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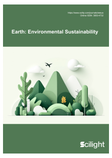

Article

Penguins as Sentinel Species for Monitoring Per- and Polyfluoroalkyl Substances (PFAS): Evaluation of Silicone Passive Samplers as a Non-Invasive Tool

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How To Cite: Montgomery, P.C.; Vanstreels, R.E.T.; Gallo, L.; et al. Penguins as Sentinel Species for Monitoring Per- and Polyfluoroalkyl Substances (PFAS): Evaluation of Silicone Passive Samplers as a Non-Invasive Tool. *Earth: Environmental Sustainability* **2026**, *2*(1), 114–132. https://doi.org/10.53941/eesus.2026.100009

Received: 11 November 2025
Revised: 4 March 2026
Accepted: 11 March 2026
Published: 23 March 2026

Abstract: Per- and polyfluoroalkyl substances (PFAS) are globally distributed anthropogenic contaminants of concern due to their environmental persistence, bioaccumulation potential, and adverse health effects. Despite increasing recognition of PFAS exposure in marine wildlife, data for penguins remain limited, in part due to challenges associated with traditional sampling methods. We present a proof-of-concept application of silicone passive samplers (SPS) as a minimally invasive approach to characterize contact-based environmental PFAS exposure in penguins. SPS bands ($n = 55$) were deployed (2–9 days) on Magellanic penguins (*Spheniscus magellanicus*) at two colonies along the Patagonian coast of Argentina across three consecutive breeding seasons. Forty PFAS were targeted and quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). PFAS were detected on the vast majority (90.7%) of SPS bands, with a mean ΣPFAS concentration of 2.26 ± 2.46 ng/g (maximum = 16.23 ng/g). Five legacy (PFPeA, PFHxA, PFHxS, PFHpA, and PFOS) and four emerging compounds (HFPO-DA, 6:2 FTS, NMeFOSAA, and NEtFOSAA) were identified. The frequency and/or concentration of PFPeA, PFHxA, PFHpA, HFPO-DA, and 6:2 FTS varied significantly among colony-season cohorts, while PFHxA, 6:2 FTS, and NMeFOSAA varied significantly with the duration of the deployment period. SPS bands provide a minimally invasive, repeatable, and practical approach for comparing relative external exposure profiles of penguins, and they could be adapted for other sensitive species to improve our understanding of PFAS and contaminant exposure in wildlife. Future studies should pair SPS deployments with biological matrices (e.g., blood) to determine how SPS-derived profiles relate to internal biological burdens and to validate the interpretation of SPS measurements.

Keywords: forever chemicals; marine pollution; seabird; silicone bands; threatened species; wildlife health; biomonitoring

1. Introduction

The global release of anthropogenic pollutants has introduced a persistent chemical burden across ecosystems [1,2]. These pollutants originate from industrial, agricultural, and urban activities, leading to a widespread dispersal of synthetic chemicals throughout the environment. Among these pollutants are per- and polyfluoroalkyl substances (PFAS), a diverse, chemically stable class of compounds characterized by strong carbon-fluorine bonds. These bonds contribute to PFAS' unique surfactant properties, high resistance to heat and degradation, and water- and oil-repellent qualities that have led to their broad use in consumer and industrial products. However, the same chemical stability that makes them desirable, also contributes to their long-term persistence and accumulation in the environment, earning them the designation of "forever chemicals" [3]. PFAS have emerged as a major global concern due to their widespread use, extreme environmental persistence, long-range transport potential, and bioaccumulative properties [3]. Over 8000 PFAS in existence can be classified into two broad categories: (i) legacy compounds, which are typically long-chain PFAS that have been phased-out of industrial use since 2000; and (ii) replacement or emerging compounds, typically short-chain or branched PFAS that have increased in use as substitutes for legacy compounds since 2000 [4,5].

PFAS contamination has been detected worldwide in virtually every environmental matrix [6], including surface waters, sediments, atmospheric aerosols, rain, snow, and the deep ocean. Their mobility in water and ability to sorb to particles allow PFAS to be transported far beyond their point of release. Remote marine environments are also affected by long-range atmospheric deposition, oceanic currents, and global water cycle dynamics [7,8]. As a result, PFAS represent a potential health risk on a planetary scale. In humans, exposure has been associated with increased blood pressure and cholesterol levels, reduced immune function, elevated risks of certain cancers and thyroid disease, and decreased infant birth weight [9,10]. In wildlife, PFAS can bioaccumulate and biomagnify, contributing to higher concentrations in upper-trophic-level predators even in regions distant from direct sources [11–14]. However, environmental exposure may not directly predict internal biological burden, because differences in uptake, distribution, metabolism, elimination, and species-specific traits can make tissue concentrations diverge from external contact concentrations. Nevertheless, exposure studies remain essential because they identify and quantify the sources, magnitude, and distribution of chemical contact that drive internal dose.

Seabirds such as penguins are well-positioned as sentinel species for assessing contaminant pressures in marine ecosystems due to their high trophic status, longevity, and colony-based life history [15–17]. Currently, 13 of 18 recognized penguin species are listed as threatened and/or their global population is declining [18]. While threats such as habitat loss, climate change, prey depletion, and fisheries bycatch are well documented [19], chemical stressors, such as PFAS, remain largely under-investigated in penguin populations [20,21]. Conceptually, PFAS exposure in penguins can be considered across three related dimensions including (1) environmental contamination (e.g., PFAS present in seawater, aerosols, coastal sediments, and microenvironments such as nest); (2) contact-based external exposure arising from direct interaction with surrounding (e.g., feather/skin surfaces, nest and substrate contact); and (3) internal biological burden resulting in uptake and accumulation driven primary by trophic transfer through contaminated prey. Their strong breeding-site fidelity and constrained spatial ecology during breeding can enable colony-level comparisons that reflect spatial and temporal variability in PFAS presence in coastal marine systems [15–17]. Despite this sentinel potential, PFAS monitoring in penguins remains limited relative to other seabirds and marine predators, leaving key gaps in baseline exposure and motivating minimally invasive approaches that can be deployed repeatedly.

Efforts to understand PFAS exposure in penguins are hindered by the practical and technical challenges of conventional biomonitoring techniques, particularly when repeated sampling is needed. PFAS detection in penguins typically relies on sampling biological matrices, such as collection of blood [22,23], tissue [24], feathers [25], and eggs [26,27], which provide valuable information on internal concentrations (i.e., biological burden) but can be invasive or logistically difficult in remote field settings. While blood and feathers can provide valuable information on contaminant burdens, their collection often requires venipuncture or plucking of feathers, which may cause some level of pain or distress or cause secondary health problems (e.g., localized bruising or infection) [24,28]. Repeated sampling for long-term monitoring is impractical under these conditions due to animal welfare considerations, and logistical challenges of fieldwork in remote colonies. Although fecal collection is not invasive, bioaccumulative chemicals are not consistently excreted, which can lead to lower detection rates or false negatives, even when the animals have been exposed to environmental contaminants [28,29]. Collectively, these constraints motivate complementary approaches that can characterize contact-based exposure with minimal disturbance, while recognizing that external exposure measurements do not directly replace tissue-based biomarkers of internal biological burden.

Silicone passive samplers (SPS) have emerged as a promising, non-invasive alternative for monitoring contact-based (external) exposure to organic contaminants, including PFAS [30,31]. SPS are soft, wearable devices that absorb a range of contaminants from air, water, and direct contact with surfaces. Once deployed, SPS provide a time-integrated record of semi-volatile and non-volatile organic compounds present in the surrounding environment during the deployment period, as chemicals diffuse into the silicone matrix until sampler reaches equilibrium or saturation [30,31]; measured profiles can vary with deployment duration, behavior, and compound-specific uptake kinetics, and can help contextualize internal biomonitoring results when paired with biological matrices. Originally developed for assessing human exposure to organic pollutants [30], SPS wristbands have since been validated in numerous human studies to detect pesticides [32], flame retardants [32–35], personal care products [36] and PFAS [33,37]. More recently, SPS applications have been expanded to include pets [38–41], honeybee colonies [31], and amphibians [42,43], demonstrating their versatility and minimal impact on animal welfare. Their portability, ease of deployment, flexibility, and low cost also make them a promising tool for studying threatened wildlife species and for repeated sampling. Wearable devices have already been successfully employed in seabird research to monitor movement, diving behavior, and environmental interactions through GPS, bio-logging technology, and wearable magnetic sensors, highlighting the potential for integrating SPS into similar ecological monitoring frameworks [44,45]. While SPS show promise, their ecological applications remain limited, and their use for characterizing PFAS exposure in penguins is poorly documented. Developing and validating these non-invasive techniques will improve our ability to generate a time-integrated record of contact exposure over the deployment window, enabling comparisons of external exposure profiles across colonies and seasons as well as evaluation of deployment-related factors. Such information can help identify when and where PFAS are most available in penguin-relevant microenvironments and prioritize targeted, paired biological sampling to evaluate internal burdens and potential health effects.

