



## The role of animal behaviour in the study of endocrine-disrupting chemicals

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Numerous chemicals are known to interfere with the endocrine systems of animals. These chemicals, commonly referred to as endocrine-disrupting chemicals (hereafter EDCs), pose a particularly severe threat to animal health. They accumulate in body tissues and are highly persistent in the environment and thus can occur at significant concentrations far from their points of origin. Ecotoxicologists have used a number of animal species to identify physiological and morphological consequences of EDC exposure. Effects of exposure have typically been measured in terms of survival, development or aspects of reproductive anatomy and physiology. In recent years, ecotoxicologists have begun using behavioural endpoints as components of standard toxicological assays. There has been a concurrent although less widespread interest among animal behaviourists in understanding and studying the effects of these contaminants on animal behaviour. The purposes of this review are four-fold. First, we provide a primer on EDCs. Second, we summarize current knowledge about endocrine disruption of animal behaviour. Third, we describe the role that we envision for behaviour in the field of ecotoxicology. Finally, we hope to stimulate a dialogue between animal behaviourists and ecotoxicologists that will enhance our understanding of these environmental contaminants and their impacts on animal populations.

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In the 1970s, biologists in southern California, U.S.A., noticed more eggs than usual in the nests of western gulls, *Larus occidentalis*. The interval at which eggs were laid in these nests was shorter than in nests with normal clutches, suggesting that multiple females were laying eggs in each nest. Detailed observations revealed that 8–14% of gull pairs in the population comprised two females, and that there was a shortage of reproductively competent males (Hunt & Hunt 1977; Fox 1992). Subsequent laboratory experiments revealed a probable cause: exposure to the pesticide *o,p'*-DDT feminizes male gull embryos and interferes with their normal reproductive behaviour (Fry & Toone 1981; Fry et al. 1987). The DDT exposure hypothesis was also supported by evidence that contaminated populations had a higher frequency of

enlarged clutches than did the uncontaminated populations (Fox 1992).

This example illustrates two general points that we discuss in this review. First, endocrine-disrupting chemicals such as DDT have profound effects on animal behaviour. Second, behavioural measures can be useful bioindicators of endocrine disruption and be important in the study of endocrine-disrupting chemicals.

More than 100 chemicals originating from agricultural, industrial and municipal sources are known to interfere with the endocrine systems of animals, and thousands more are suspected of having similar effects (reviews in Toppari et al. 1996; Keith 1997; Crisp et al. 1998). Some of these chemicals were designed specifically to disrupt normal endocrine function (e.g. pesticides, oral contraceptives), but most endocrine-disrupting chemicals produce inadvertent effects in nontarget animals as a by-product of other functions. The first endocrine-disrupting chemicals (EDCs) to be described were oestrogen mimics and were labelled environmental oestrogens. Today we know that EDCs can affect most endocrine systems, from

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reproductive to pituitary and thyroid glands; in some cases, a single chemical can have multiple effects. Effects on humans and animals include reproductive impairment and abnormalities, reduced immune function, reduced or abnormal growth, decreased cognitive abilities, increased susceptibility to disease and increased mortality.

Animals are exposed to EDCs in the air, water and in their food. EDCs enter animals' bodies through the skin, gills, and even via the mother in utero or in ovo. One of the most insidious characteristics of EDCs is that, because they are lipid-soluble, they tend to accumulate in animal body tissues. This problem is further compounded by the process of biomagnification, in which chemical concentrations increase at higher trophic levels. In aquatic birds, contaminant concentrations are often 100 times greater in body tissue than in the surrounding water (Anderson & Hickey 1976; Norstrom et al. 1976), and significant biomagnification has been observed in several species of marine mammals (Ross et al. 2000; Ikonomou et al. 2002). Top predators are essential for maintaining the integrity of food webs; thus, biomagnification of EDCs can affect entire ecosystems by harming species at the highest trophic levels.

Another significant problem with EDCs is their persistence. Heavy metals, for example, cannot be degraded or destroyed. The half-lives of DDT and polychlorinated biphenyls (PCBs) in the bodies of some vertebrates can be many years. Although PCB mixtures have declined overall in the environment since the 1970s, chemical alterations have increased the relative abundance of the more persistent and toxic congeners (Helander et al. 2002). Animals in even the most remote areas of the world (e.g. polar bears, *Ursus maritimus*; black-footed albatrosses, *Diomedea nigripes*; Kannan et al. 2001a, b) carry significant loads of industrial chemicals in their tissues. Endocrine disruptors are transported by sediments, water and air (Wilkening et al. 2000); thus, there are few places in the world not contaminated by them.

