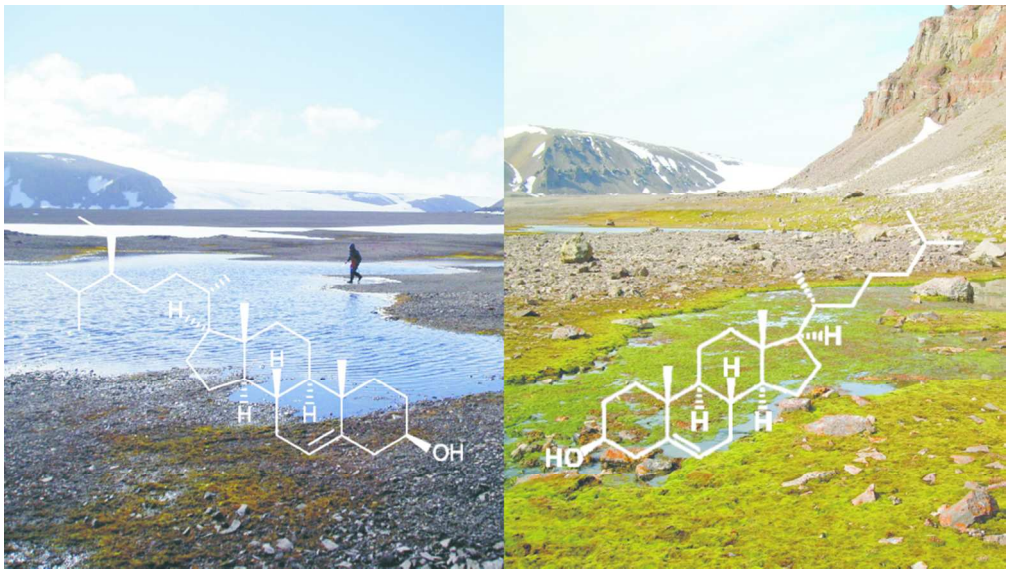
Sterols and stanols preserved in pond sediments track seabird biovectors in a High Arctic environment

Journal:	*Environmental Science & Technology*
Manuscript ID	es-2016-02767p.R1
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Cheng, Wenhan; University of Ottawa, Biology Sun, Liguang; University of Science and Technology of China, Kimpe, Linda; University of Ottawa, Biology Mallory, Mark; Acadia University, Smol, John; Paleoecological and Environmental Assessment and Research Lab (PEARL), Queen's University, Biology Gallant, Lauren; University of Ottawa, Biology Li, Jinping; Qinghai Normal University Blais, Jules; University of Ottawa, Department of Biology


H
H
H
H
OH
H
H
H
H
HO

Sterols and stanols preserved in pond sediments track seabird biovectors in a High Arctic environment

Wenhan Cheng [1], Liguang Sun [2], Linda E. Kimpe [1], Mark L. Mallory [3], John P. Smol [4], Lauren R. Gallant [1], Jinping Li [5], Jules M .Blais [1 *]

1 Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

2 Institute of Polar Environment, School of Earth and Space Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China

3 Department of Biology, Acadia University, Wolfville, Nova Scotia, B4P 2R6, Canada

4 Paleoecological Environmental Assessment and Research Lab (PEARL), Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada

5 Key Laboratory of Environment and Resources on Tibetan Plateau, Qinghai Normal University, Xining 810008, China

Corresponding author:

Jules M .Blais

Address: 30 Marie Curie Private, Ottawa, ON, K1N 6N5, Canada

Phone: +1(613) 562-5800 Ext. 6650

Fax: +1(613) 562-5486

Email: Jules.Blais@uOttawa.ca

Abstract

Seabirds are major vertebrates in the coastal ecosystems of the Canadian High Arctic, where they transport substantial amounts of marine-derived nutrients and pollutants from oceans to land by depositing guano and stomach oils to their nesting area, which often includes nearby freshwater ponds. Here we present novel indicators for evaluating the impact of seabirds on freshwater ecosystems. The ratio of cholesterol : (cholesterol + sitosterol) in pond sediments showed significant enrichment near a nesting colony of northern fulmars (*Fulmarus glacialis*) and was significantly correlated with ornithogenic enrichment of sediment as determined by sedimentary $\delta^{15}N$. The sterol ratio was also correlated with several bioaccumulative persistent organic pollutants (POPs), suggesting its usefulness in tracking biovector enrichment of contaminants. Human-derived epicoprostanol was also analyzed in the sediments, and its relationship with an abandoned, prehistoric settlement was recorded, suggesting its potential as a tracer of prehistoric human activities in the Arctic. Sterols and stanols preserved in sediments appear to be useful geochemical tools that will inform our understanding of migratory species and the presence of pre-historic human populations in the Arctic, and possibly other animal populations.

Keywords: seabirds, sediment, Arctic, cholesterol, sitosterol, biovector

21 Introduction

22

23 Most High Arctic lakes are oligotrophic with low biomass and species diversity [1, 2],
24 though some sites receive significant enrichment of nutrients and persistent organic
25 pollutants (POPs) from foraging and migrating species [3-5]. Species at higher trophic
26 levels bioaccumulate many of the halogenated POPs [6-8], and are effective in funnelling
27 these contaminants to nesting and spawning areas [9, 10]. Northern fulmars (*Fulmarus*
28 *glacialis*) are common carnivorous seabirds that feed on marine animals, and they nest on
29 coastal cliffs close to the sea [11]. As a result, their nesting areas may receive a high influx
30 of seabird input, including, but not limited to, guano, moulted feathers, stomach oils and
31 carcasses, and can be a significant source of nutrients and pollutants to the nesting areas [3,
32 4, 9, 12]. In addition, coastal areas with freshwater ponds were used by northern peoples in
33 the past, which may also have influenced the limnology of these ponds by sewage and
34 marine mammal harvesting activities (e.g., whaling) [13-16].

35

36 A variety of paleolimnological proxy indicators has been developed to track biovector
37 transport of nutrients and contaminants to lake sediments [4, 12, 17-19]. Sedimentary $\delta^{15}N$ is
38 an important indicator of ornithogenic nitrogen [19, 20] because ^{15}N is enriched in seabird
39 guano [21-24]. However, $\delta^{15}N$ could be affected by factors other than biovectors. For
40 example, $\delta^{15}N$ in lake sediment may be influenced by inorganic nitrogen input from
41 weathering and post-depositional nitrification or denitrification [25-27]. Moreover, $\delta^{15}N$ can
42 also be enriched in sediments from other biovectors in addition to birds, e.g. whales [16],
43 salmon and other migratory fish [28]. As a result, interpretation of $\delta^{15}N$ data in sediment

may benefit from independent geochemical markers of ornithogenic enrichment in sediments. For instance, many diatom species are common under distinct trophic conditions, i.e. oligotrophic or eutrophic [18, 29, 30]. Hence, the spatial and temporal variation of diatom community assemblages can be used to track seabird influence in addition to $\delta^{15}N$. Sterols and stanols may also be employed to support these more traditional indicators [5, 31, 32].

Sterols are natural, unsaturated steroid alcohols that represent an important group of compounds for biological functions and structure of biological membranes [33]. Stanols are reduction products of sterols by hydrogenation, usually by microbes either in animal gastrointestinal tracts or in the environment [34]. Sterols and stanols in animal faeces or other biological materials are readily preserved in lacustrine sediments, providing an archive of source information for nutrient and contaminant inputs to lakes [35]. Though sterols and stanols may degrade in some warm climates [36, 37], they are more resistant to degradation in cold environments and may be preserved for thousands of years [38, 39]. They may therefore be used as biomarkers in paleoecological studies, especially in polar regions [5, 31, 40].

A few of the sterols and stanols are already known to have associations with specific sources. Cholesterol is an essential component in cell membranes [33], and thus it is one of the most commonly found sterols in all organisms, especially animals (Kingdom Animalia) [41, 42]. It has long been used as an indicator for marine animal material in aquatic environments [43-46]. On the other hand, sitosterol is a common sterol in plant tissue

and wax [47, 48], and it is widely reported in phytogenic material [40, 49, 50], including algae [51, 52]. Coprostanol is widely used to trace municipal sewage input in aquatic environments [53, 54]. However, it is also reported in bird guano at a lower concentration [42, 55, 56]. Meanwhile, epicoprostanol in Arctic environments should be strongly related to human activities due to its distinctive presence in human sewage [46, 54], although it is also found in livestock and poultry waste, which are not expected in the Arctic [42].

Here we analyzed cholesterol, sitosterol, coprostanol and epicoprostanol from the sediments of 23 High Arctic ponds near a large breeding colony of northern fulmars at Cape Vera, Devon Island, NU [9, 11, 57]. A Thule archeological site was adjacent to one of the ponds, which may also have influenced the vicinal ecology and environment. The purpose of this study was to determine whether the presence of a large seabird colony and an ancient Thule settlement affected the sterol and stanol composition in sediments from ponds spanning a gradient of inputs from these sources by comparing the sterol and stanol profile to traditional biovector indicators described in previous studies from this region.