In this study, we deployed SPS on free-ranging penguins to quantify their contact-based (external) exposure to PFAS. We also evaluated SPS as a non-invasive tool for penguin-based contaminant monitoring across multiple breeding seasons. We specifically evaluated whether SPS-derived PFAS profiles varied across colony-season cohorts and whether deployment duration contributed to variability in measured concentrations. To our knowledge, this study presents the first application of SPS for pollutant monitoring in wild birds, addressing a critical data gap in PFAS exposure data for penguins while advancing non-invasive monitoring tools for wildlife conservation and environmental management. We hypothesized that PFAS profiles would vary among colony-season cohorts, reflecting spatial and temporal differences in environmental PFAS availability, and that longer deployment duration would yield higher SPS measured PFAS concentrations due to time-integrated uptake.

2. Materials and Methods

2.1. Chemicals

Analytical standards for $^{13}C_{12}$–PFAS were obtained from Wellington Labs Inc. (Guelph, ON, Canada) (Table S1). Acetonitrile (ACN), ethyl acetate (ACS grade), hexanes (HPLC), and methanol (LC/MS grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium acetate (ACS grade) and formic acid (88%, ACS grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Nanopure 18.2 MΩ water was generated using a Barnstead Nanopure Diamond system (Waltham, MA, USA).

2.2. SPS Preparation

Silicone bands (100% silicone; 24HourWristbands.com, Houston, TX, USA) were cleaned prior to deployment using the protocol described in previous literature [30]. Briefly, 65 g of bands were sequentially extracted with 800 mL of ethyl acetate:hexane (1:1, *v*:*v*; three cycles), and then with 800 mL of ethyl acetate:methanol (1:1, *v*:*v*; two cycles). Each extraction cycle lasted 2.5 h, with bands shaken on a platform shaker (Innova 2000, New Brunswick Scientific Co., Edison, NJ, USA) at 60 rpm. After cleaning, bands were air-dried in a fume hood and stored in 60-mL certified contaminant-free glass amber jars (VWR International, Radnor, PA, USA) until deployment. Procedural blanks were assessed following cleaning and prior to field deployment to verify the absence of residual PFAS contamination from materials and handling. Stainless steel wires rinsed with ethyl-acetate:methanol (1:1, *v*:*v*; three cycles) and stored in pre-baked (250 °C) aluminum foil until use. Stainless steel wire was used only as a mechanical fastener and was removed prior to extraction; the silicone segment immediately adjacent to the wire was not included in the extracted subsample. Although stainless steel is not expected to be a significant source of PFAS, it can carry traces of fluorinated residues from manufacturing or handling; therefore, wire was solvent-rinsed and stored separately to minimize this potential. Field and laboratory blanks were processed alongside samples to assess any background associated with sampler preparation, handling, transport, and storage.

2.3. Study Population and Sample Collection

Magellanic penguins (*Spheniscus magellanicus*) breed along the coast of Argentina, Chile and the Falkland/Malvinas Islands, where they form large colonies during the breeding season (September–March) [46]. Many of their colonies are relatively accessible and breeding adults can be captured at the nest and handled with minimal disturbance [47], enabling low-stress deployment and retrieval of samplers. These birds undertake consistent, well-defined foraging trips within a predictable marine environment before returning to their nests, where the SPS can be retrieved. This predictable behavior makes them ideal model species for assessing PFAS exposure during the pelagic phase at sea. SPS were deployed at two Magellanic penguin colonies in the Southwest Atlantic Ocean, on the coast of Chubut province, Argentina: San Lorenzo and Cabo Dos Bahías (Figure 1). These colonies are approximately 340 km from each other, being located in the scarcely populated region of Patagonia (human settlements represented in Figure 1 sum less than 400,000 inhabitants) [48]. San Lorenzo (42°50′ S 63°51′ W) is the species' largest colony, with over 200,000 breeding pairs, and its population has rapidly increased in recent decades [48–50]. Cabo Dos Bahías (44°54′ S 65°32′ W) is a much smaller colony, with less than 9800 breeding pairs, and its population has been decreasing in recent decades [48,50,51]. Previous tracking studies have shown that during chick-rearing, the penguins from these colonies forage over different areas comprising an important extension of the Patagonian continental shelf [52–54]. Moreover, during the non-breeding period these species inhabits the entire Patagonian shelf [55,56].

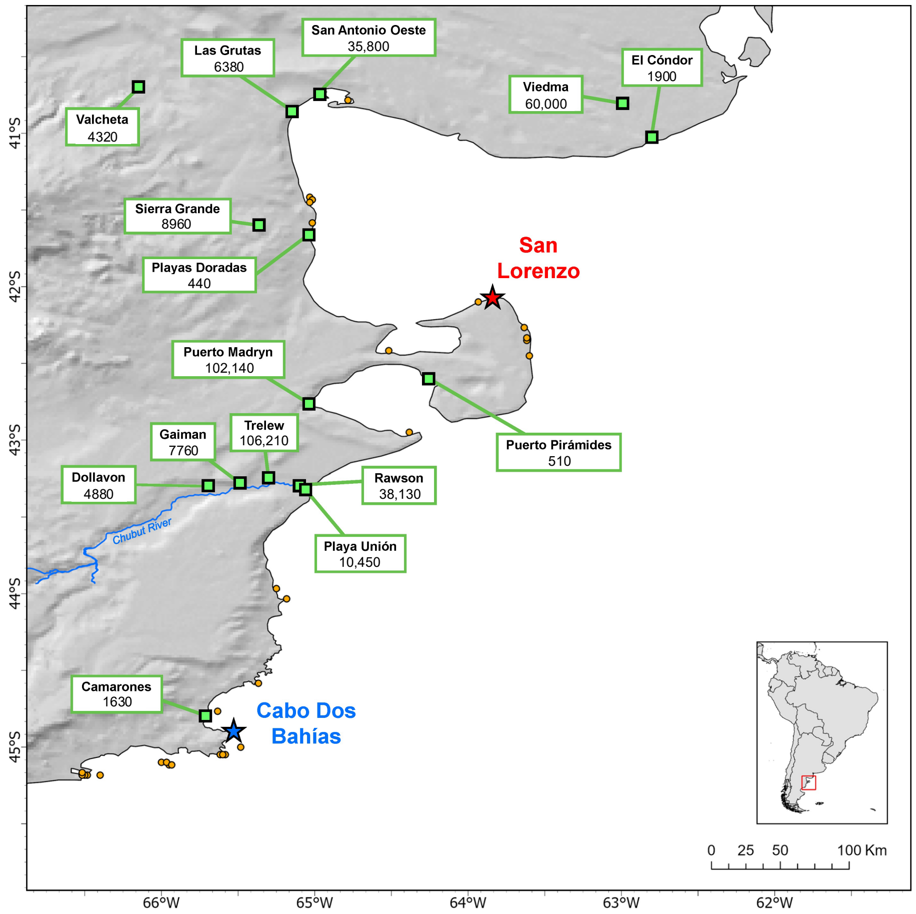

Figure 1. Map of the penguin colonies where SPS bands were deployed, San Lorenzo (red star) and Cabo Dos Bahías (blue star). Human settlements (green squares; name and population size in text boxes [49]) and other penguin colonies (orange circles [46,50]) are shown.

