Diving efficiency at depth and pre-breeding foraging effort increase with hemoglobin levels in gentoo penguins

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Abstract

Individual differences in oxygen storage and carrying capacity have been associated with differences in foraging success and fitness-related traits. For air-breathing aquatic animals, such differences can limit diving ability, and thus resource access. As many seabirds deplete their energy reserves during breeding and moulting periods, winter foraging efficiency can influence their breeding behaviour and success. Using gentoo penguins (*Pygoscelis papua*), we investigate (1) if inter-individual variation in diving efficiency is associated with indices of oxygen storage and carrying capacity (hemoglobin, hematocrit, body mass), and (2) if an individual's pre-breeding foraging effort is associated with these oxygen indices and breeding status. Hemoglobin was positively correlated with diving efficiency, particularly for deeper dives, and only penguins with high hemoglobin levels frequently dove at depth ≥ 140m. However, pre-breeding foraging effort increased with hemoglobin levels, a relationship likely explained by the fact that reaching deep offshore waters requires additional travelling compared to nearshore foraging. The relationship with hematocrit was non-linear, suggesting that commonly-used analyses may not be appropriate for this index. We found that early-laying penguins spent less time at sea prior to nesting than non-breeding penguins. Our study suggests that diving ability, and thus likely access to resources, is linked to aerobic capacity, and that lower foraging effort is associated with early laying. These associations suggest that anthropogenic changes (e.g., an increase in shallow water fisheries) may have differing impacts on individuals. Further understanding these links could help the conservation of diving species.

Running page head: Diving efficiency and effort in penguins

Keywords: Diving efficiency, oxygen storage and carrying capacity, breeding participation, gentoo penguin, hemoglobin, hematocrit, body mass, time-depth recorder

1. Introduction

Intra-specific variation in foraging and movement behaviour is ubiquitous, and can have important ecological consequences at both the individual and population levels (Bolnick et al. 2003, Ceia & Ramos 2015, Phillips et al. 2017, Shaw 2020). Such variation can be caused by complex relationships with the environment, intra- and inter-specific interactions (e.g., competition), and physiological differences (Shaw 2020). Differences in oxygen storage and carrying capacity can be particularly important, as they have been associated with variation in movement behaviour, habitat selection, and fitness-related traits such as breeding participation and arrival at breeding grounds (Crossin et al. 2013, 2015, Minias 2015). For diving animals, oxygen storage and carrying capacity can limit diving ability, and thus access to resources may be physiologically restricted (Roncon et al. 2018). For such animals, the rate of mass gain while foraging has been linked to indices of aerobic capacity (Crossin et al. 2015).

Diving animals have a range of adaptations to maximize their aerobic capacity and extend their breath-holding capability (Roncon et al. 2018). Hemoglobin is an oxygen carrying protein present in red blood cells, and thus its level is a crucial determinant of the rate of oxygen delivered to tissues (Minias 2015). Hemoglobin levels are often used as a proxy of aerobic capacity and physiological conditions in birds (Minias 2015), and is elevated in bird species that dive (Minias 2020). Prolonged breath-holding capacity is often linked to oxygen storage. Oxygen stores typically increase with hemoglobin concentration, blood volume, myoglobin concentration in skeletal muscles, muscle mass, and lung volume (Mirceta et al. 2013, Roncon et al. 2018). As larger individuals possess more blood and muscle, body mass is often positively associated with oxygen storage capacity (Ponganis & Kooyman 2000, Cook et al. 2013, Mirceta et al. 2013, Polito et al. 2015, Camprasse et al. 2017). As such, total blood hemoglobin concentration

(referred as Hb), hematocrit (percentage of red blood cell in blood, referred as Hct), and body mass can be important determinants of a breath-holding diver's physiological performance and can limit an animal's diving ability (Crossin et al. 2015, Chimienti et al. 2017).

Seabirds display high levels of individual variation in foraging and movement (Ceia & Ramos 2015, Phillips et al. 2017), and diving seabirds provide a unique opportunity to link differences in oxygen storage and carrying capacity to variation in diving and movement behaviour, and ultimately to differential breeding participation. Many seabirds severely deplete their energy reserves during breeding and moulting periods and must replenish these reserves by foraging intensively in winter (Sorensen et al. 2009, Crossin & Williams 2016). Consequently, winter foraging efficiency and success, as well as carryover effects, can influence multiple aspects of an individual bird's breeding capacity and behaviour (Alves et al. 2013, Crossin & Williams 2016). For example, individuals that breed earlier spend less time foraging in winter, and thus are assumed to be more efficient foragers (Daunt et al. 2006). Understanding how individuals vary in their foraging ability and behaviour as they prepare for breeding can help assess the possible impacts natural and anthropogenic ecosystem changes can have on populations (dit Durell 2000, Bearhop et al. 2006, Cury et al. 2011, Phillips et al. 2017).

