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# **Contaminants and energy expenditure in an Arctic seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with basal metabolic rate in a contrasted manner**

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**Abstract:** Basal metabolic rate (BMR), the minimal energetic cost of living in endotherms, is known to be influenced by thyroid hormones (THs) which are known to stimulate in vitro oxygen consumption of tissues in birds and mammals. Several environmental contaminants may act on energy expenditure through their thyroid hormone disrupting properties. However, the effect of contaminants on BMR is still poorly documented for wildlife. Here, we investigated the relationships between three groups of contaminants (organochlorines (OCs), perfluoroalkyl substances (PFASs), and mercury) with metabolic rate (MR), considered here as a proxy of BMR and also with circulating total THs (thyroxine (TT4) and triiodothyronine (TT3)) in Arctic breeding adult black-legged kittiwakes (*Rissa tridactyla*) from Svalbard, during the chick rearing period. Our results indicate a negative relationship between the sum of all detected chlordanes ( $\Sigma$ CHLs) and MR in both sexes whereas perfluorotridecanoate (PFTrA) and MR were positively related in females only. MR was not associated with mercury. Additionally, levels of TT3 were negatively related to  $\Sigma$ CHLs but not to PFTrA. The findings from the present study indicate that some OCs (in both sexes) and some PFASs (only in females) could disrupt fine adjustment of BMR during reproduction in adult kittiwakes. Importantly, highly lipophilic OCs and highly proteinophilic PFASs appear, at least in females, to have the ability to disrupt the metabolic rate in an opposite way. Therefore, our study highlights the need for ecotoxicological studies to include a large variety of contaminants which can act in an antagonistic manner.

**Keywords:** Organic contaminants; Mercury; BMR; Thyroid hormones; Black-legged kittiwake

## **1. Introduction**

Understanding the concept of energy allocation toward maintenance requirements, activity, growth and reproduction is a central goal which integrates both ecology and physiology. Usually considered as the minimal energetic cost of living, basal metabolic rate (BMR) is defined as the lowest rate of energy expenditure in a post-absorptive, adult endotherm at rest in its thermoneutral zone (Bligh and Johnson, 1973; Ellis and Gabrielsen, 2002; McNab, 1997). BMR is by far the most widely assessed parameter in vertebrate energetics (Danforth and Burger, 1984; Ellis, 1984) and has been described for a large variety of species from a wide geographical range (Bryant and Furness, 1995; Ellis, 1984; Ellis and Gabrielsen, 2002; Scholander et al., 1950). Although subject to circadian and seasonal fluctuations (Aschoff and Pohl, 1970; Kendeigh et al., 1977), a significant part of BMR variation within and between species can be attributed to adaptations either to specific environmental conditions or to particular behavioral traits of the species (Bech et al., 1999; Burton et al., 2011; Gabrielsen, 1994; Verreault et al., 2007).

Thyroid hormones (THs) are involved in a multitude of physiological traits and are known to regulate metabolism. Specifically, thyroxine (T<sub>4</sub>) and especially triiodothyronine (T<sub>3</sub>) are considered as the major controllers for the regulation of tissue oxygen consumption, thermogenesis and metabolic activity in endotherms (Bobek et al., 1977; Danforth and Burger, 1984; Freake and Oppenheimer, 1995; Hulbert, 2000). The roles of THs in the regulation of metabolism have been well documented in laboratory studies (Hulbert, 2000; Kim, 2008; Silvestri et al., 2005), and several investigations conducted in domestic as well as in free-living animals have highlighted strong and positive associations between total T<sub>3</sub> (TT<sub>3</sub>) levels and BMR (Bobek et al., 1977; Chastel et al., 2003; Elliott et al., 2013; Vézina et al., 2009; Welcker et al., 2013; Zheng et al., 2013).

Over the last few years, a significant amount of studies have led to the hypothesis that exposure to environmental contaminants could be the cause of disruption of thyroid function or BMR and several studies have reported abnormal TH concentrations and thyroid gland structure in birds exposed to contaminants under controlled laboratory conditions, as well as in free-ranging populations (Cesh et al., 2010; reviewed in Dawson, 2000; Haugerud, 2011; reviewed in McNabb, 2005; reviewed in McNabb and Fox, 2003; Melnes, 2014; Nøst et al., 2012; reviewed in Rolland, 2000; reviewed in Scanes and McNabb, 2003; Smits et al., 2002; Verreault et al., 2004, 2007, 2013; Wada et al., 2009). Besides, circulating levels of THs appear to be suitable biomarkers as measures of contaminant-associated effects in wildlife (Fox, 1993; McNabb, 2005; Peakall, 1992; Rolland, 2000). Effects of contaminants on metabolic activity are still poorly known and largely debated in the literature, since the few studies that have investigated this topic in adult birds and mammals are limited and somewhat contradictory. Briefly, decreased metabolic rate was observed in mourning doves (*Zenaida macroura*) exposed to the polychlorinated biphenyl mixture (PCB) Aroclor 1254 (Tori and Mayer, 1981) and in pigeons (*Columba livia*) fed with high doses of dichlorodiphenyltrichloroethane (DDT, Jefferies and French, 1971). Similarly, a previous study conducted in an Arctic seabird, the glaucous gull (*Larus hyperboreus*), revealed negative associations between BMR and plasma concentrations of PCBs, DDTs, and particularly chlordane and its metabolites (CHLs, Verreault et al., 2007). In contrast, other studies have reported an increased metabolic rate in response to contamination, as in DDT-exposed short-tailed shrews (*Blarina brevicauda*, Braham and Neal, 1974) and PCB-exposed white-footed mice (*Peromyscus leucopus*) (Voltura and French, 2000). High latitudes are considered as a sink for environmental pollutants due to atmospheric long-range transport and oceanic currents in combination with a cold climate (Burkow and Kallenborn, 2000). Given their properties (i.e. high volatility and/or persistence), organic pollutants and heavy metals can reach remote areas such as the Arctic (AMAP, 2010, 2011).

Once deposited in the marine ecosystem, most of those chemicals bioaccumulate in birds via food intake. Due to biomagnification, this exposure will then increase in severity throughout food webs (Atwell et al., 1998; Blévin et al., 2013; Kelly et al., 2009; Letcher et al., 2010). Several Arctic seabirds occupy relative high trophic levels and are consequently particularly exposed and sensitive to high concentrations of environmental contaminants. They are thus relevant biological models to investigate the influence of contaminant exposure on energy expenditure in wildlife (Gabrielsen and Henriksen, 2001; Letcher et al., 2010). In Svalbard, black-legged kittiwakes (*Rissa tridactyla*, hereafter “kittiwakes”), are exposed to a complex mixture of harmful halogenated compounds and heavy metals which correlates with impaired fitness and population dynamic through their endocrine disrupting properties (Goutte et al., 2015; Tartu et al., 2013, 2014, 2015, 2016). Among them are (1) toxic trace elements of both human and natural origins such as mercury (Hg) which tends to decrease in the Arctic (Cole et al., 2013); (2) the globally decreasing legacy persistent organic pollutants (POPs) which have been extensively used in the past and now banned by the Stockholm convention (Helgason et al., 2008; Rigét et al., 2010); and (3) poly- and perfluoroalkyl substances (PFASs) such as the long-chained perfluoroalkyl carboxylic acids (PFCAs) which currently are the most prevalent PFASs in Arctic biota (Butt et al., 2007, 2010; Tartu et al., 2014). Kittiwakes are thus potentially exposed to a broad cocktail of contaminants with many possible additive, synergistic, as well as antagonist effects.

