (McNabb and Fox 2003).

Laboratory studies in rats have helped to tease apart how PCBs and their metabolites meddle with thyroid hormones. PCBs do not interact with the thyroid receptor, instead they derail delivery of thyroid hormone to the cells by attaching to the thyroid transport proteins in blood, pushing off thyroid hormone. The free thyroid hormone is disposed of by deiodinases and uridine diphosphate glucuronosyltransferase (UDPGT) enzymes in the liver. PCBs also enhance activity of these disposal enzymes. The net effect is abnormally low levels of thyroid hormone in blood (Brouwer *et al.* 1998). Because thyroid function is highly conserved among vertebrates, similar mechanisms are predicted to operate in birds, but almost no data is available for UDPGT activity or thyroid transport proteins, particularly in wild birds (McNabb and Fox 2003).

Herring gulls in the Great Lakes region may also have compromised glucocorticoid status. Higher burdens of PCBs, PCDDs (polychlorinated dibenzodioxins), and PCDFs in the yolk sacs of 26 day old (two days pre-hatching) embryos were significantly linked to lower plasma corticosterone concentrations and suppressed gluconeogenic and lipogenic enzymes (Lorenzen *et al.* 1999).

No data directly link thyroid hormone or glucocorticoid disruption to fertility, behavior, or recruitment effects that could impact populations. However, when birds at the limit of their capacity to maintain thyroid status or glucose and lipid homeostasis experience additional stressors such as cold, temporary food deprivation, or even viral or parasite infections, survival could be threatened because the birds do not have sufficient thyroid hormone or corticosterone reserves to mount an appropriate metabolic response to the threat.

Current population effects

Birds of prey and fish-eating birds are still plagued by reproductive problems associated with p,p'-DDE and PCB body burdens. Some species seem to be more sensitive than others: Bald eagles near the Great Lakes still have high blood levels of PCBs and p,p'-DDE and have fewer chicks successfully leaving the nest than eagles from inland populations with lower blood levels of chemicals (Bowerman, *et al.* 2000). Higher PCB concentrations in the blood of nesting glaucous gulls (*Larus hyperboreus*) are linked to worse parental care-male

and female gulls are absent from the nest more often and for longer periods (Bustnes *et al.* 2001). In contrast, egg concentrations of PCBs 8-fold higher than those found in the most contaminated gulls had no effect on hatching or recruitment of young dippers (*Cinclus cinclus*) (Ormerod *et al.* 2000). None of the current population effects can be directly attributed to endocrine-mediated effects.

Mammals

Marine mammals throughout the world's oceans, including seals, sea lions, porpoises, dolphins, and some whales have high concentrations of organochlorine pollutants such as PCBs and pesticides stored in their blubber (Tanabe 2002, Le Boeuf *et al.* 2002, Fossi *et al* .2003). Except for plankton-feeding whales, marine mammals are top predators that accumulate the pollutants from their contaminated prey. Polar bears (*Ursus maritimus*) are also apex predators-they eat the seals and killer whales which have accumulated large body burdens of PCBs and organochlorine pesticides. As a consequence, polar bears can have very high concentrations of pollutants in their blood and fat.

Endocrine-mediated and fertility effects

Although a large body of literature documents contaminant body burdens in marine mammals, very few studies have specifically measured hormone levels or hormone-mediated processes. Body burdens of PCBs are associated with reproductive and thyroid hormone derangements in seals. Harbor seals (*Phoca vitulina*) fed organochlorine-contaminated fish from the Wadden Sea (Netherlands) during the early 1980s had normal levels of estrogen and progesterone in the blood and they ovulated, but only 30% became pregnant compared to 83% of seals fed uncontaminated fish (Reijnders, 1986). Seals fed an experimental diet of heavily PCB-contaminated fish had lower levels of thyroid hormones in their blood than seals fed mildly PCB-contaminated fish (Brouwer *et al.* 1998). Wild grey seals (*Halichoerus grypus*) off the Scottish coast have polybrominated diphenyl ethers (PBDEs, a type of flame-retardant) in their blubber and unlike the PCB case, seals with more PBDEs have higher thyroid hormone levels (Hall *et al.* 2003).

