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INTEGRATING NESTING HABITAT RESTORATION WITH AT-SEA INDIVIDUAL-BASED BIOMONITORING OF THE ENDANGERED SEABIRD *PTERODROMA CAHOW* ENDEMIC TO BERMUDA

Project number: 182520049

Target species: ***Pterodroma cahow***

BERMUDA PETREL
BIOMONITORING PROJECT

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للمحافظة على الكائنات الحية
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In this document we report the most relevant results obtained in the last six months during which we performed spatial and statistical analysis. All this information will be soon published on peer-reviewed scientific journal thus it is confidential and will be not available to the public until its publication.

Study area and species

The Bermuda petrel (*Pterodroma Cahow*) is an endangered (BirdLife International 2018) seabird species endemic to the Bermuda Islands where it nests on few of the numerous small rocky islets that fringe the eastern side of Castle Harbour Natural Reserve located at the south-eastern corner of Bermuda (32°20' N, -64°40' W) (Madeiros et al., 2012). The birds breed mostly in artificial nests that have been provided since XX to compensate for the lack of suitable natural nesting sites due to the erosion of the limestone rock of which the islets are made. Moreover, since the ‘60, a conservation and recovery programme including a translocation plan was implemented to help the recovery of the population through the creation of two new colonies (Carlile et al., 2012, Madeiros et al., 2012). Since the 1961 the Cahow breeding population has been monitored every year by recording the number of active nests and their success but it was only in 2000 when the ringing of nestlings and of new recruits started. Such a long-term population monitoring has allowed the collection of detailed information at the individual level including, age, sex, breeding status and breeding success of > 98% of the breeding population that, in 2019, comprised 139 breeding pairs (Report Madeiros 2019). Fieldwork was carried out in four nesting islets (Green Island, Horn Rocks, Long Rocks and Nonsuch Island) between the 25th of January and the 12nd of Feb (i.e., during Incubation) as well as between the 19th of March and the 11th of April 2019 (i.e., during chick-rearing period) (Madeiros et al., 2012). During these periods 60 birds were blood-sampled and 28 GPS-tracked to study their foraging movements and trophic niches. Similarly, 58 birds were blood-sampled to perform toxicological analysis and 38 eggshells were collected to analyse trace elements content.

Spatial analysis

Foraging trips characteristics and spatial niches

Although there were no significant differences in trip extension between sexes or between seasons, the amount of time spent at sea tended to be higher during incubation than during chick rearing (Table 1). The areas used for foraging in incubation and chick rearing did not coincide, and consequently there were differences in the oceanographic conditions experienced by birds, in terms of bathymetry and SST (Table 1). This is further supported by the observation of a significantly higher within-season overlap in the 50% kernel UD's in relation to between-season (overlap incubation:incubation =0.38+-0.17, incubation:chick-rearing= 0.24+-0.15 (n=17); overlap chick-rearing:chick-rearing = 0.25+-0.12 (n=7); chick-rearing:incubation = 0.23+-0.10 (n=7), LMM effect for change in overlap: ß=0.10±0.04(SE), t=2.57, P=0.017; effect of season: ß=0.07±0.05(SE), t=1.40, P=0.173). Birds spent most of their foraging time in areas beyond national jurisdiction (56.2%, n=28), followed by 34.8% in the EEZ of the Bermudas, and ca. 4.5% in waters of both Canada and of the United States. No differences between sexes or phases are significant, except for temperature and depth, where an interaction was found (in temp) and an effect of phase was found in depth

Figure 1. UD Kernel showing distribution of the Bermuda petrels according to sex and breeding periods.