SPS were deployed in the early chick-rearing stage (late November–early December) of each year. Deployment durations (2–9 days) were selected to balance sufficient time for measurable uptake with animal welfare considerations, band retention, and logistical constraints on recapturing adults during chick-rearing. Breeding adults were selected opportunistically from accessible nests meeting inclusion criteria (active chick-rearing status, general health/body condition), rather than random sampling, to maximize retrieval success. To reduce potential sex-based bias in contaminant exposure, deployments were distributed as evenly as possible between males and females; sex determination relied on bill measurements. Morphometric sexing using bill measurements has been validated against molecular sexing in *Spheniscus* penguins, with reported classification accuracy of 97% [57]. Penguins were gently restrained by an experienced field researcher to ensure safe handling and minimal disturbance. Each band was measured against the leg of the bird and cut to size to fit their tibiotarsus loosely (with a fit tolerance of at least 2–3 mm). Then, a stainless-steel wire was used to secure the silicone band above the penguins' ankle (distal tibiotarsus) allowing for full range of movement while maintaining continuous surface contact with both feathers and skin (Figure 2). The steel wire was twisted to tie the ends of the band, and the excess wire was cut off and bent to avoid sharp edges. After deployment, SPS band placement and the behavior of the bird were visually checked after 20–30 min to confirm that it neither impeded mobility nor caused any discomfort or swelling. SPS bands were successfully deployed on 55 Magellanic penguins during one breeding season at San Lorenzo (2022/2023) and three consecutive breeding seasons at Cabo Dos Bahías (2022/2023, 2023/2024, and 2024/2025; Table S2). As a result of field logistics and recapture success, sample sizes differed among colony-season cohorts (Table S2), which should be considered when interpreting between cohort comparisons. On the first breeding season, SPS bands were deployed for a short period (2 days; n = 30); following this successful initial test, medium (3–5 days; n = 5) and long (6–9 days; n = 19) deployments were made. Additionally, one deployment was classified as "very long" because the penguin could only be recaptured 17 days after deployment. There were two unsuccessful SPS band deployments: (a) one band had to be removed 30 min post-deployment because it was noted that it was improperly adjusted, apparently causing discomfort (the penguin kept its leg extended rather than standing/sitting in a normal position), and (b) another band had been lost when the penguin was recaptured after 48 h.

Figure 2. SPS bands customized for deployment on Magellanic penguins. (**A**) SPS band before and after adjustment of the stainless-steel wire. (**B**) SPS band (arrow) placed above the penguins' ankle (distal tibiotarsus). (**C**) Magellanic penguin sitting at its nest with an SPS band (arrow).

After the designated deployment periods, the penguins were recaptured and the SPS bands were retrieved, placed back into their respective jars, and stored at –20 °C. Field blanks (pre-cleaned SPS bands) were included at each colony and were handled, transported, and stored identically to deployed bands, but were not placed on birds. Field blanks were kept in their jars and opened bridely at the capture/handling area during deployment and retrievals to account for any potential contamination. Blanks remained in the field for the same sampling period as the corresponding deployments (2–9 days). All samples were shipped to the University at Buffalo and stored at –40 °C until further analysis.

SPS uptake is expected to depend on compound-specific partitioning and on the time spent in relevant microenvironments (e.g., at sea vs. at the nest). In this proof-of-concept design, we assume that uptake kinetics are broadly comparable across individuals and seasons under similar deployment durations, while acknowledging that behavior and environmental variation may contribute to between-individual variability. Deployment duration was therefore included as a factor in the statistical analyses.

2.4. PFAS Extraction

Approximately 0.3 g of band per SPS was cut and placed into a clean, pre-weighed 15 mL falcon tube. The combined mass of the SPS sample and falcon tube was recorded using an analytical balance. All the samples were spiked with 20 µL of 1 ppm $^{13}C_{12}$–PFAS surrogate standard, while two control samples were spiked with 20 µL of 1 ppm $^{12}C_{12}$–PFAS native mixture. Samples were dried for 1 h in the atmosphere prior to extraction. The PFAS extraction method was adapted from a previous publication [33]. Briefly, SPS samples underwent a solid-liquid extraction using 10 mL of 0.1% ammonium hydroxide in methanol, followed by sonication for 15 min. The extract was decanted into an additional 15 mL falcon tube, and the SPS segment underwent two additional extractions. The extraction solvents were combined and dried to completion under N_2. Samples were reconstituted in 200 µL of the liquid chromatography starting mobile phase with 20 ng of M4PFOS spiked into each sample as an instrumental internal standard. Samples were vortexed and sonicated for 10 min and transferred to 2 mL amber vials. A 20 µL aliquot of each sample was transferred to a 200 µL polypropylene vial insert for analysis.

2.5. LC-MS/MS Analysis

SPS samples were analyzed for 40 PFAS compounds (Table S1). Analysis was performed using an LC tower (Agilent, Santa Clara, CA, USA) coupled with a TSQ Quantum Ultra triple quadrupole MS system under negative electrospray ionization (ESI) mode (Thermo Scientific, Waltham, MA, USA). The details of the targeted analysis method are described in our previously published paper [58]. Briefly, chromatographic separation was performed using a Raptor C18 analytical column (100 Å pore size, 3.0 mm particle size, 2.7 mm i.d. 150 mm length; Restek, Bellefonte, PA, USA). Separation was achieved using a gradient mobile phase presented in Figure S1, which consists of 5 mM ammonium acetate in water:acetonitrile (19:1 *v*:*v*) mixture, adjusted to pH of 3.8 using acetic acid (mobile phase A) and acetonitrile:methanol (1:1 *v*/*v*) mixture (mobile phase B). The flow rate was 0.37 mL/min. All transitions and mass spectrometer parameters are presented in Table S4.

2.6. Quality Assurance and Quality Control

During sample handling and preparation, SPS bands were weighed on pre-baked (250 °C) aluminum foil to prevent contamination from work surfaces. All tools were thoroughly rinsed with methanol prior to and between processing each SPS sample. The SPS “laboratory” blanks, which are pre-cleaned SPS bands prepared for deployment, were handled under the same laboratory conditions and extraction protocol as the samples to detect any contamination introduced during laboratory procedures. Field blanks were also extracted and analyzed to identify any PFAS contamination that could occur during field handling and deployment, ensuring that PFAS detected in penguin-exposed SPS bands could be distinguished from background contamination. No PFAS were detected in laboratory or field blanks above the method’s limits of detection (LOD) (Table S5).

Method LODs were determined based on 3 times the standard deviation of the lowest observed concentration spiked in SPS band matrix (n = 7). Limits of quantitation (LOQs) were determined based on 10 times the standard deviation of the lowest observed concentration spiked in SPS band matrix (n = 7). PFAS LODs and LOQs are presented in Table S5.

Recovery experiments were performed by spiking cleaned SPS bands (~1 g; n = 3) prior to extraction with a standard solution containing 5 ng/g of each analyte and comparing them to cleaned SPS bands (n = 3) spiked after extraction. Recoveries are summarized in Table S6. Carboxylic, sulfonic, fluorotelomers, sulfonamide acetic acids, ether carboxylic acids, and ether sulfonic acid recoveries generally ranged from ~75% to 100%, with some exceptions including PFPeA, PFBS, PFNS, 4:2 FTS, PFMPA, and PFMBA. Specifically, fluorotelomer carboxylic acids exhibited notably low recoveries ranging from ~10% to 30%. Sulfonamide and sulfonamide ethanol compounds such as FOSA, NEtFOSA, NMeFOSA, NMeFOSE, and NEtFOSE were not recovered likely due to loss during the drying step. Low recoveries for fluorotelomer carboxylic acids and non-recovery of sulfonamides and sulfonamide ethanol’s indicate that concentrations for these analytes may be biased low (or not quantifiable) in SPS extracts under the current workflow. Accordingly, interpretation focuses on compounds with acceptable recoveries and consistent detection.

All data were integrated using Xcalibur’s processing software (Thermo Scientific), identifying both qualifier and quantifier ions. Chromatograms were manually reviewed to confirm correct peak integrations and retention time alignment. Compounds were considered positively identified when both the retention time and ion ratios matched the reference standards within 0.3 min and 30%, respectively. Laboratory blanks were analyzed after every 5–7 field samples to assess any potential carryover. All chromatograms were manually reviewed to confirm that no target compounds were transferred between consecutive injections. No evidence of carryover was observed in any of the instrumental analyses.