The association between PCB body burden and reproductive and thyroid disruption is consistent across species. In a heavily contaminated population of polar bears in Svalbard, Norway hormonal changes are linked with the total amount of PCBs and DDEs in the blood - female bears with higher contaminant burdens have higher blood levels of progesterone, but similar estrogen compared to females with low contaminant burdens (Haave *et al.* 2003). More heavily contaminated male bears have lower blood testosterone levels (Oskam *et al.* 2003). Regardless of age and sex, bears with higher contaminant burdens have lower blood levels of cortisol (the predominant stress hormone) and thyroid hormones (Oskam *et al.* 2004, Skaare *et al.* 2001). Statistically significant correlations between contaminant burden and hormone concentration in individual polar bears strongly suggest a dose-response relationship.

As described in laboratory rats (Brouwer *et al.* 1998), low circulating thyroid hormones in seals and polar bears could be the result of contaminant competition for thyroid transport proteins and contaminant-induced increases in thyroid hormone disposal enzymes. Neither thyroid transport proteins nor disposal enzyme activities have been investigated in marine mammals or polar bears.

Temporal changes in body burdens of PCBs, DDTs, and other organochlorines have been recorded. Total DDTs in California sea lion blubber are one-tenth the concentrations measured in 1970 (Le Boeuf *et al.* 2002) and in polar bears are one-half to one-tenth the concentrations measured in 1967 (Derocher *et al.* 2003). Conversely, PCB body burdens in polar bears have increased two to four-fold since 1967, probably due to slow transport times of contaminants into the Arctic (Derocher *et al.* 2003). Because no data on endocrine-mediated

effects are available for polar bears sampled in the late 1960s and because sea lion populations have increased even during the period of greatest DDT contamination (1948-1970), no clear evidence supports a temporal link between contaminant exposure and adverse endocrine-mediated effects in wild mammals.

Population effects

Although no causal links have been demonstrated, mass mortalities and locally declining marine mammal stocks have been attributed to high organochlorine body burdens (Le Boeuf *et al.* 2002). In direct contradiction to such an interpretation, California sea lion populations increased steadily throughout the 20th century, even though these marine mammals have some of the highest total PCB and DDT loads recorded, loads equivalent to or exceeding those found in polar bears (Le Boeuf *et al.* 2002).

The implications of the reproductive, thyroid, and adrenal hormone disruptions in the Svalbard polar bears are not fully understood, but these bears have a low reproductive rate compared to other populations (Derocher *et al.* 2003). Whether low fertility is due to contaminant-related hormone problems or to differing population age structure and harvesting pressure is a source of continuing research and debate (Haave *et al.* 2003).

REMEDIATION AND PREVENTION

Endocrine disruption in fish is strongly linked to sewage effluents containing steroidal estrogens and alkylphenol polyethoxylates, common detergent compounds. As more of the world's population becomes centralized in urban areas, the concentrations of both compounds in municipal sewage effluents are likely to increase. More advanced treatment of effluents before release to receiving waters has some potential for alleviating endocrine disruption as demonstrated by additional treatment of an estrogenic effluent in the Aire River, UK (Sheahan *et al.* 2002). Similarly, removal of endocrine-disrupting sterols and other wood-derived compounds has good potential for reducing the masculinizing and anti-reproductive effects of pulp mill effluents (Hewitt *et al.* 2002).

Many cases of endocrine disruption in wildlife are strongly associated with PCB and DDT contamination. Both types of organochlorines have been banned in North America and Europe since the 1970s and early 1980s, but their persistence and global distribution in soil, water, air and animal fat make them a particularly daunting threat to wildlife health. Even in remote areas with few or no local sources of contamination, such as Culebra Bay on the Pacific coast of Costa Rica, PCBs are detectable in marine sediments and in benthic marine worms (the sipunculid Phascolosoma perlucens) (Spongberg 2004, 2006). In addition, DDT is still used in much of the southern hemisphere for malaria control. Given the strong association between p,p'-DDE and reptile feminization, bird eggshell thinning, and polar bear reproductive hormone derangements, endocrine disruption is likely and potential wildlife population declines are possible wherever DDT is used. Balancing human and animal health concerns is and will remain a difficult social and political issue.