### Mechanisms of Endocrine Disruption

Endocrine-disrupting chemicals can interfere with the biosynthesis, transportation, metabolism or binding of hormones. In the case of steroids, for example, biosynthesis can be disrupted by reduction of steroid precursors (cholesterol) or alteration of enzyme activity. In addition, some EDCs are concentrated in glandular tissue where they cause cell death and necrosis; in this case, the tissue responsible for hormone production is destroyed or significantly reduced. EDCs may affect hormone transportation by increasing the binding affinity of carrier molecules (e.g. sex-hormone-binding globulin) or by competing with endogenous hormones for access to binding sites, both of which affect hormone concentrations in the blood plasma or haemolymph. Endocrine disruption may also occur if the rate at which hormones are metabolized is altered. Some chemicals stimulate or inhibit the activity of enzymes, such as mono-oxygenases, that are responsible for hormone degradation. Finally, EDCs can bind to hormone receptors and activate or block

them; they can also bind to inactive sites and distort receptors enough that they are rendered dysfunctional. It is difficult to predict the binding effects of a particular EDC across species, because of taxonomic differences in binding affinities of cellular receptors (Le Drian et al. 1995).

Animals are often exposed to multiple EDCs and thus subject to multiple mechanisms of endocrine disruption (Porter et al. 1999). Little is known, however, about the broad-scale effects of chemical mixtures, but additive and synergistic effects have been documented in several species (Crews et al. 1995; Bergeron et al. 1999; Rajapakse et al. 2002). For example, Bemis & Seegal (1999) found that a mixture of methylmercury and PCBs reduced dopamine levels in rat brains more than either chemical did alone. Gupta & Gill (2000) found that rats exposed to lead in the presence of ethanol suffered alterations in motor coordination, activity level and aggressiveness. There has been much debate over how widespread the synergistic effects of EDCs are, or whether they exist at all. For example, results from a highly influential study by Arnold et al. (1996) could not be replicated by other laboratories, and the study was eventually retracted (McLachlan 1997).

### STANDARD ECOTOXICOLOGICAL APPROACHES

For animal behaviourists to understand the potentially important role of behaviour in the study of endocrine-disrupting chemicals, they must first understand a little about ecotoxicology. Below we describe some standard approaches used by ecotoxicologists, as well as how the peculiar characteristics of EDCs are forcing scientists to rethink these same methods.

### Dose-Response Relations

The standard approach to studying toxicity involves exposing isolated cells (in vitro), eggs (in ovo) or whole animals (in vivo) to varying concentrations of a chemical. A dose-response curve is the quantitative relation between the dose administered (or the exposure experienced) and the incidence or extent of adverse effects. For many chemicals, this relationship is sigmoidal, such that adverse effects increase dramatically above a threshold level. This approach works well when toxicity is immediately apparent and when a simple monotonic relationship exists between dose and response. However, many EDCs differ from classical pollutants (e.g. dioxins, polycyclic aromatic hydrocarbons) in two important respects.

First, the toxicological rule that 'the dose makes the poison' is not always applicable to EDCs. Hormone-mimicking EDCs can interact with endogenous hormones such that the threshold for that hormone is automatically exceeded (Welshons et al. 2003; Tokumoto et al. 2004). Furthermore, effects of some EDCs are greater at low than at high doses. For example, Hayes et al. (2002a) showed that the gonadal development of male leopard frogs, *Rana pipiens*, was retarded at low, environmentally relevant concentrations of the popular herbicide atrazine but not

at high concentrations. Unusual dose–response relationships, including U-shaped and inverted U-shaped curves, also occur with some EDCs (vom Saal et al. 1995; Alworth et al. 2002). U-shaped dose–response curves occur when the maximal response is produced at very low and very high concentrations, but not at intermediate ones; inverted U-shaped curves are the inverse. U-shaped and inverted U-shaped curves are extremely controversial in ecotoxicology, because they contradict the long-held belief that dose–response relationships are linear. Environmentally safe levels of most chemicals are established on the basis of this assumption. Although several studies have attempted to quantify the number of chemicals that might show nonlinear relationships (Davis & Svendsgaard 1990; Calabrese et al. 1999), their statistical methodologies have been criticized (Crump 2001), making it difficult to estimate the prevalence of such relationships. Some ecotoxicologists believe that nonlinear dose–response relationships are underreported because of their deviation from toxicology dogma (Calabrese & Baldwin 2003). Increased awareness of the problem of nonlinear dose–response relationships has led to calls for more studies that test a range of physiologically active doses and that are more attentive to positive and negative controls (Welshons et al. 2003). The detection of nonlinear relationships is an area in which behavioural studies might make significant contributions to ecotoxicology. Behaviour is sensitive even to small changes in hormone levels, so behavioural endpoints should provide particularly good response variables for the detection of nonlinear relationships (vom Saal et al. 1995).