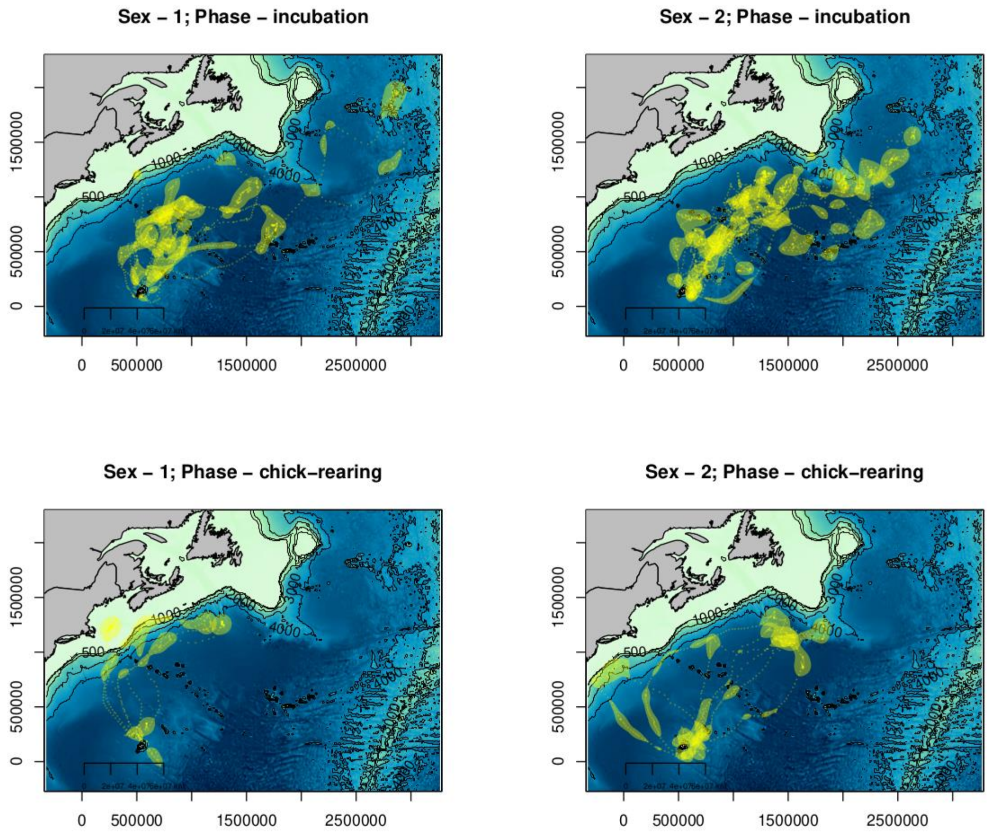


Figure 2. GPS tracks of the Bermuda petrel from 2019 presented according to sex and breeding phases overlain a SST cover (degree Celsius).

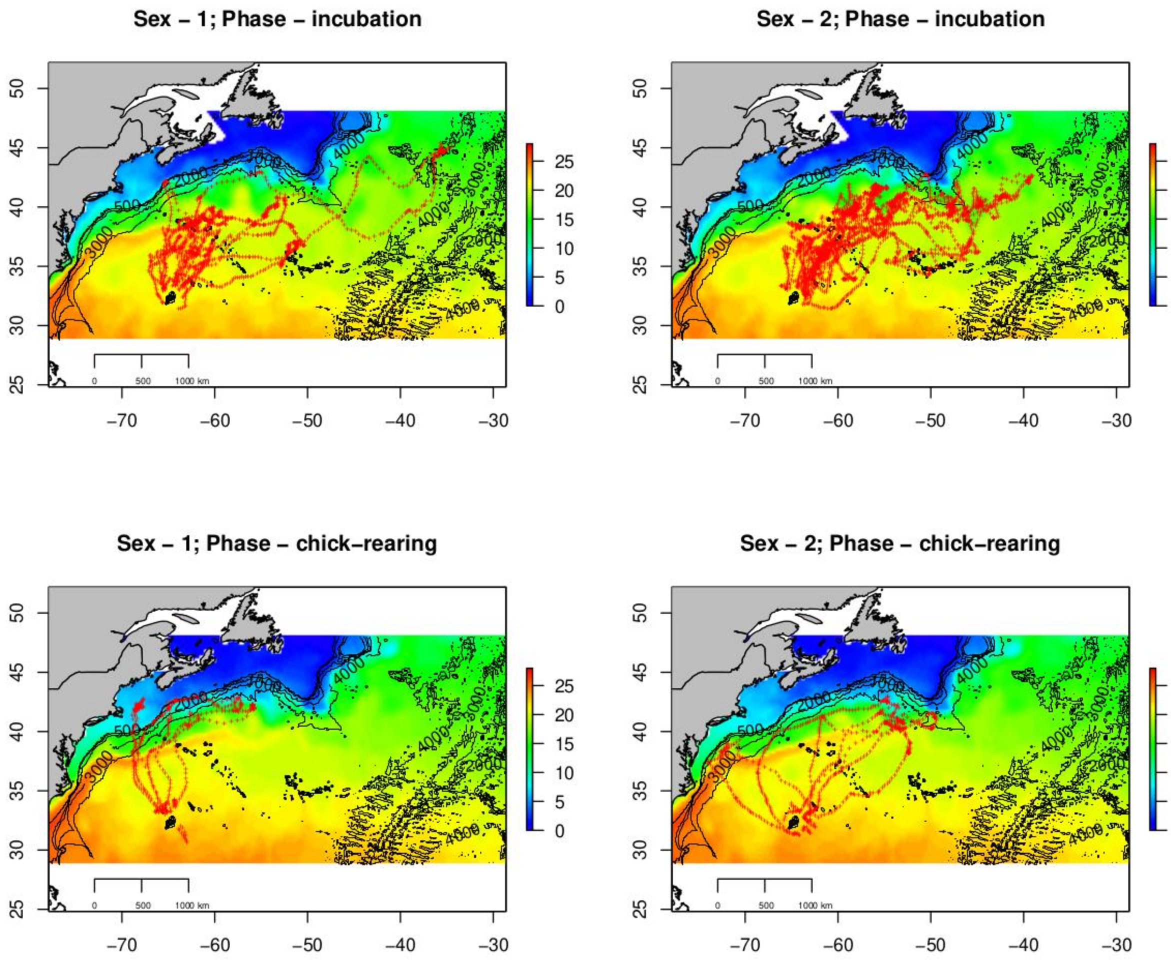


Table 1. Basic foraging movement statistics for the Bermuda petrel.

