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Demographic consequences of heavy metals and persistent organic pollutants in
a vulnerable long-lived bird, the wandering albatross

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Abstract: Seabirds are top predators of the marine environment that accumulate contaminants over a long life-span. Chronic exposure to pollutants is thought to compromise survival rate and long-term reproductive outputs in these long-lived organisms, thus inducing population decline. However, the demographic consequences of contaminant exposure are largely theoretical because of the dearth of long-term datasets. This study aims to test whether adult survival rate, return to the colony and long-term breeding performance were related to blood mercury (Hg), cadmium (Cd) and persistent organic pollutants (POPs), by using a capture–mark–recapture dataset on the vulnerable wandering albatross *Diomedea exulans*. We did not find evidence for any effect of contaminants on adult survival probability. However, blood Hg and POPs negatively impacted long-term breeding probability, hatching and fledging probabilities. The proximate mechanisms underlying these deleterious effects are likely multifaceted, through physiological perturbations and interactions with reproductive costs. Using matrix population models, we projected a demographic decline in response to an increase in Hg or POPs concentrations. This decline in population growth rate could be exacerbated by other anthropogenic perturbations, such as climate change, disease and fishery bycatch. This study gives a new dimension to the overall picture of environmental threats to wildlife populations.

Keywords: capture-recapture; *Diomedea exulans*; mercury; polybrominated diphenyl ethers (PBDE), polychlorinated biphenyl; pesticides

1. Introduction

Free-living animal populations are affected by a wide range of anthropogenic pressures. Chronic exposure to metallic and organic pollutants may compromise survival and long-term fecundity, thereby leading to population decline. For instance, mercury (Hg) is a globally distributed heavy metal of particular concern for aquatic biota, because of the harmful effects of its organic form (methyl-Hg) on embryo development, neurology, immune system, physiology and behaviour [1–3]. Another ubiquitous heavy metal, cadmium (Cd), causes irreversible renal tubular damage, leading to reduced skeletal calcium content [4]. Persistent organic pollutants (POPs), such as organochlorine pesticides (HCB, HCH, DDE, DDD and DDT), polychlorinated biphenyls (PCBs) and brominated diphenyl ethers (BDEs), can persist in the environment for decades and trigger a suite of detrimental effects in vertebrates, including endocrine disruption, immunotoxicity, embryo mortality and behavioural impairments [5–7].

Although toxic effects of heavy metals and POPs have been well described at the individual level and under controlled laboratory conditions, their population level effects have been virtually neglected in free-living vertebrates because of the dearth of long-term datasets. In aquatic birds, there is no evidence of an impact of heavy metals on adult mortality [8,9]. In two *Catharacta* skua species, breeding failure in the following year, but not adult survival rate, was positively related to Hg exposure [10], as highlighted by the use of long-term datasets and multi-state mark–recapture models (MSMR [11]). Concerning POPs, very high concentrations of organochlorine compounds were related to increased mortality in the glaucous gull *Larus hyperboreus* [12], but their effects on long-term fecundity were not explored in that study.

The present study aimed to relate demographic parameters to pollutants in wandering albatrosses *Diomedea exulans*. The effects of Hg and Cd in red blood cells and \sum POPs in

plasma on apparent probabilities of adult survival, return to the breeding colony, breeding, hatching and fledgling, were investigated by using a MSMR approach. According to recent studies on long-lived seabirds [10,12], a deleterious effect of Hg was expected on long-term breeding performances and a possible lethal effect of POPs was expected in the most contaminated wandering albatrosses. Then, population-level responses to an increase in Hg and POPs levels were assessed by establishing a life cycle for the wandering albatross and by including Hg- or POP-dependent demographic traits in the matrix population models [13].

Despite high pollution burdens in albatrosses [14–16], the effects of contaminants on demographic parameters and population growth rate are unknown. Albatrosses are among the most highly endangered of the world's birds, with 18 of 22 species considered as threatened and the remaining four species considered as near threatened [17]. In this population of wandering albatrosses, population growth rate remained relatively stable during the 1960s (about 850 breeding pairs), before a first decline between the early 1970s and 1986, and a second decline since 2003 down to 380 breeding pairs [18]. Causes of decline in albatross populations have been attributed to fishery bycatch, climate change and disease [19]. This study thus gives a new dimension to the overall picture of environmental threats in albatross populations.

2. Material and Methods

a) Study area and species

The study was conducted at Ile de la Possession in the Crozet Archipelago (46° S, 52° E), Southern Indian Ocean, where 300–400 pairs of wandering albatrosses nest each year. Adults return to their breeding grounds in December and females lay a single egg in late December–early January. Both parents incubate alternately until hatching in March and most young are fledged in November. Up to 6% of the birds that fledged a chick bred again in the following

year, and the wandering albatross is considered to be a quasi-biennial breeding species [20]. Approximately 80% of birds that failed to breed in the previous year engage in another breeding attempt in the following year. All wandering albatrosses had been ringed and sexed as part of a long-term mark–recapture programme [21]. In December, pre-breeding adults are controlled over the whole island. From mid-January to mid-February, at least three visits are carried out every 10 days at each nest to determine the identity and breeding status (egg laid/egg hatched) of each individual. In mid-April, June and August, all nests are checked to monitor the chicks' survival.

b) Blood sampling

From 21 December 2007 to 04 March 2008, 147 sexually mature adults (i.e. observed as incubating or chick-rearing at least once before or during the current breeding season) were captured. A sample of venous blood was taken from the tarsus with a 1-ml heparinized syringe and a 25-gauge needle. Only one bird was sampled per nest. The volume of the blood draws never exceeded 0.05% of the bird's body mass (8–12 kg).

c) Laboratory analyses

Hg and Cd were analysed in red blood cells at the Littoral Environnement et Sociétés (LIENSs), La Rochelle, France. POPs (PCBs: CB-28, -52, -101, -118, -138, -153 and -180; organochlorine pesticides: HCB (hexachlorobenzene), Gamma HCH (hexachlorocyclohexane), Heptachlore, 2,4' DDE (dichlorodipenyldichloroethylene), Cis-chlordane, trans-nonachlor, 4,4' DDD (dichlorodipenyldichloroethane), 2,4' DDT (dichlorodipenyltrichloroethane), 4,4' DDT, Mirex; and BDE-47) were analysed in plasma at the EPOC-LPTC, Bordeaux, France. Further details about analyses are reported in the electronic supplementary material.

d) Estimating the effects of blood heavy metals and persistent organic pollutants on breeding output during the year of sampling

Generalized linear models (GLMs) with binomial error distribution and a logit link function were used to test whether breeding success in 2007–2008 was linked to blood Hg, Cd or POP levels in individuals sampled as breeders in 2007–2008. Breeding success was coded as 1 for birds that successfully fledged a chick, and as 0 for those that failed at the egg or chick stage. Analyses were performed using R [22].

e) Estimating the effects of blood heavy metals and persistent organic pollutants on demographic parameters

The capture–recapture data of sampled individuals from 2008 to 2012 were used to evaluate the effects of blood Hg, Cd and POPs on demographic parameters¹. A MSMR model was constructed, as developed by Pardo et al. [23,24], and included eight states: dead, failed breeder on egg (FBE, defined as an individual that was observed with one egg that failed to hatch), failed breeder on chick (FBC, defined as an individual that was observed with one chick but that failed to fledge the chick), successful breeder (SB, defined as an individual that fledged one chick), observable non-breeder (ONB, defined as an individual that was observed at the colony but that was not observed with an egg or a chick) and three unobservable states (UNB) consisting of non-breeders that were observed at the colony during the previous breeding attempt (PONB), non-breeders whose previous breeding attempt failed (PFB) and non-breeders whose previous breeding attempt was successful (PSB). The state coded as ‘dead’ (†) absorbed all those individuals that had either died or permanently emigrated from the study areas. The UNBs account for temporary absence, corresponding to birds that skip breeding in one year after breeding unsuccessfully or successfully during the previous year.