2.7. Quantification

Isotope dilution was performed using a PFAS isotope mixture containing 19 $^{13}C_{12}$–PFAS, and pairing each surrogate to one of the 40 targeted native $^{12}C_{12}$–PFAS. Native compounds were paired with surrogate isotopes based on three criteria: (i) functional group similarity; (ii) molecular mass; and (iii) retention time (Table S7). Linear calibration curves, between 1 and 250 ppb, were produced for all PFAS standards, to assure that quantification fall within the linear dynamic range and was an appropriate quantification method for this analysis. Calibration curves were fit using unweighted linear regression ($R^2 \geq 0.99$ for acceptance). Unweighted fits were used because isotope dilution reduces concentration-dependent variability in response across the working range. A 50-ppb solution of both native and surrogate standards was injected at least three times during the analytical run to generate response factors (*F*). Quantification was performed using the isotope dilution relationship (Equation (1)):

$$\frac{I_X}{[X]} = F \times \frac{I_S}{[S]} \tag{1}$$

where X is the native PFAS analyte, S is the paired ^{13}C surrogate isotope (Table S7), I_X and I_s are peak areas normalized to the internal standard (M4PFOS), $[X]$ and $[S]$ are the concentrations of the native analyte and surrogate, respectively, and F is the response factor.

Results were compared to LOD and LOQ values to ensure accurate detection. Concentrations below LOQ were reported as estimated quantified values using Equation (2) and included in the statistical analysis, while values below the LOD were reported as non-detects (ND). LODs and LOQs for all targeted PFAS are provided in Table S5. Once peaks were confirmed as positive, calculated concentrations were normalized (C) to the initial mass of the SPS band sample to account for variation in sample size (Equation (2)):

$$C = \frac{[X] \times V}{m} \tag{2}$$

where $[X]$ is the calculated concentration in the resuspended extract, V is the resuspended volume (200 μL), and m is the mass of the SPS band subsample extracted. This normalization allowed for direct comparison of PFAS concentrations across different SPS bands. Reported concentrations were not corrected using compound-specific absolute recoveries, because isotope dilution with matched surrogates corrects for much of the procedural loss and matrix effects.

2.8. Statistical Analyses

Sample sizes differed among colony-season cohorts due to field logistics and recapture successes (Table S2). This imbalance may reduce power for some comparisons and can bias estimates toward cohorts with larger n. Results are interpreted as cohort-level patterns rather than definitive population-wide estimates.

Data were analyzed using R v4.4.1 [59] with the packages *FSA* v0.10.0 [60], *ggplot2* v3.5.2 [61], *plyr* v1.8.9 [62], and *rcompanion* v2.5.0 [63]. Data from 54 samples were included in the statistical analyses. The unintentionally long period (17 day) sample was excluded because it was outside the planned deployment window (2–9 days) and likely reflects different uptake dynamics, making it non-comparable to the intended exposure window. Chi-square tests with false discovery rate-corrected Fisher post-hoc comparisons were used to compare the frequency of detection of PFAS among colony-season cohorts (San Lorenzo 2022/2023, Cabo Dos Bahías 2022/2023, Cabo Dos Bahías 2023/2024, or Cabo Dos Bahías 2024/2025) and deployment periods (short, medium, or long). Kruskal-Wallis tests with Dunn-Šidák post-hoc comparisons were used to compare PFAS concentrations among colony-season cohorts and deployment periods. Given the small sample size and the failure of both the normality (Shapiro-Wilk) and homoscedasticity (Breusch-Pagan) tests, a non-parametric analytical approach was adopted. Principal component analysis (with scaled variables) was used to evaluate the association in the variability of PFAS compounds in relation to colony-season cohorts. Compounds not detected in any sample (<LOD across the dataset) were excluded. Remaining non-detects were treated as missing values (NA) prior to scaling. The significance level was 0.05 for all tests.

3. Results

Nine targeted PFAS were detected in SPS bands deployed on Magellanic penguins over three consecutive breeding seasons, comprising five legacy compounds (PFPeA, PFHxA, PFHxS, PFHpA, and PFOS) and four emerging compounds (HFPO-DA, 6:2 FTS, NMeFOSAA, and NEtFOSAA) (Figure 3 and Table S2). PFAS were detected in 90.7% of the samples (n = 54). with a mean ΣPFAS concentrations of 2.26 ng/g (SE = 0.5, SD = 2.46; Table 1). The maximum concentration was 16.23 ng/g for PFOS, corresponding to a sample that was a statistical outlier (sample 10 from Cabo Dos Bahías, 2022/2023 in Figure 3). Concentration values were considered outliers when they exceeded 5 standard deviations from mean PFAS compound concentrations in this study; statistical descriptors (mean, standard deviation, etc.) were calculated for the data with and without outliers. If this outlier sample is excluded, the mean ΣPFAS concentration was 2.00 ng/g (SE = 0.03, SD = 1.53), with a maximum concentration of 6.04 ng/g. A summary of the results including the outlier reported in Table 1. Corresponding statistics with the outlier excluded are provided in the Table 1 footnote.

Table 1. Summary of the frequency of occurrence of PFAS compounds in SPS bands deployed in Magellanic penguins at two colonies (SL = San Lorenzo, CDB = Cabo Dos Bahías) over three breeding seasons (2022/2023, 2023/2024, and 2024/2025). Deployment periods were categorized as short (2 days), medium (3–5 days), long (6–9 days). Concentration (ng PFAS/g SPS band) = mean ± SD (maximum). Frequency = percentage of samples with the compound (samples detected/samples analyzed). ND = Not detected.