Parameter	Males_Incub	Females_Incub	Males_ChickR	Females_ChickR	Two-way ANOVA (effect of sex and phase)
Total distance (km)	4743.1±2013.9	5203.5±2817.9	3436.2±1682.6	3820.7±1491.3	sex: $F_{1,25}$=0.36, P=0.553 phase: $F_{1,25}$=2.1, P=0.155
(range)	(2547.9-8690.7)	(247.3-9462.4)	(1097.6-5058)	(1605.2-5623.3)	
Maximum distance (km)	1320.4±784.6	1273.7±770.9	1018.7±551.2	1158.7±578.7	sex: $F_{1,25}$=0.01, P=0.916 phase: $F_{1,25}$=0.45, P=0.507
(range)	(587.2-2957.6)	(71-2522.4)	(199.4-1348.3)	(213.7-1675.9)	
Trip duration (h)	216.1±59.7	242.4±116.4	146.2±54.6	147.2±62	sex: $F_{1,25}$=0.48, P=0.495 **phase: $F_{1,25}$=5.6, P=0.027**
(range)	(140-294)	(23.5-384)	(67-192)	(73-239)	
Trip duration (days)	9±2.5	10.1±4.9	6.1±2.3	6.1±2.6	
(range)	(5.8-12.2)	(1-16)	(2.8-8)	(3-10)	
SST (ºC)	20.4±1.1	19.8±1.5	14.5±5.9	19.2±2	sex: $F_{1,25}$=1.7, P=0.198 **phase: $F_{1,25}$=6.4, P=0.0178**
(range)	(18.4-21.5)	(16.8-21.5)	(78.4-21.7)	(16.7-21.9)	
depth (m)	4874±183	4802±306	3345±1869	4478±478	sex: $F_{1,25}$=1.7, P=0.203 **phase: $F_{1,25}$=67.1, P=0.0135**
(range)	(4595-5056)	(4204-5191)	(791-4819)	(3683-4872)	

Sample size	7	12	4	5

Stable Isotope Analysis and diet

Breeding period-, sex- and age-related differences in stable isotopic signatures and niches

The isotopic niche (as estimated by both SEA_C and SEA_B) of incubating adult Bermuda petrels did not overlap with that of chick-rearing adults being wider in the former period. Moreover, Nitrogen ($\delta^{15}N$) isotopic values during incubation were significantly lower than those registered for the chick-rearing ($\delta^{15}N$:$F_{1,66}$: 115.6, P <0.0001) while between-period difference in Carbon ($\delta^{13}C$) signature were only marginal ($F_{1,66}$=3.95, P =0.05) (Table 2). During incubation, males showed a slightly broader isotopic niche than females (as estimated by both SEA_C and SEA_B), with the isotopic niche of females being totally overlapped by that of males (Table 2) suggesting no sex-specific partitioning in resource use. Similarly, the isotopic niche width and isotopic signatures of females and male during chick-rearing ($\delta^{15}N$: t = 0.47, df = 13, P = 0.64 and $\delta^{13}C$: t =-1.18, df = 13, P=0.26) were very similar, although their niche overlap was less extensive compared to the incubation period (Table 3). Finally, the isotopic $\delta^{15}N$ and $\delta^{13}C$ signatures of birds were unrelated to age ($\delta^{15}N$: $F_{2,64}$ = 0.01, P = 0.99 and $\delta^{13}C$: $F_{2,64}$ = 1.21, P = 0.30).

Prey species identified by barcoding analyses

Two prey species were identified by DNA barcoding in the Bermuda Petrel diet during the chick-rearing stage. One of the species was identified as Lovely hatchetfish *Argyropelecus aculeatus* (99.8% identity in the BLAST query) (Stomiiformes, Sternoptychidae) and the other as the Spothead lantern fish *Diaphus metopoclampus* (100% identity in the BLAST query) (Myctophiformes, Myctophidae). These are both mesopelagic, open ocean species which occur in the areas where the Bermuda petrels were recorded foraging during chick-rearing (Figure 1).