Models were parametrized in terms of the probability of survival (s), the probability of returning to the colony given survival (r), the probability of breeding given return to the colony (β), the probability of successful hatching given breeding (ω), the probability of

successful fledgling given hatching (γ) and the detection probability (p). Transition probabilities between states were thus modelled with a five-step procedure where s , r , β , ω and γ were considered as five successive steps in transition matrices. Parameters of the model are defined in the electronic supplementary material. We chose a MSMR approach as this allows us to take into account the probability of detecting individuals given their return to the study site, as well as the previous breeding state of individuals in order to obtain unbiased estimates of demographic parameters [11].

This MSMR model was parametrized by the survival–transition probabilities matrix:

$$\begin{array}{c}
 \text{FBE} \\
 \text{FBC} \\
 \text{SB} \\
 \text{ONB} \\
 \text{PFB} \\
 \text{PSB} \\
 \text{PONB} \\
 \dagger
 \end{array}
 \begin{bmatrix}
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & s(1-r) & \text{---} & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & s(1-r) & \text{---} & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & s(1-r) & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & \text{---} & s(1-r) & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & \text{---} & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & \text{---} & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & \text{---} & \text{---} & \text{---} & * \\
 sr\beta(1-\omega) & sr\beta\omega(1-\gamma) & sr\beta\omega\gamma & sr(1-\beta) & \text{---} & \text{---} & \text{---} & \text{---} & *
 \end{bmatrix}$$

Several constraints were made to ensure that the parameters of the model were estimable. The state ‘dead’ being explicitly included in the model but never being encountered, initial state probability was fixed at 0, transition probabilities from the state ‘dead’ to the other states were fixed at 0 and capture probability was fixed at 0 [25,26]. The probability of seeing individuals in UNBs and transitions between UNBs was constrained to 0. Since some individuals were observed breeding in the year consecutive to a successful breeding event [20], β_{SB} was not constrained to 0. To limit redundancy in survival parameters, models where survival probabilities all varied separately were not considered [27]. Because of the limited number of individual capture histories, the limited number of recapture occasions and the relatively large number of UNBs, we constrained (i) all parameters to be constant over time, (ii) r to be identical for ONB, PFB, PSB and PONB and (iii) β to be identical for PFB,

PSB and PONB. With these constraints, the initial model was full-rank. Note that the model where all demographic parameters were time- and state-dependent was highly rank deficient.

Once the best model structure was identified (Model 21, electronic supplementary material), effects of blood Hg, Cd and POPs were tested on demographic parameters to investigate whether contamination levels in one breeding season may influence the long-term survival and breeding outputs of an individual over the four following years. MSMR models were built where each demographic parameter θ was modelled as a function of an individual covariate C (standardized level of Hg, Cd or sum of POPs (log-transformed)) using a logit link function: $\text{logit}(\theta) = a + b X C_i$, where a is an intercept, b is a slope and C_i is the covariate for individual i . When $b < 0$, or $b > 0$, C has a negative or positive effect on θ , respectively.

The effect of C was first tested on each demographic parameter separately and for different states. Because some parameters were estimated as 100% [100–100%] (electronic supplementary material), we did not test the effects of C on the return probability of males previously observed as (FBE and FBC) or as (ONB, PFB, PSB and PONB), and on the breeding probability of unobservable non-breeders. The 95% confidence interval (CI) of the slope parameters was used, as well as Akaike's information criterion corrected (AICc) for small sample size [28] for inference. We considered a contaminant's effect to be statistically supported when 0 was outside the 95% CI of the mean of the slope of the relationship [29]. A composite model was then constructed by combining all the covariates that were detected to have an effect on demographic parameters. Composite models were constructed for heavy metals and POPs separately, as sample sizes differed. In composite models, an effect was not supported if the 95% CI of the slope parameter included 0 [29].

We tested the goodness-of-fit (GOF) of the time-dependent MSMR model using U-CARE [30]. All models were run under program E-SURGE 1.8.5 allowing splitting transition

probabilities between states [26]. To avoid estimating parameters at a local minimum of the likelihood function, each model was run five times with random initial values.

(f) Modelling population dynamics

To evaluate the population-level effects of contaminants, we constructed population models using pre-breeding matrices [13] structured by age and reproductive status classes. We built a two-sex age- and stage-classified matrix population model [13], because Hg and POPs concentrations affected the demographic parameters of males and females differently (see §3). Based on a detailed demographic study on wandering albatrosses [31], the model consists of five juvenile age classes, one pre-breeder class and seven stage-classes according to the breeding status (SB, FBE, FBC, ONB, PSB, PFB and PONB). Parameters entering the model were the recruitment probability, s of adult males, females and juveniles, r of SB and other states, β of SB, FB, ONB and UNB for males and females separately, ω and γ of breeders and non-breeders. We assumed a 1 : 1 sex ratio.

We first built a deterministic matrix model with no stochasticity, which included the mean estimates of the demographic parameters from our MSMR model results and from Barbraud et al. [31] for juvenile survival and recruitment probability. From these matrix analyses, we estimated the deterministic population growth rate [13]. We then focused on stochastic matrix models to estimate the stochastic growth rate. Environmental stochasticity was included in two different ways. When a vital rate had no significant relationship with levels of contaminant (C), its yearly values were sampled from a beta distribution [32], with mean and variance equal to those estimated from the MSMR model selected. When a vital rate had a significant relationship with C levels, its value was modelled as $\theta = \text{logit}^{-1}(a + b \times \hat{C})$, where \hat{C} is the mean value of the contaminant levels for all individuals. C values were sampled from a log-normal distribution for all individuals sampled. The values of a and b

were recalculated for non-standardized C values representing the absolute values for Hg and log-transformed values for Σ POPs. To assess the population-level effects of C , we estimated stochastic population growth rates according to changes in mean C levels within the range of observed C values. The matrix population models were analysed by Monte Carlo simulations (10 000 iterations) using package `popbio` [33] implemented in R [22].

3. Results

(a) Effects of blood heavy metal and persistent organic pollutants on current breeding output

Blood levels of contaminants are given in the electronic supplementary material. When considering only breeders in 2008, current breeding success was not related to Hg (d.f. = 1,105, $\chi^2 = 0.126$, $p = 0.723$), Cd (d.f. = 1,105, $\chi^2 = 0.008$, $p = 0.929$) or Σ POPs concentrations (d.f. = 1,81, $\chi^2 < 0.001$, $p = 0.993$).

(b) Demographic consequences of blood heavy metal levels

The GOF of the MSMR model was overall not significant (males: $\chi^2 = 16.327$, d.f. = 22, $p = 0.799$ and females: $\chi^2 = 7.078$, d.f. = 16, $p = 0.972$). The effects of sex and states on demographic parameters and the estimation of parameters are detailed in the electronic supplementary material.