Materials and methods

Cape Vera (76°15'N, 89°15'W; Figure 1) is located on northern Devon Island in the Canadian Arctic, ~500 km west of Greenland. The Devon Ice Cap covers the montane area behind the cliffs. Approximately 11,000 pairs of northern fulmars nest on the coastal cliff every summer [57], from where they feed on fish and marine invertebrate prey,

sometimes hundreds of kilometers away [58, 59]. A series of ponds are located at various distances below this cliff, with each receiving different amounts of ornithogenic inputs [9]. There were visible differences among these ponds, with a clear decrease in nutrients and algal biomass with increasing distance from the bird colony, which was subsequently supported by geochemical studies [18]. Following our previous work at Cape Vera [9], each pond was identified as CV (number), where CV stands for Cape Vera. All these ponds are small and shallow, less than 100 m^2 in area, maximum depth less than 1.5 m, and were located within 2 km of the coast, but no significant seawater inputs were noted based on salinity measurements [18]. Periphyton, phytoplankton, zooplankton, and chironomids were studied in all ponds [3, 4, 60].

Most of our studied ponds are located adjacent to the major fulmar colony on the eastern side of the cape (Figure 1). This group of ponds includes CV5 to CV10, CV12, CV14, CV15, CV20 and CV30. In particular, CV8, CV9, CV10 and CV30 are at the foot of the cliff, receiving the most direct inputs from the fulmar colony, and they are the most affected ponds based on nutrient concentrations and other ornithogenic indicators [4, 9, 18]. CV12 and CV14 are far from the colony among this group and isolated from any other ponds. They are the ponds least influenced by seabirds in this group [18]. Another group of three ponds, including CV16, CV17 and CV18, are located at the base of a cliff opposite the northern fulmar colony. These three ponds are used as a reference [18]. Meanwhile, CV1 and CV2 are at the far end of the cliffs located ~4 km away. CV3 and CV4 are ~2 km from the major colony. CV22, CV23 and CV24 are also located at a remote reference site ~7 km away to the north, and CV11 is ~10 km away to the southeast. These eight

ponds are far away from any modern seabird colony. No seabird activities were observed near them during our fieldwork, hence they are likely unaffected by seabirds [3, 4, 18]. CV13 is ~1 km from the major colony, where an abandoned Thule archeological site was observed. This site consisted of the remains of at least seven recognizable Thule tent rings, each of less than 2.5 m in diameter [61].

Sediment samples were collected from each pond in the summers of 2005, 2006 and 2007 [3, 18]. As the ponds are shallow, samples were collected by wading into the ponds with chest waders or from an inflatable boat. Sediment samples were collected with a gravity corer at the approximate center of each pond, and extruded on shore. A 1 cm surface sediment slice was collected using a pre-cleaned spatula, then stored in Whirlpak® bags. Caution was taken to ensure only sediments at the surface ~1 cm were collected and nothing was lost during the sample collection. Moss samples were also collected in the same way near each pond where available. Fresh guano samples were not available, because the fresh excrement usually accumulates at the nesting area, which could not be safely accessed, or dissipates in the air before reaching the ground. Instead of guano samples, stomach oil and other digestive track content samples were collected from captured fulmars at the same site, as well as at Prince Leopold Island, a nearby high Arctic fulmar colony where the diet of the birds is similar to Cape Vera fulmars [58]. Regurgitated stomach oil was collected from fulmars at Cape Vera in sterile Whirlpak bags by holding the head of fulmars over the bag allowing materials to drain. Crop proventriculus content and small intestine content were also collected from two northern fulmar carcasses, which perished by natural causes during the same field season at Prince

Leopold Island. As the prey retention time is only 10 h in northern fulmar digestion [62], gastroenteric content should represent the final composition of guano. Whale blubber samples were collected as part of a monitoring program in nearby Inuit communities on Baffin Island between 2009 and 2014. We analyzed ten whale blubber samples for sterols and stanols to determine whether traces of whale-derived biomarkers are detectable in sediments near this Thule archeological site. All samples were immediately sealed and frozen within 24 h, then transported to a laboratory and kept frozen until analysis.

Analytical methods were modified from Birk, et al. [63]. All sediment and animal samples were freeze dried before analysis. Dry sediment and moss samples (~0.1 g dry weight) were sonicated with 10 mL dichloromethane for 10 min with activated copper, which was repeated three times. All extracts were combined and concentrated to ~0.5 mL under a gentle flow of nitrogen at 35°C. The concentrated extract was transferred to a LC-Si SPE column. As we were only interested in sterols and stanols, the SPE columns were only eluted with 15 mL dichloromethane for cleanup. The eluted sterols and stanols were dried completely under a gentle flow of nitrogen, before adding 100uL 99:1 BSTFA+TMCS (N,O-Bis-(trimethylsilyl) trifluoroacetamide and Trimethylchlorosilane), then heated at 60°C for 2 h and dried completely again. We added 900 μL of toluene to dissolve the derivatized samples, and 100 μL of 5000 ng/mL 5α-cholestane were added as an internal standard.

Freeze-dried animal samples (~0.1 g dry weight), including fulmar stomach oil, digestive samples and whale blubber sample, were also extracted with dichloromethane and

159 concentrated to ~0.5 mL as sediment samples. Concentrated extracts of animal samples
160 were saponified with 5 mL 0.5 mol/mL potassium hydroxide at 70°C for 1.5 h, then
161 liquid-liquid extracted with hexane consecutively three times; 10 mL hexane was used in
162 each extraction. Hexane extracts were combined and concentrated to ~0.5 mL, and
163 transferred to a LC-Si SPE column. Subsequent steps were the same as for sediment
164 samples.
165
166 Sterols and stanols were quantified by GC-MSD with a capillary column of Agilent
167 19091J-433 HP-5 5% phenyl methyl siloxane. The initial oven temperature was set at
168 80°C and initial time was 1.5 min, then equilibrated for 0.5 min. The oven ramp was set
169 as follows: first an increase to 265°C at 12°C/min, then an increase to 288°C at
170 0.8°C/min, then an increase to 300°C at 10°C/min, and kept at that temperature for 12
171 min. The transfer line temperature was 280°C. Details of the MSD parameters are in
172 Table 1. Sterol concentrations (mean ± SD) are reported in μg/g dry weight.
173
174 For quality control, all GC-MSD results were corrected with the internal standard 5α-
175 cholestane. The calculation was done by MSD ChemStation D.02.00.275. For every five
176 samples, an experimental blank was run simultaneously. Sitosterol and epicoprostanol
177 were not quantified in any blanks, but cholesterol was detected in some blanks with
178 concentrations no more than 10% of samples. The blank values were subtracted from
179 corresponding samples. Concentration results were not recovery corrected. Limit of
180 quantification was defined as a signal to noise ratio of 3 (Table 1). Signals below that
181 ratio were regarded as not quantified and discarded.

182

183 The nitrogen stable isotope and POP data for the same sediment samples were obtained
184 from a previous study [9], where analytical methods were described in detail.

185

186 Results and discussion

187

188 Cholesterol in three fulmar samples (crop proventriculus, stomach oil content and small
189 intestine content) were 5483, 2543and 7488 μg/g, respectively, composing more than
190 99.7% of total sterols and stanols in the fulmar samples. Sitosterol concentrations were
191 14.4 μg/g, 4.5 μg/g and 6.3 μg/g in these samples, and its proportion in total sterols and
192 stanols was no more than 0.25%. This result suggests that cholesterol was the
193 overwhelmingly dominant sterol in the fulmar samples (Figure 2). The crop
194 proventriculus is one of the first parts of the alimentary system of fulmars, and it is where
195 stomach oil forms [64], hence the crop proventriculus content is the precursor of stomach
196 oil. Though no detailed investigation was available on the sterol and stanol composition
197 of alimentary content of northern fulmars, our preliminary results suggested that the
198 portion of cholesterol was increasing with the alimentary process, from crop
199 proventriculus content to stomach oil, then small intestine content (Figure 2). Therefore,
200 the cholesterol proportion of northern fulmar guano may be similar to or even higher than
201 those in alimentary samples. In contrast, cholesterol was 43.6±31.6 μg/g and sitosterol
202 was 36.8±14.2 μg/g in moss samples (n=9). Their composition in total sterols and stanols
203 was 49.7±17.2% and 50.3±17.2%, respectively. Thus the proportions of cholesterol and
204 sitosterol in moss were about equal, though cholesterol in moss was higher in the ponds

adjacent to the bird colony, namely CV8, CV20 and CV30 (Figure 2). Algae samples were not available for sterol analysis due to very low biomass in the reference ponds, but Kamenarska et al. [51] indicated that concentrations of sitosterol and cholesterol in algae are typically about the same. Therefore, cholesterol could come from algae, but its relative ratio to sitosterol is distinct from that in ornithogenic material. Meanwhile, cholesterol in whale blubber samples was 5845±3967 μg/g (n=10), comprising more than 99.9% of total sterols and stanols, with no sitosterol quantified in any whale blubber samples. These results suggest that animals, and most probably northern fulmars due to their overwhelmingly large population at this site [65], were the likely source of the cholesterol, while plants were likely the source of sitosterol, at Cape Vera.