		SL	**CDB**	**CDB**	**CDB**	**CDB**	**CDB**	**All Samples (n = 54)**
		2022/2023	**2022/2023**	**2022/2023**	**2023/2024**	**2023/2024**	**2024/2025**	
		Short	**Short**	**Medium**	**Short**	**Long**	**Long**	
PFPeA	Conc.	0.15 ± 0.22 (0.57)	0.23 ± 0.37 (1.10)	0.09 ± 0.12 (0.23)	0.46 ± 0.34 (1.22)	0.37 ± 0.27 (0.66)	0.38 ± 0.44 (1.13)	0.29 ± 0.34 (1.22)
	Freq.	40% (4/10)	40% (4/10)	40% (2/5)	90% (9/10)	75% (6/8)	54.5% (6/11)	57.4% (31/54)
PFHxA	Conc.	0.16 ± 0.26 (0.66)	0.11 ± 0.25 (0.76)	0.18 ± 0.4 (0.90)	1.48 ± 0.66 (3.23)	1.26 ± 0.51 (1.96)	0.46 ± 0.35 (1.01)	0.62 ± 0.69 (3.23)
	Freq.	30% (3/10)	20% (2/10)	20% (1/5)	100% (10/10)	100% (8/8)	72.7% (8/11)	59.3% (32/54)
PFHxS	Conc.	0.05 ± 0.16 (0.51)	0.07 ± 0.23 (0.73)	0.13 ± 0.29 (0.64)	ND	ND	ND	0.03 ± 0.15 (0.73)
	Freq.	10% (1/10)	10% (1/10)	20% (1/5)	ND (0/10)	ND (0/8)	ND (0/11)	5.6% (3/54)
PFHpA	Conc.	0.02 ± 0.07 (0.22)	0.02 ± 0.05 (0.17)	ND	0.19 ± 0.18 (0.43)	0.14 ± 0.15 (0.33)	ND	0.06 ± 0.12 (0.43)
	Freq.	10% (1/10)	10% (1/10)	ND (0/5)	60% (6/10)	50% (4/8)	ND (0/11)	22.2% (12/54)
PFOS	Conc.	0.05 ± 0.17 (0.53)	1.77 ± 5.09 (16.23) [a]	0.13 ± 0.29 (0.65)	0.03 ± 0.10 (0.33)	0.10 ± 0.29 (0.82)	ND	0.37 ± 2.21 (16.23) [b]
	Freq.	10% (1/10)	30% (3/10)	20% (1/5)	10% (1/10)	12.5% (1/8)	ND (0/11)	13.0% (7/54)
HFPO-DA	Conc.	0.73 ± 0.81 (1.88)	0.11 ± 0.35 (1.12)	0.73 ± 1.62 (3.63)	0.36 ± 0.36 (1.09)	0.29 ± 0.32 (0.79)	0.16 ± 0.37 (0.92)	0.36 ± 0.67 (3.63)
	Freq.	50% (5/10)	10% (1/10)	20% (1/5)	60% (6/10)	50% (4/8)	18.2% (2/11)	35.2% (19/54)
6:2 FTS	Conc.	ND	ND	ND	0.42 ± 0.78 (2.31)	1.16 ± 1.34 (3.34)	1.05 ± 0.36 (2.08)	0.46 ± 0.78 (3.34)
	Freq.	ND (0/10)	ND (0/10)	ND (0/5)	30% (3/10)	50% (4/8)	100% (11/11)	33.3% (18/54)
NMeFOSAA	Conc.	0.08 ± 0.17 (0.50)	ND	0.12 ± 0.16 (0.34)	ND	ND	ND	0.03 ± 0.09 (0.50)
	Freq.	20% (2/10)	ND (0/10)	40% (2/5)	ND (0/10)	ND (0/8)	ND (0/11)	7.4% (4/54)
NEtFOSAA	Conc.	ND	ND	0.11 ± 0.25 (0.57)	0.03 ± 0.10 (0.33)	0.05 ± 0.13 (0.38)	ND	0.02 ± 0.10 (0.57)
	Freq.	ND (0/10)	ND (0/10)	20% (1/5)	10% (1/10)	12.5% (1/8)	ND (0/11)	5.6% (3/54)
Legacy PFAS	Conc.	0.44 ± 0.40 (1.19)	2.20 ± 4.98 (16.23) [c]	0.52 ± 0.75 (1.77)	2.16 ± 1.04 (4.88)	1.87 ± 0.84 (3.41)	0.84 ± 0.70 (2.08)	1.39 ± 2.28 (16.23) [d]
	Freq.	70% (7/10)	60% (6/10)	60% (3/5)	100% (10/10)	100% (8/8)	90.9% (10/11)	81.5% (44/54)
Emerging PFAS	Conc.	0.80 ± 0.88 (2.38)	0.11 ± 0.35 (1.12)	0.96 ± 1.51 (3.63)	0.80 ± 0.82 (2.72)	1.49 ± 1.48 (3.64)	1.21 ± 0.53 (2.08)	0.88 ± 0.99 (3.64)
	Freq.	60% (6/10)	10% (1/10)	80% (4/5)	70% (7/10)	75% (6/8)	100% (11/11)	64.8% (35/54)
All PFAS	Conc.	1.24 ± 1.14 (3.57)	2.31 ± 4.93 (16.23) [e]	1.48 ± 1.67 (4.28)	2.97 ± 1.37 (5.97)	3.37 ± 1.67 (6.04)	2.05 ± 1.20 (4.14)	2.26 ± 2.46 (16.23) [f]
	Freq.	80% (8/10)	70% (7/10)	100% (5/5)	100% (10/10)	100% (8/8)	100% (11/11)	90.7% (49/54)

Summary statistics excluding an outlier sample that had 16.23 ng/g of PFOS: [a] 0.17 ± 0.33 (0.78), [b] 0.07 ± 0.21 (0.82), [c] 0.64 ± 0.71 (1.72), [d] 1.11 ± 1.00 (4.88), [e] 0.76 ± 0.68 (1.72), [f] 2.00 ± 1.53 (6.04).

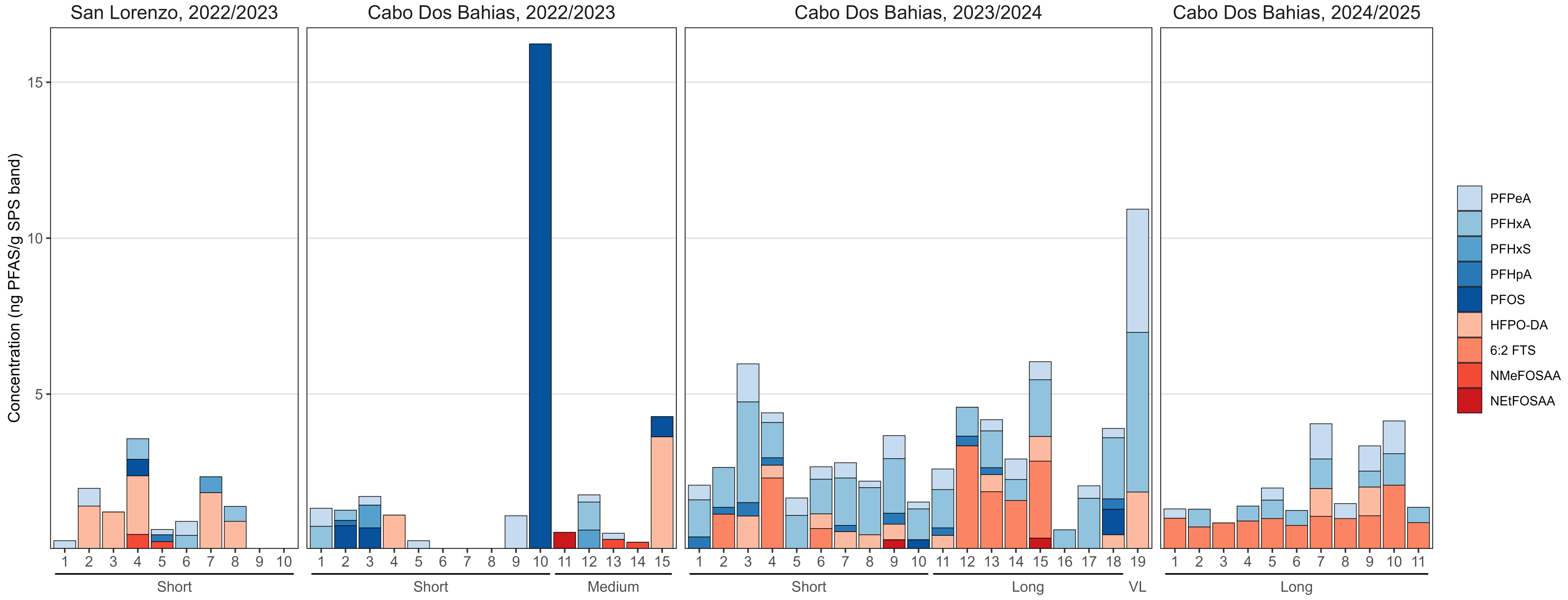

Figure 3. PFAS concentration in SPS bands (n = 55) deployed on Magellanic penguins at two colonies (San Lorenzo and Cabo Dos Bahías) over three breeding seasons (2022/2023, 2023/2024, and 2024/2025). Deployment periods were categorized as short (2 days), medium (3–5 days), long (6–9 days), and very long (VL, 17 days). PFAS compounds are colored according to their group (legacy = shades of blue, emerging = shades of red).

The following compounds varied in frequency of detection and/or concentration among colony-season cohorts (Figure 4A,C): PFPeA, PFHxA, PFHpA, HFPO-DA, and 6:2 FTS. Detections of PFPeA and especially PFHxA were higher in Cabo Dos Bahías 2023/2024 and remained elevated in the following year (2024/2025) relative to earlier cohorts. PFHpA occurred primarily in Cabo Dos Bahías 2023/2024, while HFPO-DA was most frequently detected in San Lorenzo 2022/2023 and Cabo Dos Bahías 2023/2024. Notably, 6:2 FTS showed the strongest cohort separation, shifting from non-detects in the earliest cohorts to complete detection in Cabo Dos Bahías 2024/2025, with a corresponding increase in concentration. The following compounds varied in frequency of detection and/or concentration among deployment periods (Figure 4B,D): PFHxA, 6:2 FTS, and NMeFOSAA. Specifically, PFHxA and 6:2 FTS were most frequently detected during long deployments, and 6:2 FTS concentrations were highest in long deployments, whereas NMeFOSAA was observed primarily during medium deployment. Exact p-values for all statistical comparisons are provided in Table S8. Principal component analysis (PCA) of the concentrations of nine PFAS in 54 samples yielded two principal components that accounted for 37.5% of the variance (eigenvalue = 3.38). The scores bi-plot reveals that samples can be separated in two main groups: (a) samples collected at both San Lorenzo and Cabo Dos Bahías in 2022/2023; and (b) samples collected at Cabo Dos Bahías in 2023/2024 and 2024/2025 (Figure 5A). On the other hand, the loadings bi-plot reveals that legacy and emerging PFAS compounds had nearly perpendicular vectors (i.e., they varied independently from each other); yet, the analysis revealed split patterns of variance within each of these two groups: the vectors of PFPeA, PFHxA and PFHpA were approximately opposite to those of PFHxS and PFOS, whereas the vectors of HFPO-DA and NMeFOSAA were approximately opposite to those of 6:2 FTS and NEtFOSAA (Figure 5B).