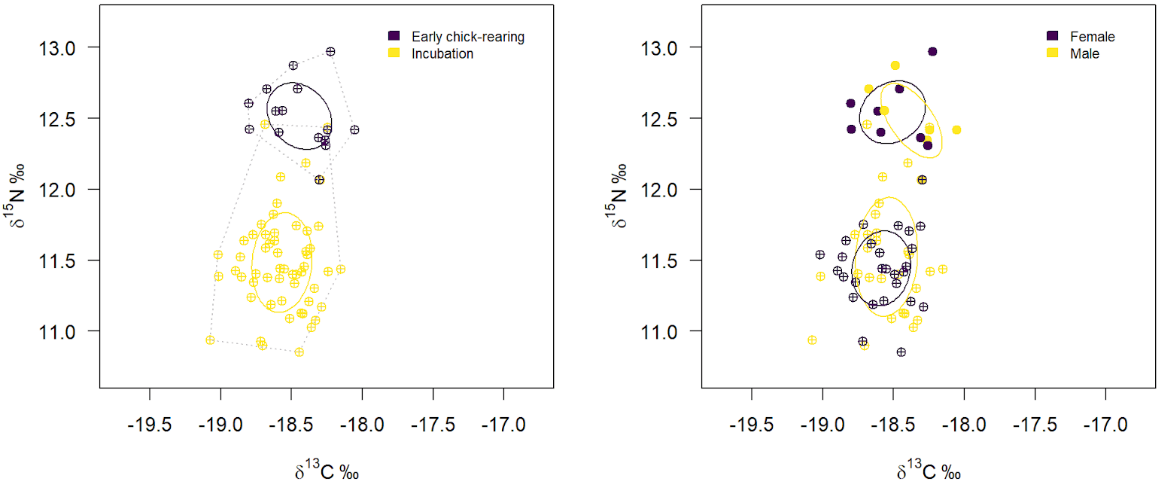
Table 2. Mean (± SD) nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic values of blood of male, and female Bermuda petrels presented by breeding periods. Sample size is given in parenthesis.

Period		**Mean ± SD**	
		$\delta^{15}N$	**$\delta^{13}C$**
Incubation	all adults (55)	11.48±0.35	-18.57±0.23
Early Chick-rearing	all adults (15)	12.52±0.23	-18.44±0.21
Incubation	female (27)	11.44±0.26	-18.59±0.21
	male (28)	11.5±0.42	-18.55±0.22
Early Chick-rearing	female (8)	12.54±0.21	-18.51±0.23
	male (7)	12.48±0.26	-18.37±0.21

Table 3. Isotopic niches of adult Bermuda petrels measured as Bayesian standard ellipse areas (SEA_B, with 95% credible intervals) and sample size-corrected standard ellipse areas (SEA_C). Niche overlap is expressed as the proportion of SEA_C of one group overlapped by its pair (males and females and vice-versa) and presented for the incubation and chick-rearing periods (all adults). Sample size is given in parenthesis.

Period		**SEA_B**	**SEA_C**	**Niche overlap**
Incubation	all adults (55)	0.225 [0.171-0.296]	0.233	0
Early chick-rearing	all adults (15)	0.145 [0.093-0.264]	0.173	0
Incubation	female (27)	0.159 [0.111-0.244]	0.174	0.96
	male (28)	0.273 [0.193-0.405]	0.293	0.57
Early chick-rearing	female (8)	0.141 [0.067-0.318]	0.182	0.58
	male (7)	0.137 [0.059-0.327]	0.169	0.62

Figure 3 Bermuda petrel carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures (dots) and isotopic niche as measured by convex hull area (dotted line) and sample size-corrected standard ellipse area (SEA_C) are presented by breeding period (a) and sex (b). SEA_C encompasses around 40% of individuals.



Trace elements analysis in eggshells

Macrominerals

During the breeding season 2019 and 2020 we collected 40 eggshells from 14 unsuccessful nests and 26 successful ones. The inorganic elements detected in eggshell are presented in Table 2. The concentration of four macrominerals (Ca, Mg, P and K) across the three groups showed different patterns. Mg concentrations were always below the detection limit, whereas Ca, P and K concentrations were consistently higher in fail eggs (early- and late-failure eggs) than in the successfully hatched ones (Figure 4). However, such a difference was statistically significant only for potassium (Kruskal-Wallis χ^2= 8.36, *df* = 2, *P* = 0.015).

Essential elements

Among the essential elements we detected Cu, Zn, Ni, Cr, Fe, Se (see Table 2) in 100% of eggshells whereas, manganese (Mn) and cobalt (Co) were detected only in 10% and 47.5% of our samples, respectively. Levels of Cr, Cu, Ni, and Zn in eggshell did not change with embryo development stages (Kruskal-Wallis $\chi2$ always < 3.9, *df* = 2, P always > 0.1) whereas, Fe concentration was significantly lower in hatched eggs (Kruskal-Wallis χ2 =7.9, df=2, P = 0.018) and Se level increased during embryo development (Figure 5).

Non-essential elements

Cadmium mercury, and strontium were detected in 100% of the eggshells while antimony, arsenic and lead were found in the 90%, 82% and 25% of samples respectively. Levels of antimony (As), Cd, Hg and Sr in eggshells did not change significantly with embryo development stages (Kruskal-Wallis χ^2 always < 3.9, *df* = 2, *P* always > 0.1, Figure 6) but median values of As and Cd were higher in early-failure eggs than the in other two groups. Moreover, we investigated the correlation between females' $\delta^{15}N$ isotopic level and non-essential element concentration we only found a significant positive correlation between the Hg concentration in the eggshells and the $\delta^{15}N$ blood levels in breeding females ($r_{(5)}$ = 0.97, *P* = 0.005).