No epicoprostanol was quantified in either fulmar (n=3) or moss (n=9) samples, but epicoprostanol was identified and quantified in three out of 10 blubber samples from bowhead whales (*Balaena mysticetus*) at concentrations of 0.5 μg/g to 1.1 μg/g.

Cholesterol (24.4±23.0 μg/g, n=23) and sitosterol (22.6±13.5 μg/g, n=23) were ubiquitous in the Cape Vera pond sediments, suggesting widespread inputs from animal and plant-derived material in this area (Table 2). Indeed, ponds with higher $\delta^{15}N$ had significantly higher cholesterol (Pearson correlation, r_{23}=0.49, P<0.02), but did not have higher sitosterol (r_{23}=0.12, P>0.5). Hence, the marine-derived ornithogenic input appears to be the dominant source of cholesterol in these ponds, confirming the earlier work done on these sites [3, 4, 9, 18]. Cholesterol concentrations in pond sediments closest to the fulmar colony, including CV8, CV9, CV10 and CV30, were among the highest (from 34 to 71

μg/g), suggesting ornithogenic enrichment of cholesterol at these locations. However, sitosterol content in these ponds was also high, from 17 to 30 μg/g, suggesting there was also high phytogenic input, likely reflecting the lush vegetation surrounding the fertilized ponds nearest to the bird colonies. In the other ponds, which were remote to the bird colony, the highest concentration of 84 μg/g of cholesterol was recorded in CV13, which might be ascribed to whale blubber or other materials that were introduced by the Thule whalers. Meanwhile, the surface sediment in CV14, one of the ponds that did not receive direct input from the fulmar colony due to its isolation from bird-affected ponds, contained high cholesterol (43 μg/g) in addition to relatively high sitosterol (44 μg/g). We suspect that this may be ascribed to different sedimentation rates, which may significantly affect absolute concentrations of indicators in sediments by diluting or concentrating them [66]. Though sedimentation rate data were not available for all ponds, the geochronologies of the sediment cores collected from these ponds revealed significant differences between bird-influenced ponds and reference ponds [67]. For example, the surface accumulation rate of reference pond CV22 was around 0.02 g cm^{-2} yr^{-1} based on ^{210}Pb chronologies, while CV8 accumulated 0.23 g cm^{-2} yr^{-1} because of direct ornithogenic input [67]. A lower sedimentation rate in reference ponds may result in a magnification of the absolute concentrations of indicators, such as sterols and stanols. Hence, the absolute concentration of sterols may not be an effective indicator of seabird impact.

Though part of the cholesterol in sediment might come from the higher plant and algal populations in the bird impacted ponds [45, 68], the majority of this compound may come

from ornithogenic input, and the ratio between cholesterol and sitosterol is distinct between phytogenic and ornithogenic material, according to this study and literature [42, 45, 51, 69]. Hence we propose a seabird impact index, defined as cholesterol : (cholesterol + sitosterol), as a proxy for relative seabird inputs to pond sediments, instead of the absolute sterol concentrations. The principle in this index was the different sterol proportions in seabird and plant materials. As most Arctic freshwater bodies are oligotrophic [1, 2], ornithogenic input of nutrients at Cape Vera was the dominant factor for vegetation growth near and inside these ponds [18]. High index values were found in every pond adjacent to the fulmar colony, including CV7, CV8, CV9, CV10 and CV30 (Figure 3A). CV14 and other remote ponds were isolated from these highly affected ponds. Reference ponds, including CV23, CV24, CV2 and CV12, had the lowest seabird impact index. Though the index in reference ponds CV1 and CV22 were not the lowest, they were still much lower than those in the impacted ponds.

The northern fulmar colony on the cliff was the obvious source of ornithogenic material at Cape Vera, but there might be multiple pathways for biovector material to reach the coastal ponds. One is that bird excrement accumulates around the nesting area [11, 57], and subsequently washes downhill by precipitation or meltwater from the glacier. Fulmars use partly digested chyme, which is referred to as stomach oil, to feed their young [64]. The stomach oil is also expelled on predators for defensive purposes or in conspecific competition [70]. The stomach oil is enriched in energy as well as cholesterol [69], which could fall into the vicinity and act as another important source of ornithogenic cholesterol in this area [71]. As a result of receiving excrement and stomach oil from the colony, the

adjacent ponds had the highest seabird impact index. On the other hand, the atmospheric transportation of ornithogenic materials was negligible at Cape Vera, as seen by the confined impact by birds within a square kilometer [4, 9, 12, 18, 72]. Therefore, ponds that did not receive direct input from the colony may have a low seabird impact. As a result, pond CV12 had a very low seabird impact index (0.36) due to local hydrology and topography, despite the fact that it was not far from the major colony. This finding was supported by previous studies at this location [4, 18, 72], which also revealed that CV12 was among the least affected by seabirds. Other reference ponds, e.g. CV23 and CV24, had the lowest seabird impact index because of their remoteness to the bird colony, despite that CV22 had a moderate index among all the ponds.

In further support of our sterol index, seabird impact (cholesterol : (cholesterol + sitosterol)) was strongly correlated with $\delta^{15}N$ (r_{23}=0.68, P<0.001. Figure 3B), which itself was strongly correlated with other indicators of ornithogenic enrichment in these same ponds [9], and it has been shown to be a clear tracer of ornithogenic enrichment in these ponds [3, 9, 72]. Previous research on the same island suggested that post-depositional processes might influence the soil $\delta^{15}N$ as much as 3.25‰ [73], which was much lower than our observed $\delta^{15}N$ range (~20‰, Figure 3B). However, this might explain some unexpected $\delta^{15}N$ results in the studied ponds, e.g. relatively low $\delta^{15}N$ in impacted pond CV7 (11.9‰) and relatively high $\delta^{15}N$ in reference ponds CV3 (18.4‰) and CV4 (15.0‰). The ecosystem at Cape Vera is very simple; northern fulmars were the dominant species and the source of high $\delta^{15}N$ and major nutrients (nitrogen and phosphorus) in these ponds [61].

297

298 A ratio of coprostanol : cholesterol > 0.5 has been suggested as evidence of human
299 sewage pollution in various studies [34, 74, 75]. However, CV5 was the only studied pond at
300 Cape Vera which had a ratio of coprostanol : cholesterol > 0.5 (Table 2). This result
301 indicates that the modern anthropogenic impact was limited at Cape Vera. In addition,
302 coprostanol was reported in non-anthropogenic materials in Antarctica, implying possible
303 limitations of its reliability as a human activity indicator [56]. Indeed, coprostanol was
304 quantified at 0.4 μg/g in crop proventriculus content sample of a northern fulmar,
305 indicating that fulmar guano may act as a source of coprostanol other than human
306 activities at Cape Vera.

307

308 Instead of coprostanol and its ratio to cholesterol, we propose epicoprostanol as an
309 anthropogenic indicator in the Arctic, due to its selective presence in human faeces [46, 54]
310 and whale carcasses (this study), which were both related to past human activities in this
311 part of the Arctic. Epicoprostanol was quantified only in sediments from ponds CV2,
312 CV6, CV8, CV9, CV10, CV13, CV14, CV20 and CV30. Because epicoprostanol is
313 reported only in municipal sewage [46, 54] and in animal waste that is not endemic to the
314 Arctic, such as pigs, poultry, and magpies [42], the presence of this compound at Cape Vera
315 may suggest the impact of human activities in these ponds. Although some baleen whales
316 are believed to produce epicoprostanol [53, 56], the whale material at Cape Vera may also be
317 related to the indigenous people because of their whaling activities. CV13, which is
318 directly adjacent to the Thule site, had the highest epicoprostanol concentration (4.6 μg/g)
319 in our studied ponds. Though the seabird impact index in CV13 was also the highest

(0.71, compared to an average of 0.47), its epicoprostanol concentration was outstanding and indicated a possible different sediment source, which coincided with field observation of the Thule settlement at this pond. Apart from CV13, other ponds showing high epicoprostanol, including CV8, CV9, CV10, CV20 and CV30, were also in close proximity to the fulmar colony. This result may suggest that human impacts were not confined to the CV13 site, or that epicoprostanol may be derived from other animal sources, e.g. biotransport of epicoprostanol by northern fulmars may have occurred from fulmars feeding on whale carcasses [11]. Nonetheless, the presence of epicoprostanol is likely evidence of human activities in this Arctic environment.

