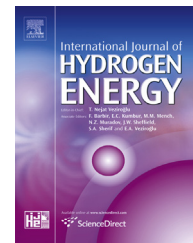


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Impacts of oil spills on seabirds: Unsustainable impacts of non-renewable energy

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ABSTRACT

Accidental spillage of oil in to the sea from shipping transport and drilling rigs results in spills that cause significant unsustainable mortality of wildlife and destroys marine ecosystem services. External oiling of seabirds causes large scale mortality within days following a spill, while survivors suffer long term chronic effects from the exposure to toxic polycyclic aromatic hydrocarbons (PAHs) present in ingested oil. Survival rates for rehabilitated oiled birds are very low despite investment of significant resources. PAHs disturb thyroid homeostasis which plays a vital role in the control of energy metabolism. In this study, plasma PAH and thyroid-stimulating hormone (TSH) were quantified as biomarkers of exposure and endocrine disruption in oiled guillemots (*Uria aalge*). Mean plasma PAH and TSH concentrations, were 98.1 ± 8.3 ng/ml and 0.13 ± 0.02 ng/ml and these parameters were found to be negatively correlated ($p < 0.01$) indicative of PAH-associated thyroid hormone suppression in more heavily oiled birds. Body condition and weight were also lower in birds that died compared with birds that were released. The data also show the value of measuring plasma TSH and PAH to monitor metabolic status and progress of decontamination of oiled birds in a rehabilitation setting.

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Introduction

Petroleum continues to be a major source of global energy despite the fact it is a non-renewable resource that has significant adverse impacts on the environment from its extraction, transport and use to its disposal, including resource depletion, habitat destruction, climate change, acid rain, ozone depletion, ecotoxicity and human toxicity [1]. Freight tankers transport large quantities of crude oil to

refinery terminals around the world. In addition to regular discharges of refined products from ships, there are also unintentional releases from tankers and drilling rigs, arising from accidents and negligence (environmental conditions, collisions, engineering failures or and/poor maintenance). In 2013, a total of 605 separate discharges of oil from vessels and offshore oil and gas installations were reported in UK waters alone [2]. Worldwide release of petroleum oils in to the marine ecosystem globally, has been estimated to range from 0.5 to 8.4 million tonnes per year,

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with shipping and drilling rigs contributing ~35% to this total [3,4].

Mass mortalities of seabirds are a common in the aftermath of oil spills with tens if not hundreds of thousands of birds stranding dead or dying [5]. Seabird populations are particularly vulnerable due to their distribution, foraging and breeding behaviour. Following a spill, seabirds come in to contact with crude oil floating on the water's surface causing them to become smothered with oil and this can cause immediate mortality via suffocation [6]. Crude oil disrupts feather integrity displacing insulating air between feathers leading to loss of water-proofing, thermal insulation and buoyancy. They become unable to dive or fly so they cannot forage to feed. Relatively quickly fat reserves are depleted and ultimately birds become severely hypothermic and emaciated causing significant mortality [7,8]. The oil that is ingested from preening and feeding results in oral exposure to hydrocarbon chemicals present in crude oil. A significant proportion of these are toxic polycyclic aromatic hydrocarbons (PAHs) which, depending on the type of oil, degree of weathering and water content, can constitute up to 30% of total hydrocarbons present [9]. It has been estimated that ~170,000 metric tons of PAHs are discharged to the aquatic environment per annum as a result of petroleum spillage and natural sources [10]. PAHs enter the circulation leading to tissue and plasma contamination [11]. PAHs are believed to be the cause of the long term chronic effects of oiling which compromise the ability of rehabilitated cleaned seabirds to recover both at the individual, and population level. The subsequent effects of "internal oiling" on seabirds are numerous and include pathological changes in the intestinal tract, lungs, liver, kidneys and salt gland [12–14] leading to dysfunction, reproductive toxicity [15], haemolytic anaemia [16,17], immunotoxicity [18,19] and endocrine disruption [20,21].