Model selection and slope estimates suggested negative effects of Hg on the breeding probability of females previously in state ONB, and on hatching probability and fledging probability of individuals previously in states FB and SB (Table 1a). There was no detectable effect of Cd on demographic parameters (Table 1b). Slope estimates obtained from the composite model (Table 1c) were -2.114 (95% CI: (-4.213; 20.015)), -0.620 (-1.234; -0.005)

and -0.807 (-1.645;0.032) for the effects of Hg on breeding probability of females previously in state ONB (Figure 1a,c), hatching probability of individuals previously in states FB and SB (Figure 1b,c) and fledging probability of individuals previously in states FB and SB, respectively. The last effect was not supported, because 95% CI included 0 in the composite model.

(c) Demographic consequences of blood Σ POP levels

Model selection and slope estimates suggested a negative effect of Σ POPs on fledging probability of individuals previously in states ONB or UNB and on breeding probability of females previously in state ONB (Table 2). Other models were not supported, because 95% CI of slope parameter values included 0 (Table 2). Slope estimates obtained from the composite model were -0.976 (-1.917; -0.035) and -0.812 (-1.551; -0.072) for the effects of Σ POPs on breeding probability of females previously in state ONB (Figure 2a,c) and on fledging probability of individuals previously in states ONB or UNB (Figure 2b,c), respectively.

(d) Modelling population dynamics

The deterministic population growth rate was 1.038, and the respective generation time was 23.8. The stochastic population growth rates were 1.008 when accounting for mean Hg effects from MSMR analyses, 1.002 when accounting for mean POPs effects and 0.991 when accounting for both mean Hg and POPs effects (Figure 3). A doubling in mean Hg concentration would decelerate the population growth rate of 0.68%. A doubling in mean Σ POPs concentration would decelerate the population growth rate of 0.11%. Doublings in mean Hg and Σ POPs concentrations would decelerate the population growth rate of 1.31% (Figure 3).

4. Discussion

Using a unique long-term dataset and MSMR models, this study explores the demographic effects of both metallic and organic pollutants in a wild vertebrate population. Contaminant levels were associated with a lower breeding probability, a higher hatching failure and a higher fledgling failure, but not with adult survival rate in the wandering albatross. At the population level, a demographic decline was projected in response to increasing Hg and \sum POP levels.

(a) Effects of contaminants on current breeding output

Contrary to previous studies [1,2,5,7], no negative effect of blood Hg, Cd and \sum POPs was detected on breeding success at the year of sampling. The lack of relationship is probably due to the sampling protocol, since blood sampling was mainly conducted during the incubating period. Some effect of contaminants on early nest desertion and skipped breeding [3] may have been missed.

(b) Survival and contaminants

Estimated demographic parameters were similar to those previously estimated in the same population using all ringed individuals [20,24]. The survival rate of wandering albatrosses was not jeopardized by Hg, Cd and POPs. An effect of POPs was detected on survival rate in one of the most polluted seabirds, the glaucous gull breeding in the Norwegian Arctic, but only the most contaminated individuals had lower survival [12]. Concerning heavy metals, these findings corroborate previous studies that did not evidence an effect of Hg and Cd on adult mortality in birds [8–10]. However, our study did not exclude the possibility that contaminants could jeopardize the survival rate of immature wandering albatrosses, as they have a higher pollution burden [16] and a lower survival rate [31] than sexually mature adults.

(c) Long-term fecundity and heavy metals

A negative effect of blood Hg was detected on breeding probability of females observed as non-breeders. Concerning the proximate mechanisms, Hg, in its methylated form, is known to disrupt reproductive hormones [1] such as the luteinizing hormone, a key pituitary hormone for the onset of breeding [3].

As found in two southern *Catharacta* skua species [10], Hg negatively impacted hatching probability of albatrosses, but only in individuals previously observed as breeders. Energetic and time-dependent costs of reproduction may have downstream consequences for reproductive investment during the following breeding season (carry-over effect [35]). Hence, Hg load may have exacerbated these carry-over effects in individuals that previously bred. Concerning the possible proximate mechanisms, Hg may have caused long-term endocrine disruption of the reproductive system and behavioural impairments [1,2]. In addition, the maternal transfer of Hg into the egg may have altered embryo development [36].

(d) Long-term fecundity and Σ POPs

POP burdens reduced the long-term breeding probability of females previously observed as non-breeders and fledging probability of individuals that were previously non-breeders. Interestingly, POPs appeared to mostly affect albatrosses that skipped the preceding breeding attempt, suggesting a possible segregation of foraging areas between breeders and non breeders. Alternatively, non-breeding females may suffer from higher deleterious effects of POPs on long-term fecundity, because their loads of POPs were not eliminated through the egg.

During the incubating and chick-rearing periods, POPs may weaken the secretion of prolactin, a hormone closely involved in the mediation of parental care, as shown in glaucous

gulls [37]. In turn, a durable Hg-induced attenuation of prolactin release may result in fledgling failure.

The link between contaminants and reduced fecundity could be a by-product of age-dependant mechanisms. However, in this population, fecundity declined in the oldest individuals (35þ years old [24,38]), while Hg levels tended to decrease with age [16] and POP levels were unrelated to age (data not shown). Moreover, age (6–48þ years old) did not affect humoral immunity, oxidative stress, antioxidant defences or hormone levels in wandering albatrosses [38]. Hence, it is unlikely that age was a confounding factor in the correlation between contaminants and physiological mechanisms underlying breeding performance.

(e) Modelling population dynamics

Translating individual-level effects of contaminants to population-level processes is a crucial and ultimate goal of modern ecological research. Our population models suggested that the actual Hg and POPs levels could decelerate the population growth rate (0.991), whereas the population growth rate would increase (1.027) with zero concentrations of blood Hg and POPs. In addition, doublings in mean blood Hg and POPs levels would decelerate the growth rate of this wandering albatross population by 1.31%. These predictions could undoubtedly be worsened by other anthropogenic perturbations. For instance, climate change can impact transport, distribution, bioavailability, bioaccumulation and effects of pollutants [39–41] and triggers steep population declines in albatrosses [19]. In that respect, a future avenue for ecotoxicological and conservation research could be dedicated to evaluating and predicting the coupled effects of climatic and chemical perturbations on wildlife population viability.

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Table 1: Modeling the effects of standardized blood heavy metals levels (A. Hg; B. Cd; and C. composite model) on demographic parameters (N = 147 individuals). The estimated slope and 95% confidence intervals (CI) for models with a lower AICc than the intercept model (Hg0 and Cd0) are given.

