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Leucocyte profiles of Arctic marine birds: correlates of migration and breeding phenology

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Most Arctic marine birds are migratory, wintering south of the limit of annual pack ice and returning north each year for the physiologically stressful breeding season. The Arctic environment is changing rapidly due to global warming and anthropogenic activities, which may influence the timing of breeding in relation to arrival times following migration, as well as providing additional stressors (e.g. disturbance from ships) to which birds may respond. During stressful parts of their annual cycle, such as breeding, birds may reallocate resources so that they have increased heterophil-to-lymphocyte ratios in their white blood cell (leucocyte) profiles. We analysed leucocyte profiles of nine species of marine birds to establish reference ranges for these species in advance of future Arctic change. Leucocyte profiles tended to cluster among taxonomic groups across studies, suggesting that reference values for a particular group can be established, and within species there was evidence that birds from colonies that had to migrate farther had higher heterophil-to-lymphocyte ratios during incubation than those that did not have to travel as far, particularly for species with high wing loading.

Key words: Heterophil, leucocyte, lymphocyte, marine bird, stress

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Introduction

Influences of environmental stressors on animals are key areas of research in ecology and conservation. A fundamental premise of this research is that a stimulus in the environment places a stress on animals, which causes a behavioural or physiological change from baseline conditions (e.g. Vleck *et al.*, 2000; Plischke *et al.*, 2010; Villanueva *et al.*, 2012). Detecting these reactions can be difficult, however, owing to a lack of baseline information for many species and because many baseline metrics, such as white blood cell (leucocyte) profiles, vary through the annual cycle and in response to environmental variation over a range of time scales (e.g. Lee, 2006; Plischke *et al.*, 2010; Dehnhard *et al.*, 2011). Consequently, sound use

of these metrics requires corresponding information on environmental conditions and stage of the annual cycle of the species, as well as baseline measures, for proper interpretation.

For many animals, breeding can be the most physiologically stressful part of their annual cycle, which can manifest itself as a trade-off between investment of body resources in self-maintenance vs. reproduction (Drent and Daan, 1980; Stearns, 1992). One place where animals might adjust investment is in their immune system. Although this system protects individuals against various diseases and parasites (Sheldon and Verhulst, 1996), the immune system may be adjusted during breeding to reallocate resources to other systems (Deerenberg *et al*., 1997; Råberg *et al*., 1998; Hanssen *et al*., 2003; Lee, 2006; Davis *et al*., 2008). Leucocytes are a key component of an animal's immune system, with different white blood cells offering protection against a variety of stressors (Maceda-Veiga *et al*., 2015). In homeotherms, acquired immunity is provided by lymphocytes, tends to be pathogen specific and leads to antibody responses. In contrast, innate immunity is provided by monocytes and granulocytes (eosinophils, basophils and, especially, heterophils in birds and neutrophils in mammals) and is usually the first line of immune defense through phagocytosis of pathogens (Roitt *et al*., 1993). During periods of stress, reallocation of immunity resources leads to increased heterophil-to-lymphocyte (H:L) ratios in avian white blood cell profiles and occurs in numerous domestic and wild birds (Shutler *et al*., 2004; reviewed by Davis, 2009). Thus, leucocyte profiles (also called white blood cell differentials) are commonly used by veterinarians to assess animal health (e.g. Charles-Smith *et al*., 2014; Wojczulanis-Jakubas *et al*., 2014). Evidence also suggests that elevated H:L ratios may be associated with deleterious effects, such as slower growth, increased risk of infection and decreased survival (Davis, 2009).

The Arctic is home to millions of migratory birds and is a relatively pristine region, which is now experiencing rapid environmental change due to a more variable climate. A warmer climate may lead to higher parasitism rates (Marcogliese, 2001; Kutz *et al*., 2004; Gaston and Elliott, 2013), and increased investment in immunity may result. Many parts of the Arctic are expected to experience increased industrial activity, shipping and tourism in the near future (ACIA, 2005; Arctic Council, 2009). Elsewhere in Arctic regions, scientists have conducted sampling to establish reference ranges for haematological parameters in marine birds, as baseline metrics by which the possible effects of environmental perturbation can be assessed (e.g. Newman *et al*., 1997; Bearhop *et al*., 1999). However, few studies have been undertaken in the Canadian Arctic (see Edwards *et al*., 2006) or Iceland; therefore, establishing current reference values is important. In this study, we had the following two objectives: (i) to generate reference ranges for leucocyte profiles of breeding Arctic marine birds in Canada and Iceland; and (ii) to assess whether differences occur within species among breeding sites. Typical environmental conditions mean that many Arctic birds have a short window of time in which to breed; indeed, the time spent gathered at a colony between arrival and initiation of breeding may be dramatically reduced compared to conspecifics breeding farther south (e.g. Mallory and Forbes, 2007). Given that physical stresses of migration can also elevate H:L ratios in birds (e.g. Owen and Moore, 2006), we predicted that for species sampled from multiple colonies that varied in (i) the distance travelled by individuals for pre-breeding migration and (ii) the distance flown by individuals from their foraging ground to their breeding colony, H:L ratios would be higher for birds that had to travel farther to reach their breeding colony.