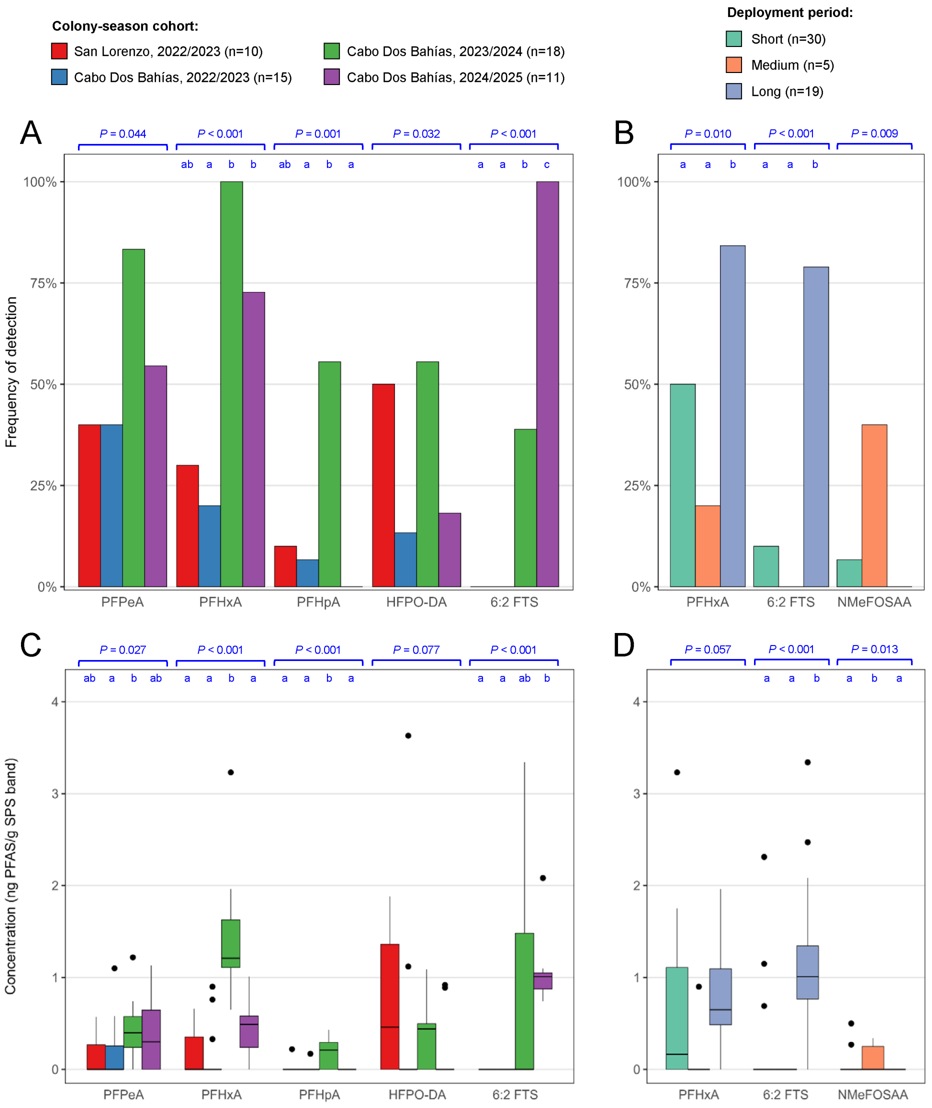

Figure 4. Comparison of the frequency of detection (**A**, **B**) and concentration (**C**, **D**) of PFAS compounds in SPS bands (n = 54) deployed on Magellanic penguins at two colonies (San Lorenzo and Cabo Dos Bahías) over three breeding seasons (2022/2023, 2023/2024, and 2024/2025). Deployment periods were categorized as short (2 days),

medium (3–5 days), long (6–9 days). *p*-values for the group comparisons (Chi-square test for frequency of detection, Kruskal-Wallis test for concentration) and the compact letter display for the post-hoc pairwise comparisons (false discovery rate-corrected Fisher tests for frequency of detection, Dunn-Šidák tests for concentration) are shown in blue above plots.

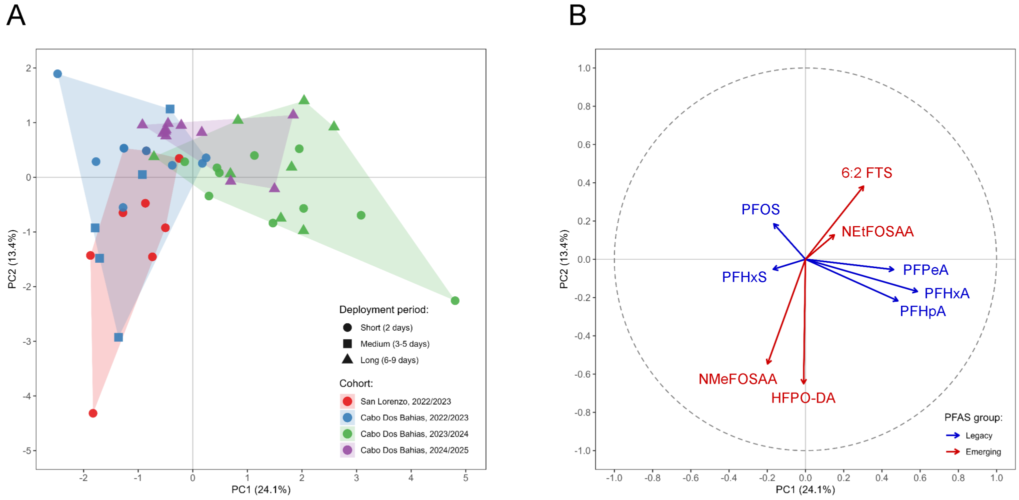

Figure 5. Scores (**A**) and loadings (**B**) bi-plots for the first two principal components of PFAS concentrations in SPS bands (n = 54) deployed on Magellanic penguins at two colonies (San Lorenzo and Cabo Dos Bahías) over three breeding seasons (2022/2023, 2023/2024, and 2024/2025).

4. Discussion

PFAS contamination has been detected across all continents, spanning environments from densely populated urban centers to some of the most remote regions on Earth, including the Arctic, Antarctic, and oceanic islands [64–66]. Although Patagonia, the southernmost portion of South America, is considered relatively pristine and scarcely human-inhabited (average <2 inhabitants/km^2), PFAS were detected in the vast majority (91%) of SPS bands deployed on Magellanic penguins along the Patagonian coast of Argentina. This finding illustrates that wildlife in remote ecosystems is nonetheless routinely exposed to these persistent contaminants.

The PFAS concentrations observed in SPS bands deployed on penguins across different colonies and breeding seasons indicate measurable contact-based exposure and are consistent with trace PFAS presence typical of remote marine environments [7,8]. Most PFAS did not show a clear pattern in how their concentrations varied among colonies or over time; however, three legacy compounds (PFPeA, PFHxA, and PFHpA) showed higher frequency and/or concentration in SPS bands deployed at Cabo Dos Bahías in 2023/2024 (Figure 4A,C). On the other hand, the emerging compound HFPO-DA, also known as “GenX”, peaked in SPS bands deployed at San Lorenzo in 2022/2023 and at Cabo Dos Bahías in 2023/2024 (Figure 4A,C). Interpretation of compound-specific patterns should also consider method performance. Fluorotelomer carboxylic acids showed low recoveries (~10–30%), and sulfonamide and sulfonamide ethanol compounds were not recovered under the current analytical workflow (Table S6). As a result, concentrations for these analytes are likely biased low, and non-detections may reflect analytical loss rather than true absence. Principal component analysis indicated that the PFAS profile was relatively similar across SPS bands deployed in 2022/2023 in both colonies, but differed considerably from the SPS bands deployed at Cabo Dos Bahías in 2023/2024 and 2024/2025 (Figure 5A). Since PC1-PC2 explained only 37.5% of total variance, these patterns are interpreted cautiously and treated as exploratory rather than definitive evidence of cohort clustering. The 2023/2024 austral summer coincided with a strong El Niño event [67], and large-scale climate variability (e.g., ENSO, Southern Annular Mode) can influence oceanographic transport and prey fields, potentially altering seabird foraging behavior and, in turn, contaminant encounter rates. However, with three breeding seasons and uneven sample sizes, our dataset cannot attribute interannual differences to specific climate drivers; longer time series paired with concurrent environmental measurements would be required to test these links. Of note, a survey of the Austral summer of 2020/2021 found relatively high PFAS concentrations in surface water samples collected off the Patagonian continental shelf, with PFPeA standing out as the most abundant compound in a sample collected ~800 km east of the studied penguin colonies [8].