Hypothesis	Model	Rank	Deviance	Δ AICc	Slope	[CI- ; CI+]
A. Effect of blood Hg on demographic parameters						
Effect of Hg on breeding probability of females previously in state ONB	Hg10	24	1442.026	0	-2.141	[-4.267 ; -0.014]
Effect of Hg on hatching success of individuals previously in states FB or SB	Hg3	24	1444.431	2.405	-0.659	[-1.271 ; -0.047]
Effect of Hg on fledgling success of individuals previously in states FB or SB	Hg1	24	1444.560	2.533	-0.844	[-1.659 ; -0.029]
Effect of Hg on breeding probability of males previously in states FBE or FBC	Hg5	24	1446.133	4.107	1.569	[-0.307 ; 3.445]
Intercept model: no effect of heavy metals on demographic parameters	Hg0	23	1449.217	4.946		
Effect of Hg on breeding probability of females previously in states FBE or FBC	Hg6	24	1448.236	6.210		
Effect of Hg on breeding probability of females previously in state SB	Hg8	24	1448.881	6.854		
Effect of Hg on return probability of individuals previously in state SB	Hg12	24	1448.955	6.929		
Effect of Hg on hatching success of individuals previously in states ONB or UNB	Hg4	24	1449.035	7.009		
Effect of Hg on breeding probability of males previously in state SB	Hg7	24	1449.159	7.133		
Effect of Hg on fledgling success of individuals previously in states ONB or UNB	Hg2	24	1449.208	7.182		
Effect of Hg on survival rate of females	Hg14	24	1449.209	7.183		
Effect of Hg on breeding probability of males previously in state ONB	Hg9	24	1449.215	7.189		
Effect of Hg on survival rate of males	Hg13	24	1449.216	7.190		
Effect of Hg on breeding probability of females previously in states PFB, PSB or PONB	Hg11	24	1449.217	7.191		
B. Effect of blood Cd on demographic parameters						
Effect of Cd on return probability of individuals previously in state SB	Cd12	24	1445.909	0	-0.350	[-0.758 ; 0.058]
Effect of Cd on hatching success of individuals previously in states ONB or UNB	Cd4	24	1446.645	0.735	0.520	[-0.204 ; 1.243]
Intercept model: no effect of of heavy metals on demographic parameters	Cd0	23	1449.217	1.063		
Effect of Cd on fledgling success of individuals previously in states ONB or UNB	Cd2	24	1447.314	1.405		
Effect of Cd on breeding probability of females previously in state ONB	Cd10	24	1447.905	1.996		
Effect of Cd on breeding probability of females previously in state SB	Cd8	24	1448.247	2.338		
Effect of Cd on breeding probability of females previously in states FBE or FBC	Cd6	24	1448.730	2.820		
Effect of Cd on hatching success of individuals previously in states FB or SB	Cd3	24	1448.744	2.835		

Effect of Cd on survival rate of females	Cd14	24	1448.794	2.885	
Effect of Cd on survival rate of males	Cd13	24	1449.048	3.139	
Effect of Cd on breeding probability of males previously in states FBE or FBC	Cd5	24	1449.054	3.145	
Effect of Cd on fledgling success of individuals previously in states FB or SB	Cd1	24	1449.129	3.220	
Effect of Cd on breeding probability of males previously in state SB	Cd7	24	1449.159	3.250	
Effect of Cd on breeding probability of males previously in state ONB	Cd9	24	1449.215	3.306	
Effect of Cd on breeding probability of females previously in states PFB, PSB or PONB	Cd11	24	1449.217	3.307	
C. Effect of blood heavy metals on demographic parameters					
Full model heavy metals (Hg10, Hg3, Hg1)	Hg15	26	1433.319	0	(cf text)
Intercept model: no effect of heavy metals on demographic parameters	Hg0	23	1449.217	9.131	

Table 2: Modeling the effects of blood Σ POP levels (log-transformed and standardized) on demographic parameters (N = 115 individuals). The estimated slope and 95% confidence intervals (CI) for models with a lower AICc than the intercept model (POP0) are given.

Hypothesis	Model	Rank	Deviance	Δ AICc	Slope	[CI- ; CI+]
Breeding probability of females previously in state SB	POP8	24	1129.266	0	2.763	[-0.205 ; 5.731]
Composite model (POP2, POP10)	POP15	25	1127.642	0.716		(cf text)
fledgling probability of individuals previously in states ONB or UNB	POP2	24	1130.764	1.498	-0.878	[-1.641 ; -0.114]
hatching probability of individuals previously in states ONB or UNB	POP4	24	1131.111	1.845	0.841	[-0.036 ; 1.719]
fledgling probability of individuals previously in states FB and SB	POP1	24	1131.312	2.046	1.226	[-0.075 ; 2.527]
hatching probability of individuals previously in states FB and SB	POP3	24	1131.434	2.168	1.006	[-0.116 ; 2.127]
breeding probability of females previously in state ONB	POP10	24	1131.826	2.560	-0.964	[-1.870 ; -0.058]
Intercept model: no effect	POP0	23	1135.079	3.488		
breeding probability of females previously in states PFB, PSB or PONB	POP11	24	1133.513	4.247		
breeding probability of males previously in state SB	POP7	24	1133.847	4.581		
breeding probability of males previously in states FBE or FBC	POP5	24	1134.095	4.829		
survival rate of males	POP13	24	1134.313	5.047		
breeding probability of females previously in states FBE or FBC	POP6	24	1134.808	5.542		
return rate of individuals previously in state SB	POP12	24	1135.034	5.768		
survival rate of females	POP14	24	1135.035	5.769		
breeding probability of males previously in state ONB	POP9	24	1135.093	5,827		

Figure 2: Effect of standardized blood Σ POPs (log-transformed) on (a) breeding probability of females previously observed as non-breeders and (b) fledgling success of individuals previously observed as non-breeders or unobserved. Dotted lines represent 95% confidence intervals estimated using the delta method [45]. Histograms represent the measured blood POP levels in the sampled individuals (c).

Figure 3: Isoclines of population growth rate (λ) of wandering albatrosses as projected with the population models, which included the responses to mercury levels (x-axis) and POPs (log-transformed) levels (y-axis) within the range of observed mercury and POPs levels

Figure 1

Figure 2:

Figure 3 :

Table 4: Modeling the effects of standardized heavy metals levels in the blood (A. Hg; B. Cd; and C. composite model) on demographic parameters (N = 147 individuals). The estimated slope and 95 % confidence intervals (CI) for models with a lower AICc than the intercept model (Hg0 and Cd0) are given.

Hypothesis	Model	Rank	Deviance	Δ AICc	Slope	[CI- ; CI+]
A. Effect of Hg on demographic parameters						
Effect of Hg on breeding probability of females previously in state ONB	Hg10	24	1442.026	0	-2.141	[-4.267 ; -0.014]
Effect of Hg on hatching success of individuals previously in states FB or SB	Hg3	24	1444.431	2.405	-0.659	[-1.271 ; -0.047]
Effect of Hg on fledgling success of individuals previously in states FB or SB	Hg1	24	1444.560	2.533	-0.844	[-1.659 ; -0.029]
Effect of Hg on breeding probability of males previously in states PFB, PSB or PONB	Hg11	24	1445.275	3.249	-36.300	[-36.300 ; -36.300]
Effect of Hg on breeding probability of males previously in states FBE or FBC	Hg5	24	1446.133	4.107	1.569	[-0.307 ; 3.445]
Intercept model: no effect of heavy metals on demographic parameters	Hg0	23	1449.217	4.946		
Effect of Hg on breeding probability of females previously in states FBE or FBC	Hg6	24	1448.236	6.210		
Effect of Hg on breeding probability of females previously in state SB	Hg8	24	1448.881	6.854		
Effect of Hg on return rate of individuals previously in state SB	Hg14	24	1448.955	6.929		
Effect of Hg on hatching success of individuals previously in states ONB or UNB	Hg4	24	1449.035	7.009		
Effect of Hg on breeding probability of males previously in state SB	Hg7	24	1449.159	7.133		
Effect of Hg on fledgling success of individuals previously in states ONB or UNB	Hg2	24	1449.208	7.182		
Effect of Hg on survival rate of females	Hg17	24	1449.209	7.183		
Effect of Hg on breeding probability of males previously in state ONB	Hg9	24	1449.215	7.189		
Effect of Hg on survival rate of males	Hg16	24	1449.216	7.190		
Effect of Hg on return rate of individuals previously in states ONB, PFB, PSB or PONB	Hg15	24	1449.217	7.191		
Effect of Hg on return rate of individuals previously in states FBE or FBC	Hg13	24	1449.217	7.191		
Effect of Hg on breeding probability of females previously in states PFB, PSB or PONB	Hg12	24	1449.217	7.191		
B. Effect of Cd on demographic parameters						
Effect of Cd on return rate of individuals previously in state SB	Cd14	24	1445.909	0	-0.350	[-0.758 ; 0.058]
Effect of Cd on hatching success of individuals previously in states ONB or UNB	Cd4	24	1446.645	0.735	0.520	[-0.204 ; 1.243]
Intercept model: no effect of of heavy metals on demographic parameters	Cd0	23	1449.217	1.063		