In kittiwakes, significant relationships between concentrations of OCs, PFASs, Hg and several hormones (e.g. THs, corticosterone) involved in energy metabolism have been observed (Ask, 2015; Tartu et al., 2014, 2015, 2016). Because THs are known to exert a strong control on the regulation of metabolic functions in kittiwakes (Elliott et al., 2013; Welcker et al., 2013), individuals exposed to high concentrations of those chemicals may experience altered metabolic activity in response to disrupted thyroid function. We tested this assumption by

investigating the relationships between three groups of contaminants (OCs, PFASs, and Hg) with metabolic rate (MR), considered here as a proxy of BMR and also with circulating concentrations of total THs (TT3 and TT4) in a kittiwake population from Svalbard (Norwegian Arctic). Because BMR is considered as a life-history component (reviewed in Burton et al., 2011), such relationships between contaminants and basal energy expenditure could potentially be related to the decreased survival rate, lower hatching success and breeding probabilities as previously reported in the most contaminated kittiwakes in Svalbard (Blévin et al., 2016; Goutte et al., 2015; Tartu et al., 2014).

## **2. Material and methods**

### **2.1. Study area and sampling collection**

Fieldwork was carried out in 2012, from July 12<sup>th</sup> to 27<sup>h</sup> in a colony of black-legged kittiwakes *Rissa tridactyla* at Kongsfjorden (78°54'N; 12°13'E), Svalbard. A total of 44 individuals (22 males and 22 females) were caught on their nest with a noose at the end of a 5 m fishing rod during the chick rearing period (when raising chicks). At capture, a 2 mL blood sample was collected from the alar vein using a heparinized syringe and a 25-gauge needle to assess contaminant concentrations, TT3 and TT4 levels (when enough blood) and to determine the sex of individuals. Blood samples were stored on ice in the field. Whole blood and both, plasma and red blood cells obtained after centrifugation were kept frozen at -20 °C until subsequent analyses in the lab.

### **2.2. MR measurements**

MR measurements were performed on 23 individuals (12 males and 11 females) among the 44 kittiwakes that have been caught in total. After capture and blood sampling, birds were kept in an opaque box and rapidly transported by boat (within 20 min) to the laboratory in NyÅlesund

to measure MR by open-flow-through respirometry measurements of at least two hours duration. Outside air was drawn into the lab and dried in indicator silica gel before entering a 41 L plexiglass chamber holding the bird. Air was drawn past the bird and into a Sable Systems FoxBox® analyzer at a rate of  $1.92 \pm 0.04$  (sd) L/min. CO<sub>2</sub> was measured by the FoxBox, after which the air was scrubbed of CO<sub>2</sub> with indicator soda lime and dried again with indicator silica gel before returning to the FoxBox where O<sub>2</sub> was measured. Prior to and following each MR measurement, the bird was weighed to the nearest 0.1 g on an electronic balance and its body temperature was taken with a Schultheis fast-reading reptile mercury thermometer accurate to 0.2 °C. During metabolic measurements, the chamber was covered with a towel to allow diffuse light while preventing the bird from observing its surroundings. This was necessary because the chamber was not in a temperature cabinet but on a lab bench where it was exposed to room temperatures. Chamber temperature (Ta) was monitored continuously by a probe connected to the FoxBox and averaged  $19.2 \pm 1.8$  °C (sd; ranged from 15.1 to 22.7 °C). Body temperature (Tb) averaged  $40.5 \pm 0.7$  °C (sd; ranged from 38.8 to 41.9 °C). Readings of all gases, flow rate, and Ta were made every 20 s by the FoxBox and recorded with a time stamp on a laptop computer. Most kittiwakes caught on their nest had been there for an unknown period of time, so it was not known if they were entirely post-absorptive. For that reason, metabolic measurements were not typically begun until about 4 h post-capture. By itself, that did not guarantee a post-absorptive condition, but the time was limited to reduce the duration the bird was away from the nest. Another consideration for BMR is that birds are in their thermoneutral zone (TNZ) when measured. Indeed, two previous studies reported the upper end of that zone to be at least 20 °C for kittiwakes (Gabrielsen et al., 1988; Humphreys et al., 2007). Because Ta was set by room temperature, seven of the birds were measured at temperatures exceeding 20 °C, though in all but two cases (21.6 and 22.7 °C)  $Ta \leq 21$  °C. For those reasons, the



measured MR herein could be used as a proxy of BMR. The effect of Ta and Tb is considered below in the Results.

### 2.3. Chemicals analyses

Organochlorines were analyzed from whole blood at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway. The following compounds were searched: the polychlorinated biphenyls (CB-28, -52, -99, -101, -105, -118, -138, -153, -180, -183, -187 and -194) hereafter referred as  $\Sigma$ PCBs and the organochlorine pesticides (*o,p'* DDT, *p,p'* DDT, *p,p'* DDE, *o,p'* DDE, *o,p'* DDD, *p,p'* DDD,  $\alpha$ -,  $\beta$ -,  $\gamma$ -HCH, HCB, *trans*-, *cis*-chlordane, oxychlordane, *trans*-, *cis*-nonachlor). Congeners detected in less than 70% of the samples were removed from the data set (Noël et al., 2009). Thereby, those remaining for further investigations were the PCBs (CB-28, -99, -105, -118, -138, -153, -180, -183, -187 and -194), the pesticides (*p,p'* DDE,  $\beta$ -HCH, HCB) and the chlordane mixture (oxychlordane, *trans*-, *cis*-nonachlor) hereafter referred as  $\Sigma$ CHLs. To a blood total sample of 0.5 to 1.5 ml, a 100  $\mu$ L of the internal standard solution was added ( $^{13}\text{C}$ -labeled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 mL of n-hexane, after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975CMSD were performed as previously described (Herzke et al., 2009). For validation of the results, blanks (clean and empty glass tubes treated like a sample) and standard reference material (1589a human serum from NIST) were run for every 10 samples. The accuracy of the method was within the 70 and 108% range.