On the other hand, the emerging compound 6:2 FTS was not detected in SPS bands deployed in 2022/2023, but showed increasing trends in both frequency and concentration in SPS bands deployed in 2023/2024 and

2024/2025 (Figure 4A,C); this gradual increase over the study period could reflect increasing environmental inputs due to market pressures, regulatory measures or altered bioavailability linked to degradation and transport processes [68,69]. It should be noted that Argentina banned the production, commerce, and use of PFOS and PFOA in 2019 in accordance with the Stockholm Convention on Persistent Organic Pollutants [70]. In this context, the detection of PFOS in 13% of SPS bands deployed on Magellanic penguins in 2022–2024 underscores the environmental persistence of these compounds. It is also worth highlighting that one of the SPS bands deployed at Cabo Dos Bahías in 2022/2023 had an unusually high PFOS concentration (16.23 ng/g); the source of this atypical high exposure is unknown, but this finding suggests there may be environmental "pockets" of PFOS input and/or accumulation in the Patagonian Sea to which wildlife may be incidentally exposed.

Although SPS measurements reflect contact-based (external) exposure rather than internal dose, PFAS concentrations remain relevant due to adverse and sub-lethal effects in birds that have been linked to internal PFOS burdens in controlled studies and risk assessments. For example, avian screening benchmarks reported effect-based reference values for PFOS on the order of ~1.7 µg/mL in serum, ~0.6 µg/g ww in liver, and ~1.7 µg/mL in eggs [71]. Experimental egg injection studies have also reported reduced hatchability at PFOS concentrations as low as ~0.1 µg/g [72], highlighting potential developmental sensitivity under some exposure scenarios. Importantly, these benchmarks are defined for biological matrices (e.g., blood, liver, eggs) and therefore cannot be directly compared to SPS concentrations (ng/g silicone), which reflect time-integrated contact exposure and depend on compound-specific uptake and deployment conditions. SPS results are best interpreted as indicators of PFAS availability in the microenvironments that penguins encounter. Pairing SPS deployments with targeted biomarker sampling would help link external exposure profiles to internal PFAS burdens, providing a stronger basis for evaluating potential health implications for penguins and other sentinel seabirds.

We did not find strong co-variation in the concentration of legacy vs. emerging PFAS (Figure 5B). However, interesting patterns of co-variation within each of these groups were observed. Among the legacy compounds, we found that the concentration variability of sulfonates (PFHxS and PFOS) was the opposite of that of carboxylates (PFPeA, PFHxA, and PFHpA). This pattern may be explained by the differing physicochemical properties of these compounds, as PFOS and PFHxS are stronger acids (lower pKa), less water-soluble, and exhibit greater sorption to organic matter than shorter-chain carboxylates leading to contrasting environmental distribution [73–75]. Among the emerging compounds, we observed that the concentration of HFPO-DA and NMeFOSAA varied in an opposite pattern to that of 6:2 FTS and NEtFOSAA. This contrasting behavior may be attributed to differences in the chemical properties of these compounds, as HFPO-DA and NMeFOSAA have considerably high polarity, greater water solubility, and lower sorption potential. Differences in their sources and uses could also be involved, since 6:2 FTS and NEtFOSAA are commonly associated with firefighting foams applications, whereas HFPO-DA and NMeFOSAA are not [76,77].

Comparisons with other marine sentinel studies highlight both consistencies and novel observations. Across fish and most marine environments, multiple studies report increasing contributions from short-chain and emerging PFAS, reflecting global transitions away from legacy PFAS and toward newer chemistries [76]. These patterns are evident in recent marine environmental and biota surveys, including invertebrates, sharks, avian eggs, pinnipeds and odontocetes [78–80], as well as in polar regions [81], indicating long-range transport and trophic transfer of emerging PFAS to remote regions. Broadly speaking, the concentration of PFAS in this study (mean ΣPFAS concentration = 2.3 ng/g, or 2.0 ng/g if an outlier sample with high PFOS concentration is excluded) was comparable to results of previous studies using blood or feather biomarkers of penguins. For example, little penguins (*Eudyptula minor*) sampled in Australia (2021/2022 and 2022/2023) had mean concentrations of approximately 0.9 ng/mL for PFHxS and 2.6 ng/mL for PFOS in plasma [20]. King penguins (*Aptenodytes patagonicus*) sampled in the Southern Indian Ocean (2014/2015) had PFOS concentrations ranging from 2.6 to 7.5 ng/g in plasma, and generally low or no detection of short-chain PFAS [21]. Gentoo penguins (*Pygoscelis papua*) sampled at the Antarctic Peninsula (2014/2015) showed PFOA concentrations up to 1.5 ng/g in feathers, whereas short-chain PFAS were not detected [28]. In this context, our results suggest that SPS bands are well-suited for capturing contact-based exposure to short-chain and highly mobile PFAS over the deployment window (e.g., HFPO-DA, PFPeA, PFHxA, PFHxS). Importantly, biological matrices (e.g., blood, feathers, eggs) represent internalized body burdens resulting largely from dietary uptake and are therefore not directly equivalent to SPS-derived concentrations. Direct, paired comparisons between SPS and biological samples are needed to evaluate detection sensitivity and cross-matrix relationships.

On the other hand, studies on excreta samples generally yield higher PFAS concentrations, especially for longer-chain compounds likely due to their greater hydrophobicity. Excreta from gentoo penguins sampled at the Antarctic Peninsula (2010/2011 and 2012/2013) had concentrations of 1.6 ng/g for PFHxA, 2.5 ng/g for 6:2 FTS, and 0.7 ng/g for PFOS [24]. Similarly, eggs of African penguins (*Spheniscus demersus*) sampled in South Africa

(2011/2012) had generally high concentrations of long-chain compounds, with 4.5 ng/g for PFOS, 3.7 ng/g for PFNA, 2.6 ng/g for PFUdA, 1.9 ng/g for PFOA, and 0.8 ng/g for PFDA [26]. These data and similar studies emphasize the complementary value of SPS with traditional biomarkers to draw a more complete picture of the PFAS exposure in these species.

Portable SPS were previously deployed as wristbands in humans [30,31,37], collar tags in pets [38–41], and cutaneous implants in amphibians [43]. To our knowledge, this is the first study to employ portable SPS in wild birds to measure contaminant exposure. Due to the unique anatomy of penguins, we used a leg-band design with a diameter adjustable to the tarsus width of each individual, an approach derived from early mark-recapture studies [82] and recent geolocator studies [55,83]. It should be noted that although a flipper band design would likely have allowed for even longer deployment periods and reduced wear, we avoided this approach as flipper bands cause significant hydrodynamic drag and can influence the long-term survival and breeding success of penguins [84,85]. The SPS bands were deployed and recovered with a high success rate (55 out of 57 attempted deployments = 96%) for periods up to 9 days. Considering that band deployment and recovery are rapid (1–2 min/bird) and painless procedures, our results demonstrate that SPS leg-bands are a safe and minimally invasive approach for pollutant exposure monitoring in penguins. Furthermore, while matrices based on biological samples (organs, blood, feathers, eggs, etc.) can be affected by physiological processes (digestion, absorption, metabolism, excretion, etc.) [86,87], SPS offer a better representation of the penguin's surrounding environment, including the water column when bands are used in combination with bio-loggers. An additional consideration is that the transport of samples for traditional biomarkers often have burdensome requirements in terms of logistics (e.g., cold-chain, packaging) and authorizations (e.g., Nagoya Protocol, CITES, import/export permits), whereas the logistics and authorizations involved in the transport of SPS bands are generally more flexible.