The other way in which effects of EDCs differ from those of classical pollutants is that such effects are not always immediate. Exposure to EDCs during critical stages of development can produce permanent, organizational changes in anatomy, the consequences of which might not become apparent until adulthood. In addition, some effects of EDCs can be transgenerational: developing fetuses or eggs might be exposed to EDCs through parental exposure. Alternatively, EDCs can be transmitted directly from mother to offspring, either via egg yolk or breast milk. The dynamics of transfer have been well documented in some species (American kestrel, *Falco sparverius*: Fernie et al. 2000; grey seals, *Halichoerus grypus*: Debier et al. 2003). Such transgenerational effects should sound an alarm for behaviourists, who are well aware of the importance of parental loading of egg yolks with variable doses of gonadal steroids in birds and reptiles (e.g. Schwabl 1993). The relation between contaminant exposure and these maternal effects is only beginning to be explored (French et al. 2001).

## Common Ecotoxicological Endpoints

### *LD<sub>50</sub> and LOEC*

Two standardized measurements widely used by ecotoxicologists to quantify the effects of EDCs are the LD<sub>50</sub> and the LOEC. The LD<sub>50</sub> is the dosage that is lethal to 50% of exposed organisms. The LOEC is the lowest concentration that produces observable effects, which traditionally have

been endpoints such as morphological deformities, but which can also refer to behavioural impairments. LD<sub>50</sub> and LOEC have helped scientists to compare relative toxicity of chemical compounds and have guided them in choosing dosages for future experiments. EDCs usually show adverse endocrine effects well below LD<sub>50</sub> levels, so the LOEC may be particularly useful in establishing common ground between traditional toxicology studies and those focused on behavioural endpoints.

### *Development*

Researchers interested in sublethal doses of EDCs often focus on developmental endpoints because animals are most sensitive to perturbation in the earliest stages of life. The effects of EDCs on the processes of gastrulation, neurulation, early organogenesis and skeletal mineralization have been studied in numerous species (reviewed in Colborn et al. 1993; Iguchi et al. 2001). EDCs are known to interfere with metamorphosis of amphibians (Rosenshield et al. 1999; Boone et al. 2001) and may play a significant role in global declines in amphibian populations (Bridges & Semlitsch 2000). Growth rate is also affected by EDCs (Willingham 2001). Some pesticides act as endocrine disrupters of development in arthropods. Relatively new pesticides collectively called ‘growth regulators’ act by inhibiting the formation of chitin (necessary for the formation of a new exoskeleton) or by mimicking ecdysone (required for shedding an old exoskeleton) or juvenile hormone (involved in metamorphosis) and thus competitively bind those receptors (Audsley et al. 2000). The majority of pesticides, however, including the organochlorines, organophosphates and carbamates, are less taxonomically specific because they inhibit nerve impulse transmission. Once released into the environment, these ‘broad spectrum’ pesticides often act as EDCs on non-target species, including vertebrates.

### *Reproduction*

Gonadal steroids mediate many reproductive processes in vertebrates, from the development of sex organs to mating and reproduction. Thus, the reproductive system is a likely target for endocrine disruption, particularly by oestrogenic endocrine disrupters. The most frequently reported effects of EDCs are on sex determination, secondary sexual characters, oogenesis, spermatogenesis and the onset of sexual maturation (reviewed in Crisp et al. 1998). Such effects can occur through either direct exposure or indirect, transgenerational effects.

Some of the most significant field studies of endocrine disruption have focused on reproductive parameters. Eggshell thinning caused by DDT exposure led to the famous population declines in the 1970s in osprey, *Pandion haliaetus*, bald eagles, *Haliaeetus leucocephalus*, and other piscivorous birds in the U.S.A. (Wiemeyer et al. 1975). Other notable cases include the discoveries of both male and female reproductive structures in molluscs exposed to tributyltin from antifouling paints (Matthiessen & Gibbs 1998), altered sexual differentiation and reduced fertility in fish living downstream of sewage effluent and pulp mills (Howell et al. 1980; Purdom et al.