As evidenced by the PCB and DDT stories, banning known endocrine disrupters can reduce adverse effects on wildlife health and populations, but cannot immediately eliminate the problem. Persistent compounds with long half-lives in environmental media and biological tissues will continue to cause harm. Identifying and banning endocrine disrupting compounds with short half-lives is more likely to provide rapid improvement in animal health. To this end, international efforts at chemical screening and testing such as those coordinated by the Organization for Economic Cooperation and Development the USA EPA and the Japanese Ministry of the Environment seek to identify currently manufactured chemicals that act on the endocrine system and cause adverse health effects in wildlife or humans, then advise governments whether restrictions

or bans should be placed on these chemicals to protect wildlife and human health.

CONCLUSION

Applying causal criteria across vertebrate taxa confirms the difficulty in ascribing population level effects to a single cause such as contaminant-induced endocrine disruption (Table 1). In fish, the possibility of population level effects is inferred from impaired individual fertility. Substantial evidence directly links fertility impairment to endocrine disruption. In amphibians, population declines are occurring worldwide and causes may vary among regions,

TABLE 1

Applying causal criteria to evidence for endocrine-mediated, fertility, and population effects of contaminants

Effect	Contaminant	Strength of association	Consistency of association	Dose response	Mechanism	Temporal association
Fish						
Feminization	sewage effluent					
intersex gonad		roach	flounder common carp marbled sole white perch sand goby	roach	estrogen receptor	•
sex steroids		roach				
VTG induction		roach	flounder common carp marbled sole white perch sand goby	roach	estrogen receptor	strong
fecundity		•	•	•	•	•
fertility		roach	•	roach	•	•
fry survival		•	•	•	•	•
recruitment		•	•	•	•	•
abundance		•	•	•	•	•
Masculinization	kraft mill effluent					
secondary sex charac.		mosquitofish	least killifish	•	androgen receptor	•
sex ratio		eelpout	•	•	•	strong
fecundity		mosquitofish - no	•	•	•	•
fertility		•	•	•	•	•
fry survival		•	•	•	•	•
recruitment		•	•	•	•	•
abundance		•	•	•	•	•
Suppression	kraft mill effluent					
secondary sex charac.		white sucker			androgen receptor	•

TABLE 1 (*Continued*)
Applying causal criteria to evidence for endocrine-mediated, fertility, and population effects of contaminants

Effect	Contaminant	Strength of association	Consistency of association	Dose response	Mechanism	Temporal association
sex steroids	white sucker		lake whitefish			strong
			longnose sucker		estrogen receptor	
			largemouth bass	largemouth bass	Ah receptor	
			longear sunfish	longear sunfish	androgen receptor	
			perch			
			roach			
			mummichog	mummichog		
fecundity						
no effect		white sucker	longnose sucker roach	•	•	•
decrease		lake whitefish	perch	•	•	•
fertility		•				
fry survival impaired		largemouth bass	•	largemouth bass	•	•
recruitment		•	•	•	•	•
abundance		•	•	•	•	•

The five criteria are strength of association between contaminant exposure and the effect, consistency of association across species, demonstrated dose-response relationship between contaminant and effect, a potential physiological mechanism by which the contaminant induces the effect, and whether there is a temporal association between contaminant exposure and a particular effect. Only one species is listed under strength of association while multiple species are listed under consistency of association to show how frequently the particular effect has been documented. All species for which dose-response studies were available are listed. Mechanisms listed are not hypothetical, but those supported by published experimental data from wildlife species. (•) indicates no data available. NE, not evaluated (beyond scope of this review).

with contaminant-induced sexual disruption being one of many insults that stymie population growth. In reptiles, local populations of alligators have been dramatically reduced by direct toxicity and recovery has probably been slowed by sexual disruption, particularly feminization induced by chronic contaminant exposure. In contrast, heavily contaminated snapping turtles display feminized secondary sex characteristics, evidence of disrupted sexual development, but populations are densest at the most heavily contaminated site. In birds, population decline and recovery have been directly linked to p,p'-DDE-mediated eggshell thinning and subsequent banning of the parent compound DDT in the USA and Europe. Regional populations of bald eagles still suffer chick mortality related to PCB and DDT burdens, but no causal link with endocrine-mediated processes has been demonstrated. In mammals, contaminant-induced hormonal derangements in seals and polar bears have been documented, but no clear evidence links endocrine effects to population declines.