Materials and methods

We collected data on marine bird leucocyte profiles in field seasons between 2004 and 2013 (Table 1). In Canada, northern fulmars (*Fulmarus glacialis*; hereafter fulmars) and black guillemots (*Cepphus grylle*; hereafter guillemots) were sampled at Cape Vera, Nunavut (76°15′ N, 89°15′ W; Fig. 1). Common eiders (*Somateria mollissima*; hereafter eiders) and Arctic terns (*Sterna paradisaea*; hereafter terns) were sampled at East Bay, Nunavut (64°01′ N, 81°47′ W). Terns, Sabine's gulls (*Xema sabini*) and eiders were sampled at Nasaruvaalik Island, Nunavut (75°49′ N, 96°18′ W), and eiders were also sampled at colonies around Nova Scotia (Eastern Shore Islands, 44°54′ N, 62°15′W; Bon Portage Island, 43°28′ N, 65°45′ W; and John's Island, 43°32′ N, 65°47′ W). Thick-billed murres (*Uria lomvia*; hereafter murres) were sampled at both Coats Island (62°57′ N, 82° W) and Digges Sound (62°33′ N, 77°43′ W), Nunavut. In Iceland, guillemots, fulmars, eiders and Atlantic puffins (*Fratercula arctica*; hereafter puffins) were sampled at Flatey (65°22′ N, 22°55′ W), while great skuas (*Stercorarius skua*; hereafter skuas) were sampled at Breiðamerkursandur (63°29′ N, 16°21′ W). In Alaska, black-legged kittiwakes (*Rissa tridactyla*) were sampled at Middleton Island (63°29′ N, 16°21′ W). All eiders sampled were nesting females, although we captured both males and females of other species (sex not determined). To minimize bias associated with seasonal variations in leucocyte profiles (e.g. Owen and Moore, 2006), all birds were captured during mid-incubation (approximately second or third week) at their nests by net or noose pole, except for some eiders in Nova Scotia caught at their nest by trained dogs. Birds nesting at each colony are fairly synchronous in the timing of breeding, particularly in the Arctic (e.g. Mallory and Forbes, 2007), and we sampled typically over a few days in mid-incubation to minimize the risk of abandonment due to disturbance at the nest and to reduce variation resulting from possible changing stress levels during incubation. Fulmars, murres and puffins typically lay one egg, guillemots, terns and skuas lay two eggs, gulls generally lay two to three eggs, and eiders generally lay more than three eggs; however, we did not control for clutch size in this study. Blood was extracted from the brachial, jugular or tarsal vein within 3 min of capture using a 22 gauge, 0.7 mm needle and a 3 ml syringe. One drop of blood was then put onto a microscope slide, and a smear was made following standard procedures (Bennett, 1970). Slides were left to air dry, then dipped in alcohol and again left to air dry before being stored.