1994; Jobling et al. 1998), and demasculinization of male American alligators, *Alligator mississippiensis*, caused by exposure to DDT and its metabolites (Guillette et al. 1994). Hayes et al. (2002a) reported demasculinization of leopard frogs from exposure to atrazine.

The production of the egg yolk protein vitellogenin (which forms the basis of egg yolks) in males has proven to be a useful and reliable bioassay for the presence of oestrogenic compounds in fish and amphibians (Sumpter et al. 1996; Shelby & Mendoca 2001). Although much attention has focused on the feminization of male anatomy and physiology by EDCs, the prominent role that oestrogen plays in female reproduction all but guarantees that we have underestimated endocrine disruption of reproduction in females. Sexual differentiation has become another widely used metric of endocrine disruption (Guillette et al. 1994; Hayes et al. 2002b), but the non-linear relation between hormone levels and degree of differentiation in some species may make interpretation difficult (Hayes 1998).

## Model Systems

Animals used in endocrine disruption research are selected because they are convenient to maintain in the laboratory, their genetics or development have been well characterized, they are economically important or they are indicators of environmental quality. Zala & Penn (2004) pointed out several key confounds that can arise from laboratory studies. Examples of common model systems used in ecotoxicology include molluscs (mussels, *Mytilus* spp.; oysters, *Crassostrea* spp.), crustaceans (cladocerans, *Daphnia* spp.; grass shrimp, *Palaemonetes pugio*), fish (fathead minnows, *Pimephales promelas*; rainbow trout, *Oncorhynchus mykiss*; Japanese medaka, *Oryzias latipes*; guppies, *Poecilia reticulata*; eastern mosquitofish, *Gambusia affinis holbrooki*), amphibians (African clawed frog, *Xenopus laevis*, on which the widely used frog embryo teratogenesis assay-*Xenopus*, FETAX, is based; ranid frogs, *Rana* spp.), reptiles (red-eared slider, *Trachemys scripta elegans*; American alligator, *Alligator mississippiensis*), birds (Japanese quail, *Coturnix coturnix japonica*) and mammals (Norway rat, *Rattus norvegicus*; mouse, *Mus domesticus*). Some species, such as polar bears, cetaceans and piscivorous birds, are studied because their diet puts them at particular risk of exposure to EDCs (Hebert et al. 2000; Kannan et al. 2001a, b; Debier et al. 2003).

Although the use of model systems has its advantages insofar as it allows researchers to understand well the mechanisms of effects on a particular animal, it is not without its problems. Even taxonomically similar species can differ in how they are affected by the same EDC. For example, closely related species of frogs differ in the effects of EDCs on predator-induced stress (Relyea 2003). Some of this variation may be from taxonomic differences in the binding affinities of cellular receptors (Le Dren et al. 1995). In addition, the behaviour of model species is often not representative of their taxonomic group. For example, guppies and mosquitofish are live bearing, not oviparous like the vast majority of fish. *Xenopus laevis* is

unusual among amphibians by being entirely aquatic throughout its life cycle. Japanese quail, commonly used in studies of reproduction, are among the minority of bird species that are polygynous. Finally, many model systems used in toxicological research have been selected for characteristics that make them conducive to laboratory research. Some of these characteristics (e.g. large litter size in mice: Spearow et al. 1999), however, may confound the results of toxicity studies with behavioural endpoints (e.g. maternal care).

Although interspecific variation creates problems for the model systems approach, there can also be considerable intraspecific variation (between different age classes, sexes or different populations) in susceptibility to EDCs. For example, young animals may be particularly susceptible to the effects of endocrine disruptors when exposed during developmentally sensitive periods (Bergeron et al. 1999). In addition, hormones, most notably the sex steroids, differentially affect the physiology of males and females. Therefore, the effects of EDCs can be sex specific (Weiss 2002). However, the effects of EDCs on even highly stereotyped behaviour patterns such as swimming can show significant variation between individuals of the same age and sex (Kolok et al. 1998). Finally, genetically different strains, inbred lines or even wild populations of the same species may vary in the degree to which they are influenced by the same EDC. For example, strains of mice differ in the degree to which they are susceptible to the oestrogenic effects of 17 $\beta$ -oestradiol (Spearow et al. 1999). Although such intraspecific variation may be problematic for those trying to predict whether a particular chemical poses a threat to a particular group of animals, intraspecific variability also offers cause for hope, because genetic variation in susceptibility is a prerequisite for the evolution of resistance (Crews et al. 2000). Ironically, although genetic variability may allow some species to evolve resistance to environmental contaminants (Nacci et al. 2002), resistance leads to an increase in the number of chemicals that humans release into the environment. More than 600 species of arthropod pests have evolved resistance to pesticides since 1950 (Georghiou 1986), resulting in an acceleration in the development and application of new chemicals.