PFASs were analyzed from plasma at NILU. The following compounds were searched: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), linear perfluorooctanesulfonate (PFOSlin), perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA),

perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA), and perfluorotetradecanoate (PFTeA). The compounds detected in >70% of the samples were PFOSlin, PFNA, PFDcA, PFUnA, PFDoA, and PFTrA. Mean concentrations  $\pm$  standard errors, ranges and limits of detections (LODs) of the other PFASs are detailed in Supplementary materials. Briefly, a sample (0.5 mL) spiked with internal standards was extracted in acetonitrile (1 mL) by repeated sonication and vortexing. The supernatant was cleaned-up using ENVICarb graphitized carbon absorbent and glacial acetic acid. Extracts were analyzed by UPLC/MS/MS. Recovery of the internal standards ranged between 50% and 120%. The deviation of the target concentrations in the SRMs (NIST Human serum 1958; NIST, 2015) were within the laboratory's accepted range (76–105%; n=3). All blanks contained concentrations below the instrument detection limits. The presented PFAS concentrations were corrected for recovery.

Total Hg analyses were performed at the Littoral Environment et Sociétés laboratory (LIENSs) in La Rochelle, France from freeze-dried and powdered red blood cells placed in an Advanced Hg Analyzer Spectrophotometer (Altec AMA 254, as detailed in Bustamante et al. (2006)). Hg is essentially associated to the red blood cells and thus, represents well the bird Hg exposure. All analyses were repeated at least two times on aliquots ranging from 5 to 10 mg of red blood cells until having a relative standard deviation <5%. Accuracy was regularly checked using certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified value  $0.27 \pm 0.06$   $\mu\text{g/g dw}$ ). Blanks were analyzed at the beginning of each set of samples and the detection limit of the method was  $0.005$   $\mu\text{g/g dw}$ .

## **2.4. Molecular sexing and hormone assays**

Molecular sexing and hormone assays were performed at the Centre d'Etudes Biologiques de Chizé (CEBC), France. The sex of the individuals was determined from red blood cells by

polymerase chain reaction amplification of part of two highly conserved genes (CHD) present on sexual chromosomes following Fridolfsson and Ellegren (1999).

TT3 and TT4 analyses were performed by radioimmunoassay (RIA) on 35 and 33 kittiwakes, respectively (TT3: 15 males and 20 females; TT4: 15 males and 18 females). Total TH levels were assessed in duplicates without extraction. 25  $\mu$ L of plasma were incubated during 24 h with 10000 cpm of the appropriate  $^{125}$ I-hormone (Perkin Elmer, US) and polyclonal rabbit antiserum supplied by Sigma company (US). The bound fraction (hormone linked to antibody) was then separated from the free fraction by addition of a sheep anti-rabbit antibody and centrifugation. After overnight incubation and centrifugation, bound fraction activity was counted on a wizard 2 gamma counter (Perkin Elmer, US). Cross-reactions of T3 antiserum were defined as follows by Sigma: triiodoD-thyroacetic acid 6%, L-thyroxine 0.2%, diiodo-L-thyrosine <0.01%, moniodo-L-thyrosine <0.01%. Cross-reactions of T4 antiserum were defined as follows by Sigma: triiodothyronine 4%, diiodo-L-thyrosine <0.01%, moniodo-L-thyrosine <0.01%. Inter- and intra-assay variations were respectively 15.9% and 7.5% for TT3, 19.4% and 12.2% for TT4. The lowest TT3 detectable concentration was 0.34 ng/mL and it was 0.51 ng/mL for TT4. Two samples were serially diluted in the assay buffer and their displacement curves were parallel to the standard curve.

## **2.5. Statistical analyses**

Statistical tests were performed using R 3.3.1 (R Core Team, 2016). Relationships between continuous variables were tested by the Pearson correlation test. The influence of contaminant levels on MR were investigated with linear models. Biologically relevant models were constructed by incorporating one contaminant and its interaction with the sex when possible. The best models were selected based on the bias adjusted Akaike's Information Criterion (AICc), which is a small-sample size bias adjustment (Burnham and Anderson, 2002). As a

general guideline, if AICc values differ by more than 2, the lowest AICc is the most accurate, whereas models with AICc values differing by less than 2 have a similar level of support in their ability to describe the data. Additionally, the Akaike weight ( $W_i$ ) was estimated and can be interpreted as the approximate probability that the model  $i$  is the best one for the observed data, given the candidate set of models (Burnham and Anderson, 2002; Johnson and Omland, 2004). Diagnostic plots were finally assessed to test whether the data sufficiently met the assumption of the linear model and data were log-transformed when necessary (Zuur et al., 2009). A significance level of  $\alpha < 0.05$  was used in this study.

### **3. Results**

#### **3.1. OCs, PFASs and Hg**

OCs, PFASs and Hg mean concentrations  $\pm$  standard errors and limits of detection (LODs) in female and male chick-rearing adult kittiwakes are listed in Table 1. OC concentrations were not related to sex ( $p \geq 0.245$  for all tests; Table 1) whereas Hg and all PFASs except PFNA and PFTrA significantly differed between sexes, with males having higher concentrations than females (Table 1). Such sex-related differences of PFAS and Hg concentrations could be attributed to the ability of females to transfer elevated amounts of contaminants into their eggs (Becker, 1992; Gebbink et al., 2012; Helgason et al., 2011). Because of the sex-related differences for PFAS and Hg concentrations, including the factor “sex” and the variables PFAS or Hg within the same model could induce multicollinearity problems and lead to biased results (Graham, 2003). Consequently, sexes were analyzed separately in further statistical analyses for Hg and PFASs only.

### 3.2. MR and total THs

Body mass and MR mean concentrations  $\pm$  standard errors for male and female chick-rearing adult kittiwakes are presented in Table 2. Since body mass was positively related to non-mass-specific MR ( $F_{1,21} = 17.01$ ;  $p < 0.001$ ) and since males were significantly heavier than females (Table 2), non-mass-specific MR tended to be higher in males than in females (Table 2). By contrast, mass-specific MR did not differ between sexes (Table 2) and is not related to body mass ( $F_{1,21} = 0.99$ ;  $p = 0.330$ ). Consequently, mass-specific MR (“MR” hereafter) was used in further statistical analyses in order to control for the effect of body mass on MR.