Table 1: Leucocyte profiles of Arctic marine bird species

Common name	Scientific name	Region	Colony	Year	*n*	Mean (SEM) proportion of leucocytes per 100 counted					Mean (SEM), CV H:L ratio
						Heterophils	Eosinophils	Basophils	Lymphocytes	Monocytes	
Northern fulmar	*Fulmarus glacialis*	High	Cape Vera	2004	31	32.0 (2.5)	13.5 (2.0)	2.2 (0.3)	61.4 (2.6)	4.4 (0.4)	0.61 (0.07), 65
		High	Cape Vera	2006	16	35.0 (4.5)	4.0 (0.9)	2.0 (0.5)	55.1 (4.0)	3.9 (0.7)	0.95 (0.33), 138
		Iceland	Flatey	2007	21	25.3 (1.7)	1.1 (0.5)	10.7 (2.0)	57.1 (2.5)	5.7 (0.6)	0.47 (0.04), 40
Common eider	*Somateria mollissima*	Iceland	Flatey	2007	18	28.3 (2.7)	14.4 (2.6)	0.7 (0.2)	50.2 (3.0)	6.4 (0.7)	0.63 (0.08), 57
		Low	East Bay	2004	46	56.8 (1.9)	2.2 (0.4)	2.2 (0.5)	36.5 (1.8)	2.2 (0.2)	1.81 (0.15), 56
		Low	East Bay	2008	19	47.2 (1.6)	8.8 (1.2)	0.3 (0.1)	32.9 (2.2)	10.8 (1.2)	1.56 (0.11), 32
		High	Nasaruvaalik	2008	19	55.0 (2.3)	2.5 (0.4)	0 (0)	35.8 (1.9)	6.6 (0.7)	1.70 (0.19), 49
		Maritimes	Various	2013	89	44.1 (1.6)	3.6 (0.4)	1.1 (0.2)	44.3 (1.4)	5.0 (0.4)	1.18 (0.08), 64
Great skua	*Stercorarius skua*	Iceland	Breiðamerkursandur	2008	15	36.7 (3.0)	4.1 (0.6)	0.4 (0.2)	52.5 (3.3)	6.2 (0.8)	0.85 (0.18), 81
Black-legged kittiwake	*Rissa tridactyla*	Alaska	Middleton	2012	31	51.4 (2.9)	2.7 (0.5)	0.2 (0.1)	42.0 (3.0)	3.8 (0.6)	1.91 (0.41), 120
Sabine's gull	*Xema sabini*	Low	East Bay	2007	6	39.7 (6.5)	1.7 (0.8)	5.0 (1.7)	44.5 (3.7)	9.1 (2.8)	0.99 (0.24), 59
		High	Nasaruvaalik	2008	12	45.9 (4.0)	3.4 (1.1)	0.3 (0.2)	41.7 (3.5)	8.6 (1.1)	1.28 (0.20), 56
Arctic tern	*Sterna paradisaea*	Low	East Bay	2007	19	32.9 (3.5)	8.1 (1.1)	3.4 (0.5)	51.1 (3.2)	4.4 (0.6)	0.79 (0.14), 78
		High	Nasaruvaalik	2007	14	25.7 (2.5)	0.7 (0.3)	1.1 (0.4)	61.2 (2.5)	11.2 (1.5)	0.45 (0.06), 49
		Low	East Bay	2008	19	34.0 (2.6)	7.2 (0.9)	0.3 (0.1)	45.3 (2.7)	13.3 (1.6)	0.86 (0.11), 56
		High	Nasaruvaalik	2008	23	39.2 (2.3)	8.6 (1.0)	0.3 (0.1)	44.5 (2.2)	7.4 (0.9)	1.00 (0.12), 59
Thick-billed murre	*Uria lomvia*	Low	Digges	2008	15	49.7 (2.6)	7.7 (0.9)	0.2 (0.1)	28.1 (2.4)	14.2 (1.5)	2.18 (0.36), 64
		Low	Coats	2008	62	43.2 (1.0)	6.4 (0.5)	1.0 (0.6)	39.1 (0.9)	10.3 (0.7)	1.19 (0.07), 47
		Low	Coats	2011	53	55 (0.5)	5.6 (4.0)	0.5 (1.1)	25.7 (10.5)	13.0 (5.8)	2.97 (0.49), 120
Black guillemot	*Cepphus grylle*	Iceland	Flatey	2007	16	32.8 (2.7)	1.8 (0.5)	0.3 (0.2)	54.3 (2.5)	10.8 (1.2)	0.74 (0.12), 63
		High	Cape Vera	2006	25	38.2 (1.2)	3.6 (0.5)	2.2 (0.4)	44.3 (1.4)	11.7 (1.1)	0.90 (0.05), 27
Atlantic puffin	*Fratercula arctica*	Iceland	Flatey	2007	9	45.1 (5.0)	0.7 (0.3)	0.5 (0.2)	47.2 (4.4)	6.4 (1.6)	1.13 (0.25), 66

Regions are generalized as High (Canadian High Arctic), Low (Canadian Low Arctic), Iceland, Maritimes (Canada) and Alaska; within these regions, colony is the specific colony location where samples were taken. Abbreviations: CV (%), coefficient of variation; and H:L ratio, heterophil-to-lymphocyte ratio.

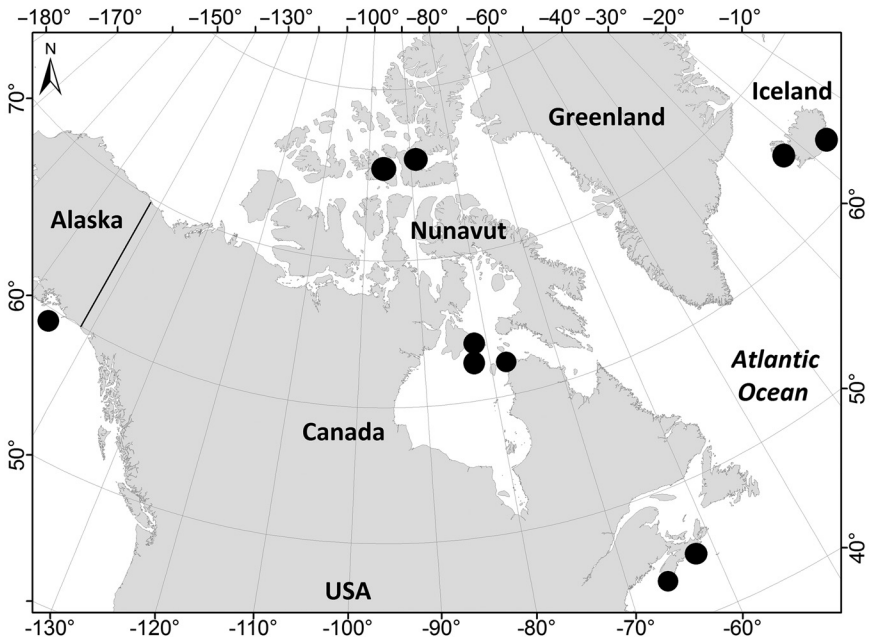

Figure 1: Colony locations (black dots) for all marine birds sampled in this study.