Effect of Cd on fledgling success of individuals previously in states ONB or UNB	Cd2	24	1447.314	1.405	
Effect of Cd on breeding probability of females previously in state ONB	Cd10	24	1447.905	1.996	
Effect of Cd on breeding probability of females previously in state SB	Cd8	24	1448.247	2.338	
Effect of Cd on breeding probability of females previously in states FBE or FBC	Cd6	24	1448.730	2.820	
Effect of Cd on hatching success of individuals previously in states FB or SB	Cd3	24	1448.744	2.835	
Effect of Cd on survival rate of females	Cd17	24	1448.794	2.885	
Effect of Cd on breeding probability of males previously in states PFB, PSB or PONB	Cd11	24	1449.043	3.133	
Effect of Cd on survival rate of males	Cd16	24	1449.048	3.139	
Effect of Cd on breeding probability of males previously in states FBE or FBC	Cd5	24	1449.054	3.145	
Effect of Cd on fledgling success of individuals previously in states FB or SB	Cd1	24	1449.129	3.220	
Effect of Cd on breeding probability of males previously in state SB	Cd7	24	1449.159	3.250	
Effect of Cd on breeding probability of males previously in state ONB	Cd9	24	1449.215	3.306	
Effect of Cd on breeding probability of females previously in states PFB, PSB or PONB	Cd12	24	1449.217	3.307	
Effect of Cd on return rate of individuals previously in states FBE or FBC	Cd13	24	1449.217	3.307	
Effect of Cd on return rate of individuals previously in states ONB, PFB, PSB or PONB	Cd15	24	1449.217	3.307	
C. Effect of heavy metals on demographic parameters					
Full model heavy metals (Hg10, Hg3, Hg1)	Hg18	26	1433.319	0	(cf text)
Intercept model: no effect of heavy metals on demographic parameters	Hg0	23	1449.217	9.131	

Table 5: Modeling the effects of POP levels in the blood (log-transformed and standardized) on demographic parameters (N = 115 individuals). The estimated slope and 95 % confidence intervals (CI) for models with a lower AICc than the intercept model (POP0) are given.

Hypothesis	Model	Rank	Deviance	Δ AICc	Slope	[CI- ; CI+]
Effect of POPs on return rate of individuals previously in states ONB, PFB, PSB, or PONB	POP15	24	1117.770	0	-12.740	[-31.840 ; 6.359]
Effect of POPs on breeding probability of females previously in state SB	POP8	24	1129.266	11.496	2.763	[-0.205 ; 5.731]
Composite model (POP2, POP10)	POP18	25	1127.642	12.212		(cf text)
Effect of POPs on fledgling success of individuals previously in states ONB or UNB	POP2	24	1130.764	12.994	-0.878	[-1.641 ; -0.114]
Effect of POPs on hatching success of individuals previously in states ONB or UNB	POP4	24	1131.111	13.341	0.841	[-0.036 ; 1.719]
Effect of POPs on fledgling success of individuals previously in states FB and SB	POP1	24	1131.312	13.542	1.226	[-0.075 ; 2.527]
Effect of POPs on hatching success of individuals previously in states FB and SB	POP3	24	1131.434	13.664	1.006	[-0.116 ; 2.127]
Effect of POPs on breeding probability of females previously in state ONB	POP10	24	1131.826	14.056	-0.964	[-1.870 ; -0.058]
Effect of POPs on breeding probability of males previously in states PFB, PSB or PONB	POP11	24	1132.429	14.659	-2.494	[-7.322 ; 2.335]
Intercept model: no effect of POPs on demographic parameters	POP0	23	1135.079	14.984		
Effect of POPs on breeding probability of females previously in states PFB, PSB or PONB	POP12	24	1133.513	15.743		
Effect of POPs on breeding probability of males previously in state SB	POP7	24	1133.847	16.077		
Effect of POPs on breeding probability of males previously in states FBE or FBC	POP5	24	1134.095	16.325		
Effect of POPs on survival rate of males	POP16	24	1134.313	16.543		
Effect of POPs on breeding probability of females previously in states FBE or FBC	POP6	24	1134.808	17.038		
Effect of POPs on return rate of individuals previously in state SB	POP14	24	1135.034	17.264		
Effect of POPs on survival rate of females	POP17	24	1135.035	17.265		
Effect of POPs on return rate of individuals previously in states FBE or FBC	POP13	24	1135.079	17.309		
Effect of POPs on breeding probability of males previously in state ONB	POP9	24	1135.093	17.323		
Effect of POPs on breeding probability of males previously in state SB	POP7	24	1133.847	16.077		