## INTEGRATING BEHAVIOUR AND ECOTOXICOLOGY

### Why Study Behavioural Effects of EDCs?

For decades, direct mortality has been the primary means of assessing chemical contamination of ecosystems. Endocrine-disrupting chemicals have taught us that contaminants can have myriad nonlethal effects on animal populations. Abnormal behaviour is one of the most conspicuous endpoints produced by EDCs, but until recently it has been underused by ecotoxicologists (Dell'Omo 2002). Warner et al. (1966) were among the first to recognize the utility of behavioural measures because such measures are relatively easy to evaluate and can be more sensitive than other endpoints. Behavioural

measures have great potential as bioindicators of endocrine disruption, because behaviour is the physical manifestation of an animal's integrated physiological response to its environment. In short, an animal's behavioural integrity is a proxy of its health. Behavioural measures have another indisputable advantage over physiological or morphological bioindicators: the tools needed to evaluate behaviour are relatively inexpensive. However, some researchers have argued that behavioural endpoints have yet to live up to their potential as bioindicators of contamination, although this topic has been widely debated (Doving 1991; Peakall 1992, 1996; Cohn & MacPhail 1996; Weis et al. 2001; Dell'Omo 2002).

### Individual- and Population-level Effects of EDCs

Here (Table 1) and elsewhere (Zala & Penn 2004), researchers have amassed considerable evidence of behavioural effects of EDCs. With a few exceptions, however, the behaviours studied have been limited to the level of the individual. Frequent examples include changes in activity level, foraging success, courtship behaviour, nest attentiveness and cognitive performance (Table 1). Relatively few of these studies have measured endpoints directly associated with fitness, such as Park and colleagues did with chemical communication in red-spotted newts, *Notophthalmus viridescens*. They found that even low doses of the pesticide endosulfan reduced pheromone gland size in males and females, and that exposed newts were then less responsive to conspecific chemical signals, which ultimately reduced individual mating success (Park et al. 2001; Park & Propper 2002).

Most biologists intuitively understand the link between individual behaviour and population-level processes (Sutherland 1996), but surprisingly few EDC studies have documented effects of altered behaviour on measures of offspring production and recruitment. Notable exceptions are the studies on western gulls cited earlier (Hunt & Hunt 1977; Fry & Toone 1981; Fry et al. 1987; Fox 1992), and studies on tree swallows, *Tachycineta bicolor* (McCarty & Secord 1999a, b; Secord et al. 1999). Secord et al. (1999) reported that PCB concentrations in tree swallow eggs and nestlings collected along the Hudson River (New York, U.S.A.) were up to 15 times higher than in PCB-contaminated populations in the Great Lakes region, U.S.A. Birds at contaminated sites built smaller, poorer-quality nests, and abandoned these nests more frequently, than did the birds at reference sites (McCarty & Secord 1999a, b). Largely because of the rate of nest abandonment, reproductive success was lower at the contaminated sites (McCarty & Secord 1999b). We believe that animal behaviourists can contribute greatly to the further integration of behavioural studies into ecotoxicology by helping to bridge the gap between behavioural impairment and the changes in reproductive success that are likely to lead to population declines.

### Community-level Effects of EDCs

Changes in some types of behaviour, particularly foraging and antipredator behaviour, can have dramatic consequences not only for individual fitness, but for entire communities. Ecologists are only beginning to examine community-level effects of exposure to pollutants, and it is unknown whether pollution alters competitive and predatory interactions between species or affects some community members more than others (Clements et al. 1988; Lefcort et al. 1999). Most studies of contaminants and predator-prey systems suggest that the effects of EDCs on multispecies interactions are difficult to predict. For example, when Bridges (1999) exposed both leopard frog tadpoles (prey) and red-spotted newts (predator) to the pesticide carbaryl, there was no overall effect because both the predator's and prey's behaviours were compromised. Another good example comes from work on mummichogs, *Fundulus heteroclitus*, by Weis and colleagues. Fish from areas of New Jersey, U.S.A., highly contaminated by heavy metals showed lower prey capture success under laboratory conditions than did fish collected from reference sites (Smith & Weis 1997). Fish collected from reference sites also showed significant declines in prey (grass shrimp) capture rates when housed in tanks with contaminated sediments (Smith & Weis 1997). The causal mechanism for this decreased performance appears to be altered levels of neurotransmitters or thyroid hormones (reviewed in Weis et al. 2001). Although the researchers did not measure reproductive success, contaminated fish were more vulnerable to predation by crabs (Smith & Weis 1997; Weis et al. 1999), suggesting a double threat by heavy metals to mummichog populations and the possibility that contaminant effects extend across three trophic levels.