POPs tend to accumulate in polar areas via transport by air and oceans [76, 77]. Fulmars are known to be important biovectors of POPs to their nesting areas because they accumulate high amounts of POPs due to their high position in the food web [9, 12, 60, 72]. We compared our seabird impact index i.e. cholesterol:(cholesterol + sitosterol) to sediment POP data from CV1 to CV11 (Figure 4) to test the performance of our seabird impact index against the presence of contaminants shown previously to be enriched by the fulmar colony. There were significant correlations between our seabird impact index in pond sediments and dieldrin (r_{11}=0.83, P<0.001), total polychlorinated biphenyls (PCBs) (r_{11}=0.69, P<0.02), hexachlorobenzene (HCB) (r_{11}=0.65, P<0.05), total dichlorodiphenyltrichloroethane (DDT) (r_{11}=0.63, P<0.05) and methoxychlor (r_{11}=0.60, P<0.05), indicating an important role of ornithogenic transport in their sources at Cape Vera. These results further confirm the ornithogenic enrichment of POPs in ponds with a high seabird enrichment index. As one of the most stable metabolized products of the

most produced congener p,p-DDT [78], the concentrations of p,p'-DDE were also much higher than those of DDT in sediments at Cape Vera, which corroborates previous studies on northern fulmars [79, 80] and other Arctic seabirds [81]. p,p'-DDE concentrations were also strongly correlated with our seabird impact index (Pearson correlation, r_{11}=0.75, P<0.01), but the sum of p,p'-DDT and o,p-DDT was not significantly correlated with the index (Pearson correlation, r_{11}=0.22, P>0.5). p,p'-DDE was the most persistent and stable metabolite of DDTs in the environment, and comprised more than 85% of total DDTs in seabird tissues and eggs [82, 83], probably due to both accumulation from diet and *in vivo* metabolism of DDT [81]. Unlike the seabird impact index, no significant correlation (all P>0.20, except dieldrin P>0.10 n=5) was found between POP concentrations and epicoprostanol. This result was not unexpected, as the Thule people pre-dated the development of the POPs analysed in this project [14, 15].

Despite the advantages of sterols and stanols as environmental indicators for seabird and human influence on ecosystems, there are limitations to consider with their application. For example, cholesterol is ubiquitous in biological material [33], and sitosterol is common in many phytogenic sources [48, 84], which makes their presence less diagnostic in tracing material sources. Furthermore, sterol and stanol sources in temperate regions could be much more complex than in Arctic regions, raising challenges in interpreting the results there. In the meantime, these compounds may degrade within a few years in aquatic sediment under warmer climate [85]. Hence, the prime application of sterol and stanol source identification may be in simple ecosystems in cold environments, such as polar or

montane regions. Nonetheless, these indicators could be applied in other environments with controls and cautions.

Here we show that steroid compounds and their ratios can inform studies on seabird influences in coastal areas, and may also be used to help differentiate other biovector sources, as indicated by our preliminary data from the Thule archaeological site. We propose that similar sterol and stanol indicator approaches should be developed to track the effects of other species not limited to birds, providing more holistic interpretations of the influences of past biota on ecosystem changes.

Acknowledgements

We gratefully thank Emily Choy and Lisa Loseto at Fisheries and Oceans Canada for providing bowhead whale blubber samples. This project was funded by an NSERC Discovery Grant to JMB, the NSERC Brockhouse Canada Prize awarded to JMB and JPS, an NSERC Strategic Projects Grant awarded to JMB and JPS, and logistical support to JMB, JPS, and MLM from the Polar Continental Shelf Program. Work at Cape Vera was licensed under permits NUN-SCI-06-01, 006PNR021, land use 591/7-2-2, WL000851, and Scientific Licence 0204405N-M. Whale blubber samples were obtained under the Marine Mammal Transportation Licence No. B0698 from Fisheries and Oceans Canada.

Figure Headings:

Figure 1. Map of the study ponds. Left: Map showing the study ponds across the entire study site. Most ponds are close to the colony and remote reference ponds are indicated with numbers. Right: Detailed map of the ponds at the major fulmar colony. Ponds are indicated with numbers and relative positions of these ponds are shown. Dots are plotted at the center of each represented pond. Right insert: Overview maps showing the location of the study site by the square with respect to the Canadian Arctic.

Figure 2. Cholesterol and sitosterol in moss samples and fulmar samples, with cholesterol shown in dense slash shade and sitosterol shown in coarse slash shade. A) Absolute concentration of cholesterol and sitosterol. The Y axis is broken to show the high concentrations of cholesterol in fulmar samples. B) Relative proportions of cholesterol and sitosterol. The Y axis is broken to show the low cholesterol proportions of sitosterol in the fulmar samples. CP = crop proventriculus content, SO = stomach oil, and SI = small intestine content.

Figure 3. Seabird impact index, calculated as cholesterol / (cholesterol + sitosterol), with higher index values indicating higher seabird impact. A) Seabird index value sorting of the study ponds from low (left) to high (right). Ponds close to the bird colony with high ornithogenic input were generally in the right, while reference ponds with limited ornithogenic input were generally in the left; B) Correlation between the seabird index and $\delta^{15}N$ ‰. Solid line shows the least square regression line.

406 Figure 4. Correlations between the seabird impact index and persistent organic pollutants.
407 Solid lines show the least square regression line. Note the scales of Y axis vary among
408 different compounds. A) Hexachlorobenzene (HCB); B) Total polychlorinated biphenyls
409 (total PCBs); C) Dieldrin; D) Methoxychlor; E) Total dichlorodiphenyltrichloroethane
410 (total DDT, including p,p'-DDT, p,p'-DDE, p,p'-DDD and o,p'-DDT); F)
411 dichlorodiphenyldichloroethylene (p,p'-DDE).

412 Table 1. Method parameters of mass selective detector. LOQ (limit of quantification) was
413 defined as a signal to noise ratio of 3. 5α-cholstane was used as internal standard,
414 therefore no LOQ was determined for it. Refer to text for more details in method.
415

Compound name	Retention time (min)	Ion fragments (m/z)	LOQ (ng/g dry weight)
epicoprostanol	30.662	370, 355	5
coprostanol	29.957	370, 355	10
cholesterol	32.538	329, 368	20
sitosterol	38.934	357, 396	250
5α-cholestane	25.514	357, 372	N/A

416
417
418

419 Table 2. Sterol and stanol concentrations in dry weight and $\delta^{15}N$ of the study ponds. N.D.
420 = not detected and N/A = data not available. The 'Seabird Index' is defined as
421 cholesterol:(cholesterol + sitosterol).

Pond	Cholesterol (μg/g)	Sitosterol (μg/g)	Coprostanol (μg/g)	Epicoprostanol (μg/g)	$\delta^{15}N$ (‰)	'Seabird Index'	Coprostanol/ Cholesterol
CV1	10.7	15.3	N.D.	N.D	6.6	0.41	N/A
CV2	6.3	11.6	2.8	0.5	4.4	0.35	0.44
CV3	12.4	15.8	1	N.D	18.4	0.44	0.08
CV4	4.1	4.7	0.4	N.D	15.0	0.47	0.10
CV5	3.6	5.2	2.3	N.D	9.0	0.41	0.64
CV6	21.5	30.2	4.5	0.1	8.0	0.42	0.21
CV7	6.9	5.4	0.2	N.D	11.9	0.56	0.03
CV8	34.8	17.4	2.8	1.0	16.3	0.67	0.08
CV9	55.8	28.1	7	2.7	13.7	0.67	0.13
CV10	71.0	30.3	7.7	3.1	13.3	0.70	0.11
CV11	12.2	13.2	1	N.D	0.7	0.48	0.08
CV12	15.1	26.6	0.7	N.D	3.0	0.36	0.05
CV13	84.1	35.1	5.1	4.6	19.4	0.71	0.06
CV14	42.9	44.1	3.2	0.4	8.2	0.49	0.07
CV15	14.4	19.0	1.9	N.D	16.3	0.43	0.13
CV16	26.2	26.3	3	N.D	6.0	0.50	0.11
CV18	10.0	17.3	1.4	N.D	6.5	0.37	0.14
CV20	34.1	50.2	9.9	4.0	7.4	0.40	0.29
CV22	12.5	13.8	1.4	N.D	2.8	0.48	0.11
CV23	12.8	51.1	2.2	N.D	1.6	0.20	0.17
CV24	8.6	23.4	1.1	N.D	1.6	0.27	0.13
CV30	57.9	28.0	4.6	2.1	16.9	0.67	0.08
CV31	5.2	7.9	N.D	N.D	12.0	0.40	N/A