In preparation for conducting leucocyte differential counts, slides were stained using Wright's staining method. Leucocyte counts were conducted at ×1000 magnification using oil immersion. To assess ratios of leucocyte cell types, we counted 100 leucocytes for each blood smear and categorized leucocytes using morphological characteristics (Lucas and Jamroz, 1961). Heterophils, eosinophils, basophils, lymphocytes and monocytes were all included in the counts, but thrombocytes were excluded. Slides were counted principally by C.M.L. (68% of total) and E.S.B. (15%), both of whom were trained by D.S., although kittiwakes and 2011 murres (17%) were counted by K.H.E. E.S.B. recounted a subset of slides ($n = 39$) initially counted by C.M.L. to assess repeatability; recount values for heterophils and lymphocytes were within 4.0 ± 0.7% SEM and 3.5 ± 0.7% SEM, respectively. Other blood cell types were less common and repeatability counts were less reliable (i.e. error between counts >50% of mean occurrence), and thus we present proportional occurrence data but do not include these in analyses. Collectively, we obtained 578 readable slides from nine marine bird species.

We compared natural logarithmically transformed H:L ratios among species and among locations using Welch's *t*-tests or Mann–Whitney Wilcoxon tests for pairwise comparisons, or Kruskal–Wallis tests (followed by Dunn's multiple comparison test) or ANOVA (followed by Tukey–Kramer multiple comparisons test; GraphPad Software, Inc., 2009). We also completed a principal component analysis on all arcsine–square root-transformed leucocyte profile data from northern seabirds to examine overall patterns among species and functional groups (Buehler *et al.*, 2011). Means are presented ±SEM.

Results

Comparisons within species

The proportion of different leucocytes exhibited considerable variation within species and years (Table 1). For example, common eiders sampled at four colony locations exhibited significantly different H:L ratios (Table 1 and Fig. 2; ANOVA, $F_{3,187} = 14.5$, $P < 0.001$), with eiders at each location having different mean H:L ratios from all other locations (all $P \leq 0.05$), except for eiders at Nasaruvaalik Island and East Bay, which did not differ significantly from each other. When we compared H:L ratios with migration distance, birds that migrated farther had higher H:L ratios ($r_{s188} = 0.48$, $P < 0.001$). Moreover, we saw some consistency among years for eiders; H:L ratios at Easy Bay in 2005 (1.56 ± 0.49) were similar to those from 2008 (1.84 ± 0.91; $t_{58} = 1.6$, $P = 0.12$). Murres also exhibited intercolony differences, with H:L ratios higher at Digges (2.17 ± 0.36) than Coats (1.19 ± 0.06, $t_{90} = 4.51$, $P < 0.001$) and higher at Coats in 2011 than 2008 (2.97 ± 0.49, $t_{128} = 8.29$, $P < 0.001$). Guillemots breeding in the high Arctic had a higher mean H:L ratio than those breeding in Iceland (Table 1; Welch's *t*-test, $t_{24} = 3.0$, $P = 0.006$). For Arctic terns sampled at East Bay and Nasaruvaalik Island in 2007 and 2008, significant differences in H:L ratios were found (Table 1; $F_{3,71} = 3.3$, $P < 0.05$), but this was attributable to low ratios (and low variation) in 2007 and high values in 2008 at Nasaruvaalik Island ($P < 0.05$). However, for the other species, H:L ratios did not differ statistically among locations (Table 1; Sabine's gulls: $t_{12} = 0.9$, $P = 0.37$), despite a twofold difference in mean H:L ratios for fulmars between

Figure 2: Mean (SEM) heterophil-to-lymphocyte ratios for common eiders (*Somateria mollissima*) sampled across the western North Atlantic and Canadian Arctic as follows: IS, Iceland; NS, Nova Scotia (Canada); LA, Canadian Low Arctic; and HA, Canadian High Arctic. The right-hand *y*-axis is the approximate migration distance (in kilometres) from wintering to breeding grounds, represented by the line in the graph; sample sizes are above bars. Bars significantly different from each other denoted with different letters.

some site-years (Kruskal–Wallis test, KW = 1.2, $P = 0.54$), presumably because of high annual, within-site variation.

Comparisons among species

The proportion of different leucocytes also exhibited high variation among species, although there were some overall similarities (Table 1). For most species, proportions of basophils were low (medians of 0–1%); fulmars had the highest median proportion of 5%. For eosinophils, median proportions differed among species, with the highest values for murres and terns (6%) and lowest for puffins (1%). Median proportions of monocytes were high in murres and guillemots (11%), with other species having proportions between 5 and 8% (Fig. 3). Overall, H:L ratios among species were fairly variable; the mean coefficient of variation for species' H:L ratios was 66 ± 28%. We found significant differences in H:L ratios among marine bird species (Table 1; KW = 96.4, $P < 0.001$). Puffins, murres, eiders, kittiwakes and Sabine's gulls all had mean H:L ratios >1 (Fig. 3). Fulmars had the lowest H:L ratios, which were significantly lower than those of murres, eiders and Sabine's gulls (all $P < 0.001$). Murres also had higher H:L ratios than terns ($P < 0.001$), guillemots ($P < 0.01$) and skuas ($P < 0.05$), and eider H:L ratios were also higher than those of terns ($P < 0.001$). Collectively, these analyses allowed us to produce reference ranges (mean ± 2SD; Fig. 3) for the main leucocytes and H:L ratios for these species, against which future comparisons can be made.