Because some MR measurements were made at  $T_a$  exceeding 20 °C, we investigated the effect that such  $T_a$  might have. Although  $T_b$  was dependent on  $T_a$  ( $R^2 = 0.26$ ,  $p = 0.009$ ), only one bird had a body temperature exceeding the mean  $\pm 1$  sd ( $> 40.5 \pm 0.7$  °C). However, we reanalysed all MR tests below, removing any birds whose  $T_a$  exceeded 20 °C and we found no change in results. More important was an effect on MR directly. We specifically examined the MR of all birds measured above 20 °C. Three of those birds had MRs above the mean  $\pm 1$  sd ( $> 1.57 \pm 0.16$  mL O<sub>2</sub>/g h). We removed those birds from analyses and again found no differences from the entire data set. Moreover, there does not appear to be relationships between  $T_a$  and MR within the whole data set ( $R^2 = 0.03$ ,  $p = 0.43$ ,  $n = 23$ ), nor within individuals above 20 °C ( $R^2 = < 0.001$ ,  $p = 0.98$ ,  $n = 7$ ). Finally,  $T_b$  was not related to MR within the whole data set ( $R^2 = 0.044$ ,  $p = 0.35$ ). This partly excludes a potential effect of  $T_a$  and  $T_b$  on metabolic activity in this study and we believe that MR of those individuals closely approximates their BMR. We consequently kept all oxygen measurements in our data set for the statistical analyses below.

Mean concentrations  $\pm$  standard errors of TT3 and TT4 levels are reported in Table 2. Although TT4 tended to be higher in males than in females, total TH concentrations did not differ between sexes (Table 2). While TT3 was significantly and positively correlated with TT4 when both

sexes were pooled ( $r = 0.46$ ;  $p = 0.006$ ), no significant correlations were found between total THs and BMR (TT3:  $r = 0.16$ ;  $p = 0.530$  and TT4:  $r = 0.38$ ;  $p = 0.118$ ).

Finally, since BMR is potentially related to individual quality (Blackmer et al., 2005; reviewed in Burton et al., 2011), we checked for a potential confounding effect of the reproductive investment of the kittiwakes (i.e. the number of raised chicks at the time of sampling) on MR and TH levels. However, we did not find any significant relationships between the number of raised chicks at the time of sampling (i.e. 1 or 2 chick size clutch) and MR ( $F_{1,20} = 1.62$ ;  $p = 0.22$ ), nor the TH levels (TT3:  $F_{1,31} = 0.06$ ;  $p = 0.80$ , TT4:  $F_{1,29} = 1.92$ ;  $p = 0.18$ ).

### **3.3. Relationships between contaminants, MR and total THs**

The AICc model selection to explain MR variations based on OC levels for male and female chick-rearing adult kittiwakes is presented in Table 3. Among the OCs considered in this study, the model including  $\Sigma$ CHLs as an explanatory variable of MR showed the best fit to the data (Table 3). We observed a significant and negative relationship between  $\Sigma$ CHLs and MR in both sexes (Fig. 1; slope =  $-4.05 \times 10^{-5}$ ;  $r = -0.51$ ;  $p = 0.012$ ). Additionally,  $\Sigma$ CHLs was negatively and significantly related to circulating TT3 (Fig. 2;  $r = -0.38$ ;  $p = 0.027$ ) but not to TT4 concentrations ( $r = -0.14$ ;  $p = 0.423$ ). AICc model selection to explain MR variations based on PFASs and Hg levels is presented in Table 4. Considering all the PFASs investigated in the present study, only PFTrA significantly explained MR variations in females, but it did not in males where no valuable models were retained (Table 4). We found a significant and positive relationship between PFTrA and MR (Fig. 3; slope =  $7.61 \times 10^{-5}$ ;  $r = 0.75$ ;  $p = 0.008$ ), however, PFTrA and total TH concentrations were not related (TT3:  $r = 0.32$ ;  $p = 0.175$  and TT4:  $r = 0.08$ ;  $p = 0.742$ ). Finally, Hg was not related to MR neither in males nor in females (Table 4). The two explanatory variables,  $\Sigma$ CHLs and PFTrA were significantly and negatively related ( $r =$

-0.51;  $p = 0.015$ ) in females. In other words, individuals with high concentrations of  $\Sigma$ CHLs had low PFTrA levels, and conversely.

#### **4. Discussion**

Our results show that among all tested contaminants,  $\Sigma$ CHLs in both adult males and females (sexes are pooled), and PFTrA only in adult females were the best predictors to explain MR variations in adult breeding kittiwakes, although in opposite ways. We found a significant and negative relationship between  $\Sigma$ CHLs and MR in both sexes whereas PFTrA and MR were significantly and positively related in females only. This suggests a contrasted effect of organochlorine pesticides and PFASs on MR variation, with  $\Sigma$ CHLs possibly inducing a decrease in MR and PFTrA possibly leading to an increase in basal metabolic activity. Additionally, the present study suggests some possible underlying mechanisms linking  $\Sigma$ CHLs and MR in kittiwakes since we reported a significant and negative relationship between  $\Sigma$ CHLs and TT3 levels in both sexes.

In Svalbard, kittiwakes are exposed to a complex mixture of potential harmful halogenated compounds but only  $\Sigma$ CHLs and PFTrA appear to disrupt their basal energy expenditure. Yet, concentrations of  $\Sigma$ CHLs and PFTrA represent only a small proportion (5% and 29%, respectively) of the total concentrations of  $\Sigma$ OCs and  $\Sigma$ PFASs in our kittiwakes. Consequently, this study highlights a metabolic disruption of those specific contaminants despite their relatively small proportion compared to other more abundant compounds which are not related to MR (e.g.  $\Sigma$ PCBs: 81% of  $\Sigma$ OCs).

#### **4.1. Relationships between contaminants and MR**

Effects of contaminants on metabolic activity are still poorly investigated and are largely debated in literature. Thus, this study partly fills the gap of knowledge about the effects of environmental contaminants on energy expenditure in endotherms. Although some experimental designs conducted in adult mammals reported conflicting observations with increased (Braham and Neal, 1974; Voltura and French, 2000) or unchanged (French et al., 2001; Gordon et al., 1995) BMR in response to OCs exposure (PCBs, DDTs and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin), other studies conducted in wild birds corroborate our results. Indeed, decreased metabolic rate was observed in captive doves and pigeons exposed to Aroclor 1254 and DDT, respectively (Jefferies and French, 1971; Tori and Mayer, 1981). Similar to our findings, a study conducted on free-living Arctic-breeding glaucous gulls revealed negative associations between BMR and concentrations of PCBs, DDTs, and especially CHLs (Verreault et al., 2007). Finally, two previous studies conducted in humans also support our findings since they highlighted lower metabolic activity in response to increasing plasma concentration of OCs mixture (including CHLs; Pelletier et al., 2002; Tremblay et al., 2004). Consequently, this study in combination with previous works points-out a possible negative effect of CHLs on BMR.

