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Pollutants affect development in nestling starlings *Sturnus vulgaris*

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Summary

1. Pollutants as endocrine disrupting chemicals (EDCs) are of worldwide concern, due to their detrimental effects on the physiology and behaviour of wildlife. One major source of EDCs is sewage treatment works which serve as foraging grounds for many bird species. However, the risks of consuming EDCs to wild birds at these sites have been largely overlooked.

2. We have previously quantified contamination levels of invertebrates from within filter beds of sewage treatment works and the intake rates of these invertebrates by wild European starlings feeding at these sites. Our work to date has shown that environmentally-relevant EDC exposure results in reduced immunocompetence, increased song behaviour and neural development in adult starlings.

3. In the present study, we hypothesized that nestling starlings exposed to similar EDC levels from contaminated prey, from parental feeding at sewage treatment works, would show altered growth and physiology.

4. Our findings show that EDC exposure results in reduced growth and immunocompetence in nestling birds. However, there was no effect on corticosterone or haematocrit levels. As growth rates and immunocompetence are likely to be related to survival rates, we suggest that these exposure levels are likely to adversely affect recruitment into the adult population.

5. *Synthesis and applications.* These results highlight the potential transfer of EDCs between semiaquatic or aquatic organisms and terrestrial organisms up the food chain at sites of sewage treatment works, broadening the scope of potential routes of exposure. Our findings suggest that birds foraging in these sites are at risk of physiological manipulation from EDCs and reduced body condition as a result. We advocate further work to evaluate the potential for EDCs from sewage treatment works to bioaccumulate and modulate the condition of wild organisms feeding on contaminated prey. Our results suggest that changes in management practices of sewage treatment works are required. We support measures to reduce EDC levels

in sewage treatment works and that these sites should be designed to exclude or deter wildlife from foraging on contaminated prey. The management implications of such policy – oriented steps will ensure the health of wildlife foraging at sewage treatment works.

Introduction

In the last 20 years there has been significant concern over a group of natural and synthetic

pollutants which act as endocrine disrupting chemicals (EDCs) (Colborn, Saal & Soto 1993;

Kavlock *et al*. 1996; Jobling *et al*. 1998). These chemicals alter the normal function of the

endocrine system (Tabb & Blumberg 2006), with knock-on effects for other physiological

functions (e.g. immune system; Hamed 2000). Because of their specificity for hormone

receptors, some EDCs can elicit physiological changes in aquatic organisms at very low

concentrations of only a few nanograms/L of water (Routledge *et al*. 1998). The main EDC

sources are anthropogenic, such as discharges from agricultural, municipal and industrial

wastewaters (Dosis & Kamarianos 2007). To date most studies on the physiological effects of

EDC exposure have focused on aquatic organisms (e.g. Sumpter, Jobling & Tyler 1996; Mills

& Chichester 2005).

Chemicals which manipulate the endocrine system by binding to hormone receptors for a

range of steroid hormones have been shown to alter reproductive anatomy (Jobling *et al*.

1998; Clotfelter, Bell & Levering 2004), reduce reproduction rates (Iguchi & Katsu 2008),

result in somatic anatomical malformation (e.g. Sower, Reed & Babbitt 2000) and change the

body condition of aquatic organisms (Zhong *et al*. 2005). However, as exposure levels of wild

organisms to EDCs are often hard to quantify, predicting the environmentally-relevant

detrimental effects is often difficult, if not impossible (Denslow & Sepúlveda 2007) and often

a causal link between exposure to EDCs and the physiological and behavioural effects of

endocrine disruption in some wild organisms is difficult to demonstrate (Jobling & Tyler

2006).

Detrimental effects of EDCs should be even more pronounced in young animals, which

have lower body mass, a faster cell division rate than adults, and are in the middle of their

developmental process (see Grote *et al*. 2009). In addition, such effects may have long lasting

consequences as these developing animals may produce markers of developmental stress (Buchanan *et al.* 2003). To date, most evidence of any effects in young terrestrial animals comes from laboratory studies, for example, exposure to the estrogen 17 alpha-ethinylestradiol during development altered reproductive physiology and behaviour in adult female Sprague-Dawley rats (Della Seta *et al*. 2008). Experimental effects of ecologically - relevant levels of EDCs on young wildlife remain largely untested.

We have recently shown that invertebrates such as earthworms *Eisenia fetida* living within the biofilm of the sewage filter beds bioaccumulate a range of EDCs (Markman *et al*. 2007). In the sewage sites sampled, *E. fetida* was found to contain substantially higher levels of 17 β estradiol (E2), dibutyl phthalate, dioctyl phthalate and bisphenol A compared to *E. fetida* sampled in organic compost sites (Markman *et al*. 2007). This is of concern because the rich invertebrate macrofauna of the sewage treatment works attract terrestrial species from higher trophic levels such as birds (Fuller & Glue 1981; Frederick & McGehee 1994) and bats (Park & Cristinacce 2006; Kalcounis-Rueppell *et al*. 2007). These terrestrial vertebrate predators feed on the diverse range of invertebrates, which either spend part (e.g. dipterans) or their entire lifecycle (e.g. oligochaeta) within the sewage biofilm (Learner & Chawner 1998).