422

423 References
424
425 1. Sierszen, M. E.; McDonald, M. E.; Jensen, D. A., Benthos as the basis for arctic lake food
426 webs. *Aquatic Ecology* **2003,** *37*, (4), 437-445.
427 2. Smol, J. P.; Douglas, M. S., From controversy to consensus: making the case for recent
428 climate change in the Arctic using lake sediments. *Frontiers in Ecology and the Environment*
429 **2007,** *5*, (9), 466-474.
430 3. Brimble, S. K.; Blais, J. M.; Kimpe, L. E.; Mallory, M. L.; Keatley, B. E.; Douglas, M. S.; Smol,
431 J. P., Bioenrichment of trace elements in a series of ponds near a northern fulmar (*Fulmarus*
432 *glacialis*) colony at Cape Vera, Devon Island. *Canadian Journal of Fisheries and Aquatic Sciences*
433 **2009,** *66*, (6), 949-958.
434 4. Brimble, S. K.; Foster, K. L.; Mallory, M. L.; Macdonald, R. W.; Smol, J. P.; Blais, J. M., High
435 Arctic ponds receiving biotransported nutrients from a nearby seabird colony are also subject to
436 potentially toxic loadings of arsenic, cadmium, and zinc. *Environmental Toxicology and*
437 *Chemistry* **2009,** *28*, (11), 2426-2433.
438 5. Huang, J.; Sun, L.; Wang, X.; Wang, Y.; Huang, T., Ecosystem evolution of seal colony and
439 the influencing factors in the 20th century on Fildes Peninsula, West Antarctica. *Journal of*
440 *Environmental Sciences* **2011,** *23*, (9), 1431-1436.
441 6. Borgå, K.; Fisk, A. T.; Hoekstra, P. F.; Muir, D. C., Biological and chemical factors of
442 importance in the bioaccumulation and trophic transfer of persistent organochlorine
443 contaminants in arctic marine food webs. *Environmental Toxicology and Chemistry* **2004,** *23*,
444 (10), 2367-2385.
445 7. Atwell, L.; Hobson, K. A.; Welch, H. E., Biomagnification and bioaccumulation of mercury
446 in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of*
447 *Fisheries and Aquatic Sciences* **1998,** *55*, (5), 1114-1121.
448 8. Braune, B.; Chételat, J.; Amyot, M.; Brown, T.; Clayden, M.; Evans, M.; Fisk, A.; Gaden, A.;
449 Girard, C.; Hare, A., Mercury in the marine environment of the Canadian Arctic: Review of recent
450 findings. *Science of The Total Environment* **2015,** *509*, 67-90.
451 9. Blais, J. M.; Kimpe, L. E.; McMahon, D.; Keatley, B. E.; Mallory, M. L.; Douglas, M. S.;
452 Smol, J. P., Arctic seabirds transport marine-derived contaminants. *Science* **2005,** *309*, (5733),
453 445-445.
454 10. Krümmel, E.; Macdonald, R.; Kimpe, L.; Gregory-Eaves, I.; Demers, M.; Smol, J.; Finney,
455 B.; Blais, J., Aquatic ecology: delivery of pollutants by spawning salmon. *Nature* **2003,** *425*,
456 (6955), 255-256.
457 11. Mallory, M. L., The northern fulmar (*Fulmarus glacialis*) in Arctic Canada: ecology,
458 threats, and what it tells us about marine environmental conditions. *Environmental Reviews*
459 **2006,** *14*, (3), 187-216.
460 12. Michelutti, N.; Liu, H.; Smol, J. P.; Kimpe, L. E.; Keatley, B. E.; Mallory, M.; Macdonald, R.
461 W.; Douglas, M. S.; Blais, J. M., Accelerated delivery of polychlorinated biphenyls (PCBs) in
462 recent sediments near a large seabird colony in Arctic Canada. *Environmental pollution* **2009,**
463 *157*, (10), 2769-2775.
464 13. Douglas, M. S.; Smol, J. P.; Savelle, J. M.; Blais, J. M., Prehistoric Inuit whalers affected
465 Arctic freshwater ecosystems. *Proceedings of the National Academy of Sciences of the United*
466 *States of America* **2004,** *101*, (6), 1613-1617.
467 14. Hadley, K. R.; Douglas, M. S.; Blais, J. M.; Smol, J. P., Nutrient enrichment in the High
468 Arctic associated with Thule Inuit whalers: a paleolimnological investigation from Ellesmere
469 Island (Nunavut, Canada). *Hydrobiologia* **2010,** *649*, (1), 129-138.

15. Hadley, K. R.; Douglas, M. S.; McGhee, R.; Blais, J. M.; Smol, J. P., Ecological influences of Thule Inuit whalers on high Arctic pond ecosystems: a comparative paleolimnological study from Bathurst Island (Nunavut, Canada). *Journal of Paleolimnology* **2010,** *44*, (1), 85-93.
16. Michelutti, N.; McCleary, K. M.; Antoniades, D.; Sutherland, P.; Blais, J. M.; Douglas, M. S.; Smol, J. P., Using paleolimnology to track the impacts of early Arctic peoples on freshwater ecosystems from southern Baffin Island, Nunavut. *Quaternary Science Reviews* **2013,** *76*, 82-95.
17. Sun, L.; Emslie, S.; Huang, T.; Blais, J.; Xie, Z.; Liu, X.; Yin, X.; Wang, Y.; Huang, W.; Hodgson, D., Vertebrate records in polar sediments: biological responses to past climate change and human activities. *Earth-Science Reviews* **2013,** *126*, 147-155.
18. Keatley, B. E.; Douglas, M. S.; Blais, J. M.; Mallory, M. L.; Smol, J. P., Impacts of seabird-derived nutrients on water quality and diatom assemblages from Cape Vera, Devon Island, Canadian High Arctic. *Hydrobiologia* **2009,** *621*, (1), 191-205.
19. Huang, T.; Sun, L. G.; Wang, Y. H.; Chu, Z. D.; Qin, X. Y.; Yang, L. J., Transport of nutrients and contaminants from ocean to island by emperor penguins from Amanda Bay, East Antarctic. *Science of the Total Environment* **2014,** *468*, 578-583.
20. Kallenborn, R.; Blais, J. M., Tracking contaminant transport from biovectors. In *Environmental Contaminants*, Springer Netherlands: 2015; pp 461-498.
21. Kling, G. W.; Fry, B.; O'Brien, W. J., Stable isotopes and planktonic trophic structure in arctic lakes. *Ecology* **1992,** *73*, (2), 561-566.
22. Dahl, T.; Falk-Petersen, S.; Gabrielsen, G.; Sargent, J.; Hop, H.; Millar, R., Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: a trophic study from an Arctic fjord. *Marine Ecology Progress Series* **2003,** *256*, 257-269.
23. Emslie, S. D.; Patterson, W. P., Abrupt recent shift in δ13C and δ15N values in Adélie penguin eggshell in Antarctica. *Proceedings of the National Academy of Sciences* **2007,** *104*, (28), 11666-11669.
24. Liu, X.; Li, H.; Sun, L.; Yin, X.; Zhao, S.; Wang, Y., δ 13 C and δ 15 N in the ornithogenic sediments from the Antarctic maritime as palaeoecological proxies during the past 2000 yr. *Earth and Planetary Science Letters* **2006,** *243*, (3), 424-438.
25. Mariotti, A.; Germon, J.; Hubert, P.; Kaiser, P.; Letolle, R.; Tardieux, A.; Tardieux, P., Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant and Soil* **1981,** *62*, (3), 413-430.
26. Wenk, C. B.; Zopfi, J.; Blees, J.; Veronesi, M.; Niemann, H.; Lehmann, M. F., Community N and O isotope fractionation by sulfide-dependent denitrification and anammox in a stratified lacustrine water column. *Geochimica et Cosmochimica Acta* **2014,** *125*, 551-563.
27. Thibodeau, B.; Hélie, J.-F.; Lehmann, M. F., Variations of the nitrate isotopic composition in the St. Lawrence River caused by seasonal changes in atmospheric nitrogen inputs. *Biogeochemistry* **2013,** *115*, (1-3), 287-298.
28. Selbie, D. T.; Lewis, B. A.; Smol, J. P.; Finney, B. P., Long-term population dynamics of the endangered Snake River sockeye salmon: evidence of past influences on stock decline and impediments to recovery. *Transactions of the American Fisheries Society* **2007,** *136*, (3), 800-821.
29. Smol, J. P.; Stoermer, E. F., *The diatoms: applications for the environmental and earth sciences*. Cambridge University Press: 2010.
30. Douglas, M. S.; Smol, J. P., Freshwater diatoms as indicators of environmental change in the High Arctic. *The diatoms: applications for the environmental and earth sciences* **2010**, 249.
31. Wang, J.; Wang, Y.; Wang, X.; Sun, L., Penguins and vegetations on Ardley Island, Antarctica: evolution in the past 2,400 years. *Polar Biology* **2007,** *30*, (11), 1475-1481.