There are considerable efforts to recover, clean and rehabilitate stranded oiled sea birds and re-introduce them to the wild. Unfortunately, post-release survival of oiled sea birds is extremely low (<1%) in common guillemots, which tend to be most severely affected species following oil spills reaching the British coast [22]. Lack of weight gain and chronic toxicity of PAHs are invariably believed to be the major cause of mortality [23,24]. Thyroid hormones, under the control of the hypothalamus-pituitary-thyroid (HPT) axis, are critical to metabolism, weight gain, thermoregulation, reproduction and development in birds [25]. Any disruption of the HPT axis by oil exposure may exacerbate emaciation, hypophagia and hypothermia in oiled birds. There is good body of evidence that polyaromatic hydrocarbon chemicals such as PAHs, and halogenated varieties (e.g. polychlorinated biphenyls (PCBs), dioxins, organochlorine pesticides) disrupt thyroid hormone homeostasis by altering the synthesis, secretion and transport of thyroid hormones in avian wildlife [20,26–31]. New monitoring approaches are required in wildlife rehabilitation to improve practice and improve post-release survival of rehabilitated oiled seabirds. The methods must address the diverse logistics and issues affecting wildlife centres practices such as technical and financial resource limitations, ethical constraints, crowding during oil spill events.

Furthermore, only small blood plasma sample volumes are feasible from severely emaciated, dehydrated and hypothermic oiled birds, especially from guillemots. Plasma thyroid-stimulating hormone (TSH) concentration can be used to diagnose HPT status where sample volumes are insufficient for T3 (tri-iodothyroxine) & T4 (thyroxine) testing, since TSH is their precursor and vital to thyroid homeostasis. The monitoring of plasma PAH concentrations provides good evidence of internal exposure status [11]. Here, a scoping study was undertaken to quantify PAH and TSH concentrations in plasma samples from guillemots oiled by the MV *Tricolour* spill in the French Channel. The relationship between these two parameters would be assessed as a means of monitoring PAH-mediated thyroid hormone disruption. The testing methods were selected and adapted for use with micro-volume plasma samples, to suit a rehabilitation setting.

Materials and methods

RaPID[®] carcinogenic PAH test kit was obtained from *Strategic Diagnostics Inc.* (Delaware, USA). Certified high-purity PAH standards were obtained from *Sigma–Aldrich Inc.* (Missouri, USA): acenaphthene, phenanthrene, acenaphthalene, pyrene, fluorene, chrysene, naphthalene, anthracene, benzo(a)anthracene, fluoranthene, benzo(a)pyrene (BaP) and phenanthrene-D₁₀ (internal standard; IS). Solvents were of high-purity glass distilled grade. All other chemicals were purchased from *Sigma–Aldrich Inc.*

Sampling

The birds in this study were common adult guillemots (*Uria aalge*) stranded live on the South coast and East Coast of England in the winter of 2002–03, as a result of oiling following the MV *Tricolor* oil spill. The spill occurred as a result of a shipping lane collision involving the MV *Tricolor* with other vessels causing 170 tonnes of crude oil to be released in to the French Channel [32]. Approximately 19,000 seabirds were oiled and subsequently stranded (alive or dead) along the French, Belgian, Dutch and English coastlines, of which nearly 90% were common guillemots (*U. aalge*) [33,34].

Only samples from stranded guillemots admitted to East Winch Wildlife Centre (RSPCA) were available for this study and samples from healthy guillemots for use as "controls" were unavailable. Blood samples were collected from 50 birds. Information on exact age and sex of the bird was only available for a handful of the birds studied as there were insufficient resources to undertake the detailed examinations required to age and sex individuals of this species. Body weights were recorded on admission and eventual outcome recorded (dead, euthanized or released). Overall body condition was determined by of examination of the fat reserves and scored as follows; emaciated (0), lean (1), fair (2) and good (3). External oil coverage of plumage was determined by examination of the feathers and scored as follows; unoiled (0), lightly oiled (1), moderately oiled (2) and severely oiled (3). Prior to blood sampling all birds were washed according to

standard RSPCA procedures to prevent further oil exposure. Birds were sampled as soon as possible within a maximum of 24 hrs of admission. Whole blood was collected in EDTA-treated paediatric (0.5 ml) blood tubes by veni-puncture of the medial metatarsal vein. Samples were centrifuged for 5 mins to obtain the plasma and stored at -20°C until analysis.