To date and to the best of our knowledge, our study is the first one to investigate relationships between PFASs and MR in vertebrates. Therefore, no comparisons with previous studies are possible and our findings can only suggest a possible positive effect of PFTrA on MR in female kittiwakes. Regarding the contrasted observations of contaminant effects on metabolic activity reported here and the discrepancy in the results between studies previously conducted, the effects of environmental contaminants on BMR appear to be complex. Such differences could be related to dissimilarities in methodological designs which are known to affect BMR values (Ellis and Gabrielsen, 2002). Moreover, it cannot be excluded that BMR variation in response



to contaminant exposure is dependent on the taxa considered (e.g. mammals vs birds; Peakall, 1992) and tightly linked to the level of contamination (dose-dependence effect). Finally, since OCs and PFASs are structurally opposed, with OCs being lipophilic (Frindlay and Defretas, 1971) and PFASs having a high affinity for proteins (Giesy and Kannan, 2002), further investigations focusing on structurally different chemicals may enable to clarify some of our results.

#### **4.2. BMR and THs**

A hormonal cascade along the hypothalamic-pituitary-thyroid axis will lead to the production and release in blood of T4 from the thyroid gland. The transport of T4 will then be ensured by serum binding proteins such as albumin and transthyretin (TTR). T4 is then converted by peripheral deiodination to T3 under the control of deiodinase enzymes (McNabb, 2007; Silvestri et al., 2005). Although the detailed pathways of how THs can affect energy expenditure are still unclear (Hulbert, 2000), it is now well established under laboratory conditions that plasma concentrations of THs increase the BMR of birds and mammals (Hulbert, 2000; Kim, 2008; Silvestri et al., 2005). This is particularly true for T3, which is considered as the primary metabolically active THs controlling BMR (Bobek et al., 1977; but see Silvestri et al., 2005). Besides, several studies conducted in domestic as well as in free-living animals, including kittiwakes from the same Svalbard population as in the present study, have highlighted strong and positive associations between TT3 concentrations and BMR (Bobek et al., 1977; Chastel et al., 2003; Elliott et al., 2013; Vézina et al., 2009; Welcker et al., 2013; Zheng et al., 2013). Thus, one possible underlying mechanism that could explain relationships between contaminants and BMR in kittiwakes could be induced by endocrine-disrupting properties of those chemicals. MR and TT3 concentrations were not related in our study, nevertheless we only measured total THs and not the metabolically active free fraction

of THs. Furthermore, since MR in the present study was measured few hours after blood sampling used for total THs quantification, plasma THs at the time of MR measurement might be slightly different (Verreault et al., 2007; but see Welcker et al., 2013).

#### **4.3. Relationships between contaminants and total THs**

Within the past decades, a wide body of evidence suggested a possible disruption of THs in response to OCs exposure in vertebrates and several studies conducted in free-living animals have reported reduced TH concentrations with increasing OC levels (Braathen et al., 2004; Brouwer et al., 1998; Cesh et al., 2010; Dawson, 2000; Jenssen, 2006; McNabb, 2005; McNabb and Fox, 2003; Peakall, 1992; Rolland, 2000; Scanes and McNabb, 2003). For example, a previous study conducted in another Arctic seabird, the glaucous gull, stated that among 18 different congeners, oxychlordan (included here in  $\Sigma$ CHLs) and HCB appeared to be the most prominent OCs in terms of their negative effect on the variation of the TT4:TT3 ratio (Verreault et al., 2004). Moreover, Smits and Fernie (2013) showed negative relationships between plasma total THs (TT3 and TT4) and several organohalogenated contaminants, including CHLs, in peregrine falcon nestlings (*Falco peregrinus*). Similarly, an experimental study conducted in American kestrels (*Falco sparverius*) reported depressed TT3 levels in PCB-exposed birds (Smits et al., 2002). Here, we reported a significant negative relationship between  $\Sigma$ CHLs and TT3 but not with TT4, while TT3 and TT4 were significantly and positively related. This suggests that  $\Sigma$ CHLs could possibly alter circulating TH levels and specifically T3 levels by affecting either the conversion of T4 to T3 and/ or the transport of circulating T3. Indeed, inhibition of mono-deiodinase activity can skew the concentration of the biologically active T3 (review in Brouwer et al., 1998). This has been reported in white Leghorn chick (*Gallus gallus*) embryos, where administration of PCB mixtures induced decreased hepatic deiodinase activities (Gould et al., 1999). Therefore, our study highlights the potential endocrine disrupting

properties of CHLs on circulating TH levels in an Arctic seabird.

To date, the effects of PFASs on THs have received much less attention and are somewhat contradictory (review in DeWitt, 2015). While some studies conducted in murine and primate models indicated negative associations between PFASs and TH levels (Lau et al., 2003; Seacat et al., 2002), Liu et al. (2011) reported increased TT3 levels in response to experimental PFASs exposure in the zebrafish (*Danio rerio*). Additionally, recent studies conducted on Arctic seabirds, the glaucous gull (Haugerud, 2011; Melnes, 2014), the northern fulmar (*Fulmarus glacialis*; Braune et al., 2011; Nøst et al., 2012), the Arctic skua (*Stercorarius parasiticus*), and kittiwakes (Ask, 2015; Nøst et al., 2012) have shown significant and positive associations between PFASs and total TH levels. Besides, the study of Ask (2015) was conducted on the same population as ours and indicated a positive relationship between PFTrA and TT3 only in females. This could indicate a hormetic response of the thyroid hormone system to PFASs exposure, but underlying mechanisms are still unclear and need to be further investigated (e.g. Videla, 2010). Firstly, it has been proposed that PFASs increase the expression of transcripts of hepatic transporters (e.g. OAPT2) in rats, which in turn, increases uptake of THs into the liver (Yu et al., 2011). Another disruptive effect of PFASs is displacing THs from binding proteins (Mortensen, 2015; Weiss et al., 2009). Finally, PFASs could act directly on the thyroid gland itself (Coperchini et al., 2015). Although there are several reports showing a positive association between PFASs and total TH levels in seabirds, we did not observe a relationship between PFTrA and TT3. Therefore, our study does not confirm the potential thyroid-disrupting properties of PFTrA previously reported in Arctic seabirds and further investigations conducted on a larger sample size in combination with experimental dose-dependence effect of PFASs on TH levels are needed.