32. Huang, W.-Y.; Meinschein, W., Sterols as ecological indicators. *Geochimica et Cosmochimica Acta* **1979,** *43*, (5), 739-745.
33. Bretsche, M. S., Membrane structure - some general principles. *Science* **1973,** *181*, (4100), 622-629.
34. Reeves, A.; Patton, D., Measuring change in sterol input to estuarine sediments. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere* **2001,** *26*, (9), 753-757.
35. Huang, W.-Y.; Meinschein, W., Sterols as source indicators of organic materials in sediments. *Geochimica et Cosmochimica Acta* **1976,** *40*, (3), 323-330.
36. Carreira, R. S.; Wagener, A. L. R.; Readman, J. W., Sterols as markers of sewage contamination in a tropical urban estuary (Guanabara Bay, Brazil): space–time variations. *Estuarine, Coastal and Shelf Science* **2004,** *60*, (4), 587-598.
37. Campos, V.; Fracácio, R.; Fraceto, L. F.; Rosa, A. H., Fecal sterols in estuarine sediments as markers of sewage contamination in the Cubatão area, São Paulo, Brazil. *Aquatic Geochemistry* **2012,** *18*, (5), 433-443.
38. Ogura, K.; Machihara, T.; Takada, H., Diagenesis of biomarkers in Biwa Lake sediments over 1 million years. *Organic Geochemistry* **1990,** *16*, (4), 805-813.
39. Nishimura, M., Geochemical characteristics of the high reduction zone of stenols in Suwa sediments and the environmental factors controlling the conversion of stenols into stanols. *Geochimica et Cosmochimica Acta* **1978,** *42*, (4), 349-357.
40. Fu, P.; Kawamura, K.; Barrie, L. A., Photochemical and other sources of organic compounds in the Canadian high Arctic aerosol pollution during winter− spring. *Environmental science & technology* **2008,** *43*, (2), 286-292.
41. Volkman, J. K., A review of sterol markers for marine and terrigenous organic matter. *Organic Geochemistry* **1986,** *9*, (2), 83-99.
42. Leeming, R.; Ball, A.; Ashbolt, N.; Nichols, P., Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. *Water Research* **1996,** *30*, (12), 2893-2900.
43. Wakeham, S. G.; Canuel, E. A., Organic geochemistry of particulate matter in the eastern tropical North Pacific Ocean: Implications for particle dynamics. *Journal of Marine Research* **1988,** *46*, (1), 183-213.
44. Rielley, G.; Collier, R.; Jones, D.; Eglinton, G., The biogeochemistry of Ellesmere Lake, UK—I: source correlation of leaf wax inputs to the sedimentary lipid record. *Organic Geochemistry* **1991,** *17*, (6), 901-912.
45. Tolosa, I.; LeBlond, N.; Copin-Montégut, C.; Marty, J.-C.; de Mora, S.; Prieur, L., Distribution of sterol and fatty alcohol biomarkers in particulate matter from the frontal structure of the Alboran Sea (SW Mediterranean Sea). *Marine Chemistry* **2003,** *82*, (3), 161-183.
46. Derrien, M.; Cabrera, F. A.; Tavera, N. L. V.; Manzano, C. A. K.; Vizcaino, S. C., Sources and distribution of organic matter along the Ring of Cenotes, Yucatan, Mexico: Sterol markers and statistical approaches. *Science of the Total Environment* **2015,** *511*, 223-229.
47. Ryan, E.; Galvin, K.; O'Connor, T.; Maguire, A.; O'Brien, N., Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods for Human Nutrition* **2007,** *62*, (3), 85-91.
48. Peng, L.; Kawagoe, Y.; Hogan, P.; Delmer, D., Sitosterol-β-glucoside as primer for cellulose synthesis in plants. *Science* **2002,** *295*, (5552), 147-150.
49. Pautler, B. G.; Austin, J.; Otto, A.; Stewart, K.; Lamoureux, S. F.; Simpson, M. J., Biomarker assessment of organic matter sources and degradation in Canadian High Arctic littoral sediments. *Biogeochemistry* **2010,** *100*, (1-3), 75-87.

564 50. Andersson, R. A.; Meyers, P. A., Effect of climate change on delivery and degradation of
565 lipid biomarkers in a Holocene peat sequence in the Eastern European Russian Arctic. *Organic*
566 *Geochemistry* **2012,** *53*, 63-72.
567 51. Kamenarska, Z. G.; Dimitrova-Konaklieva, S. D.; Stefanov, L. K.; Popov, S. S., A
568 comparative study on the sterol composition of some brown algae from the Black Sea. *Journal of*
569 *the Serbian Chemical Society* **2003,** *68*, (4-5), 269-275.
570 52. Ribeiro, N.; Désaubry, L.; Gotoh, M.; Nakatani, Y., *Biomimetic Synthesis and Properties of*
571 *Polyprenoid*. INTECH Open Access Publisher: 2011.
572 53. Venkatesan, M. I.; Kaplan, I. R., Sedimentary coprostanol as an index of sewage addition
573 in Santa Monica Basin, Southern California. *Environmental science & technology* **1990,** *24*, (2),
574 208-214.
575 54. Vane, C. H.; Kim, A. W.; McGowan, S.; Leng, M. J.; Heaton, T. H.; Kendrick, C. P.; Coombs,
576 P.; Yang, H.; Swann, G. E., Sedimentary records of sewage pollution using faecal markers in
577 contrasting peri-urban shallow lakes. *Science of the Total Environment* **2010,** *409*, (2), 345-56.
578 55. Bethell, P. H.; Goad, L. J.; Evershed, R. P.; Ottaway, J., The study of molecular markers of
579 human activity: the use of coprostanol in the soil as an indicator of human faecal material.
580 *Journal of Archaeological Science* **1994,** *21*, (5), 619-632.
581 56. Venkatesan, M.; Ruth, E.; Kaplan, I., Coprostanols in Antarctic marine sediments: a
582 biomarker for marine mammals and not human pollution. *Marine Pollution Bulletin* **1986,** *17*,
583 (12), 554-557.
584 57. Gaston, A. J.; Mallory, M. L.; Gilchrist, H. G.; O'Donovan, K., Status, trends and
585 attendance patterns of the northern fulmar *Fulmarus glacialis* in Nunavut, Canada. *Arctic* **2006,**
586 *59*, (2), 165-178.
587 58. Mallory, M.; Karnovsky, N.; Gaston, A.; Hobson, K.; Provencher, J.; Forbes, M.; Hunt, G. J.;
588 Byers, T.; Dick, T., Temporal and spatial patterns in the diet of northern fulmars (*Fulmarus*
589 *glacialis*) in the Canadian High Arctic. *Aquatic Biology* **2010,** *10*, (2), 181-191.
590 59. Mallory, M. L.; Akearok, J. A.; Edwards, D. B.; O'Donovan, K.; Gilbert, C. D., Autumn
591 migration and wintering of northern fulmars (*Fulmarus glacialis*) from the Canadian high Arctic.
592 *Polar Biology* **2008,** *31*, (6), 745-750.
593 60. Blais, J. M.; Macdonald, R. W.; Mackay, D.; Webster, E.; Harvey, C.; Smol, J. P.,
594 Biologically mediated transport of contaminants to aquatic systems. *Environmental science &*
595 *technology* **2007,** *41*, (4), 1075-1084.
596 61. Keatley, B. E.; Blais, J. M.; Douglas, M. S.; Gregory-Eaves, I.; Mallory, M. L.; Michelutti, N.;
597 Smol, J. P., Historical seabird population dynamics and their effects on Arctic pond ecosystems: a
598 multi-proxy paleolimnological study from Cape Vera, Devon Island, Arctic Canada. *Fundamental*
599 *and Applied Limnology* **2011,** *179*, (1), 51-66.
600 62. Hilton, G. M.; Furness, R. W.; Houston, D. C., A comparative study of digestion in North
601 Atlantic seabirds. *Journal of Avian Biology* **2000,** *31*, (1), 36-46.
602 63. Birk, J. J.; Dippold, M.; Wiesenberg, G. L.; Glaser, B., Combined quantification of faecal
603 sterols, stanols, stanones and bile acids in soils and terrestrial sediments by gas
604 chromatography–mass spectrometry. *Journal of Chromatography A* **2012,** *1242*, 1-10.
605 64. Warham, J. In *The incidence, functions and ecological significance of petrel stomach oils*
606 Proceedings (New Zealand Ecological Society), 1977; JSTOR: 1977; pp 84-93.
607 65. Black, A. L.; Gilchrist, H. G.; Allard, K. A.; Mallory, M. L., Incidental observations of birds
608 in the vicinity of Hell Gate Polynya, Nunavut: species, timing, and diversity. *Arctic* **2012,** *65*, (2),
609 145-154.