Association between elements

When we tested for the presence of specific associations as between Ca and Sr (r=0.23, *P*= 0.17), Pb (r= -0.21, *P*= 0.56) and Cd we found that only this latter was significantly correlated to Ca (r=0.68, *P* <0.0001). Moreover, we found significant and positive correlations between Ca and As (r=0.43, *P* = 0.007), Ca and Cu (r=0.47, P<0.0001), Ca and Ni (r=0.86, *P* <0.0001) and Cd and Ni (r=0.58, *P*= 0.0001).

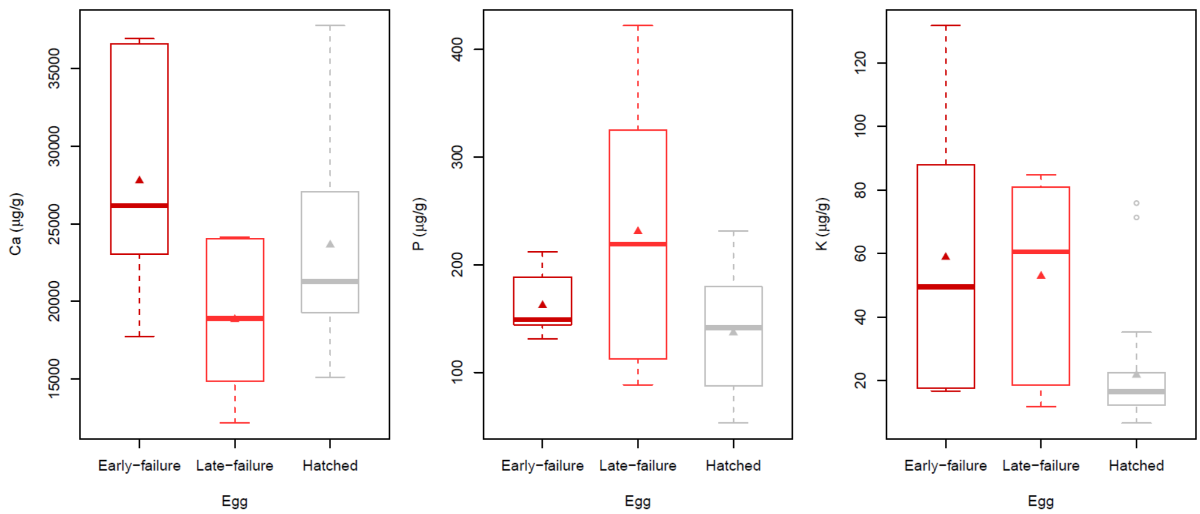


Figure 4. Macrominerals (Ca, P, K) concentration presented according to the development stage of embryo and hatching success of 38 Bermuda petrel eggs collected in 2019 and 2020.