#### **4.4. Possible consequences on individual fitness**

Individual BMR variations may influence fitness because self-maintenance and reproduction are considered as two key life-history components (review in Burton et al., 2011; Stearns, 1992). In female kittiwakes, adaptive decrease in mass-specific BMR prior to hatching has been suggested as a mean to reduce self-maintenance and to increase chick food provisioning (Bech et al., 2002; Rønning et al., 2008). However, energetic balance is a sensitive system and additional cost from CHLs expressed here as a reduction of BMR could have some consequences on fitness. In that case, self-maintenance could be impacted, leading individuals to be less able to cope with reproduction. Indeed, two recent studies conducted in the same kittiwake population reported decreased adult survival rate, reduced telomere length (a predictor of survival), and lower breeding probability (Blévin et al., 2016; Goutte et al., 2015) in response to CHLs contamination. Despite some inconstancies, this has already been demonstrated experimentally and under field conditions in mammals that individuals with lower BMR tend to survive less (Burton et al., 2011; Speakman et al., 2004; but see Burton et al., 2011). By contrast, exposure to PFTrA may disrupt the ability of female kittiwakes to adaptively decrease BMR, thus might possibly explain the lower hatching success in most PFASs-contaminated adults (Tartu et al., 2014). In female kittiwakes, the BMR during the incubation is a good predictor of the BMR during the chick-rearing period (Bech et al., 1999). Consequently, it is likely that exposure to PFASs during the incubation period could have significant consequences for the metabolic adjustments required for the chick rearing period. In that case, most PFTrA-contaminated female kittiwakes would allocate more energy for self-maintenance rather than for reproduction. Thus, an indirect positive effect of PFASs exposure might occur on survival rate in adult kittiwakes, at the expense of reproduction. However, this statement needs to be confirmed with capture-mark-recapture (CMR) investigations since the present study did not report a relationship between the number of raised chick and MR.

#### **4.5. Limitations of the study and other potential confounding factors**

Given the absence of information in literature about PFAS effects on BMR and because our study did not report any relationships between PFTrA and total THs, we cannot conclude with certainty about effects of PFASs on basal energy expenditure. The positive relationship between PFTrA and MR in female kittiwakes may rely on other potential confounding factors. Indeed, since food ingestion is the main route for PFASs exposure, further investigations focusing on ecological variables directly linked to feeding ecology (e.g. via stable isotopes, protein amounts analysis) of kittiwakes should be included as predictors of MR. Additionally, the negative relationship between  $\Sigma$ CHLs and PFTrA in female kittiwakes is a crucial point that could lead to misleading interpretations. Similarly, although this study is considering three different groups of contaminants, kittiwakes are obviously exposed to an additional mixture of chemicals which are not included in this study and which could act on energy expenditure too. Finally, our study was conducted on a limited number of individuals and to fully validate our findings, future investigations focusing on the effects of PFASs on BMR should be conducted experimentally, with a laboratory avian model.

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#### **Conflict of interest**

The authors declare no conflicts of interest.

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**Table 1.** OCs (ng/ mL ww), PFASs (ng/ mL ww) and Hg (µg/g dw) mean concentrations ± standard errors and limits of detection (LODs) of female and male chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. OCs have been measured in whole blood, PFASs in plasma and Hg in red blood cells.

		Males (n = 22)			Females (n = 22)				
	LODs	Mean ± SE	Median	Range	Mean ± SE	Median	Range	<i>F</i> <sub>1,42</sub>	P-value
OCs									
ΣPCBs <sup>a</sup>	18.5 10 <sup>−3</sup>	82.616 ± 7.385	75.618	[23.911; 169.216]	71.176 ± 6.778	59.342	[30.878; 137.262]	1.351	0.252
ΣCHLs <sup>b</sup>	2.7 10 <sup>−3</sup>	4.585 ± 0.314	4.651	[2.147; 7.091]	4.773 ± 0.470	4.781	[2.082; 10.032]	0.002	0.970
HCB <sup>c</sup>	0.2 10 <sup>−3</sup>	5.409 ± 0.271	5.280	[3.160; 7.370]	5.491 ± 0.329	5.466	[3.280; 10.000]	0.037	0.848
β-HCH <sup>d</sup>	53.5 10 <sup>−3</sup>	0.274 ± 0.030	0.251	[0.080; 0.636]	0.331 ± 0.046	0.324	[0.130; 1.190]	1.393	0.245
p,p′-DDE <sup>e</sup>	23.2 10 <sup>−3</sup>	8.377 ± 2.009	5.960	[1.430; 44.700]	6.216 ± 0.818	6.121	[0.832; 16.000]	0.241	0.626
PFASs									
PFOSlin <sup>f</sup>	704 10 <sup>−3</sup>	10.847 ± 0.574	10.352	[6.755; 15.191]	8.922 ± 0.676	8.108	[4.440; 14.394]	5.813	0.020
PFNA <sup>g</sup>	40.9 10 <sup>−3</sup>	1.210 ± 0.099	1.210	[0.478; 2.593]	1.081 ± 0.144	0.902	[0.383; 3.047]	1.926	0.173
PFDcA <sup>h</sup>	61.9 10 <sup>−3</sup>	2.193 ± 0.120	2.269	[1.104; 3.123]	1.625 ± 0.122	1.410	[0.886; 2.894]	11.000	0.002
PFUnA <sup>i</sup>	83 10 <sup>−3</sup>	12.110 ± 0.641	12.110	[6.487; 17.546]	9.383 ± 0.688	8.390	[5.499; 17.313]	8.402	0.006
PFDcA <sup>j</sup>	109 10 <sup>−3</sup>	2.541 ± 0.136	2.468	[1.394; 3.815]	1.995 ± 0.160	1.821	[0.926; 4.015]	8.744	0.005
PFTTrA <sup>k</sup>	360 10 <sup>−3</sup>	11.618 ± 1.410	9.819	[2.780; 23.055]	9.675 ± 1.521	6.650	[2.711; 29.735]	1.574	0.217
Trace elements									
Hg <sup>l</sup>	5 10 <sup>−3</sup>	1.14 ± 0.07	1.099	[0.646; 1.771]	0.89 ± 0.05	0.871	[0.505; 1.641]	7.666	0.008

Significant p-values are in bold.

<sup>a</sup> ΣPCBs (ΣPolychlorinated biphenyls): CB-28, 99, 105, 118, 138, 153, 180, 183, 187, 194.

<sup>b</sup> ΣCHLs (ΣChlordanes): Oxychlordane, *cis*-nonachlor and *trans*-nonachlor.

<sup>c</sup> HCB: Hexachlorobenzene.

<sup>d</sup> β-HCH: β-hexachlorocyclohexane.

<sup>e</sup> *p,p'*-DDE: Dichlorodiphenyldichloroethylene.

<sup>f</sup> PFOSlin: Perfluorooctane sulfonate.

<sup>g</sup> PFNA: Perfluorononanoate.

<sup>h</sup> PFDcA: Perfluorodecanoate.

<sup>i</sup> PFUnA: Perfluoroundecanoate.

<sup>j</sup> PFDcA: Perfluorododecanoate.

<sup>k</sup> PFTTrA: Perfluorotridecanoate.

<sup>l</sup> Hg: Mercury.



**Table 2.** Body mass (g), non-mass-specific BMR (mL O<sub>2</sub>/h), mass-specific BMR (mL O<sub>2</sub>/g·h), total T3 and total T4 (ng/ mL) mean concentrations  $\pm$  standard errors for female and male chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard.