66. Chen, Z.; Saito, Y.; Kanai, Y.; Wei, T.; Li, L.; Yao, H.; Wang, Z., Low concentration of heavy metals in the Yangtze estuarine sediments, China: a diluting setting. *Estuarine, Coastal and Shelf Science* **2004,** *60*, (1), 91-100.
67. Michelutti, N.; Blais, J.; Liu, H.; Keatley, B.; Douglas, M.; Mallory, M.; Smol, J., A test of the possible influence of seabird activity on the 210Pb flux in high Arctic ponds at Cape Vera, Devon Island, Nunavut: implications for radiochronology. *Journal of Paleolimnology* **2008,** *40*, (3), 783-791.
68. Siere, M.-A.; Tian, R.; Broyelle, I.; Saliot, A., Aquatic distribution of 4-desmethyl sterols in the Chang Jiang Estuary, China. *Marine Chemistry* **1993,** *42*, (1), 11-24.
69. Warham, J.; Watts, R.; Dainty, R., The composition, energy content and function of the stomach oils of petrels (order, Procellariiformes). *Journal of Experimental Marine Biology and Ecology* **1976,** *23*, (1), 1-13.
70. Clarke, A., Contamination of peregrine falcons (*Falco peregrinus*) with fulmar stomach oil. *Journal of Zoology* **1977,** *181*, (1), 11-20.
71. Foster, K. L.; Wang, S. W.; Mackay, D.; Mallory, M. L.; Blais, J. M., Preliminary assessment of avian stomach oils: a vector of contaminants to chicks and potential for diet analysis and biomonitoring. *Environmental science & technology* **2010,** *44*, (17), 6869-6874.
72. Foster, K. L.; Kimpe, L. E.; Brimble, S. K.; Liu, H.; Mallory, M. L.; Smol, J. P.; Macdonald, R. W.; Blais, J. M., Effects of seabird vectors on the fate, partitioning, and signatures of contaminants in a high Arctic ecosystem. *Environmental science & technology* **2011,** *45*, (23), 10053-10060.
73. Chapin, D. M., Nitrogen mineralization, nitrification, and denitrification in a high arctic lowland ecosystem, Devon Island, NWT, Canada. *Arctic and Alpine Research* **1996,** *28*, (1), 85-92.
74. Mudge, S. M.; Bebianno, M. J., Sewage contamination following an accidental spillage in the Ria Formosa, Portugal. *Marine Pollution Bulletin* **1997,** *34*, (3), 163-170.
75. Furtula, V.; Osachoff, H.; Derksen, G.; Juahir, H.; Colodey, A.; Chambers, P., Inorganic nitrogen, sterols and bacterial source tracking as tools to characterize water quality and possible contamination sources in surface water. *Water Research* **2012,** *46*, (4), 1079-1092.
76. Wania, F.; Mackay, D., Tracking the distribution of persistent organic pollutants. *Environmental science & technology* **1996,** *30*, (9), 390A-396A.
77. Wania, F.; Mackay, D., A global distribution model for persistent organic chemicals. *Science of the Total Environment* **1995,** *160*, 211-232.
78. Heath, R. G.; Spann, J. W.; Kreitzer, J., Marked DDE impairment of mallard reproduction in controlled studies. *Nature* **1969,** *224*, 47-48.
79. Knudsen, L. B.; Borga, K.; Jorgensen, E. H.; van Bavel, B.; Schlabach, M.; Verreault, J.; Gabrielsen, G. W., Halogenated organic contaminants and mercury in northern fulmars (*Fulmarus glacialis*): levels, relationships to dietary descriptors and blood to liver comparison. *Environmental pollution* **2007,** *146*, (1), 25-33.
80. Buckman, A. H.; Norstrom, R. J.; Hobson, K. A.; Karnovsky, N. J.; Duffe, J.; Fisk, A. T., Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. *Environmental pollution* **2004,** *128*, (3), 327-38.
81. Borga, K.; Hop, H.; Skaare, J. U.; Wolkers, H.; Gabrielsen, G. W., Selective bioaccumulation of chlorinated pesticides and metabolites in Arctic seabirds. *Environmental pollution* **2007,** *145*, (2), 545-53.
82. Gabrielsen, G. W.; Skaare, J. U.; Polder, A.; Bakken, V., Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. *Science of the Total Environment* **1995,** *160*, 337-346.

83. Braune, B.; Donaldson, G.; Hobson, K., Contaminant residues in seabird eggs from the Canadian Arctic. Part I. Temporal trends 1975–1998. *Environmental pollution* **2001,** *114*, (1), 39-54.
84. Puglisi, E.; Nicelli, M.; Capri, E.; Trevisan, M.; Del Re, A. A., Cholesterol, β-sitosterol, ergosterol, and coprostanol in agricultural soils. *Journal of Environmental Quality* **2003,** *32*, (2), 466-471.
85. Santos, E. S.; Carreira, R. d. S.; Knoppers, B. A., Sedimentary sterols as indicators of environmental conditions in southeastern Guanabara Bay, Brazil. *Brazilian Journal of Oceanography* **2008,** *56*, (2), 97-113.

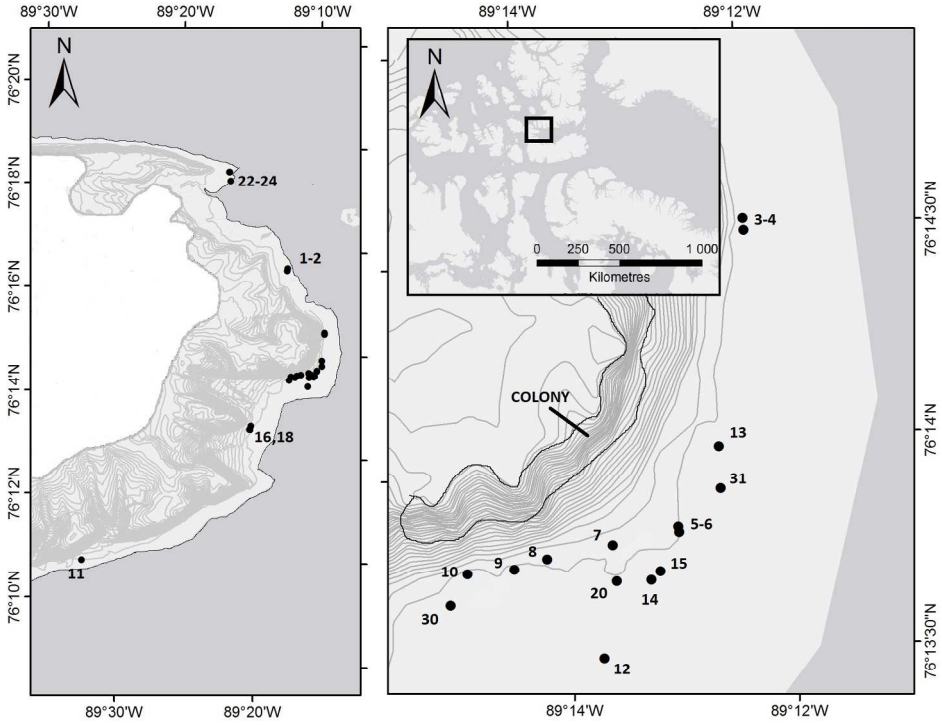


Figure 1. Map of the study ponds. Left: Map showing the study ponds across the entire study site. Most ponds are close to the colony and remote reference ponds are indicated with numbers. Right: Detailed map of the ponds at the major fulmar colony. Ponds are indicated with numbers and relative positions of these ponds are shown. Dots are plotted at the center of each represented pond. Right insert: Overview maps showing the location of the study site by the square with respect to the Canadian Arctic.

279x215mm (200 x 200 DPI)