Strong associations between endocrine disruption and population effects exist for alligators and birds, but have not been demonstrated conclusively for fish, amphibians,

or mammals. Endocrine disruption is entirely plausible as a cause of slow or declining population growth in contaminated mammals such as polar bears since thyroid and glucocorticoid hormones mediate metabolic responses necessary for survival and sex steroids regulate sexual development, breeding behavior, and gamete production.

How much data will be enough for biologists to insist that endocrine disruption is a serious threat to wildlife? Do we wait until catastrophic population declines like those of predatory birds in the 1970s and 1980s "prove" that endocrine disruption has adverse effects on wild animals? Although endocrine disruption will rarely be the sole cause of failing recruitment or decreased abundance, it may subtly sabotage sexual development, sex ratio, and metabolic compensation for environmental stress. Endocrine disruption is unlikely to extirpate a species over its entire range, but in combination with other stressors such as habitat loss, over-harvesting, and global climate change, may contribute to local extinctions.

This review is based, in part, on a conference by the author on ocassion of the celebrations of the XXVth Anniversary of the Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, on April 21-22, 2006. The conference was sponsored by the Costa Rica-United States of America (CR-USA) Foundation for Cooperation thru a grant to José A. Vargas (CIMAR).

RESUMEN

Qué tan importante es la disrupción endocrina como amenaza para las poblaciones de vida silvestre? Esta revisión aplica criterios causales a estudios de poblaciones silvestres de peces, anfibios, reptiles, aves, y mamíferos para contestar tres preguntas: (1) Han sido documentados efectos, mediados por via endocrina, debido a exposición por contaminantes? (2) Han sido documentados efectos individuales adversos que pueden llevar a efectos en poblaciones? (3) Han sido documentados efectos a nivel de poblaciones?. En peces, la posibilidad de efectos a nivel poblacional es inferida por la disminución de la fertilidad individual. Evidencia sustancial vincula directamente la disminución de la fertilidad con la disrupción endocrina. En anfibios, a nivel mundial esta ocurriendo una disminución de las poblaciones y sus causas varían entre regiones, con la disrupción endocrina inducida por contaminantes como una de las causas que frustra el crecimiento poblacional. En reptiles, las poblaciones locales de lagartos han sido reducidas drásticamente por exposición directa a sustancia toxicas y su recuperación ha sido demorada probablemente por disrupción sexual, particularmente por la feminización inducida por exposición crónica a contaminantes. En contraste, tortugas lagarto altamente contaminadas muestran características sexuales secundarias feminizadas, evidencia de desarrollo sexual alterado, pero las poblaciones son más numerosas en los sitios más contaminados. En aves, el decrecimiento de las poblaciones y su recuperación han sido directamente ligadas con la exposición a residuos de p, p-DDE a través del adelgazamiento de la cáscara del huevo y con la prohibición del uso del compuesto DDT en USA y Europa. Aves depredadoras regionales todavía sufren mortalidad de polluelos relacionada con cargas de PCB y DDT, pero no han sido demostrados vínculos causales con procesos mediados via endocrina. En mamíferos, han sido documentados desórdenes hormonales inducidos por contaminantes en focas y osos polares, pero no hay evidencia directa que ligue efectos endocrinos con la disminución de las poblaciones. No obstante que la disrupción endocrina raramente es la única causa de fallos en reclutamiento o disminución de las abundancias, puede ser un saboteador sutil del desarrollo sexual, tasa de sexos, y compensación metabólica ante el estrés ambiental. La disrupción endocrina es poco probable que llegue a extirpar una especie de su ámbito entero, pero en combinación con otros tensores como pérdida de habitat, extracción excesiva, y cambio climático global, podría contribuir a extinciones locales.

Palabras clave: disrupción endocrina, peces, anfibios, reptiles, aves, mamíferos, tiroides, adrenales.

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