The study of animal behaviour has a rich tradition of combining mechanistic studies, often conducted in the laboratory, with field estimates of reproductive success in an effort to draw connections between the behaviour of individuals and the populations and communities to which they belong. This combination of proximate and ultimate approaches is critical to dissecting correlational from causal relations in ecotoxicology (Blus & Henny 1997). The challenge facing researchers is to make these connections in the context of endocrine disruption, where the biological rules have been changed in a few short decades.

### What Can Animal Behaviourists Do?

Several of the experimental protocols used in the studies listed in Table 1 were widely used by animal behaviourists long before they were applied to the study of endocrine disruption, including the intruder-response paradigm, the open field test, copulatory behaviour assays (e.g. lordosis in rodents) and spatial learning tests. Other widely used behavioural tests (e.g. foraging under predation risk, responding to playback calls, mate choice trials), although less standardized, could also be used by ecotoxicologists interested in behaviour. Experimental designs that are commonly used in behavioural ecology, and increasingly in ecotoxicology, include using individuals as their own

**Table 1.** Effects of endocrine-disrupting chemicals (EDCs) on animal behaviour (see also text and Zala & Penn 2004)

Behavioural effect	Taxa	Endocrine disrupter	References
Activity, locomotion			
Induced ataxia	Egret ( <i>Ardea albus</i> )	Methylmercury	Bouton et al. 1999
Impaired balance, righting response	Gull ( <i>Larus argentatus</i> )	Lead	Burger & Gochfeld 2000
Decreased activity	Frog ( <i>Xenopus laevis</i> )	Aroclor 1254 (PCB)	Jelaso et al. 2002
Decreased activity in females	Rat ( <i>Rattus norvegicus</i> )	Bisphenol A	Kubo et al. 2001
Increased activity (some hyperactive)	Goldfish ( <i>Carassius auratus</i> )	DDT	Weis & Weis 1974
	Goldfish	Atrazine	Saglio & Trijasse 1998
	Dove ( <i>Streptopelia risoria</i> )	DDE/PCB mixture	McArthur et al. 1983
	Rat	Tributyltin	Ishido et al. 2002
	Rat	DES, bisphenol A	Kubo et al. 2003
Increased 'stress'	Platyfish ( <i>Xiphophorus maculatus</i> ), Swordtail ( <i>X. helleri</i> )	Nonylphenol, methoxychlor	Magliulo et al. 2002
Decreased locomotor performance	Minnnow ( <i>Pimephales promelas</i> ) Frogs ( <i>Rana clamitans</i> , <i>R. pipiens</i> )	Heavy metals* PCB 126	Kolok et al. 1998 Rosenshield et al. 1999
Feeding			
Reduced prey capture, foraging efficiency	Mummichog ( <i>Fundulus heteroclitus</i> )	Heavy metals†	Smith & Weis 1997; Weis et al. 2001
	Bass ( <i>Micropterus salmoides</i> )	Pentachlorophenol	Mathers et al. 1985; Brown et al. 1987
	Trout ( <i>Oncorhynchus mykiss</i> )	DEF, 2,4-DMA	Little et al. 1990
	Salmon ( <i>Salmo salar</i> )	Fenitrothion	Morgan & Kiceniuk 1990
	Frog ( <i>R. catesbeiana</i> ) tadpoles	Heavy metals	Rowe et al. 1996
Antipredator			
Reduced fear response	Mouse	Coumesterol	Garey et al. 2001
Increased fear response	Mallard ( <i>Anas platyrhynchos</i> )	Methylmercury	Heinz 1979
Increased vulnerability to predation	Mayfly ( <i>Baetis</i> ) larvae	Azinphomethyl fenvalerate	Schulz & Dabrowski 2001
	Crab ( <i>Uca pugnax</i> )	Temefos	Ward et al. 1976