The fact that SPS bands accumulated PFAS reliably across all three breeding seasons in this study demonstrates their suitability for monitoring environmental contaminant exposure. While deployment periods varied among individuals, for most compounds there was no clear association between how long the SPS bands were deployed and the PFAS concentrations. This suggests that differences in uptake were minimal for the range of deployment periods used in this study, and SPS bands were able to capture representative PFAS exposure profiles even when worn for shorter periods within the tested range. However, considering that longer deployment periods were associated with high frequency of detection and/or concentration of PFHxA, 6:2 FTS, and NMeFOSAA (Figure 4B,D), it might be desirable to deploy SPS bands for one week or more (when feasible and safe to do so) to maximize PFAS uptake.

While SPS offers clear advantages, some limitations require consideration. The passive diffusion process is influenced by environmental parameters, and temperature or water interaction could affect uptake kinetics [31,88]. Increased temperatures can raise diffusion coefficients generally increasing uptake rates, thus deployment in warm areas could potentially lead to higher recorded PFAS levels than in colder areas [89]. Water interaction can both enhance or diminish PFAS uptake due to the individual PFAS properties and partitioning with water [89,90]. Due to the arid climate of the Argentine Patagonian coast, it is unlikely that air humidity or precipitation interfered with PFAS absorption in this study; however, this could be a significant factor when deploying SPS bands in regions with more humid or variable weather. However, the proportion of time spent in water can also be determined by the behavior of the animals in response to factors such as sex, individual experience, number of chicks, prey density and distribution at sea, among others [52,91,92]. GPS trackers or wet-dry sensors could help control for these factors when interpreting PFAS results in SPS bands; however, caution is warranted with regards to the adhesives and tapes used to secure such devices acting as a potential source of PFAS contamination. Alternatively, trap cameras could be deployed facing the penguin nests to estimate the proportion of time spent at the nest in each SPS deployment without the need for animal-borne devices. The deployment of SPS to sample the penguin colony environment (e.g., beach, walkways, nests) could help untangle the amount of pollutant exposure occurring locally at the colony vs. at sea.

The demonstration of the feasibility of SPS as non-invasive monitoring tools to monitor PFAS exposure in Magellanic penguins provides proof-of concept for applying similar approaches to more vulnerable or threatened penguins and other diving seabirds. Specifically for penguins, the critically endangered African penguin (*Spheniscus demersus*) from southern Africa, the endangered yellow-eyed penguin (*Megadyptes antipodes*) from New Zealand and the little penguin (*Eudyptula minor*) from Australia and New Zealand stand out as examples of species that often breed in urban or peri-urban environments [19,93] and could benefit from the use of SPS bands to non-invasively monitor their exposure to pollutants such as PFAS. In remote areas (e.g., Antarctica and sub-Antarctic islands), penguins wearing SPS bands could also be used as biosensors to monitor PFAS pollution in the ocean, as has been done through the study of mercury levels in penguin blood and feathers [15,19]. Developing strategies to deploy and recover SPS in other marine and terrestrial wildlife species could prove valuable for pollution monitoring on a larger scale, especially where invasive sampling is ethically or logistically challenging. Implementing SPS across multiple species and geographic regions could yield a comprehensive baseline for contaminant exposure, guiding remediation strategies and policy intervention. Furthermore, although we focused on PFAS in this study, it should be noted that SPS can also be used to detect and quantify a broad range of semi-volatile organic compounds such as flame retardants, pesticides, phthalates, PAHs, among others [30–33].

5. Conclusions

SPS provides a minimally invasive approach to characterize contact-based (external) exposure to PFAS and other contaminants in wildlife. This study provides a proof-of-concept application of SPS for monitoring pollutant exposure in wild birds, adding to the ever-expanding variety of wearable technologies being developed to monitor marine environments and their inhabitants [44]. SPS bands deployed on the ankles of Magellanic penguins during the early chick-rearing stage demonstrated a safe and effective approach for detecting both legacy and emerging PFAS, with measurable differences among colony-season cohorts. Importantly, SPS-derived concentrations reflect external exposure conditions over the deployment window (2–9 days) but are constrained to point-of-time analysis, and therefore complement traditional tissue-based matrices that quantify internal biological burden. Accordingly, future work should pair SPS with established biomonitoring matrices (e.g., blood and/or feathers) and relevant physiological endpoints to evaluate the relationship between PFAS external exposure profiles, internal biological burdens, and potential health relevance. Incorporating SPS into long-term wildlife monitoring programs offers a powerful approach for characterizing time-integrated PFAS exposure while minimizing disturbance to study animals. Their deployment enables repeated sampling to detect shifts in PFAS occurrence and profiles, especially when combined with environmental measurements (e.g., temperature, air humidity, precipitation) and animal behavior (e.g., GPS tracking, wet-dry sensors), thereby improving PFAS risk assessment and source attribution.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2603231336517199/EESUS-25110070-SM.pdf. Figure S1: LC-MS/MS mobile phase gradient. The gradient starts at 50% mobile phase A (5 mM ammonium acetate in water: acetonitrile (19:1)) and 50% mobile phase B (acetonitrile: methanol (1:1 *v*/*v*)). Mobile phase B increases to 95% (0–8 min) and is held constant for 3.5 min. Then, the mobile phase B is returned to its starting condition of 50% (11.5–18 min). The phases were held constant for 5 min to allow for column equilibration. Table S1: List of 40 PFAS standards with their abbreviation, IUPAC name, CAS number, supplier information, and precursor ion. Table S2: Wristband deployment and penguin identification. Table S3: PFOS concentration (ng PFAS/g SPS band) in wristbands deployed on penguins. Table S4: Mass spectrometry parameters and transitions. The source conditions include capillary temperature 220 °C, vaporizer temperature 210 °C, sheath gas 30 psi, aux gas pressure 2 psi, and spray voltage –3000 V. Table S5: List of PFAS limits of detection (LOD) and quantitation (LOQ). Table S6: PFAS extraction efficiencies from the recovery study. Table S7: Response factor pairing for PFAS quantification. Table S8: *p*-values for statistical comparisons.

Author Contributions

P.C.M.: data curation, formal analysis, writing—original draft preparation, investigation; R.E.T.V.; resources, data acquisition, formal analysis, writing—review and editing, interpretation of results; L.G., G.S.B. and F.R.Q.: writing—review and editing; M.M.U.: supervision, writing—review and editing; K.K.: supervision, writing- review and editing; D.S.A.: supervision, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the National Science Foundation PIRE grant (Award No. OISE 2230728) awarded to DSA. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the NSF.

Institutional Review Board Statement

Field procedures were conducted under permits issued by Dirección de Fauna y Flora Silvestre, Ministerio de Agricultura Ganadería Industria y Comercio (DFyFS-MAGIyC 079/2022, 2499/2022, 128/2024) and Subsecretaría de Conservación y Áreas Protegidas (SsCyAP 076/22, 084/23, 083/24) and with the approval of the Institutional Animal Care and Use Committee of the University of California—Davis (IACUC 21806).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data will be made available upon request.

Acknowledgments

We are grateful to Hilton Entringer Jr, Ariel Serra, Agustina Donini, Martin Brogger, Alexis Durant, and Elizabeth Ashley for their field assistance. Additionally, we are thankful for Tyrone Enova at the University at Buffalo for his laboratory assistance. The first author also thanks Jake Burchfield for his support and encouragement throughout this work. We would like to express our gratitude to Guillermo Paats and Sebastian Machinea and all the staff of the Estancia San Lorenzo for supporting research at San Lorenzo. We especially thank P. Riger (R.I.P.), R. Bumpus, M. Parker, T. Rhoades and B. Jones from Houston Zoo for their long-term commitment and support towards saving marine wildlife from pollution.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Grammarly for post-draft spell-checking and grammar, and used ChatGPT to assist in developing the concept for the graphical abstract. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article. The final graphical abstract was created by the authors in PowerPoint.

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