Quantification of PAHs

PAH concentrations were determined using an adapted immunoassay previously adapted for quantification of PAHs in guillemot plasma [35]. PAHs were extracted from 50 μl plasma samples by addition of 100 μl methanol and 100 μl PBS (10 mM phosphate buffered saline, containing 150 mM NaCl, 0.1% w/v sodium azide, pH 7.4) followed by centrifugation at 4000 rpm at 4°C for 10 mins to obtain a clear protein-free extract. Extracts were analysed in triplicate by immunoassay, according to a published method based on the RaPID[®] carcinogenic PAH test kit (Strategic Diagnostics Inc. USA) adapted for analyses of micro-volume biological samples (plasma) in 96 well micro-plates and validated by Gas Chromatographic (GC–MS) parallel analysis. Internal standard (phenanthrene- D_{10}) was added to all samples to monitor recovery. ΣPAH concentrations in samples were calculated using the equation of the linear curve of log BaP concentration versus [observed absorbance of a standard/absorbance of zero standard (negative control)] $\times 100$ (%B/B₀). Values were corrected for dilution accordingly. The specificity of cRaPID[®] anti-PAH antibody (raised against BaP immunogen) was established by comparing the BaP concentration required for 50% inhibition of colour formation (i.e. 50% B/B₀ or I₅₀) with respective values for 10 parent PAHs and 10 metabolites in the same diluent (50:50 v/v methanol: PBS washing buffer). Cross reactivity (CR) values were calculated as follows: 50% B/B₀ PAH_x/50% B/B₀ BaP. The linear range of the assay was 0.1–20 ng/ml ($r^2 = 0.99$, $p < 0.001$) with a limit of detection (assay sensitivity) of 0.1 ng/ml, as BaP equivalents. Within assay precision (mean coefficient of variation (%CV) was $6.8 \pm 1.8\%$ (within assay) inter-assay reproducibility (% CV = $12.3 \pm 2.7\%$) and BaP % recovery (range: 80–105%) were all within acceptable limits.

Quantification of TSH

There are no commercially-available avian specific TSH capture antibodies or immunoassay kits and this was a necessary requirement for rehabilitation centres (ease of purchasing and use). Consequently, TSH concentrations were measured using an immunoassay kit based on a capture antibody raised against mammalian TSH available from IBL International (Hamburg, Germany). Homology of TSH antibody binding domains are highly conserved in vertebrate species, with 68–86% homology in amino acid sequences reported for avian and mammalian TSH-beta (the sub-unit conferring thyroid activity) [36,37]. The TSH assay was conducted according to the manufacturer's instructions. Briefly, 100 μl of plasma sample/standard/control were added to micro-well plate pre-coated with capture antibody (polyclonal goat anti-human TSH) and incubated at 37°C for 1hr. The plate was inverted

to remove unbound reagents and wells washed 3 times with washing buffer (50 mM tris-phosphate buffered saline (PBS), 0.05% (v/w) tween 20, pH 8). To all wells, 100 μl of secondary antibody solution (polyclonal HRP-conjugated mouse anti-human TSH in PBS, 2% w/v bovine serum albumin (BSA), pH 8) were added and the plate incubated at 37°C for 1hr. Wells were washed twice with washing buffer as before and 100 μl of enzyme substrate solution (TMB and peroxidase solution) added to all wells. Following a final incubation at 37°C for 15 mins, reaction was terminated by 50 μl of stop solution (2.5 M H_2SO_4) and absorbance read at 450 nm in an ELISA plate reader. Sample TSH concentrations were determined by extrapolation from the calibration curve of pre-diluted TSH standards provided (25, 10, 4, 1, 0.4, 0.1 and 0 ng/ml). The limit of detection was 0.01 ng/ml. Within assay precision (mean coefficient of variation (%CV) was $5.5 \pm 4.1\%$ (within assay) inter-assay reproducibility (%CV = $11.7 \pm 4.9\%$) were all within acceptable limits.

Results and discussion

Typical of guillemot rehabilitation scenarios, the majority of the birds admitted were euthanized on admission ($n = 34$) and roughly half the birds sent for rehabilitation were successfully released (died $n = 7$; released $n = 9$) (Fig. 1). Causes of mortality for birds that died was mainly starvation, sometimes combined with secondary chronic effects of oil inhalation and ingestion (pulmonary edema, intestinal haemorrhaging) and are commonly reported in the aftermath of guillemot mass mortalities post oil spill [6,8,13,38]. Furthermore, birds which died later following admission during the in rehabilitation process, often died due to further complications such as foot hock lesions from standing on inappropriate substrates for extended periods) and/or Aspergillosis lung infections (prevalent when large numbers of captive birds are held indoors in a rehabilitation setting [13]. The mean plasma PAH concentration observed for the 50 birds was 98.1 ± 8.3 ng/ml (wet weight, range 18.8–345 ng/ml). Plasma PAH levels were in agreement with levels reported in other similar studies of PAH exposure