Starlings often feed at sewage works and use earthworms (*E. fetida*) as their prey items (Feare 1984). Using contamination levels calculated from field sampling (Markman *et al*. 2007), we recently tested the impact of the suite of estrogenic chemicals identified in our earthworm samples on the physiology and behaviour of wild caught adult European starlings *Sturnus vulgaris* Linnaeus. Our results demonstrated that adult male starlings, experimentally dosed with the full spectrum of chemicals identified in the earthworm samples, showed reduced immunocompetence, but enhanced song complexity due to hypermasculinisation of the brain, compared to birds in the control group (Markman *et al.* 2008). As starlings also feed their young with invertebrates from contaminated areas such as sewage works during the

breeding season (Root 1990; S. Markman unpublished data), in the present study we sought to test the effects of the same chemicals on nestling development. We hypothesized that nestlings fed the same suite of EDCs, at an age - adjusted environmentally - relevant dose levels as calculated in our previous work on adults (Markman *et al*. 2008), would show reduced physiological condition and growth compared to control birds. Specifically, we predicted that reduced physiological condition would be represented by a reduction in haematocrit levels, immunocompetence, growth, and increased corticosterone production in experimental nestlings, compared to control nestlings. Corticosterone is the principle avian stress hormone and elevated body levels are indicative of a physiological challenge to homeostasis. Therefore, we further predicted an increase in basal levels of corticosterone as an indicator of chronic stress, as our manipulations sought to expose birds to an EDC dose twice daily as an environmental stressor during early development (see details below).

Materials and methods

STUDY SITE

This study was carried out in 2006 in Cambridgeshire, England, U.K.[Give latitude & longitude] Starling nest boxes were identified and monitored for reproductive activity from 12 April 2006, before egg laying. Breeding starlings were documented foraging up to 2 km away from their nest, even when the availability of suitable foraging habitat was low [do you mean low? You would expect them to forage further away if the area round the nest was low quality. Please check sentence and rephrase as appropriate.] around their nest area (Feare 1984; Bruun & Smith 2003). Therefore, all nests included in the study, were at least 5 km away from active sewage treatment works, reducing the risk of parents feeding on contaminated prey items during the experiment. We tested the effects of EDC exposure on nestling development using 10 broods of starling nestlings (N = 48 nestlings). Before and

during the experimental dosing, all nestlings were weighed from hatching until 15 days of age, which is a few days before fledging at 21 days of age, to avoid premature fledgling. Nestlings were individually marked on their claws by different colours of nail varnish. At 14 days of age, all nestlings were individually colour ringed.

CHEMICAL ANALYSIS AND DOSING

Dose rates were set following the calculation of chemical contamination levels of invertebrate prey from 20 sewage treatment works across south-west UK in 2003/4 (Markman *et al*. 2007). In this study, we found that the main prey item taken by adult starlings was the earthworm *E. fetida* and it is well established that earthworms are included as a prey item of nestling starlings (Feare 1984; Wright *et al*. 1998). Individual adult starlings took in on average 14.4 g day $^{-1}$ wet weight of invertebrates from the sewage filter beds (Markman *et al*. 2008). As the daily food intake of invertebrates for adult starlings is approximately 30 g day $^{-1}$ wet weight (Feare 1984) during the period when they are feeding nestlings, food intake from sewage filter beds represents ca – 50 % of their daily food intake (Markman *et al*. 2008). To be conservative in our dosing regime of starling nestlings in the present study, we assumed that their parents brought 50 % of prey items from sewage filter beds. Doses were adjusted according to daily food intake by a nestling starling. The mean daily food intake of a single starling nestling between 1-5 days old is ca 25 g wet weight day $^{-1}$, between day 6-10 ca 50g wet weight day $^{-1}$ and between day 11-15 ca 45g wet weight day $^{-1}$ (Tinbergen 1981). By multiplying half of each of these food intakes by the levels of identified EDCs per gram of invertebrates based on data in Markman *et al*. (2008), we calculated the following daily dose levels to be administered to the nestlings: 1 – 5 days old, 125 ng of 17 β estradiol (E2), 75 ng dibutyl phthalate, 325 ng dioctyl phthalate and 50 ng bisphenol A in 10 μL of sunflower oil. From day 6 - 10, 250 ng of 17 β estradiol (E2), 150 dibutyl phthalate, 650 ng dioctyl phthalate and 100 ng bisphenol A, and from day 11 - 15, 225 ng of 17 β estradiol (E2), 135 ng dibutyl

phthalate, 585 ng dioctyl phthalate and 90 ng bisphenol A in 10 µL of sunflower oil. From days 1-5, doses were administered by a gavage needle and from days 6-15, doses were administered via oil-injected mealworm *Tenebrio molitor* Linnaeus. We dosed the nestlings twice daily (early morning and late afternoon to mimic daily nestling feeding peaks and to aid absorption in the gut), by dividing the above doses into two. Control nestlings received 10 µL of sunflower oil only via gavage (days 1-5) or inside a mealworm (days 6-15), in similar ways to the treatment nestlings. We alternated the order of daily dosing each brood to balance for the time of the day effect across the different nests. Parents resumed provisioning their nestlings usually within a few minutes of dosing.

EXPERIMENTAL PROCEDURE

We determined initiation of egg laying, clutch size and hatching dates through daily visits to nest boxes. Once the nestlings had hatched, nestlings within a nest were paired, matched for body size and each member of the pair was assigned to one of two treatment groups: control or treatment (EDC dosed). In the case of odd number of nestlings in a given brood, we alternated the treatment of the odd nestling between broods. Any potential minor variation in nestling size/body mass within these pairings was balanced within broods by swapping the treatment for the largest nestling in each case. This design of within-nest manipulation allowed control for differences in parental and territorial quality between the different pairs of parents.

