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Variability in the health and condition of breeding Northern Gannets (*Morus bassanus)*.

Micheál Fitzgerald



A thesis submitted to University College Cork

In candidature of the degree of Master of Science

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This thesis was conducted in the School of Biological, Earth & Environmental Sciences,

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Head of Department Prof Andy Wheeler

Supervisors: Dr Mark Jessopp & Dr Sharon Lynch

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ABSTRACT

Seabird populations are in global decline, with impacts from fisheries and invasive species on seabird mortality being well described. Physiological condition can affect survival and reproductive success at the individual level, but effects of physiological condition at the population level are less well understood. This study identifies patterns of variability in the physiological condition of a population of breeding northern gannets (*Morus bassanus)* between years, nesting stages, and fasting levels. Blood samples were collected from breeding birds attending chicks in 2018-2020, with blood smears used to quantify blood cell types indicative of chronic stress, infection, disease, and immunocompetence. While no blood parasites were observed, elevated Heterophil to Lymphocyte (H:L) ratios, eosinophils, and monocytes suggest that there was a higher prevalence of infection in 2018 and 2020 than in 2019. Standardised body condition was lowest during early chick-rearing, and chronic stress levels, indicated by high H:L ratio, were elevated in both incubation and early chick-rearing compared to the late chick-rearing stages. Interestingly, birds returning from foraging trips were less stressed than those departing, suggesting an effect of fasting on stress levels. However, sample size was too small to determine whether this difference was statistically significant. This study highlights the value of haematology as a tool for identifying patterns of variability in the health and condition of seabird populations; however further research is needed in order to better understand the processes driving this variation and its effect on populations.

INTRODUCTION

The influence of physiological condition on survival and reproductive success has been well established across numerous taxa including mammal, fish, and avian species (Atkinson and Ramsay, 1995; Susdorf *et al*., 2017; Grilli *et al.*, 2018). Poor physiological condition has been linked to increased susceptibility to infection (Chaudry and Ayala, 1993; Krist *et al*., 2003; Beldomenico and Begon, 2010), as well as reduced foraging ability (Goater *et al*., 1993; Venesky *et al*., 2010; Benaets *et al*., 2017) and reproductive success (Atkinson and Ramsay, 1995; Milenkaya *et al*., 2015; Grilli *et al*., 2018). The mean physiological condition of individuals within a given population, can be influenced, for example, by altered food availability, changes in climatic conditions, or disturbance from humans (Post *et al*., 1997). This population-level condition can have a significant impact on population growth and stability (Post and Strenseth, 1999; Lenarz *et al*., 2009; Fefferman and Romero, 2013; Grilli *et al.*, 2018). Therefore, if wild animal populations are to be effectively managed and conserved, knowledge of patterns of variability in their physiological condition, and thresholds where impacts occur is important.

The physiological condition of both individuals and populations has been quantified for numerous taxa, including Adelie Penguins (*Pygoscelis adeliae*) and Little Auks (*Alle alle)*, using the analyses of parameters such as energy reserves, stress levels, and immunological profiles (Vleck *et al.,* 2000; Fairhust *et al*., 2015; Jones, 2015; Labocha and Hayes, 2012; Mallory *et al*., 2015; Malvat *et al*., 2020). Stress levels, typically quantified using haematological methods, have been found to predict survival in Eurasian Rabbits (*Oryctolagus cuniculus*) and Eurasian Kestrels (*Falco tinnunculus)* (Cabezas *et al*., 2007; Muller *et al*., 2011), and therefore may be an effective early indicator of declining physiological condition, and a valuable tool for the assessment of health at a population level (Romero and Wikelski, 2001). Haematological methods may be used not only to assess stress levels (Mallory *et al*., 2000; Malvat *et al*., 2020), but also, to identify cryptic health issues (Clark *et al*., 2009), making it a particularly valuable tool in the assessment of physiological condition in avian species, which often disguise health issues that can potentially impact reproductive success and foraging ability (Clark *et al*., 2009).

Determining physiological condition may be particularly relevant in the case of seabirds, which are in global decline, and many species of which are of conservation concern (Dias *et al*., 2019). Therefore, the monitoring of seabird populations' health in order to detect patterns and causes of reduced condition, has been advocated (Mallory *et al*., 2015). While some research has established baseline reference values of physiological parameters including chronic stress during the breeding season (Mallory *et al*., 2015, Malvat *et al*., 2020), most studies are limited to relatively short time periods within a single nesting stage or a single breeding season (Cockrem *et al*., 2016; Franci *et al*., 2014; Malvat *et al.*, 2020). While this might largely be due to logistical difficulties of accessing colonies, further studies investigating how physiological condition changes within and between nesting seasons are needed.

Some previous studies have observed the condition of seabirds to vary markedly both between (Weimerskirsch *et al*., 2001; Will *et al*., 2015) and within years (Vleck *et al*., 2000). Interannual variability in aspects of physiological condition including body mass and stress levels has been observed in seabird taxa, and is often attributed to variable oceanographic conditions and resulting alterations to prey availability (Weimerskirsch *et al*., 2001; Warwick-Evans *et al.*, 2016). Minor variation in diet may cause increases in nutritional stress (Will *et al*., 2015), and with the abundance and distribution of prey species being subject to substantial spatial and temporal variability (Warwick-Evans *et al*., 2016), seabirds will experience years of both good and bad food availability and quality. This could have significant consequences for the condition of entire populations (Warwick-Evans *et al*., 2016; Storey *et al*., 2017). Reduced prey availability could also force seabirds to increase foraging trip durations and distances, negatively impacting nest and chick attendance, as well as bringing birds into increasing contact with fishing vessels and other forms of anthropogenic activity (Warwick-Evans *et al*., 2016). Almost half of all seabird species are known to rely to some extent on supplemental food through the use of fishery discards in the form of undersized fish, offal, or non-commercial species (Furness 2003, Oro *et al*. 2013). Furthermore, interannual variability in discard consumption has been linked to negative effects on body condition and breeding success (Le Bot *et al*., 2019). With greater interannual variability in climatic conditions and prey availability predicted to occur under various climate scenarios (Gremillet and Boulinier, 2009; Sydeman *et al*.,

2012), long-term assessment of the health and condition of seabird populations will be crucial to ensure early detection of negative impacts within marine ecosystems.

Studies have also found elements of physiological condition in seabirds, such as body condition and chronic and acute stress levels, to vary within breeding seasons. This has typically been attributed to factors such as altered energy requirements due to parental care (Salamolard and Weimerskirch, 1993), prolonged periods of fasting (Vleck *et al*., 2000), and exposure to aggressive interactions during competition for nest space (Hunt and Hunt, 1976; Pius and Leberg, 1997; Vleck *et al*., 2000; Wojczulanis-Jakubas *et al*., 2014). Effects also vary dramatically between species (Vleck *et al*., 2000) and sexes (Grilli *et al*., 2018). For example, the body mass of Little Auks was found to be lowest in the chick-rearing period of the breeding season (Wojczulanis-Jakubas *et al*., 2018). However, adult body mass remained stable throughout the breeding season in female Red Footed Boobies (*Sula sula*), despite decreasing markedly during the chick-rearing stage in males (Lommee *et al.*, 2003). Chronic stress levels, indicated by heterophil to lymphocyte (H:L) ratios, were found to be highest in the chick-rearing stage of the breeding season in Adelie Penguins (*Pygoscelis adeliae*) (Vleck *et al*., 2000), while in Brown Skuas (*Stercorarius antarcticus*), H:L ratios were found to be higher in females than in males, but remained consistent across stages (Grilli *et al*., 2018). Unfortunately, many studies investigating the condition of seabirds during the breeding season have been restricted to a single nesting stage (Cockrem *et al*., 2016; Franci *et al.*, 2014; Malvat *et al*., 2020), and thus the changing condition of most seabird species throughout their breeding periods is largely unknown.