Using data from this study (Table 1) and other studies on northern marine birds (Table 2), we ran a principal component analysis on the five leucocyte types, which separated bird groups by study and species. The first principal component explained 82.5% of the variance and the second explained 11.0% (Fig. 4). The proportion of heterophils and lymphocytes loaded heavily on the first axis (Fig. 4). Several groups, including small Pacific auks, fulmars, Alcini, *Sterna* terns, puffins and large gulls tended to cluster together, which may represent similarity of leucocyte profiles due to ancestry and/or environment. Others, notably guillemots and especially eiders, did not cluster.

Discussion

Our study is the largest reported haematological sampling of marine birds in northern waters to date, and our results share some similarities with earlier work. For example, fulmars appear to have consistently low H:L ratios compared with other species (Newman *et al.*, 1997; Edwards *et al.*, 2006; present study), which may be characteristic of the family Procellariidae (Work, 1996; Uhart *et al.*, 2003); however, albatrosses appear to have high proportions of heterophils (e.g. Work, 1996), and thus low H:L ratios are not consistent within the entire Order Procellariiformes. Across many marine birds, basophils tend to be the least common of the leucocytes, consistent with our results, but among our species we found median proportions of 5–11% monocytes, generally higher than reported elsewhere (reviewed by Davis *et al.*, 2008; Davis, 2009). We also observed high variation among individuals within a species, as has been reported by Newman *et al.* (1997). Moreover, we found considerable variation among studies, even for the same species or closely related species. As pointed out by Bearhop *et al.* (1999), sex, age, stage of season and various environmental differences all influence bird blood metrics, making comparisons among studies challenging. Nonetheless, many taxonomic groups clustered together across studies and sites (Fig. 4), suggesting that with additional data our reference values for a particular group can be verified. Exceptions were common eiders and guillemots that had particularly large latitudinal ranges; perhaps one reason that those species could exist in such variable climates is that their physiology is particularly adaptable.

Despite these challenges, our first priority was to generate a set of reference values for the various species, as a baseline against which future samples can be compared. This rationale has been adopted in other studies where there is potential for future industrial activity that could affect marine birds (e.g. Newman *et al.*, 1997; Bearhop *et al.*, 1999). In other cases, haematological parameters have been applied as one metric by which to measure long-term effects of marine pollution accidents on local bird populations (e.g. Seiser *et al.*, 2000). In the present study, birds sampled from High and Low Arctic populations are effectively naïve to industrial activity during their breeding season, but that will change in the near future with proposed increases in shipping activity as part of industrial expansion for ore extraction, supply shipping and tourism (Arctic Council, 2009). In generating these reference values, it is clear that there is variation in mean blood cell counts among specific sampling sites or regions, and thus applying average