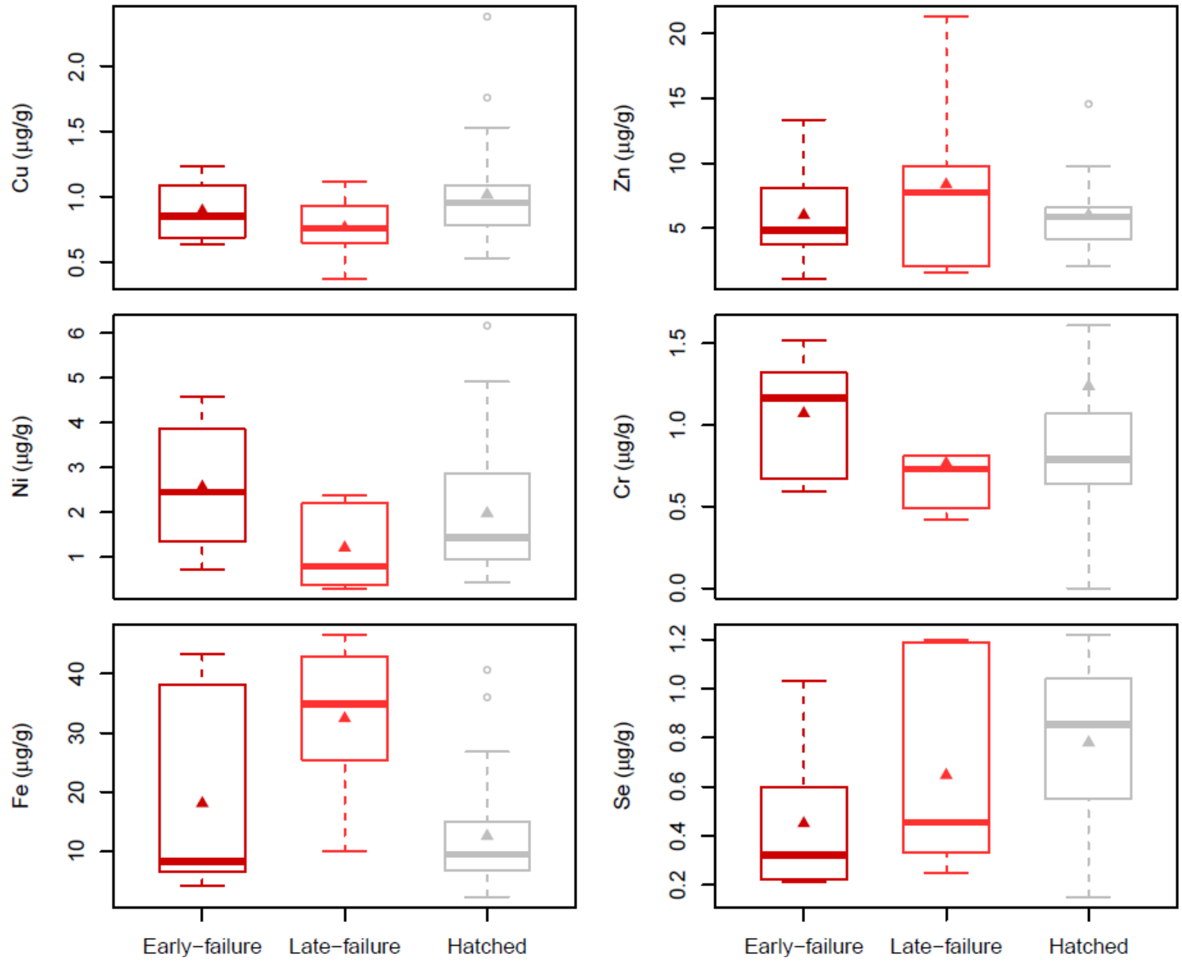


Figure 5. Essential elements concentration presented according to the development stage of embryo and hatching success of 38 Bermuda petrel eggs collected in 2019 and 2020.

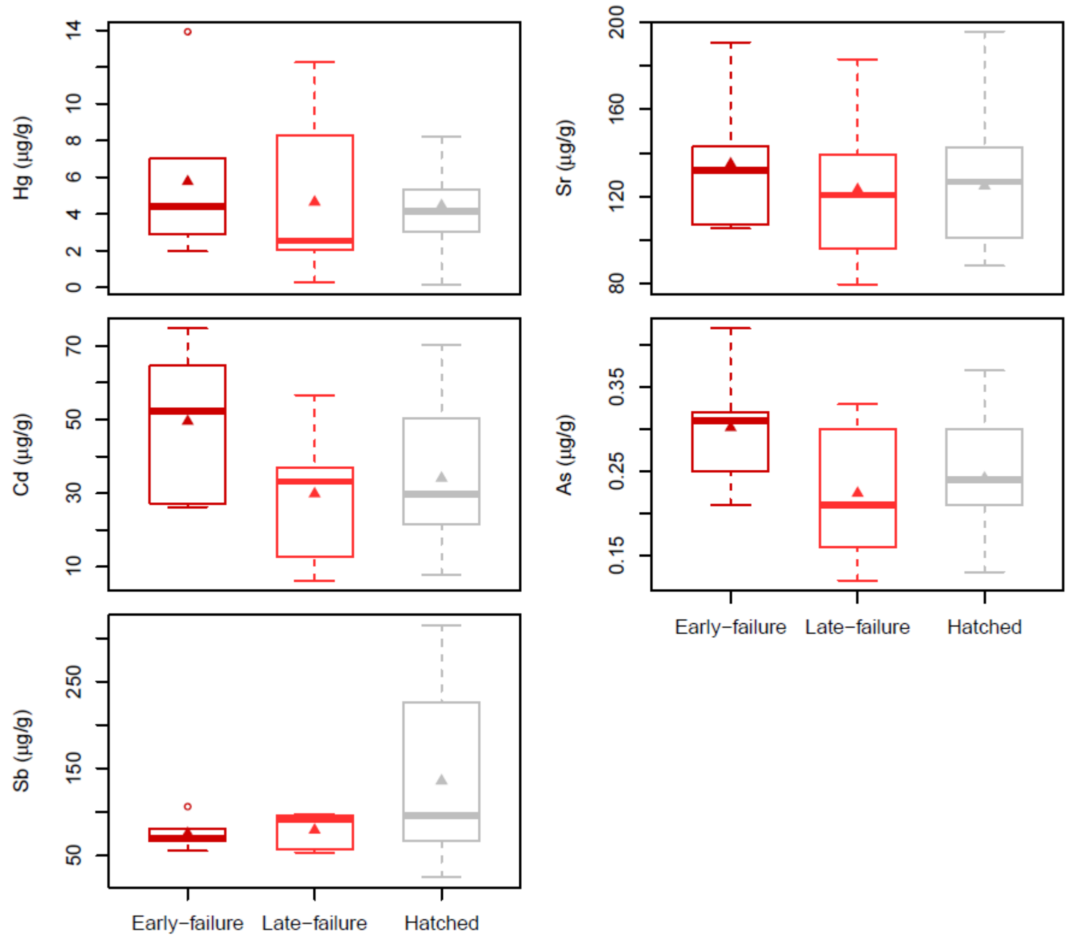


Figure 6. Non-essential elements concentration presented according to the development stage of embryo and hatching success of 38 Bermuda petrel eggs collected in 2019 and 2020.