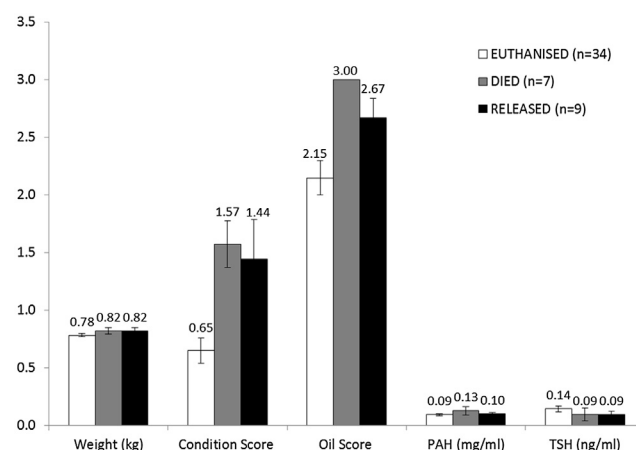


Fig. 1 – Mean (\pm std. error) values for measured parameters by outcome.

in oiled birds [11]. In this study, birds that died during rehabilitation were found to have significantly higher oil coverage scores compared than the other birds according to student t-test ($p < 0.01$) (Fig. 1). These birds had longer to preen and ingest oil from their feathers, thereby exacerbating their condition while reducing their external oil coverage. This interpretation is supported by the higher plasma PAH concentrations observed in these birds (Fig. 1). Clearly, more data are needed to improve the statistical reliability of this interpretation. This is reflected in the data in this study as PAH and TSH concentrations were not significantly different between groups of birds most likely due to unequal distribution of birds in each oil and body condition category. PAH concentrations did not differ significantly between oil score categories, likely due to the variation in preening time prior to rescue and other sources of PAH exposure (food chain) and agrees with observations in previous studies [11].

Mean body weight of adult guillemots in this study was $792 \text{ g} \pm 11 \text{ g}$ (range: 642–938 g). This corresponds with body weight ranges reported for oiled adults of this species, which are unsurprisingly lower than the weight range (925–1250 g) reported for healthy unoiled adults [8]. The most severely emaciated oiled birds have a poor prognosis and are unlikely to survive rehabilitation so it is common practice to euthanize these birds on admission to prevent further suffering [24]. In our study, euthanized birds were indeed found to have significantly poorer body condition scores than other birds which later died in rehabilitation (EUTH < DIED; $p < 0.001$) or were released (EUTH < REL; $p < 0.01$) (Fig. 1). As one would expect, the mean body weight of birds with body condition score 0 were lower than those with higher body condition scores. It is a common finding in studies of oiled guillemots and other seabird species affected by oil spills, that body condition and body weight are significantly reduced by oiling [8,13,38–41]. Failure to gain weight and improve body condition is believed to be the primary cause of high mortality rates and poor rehabilitation success in oiled seabirds [7]. It has been proposed that the probability of survival is increased by 10% for every 127 g gain in release weight in seabirds over a 20 month rehabilitation period [40]. Controlled crude oil dosing laboratory studies confirm field observations [20,26]. It has been reported, an average 40% loss of normal body weight occurs in stranded oiled guillemots and that this is likely the threshold beyond which death occurs [8].

In this study mean TSH concentration in plasma was found to be $0.13 \pm 0.02 \text{ ng/ml}$ (range: <0.01 – 0.5 ng/ml). Whether data deviated from the normal physiological range could not be ascertained as data for healthy, unexposed (control) birds are not available in the literature. For comparison, reference ranges for rats and dogs are 0.85 – 3.23 ng/ml and 0.03 – 2.19 ng/ml , respectively [42]. The data showed a weak but statistically-significant negative relationship between plasma PAH and TSH concentration ($r^2 = 0.21$, $p < 0.001$; Fig. 2). The normal thyroid response to starvation in birds and mammals is a reduction in secretion of TSH from the pituitary which, in turn, reduces plasma concentrations of the active thyroid hormone T3. This is the normal thyroid response to starvation in an effort to conserve energy [43]. Considering this, any exposure-related suppression of TSH secretion could interfere with homeostatic set points for controlling metabolic rate,