	Grass shrimp ( <i>Palaemonetes pugio</i> )	HgCl or MeHg	Kraus & Kraus 1986
	Grass shrimp	Methyl-, ethylparathion	Farr 1977
	Mummichog	Methylmercury	Weis et al. 1999
	Mosquitofish ( <i>Gambusia affinis holbrooki</i> )	Mercury	Kania & O'Hara 1974
	Minnnow	Cadmium	Sullivan et al. 1978
	Stickleback ( <i>Gasterosteus aculeatus</i> )	Bis(tributyltin)oxide	Wibe et al. 2001
	Trout ( <i>O. mykiss</i> )	Carbaryl, pentachlorophenol	Little et al. 1990
	Salmon ( <i>S. salar</i> ) parr	Sumithion (fenitrothion)	Hatfield & Anderson 1972
Decreased response to predator cues	Snail ( <i>Physella columiana</i> )	Heavy metals	Lefcort et al. 2000
	Goldfish	Atrazine, diuron	Saglio & Trijasse 1998
	Salmon ( <i>O. tshawytscha</i> )	Diazinon	Scholz et al. 2000
	Frog ( <i>R. luteiventris</i> )	Heavy metals	Lefcort et al. 1999
Increased feeding under predation risk	Stickleback	Ethinyl oestradiol	Bell 2004
	Frog ( <i>H. versicolor</i> ) tadpoles	Carbaryl	Bridges 1999
Communication			
Reduced pheromone production	Newt ( <i>Notophthalmus viridescens</i> )	Endosulfan	Park & Propper 2002
Decreased response to maternal calls	Mallard	Methylmercury	Heinz 1979
Courtship, mating			
Separation of precopulatory pairs	Amphipod ( <i>Hyalella azteca</i> )	Lindane	Pascoe et al. 1994; Blockwell et al. 1998
	Amphipod ( <i>Gammarus pulex</i> )	Ethinyl oestradiol, bisphenol A	Watts et al. 2001
Decreased or impaired male courtship	Cichlid ( <i>Sarotherodon mossambicus</i> )	Endosulfan	Matthiessen & Logan 1984
	Goldfish ( <i>C. auratus</i> )	17 $\beta$ -oestradiol	Bjerselius et al. 2001
	Medaka ( <i>Orzyzias latipes</i> )	Octyphenol, 17 $\beta$ -oestradiol	Gray et al. 1999
	Medaka	Phenol	Colgan et al. 1982
	Guppy ( <i>Poecilia reticulata</i> )	17 $\beta$ -oestradiol	Oshima et al. 2003
	Dove ( <i>Zenaida macroura</i> )	PCBs	Tori & Peterle 1983
Decreased male mating behaviour	Quail ( <i>Coturnix coturnix japonica</i> )	Ethinyl oestradiol, DES	Halldin et al. 1999
	Quail	Vinclozolin	McGary et al. 2001
	Quail	Methoxychlor	Ottinger et al. 2001
	Rat	Bisphenol A	Farabollini et al. 2002
	Rat	TCDD	Bjerke et al. 1994

(continued)

Table (continued)

Behavioural effect	Taxa	Endocrine disrupter	References
Increased female reproductive behaviour	Rat	<i>o,p'</i> -DDT	Etgen 1982
Increased male mating behaviour	Kestrel ( <i>Falco sparverius</i> )	PCBs	Fisher et al. 2001
Parental behaviour			
Reduced nest attentiveness, maintenance	Minnow Dove ( <i>S. risoria</i> ) Gull ( <i>Larus hyperboreus</i> ) Falcons ( <i>F. mexicanus</i> , <i>F. columbarius</i> )	Lead DDE/PCB mixture PCBs† DDT, DDE†	Weber 1993 McArthur et al. 1983 Bustnes et al. 2001 Fyfe et al. 1976
Reduced, impaired incubation	Dove Tern ( <i>Sterna forsteri</i> ) Gull ( <i>L. argentatus</i> )	DDE/PCB mixture PCBs, dioxins† PCBs, dioxins†	McArthur et al. 1983 Kubiak et al. 1989 Fox et al. 1978
Social behaviour			
Increased response to intruder	Rat	Bisphenol A	Farabollini et al. 2002
Increased rough play	Macaque ( <i>Macaca mulatta</i> )	TCDD	Schantz et al. 1992
Increased rough play in females	Rat	Vinclozolin	Hotchkiss et al. 2002
Altered sociosexual behaviour	Rat	Bisphenol A	Dessi-Fulgheri et al. 2002
Learning, memory			
Reduced associative learning	Frog ( <i>R. catesbeiana</i> ) tadpoles Macaque	Lead PCBs	Strickler-Shaw & Taylor 1991 Rice 1997
Reduced spatial learning, memory	Rat	PCBs	Roegge et al. 2000