Haematological analysis has been shown to be a reliable method of assessing the physiological condition of captive and free-living birds (Boross et al., 2012; Campbell, 1995; Davis *et al*., 2008; Muller *et al*., 2011). The counting, identification of developmental stages, and morphological assessment of various blood cells provides information on the stress levels (Davis *et al*., 2008; Vleck *et al*., 2000) and immunological condition (Owen and Moore, 2006) of individuals, while also indicating the presence of diseases (Marinho, 2012) and infection, for example by blood parasites (Davis *et al.*, 2004). Packed cell volume (PCV), total erythrocyte (oxygen transport) and leucocyte (immune response) counts, leucocyte differentials, and the staging of erythrocytes are most commonly employed (Jones, 2015). Reduced

PCV counts, as well as numbers of immature erythrocytes and/or microcytes (smaller erythrocytes with reduced O^2 carrying capacity) in excess of 5%, are indicative of anaemia (Martinho, 2012). A reduction in the number of mature erythrocytes is typically as a result of blood loss, haemorrhage, parasitism, or toxicosis (Campbell, 1995; Jones, 2015). Elevated leucocyte levels, or leucocytosis, can be indicative of inflammation, due to infection, injury, or toxicosis (Davis *et al*., 2008). The heterophil to lymphocyte ratio (H:L) is commonly used to assess chronic stress, with elevated ratios typically being caused by stress due to starvation, injury, infection, or disturbance, (Gross and Siegel, 1983; Vleck *et al*., 2000). More sensitive to subtle, long-term stressors than steroid hormones, the H:L ratio can be used to assess the stress response of birds to interannual and seasonal environmental changes, as well as to conditions imposed by reproductive cycles, such as fasting and overcrowding (Muller *et al*., 2011; Wingfield, 1994; Vleck *et al*., 2000).

The Northern Gannet (*Morus bassanus*) is a pelagic seabird breeding in colonies throughout the North Atlantic (Nelson, 2002). A significant proportion of the population occurs on offshore islands around Britain and Ireland (Mitchel *et al*., 2004), with the latter supporting approximately 48,000 breeding pairs (Newton *et al*., 2015). In western Atlantic populations, the nesting season begins with egg laying from April to May, followed by 40-50 days of incubation by both parents before hatching peaks in mid-June (Nelson, 2002). Chicks grow from approximately 60g at hatching to reach adult weights of approximately 3kg within 6 weeks (Mowbray, 2002). Chicks fledge at 13 weeks of age in late August/early September after attaining fledging weight of approximately 4kg (Nelson, 1964). Between-sex variation in parental care has been observed, with Nelson (1978) finding that mean incubation and brooding times in male Gannets on Bass Rock, Scotland, were significantly longer than those of females. In a precursor to the current study, Malvat *et al* (2020) found both male and female breeding Gannets on Great Saltee Island, Ireland, to be highly stressed, indicated by high H:L ratios. However, this study was limited to the early chick-rearing period, and only sampled birds that had been fasting. Thus, information regarding the changing physiological condition of Northern Gannets throughout the nesting season, and at different starvation levels, is limited. Unlike most seabird species, the world gannet population is increasing (Pettex *et al.*, 2015), potentially due to food subsidies from fishing vessels, for which they can outcompete other seabird species (Tasker *et al.*,

2000). However, Gremillet *et al.,* (2020) found decreased survival of gannets on Rouzic Island, France, in recent years, which could be an early indication of future population declines.

The present study aimed to (1) investigate interannual variability over three years in physiological parameters indicative of health, as well as effects of sex; (2) investigate variability in physiological condition across the breeding season in a single year and between sexes; (3) investigate the effect of time spent on the nest between foraging trips, or fasting, on chronic stress levels. Findings of this study will support the use of haematological methods as a means of assessing the health of seabird populations, and for the first time, identify patterns of variability in the health status of breeding northern gannets, between and within nesting seasons.

MATERIALS AND METHODS

Bird Sampling

All bird handling and sampling were approved by the UCC Animal Ethics Committee and conducted under permits issued to Dr Mark Jessopp, by the British Trust for Ornithology, National Parks and Wildlife Service, and the Irish Health Products Regulatory Authority. Throughout 2020, government mandated restrictions on travel and closure of the university campus due to Covid-19 severely limited sampling opportunities and access to laboratories in order to process and analyse samples.

Breeding Northern Gannets on Great Saltee Island, Wexford, Ireland (Lat: 52.109245, Long: -6.621879, Figs 1 & 2), were caught using a 7-10m pole fitted with a crook while attending 3 to 5 week old chicks in July of 2018, 2019 and 2020. Birds were sampled immediately prior to their departure on a foraging trip, identified by a changeover of parents at the nest, and in July 2020 six birds were sampled on return to the colony. In 2020 birds were also randomly sampled from the colony during incubation (June), early chick-rearing (July) and late chick-rearing (September) (Table 1). Randomly sampled birds are therefore likely to be a mix of birds from those that have recently returned from foraging trips to those that are due to leave soon. Within 5 minutes of capture, <2ml blood was taken from the tarsal vein of each bird using a 25G needle (Fig 3). A 60µl subsample was mixed with an equal volume of the anti-coagulant and preservative ethylenediaminetetraacetic acid (EDTA) and used to make duplicate 60µl blood smears using the two-slide wedge technique of Coles (1986) before being allowed to air dry.



Fig. 1: Map of Great Saltee, Wexford, Ireland. Box indicates approximate area in which fieldwork was conducted.

All birds were weighed (±0.05kg), fitted with a metal BTO identification ring, and 2-3 breast feathers were taken for genetic identification of sex. In 2020 wing lengths were also measured using a wing rule to obtain a measure of body condition by dividing weight by wing length (Labocha and Hayes, 2012). Departing birds were fitted with GPS tracking devices (i-gotu GT120, mobile action technology), attached using waterproof Tesa tape to 2-3 central tail feathers, and set to record locations at 3-minute intervals.



Fig. 2: Study area in which northern gannets were caught and sampled, on Great Saltee, Wexford, Ireland, 2020.

Table 1: Sex ratios and mean weight of all northern gannets caught in 2018, 2019, and 2020 on Great Saltee. M, F, U indicate male, females, unknown, respectively. Brackets indicate birds from which adequate blood samples could be acquired. Weight values indicate mean ± standard deviation.

Year	Breeding Stage	Sample Time	N	M,F,U	Weight Males	Weight Females
2018	Early Chick	Departure	28 (27)	14,13,1 (13,13,1)	2.96 ± 0.31	3.06 ± 0.22
2019	Early Chick	Departure	30 (22)	10,17,3 (9,10,3)	2.74 ± 0.13	3.04 ± 0.27
2020	Incubation	Random	21 (12)	14,6,1 (5,7)	**3.125 ± 0.32**	3.29 ± 0.22
2020	Early Chick	Departure	14 (10)	4,8,2 (3,5,2)	2.82 ± 0.06	3.01 ± 0.21
2020	Early Chick	Return	6 (5)	1,5 (1,4)	2.6 ± 0	**3.31 ± 0.32**
2020	Early Chick	Random	16 (11)	10,4,2 (9,2)	2.84 ± 0.34	2.91 ± 0.11
2020	Late Chick	Random	19 (18)	7, 12 (6,12)	3 ± 0.38	3.1 ± 0.21

*Largest weight values for each sex highlighted in bold font.



Fig. 3: Blood sampling procedure performed on a northern gannet, on Great Saltee, Wexford, Ireland, 2020.