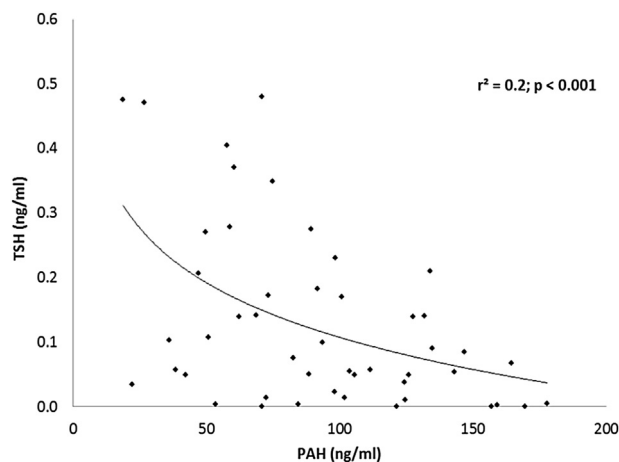


Fig. 2 – PAH versus TSH concentrations in oiled guillemot plasma.

eating behaviour and subsequent weight gain. A negative association was also observed between body weight and plasma PAH concentration ($r^2 = 0.13$, $p < 0.015$; Fig. 3) supporting this interpretation. Failure to re-gain normal body weight during the rehabilitation process is the major factor influencing rehabilitation success with oiled guillemots [13,23,24,38–41]. Although this is primarily due to the damage oil ingestion causes on intestinal epithelia such as poor nutrient absorption and diarrhoea [12,13,38,44], PAH exposure via oil ingestion would exacerbate emaciation in oiled. Hypothyroid responses from exposure to polycyclic aromatic hydrocarbons (PAH) have also been observed in other studies of wild birds. Controlled dosing with polychlorinated biphenyls (PCB) and crude oils has been reported to induce an hypothyroid state in guillemots and other avian species, evidenced by pathological changes in the thyroid diagnostic of a state of thyroid involution and inactivity (colloid accumulation, increased thyroid gland and follicular size) with concomitant alterations in thyroid hormone secretion [20,26]. Unfortunately pituitary and thyroid pathological data were not available in this study but would have been useful to confirm these phenomena.

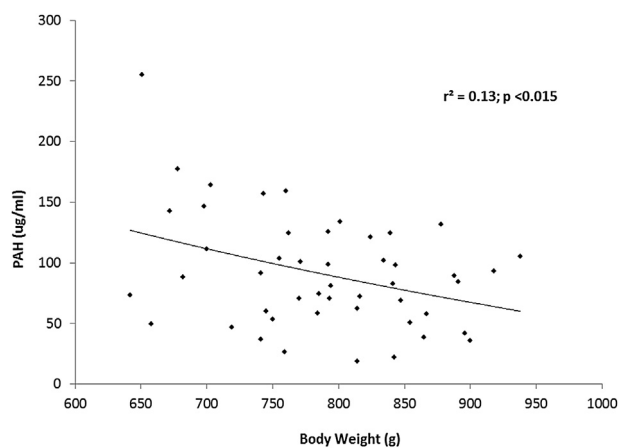


Fig. 3 – Plasma PAH concentration versus body weight of guillemots.

The toxicological mechanisms proposed to explain contaminant-induced hypothyroidism in birds includes changes to pituitary and thyroid glands pathology resulting in reduced TSH secretion [20,26] and displacement of T3 and T4 from albumin and transthyretin (TTR) plasma binding sites by parent and hydroxylated metabolites of contaminants. *In vitro* studies have demonstrated that hydroxylated planar metabolites of polyaromatic organic chemicals, such as PAHs and PCBs, have a higher affinity for TTR than endogenous ligands T3 and T4, potentially leading to a reduction in availability of thyroid hormone binding sites reducing transfer of T4 to target tissues where it can be converted to active T3 by deiodinase enzymes [45,46]. This can lead to increased concentrations of circulating T4 which exerts negative feedback on the pituitary to reduce TSH secretion [20,26]. Previous research has indeed shown that hydroxylated PAH metabolites are present in the plasma of oiled guillemots in appreciable concentrations [11]. In practice, wild birds are also exposed to a range of other bioaccumulated pollutants from the food chain which can also interfere with thyroid homeostasis, raising concerns regards synergistic mixture effects on thyroid toxicity.