We measured the nestlings at hatching and then at 5, 10 and 15 days old. These measurements included: body mass, tarsus length, and wing length (at day 15 only). On day 11, the cell-mediated immune response of the nestlings was tested by using an injection of phytohaemagglutinin (PHA) into both wings webs. PHA is a plant lectin, which promotes a hypersensitivity reaction and has been used extensively to test avian immune function (Lochmiller, Vestey & Boren 1993). The thickness of both wing webs was measured (taking

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an average of 3 measurements) at the same location on the wing before injection and 24 hours after injection, using callipers (Moore & Wright™; to 0.1 mm). PHA (Sigma L-8754) in phosphate buffered saline (PBS; 0.150µg PHA in 50 µL PBS in each wing web) was injected into both wings webs of each bird (following Lochmiller, Vestey & Boren 1993; Smits, Bortolotti & Tella 1999; Granbom, Raberg & Smith 2005). Blood samples were obtained for basal corticosterone levels (samples taken within 3 minutes of nest disturbance) and haematocrit levels on day 15 post-hatch. Fifty microlitres of blood were taken by venepuncture in capillary tubes and spun for 5 min at 13,000 r.p.m. in a centrifuge (Jouan A13, VA, USA). The resultant packed cell length was measured to the nearest mm and the percentage of blood cells in each sample was calculated.

For the determination of corticosterone levels, blood samples (100 µL) were collected in heparinized capillary tubes after puncture of the brachial vein with a 25 gauge needle, centrifuged and the plasma stored at -20 ºC for later hormone assay. Corticosterone concentrations were measured after extraction of 20 µl aliquots of plasma in diethyl ether, by radioimmunoassay (Wingfield 1994) using anti-corticosterone antiserum (code B21-42, Endocrine Sciences, Tarzana, CA) and [1,2,6,7-3H] - corticosterone label (Amersham, UK). The extraction efficiency was 75-90%. Samples were run in one assay with 50% binding at 123 pg per tube, and the detection limit (for 7.3 µL aliquots of extracted plasma) at 0.45 ng mL^{-1}.

STATISTICAL ANALYSIS

All data were reduced to single parameter estimates for each nestling prior to analyses. Five nestlings died before day 15 (1 experimental, 4 control), so the statistical analysis was conducted on the basis of the 43 surviving nestlings in the following brood sizes: (i) two chicks (n= number of broods, n=1), (ii) three chicks (n=2), (iii) four chicks (n=2), (iv) five chicks (n=3) and (v) six chicks (n=2).

We used repeated measures ANOVA, with nestling age (i.e. 0 = hatching date, 5, 10 and 15 days old) and treatment within nest (i.e. control nestlings versus EDC dosed nestlings) as within-subjects effects. To analyze the type of data that we had, while using these two within-subjects effects in a repeated-measures ANOVA, we had to use the average value of each dependent variable across all the chicks of one treatment in a given brood as one datum point. This meant that we had two average values of each dependent variable for each brood for a given age, one for the control nestlings and the second for the EDC dosed nestlings. In that way, we could perform repeated measures ANOVA using both nestling age and treatment within a nest as within-subjects effects including the interaction term between these two repeated measures. We then explored the linearity of nestling age effect by using polynomial contrasts. We used brood size as a between-subject effect in the above mentioned repeated measures ANOVA. Where dependent variables were recorded only once, we used repeated measures ANOVA only with treatment within nest as the repeated measure and brood size as between-subjects effects. However, as brood size effects were not significant across the analysis of all the dependent variables (all P values > 0.05), we excluded brood size as a possible effect from all the repeated measures ANOVA models that are reported later. We used SPSS version 16 (SPSS Inc., Chicago, IL, USA) for all our statistical analyses.

Results

At hatching there was no significant difference between the treatment groups in nestling body mass (repeated measures ANOVA, $F_{1, 8} = 0.184$, $P = 0.679$), or tarsus length (repeated measures ANOVA, $F_{,} = 0.0$, $P = 0.$).

Nestlings allocated to the experimental treatment group grew slower in terms of body mass during the experimental period, compared to control nestlings (repeated-measures ANOVA, $F_{1, 8} = 23.84$, $P = 0.001$; Fig. 1). There was also a significant non-linear increasing

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effect of age on body mass along the experimental manipulation (repeated-measures ANOVA, $F_{3, 24} = 2430.68$, $P<0.001$; cubic contrasts within repeated-measures model: $F_{1, 8} = 110.35$, $P<0.001$), and a significant interaction between treatment and age (repeated-measures ANOVA, $F_{3, 24}=6.60$, $P=0.002$). This is in part because while at hatching there was a non-significant difference in body mass between the two treatment groups, the mass of the nestlings in these groups deviated when they grew older (Fig. 1). There was a significant effect of treatment on body mass at the end of the manipulation (15 days of age): nestlings fed with EDCs were lighter than their control nest mates (repeated-measures ANOVA, $F_{1, 8} = 35.56$, $P < 0.001$).

There was a significant non-linear increasing effect of age on tarsus growth (repeated-measures ANOVA, $F_{3, 24}=273.49$, $P<0.001$; quadratic contrasts within repeated-measures model: $F_{1, 8} = 88.69$, $P<0.001$), no effect of treatment (repeated-measures ANOVA, $F_{1, 8} = 2.63$, $P = 0.143$) and a non-significant interaction between age and treatment (repeated measures ANOVA, $F_{3, 24} = 0. 73$, $P=0.457$). Treatment did not affect wing length at the end of the experiment (repeated measures ANOVA, $F_{1, 8} = 0.21$, $P = 0.655$).