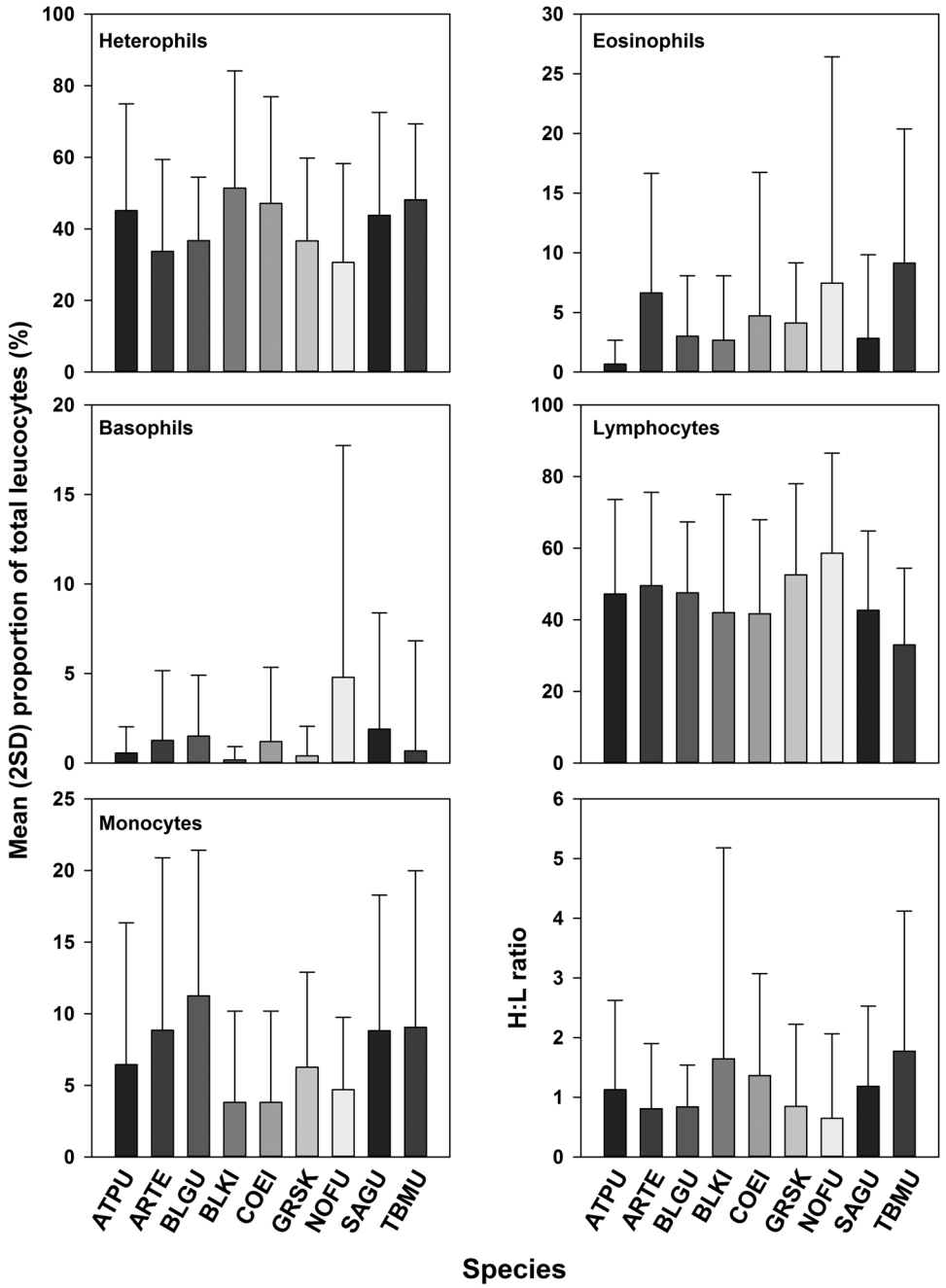

Figure 3: Mean (2SD) reference ranges of proportions of heterophils, eosinophils, basophils lymphocytes, monocytes and heterophil-to-lymphocyte ratios for nine different marine bird species sampled across the western North Atlantic and Canadian Arctic, as follows: Atlantic puffin (*Fratercula arctica*; ATPU), Arctic tern (*Sterna paradisaea*; ARTE), black guillemot (*Cepphus grylle*; BLGU), black-legged kittiwake (*Rissa tridactyla*; BLKI), common eider (*Somateria mollissima*; COEI), great skua (*Stercorarius skua*; GRSK), northern fulmar (*Fulmarus glacialis*; NOFU), Sabine's gull (*Xema sabini*; SAGU) and thick-billed murre (*Uria lomvia*; TBMU). Sample sizes are given in Table 1.

values from a different study, or even a different colony, may not be entirely appropriate, although we believe that this may vary by taxon (see above). We propose that the variation may be due, at least in part, to migration distances and resulting time constraints experienced by northern birds.

We found some support for our hypothesis that H:L ratios would be elevated at colonies where individuals migrated or commuted farther than conspecifics at other colonies. Eiders that had the longest migrations (High and Low Arctic birds, >1500 km; Mosbech *et al.*, 2006) had higher H:L ratios than

Table 2: Leucocyte profiles of species from selected studies on species in the northern hemisphere

Common name	Scientific name	*n*	Mean proportion of leucocytes					H:L ratio	Source
			Heterophils	Eosinophils	Basophils	Lymphocytes	Monocytes		
Northern fulmar	*Fulmarus glacialis*	5	29.0	13.0	0.0	56.0	2.0	0.52	Newman *et al.* (1997)
Black-legged kittiwake	*Rissa tridactyla*	10	30.0	5.0	0.0	62.0	2.0	0.48	Newman *et al.* (1997)
Glaucous-winged gull	*Larus glaucescens*	8	53.0	3.0	0.0	43.0	1.0	1.23	Newman *et al.* (1997)
Great black-backed gull	*Larus marinus*	34	35.2	0.7	2.0	61.0	2.2	0.58	Averbeck (1992)
Herring gull	*Larus argentatus*	103	35.5	0.9	2.0	60.9	1.3	0.58	Averbeck (1992)
		152	56.7	0.3	2.9	35.3	4.8	1.61	Grasman *et al.* (2000)
Sooty tern	*Onychoprion fuscatus*	34	24.7	5.8	1.3	67.3	0.8	0.37	Work (1996)
Common tern	*Sterna hirundo*	33	38.0	9.1	0.0	51.0	5.6	0.75	Fiorello *et al.* (2009)
Ancient murrelet	*Synthliboramphus antiquus*	9	21.0	9.0	1.0	69.0	2.0	0.30	Newman *et al.* (1997)
Crested auklet	*Aethia cristatella*	7	22.0	4.0	1.0	70.0	4.0	0.31	Newman *et al.* (1997)
Horned puffin	*Fratercula corniculata*	18	32.0	7.0	1.0	57.0	4.0	0.56	Newman *et al.* (1997)
Marbled murrelet	*Brachyramphus marmoratus*	1	26.0	9.0	2.0	63.0	1.0	0.41	Newman *et al.* (1997)
Parakeet auklet	*Aethia psittacula*	21	37.0	2.0	0.0	59.0	2.0	0.63	Newman *et al.* (1997)
Pigeon guillemot	*Cepphus columba*	9	47.0	5.0	0.0	47.0	1.0	1.00	Newman *et al.* (1997)
		17	61.0	0.7	1.1	37.0	0.0	1.65	Seiser *et al.* (2000)
Tufted puffin	*Lunda cirrhata*	30	37.0	4.0	1.0	57.0	3.0	0.65	Newman *et al.* (1997)
Dovekie	*Alle alle*	23	37.0	9.0	5.0	38.0	8.0	0.97	Jakubas *et al.* (2008)
Great skua	*Stercorarius skua*	49	44.9	1.4	2.8	34.4	5.5	1.31	Bearhop *et al.* (1999)