Staining and Analysis of Blood Smears

Blood smears were soaked in methanol for 2 minutes, before being dipped in One Step Wright's Stain, followed by rinse solution no.2 (Sigma Aldrich), and finally in de-ionized water, each for 25 seconds. Slides were then air dried for 5 minutes,

coverslipped using Eukitt® quick-hardening mounting medium (Sigma-Aldrich), and left to stand for 5 days, in order for the mounting medium to dry. Once dried, blood smear analysis was performed, with classification of avian blood cell types following Campbell (1995) (Appendix B). In order to determine the total WBC count, leucocytes were counted at 40x magnification in 10 fields using the modified battlement technique of Bain (2015), with the mean number of leucocytes per slide then being multiplied by 2000 (Russo et al., 1986; Walberg, 2001). To calculate the WBC differential, the percentage of heterophils, lymphocytes, eosinophils, basophils, and monocytes per 100 leucocytes were counted at 100x magnification, with oil immersion (Samour, 2006). To determine the H:L ratio, heterophils and lymphocytes were counted at 100x magnification with oil immersion until the total reached 100, and the number of heterophils counted was divided by the number of lymphocytes (Vleck et al., 2000). Using 40x magnification, the percentage of immature erythrocytes and microcytes per 100 erythrocytes were counted, averaging across 3 replicates per slide.

Percentages of immature erythrocytes in excess of 6.78% were considered to be indicative of regenerative anaemia, while percentages of microcytes in excess of 5% were considered to be indicative of microcytic anaemia (Martinho, 2012).

Genetic Sexing

The sex of each bird was determined genetically, using feather samples, following the method described by Griffiths et al (1998). The Qiagen DNeasy™ Blood & Tissue Kit was used to extract DNA from the calamus of each feather, which was then eluted in 30µl of double distilled water. The polymerase chain reaction (PCR) was conducted in 5µl reaction volumes of 2.5µl of 2X TopBIO Combi PP™ mastermix, 1µM of both P2 and P8 primers, and 1µl of DNA. In order to visualise products on an ABI3500xl DNA analyser, the P2 was labelled with 6-Carboxyfluorescein (6-FAM). Products were diluted in water three times, and prepared in Hi-Di™ Formamide, using GeneScan™ 600 LIZ™ Dye Size Standard (ThermoFisher Scientific) as a size ladder. An ABI3500xl DNA analyser was used to conduct electrophoresis. Males were signified by the presence of only a CHD-Z band, while females were signified by the presence of both CHD-Z and CHD-W bands.

Statistical Analysis

All data were analysed using R statistical software (Version 4.02, RStudio Team, 2020). As wing lengths were not measured in 2018 and 2019, weight was used in interannual comparisons of body condition. All data were tested for normality and equality of variances using the Shapiro-Wilk and Levene's tests. When assumptions of parametric statistical methods were not met, non-parametric tests were used. Inter and intra-annual differences in haematological parameters and body condition were tested using general linear models or the non-parametric Kruskal Wallis H test, with either post-hoc Tukey or the *kruskalmc()* function in the R package *pgirmess* (Giraudoux *et al.,* 2018) being used for pairwise comparisons. Bird sex was included as a factor in all tests. Analysis of departing and returning birds sampled in July 2020 were conducted using Mann-Whitney U tests. Relationships between variables were tested using Pearson's and Spearman's correlation tests for parametric and non-parametric data.

Tracking data were analysed to investigate the relationship between H:L ratio and time spent at the nest or fasting. In the GPS data, any time that gannets spent beyond 1.5km from the colony was interpreted as a foraging trip, anything less was considered to be behaviour associated with the colony such as rafting or circling the colony (Carter *et al.,* 2016). For each bird, each foraging trip was identified, and the time between each trip calculated, in order to identify between trip fasting times at the nest. For each bird, mean between-trip (fasting) times were calculated, so the relationship between fasting times and H:L ratios could be investigated using regression analysis.

RESULTS

Interannual differences in body condition and haematological parameters

Analysis of interannual variability in body condition and haematological parameters only involved birds sampled at departure from the colony from each year. Birds weighed the same among years ($F = 1.72$, $P = 0.19$, $df = 2$, $n = 52$), however there was a significant difference between sexes ($F = 9.21$, $P = 0.004$, $df = 1$, $n = 52$) when data from all years were combined. In 2019 females were also significantly heavier than males ($U = 98$, $P = 0.014$, $n = 23$), however there was no statistically significant difference in the weights of birds between sexes in 2018 or 2020 (All Mann-Whitney U-tests $P > 0.05$).

Mature and immature erythrocytes, heterophils, lymphocytes, and eosinophils were present in all blood smears in each year, while monocytes and microcytes were only present in some blood smears in each year (see Appendix A). No statistically significant difference was found between the sexes for any haematological parameters in any year, nor when data from all years were pooled together (see Appendix C). Significant interannual differences were found in a number of leukocyte counts between years (Table 2). There were significant differences in H:L ratios between years ($H = 28.405$, $P < 0.001$, $df = 1$, $n = 52$), with pairwise comparisons showing birds sampled in 2019 having significantly lower H:L ratios than 2018 and 2020 ($P < 0.001$) but no difference between 2018 and 2020 ($P > 0.05$) (Figure 4). White blood cell (WBC) counts did not differ significantly among years ($H = 3.065$, $P = 0.216$, $df = 2$, $n = 52$). Interannual differences occurred in the percentages of eosinophils ($H = 41.722$, $P < 0.001$, $df = 2$, $n = 3$) and monocytes ($H = 13.25$, $P < 0.001$, $df = 2$, $n = 52$). Pairwise comparisons showed that birds in 2018 had significantly higher eosinophil percentages than those of 2019 and 2020 ($P < 0.001$), while the difference between birds sampled in 2019 and 2020 was not significant ($P > 0.05$). In 2020, birds had higher monocyte percentages than both 2019 ($P < 0.05$) and 2018 ($P < 0.05$), however the difference between birds in 2019 and 2018 was not significant ($P > 0.05$).

Similar to leukocytes, there were significant interannual differences in a number of erythrocyte parameters (Table 2). Percentages of immature erythrocytes were found to differ between years ($H = 20.01$, $P < 0.001$, $df = 2$, $n = 52$), with pairwise comparisons highlighting birds sampled in 2018 with higher percentages than those in

2019 ($P < 0.001$) and 2020 ($P < 0.001$). However, there was no significant difference between birds in 2019 and 2020 ($P > 0.05$). Microcyte percentages were not found to differ between years ($H = 4.52$, $P = 0.105$, $df = 2$, $n = 52$). Anaemic birds were present in each year. Regenerative anaemia was found in two birds in 2018 and one bird in both 2020 and 2019, while one bird was found to be suffering from microcytic anaemia in 2019 and two in 2020.

Table 2: Blood parameters (Mean ± Standard Deviation) of Northern Gannets, departing on foraging trips in July 2018, 2019, and 2020.

Parameter	2018 (n = 28)	2019 (n = 27)	2020 (n = 13)
H:L	4.073 ± 1.745	1.856 ± 0.987	**4.13 ±1.216**
Heterophil	58.037 ± 8.117	54.750 ± 12.074	**74 ± 3.924**
Lymphocyte	16.074 ± 4.678	**37.1 ± 13.996**	18.5 ± 4.566
Eosinophil	**24.815 ± 7.822**	5.8 ± 2.542	4.3 ± 1.187
Monocyte	1.074 ± 1.562	0.65 ± 1.014	**3.2 ± 1.887**
WBC	**4814 ± 1827**	3890 ± 1087	4540 ± 2913
I.E	**5.185 ± 1.262**	3.848 ± 1.493	2.933 ± 0.49
Microcyte	**0.852 ± 1.170**	0.515 ± 1.122	0.7 ± 1.67

* Largest values for each parameter highlighted in bold font.