Nestlings in the treatment group suffered reduced immunocompetence, showing a lower response to PHA injection as compared to control nestlings (repeated measures ANOVA, $F_{1, 8} = 18.14$, $P = 0.003$; Fig. 2). There was no effect of treatment (repeated measures ANOVA, $F_{1, 8} = 1.37$, $P = 0.274$) on haematocrit levels. There was no effect of treatment (Repeated measures ANOVA, $F1_{, 8} = 0.001$, $P = 0.982$) on basal corticosterone titre.

Discussion

Anthropogenic change such as pollution has profound effects on avian populations (Fry 1995; Bowerman *et al*. 2000). One of the most difficult effects to define is the importance of exposure to endocrine disrupting chemicals (EDCs). Within aquatic environments EDCs are

thought to pose one of the most significant threats to the health and welfare of wildlife (Feyk & Giesy 1998; Jenssen 2006) through immersion in polluted water (Gross - Sorokin *et al*. 2003). In our previous studies, we have demonstrated that EDCs appear to bioaccumulate in invertebrates living and developing in filter beds of sewage treatment plants (Markman *et al*. 2007; Park *et al*. 2009). We further demonstrated that adult starlings are affected by ecologically-relevant experimental doses of EDCs (Markman *et al*. 2008). To the best of our knowledge, the present study is the first to experimentally demonstrate that developing wild terrestrial birds are potentially at risk from the effects of environmental levels of EDCs originated from sewage.

We have demonstrated that artificial dosing with the suite of EDCs identified as occuring in the starling's prey (Markman *et al.* 2007) resulted in reduced growth and reduced immunocompetence in nestling starlings. Reduced body mass at fledging and reduced immune function are known to be negatively associated with survival rate of young birds (Horak *et al*. 1999; Christe *et al*. 2001; Menu, Gauthier & Reed 2005). However, Mayne *et al*. (2004) determined the relative effects of pesticides in current use and of persistent residues of p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE), a known endocrine disrupting chemical, on tree swallows *Tachycineta bicolor*, and found no significant differences in body mass of nestlings sampled from sprayed orchards and reference sites. As opposed to body mass differences between EDC-dosed nestlings and control nestlings, treatment with EDC had no effect on tarsus growth or wing length. It appeared that the structural growth of the nestlings was not affected; but that control nestlings were in better condition (namely higher body mass but similar body size) compared to the EDC - dosed nestlings.

Our results are consistent with previous findings that adult male starlings, dosed experimentally with the same suite of EDCs, suffered reduced cellular and humoral immune function (Markman *et al*. 2008). However, our previous work did not find any detrimental

effect of chemical dosing on body mass, although these birds had attained their adult mass before the start of the study. It seems likely therefore that mass gain during development is more susceptible to the effects of endocrine disrupters than the maintenance of adult mass.

There is evidence that EDCs result in immunosuppression via various general mechanisms such as changes in antibody production, nitric oxide synthesis, cytokine synthesis, and changes to the allergic response (Chalubinski & Kowalski 2006) as well as cellular effects such as reduction in T cell function. In part, this is reflected by a reduced response to PHA immune challenge (Ahmed 2000), which has been found in the present study. Some EDCs, such as the herbicide triazine, have been shown to alter growth in young animals such as in Japanese quail *Coturnix japonica* chicks; however exposure levels of these animals were above ecologically relevant levels (Wilhelms *et al*. 2006). [see paper by Poulin et al., above] The potential multiple mechanisms that result in these slower growth rates are not fully understood.

Our study detected no significant treatment effect on either hematocrit or basal corticosterone levels, in agreement with our previous study on adult starlings. This may be because these physiological indices were not affected within our sampling time points, or because no such effects exist. Even though basal levels of corticosterone may appear unaffected by our EDC treatment, a stress-induced response may possibly have shown a difference in corticosterone levels. However, our main aim was to test whether EDCs cause a long term chronic effect on condition, not whether they potentially can affect acute corticosterone production. Previous studies have indicated that increased basal corticosterone levels, peak corticosterone response and/or response to ACTH (adrenocorticotrophic hormone) challenge and greater thymic lymphocyte density, cortical/medullary ratios and significant splenic B-cell hyperplasia may result from exposure to pesticides, such as *p,p'*-

DDE, with known endocrine disruptive effects, in tree swallows *Tachycineta bicolor* and Eastern bluebirds *Sialia sialis* (Martinovic *et al*. 2003; Mayne *et al*. 2004).

As EDCs may directly or indirectly affect different physiological systems other than the endocrine system, such as the reproductive system, immune system, cardiovascular and nervous system (Vos *et al*. 2000; Guillette 2006; Chiu *et al*. 2010), our results suggest that when testing the effects of EDCs on wildlife, multiple end-points should be recorded to fully assess the potential effects of these chemicals. Our results do not confirm that these chemicals have caused endocrine disruption within our experimentally treated birds, as the mechanisms underlying the observed effects were not quantified. However, as these chemicals are all known to alter endocrine function, the most parsimonious explanation is that endocrine modulation is involved in the physiological changes observed as a result of EDC consumption. Future work can now focus on the mechanisms underlying the functional response of the endocrine system as well as other body systems to the EDC exposure.