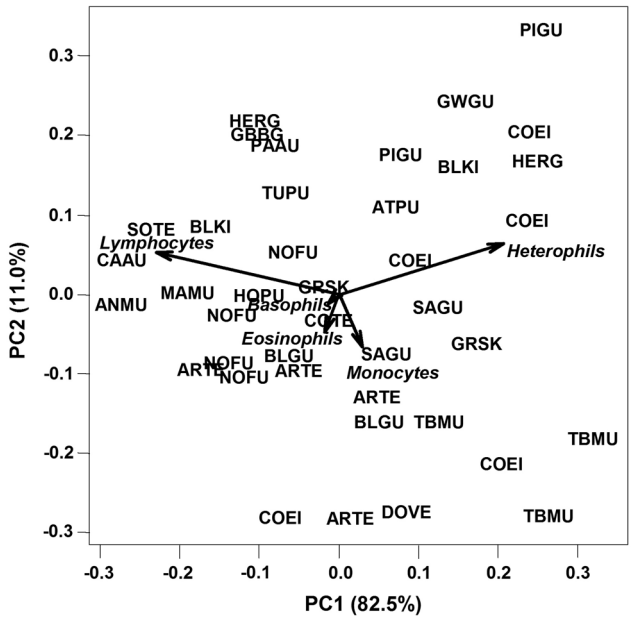

Figure 4: Principal component analysis of average seabird leucocyte profiles. Each represents the average value for a particular study, from Tables 1 and 2. Guilds were grouped as follows: puffins [ATPU, Atlantic puffin; TOPU, tufted puffin (*Fratercula cirrhata*); and HOPU, horned puffin (*F. corniculata*)]; small Pacific auks [PAAU, parakeet auklet (*Cyclorrhynchus psittacula*); MAMU, marbled murrelet (*Brachyramphus marmoratus*); ANMU, ancient murrelet (*Synthliboramphus antiquus*); and CAAU, crested auklet (*Aethia cristatella*)]; Alcini [TBMU, thick-billed murre; and DOVE, dovekie (*Alle alle*)]; guillemots [BLGU, black guillemot; and PIGU, pigeon guillemot (*Cepphus columba*)]; Procellariformes [NOFU, northern fulmar]; ducks [COEI, common eider]; terns [ARTE, Arctic tern; COTE, common tern (*Sterna hirundo*); and SOTE, sooty terns (*Onychoprion fuscatus*)]; and gulls [HERG, herring gull (*Larus argentatus*); BLKI, black-legged kittiwake (*Rissa tridactyla*); GBBG, great black-backed gull (*Larus marinus*); GWGU, glaucous-winged gull (*Larus glaucescens*); SAGU, Sabine's gull (*Xema sabini*); and GRSK, great skua (*Stercorarius skua*)].

those that had shorter migrations (Nova Scotia, 700 km; M. L. Mallory, unpublished data). These, in turn, had higher ratios than those that hardly migrated at all (Iceland, ~100 km; Petersen, 1998). Likewise, High Arctic guillemots, presumably migrating as far as the eiders due to ice patterns, had higher ratios than guillemots in Iceland (some of which migrate to Greenland but many of which do not; Petersen, 1977). However, we found no significant differences in H:L ratios for birds from different colonies of Sabine's gulls, fulmars or terns, despite an approximate 3000 km difference in average migration distance between the locations that we sampled (Mallory *et al.*, 2008; Egevang *et al.*, 2010; Davis, 2015). Unlike eiders and guillemots, however, these species are all long-distance migrants; the 3000 km difference represents ~20% or less of the total migration distance from wintering to breeding sites of each of these species compared with an increase of 1500 km (two to 10 times greater than the migration distance of eiders from the other colonies). Eiders and guillemots also have higher wing loadings and are less efficient fliers than the other species (e.g. Greenwalt, 1962; Alerstam *et al.*, 2007; Elliott *et al.*, 2013); an increase of 1500 km may create greater stress for those species. In addition, H:L ratios were higher at the larger Digges Island colony, where birds commute almost twice as far due to stronger intraspecific competition (Gaston *et al.*, 2013). Murres have the highest relative flight costs of any bird studied to date (Elliott *et al.*, 2013) and would therefore be expected to be particularly susceptible to activity stress. Thus, we suggest that efficiency of flight and the distance flown must influence stress levels in these species, which may be reflected in increased H:L ratios during incubation.