Analysis of persistent organic pollutants (POPs)

The toxicological analyses we carried out on 58 blood samples looking for persistent organic pollutants highlighted that the Bermuda petrel has not being longer exposed to *p,p'*-DDT in recent years being its mean concentration (0.80±0.20 ng/g w.w.) of this insecticide significantly lower the concentration found 50 years ago in eggs and chicks (6440 ng/g w.w.)(Wurster and Wingate 1968). No relationship has been found between *p,p'*-DDT or *p,p'*-DDE and hatching success. Among the POPs we analysed 25 organochlorine compounds (OCPs), namely 7 polychlorinated biphenyls (PCBs), and 8 polybrominated diphenyl ethers (PBDEs), the latter one has not been detected while the former has been found but at low concentration. High correlations between OCPs indicates that BP's exposure to contaminates may occur through the diet. In fact, we found that the concentration of almost all POP groups analysed decrease with increasing $\delta^{15}N$ and increase in less offshore feeders (Table 4). An important output we found is that eggs laid by birds with higher ∑PCBs burdens have lower probability to hatch (Table 5 and Figure 7). This may suggest that recurrent sublethal exposure to PCBs may contribute to reduce hatching success.

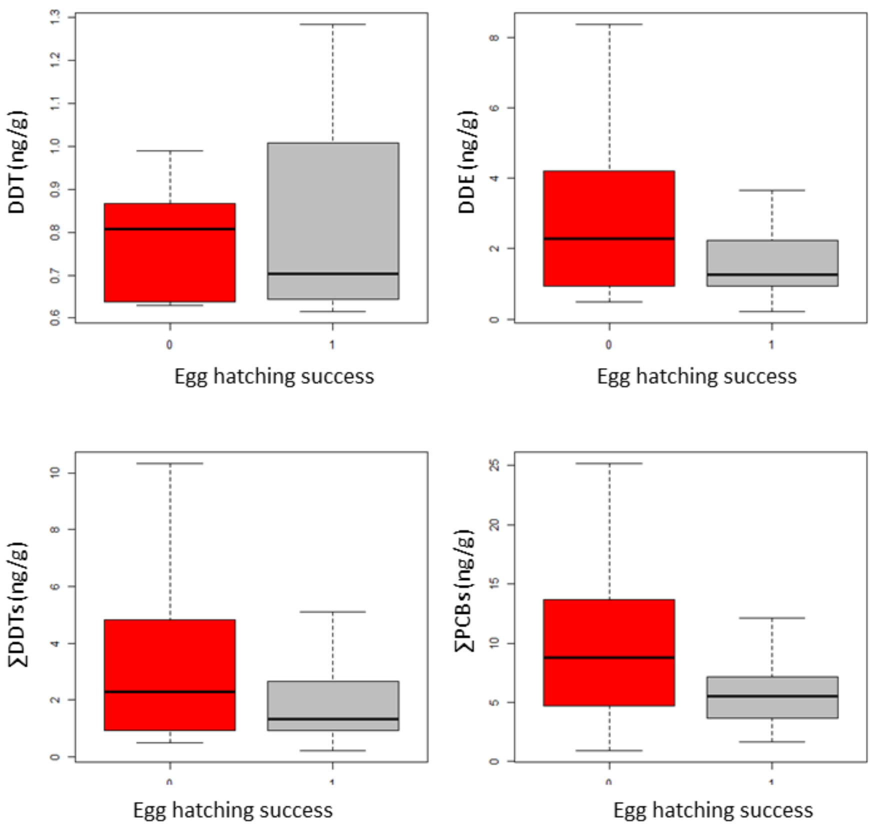
Table 4. Model selection of linear mixed-effects models used to explain variation of POPs and PHAs concentration in Bermuda petrels. For selected models (ΔAIC ≤ 2), we show: number of estimated parameters (K), AICc, ΔAICc and weight values.