Whilst we suggest that these chemicals can have considerable effects on nestling bird development, our results should be interpreted with caution. Population level effects will depend on the broad applicability of the EDC contamination levels and intake rates. Starlings may feed on other EDC contaminated food items outside sewage treatment works such as at agricultural areas. Further work is needed to test the variation in EDC contaminated prey types taken from non-sewage sites. If the levels of EDCs found in this study are broadly representative of the situation in the wild then our results may have implications at the population level. In addition, earlier work has shown that female starlings preferred the song, of males fed EDC contaminated prey (Markman *et al*. 2008). As these males were in poorer condition compared to control males, it seems likely that this will negatively affect their parental care levels, breeding success and survival. As both nestling and adult birds suffer from harmful effects while feeding at sewage treatment works, the birds' direct physiological

damage may be exacerbated by skewed mate choice preferences toward the less healthy individuals within a population.

The starling has shown considerable population declines in recent years in many parts of the world, including the U.K (over 50% decrease in the last forty years) (Robinson, Siriwardena & Crick 2005). The reasons underlying this change are complex and undoubtedly include factors such as habitat fragmentation, change in farm management practices, changes in food resources, pesticide use and loss of nest sites (see review Cheek 2006). However, exposure to EDCs and their effects as found in the present study may also play an important role in starling population decrease.

Most of the correlative evidence for the effects of EDCs {e.g. p, p'-DDE and polychlorinated biphenyls (PCBs)} in wild birds comes from fish-eating bird species (e.g. bald eagles *Haliaeetus leucocephalus*) that feed on contaminated fish and suffer from thyroid disorders, reproductive and teratogenic effects (Bowerman *et al*. 2000). Varying levels of EDCs {e.g. PCBs, DDT and polybrominated diphenyl ethers (PBDEs)} found in plasma and eggs of glaucous gulls *Larus hyperboreus*)from the Norwegian Arctic, were correlated with alterations in circulating reproductive hormones (Verreault *et al*. 2006) and smaller eggs and of varying composition (Verboven *et al*. 2009a). Furthermore, behavioural changes such as reduced nest site attendance in male glaucous gulls with increasing EDC levels in their plasma, suggest suboptimal thermal conditions for embryo development and possibly increased egg predation risk (Verboven *et al*. 2009b).

In addition, historical studies in the USA and Europe have shown that some raptor species suffered from eggshell thinning following exposure to pesticides which act as endocrine disrupters such as *p, p'*-DDE (for reviews, see Botham *et al*. 1999; Dawson 2000; Giesy *et al*. 2003). However, following a ban on the parent compound DDT in the USA and Europe, eggshell thinning declined (Dawson 2000) although other pollutants may still cause this

phenomenon (Cheek 2006; Bouwman *et al.* 2008). Few studies have experimentally tested the effects of ecologically relevant levels of EDCs on young wild terrestrial vertebrate (e.g. Rochester *et al*. 2009).

Many EDCs are assumed to be unstable and are not throught to bioaccumulate in the environment (Munkittrick 2001). In addition, there are relatively few routes for these chemicals to move from an aquatic to a terrestrial environment (Dawson 2000). We suggest that more research is needed to quantify contamination levels for prey associated with sewage processing and the potential for food chain effects. Starlings are among many species that forage behaviour in this environment (Fuller & Glue 1978; Fuller & Glue 1980), particularly in the winter months when alternative food supplies may be lacking. Our results therefore highlight the potential negative effects associated with allowing wildlife access to bioconcentrated levels of EDCs through the sewage treatment system.

Given the world-wide loss of wetlands and the extended use of sewage treatment facilities for water reuse and increased sanitation needs as the world population increases, the importance of sewage treatment works as foraging sites of wildlife is predicted to increase (Murray & Hamilton, 2010). Given our results, we advocate an approach that seeks to exclude or deter wildlife from foraging at sewage treatment works with high levels of contamination by EDCs. This could be achieved through design changes to new beds to deter birds from walking on the substrate. However, in recent years filter beds have become less common in Europe and activated sludge processing plants combined with settlement ponds are now favoured. Some approaches may be cost effective and easily applied, such as the netting of settlement lagoons to prevent access of aquatic birds, as for artificial fish ponds (Nemtzov & Olsvig-Whittaker 2003). However, minimising the exposure of aerial predators (birds and bats) feeding on emerging aerial insects will be more problematic and may involve the use of deterrents (acoustic or visual), in combination with efforts to reduce contamination levels at

specific sites. Waste water companies should monitor water levels of EDCs and other pollutants as well as levels of bioaccumulation of these pollutants within invertebrates, in order to responsibly protect local wildlife communities. These measures are likely to be instrumental in minimising the health risks to organisms such as wild birds and bats (Park *et al*. 2009).

Ed. – how does monitoring help to reduce the risk? It needs to be alongside with exclusion or deterrence measures.

Acknowledgments

References

Ahmed, S.R. (2000) The immune system as a potential target for environmental estrogens (endocrine disrupters): a new emerging field. *Toxicology*, **150,** 191-206.

Botham, C., Holmes, P. & Harrison, P. (1999) Endocrine disruption in mammals, birds, reptiles and amphibians. *Endocrine Disrupting Chemicals*. (eds R.E. Hester & R.M. Harrison), pp. 61–82. Issues in Environmental Science and Technology, no. 12. Royal Society of Chemistry. Cambridge, United Kingdom.

Bouwman, H., Polder, A., Venter, B. & Skaare, J.U. (2008) Organochlorine contaminants in cormorant, darter, egret, and ibis eggs from South Africa. *Chemosphere*, **71**, 227-241.

Bowerman, W.W., Best, D.A., Grubb, T.G., Sikarskie, J.G. & Giesy, J.P. (2000) Assessment of environmental endocrine disruptors in bald eagles of the Great Lakes. *Chemosphere*, **41**, 1569-1574.