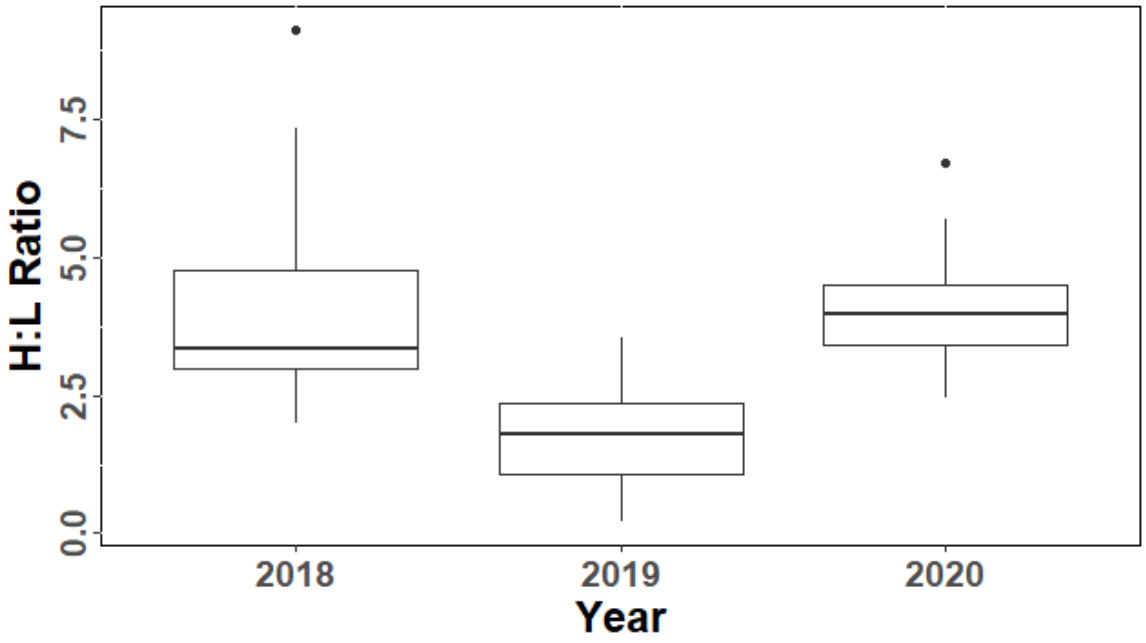


Fig. 4: H:L ratios of gannets departing on foraging trips in July 2018, 2019, and 2020.

Breeding stage differences

Analysis of body condition and haematological parameters *within* the breeding season only involved birds sampled randomly in the colony from each breeding stage (Table 3). Regression analysis revealed no relationship between body condition and chronic stress, measured by H:L ratios ($R^2 = 0.039$, P = 0.097, df = 1, n = 57). Body condition (weight/wing) varied according to breeding stage (F = 5.745, P = 0.006, Fig. 5) but not sex (F = 1.432, P = 0.249). Birds in the early chick-rearing stage were in significantly poorer condition than those in the incubation (P < 0.001) and late chick-rearing stages (P = 0.025), however there was no difference between incubating and late chick-rearing birds (P = 0.360).

Table 3: Mean values of haematological parameters quantified using blood samples taken from northern gannets sampled randomly throughout the colony on Great Saltee, during incubation, early, and late chick-rearing stages of the 2020 nesting season. Values indicate mean ± standard deviation.

Parameter	Incubation	Early Chick-rearing	Late Chick-rearing
H:L Ratio	**1.911 ± 1.164**	1.737 ± 0.466	0.817 ± 0.531
Heterophil	56.222 ± 17.055	**58.455 ± 5.837**	37.889 ± 14.422
Lymphocyte	38.111 ± 17.221	35 ± 5.657	**55.903 ± 14.829**
Eosinophil	4.403 ± 1.9	**4.812 ± 0.936**	3.764 ± 2.324
Monocyte	1.847 ±1.304	**2.545 ± 1.157**	2.444 ± 1.891
WBC	2966.667 ± 979.229	**4672.727 ± 2415.515**	2944.444 ± 1167.724
I.E	**4.584 ± 1.225**	2.939 ± 0.897	3.5 ± 0.951
Microcyte	0.333 ± 0.491	**1.121 ± 1.876**	0.204 ± 0.275

*Largest values for each parameter highlighted in bold font.

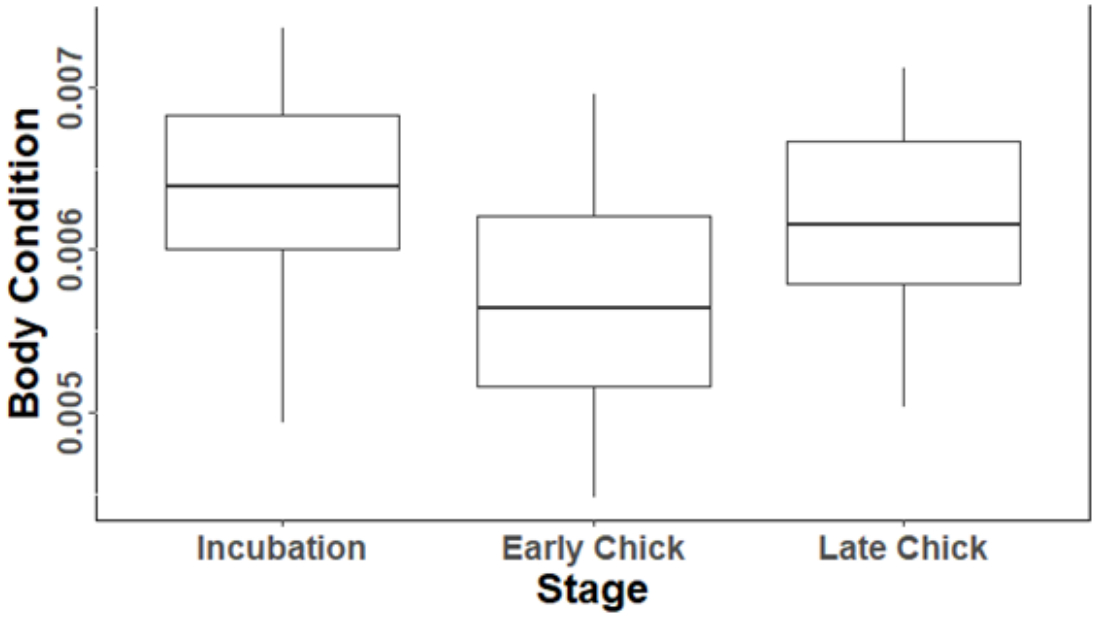


Fig. 5: Relationship between body condition (weight/wing length) and breeding stage for gannets breeding on Great Saltee in 2020.

A general linear model including breeding stage and sex highlighted a significant difference in H:L ratios according to breeding stage (F = 6.922, P = 0.003, df = 2, n = 41) but not sex (F = 0.016, P = 0.900, df = 1, n = 41). Pairwise comparisons showed no difference between the H:L ratios of birds in the incubating and early chick-rearing stages (P = 0.601), while H:L ratios of birds in the late chick-rearing stage were significantly lower than those in the incubation (P = 0.002) and early chick-rearing stages (P = 0.006) (Fig 6). Birds sampled during early chick-rearing had higher WBC's than those sampled during incubation (P = 0.024) and late chick-rearing (P = 0.024), suggesting a higher prevalence of injury, infection, or stress during this period. The difference in WBC's between incubation and late chick-rearing was not statistically significant (P = 0.971). There was also no effect of breeding stage on the percentage of eosinophils (F = 0.62, P = 0.55, df = 2, n = 43) or monocytes (F = 0.74, P = 0.48, df = 2, n = 43).