Pollutant	Model	K	AICc	ΔAICc	weight
p,p'-DDE	Body mass	4	51.24	0.00	0.31
	Intercept	3	51.28	0.04	0.30
	Age+ Body mass	5	51.67	0.42	0.25
	$\delta^{15}N$	4	50.98	0.00	0.45
	Intercept	3	51.28	0.30	0.36
∑PCBs	Body mass	4	117.26	0.00	0.36
	Age + Body mass	5	117.74	0.48	0.28
	Intercept	3	118.56	1.30	0.19
	Age	4	119.16	1.90	0.14
	$\delta^{15}N$	4	110.91	0.00	0.50
	$\delta^{13}C$	4	111.35	0.45	0.40
∑OCPs	Age + Body mass	5	115.48	0.00	0.69
	$\delta^{15}N$	4	111.31	0.00	0.62
	$\delta^{13}C$	4	112.97	1.66	0.27
∑HPAs	Age + Body mass	4	59.74	0.00	0.46
	Intercept	2	61.10	1.36	0.23
	$\delta^{15}N$	4	55.80	0.00	0.50
	$\delta^{13}C$	4	57.09	1.29	0.26

Table 5. Model selection of generalised mixed-effects models used to explain variation in hatching success according to POPs and PHAs burdens in Bermuda petrels. For selected models (ΔAIC ≤ 2), we show: number of estimated parameters (K), AICc, ΔAICc and weight values.

Class of pollutant	K	Model	AICc	ΔAICc	weight
p,p′-DDE	3	*p,p*′-DDE	77.95	0.00	0.67
∑PCBs	3	∑PCBs	75.82	0.00	0.73
∑OCPs	3	∑OCPs	75.00	0.00	0.72
∑HPAs	2	Intercept	81.48	0.00	0.71

Figure 7. Blood concentration of *p,p*′-DDT, *p,p*′-DDE ∑DDTs and ∑PCBs in Bermuda petrels with successfully and unsuccessfully hatched eggs. Concentration are in ng/g w.w..



Conclusions

Our project has provided substantial support and novel information useful to the local and international conservation of the Bermuda petrel (BP). For the first time ever, we GPS-tracked the foraging movements of the Bermuda petrels during two breeding seasons unravelling the most important oceanic areas for the species. Specifically, we pointed out that the Bermuda petrels spent most of their foraging time in areas beyond national jurisdiction (56.2%) followed by 34.8% in the Exclusive Economic Zone of the Bermuda Islands, and ca. 4.5% in waters of both Canada and of the United States. This latter insight provides empirical evidence needed to submit a request to the COSEWIC (Committee on the Status of Endangered Wildlife in Canada) to evaluate and list the BP as a Canadian (wintering) species. Currently, the BP has been nominated for assessment and in the next months it will be evaluated for being listed. The positive response by COSEWIC may represent an important improvement to the international conservation of the species. Similarly, our spatial data have been provided to implement the Bermuda Ocean Prosperity Programme aiming at fully protect 20% of Bermuda EEZ waters by 2022. This kind of measure will increase the level of protection when the birds are within the Bermuda EEZ.

The toxicological analyses we carried out on BP blood looking for persistent organic pollutants highlighted that the species was no longer exposed to *p,p′*-DDT in recent years being the mean concentration of this insecticide significantly lower the concentration found in the 1967. No relationship has been found between *p,p′*-DDT or *p,p′*-DDE and hatching success. However, the polychlorinated biphenyls (PCBs) were the most ubiquitous compounds in terms of frequency of detection and levels of concentration. The more chlorinated congeners (#138, #153 and #180) were detected in more than 50 samples at levels ranging from 0.56 to 10.25 ng/g w.w.. High correlations between PCBs suggests that BP's exposure to pollutants occurs through the diet. An important output we found is that eggs laid by birds with higher PCBs burden have lower probability to hatch. This may suggest that recurrent sublethal exposure to PCBs may contribute to reduce hatching success. Although further long-term monitoring is needed to confirm this hypothesis, it is important to take it into account when planning future conservation actions. We also provided new evidence regarding the use of mesopelagic prey by BP with an important change in trophic levels between years and periods (incubation vs. chick-rearing). Considering the relationships, we found between $\delta^{15}N$ and $\delta^{13}C$ and several contaminant levels, it is important keeping monitoring the diet to understand the risk of exposure to chemical pollutants. Lastly, trace elements monitoring in eggshells suggests that mercury transfer from female body to egg (yolk and albumen) may be important and for this reason it needs to be further investigated. Moreover, BPs seem to be exposed to high concentration of antimony (Sb), a non-essential persistent element used in numerous anthropogenic activities (e.g., fire retardant is the most important and in the past as element used in the manufacture of pesticides and/or herbicides). However, higher concentrations have been found in successfully hatched eggs suggesting that during embryo formation this potentially toxic non-essential element is gradually transferred from the albumen/yolk to the eggshell. To conclude, our project highlighted the presence of overlooked and unknown risks faced by the species when feeding at sea. Both direct and indirect impacts of anthropogenic activities can have important implications to the recovery of the Bermuda petrel population. We also highlighted that to ensure a successful recovery of this endemic and endangered pelagic seabird it is critical to integrate on land monitoring with at sea information.