Unless otherwise indicated, chemicals were administered in the laboratory via injection, water or food consumption or waterborne exposure.  
\*Laboratory-reared fish exposed to contaminated sediments.

controls, cross-fostering and common garden experiments. Animal behaviourists can contribute directly to the burgeoning interest in EDCs and behaviour by continuing to develop and standardize behavioural assays that can be used across a range of taxa (Dell'Omo 2002).

Behavioural paradigms such as copulatory assays and maze tests in rodents have contributed much to our understanding of EDCs, but they are based on stereotypy and repeatability, not on a thorough understanding of the factors that ultimately affect fitness. Animal behaviourists need to lend their expertise to the design of behavioural assays that are grounded in natural history and an understanding of life histories; these assays will help to generate testable hypotheses that can be applied to field studies (Sandheinrich & Atchison 1990). Parmigiani et al. (1998) referred to this as the 'etho-toxicological' approach. Feeding, antipredator and reproductive behaviour patterns are all fertile areas for the development of new behavioural assays and could provide excellent opportunities for collaboration between animal behaviourists and ecotoxicologists. For example, several researchers have successfully developed a test for two species of amphipod, *Hyalella azteca* and *Gammarus pulex*, based on their pre-copulatory mate-guarding behaviour (Pascoe et al. 1994; Watts et al. 2001). These assays may not be good predictors of EDC effects on humans, but they have tremendous potential for rapid in situ assessments of contamination (Blockwell et al. 1998).

Animal behaviourists can contribute to the increased interest in behavioural endpoints by ecotoxicologists by fostering connections with their colleagues in this area.

Collaborative research projects, peer review of grant proposals and journal articles, sharing of field data and reporting of anomalous behaviour are all important steps that we can take to help integrate these formerly disparate disciplines. Whenever possible, animal behaviourists should report environmental or laboratory conditions that may be relevant to ecotoxicologists (e.g. pH, dissolved oxygen), and that may allow them to link their work more readily with behavioural studies.

### Challenges that EDCs Present for Field and Laboratory Research

Just as ecotoxicologists are becoming aware of the importance of behaviour in assessing the effects of endocrine disruption, animal behaviourists must become aware of the effects that EDCs might have on their study subjects. We have described many avenues of EDC exposure for animals in the field, but laboratory animals are at significant risk as well. For example, municipal water sources can contain trace levels of many contaminants, including lead, arsenic and atrazine. The use of treated water (e.g. reconstituted reverse osmosis water) can help to reduce the cumulative effects of these contaminants. More significantly, EDCs are known to leach from numerous laboratory-grade plastics, including polyvinyl chloride (phthalates, DEHA; Jaeger & Rubin 1972), polystyrene (nonylphenol; Soto et al. 1991) and polycarbonate (bisphenol A; Krishnan et al. 1993). Flexible and recycled plastics are particularly likely sources of EDC exposure.

Leaching increases when plastics are heated, but Howdeshell et al. (2003) recently found that bisphenol A leached from polycarbonate animal cages immersed in water at room temperature. Alternatives to plastics that are more inert (e.g. glass) or less likely to leach EDCs (e.g. silicone) can help to reduce the problem. Laboratory animal feed represents another heretofore underappreciated route of EDC exposure. Thigpen et al. (1999) described the phytoestrogen content of several commercial rodent feeds, but more work needs to be done in this area.

Recent years have seen tremendous interest within the animal behaviour community in applying behavioural research to conservation problems (Clemmons & Buchholz 1997; Caro 1998; Sutherland 1998). The problem of endocrine-disrupting chemicals provides the perfect opportunity for animal behaviourists to put that ambition into action. In this review, we have summarized two primary areas where behavioural expertise is needed in the field of ecotoxicology: (1) the development and standardization of behavioural assays that can be used as bioindicators of EDC contamination and (2) a better understanding of how behavioural impairment from EDC exposure can have population- and community-level consequences. We hope that animal behaviourists will meet this challenge and help to address environmental problems that ultimately concern us all.

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