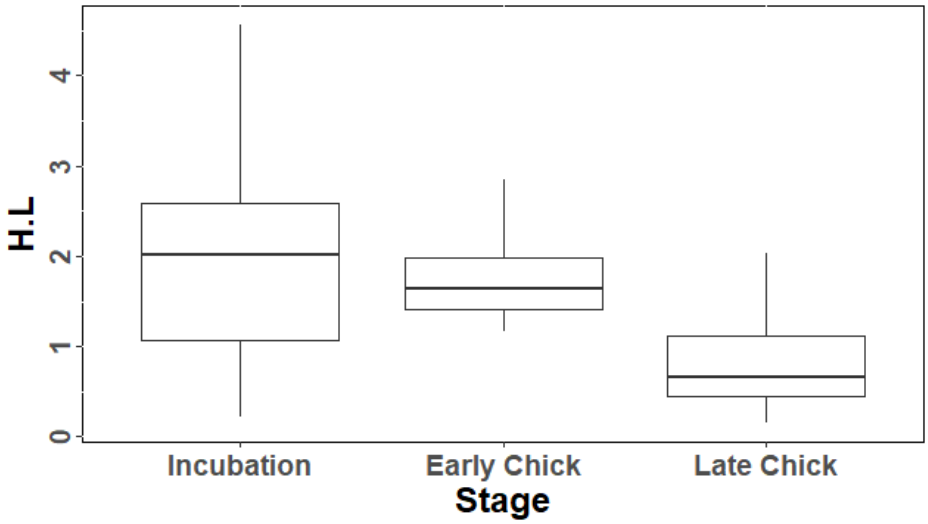


Fig. 6: H:L ratios of gannets sampled randomly throughout the colony in June (Incubation), July (Early Chick), and September (Late Chick), 2020.

Immature erythrocyte percentages were found to vary between nesting stages ($F = 9.728$, $P < 0.001$, $df = 2$, $n = 43$), being higher in incubation than both early ($P < 0.001$) and late chick-rearing ($P = 0.008$). The difference between early and late chick-rearing was not statistically significant ($P = 0.138$). One male bird sampled during incubation had an immature erythrocyte percentage of 7.33%, indicating it was suffering from regenerative anaemia. There was no significant difference in microcyte counts between any of the three nesting stages ($F = 1.841$, $P = 0.169$, $df = 2$, $n = 43$), however two birds in early chick-rearing, one male and one female, were found to have high proportions of microcytes, indicating that they were suffering from microcytic anaemia.

Effect of chick guarding on stress

In early chick-rearing of 2020, blood samples were obtained from thirteen birds that were departing from the colony, three of which were male, eight female, and 2 unsexed. Five birds were also blood sampled that had returned from foraging trips, one of which was male and four of which were female (Table 1). One returning bird was suffering from microcytic anaemia, and as this was associated with high stress levels, was excluded from analysis of stress levels between departing and returning birds. Although H:L ratios were higher in departing birds (Fig 7), this difference was not statistically significant ($U = 37$, $P = 0.06$). However, the lack of significance is

likely due to low statistical power associated with small sample size, particularly given a P value approaching 0.05. To further explore the effect of chick guarding on stress levels, tracking data from 31 individuals that had performed multiple foraging trips were used to determine the time spent in the colony between trips. Assuming individual consistency in foraging trip durations and time spent in the colony between trips, a relationship between time in colony (starvation duration) and H:L ratio would be expected. While a positive relationship was noted (Fig 8), the relationship was not significant (R^2 = 0.042, P = 0.153).

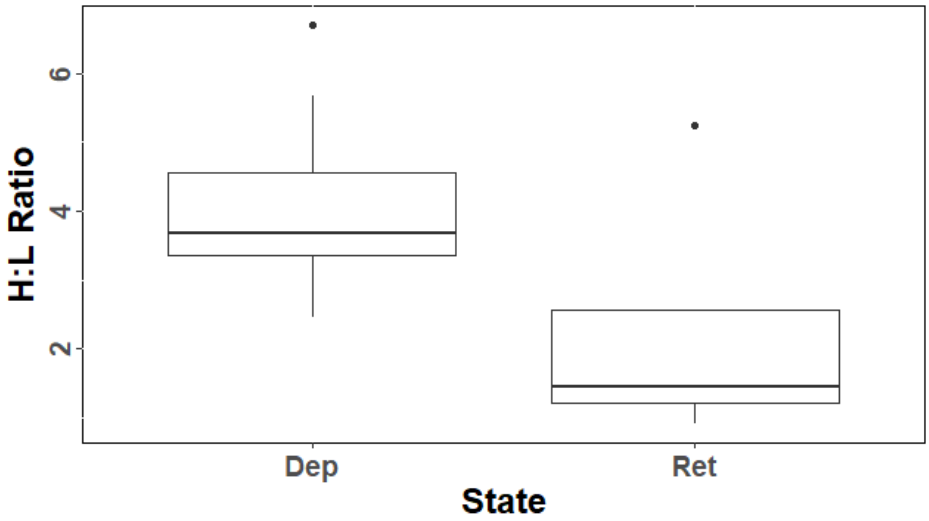


Fig. 7: H:L ratios of northern gannets sampled while departing from and returning to the colony in July 2020.

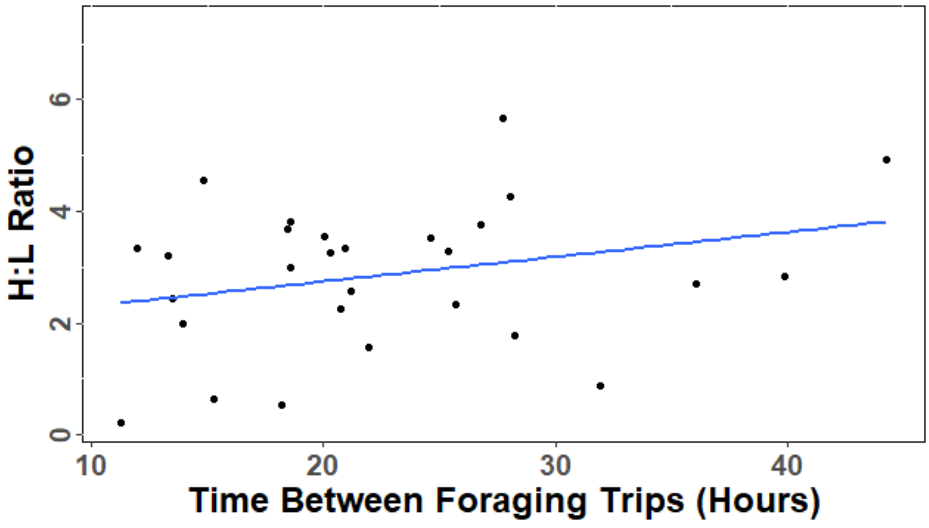


Fig. 8: Relationship between the time gannets spent in the colony between foraging trips and H:L ratios, in 2018 and 2019 (R^2 = 0.042, P = 0.153).

DISCUSSION

This study has examined variability in the physiological condition of northern gannets between years, nesting stages, and fasting durations. Blood cell counts indicate significant interannual variability in the health status of breeding northern gannets in this Irish colony. Additionally, a marked effect of breeding stage on the body condition, chronic stress levels, and prevalence of inflammation in birds was detected, with a particularly negative effect on health of both sexes during early chick-rearing. While sample sizes are admittedly small, the study also provides evidence for the influence of the fasting period on chronic stress levels in breeding seabirds.