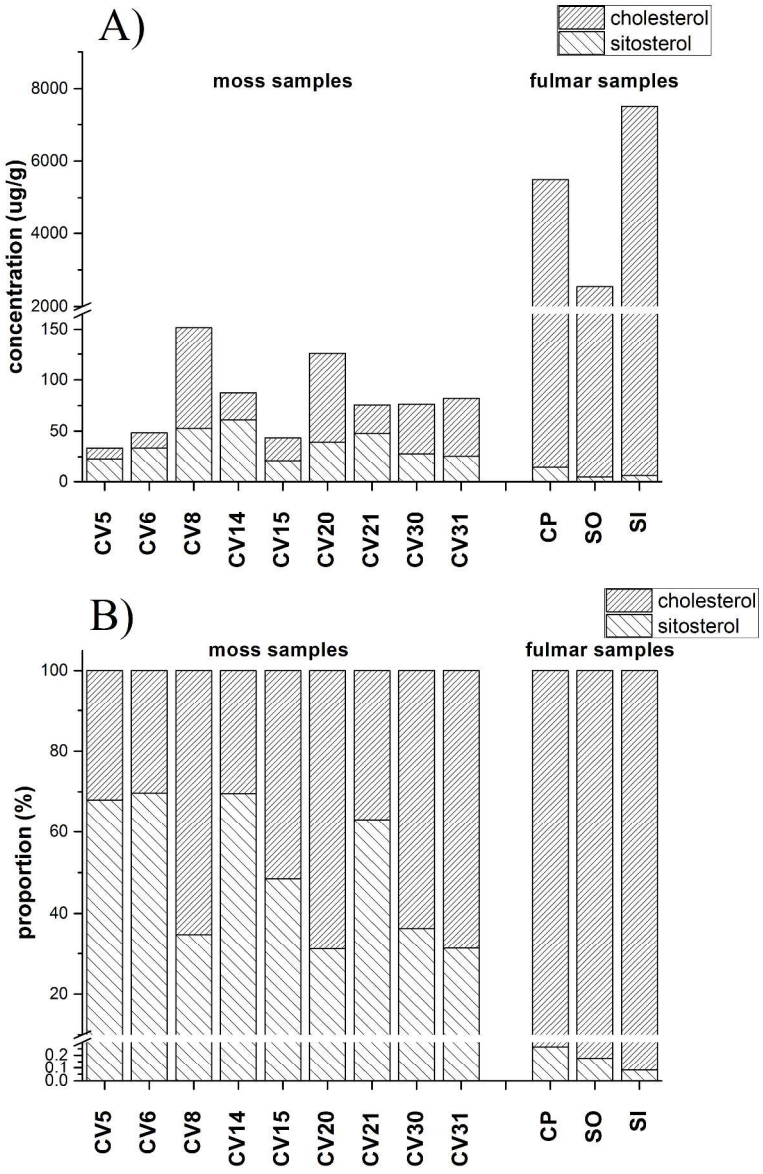


Figure 2. Cholesterol and sitosterol in moss samples and fulmar samples, with cholesterol shown in dense slash shade and sitosterol shown in coarse slash shade. A) Absolute concentration of cholesterol and sitosterol. The Y axis is broken to show the high concentrations of cholesterol in fulmar samples. B) Relative proportions of cholesterol and sitosterol. The Y axis is broken to show the low cholesterol proportions of sitosterol in the fulmar samples. CP = crop proventriculus content, SO = stomach oil, and SI = small intestine content.

927x1311mm (96 x 96 DPI)

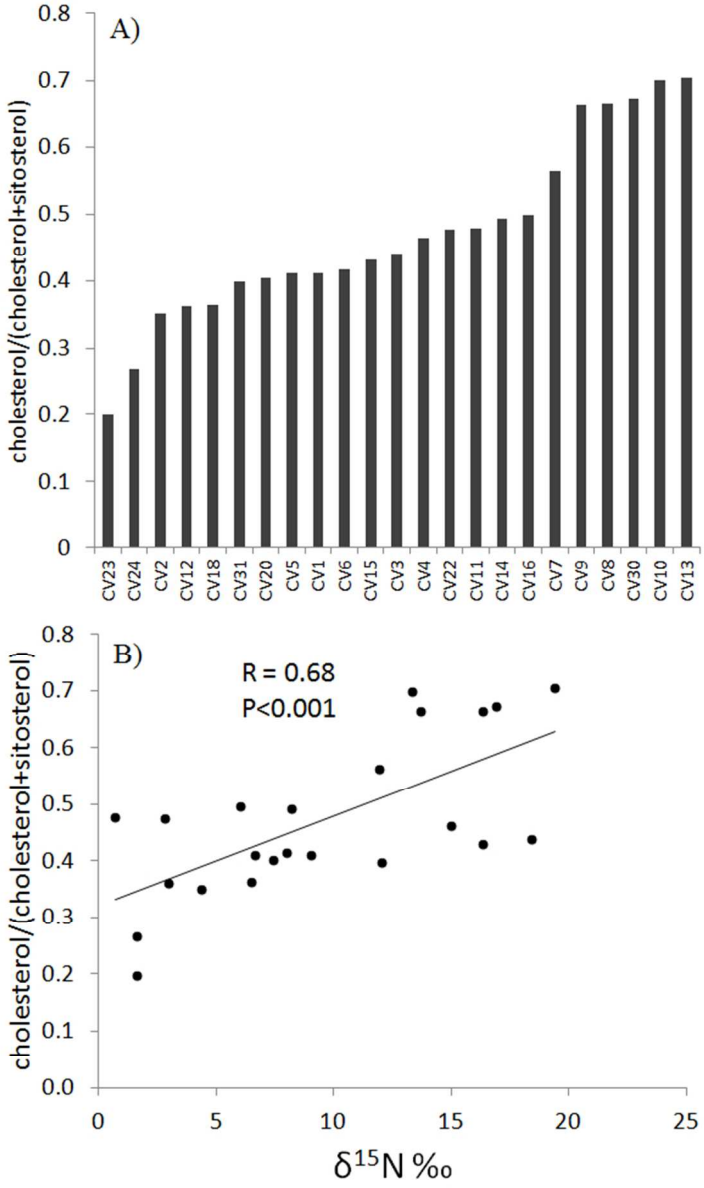


Figure 3. Seabird impact index, calculated as cholesterol / (cholesterol + sitosterol), with higher index values indicating higher seabird impact. A) Seabird index value sorting of the study ponds from low (left) to high (right). Ponds close to the bird colony with high ornithogenic input were generally in the right, while reference ponds with limited ornithogenic input were generally in the left; B) Correlation between the seabird index and δ15N ‰. Solid line shows the least square regression line.

160x260mm (96 x 96 DPI)

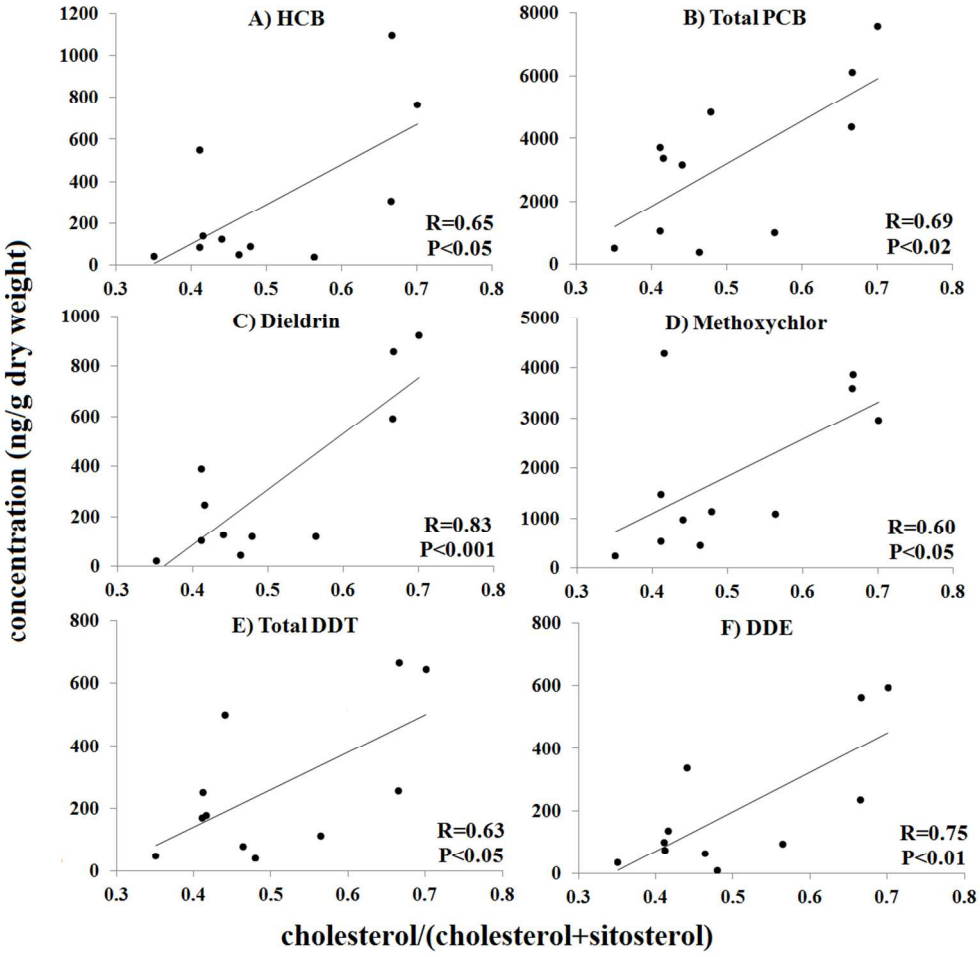


Figure 4. Correlations between the seabird impact index and persistent organic pollutants. Solid lines show the least square regression line. Note the scales of Y axis vary among different compounds. A) Hexachlorobenzene (HCB); B) Total polychlorinated biphenyls (total PCBs); C) Dieldrin; D) Methoxychlor; E) Total dichlorodiphenyltrichloroethane (total DDT, including p,p'-DDT, p,p'-DDE, p,p'-DDD and o,p'-DDT); F) dichlorodiphenyldichloroethylene (p,p'-DDE).

324x311mm (96 x 96 DPI)