Bruun & Smith (2003) Landscape composition affects habitat use and foraging flight distances in breeding European starlings. *Biological Conservation*, **114**, 179-187.

Buchanan, K.L., Spencer, K.A., Goldsmith, A.R. & Catchpole, C.K. (2003) Song as an honest signal of past developmental stress in the European starling (Sturnus vulgaris). *Proceedings of the Royal Society of London Series B Biological Sciences*, **270**, 1149-1156.

Chalubinski, M. & Kowalski, M.I. (2006) Endocrine disrupters – potential modulators of the immune system and allergic responses. *Allergy*, **61**, 1326-1335.

Cheek, A.O. (2006) Subtle sabotage: endocrine disruption in wild populations. *Revista De Biological Tropical*, **54**, 1-19.

Chiu, T.Y., Paterakis, N., Cartmell, E., Scrimshaw, M.D., Lester, J.N. (2010) A Critical review of the formation of mono- and dicarboxylated metabolic intermediates of alkylphenol polyethoxylates during wastewater treatment and their environmental significance. *Critical Reviews in Environmental Science and Technology*, **40**, 199-238

Christe, P., de Lope, F., Gonzalez, G., Saino, N. & Moller, A.P. (2001) The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (Delichon urbica). *Oecologia*, **126**, 333-338.

Clotfelter, E.D., Bell, A.M. & Levering, K.R. (2004) The role of animal behaviour in the study of endocrine-disrupting chemicals. *Animal Behaviour*, **68**, 665-676.

Colborn, T., Saal, F.S.V. & Soto, A.M. (1993) Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans. *Environmental Health Perspectives*, **101** 378-384.

Dawson, A. (2000) Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review. *Ecotoxicology*, **9**, 59-69.

Della Seta, D., Farabollini, F., Dessi-Fulgheri, F., Dessi-Fulgheri, F. & Fusani, L. (2008) Environmental-like Exposure to low levels of estrogen affects sexual behavior and physiology of female rats. *Endocrinology*, **149**, 5592-5598.

Denslow, N. & Sepúlveda, M. (2007) Ecotoxicological effects of endocrine disrupting compounds on fish reproduction. *The Fish Oocyte: From Basic Studies to Biotechnological Applications*. Springer, Netherlands.

Dosis, I. & Kamarianos, A. (2007) Environmental toxic Endocrine Disrupting Compounds (EDCs): Effects on environment, animal production and human. *Journal of the Hellenic Veterinary Medical Society,* **58**, 321-334.

Eens, M., Pinxten, R. & Verheyen, R.F. (1991) Male song as a cue for mate choice in the European starling. *Behaviour*, **116**, 210-238.

Feare, C. (1984) The straling. Oxford: Oxford University Press.

Feyk, L.A. & Giesy, J.P. (1998). Xenobiotic modulation of endocrine function in birds. Principles and Processes for Evaluating Endocrine Disruption in Wildlife (eds Kendall, R., Dickerson, R., Giesy, J., Suk, W.), pp. 121–140. Technical Bulletin, Society of Environmental Toxicology and Chemistry, Pensacola, Florida.

Frederick, P.C. & McGehee, S.M. (1994) Wading Bird Use of Wastewater Treatment Wetlands in Central Florida, USA. *Colonial Waterbirds,* **17,** 50-59.

Fry, D. M. (1995) Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives*, **103**, 165-171.

Fuller, R.J. & Glue, D.E. (1978) Seasonal activity of birds at sewage works. *British Birds*, **71**, 235–244.

Fuller, R.J. & Glue, D.E. (1980) Sewage works as bird habitats in Britain. *Biological Conservation*, **17**, 165–181.

Fuller, R.J. & Glue, D.E. (1981) The impact on bird communities of the modernization of sewage-treatment works. *Effluent Water Treatment Journal*, **21**, 27–31.

Giesy, J. P., Feyk, L.A., Jones, P.D., Kannan, K. & Sanderson, T. (2003) Review of the effects of endocrine-disrupting chemicals in birds. *Pure and Applied Chemistry,* **75**, 2287–2304.

Granbom, M., Raberg, L., & Smith, H.G. (2005) The spatial and temporal repeatability of PHA-responses. *Behavioral ecology*, **16**, 497-498.

Gross-Sorokin, M.Y., Grist, E.P.M., Cooke M. & Crane M. (2003) Uptake and depuration of 4-monylphenol by the benthic invertebrate Gammarus pulex: How important is feeding rate? *Environmental Science & Technology*, **37**, 2236-2241.

Grote, K., Hobler, C., Andrade, A.J.M., Grande, S.W., Gericke, C., Talsness, C. E, Appel, K. E. & Chahoud, I. (2009) Sex differences in effects on sexual development in rat offspring after pre- and postnatal exposure to triphenyltin chloride. *Toxicology*, **260**, 53-59

Guillette, L.J. (2006) Endocrine disrupting contaminants - Beyond the dogma. *Environmental Health Perspectives*, **114**, 9-12.

Horak, P., Tegelmann, L., Ots, I. & Moller, A.P. (1999) Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia,* **121**, 316-322.

Iguchi, T. & Katsu, Y. (2008) Commonality in Signalling of Endocrine Disruption from Snail to Human. *Bioscience*, **58,** 1061-1067.

Jenssen, B.M. (2006) Endocrine-disrupting chemicals and climate change: A worst-case combination for arctic marine mammals and seabirds? *Environmental Health Perspectives*, **114**, 76-80.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G. & Sumpter, J.P. (1998) Widespread sexual

disruption in wild fish. *Environmental Science & Technology*, **32**, 2498–2506.