Interannual differences

H:L ratios were found to be significantly higher in birds in 2018 and 2020 than in 2019. As this parameter is known to increase due to both stress and infection, it must be interpreted in the context of the leukocyte differential as a whole, in order to accurately identify the cause of its response (Davis *et al.*, 2008). Typically, eosinophil counts are inversely related to stress; however, they may increase as a result of parasitic infection or inflammation (Jain, 1986; Davis *et al.*, 2008). Eosinophil counts are therefore an effective means of differentiating infection and chronic stress as causes of elevated H:L ratios (Jain, 1986; Davis *et al.*, 2008). Similarly, monocyte counts may increase in order to combat parasitic and bacterial infections (Davis *et al.*, 2008). Considering that eosinophil counts were significantly higher in 2018, and monocytes in 2020, it is possible that a higher prevalence of infection in the colony was at least partially responsible for elevated H:L ratios in those years. However, no parasites or bacteria were observed in the blood smears, suggesting that any potential infections were not blood-born. Other causes such as injury may have caused inflammation or infection, which may have led to subsequent increases in eosinophils and monocytes (Davis *et al.*, 2008). However, infection is likely to have caused subsequent increases in the stress levels of affected individuals (Davis *et al.*, 2008), which may have been exacerbated further by sub-optimal environmental conditions, such as reduced food availability or un-favourable climatic conditions (Vleck *et al.*, 2000). Further understanding of how these environmental factors vary, as well as any relationship they may have with the health status of this species is needed in order to

definitively identify the causal processes behind interannual variability in the stress levels and overall health status of this seabird population.

Data pooled from all three years, as well as in 2019 alone, indicated that females were significantly heavier than males, consistent with studies elsewhere (Nelson, 1978). However, it is likely that a sex based weight difference was present in 2018 and 2020, but was not detected due to the relatively small difference in weight (in the region of 150g or approx. 6% of average body mass) and limited sample sizes. The lack of a significant sex difference in any blood parameters, in any of the three years, would suggest that there are similar pressures on both parents during chick-rearing. Despite evidence that male gannets brood and incubate chicks for longer, while females play a greater role in provisioning (Nelson, 1978, Montevecci *et al.,* 1984), both parents still must spend a sufficient amount of time incubating, brooding and chick/nest guarding in order to become sufficiently stressed. Such stress is most likely due to starvation, but exposure to aggression from conspecifics may also play a role. This lack of a sex based difference in blood parameters is in agreement with studies of northern gannets elsewhere (Brousseau-Fournier *et al.*, 2014).

While immature erythrocyte counts were found to be significantly higher in 2018, very few birds were found to have counts high enough to be indicative of anaemia; two individuals in 2018, one in 2019, and one in 2020. Microcyte counts did not differ between years, and similar to immature erythrocytes, few birds were above levels that would suggest they were suffering from anaemia; none in 2018, one bird in 2019, and two birds in 2020. Anaemia is known to occur as a result of blood loss in injured animals (Campbell, 1995), and thus its occurrence may be a result of the common intraspecific aggression that occurs between gannets at the colony. However, anaemia may also be caused by parasitism, disease, toxicosis, and other factors (Campbell, 1995; Jones, 2015) which cannot be conclusively ruled out. Despite finding no blood-borne parasites in the samples of any bird, the presence of other parasites in the colony capable of causing anaemia, such as ectoparasitic species including ticks (Norte *et al.,* 2013), is a possibility. To date, no research has investigated the occurrence of macro-parasitic species in the gannetry at Great Saltee, however the results of this study suggest that such work would be of value, in order to fully understand causes of interannual variability in the health status and physiological condition of this population. Additionally, although certain individuals were suffering from anaemia,

they were still attending chicks, and thus it is unlikely that such health defects were compromising parents' ability to raise young within the season. However, anaemia, disease, or other negative health effects could potentially have carry-over effects, for example, increasing risk of overwintering mortality or reducing condition to attempt breeding in following years (Newman *et al.,* 2007). Such effects could significantly impact the lifetime reproductive success of affected individuals. Future studies should consider attempting to re-sight birds which have had compromised health in previous years, in order to provide evidence of increased mortality due to such factors.

Breeding stage differences

Significantly poorer body condition of birds as well as elevated stress levels and evidence of inflammation in early chick-rearing suggests this stage of the breeding season is the most physiologically demanding. Body condition likely decreased during this stage as breeding birds are required to undergo periods of starvation while guarding their chicks during their partner's foraging trips, while a significant portion of the food they do obtain must also contribute to chick growth (Nelson, 1978; Nelson, 2002). Conversely, while incubating eggs, gannets have no need to sacrifice food in order to provision young, while in the late chick-rearing stage, the increased size of chicks makes them less vulnerable to predation, or aggression from other adult gannets (Nelson, 2002), enabling parents to forage simultaneously. Both factors will reduce the likelihood of nutritional stress (Vleck *et al.,* 2000). While sampling in September, it was noted that many well-developed chicks were unsupervised (*Personal observation*), supporting the hypothesis that parents are able to forage simultaneously later in the season. However, this may also be due to increased food demands of chicks that cannot be met by alternate foraging trips (Nelson, 2002). A lack of abandoned nests in early and late chick-rearing (*Personal observation*) suggests that, despite poor condition in early chick-rearing, birds were still successfully raising chicks. However, effects of poor body condition and high stress on overwintering mortality, and future reproductive ability of northern gannets are unknown. Stress levels and body condition have been shown to influence survival and reproductive success in many taxa (Atkinson and Ramsay, 1995; Milenkaya et al., 2015; Grilli et al., 2018), and some gannet populations have shown a reduction in annual survival in recent years (Gremillet et al., 2020). Assessments of health and condition in these populations may help to identify important links between breeding condition and subsequent

survival/reproduction rates. Such research could thus identify thresholds of poor condition or chronic stress beyond which mortality and population declines are likely to occur.

Chronic stress levels, indicated by H:L ratios, were significantly lower in late chick-rearing than earlier stages. Despite the poorer body condition of birds during early chick-rearing, chronic stress levels in this stage were not significantly different from incubation. In fact, chronic stress levels were not correlated with body condition at all, with H:L ratios actually being highest in the incubation stage, when birds body condition was best. Chronic stress levels are determined by a combination of factors (Davis *et al*., 2008), and one explanation for this absence of a correlation with body condition may be the additional effect of colony density on stress levels. An increase in the frequency of agonistic interactions at greater colony densities has been proposed to influence H:L ratios in seabirds (Vleck *et al.,* 2000). Given the aggression and territoriality of breeding gannets (Nelson, 2002), a denser colony in the incubation and early chick-rearing stages, when parents must also defend the nest site from prospecting birds, may result in a higher frequency of fights and disturbance. WBCs were significantly higher in the early chick-rearing stage, suggesting that there was a greater prevalence of inflammation among birds during this period (Jain, 1986; Latimer *et al.*, 1988; Davis *et al.*, 2008) which may have been caused by injury. However, the higher monocyte counts in this year compared to 2019 suggests it is also possible that the increased occurrence of inflammation may have been influenced by infection (Jain, 1986). Additionally, it is possible that variability in other factors between nesting stages, such as increased temperatures, could have contributed to the changing stress response of birds throughout the season (Vleck *et al*., 2000; Xu *et al.*, 2018). While no obvious differences in climate/temperature were noted between sampling periods the potential impact of various environmental factors on the condition of this gannet population should be the focus of future research.