Other than simply the stress of having to migrate farther, differences in environmental conditions on the breeding grounds and migration distance among colonies may have indirect effects on H:L ratios of eiders and other High Arctic nesters; birds at the High Arctic colony may arrive with less time before they start laying. This could leave birds with less time to invest resources in immunity on the breeding grounds (i.e. to reallocate resources that have been shunted to deal with energetic needs of migration) and would thus result in H:L ratios that may be diminishing, but would be relatively high post-migration compared with those of birds at southern colonies that arrived long before breeding. We do not know how long it takes H:L ratios to return to 'normal' after prolonged stress in these wild species, but in one experimental study it took 10 days for quail H:L ratios to return to normal after short-term exposure to a corticosteroid (Aengwanich and Chinrarsi, 2003). Arctic birds have a short window of time in which to breed and, moreover, may arrive when there is still ice and snow covering their breeding habitats. Thus, they often adjust their breeding schedules to accommodate annual variation. In fulmars, pre-breeding activities at High Arctic colonies are markedly reduced compared with fulmars elsewhere in the range, including Alaska (Mallory and Forbes, 2007). Newman *et al.* (1997) found that mean H:L ratios in Alaskan marine birds, including many alcids, were generally less than one (Table 2). Those sites in Alaska are generally ice free, and some of the species have very short migrations (e.g. crested auklet, *Aethia cristatella*). In contrast, Jakubas *et al.* (2008) found H:L ratios near one in dovekies (*Alle alle*) from Svalbard, a location where birds would have to deal with sea ice, at least early in the season. In our study, the only diving bird with mean H:L ratios less than one were guillemots, even the High Arctic birds (Table 1). That High Arctic colony may be a special case, however, because it was situated in a polynya where birds may exploit the early open water and arrive at the breeding grounds months before breeding (Prach and Smith, 1992).

Collectively, we believe that our data provide key baseline references for colony-level comparisons of stress in the various marine bird species. Dehnhard *et al.* (2011) suggested that leucocyte profiles were blunt tools, but were sufficient to look at population (colony) level changes in stress, and we suggest a similar interpretation of our data. If researchers gather basic data on marine bird health regularly as part of standard protocols (e.g. Mallory *et al.*, 2010), annual variation in leucocyte

profiles may be detected and factors contributing to increased stress indicators can be more easily identified. In particular, reductions in sea ice leading to earlier nesting by some species (e.g. Gaston *et al.*, 2009) will change the dynamic between migration departure and nest initiation, presumably altering residual effects of migration stress on breeding birds and their H:L ratios proposed in this study. As evidence, the highest H:L ratios in the present study were from murres near their southern range limit, at Coats Island in 2011. That year was a particularly warm year and a period of high stress when polar bears (*Ursus maritimus*) and mosquitoes combined caused a 20% reduction in reproduction (Gaston and Elliott, 2013). Furthermore, water loss due to heat stress and mosquitoes caused very low haematocrits (Gaston and Elliott, 2013). Conducting such observations for 3 weeks was logistically difficult and time consuming; H:L ratios may be a simple and quick method for assessing population stress in response to a warmer climate. We believe that future investigations should test the following predictions: (i) across colonies and among individuals at a colony, the duration of pre-breeding periods at a colony is negatively related to H:L ratios during incubation; (ii) H:L ratios are lower for individual birds that initiate nesting later in the breeding season (controlling for body condition); and (iii) a progressive increase in H:L ratios during incubation will occur at Arctic colonies through time, as birds try to initiate nesting earlier to keep pace with earlier seasonal phenology of food supplies (e.g. Gaston *et al.*, 2009).

Our research focused on leucocyte profiles and stress, but there are many other metrics of immune function (Matson *et al.*, 2005; Millet *et al.*, 2007; Liebl *et al.*, 2009) and many other questions in the field of ecological immunology (Ardia and Schat, 2008; Martin *et al.*, 2011; Pedersen and Babayan, 2011). Blood smears are one of the easiest metrics to collect in field conditions, and leucocyte profiles are one of the most inexpensive metrics to use. For some investigations, leucocytes have drawbacks in interpretation (Norris and Evans, 2000), but H:L ratios are quite robust to evaluation (Davis *et al.*, 2008). In any case, future work should compare other metrics of immune function with results generated by blood smears.

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