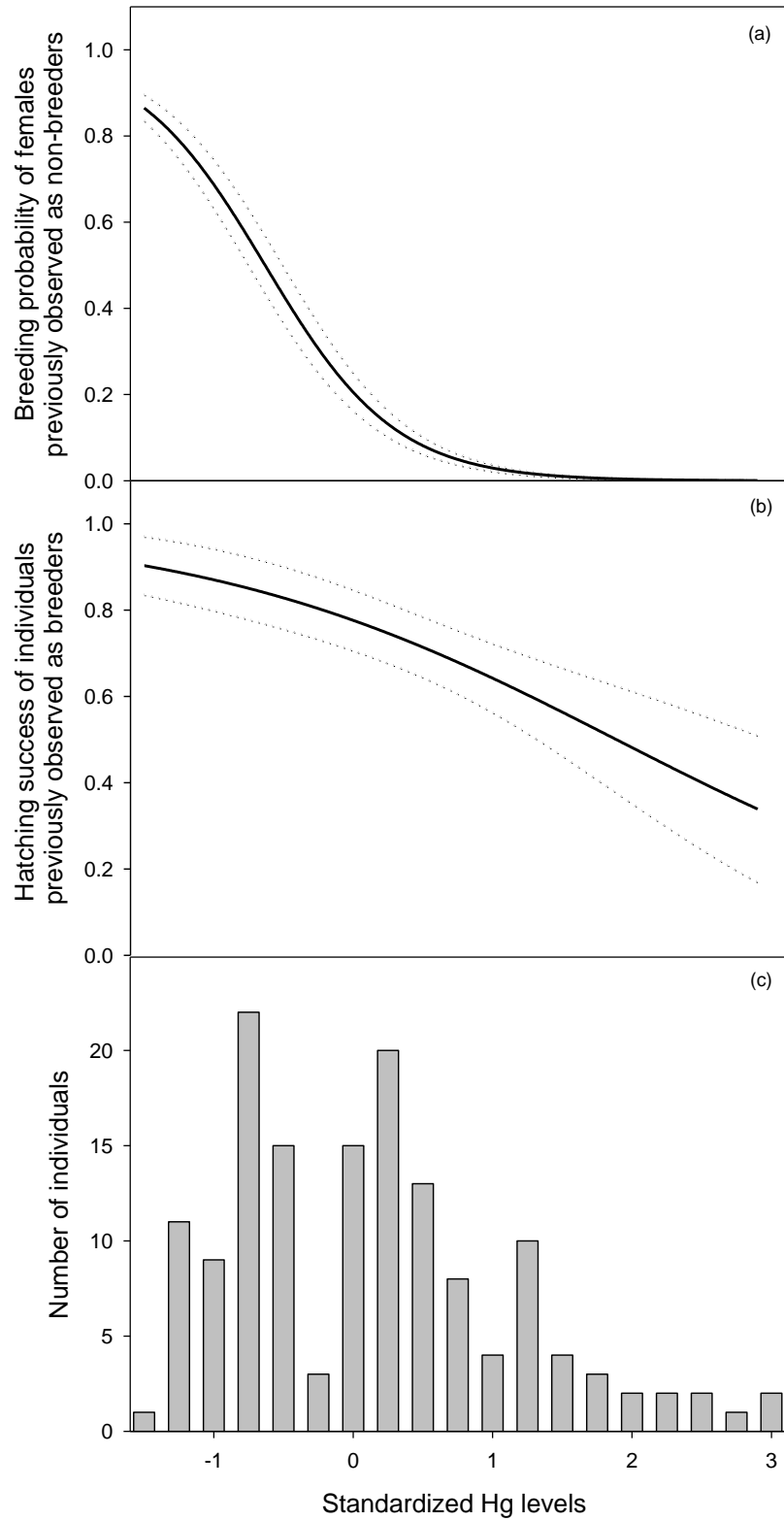


Figure 1: Effect of standardized blood Hg levels on (a) breeding probability of females previously observable as non-breeders (ONB), and (b) hatching probability of individuals previously observed as breeders. Dotted lines represent 95% CIs estimated using the delta method [34]. Histograms represent the measured blood Hg levels (c).

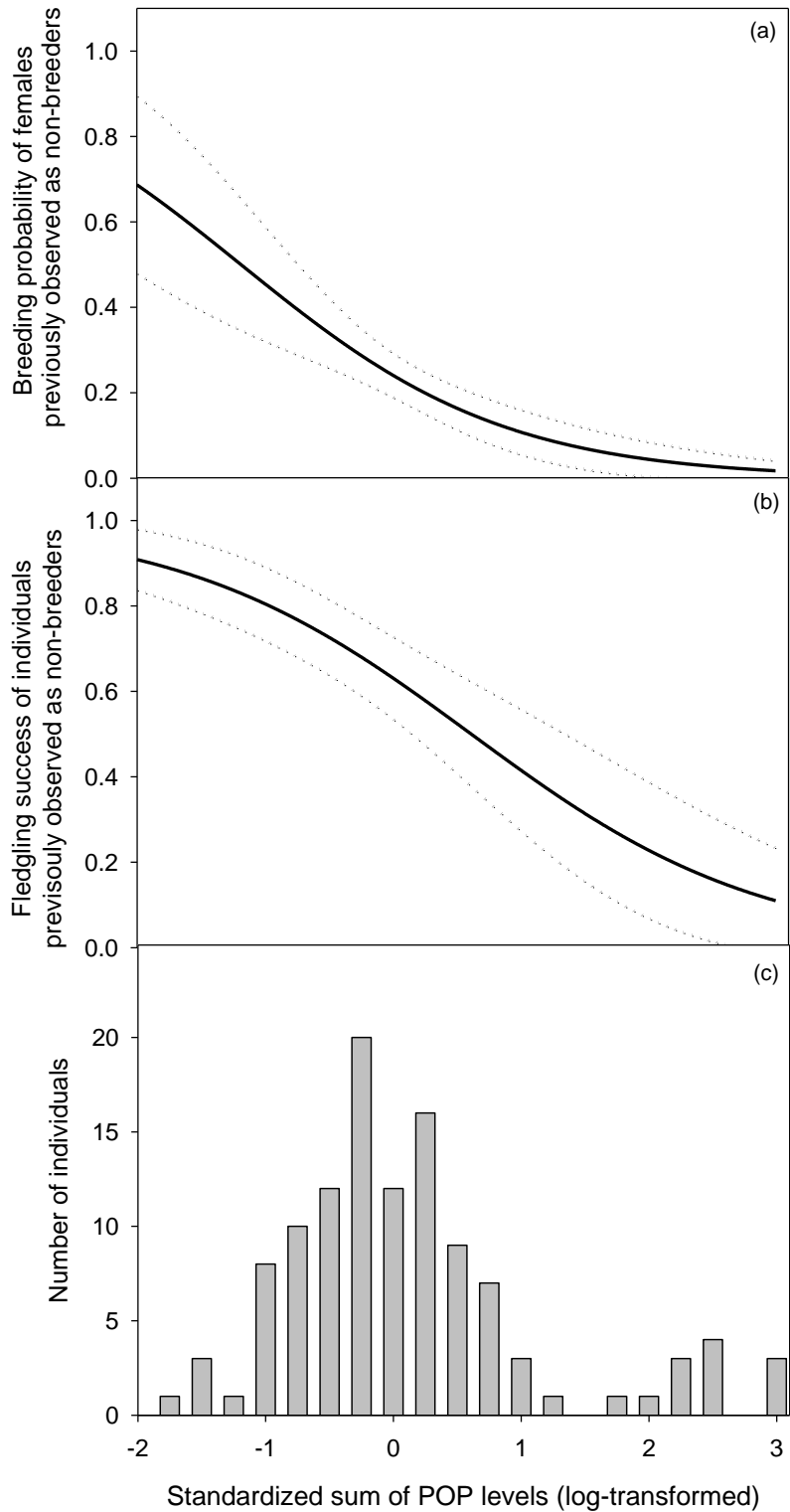


Figure 2: Effect of standardized \sum POPs (log-transformed) on (a) breeding probability of females previously observed as non-breeders and (b) fledgling probability of individuals previously observed as non-breeders or unobserved. Dotted lines represent 95% confidence intervals estimated using the delta method [34]. Histograms represent the measured blood \sum POP levels (c).