Circulating corticosterone, a measurement of acute stress, has been related to short term, severe, stressors in birds, for example, predicting survival in Eurasian kestrel (*Falco tinnunculus*) nestlings (Muller *et l.,* 2011). Relationships between chronic and acute stress have also been observed, with for example, the downregulation of the acute stress response occurring in more chronically stressed individuals in European starlings (*Sturvus vulgaris)* (Rich and Romero, 2005). In the case of the current study

population, information regarding any such relationship would be useful. For instance, an improved understanding of between-stage variability in the magnitude of the stress response of gannets to acute stressors, could allow researchers to dictate when in the season sampling should occur. While blood was collected for hormone assays to quantify acute stress levels, laboratory closures and inaccessibility of necessary equipment due to the Covid-19 crisis prevented this from being conducted. Future studies investigating the relationship between corticosterone as an indicator of acute stress, and chronic stress, would be of value.

Increases in immature erythrocyte counts are known to occur during and after egg-laying in birds (Wagner *et al.,* 2008). Thus, elevated immature erythrocyte counts in females that had laid eggs may explain the significantly higher proportion of immature red blood cells during the incubation stage of this study. Additionally, anaemic birds were present in both the incubation and early chick-rearing stages of the nesting season, but not in the late chick-rearing stage. As only nineteen birds were caught in late chick-rearing, it is possible that a low prevalence of anaemic birds within the population would result in any anaemic individuals being missed, particularly if they are more likely to forage once the constraints of chick/nest guarding are lifted in order to recover condition.

Effects of fasting

Although the difference was not statistically significant, chronic stress levels of departing birds were, with the exception of one outlier, all high compared to returning ones. While the lack of significance is likely due to low statistical power caused by small sample size, the difference noted potentially reflects the stress caused by starvation (Gross and Siegel, 1986). In the case of two birds, blood samples were obtained both prior to departure and upon return from foraging trips. Bird 1432805 (Appendix A), whose H:L ratio decreased from 4.268 to 0.923, gained 0.2kg, suggesting that it fed successfully, and supporting the hypothesis that stress levels in these birds are at least partially reliant upon their ability to forage. Conversely, bird 1432813's H:L ratio actually increased marginally from 4.263 to 4.556. However, this individual was found to be suffering from microcytic anaemia, which most likely contributed significantly to its high stress levels. It's compromised health may also have also had a negative impact on this bird's ability to forage, as upon returning to

the colony this bird's weight was the same as on departure. Furthermore, using tracking data obtained from gannets caught in 2018 and 2019, no relationship was found between chronic stress levels and the amount of time birds spent at the nest between foraging trips. This provides further evidence that the stress levels of these birds are reliant not only upon nutrition, but upon a combination of factors, possibly including infection and injury. It should be noted however, that time spent at the nest inferred from foraging trips, did not correspond to the period prior to blood sampling, but rather after birds were blood sampled. Analysis therefore assumed that birds are relatively consistent in foraging trip durations. In gannets, although repeatability has been observed in bearing from the colony, foraging site use, and other at sea behaviours, significant variability has been observed in foraging trip durations (Hamer *et al.*, 2001; Hamer *et al.*, 2007). Therefore, in order to conduct more robust analysis on the relationship between time spent at the nest and chronic stress levels, future studies should sample birds upon return from foraging trips, and again at departure, after a known fasting period.

Conclusions

This study highlights the usefulness of haematological methods in identifying patterns in the physiological condition of breeding seabirds. Interannual variability in physiological condition is likely the result of wider ecosystem processes affecting the quality and abundance of food, while fasting and colony density are likely to influence physiological condition within breeding seasons. These findings suggest that the disparity in chronic stress levels in northern gannets noted between past studies (Brousseau-Fournier *et al.*, 2014; Malvat *et al.*, 2020) are likely due to differences in sampling times during the breeding season, as well as the use of departing birds that had likely been fasting. This highlights the importance of accounting for such variation when comparing studies or populations. Despite the poorer condition of some gannets, particularly in the early chick-rearing stage, it should be noted that birds were still successfully raising chicks. While there is no evidence that the Great Saltee gannet population is decreasing, future studies should investigate the potential for carry-over effects including increased overwintering mortality or reduced breeding success in subsequent years. Assessment of physiological health at the Rouzic colony could also help identify links between physiological condition and stress with the reduced survival noted at this location in recent years. The variability observed in

haematological parameters and physiological condition suggests a measurable individual response to environmental perturbation that may make this a more informative monitoring metric than breeding success, and a possible early indicator of population declines.

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APPENDICES

Appendix A – Raw data collected from blood counts in 2020.

Table A1: Measurements of haematological variables, of northern gannets caught in the 2020 nesting season on Great Saltee. I.E (%) = percentage of immature erythrocytes, Micro (%) = percentage of microcytes. Values indicating potential anaemia or immunocompromised individuals are highlighted in bold.

BTO ring	Sex	Stage	State	H:L	Eosino	Mono	WBC	I.E. (%)	Micro (%)
1432780	male	Incubation	Random	2.556	3	1	2800	**7.333**	1.667
1432783	female	Incubation	Random	1.174	6	2	2400	4.333	1
1432788	male	Incubation	Random	2.125	0	0	3200	6.333	0
1432789	female	Incubation	Random	1.222	3	3	3000	3.337	0
1432790	male	Incubation	Random	2	4	2	2400	4	0
1432791	female	Incubation	Random	0.222	4.167	4.167	2000	3.333	0
1432793	female	Incubation	Random	2.032	5	1	2600	**5.333**	0.333
1432795	male	Incubation	Random	0.613	3	2	3800	3.333	0
1432796	male	Incubation	Random	0.724	5	2	3600	4.667	0.333
1432797	female	Incubation	Random	4.556	6	1	5600	4	0.333
1432798	male	Incubation	Random	2.704	7	4	2400	3.667	0.333
1432799	male	Incubation	Random	3	6.667	0	1800	5.333	0
1432800	male	Early Chick	Ret	1.222	5	0	4800	4.333	0
1432801	female	Early Chick	Ret	2.571	4	2	3600	3.667	0.333
1432805	female	Early Chick	Ret	0.923	4	0	4200	4.333	0.333
1432807	female	Early Chick	Ret	1.439	6	5	2600	4	0
1432813	female	Early Chick	Ret	4.556	4	2	7400	3	7
1432803	male	Early Chick	Dep	3.348	5	0	2000	3	0
1432804	female	Early Chick	Dep	**6.692**	4	4	2800	3.667	0.333
1432805	female	Early Chick	Dep	4.263	4	3	2600	2.333	0
1432806	male	Early Chick	Dep	4.556	5	3	2600	2.333	0.667
1432808	male	Early Chick	Dep	**5.667**	6	1	2400	3.333	0
1432809	female	Early Chick	Dep	2.448	3	2	3200	3	0
1432812	Na	Early Chick	Dep	3.545	4	5	6000	3.667	0
1386153	Na	Early Chick	Dep	2.846	2	4	9000	3	0.333

1432813	female	Early Chick	Dep	4.263	6	**7**	**10800**	2.667	**5.667**
1432814	female	Early Chick	Dep	3.667	4	3	4000	2.333	0.333