	<b>Males</b>	<b>Females</b>			
	<b>Mean <math>\pm</math> SE</b>	<b>Mean <math>\pm</math> SE</b>	<b><i>df</i></b>	<b><i>F</i></b>	<b>P-value</b>
Body mass	389.75 $\pm$ 4.18	342.08 $\pm$ 6.72	1,22	34.72	<b>&lt; 0.001</b>
Non-mass-specific BMR	576.03 $\pm$ 24.15	517.21 $\pm$ 17.89	1,21	3.831	0.064
Mass-specific BMR	1.57 $\pm$ 0.05	1.57 $\pm$ 0.04	1,21	0.012	0.916
Total T3	2.29 $\pm$ 0.25	2.24 $\pm$ 0.17	1,33	0.036	0.850
Total T4	17.39 $\pm$ 1.57	22.35 $\pm$ 1.91	1,31	3.808	0.060

Significant p-values are in bold

**Table 3.** AICc model selection to explain MR variations based on OC concentrations in female and male chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. OCs have been measured in whole blood. Sexes are pooled (n = 23, 12 males and 11 females).

<b>Models<sup>a</sup></b>	<b>AICc</b>	<b><math>\Delta</math>AICc<sup>b</sup></b>	<b><math>W_i^c</math></b>
$\Sigma$ CHLs	-21.23	0.00	0.47
$\Sigma$ CHLs : sex	-18.67	2.56	0.13
$\Sigma$ PCBs	-18.55	2.68	0.12
HCB	-17.44	3.78	0.07
<i>p,p'</i> -DDE	-17.10	4.13	0.06
null	-16.87	4.36	0.05

Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values;  $W_i$ , AICc weights.

<sup>a</sup> Only the five best ranked and the null models are presented.

<sup>b</sup> Scaled  $\Delta$ AICc;  $\Delta$ AICc = 0 is interpreted as the best fit to the data among the models.

<sup>c</sup> Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

**Table 4.** AICc model selection to explain MR variations based on PFASs and Hg concentrations in female and male chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. PFASs have been measured in plasma and Hg in red blood cells. Sexes are analyzed separately.

Models <sup>a</sup>	AICc	$\Delta$ AICc <sup>b</sup>	$W_i$ <sup>c</sup>
<b>Males</b>			
null	-2.91	0.00	0.45
PFNA	0.22	3.13	0.09
PFOSlin	0.33	3.23	0.09
PFDCa	0.45	3.36	0.08
PFTTrA	0.65	3.57	0.07
PDFoA	0.74	3.64	0.07
<b>Females</b>			
PFTTrA	-13.93	0.00	0.61
PFOSlin	-10.75	3.18	0.12
PFUnA	-9.52	4.41	0.07
PFDCa	-9.21	4.71	0.06
Hg	-8.84	5.09	0.05
null	-8.80	5.13	0.05

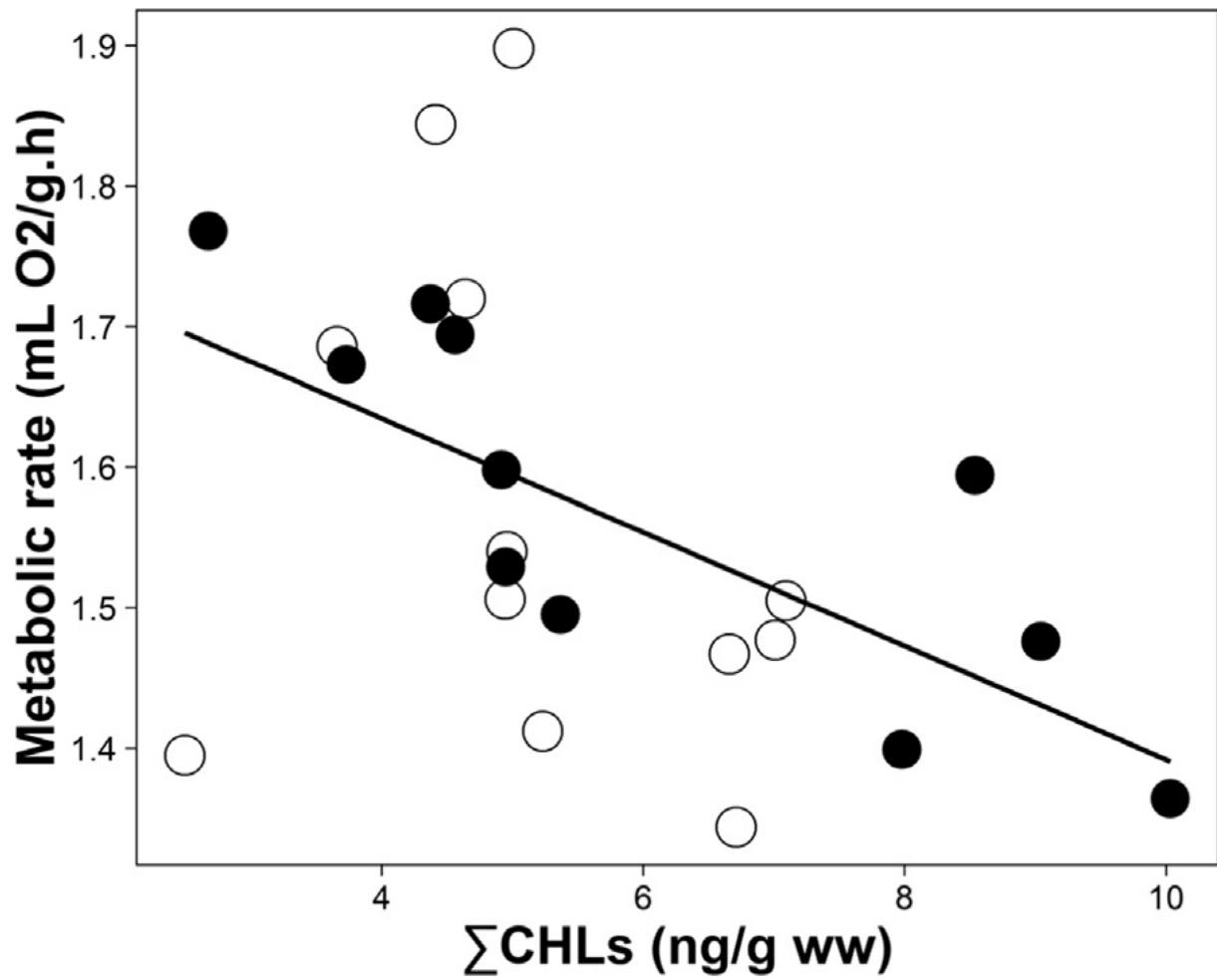
Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values;  $W_i$ , AICc weights.

<sup>a</sup> Only the five best ranked and the null models are presented.