Jobling, S. & Tyler, C.R. (2006) Introduction: The ecological relevance of chemically

induced endocrine disruption in wildlife. *Environmental Health Perspectives*, **114**, 7-8.

Kalcounis-Rueppell, M.C., Payne, V.H., Huff, S.R. & Boyko, A.L. (2007) Effects of

wastewater treatment plant effluent on bat foraging ecology in an urban stream system.

Biological Conservation, **138**, 120-130.

Kavlock, R.J., Daston, G.P., DeRosa, C., FennerCrisp, P., Gray, L.E., Kaattari, S., Lucier, G.,

Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan,

D.M., Sinks, T. & Tilson, H.A. (1996) Research needs for the risk assessment of health

and environmental effects of endocrine disruptors: A report of the US EPA-sponsored

workshop. *Environmental Health Perspectives*, **104**, 715-740.

Learner, M.A. & Chawner, H.A. (1998) Macro-invertebrate associations in sewage filter-beds

and their relations to operational practice. *Journal of Applied Ecology,* **35**, 720–747.

Lochmiller, R.L., Vestey, M.R. & Boren, J.C. (1993) Relationship between Protein

Nutritional-Status and Immunocompetence in Northern Bobwhite Chicks. *Auk,*

110, 503–510.

Markman, S., Guschina, I., Barnsley, S., Buchanan, K., Pascoe, D. & Müller, C.T. (2007)

Endocrine disrupting chemicals accumulate in earthworms exposed to sewage effluent.

Chemosphere, **70**, 119–125.

Markman, S., Leitner, S., Catchpole, C., Barnsley, S., Müller, C.T., Pascoe, D. & Buchanan,

K. L. (2008) Pollutants increase song complexity and the volume of the brain area HVC in

a songbird. *Plos One,* **3**, 1-6.

Martinovic, B., Lean, D., Bishop, C.A., Birmingham, E., Secord, A., Jock, K. (2003). Health

of tree swallow (Tachycineta bicolor) nestlings exposed to chlorinated hydrocarbons in

the St. Lawrence River Basin. Part II. Basal and stress plasma corticosterone concentrations. *Journal of Toxicology & Environmental Health A*, **66**, 2015-2029.

Mayne, G.J., Martin, P.A., Bishop, C.A. & Boermans, H.J. (2004). Stress and immune responses of nestling tree swallows (Tachycineta bicolor) and Eastern bluebirds (Sialia sialis) exposed to nonpersistent pesticides and p,p'-dichlorodiphenyldichloroethylene in apple orchards of Southern Ontario, Canada. *Environmental Toxicology & Chemistry*, **23**, 2930-2940.

Menu, S., Gauthier, G. & Reed, A. (2005) Survival of young greater snow geese (Chen caerulescens atlantica) during fall migration. *Auk*, **122**, 479-496.

Mills, L. J. & Chichester, C. (2005) Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? *Science of the Total Environment*, **343**, 1-34.

Munkittrick, K. R. (2001) Assessment of the effects of endocrine disrupting substances in the Canadian environment. *Water Quality Research Journal of Canada*, **36,** 293-302.

Murray, C. G & Hamilton, A. J. (2010) Perspectives on wastewater treatment wetlands and waterbird conservation. *Journal of Applied Ecology*, **47**, 976-985.

Nemtzov, S. C. & Olsvig-Whittaker, L, (2003) The use of netting over fishponds as a hazard to waterbirds. *Waterbirds*, **26**, 416-423.

Park, K.J. & Cristinacce, A. (2006) Use of sewage treatment works as foraging sites by insectivorous bats. *Animal Conservation*, **9**, 259-268.

Park, K.J., Müller, C.T., Markman, S., Swinscow-Hall, O., Pascoe, D. & Buchanan, K.L. (2009). Detection of endocrine disrupting chemicals in aerial invertebrates at sewage treatment works. *Chemosphere*, **77**, 1459-1464.

Robinson, R.A., Siriwardena, G.M. & Crick, H.Q.P. (2005) Status and population trends of Starling Sturnus vulgaris in Great Britain. *Bird* Study, **52**, 252–260.

Rochester, J.R., Klasing, K.C., Stevenson, L., Denison, M.S., Berry, W. & Millam, J.R. (2009) Dietary red clover (Trifolium pratense) induces oviduct growth and decreases ovary and testes growth in Japanese quail chicks. *Reproductive Toxicology*, **27,** 63-71.

Root, M. (1990) Biological monitors of pollution. *Bioscience*, **40**, 83-86.

Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M. & Sumpter, J.P. (1998) Identification of estrogenic chemicals in sewage treatment works effluent. 2. In vivo responses in trout and roach. *Environmental Science & Technology*, **32,** 1559-1565.

Smits, J.E., Bortolotti, G.R. & Tella, J.L. (1999) Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology*, **13**, 567–572.

Sower, S.A., Reed, K.L. & Babbitt, K.J. (2000) Limb malformations and abnormal sex hormone concentrations in frogs. *Environmental Health Perspectives,* **108**, 1085–1090.

Sumpter, J.P., Jobling, S. & Tyler, C.R. (1996) Oestrogenic substances in the aquatic environment and their potential impact on animals, particularly fish. (eds E. Taylor), *Toxicology of Aquatic Pollution: Physiological, Molecular and Cellular Approaches*. pp. 205–224. Cambridge, UK.

Tabb, M.M. & Blumberg, B. (2006) New modes of action for endocrine-disrupting chemicals. *Molecular Endocrinology*, **20**, 475–482.