BTO ring	**Sex**	**Stage**	**State**	**H:L**	**Eosino**	**Mono**	**WBC**	**I.E. (%)**	**Micro (%)**
1432815	male	Early Chick	Random	1.703	4	3	5000	3.333	0
1432817	male	Early Chick	Random	1.381	3	1	**10800**	2.333	0
1432818	male	Early Chick	Random	2.03	3	1	2400	1	0
1432819	male	Early Chick	Random	1.564	3	2	3000	3.667	0.333
1432824	male	Early Chick	Random	2.172	6	3	3000	3	0.333
1432825	male	Early Chick	Random	1.222	5	2	4600	2.333	1
1432826	female	Early Chick	Random	1.174	5	4	3600	2.667	0
1432827	male	Early Chick	Random	2.846	4	2	4200	4.333	1
1432828	female	Early Chick	Random	1.439	4	3	8000	2.333	0.6667
1432829	male	Early Chick	Random	1.632	4	2	3200	3.333	2.333
1432830	male	Early Chick	Random	1.941	5	5	3600	4	**6.667**
1432841	female	Late Chick	Random	0.266	3	4	3600	3.667	0
1432842	female	Late Chick	Random	2.03	4	4	1800	5.667	0
1432843	male	Late Chick	Random	0.923	2	1	3800	3	0
1432844	female	Late Chick	Random	1.211	**11.76**	5.99	3200	3.667	0.667
1432845	female	Late Chick	Random	0.163	4	5	1800	5	0.667
1432846	female	Late Chick	Random	0.25	3	3	5000	3	0.333
1432847	female	Late Chick	Random	0.25	1	1	1600	3	0.333
1432848	male	Late Chick	Random	0.515	3	2	2200	4	0
1432849	female	Late Chick	Random	1.632	5	0	5400	4	0
1432850	female	Late Chick	Random	1.703	4	0	1800	2.333	0
1432951	male	Late Chick	Random	0.439	3	1	3200	3	0
1432952	female	Late Chick	Random	0.471	3	1	2600	2.666	0.666
1432953	female	Late Chick	Random	0.639	4	4	1600	3	0
1432954	male	Late Chick	Random	0.887	3	2	3000	2.333	0
1432956	female	Late Chick	Random	1.174	3	2	3600	3.333	0.333
1432957	female	Late Chick	Random	0.923	2	1	4600	4.333	0
1432958	male	Late Chick	Random	0.667	2	**6**	1800	3.333	0
1432959	male	Late Chick	Random	0.563	**7**	1	2400	4.667	0.667

Appendix B – Blood cells

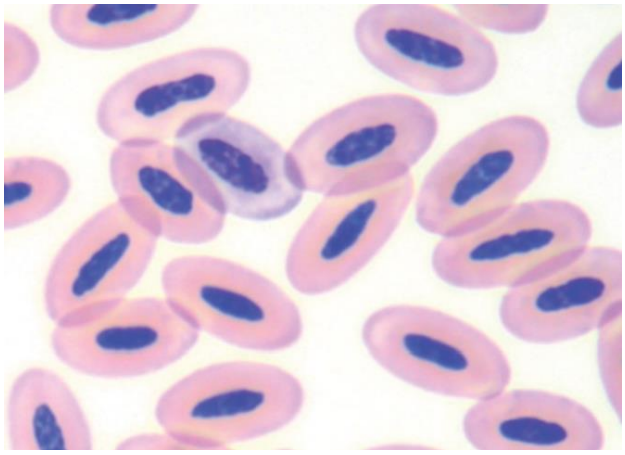


Fig. C1: Mature erythrocytes (Clark *et al.*, 2009).

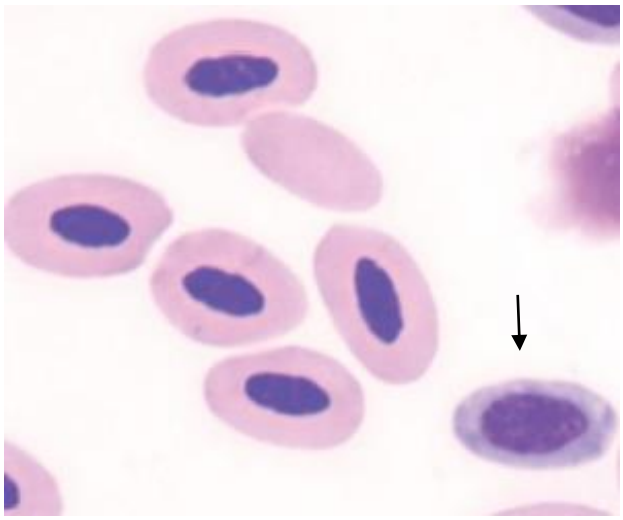


Fig. C2: Immature erythrocyte (Arrow) (Clark *et al.*, 2009).

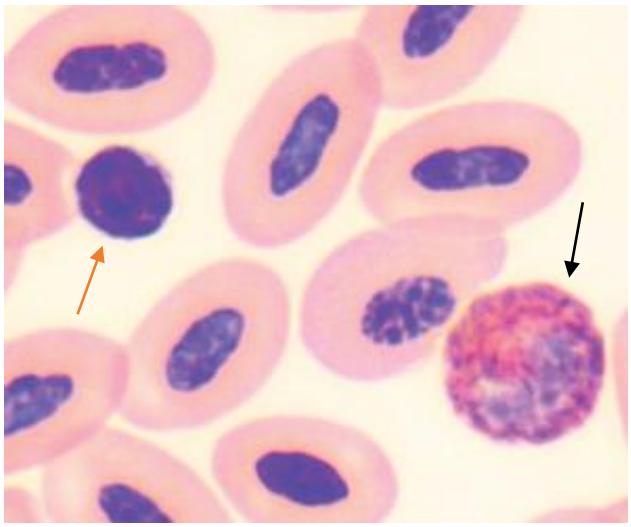


Fig. C3: Heterophil (Black arrow) and lymphocyte (Red arrow).

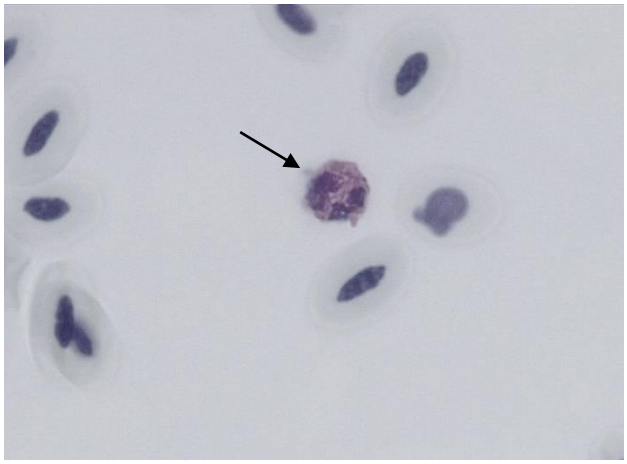


Fig. C4: Eosinophil. Image taken at 100x magnification with oil immersion.

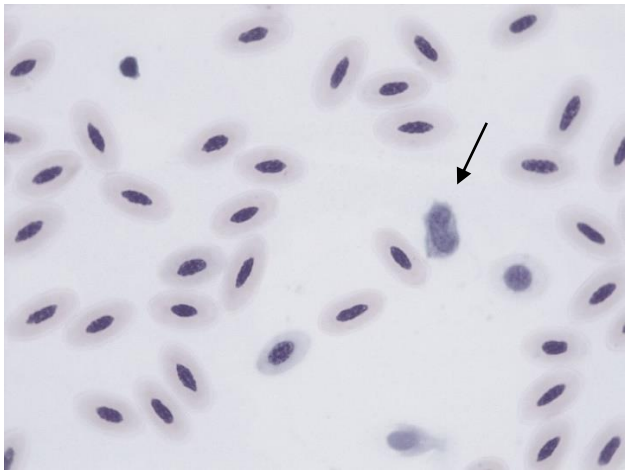


Fig. C5: Monocyte. Image taken at 100x magnification with oil immersion.

Appendix B – Comparisons of blood parameters between sexes

Table A2: P values of Mann Whitney U tests comparing blood parameters between sexes in 2019 and 2020, as well as using pooled data from all years.

	Years		
Parameter	**Pooled**	**2019**	**2020**
H:L	0.74	0.73	0.9
WBC	0.76	0.33	0.38
Monocyte	0.37	0.76	0.26
Eosinophil	0.81	0.62	0.38
I.E	0.22	0.14	0.43
Microcyte	0.61	0.07	0.75