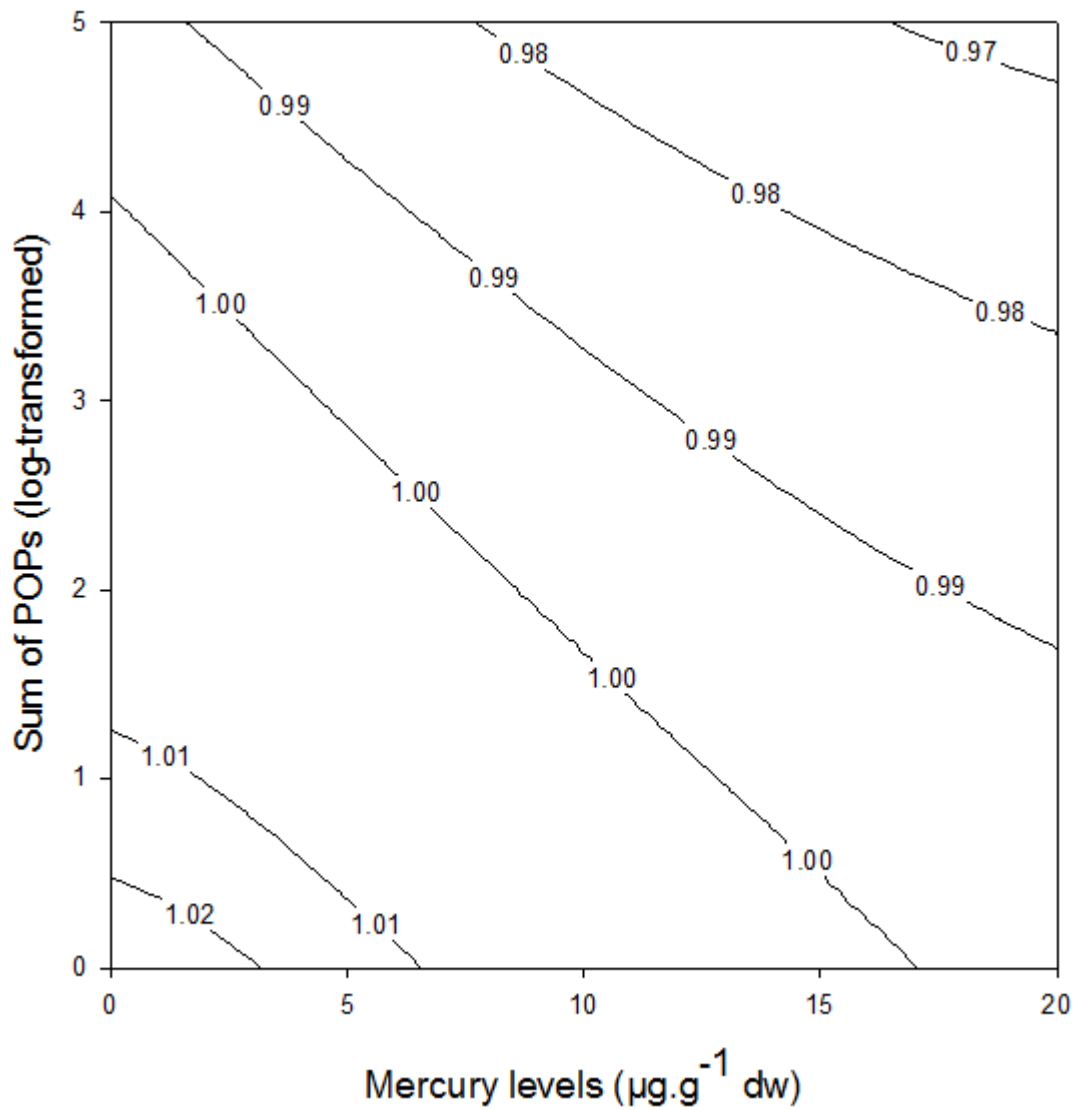


Figure 3. Isoclines of population growth rate in wandering albatrosses as projected with the population models, which included the responses to blood Hg levels and blood Σ POPs (log-transformed) within the range of observed Hg and Σ POP levels.

Supplementary material

1. Laboratory analyses

(i) Analysis of heavy metals concentrations

Total Hg was measured at the laboratory Littoral ENvironnement et Sociétés (LIENSs) from lyophilized red blood cells (N = 147 samples) with an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254). At least two aliquots ranging from 5 to 10 mg dry weight were analyzed for each individual and measurement quality was certified by reference material, as described by Bustamante et al. (2006). Accuracy was checked using a certified reference material (CRM, Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: $0.27 \pm 0.06 \mu\text{g g}^{-1}$ dry mass). Mass of CRM was adjusted to represent the same amount of Hg introduced in the AMA compared to that in blood samples. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was $0.005 \mu\text{g g}^{-1}$ dry mass.

Concerning Cd assays, two aliquots of lyophilized red blood cells (~300 mg of dry sample) were digested with 3 ml of 65% HNO₃ and 2 ml of 37% HCl (both from Merck and suprapur quality). Acidic mineralization was performed at room temperature overnight, then in a microwave during 30 min with increasing temperature until 105°C, and 15 min at 105°C (1200 W). After the mineralization process, each sample was diluted to 30 with milli-Q quality water. Cd was analysed using a Varian Vista-Pro ICP-OES and a Thermo Elemental Series II Varian ICP-MS. Data of Hg and Cd concentrations are presented relative to the dry weight (dw).

(ii) Analysis of POPs concentrations

POPs were analysed from plasma (N = 115 samples) at the EPOC-LPTC, Université Bordeaux 1, France. The targeted compounds included 7 indicator PCBs (CB-28, -52, -101, -118, -138, -153 and -180), 10 organochlorine pesticides (HCB, Gamma HCH, Heptachlore, 2,4' DDE, Cis-chlordane, trans-nonachlor, 4,4' DDD, 2,4' DDT, 4,4' DDT, Mirex) and BDE-47. Certified solutions containing all analytes at 2 ng μL^{-1} each were obtained from LGC Standards (Molsheim, France). To a plasma sample of 100 μL , internal standards (1 ng each) were added gravimetrically: PCBs 30, 103, 155 and 198 were used to quantify PCBs, p,p'-DDT-d8 was used to quantify pesticides, and F-BDE-47 was used to quantify PBDE; standards were provided by either Dr Ehrenstorfer GmbH or Cambridge Isotope Laboratory (via Cluzeau Info Labo, Sainte-Foy-La-Grande, France). POPs were extracted with 1 mL of pentane/dichloromethane (90/10; v/v); after centrifugation (2000 rpm, 2 min at 4°C), the organic layer was collected and the operation was repeated. Both extracts were combined and purified on an acid silica gel column (40% H₂SO₄). After extract loading, analytes were eluted with 3 x 5 mL of pentane/dichloromethane (90/10; v/v). The so-obtained extract was then concentrated using a RapidVap vacuum evaporation system from Labconco (Kansas City, MO, USA) to a volume of 1 mL; it was then further concentrated under a gentle stream of nitrogen (40°C) after addition of 100 μL of isooctane as solvent keeper. A syringe standard (octachloronaphtalene, 1 ng) was finally added to quantify internal standards and to assess their recovery rate for each sample (68-108%). Final extracts were analysed by gas chromatography coupled with electron capture detection (GC-ECD) as described elsewhere (Tapie et al., 2011).

Quality control consisted in the analysis of procedural blanks (clean and empty glass tubes treated like a sample, one run for 8 samples). Chicken plasma samples (Sigma-Aldrich, St Quentin Fallavier, France) spiked at 3 ng g⁻¹ were analysed; the recovery rates of PCBs

and organochlorine pesticides were in the range 77-103% with coefficients of variation lower than 17% (n=5), except for CB-52 (22%) and mirex (29%). POPs levels were blank corrected and the detection limit (LoD) was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3. Overall, LoDs ranged from 0.09 to 0.76 ng g⁻¹wet weight. Additionally, plasma total lipids were measured on an aliquot of 10 μ L by the sulfo-phospho-vanillin (SPV) method for colorimetric determination (Frings et al., 1972).