From a rehabilitation perspective, until metabolised and excreted, PAHs and their metabolites may continue to disturb HPT homeostasis and dependent physiological processes. Furthermore stress, induced by emaciation, hypothermia and rehabilitation (handling & captivity) induces release of corticosterone in oiled birds. Elevated corticosterone, in turn signals hypothalamic suppression and concomitant decrease in plasma TSH concentrations, which would serve to exacerbate any hypothyroid effect induced by PAH exposure [43]. Given the vital role of by thyroid hormones in energy metabolism and the potential impacts of PAH exposure shown and discussed here, there is value in the routine measurement of plasma TSH and PAH to monitor metabolic status and progress of decontamination of oiled birds in rehabilitation. However, rehabilitators are also concerned with other impacts of oiling and PAH toxicity on exposed birds and will view thyroid disruption within the context of these. For example, PAH-induced immune suppression and damage to the lungs (pulmonary edema and epithelial damage) caused by the inhalation of crude oil toxic fumes (benzene & toluene), are both likely to compromise ability of these birds to combat lung infections (e.g. *Aspergillus* spp, pneumonia) which are prevalent in wildlife centres where large numbers of birds are held together indoors [8,18,19,44]. Also of importance are haemolytic anaemia and damage to intestinal epithelia, liver, kidneys and salt gland which also affect recovery of oiled birds [8,12–14,16,17,44].

In the long term guillemot populations in the wild are impacted by reproductive [15,21] and immune toxicity [18,19] of PAHs which would compromise recruitment and recovery of populations following mortality in the wild due to various factors such as epizootics, reduced prey density, habitat destruction and climate change. Studies have shown a doubling of adult guillemot mortality in years coinciding with an oil spill for North Atlantic populations and these have been worsened by the warming of surface waters [47,48]. Mass mortality events of the size and frequency reported following oil spills for seabird populations, as reviewed by Castege et al.

[5], are clearly unsustainable. Unsurprisingly, guillemots are recognised as sentinels for the state of oil pollution impacts in the N.E. Atlantic marine ecosystem by the OSPAR Convention. Compliance with OSPAR *ecological quality objectives* (EcoQO) requires that the “average proportion of oiled common guillemots stranding in winter months (November–April) should be 20% or less by 2020 and 10% or less by 2030 of the total found dead in 15 designated areas of the North Sea, over a period of at least 5 years” [49]. Amidst policy uncertainties, international agreements, harmonisation and cross-border issues, policy drivers, have not been very effective at mitigating the impact of oil spills on seabird populations. Wildlife rehabilitators and non-profit organisations regularly deal with the day to day reality of mass strandings and animal suffering, with limited resources and minimal government support, and provide the scientific and conservation community with invaluable data and biological material to improve our understanding of the impacts of oil spills on marine wildlife. Presently, the economies of scale are driving up the size of container ships, with 150,000 tonne vessels not uncommon, which is 3 times the size of *MV Tricolour*. Until there is a transition to a sustainable energy economy, the severity of future oil spills will continue to increase and contingency planning and investment should be scaled up in anticipation for inevitable future energy-driven disasters, ranging from accidents in ever busier shipping lanes to future engineering safety failures in offshore rigs. In the aftermath of the Deep Water Horizon blow-out in 2010 (Gulf of Mexico), there is a growing international consensus that petroleum as an energy source has unsustainable environmental impacts on wildlife, marine ecosystem services and considerable socio-economic costs that will continue to rise [1,50,51]. Until there is a paradigm shift globally towards dependence on renewable clean energy, significant resources will continue to be depleted in an unsustainable manner.

Conclusion

This study reports a negative relationship between PAH exposure and TSH concentration in oiled guillemots. A possible consequence of this is a hypothyroid condition which could interfere with homeostatic set points for controlling metabolic rate, eating behaviour and subsequent weight gain. PAH thyroid disruption may exacerbate weight loss in already emaciated birds, hampering efforts to improve weight gain of birds in rehabilitation. The data here provide valuable preliminary information to justify further study of the influence of PAH exposure on thyroid hormone status in oiled birds, ideally employing a larger sample size representing control unexposed healthy individuals. This study contributes to the large body of evidence of endocrine disrupting effects of environmental chemicals in wildlife, in a species which has been significantly impacted by oil spills. Beyond oil pollution, research is also needed to address how endocrine disruptors impact wild bird populations after taking into account other physiological factors (age, sex, reproductive status, condition) and anthropogenic stressors such as climate change, habitat destruction and over-fishing.

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