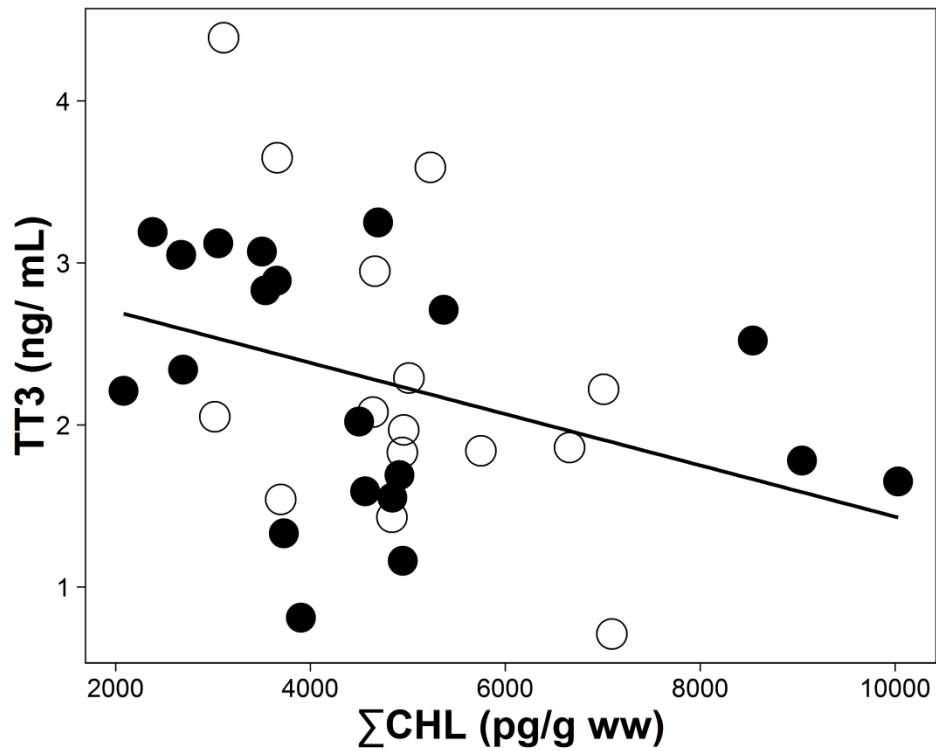
<sup>b</sup> Scaled  $\Delta$ AICc;  $\Delta$ AICc = 0 is interpreted as the best fit to the data among the models.

<sup>c</sup> Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

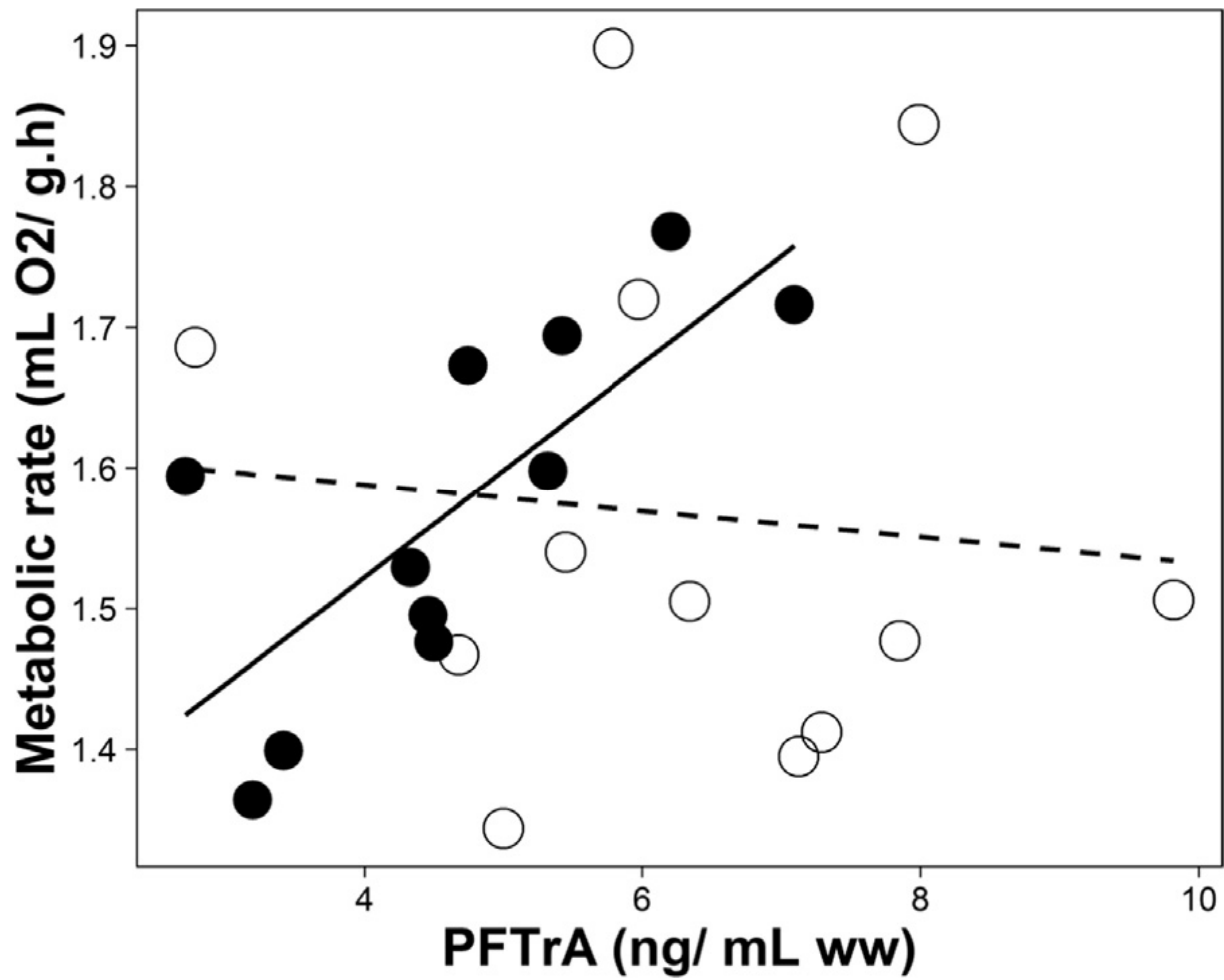




**Fig 1.** Relationship between  $\Sigma$ CHLs concentrations and MR in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Males ( $n = 12$ ) are represented with empty circles and females ( $n = 11$ ) with filled circles. CHLs have been measured in whole blood.



**Fig 2.** Relationship between  $\Sigma\text{CHLs}$  concentrations and TT3 in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Males ( $n=15$ ) are represented with empty circles and females ( $n=20$ ) with filled circles. CHLs have been measured in whole blood and TT3 in plasma.



**Fig 3.** Relationships between PFTTrA concentrations and MR in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Males ( $n = 12$ ) are represented with empty circles and dashed line and females ( $n = 11$ ) with filled circles and solid line. PFTTrA has been measured in plasma.