2. Definition of parameters used in the multistate mark–recapture model

Parameter	Definition
s_s^t	Probability that an individual in state s at time t survives to time $t + 1$ and does not permanently emigrate from the study area
r_s^t	Probability that an individual in state s at time t returns at the colony to time $t + 1$ given that it survives to $t + 1$
β_s^t	Probability that an individual in state s at time t breeds at time $t + 1$ given that it survives to and returns at the colony at time $t + 1$
γ_s^t	Probability that an individual in state s at time t incubates successfully at time $t + 1$ given that it survives to, returns at the colony and breeds at time $t + 1$
δ_s^t	Probability that an individual in state s at time t raises successfully one chick at time $t + 1$ given that it survives to, returns at the colony and incubates successfully at time $t + 1$
p_s^t	Probability that an individual in state s at time t is encountered at time t

3. Testing for the effects of sex and states on demographic parameters

Initial model (# 1) considers sex-difference and no difference among states on survival (s); sex-difference and difference among failed breeder at the egg stage FBE, failed breeder at the chick stage FBC, successful breeder SB, and (observable non breeder ONB, post failed breeder PFB, post successful breeder PSB, post observable non-breeders PONB) on return at the colony (r); sex-difference and differences among FBE, FBC, SB, ONB, and (PFB PSB PONB) on breeding (β); sex-difference and differences among FB, SB, ONB, UNB on hatching (ω); sex-difference and differences among FB, SB, ONB, UNB on fledgling (γ); sex-difference and differences among FBE, FBC, SB and ONB on detection (p). The

parentheses indicate that the demographic parameter was identical for all states into the parentheses. UNB indicates that all unobservable states (i.e. PFB, PSB and PONB) were constrained to have the same demographic parameter.

Testing for the effects of sex on s, r, β, ω, γ, and p	#	Rank	Deviance	Δ AICc
Sex-differences in s and β	7	31	1628.94	0
Sex-differences in s , β and γ	6	35	1624.95	5.24
Sex-differences in s , β , ω and γ	4	39	1624.90	14.57
Sex-differences in r , β , ω and γ	3	42	1626.13	22.95
Sex-differences in s , r , β , ω and γ	2	43	1622.89	22.12
Sex-differences in s , r , β , ω , γ , and p	1	47	1618.47	27.42
Sex-differences in s , ω and γ	5	34	1666.45	44.42
Testing for the effects of states on p	#	Rank	Deviance	Δ AICc
Differences among (FBE FBC), SB and ONB	8	30	1629.69	0
Differences among FBE, FBC, SB and ONB	7	31	1628.94	1.53
Similitude among FBE, FBC, SB and ONB	9	28	1667.71	33.49
Testing for the effects of states on r	#	Rank	Deviance	Δ AICc
Differences among (FBE FBC) SB, and (ONB PFB PSB PONB)	10	29	1629.97	0
Differences among FBE,FBC,SB, and (ONB PFB PSB PONB)	8	30	1629.69	1.99
Differences among (FBE FBC SB) and (ONB PFB PSB PONB)	11	28	1691.79	59.56
Similitudes among FBE FBC SB ONB PFB PSB PONB	12	27	1737.14	102.66
Testing for the effects of states on β	#	Rank	Deviance	Δ AICc
Differences among (FBE FBC), SB, ONB, and (PFB PSB PONB)	13	27	1634.18	0
Differences among FBE, FBC, SB, ONB, and (PFB PSB PONB)	10	29	1629.97	0.30
Differences among (FBE, FBC, SB), ONB, and (PFB PSB PONB)	14	25	1673.25	34.60
Differences among (FBE FBC), SB, and (ONB PFB PSB PONB)	15	25	1713.04	74.39
Similitudes among FBE, FBC, SB, ONB, PFB, PSB, PONB	16	21	1733.53	86.05
Testing for the effects of states on ω	#	Rank	Deviance	Δ AICc
Differences between (FB SB) and (ONB UNB)	18	25	1636.16	0
Differences among FB, SB and (ONB UNB)	17	26	1635.98	2.06
Differences among FB, SB, ONB, UNB	13	27	1634.18	2.49
Similitudes among FB, SB, ONB, UNB	19	24	1651.54	13.16
Testing for the effects of states on γ	#	Rank	Deviance	Δ AICc
Differences between (FB SB) and (ONB UNB)	21	23	1636.55	0
Differences among FB, SB and (ONB UNB)	20	24	1636.30	1.96
Differences among FB, SB, ONB, UNB	18	25	1636.16	4.04
Similitudes among FB, SB, ONB, UNB	22	22	1651.45	12.70

The best model according to AICc (model #21) suggested that males and females differed in survival rate and breeding probability, but not in return probability, hatching success and fledgling success. Return probabilities differed between individuals previously (i.e. the preceding year) in states failed breeder (FB = FBE and FBC), SB and non-breeder (ONB, PFB, PSB and PONB). Breeding probability differed between individuals previously in states FB, SB, ONB and unobservable non-breeders (UNB). Hatching success and fledgling success differed among individuals previously in states breeders (FB and SB) and non-breeders.

4. Estimation of parameters (mean and CI) calculated from the best model (model # 21).

State	Survival probability (%)	Return probability (%)	Breeding probability (%)	Hatching probability (%)	Fledging probability (%)	Detection probability (%)
SB male	91.8 [87.3 ; 94.8]	34.8 [26.6 ; 43.9]	14.0 [5.2 ; 32.5]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	98.8 [56.4 ; 100]
SB female	95.9 [92.1 ; 97.9]	34.8 [26.6 ; 43.9]	28.0 [14.2 ; 47.6]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	98.8 [56.4 ; 100]
FBE male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	79.2 [60.0 ; 90.6]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	75.4 [53.3 ; 89.2]
FBE female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	86.6 [68.5 ; 95.0]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	75.4 [53.3 ; 89.2]
FBC male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	79.2 [60.0 ; 90.6]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	75.4 [53.3 ; 89.2]
FBC female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	86.6 [68.5 ; 95.0]	61.7 [49.1 ; 72.8]	60.6 [43.7 ; 75.3]	75.4 [53.3 ; 89.2]
ONB male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	20.0 [12.8 ; 29.7]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	55.7 [43.8 ; 66.9]
ONB female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	82.1 [60.9 ; 93.1]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	55.7 [43.8 ; 66.9]
PSB male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	100 [100 ; 100]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	
PSB female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	96.9 [57.5 ; 99.9]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	
PFB male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	100 [100 ; 100]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	
PFB female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	96.9 [57.5 ; 99.9]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	
PONB male	91.8 [87.3 ; 94.8]	100 [100 ; 100]	100 [100 ; 100]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	
PONB female	95.9 [92.1 ; 97.9]	100 [100 ; 100]	96.9 [57.5 ; 99.9]	87.6 [80.4 ; 92.4]	92.2 [85.4 ; 96.0]	

5. Blood heavy metals and POP concentrations in the wandering albatross.

Mean and standard deviation are given. Nm indicates the sample size of males and Nf indicates the sample size of females. Generalized linear models (GLMs) with normal error and identity link function were used to test sex-differences.

Contaminants	Nm	Males	Nf	Females	Statistics
Hg in red blood cells (in $\mu\text{g g}^{-1}$ dw)	90	6.2 ± 3.0	57	10.7 ± 0.5	$F_{1,141} = 63.630, p < 0.001$
Cd in red blood cells (in $\mu\text{g g}^{-1}$ dw)	90	0.05 ± 0.03	57	0.07 ± 0.04	$F_{1,141} = 12.121, p < 0.001$
Σ POPs in plasma (in ng g^{-1} lw)	72	6363 ± 10002	39	2726 ± 4398	$F_{1,109} = 10.514, p = 0.002$

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