Tinbergen, J.M. (1981) Foraging decisions in starlings (*STURNUS VULGARIS L.*). *Ardea* **69,** 1-67.

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W. & Evans, N.P. (2009a) Differential Investment in Eggs by Arctic-Breeding Glaucous Gulls (*Larus hyperboreus*) Exposed to Persistent Organic Pollutants. *Auk*, **126**, 123-133.

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W. & Evans, N.P. (2009b) Nest

temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to

persistent organic pollutants. *Animal Behaviour*, **77**, 411-418.

Verreault, J., Letcher, R.J., Ropstad, E., Dahl, E. & Gabrielsen, G.W. (2006) Organohalogen

contaminants and reproductive hormones in incubating glaucous gulls (Larus

hyperboreus) from the Norwegian Arctic. *Environmental Toxicology and Chemistry*, **25**,

2990-2996.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. &

Vethaak, A.D. (2000) Health effects of endocrine-disrupting chemicals on wildlife, with

special reference to the European situation. *Critical Reviews in Toxicology*, **30**, 71-133.

Wilhelms, K.W., Fitzpatrick, K.F., Scanes, C. G. & Anderson, L.L (2006) In ovo exposure to

a triazine herbicide: Effects of atrazine on circulating reproductive hormones and gonadal

histology in young Japanese quail. *Archives of Environmental Contamination and*

Toxicology, **51**,117-122.

Wingfield, J.C. (1994) Modulation of the adrenocortical response to stress in birds. In

Perspectives in Endocrinology (eds. K. Davey, R. Peter & S. Tobey), pp. 520-528.

Ottawa: National Research Council of Canada.

Wright, J., Both, C., Cotton, P.A. & Bryant, D. (1998) Quality vs. quantity: energetic and

nutritional trade-offs in parental provisioning strategies. *Journal of Animal Ecology*, **67**,

620-634.

Zhong, X., Xu, Y., Liang, Y., Liao, T. & Wang, J. (2005) The Chinese rare minnow

(*Gobiocypris rarus*) as an in vivo model for endocrine disruption in freshwater teleosts: a

full life-cycle test with diethylstilbestrol. *Aquatic Toxicology*, 71**,** 85-95.

Figure legends:

Fig. 1. The effect of treatment on body mass (± s.e) from 1 to 15 days of age in nestling

starlings [open circles and dashed line - nestlings dosed with endocrine disrupting chemicals

(EDCs), and closed triangles and black line - control nestlings].

Fig. 2. Cellular immune function (+s.e.) in nestling starlings: open bar- nestlings dosed with

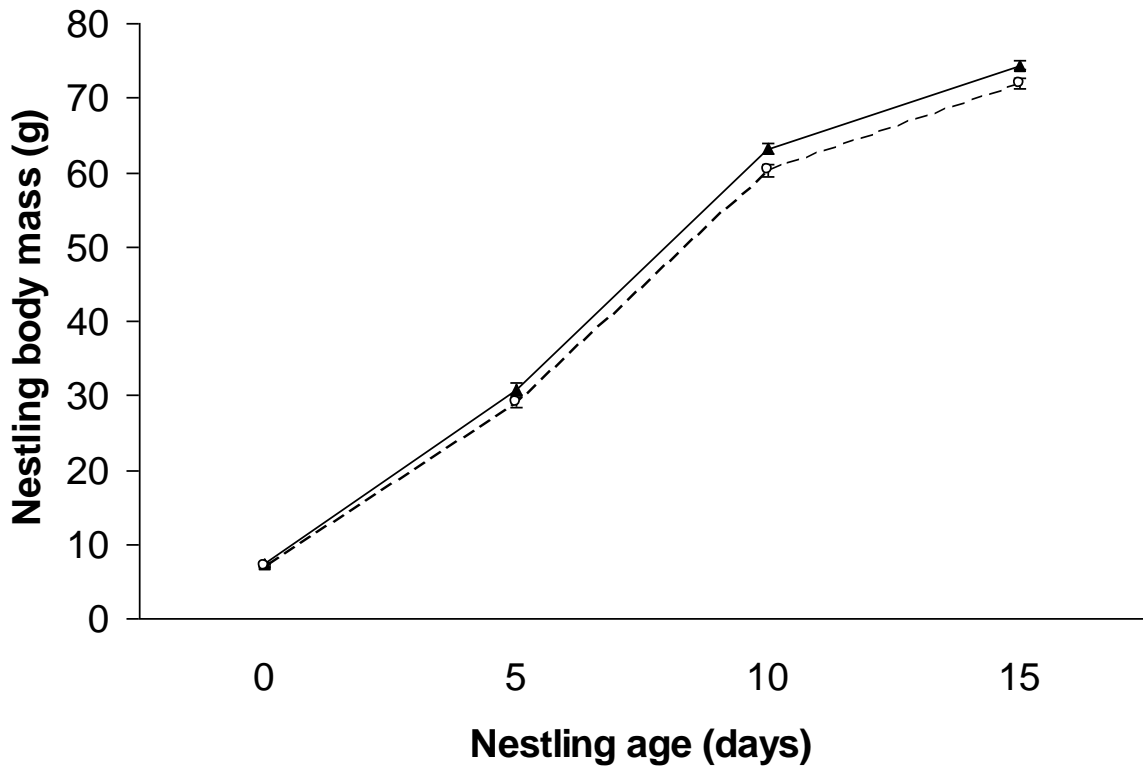endocrine disrupting chemicals (EDCs) and black bar - control nestlings.

Fig. 1.

Fig. 2.