Here, using a population of gentoo penguin (*Pygoscelis papua*), we explore how individual variation in winter diving behaviour relates to indices of oxygen storage and carrying capacity, and how these relate to their breeding status. We use gentoo penguin as a case study because they show considerable individual variation in winter movement behaviour (Baylis et al. 2021). They have been identified as Type B generalists, meaning that gentoo penguins exist as generalist populations composed of specialized individuals (Polito et al. 2015, Camprasse et al. 2017, Handley et al. 2017, Herman et al. 2017). Gentoo penguins consume a wide variety of

locally available prey found at a range of depths down to approximately 200 m (Tanton et al. 2004, Thiebot et al. 2011, Camprasse et al. 2017). There is a high likelihood of intraspecific competition for prey around colonies, as gentoo penguins are not migratory and forage close-to-shore year-round (Clausen et al. 2005, Bearhop et al. 2006, Kokubun et al. 2010). The number of breeding pairs in the Falkland Islands fluctuates drastically, with changes of 50 % occurring in a single year (Pistorius et al. 2010, Stanworth & Crofts 2019). Changes in population breeding success are thought to be linked to prey availability (Pistorius et al. 2010) and broad-scale climatic variation (Baylis et al. 2012).

To understand the link between oxygen storage and carrying capacity, pre-breeding diving and foraging behaviour, and breeding activity, we investigate (1) if diving efficiency is associated with indices of oxygen storage and carrying capacity (Hb, Hct, body mass), and (2) if pre-breeding foraging effort differs between individuals based on their breeding status and indices of oxygen storage and carrying capacity. We use time-depth recorders (TDRs) to quantify diving efficiency and foraging effort. As attaching such devices to animals can add drag and affect their performance, behaviour, and reproductive success (Taylor et al. 2001, Beaulieu et al. 2010, van der Hoop et al. 2014, Wilson et al. 2015), we also evaluate whether tagged individuals differ from control birds in terms of breeding activity, Hb, Hct, and mass.

2. Methods

2.1 Data collection

We captured 66 adult gentoo penguins post-moult (April 4 – 10, 2018) from two colonies in the Falkland Islands: Rookery Sands, Race Point (51.4345°S, 59.1081°W, n=33) and Tamar Point, Pebble Island (51.3241°S, 59.4523°W, n=33, Fig. 1). We weighed each captured penguin

using a 10 kg Pesola scale, took a blood sample (< 1.5 ml) from the left brachial vein using heparinized syringes fitted with 25-gauge needles, and marked them with black hair dye (L'Oreal Preference) to facilitate re-identification. We equipped each penguin with a Sirtrack K2G 173A SWS KIWISAT 202B (34g, 55 x 27 x17 mm, Sirtrack, Havelock North, New Zealand) and Lotek LAT1800 TDRs (13.6 g, 62 x 13 x 13 mm, Lotek Wireless Inc, St. John's, Canada). We attached the two devices to midline back feathers using TESA® tape (Beiersdorf AG, GmbH, Hamburg, Germany) and cyanoacrylate glue on tape seams.

During the breeding season (September 27 - October 21, 2018), we recaptured 35 of the previously tagged penguins and 35 unmarked penguins, the latter serving as controls in our analysis of the potential effects of tagging. As described above, we weighed each penguin, took a blood sample, and marked the control bird with hair dye. We retrieved any devices still attached to the penguins. Only one of the recaptured penguins had an Argos tag still attached, but 21 had TDR devices still attached (7 Race Point, 14 Pebble Island). We note that we were unable to collect sufficient blood samples from a few birds, resulting in only 19 of the individuals with TDRs also having blood data from the breeding period (5 Race Point, 14 Pebble Island).

The research was conducted under Falkland Islands Scientific Research Licence (R12/2017) and conformed to guidelines from the Canadian Committee on Animal Care (University of British Columbia Animal Care Permit A17-0243 and Dalhousie University Animal Care Permit 17-100).

2.2 Indices of oxygen storage and carrying capacity

Using the blood samples from the breeding period, we used hematocrit (Hct) and hemoglobin (Hb) as physiological indicators of blood oxygen storage and carrying capacity. As Hct is based on blood cytology and Hb is based on blood biochemistry, each measure provides

different insights into the aerobic capacity of individuals (Fair et al. 2007, Kaliński et al. 2011, Minias 2015). Hct (percent packed red blood cell volume) was determined from fresh whole blood in heparinized capillary tubes centrifuged for 5 m (Centrifuge; Brinkmann Instruments, Ontario, Canada) at 10000 g and measured using digital callipers (± 0.01 mm). Hb (total blood hemoglobin concentration in g/dL) was determined using the cyanomethemoglobin method (Drabkin & Austin 1932), where 5 µl of fresh whole blood was pipetted (Gilson Pipetman P2; Gilson Medical Electronics, France) into 1.25 ml Drabkin's reagent (D541-6V, Brij 35 solution B4184-100ml; Sigma-Aldrich, Missouri, US). We ran triplicate samples and read absorbance at 540 nm with a microplate spectrophotometer (BioTek PowerWave 340, Vermont, USA). The inter-assay coefficient of variation was 1.70 % between plates.

We used the body mass measured during the breeding period as a proxy of oxygen storage capacity. We did not scale body mass to structural body size to generate a morphometric index because (1) oxygen stores (e.g., blood volume) and dive duration have been shown to increase with body mass itself (e.g., Hassrick et al. 2010), and (2) mass alone has been shown to be a more reliable measure of body condition in birds (Schamber et al. 2009, Labocha & Hayes 2012). We identified the sex of the individuals by sending blood samples to Viaguard (https://www.accu-metrics.com/animal-services/p/avian-dna-bird-sexing-test), which performs DNA testing. We note that sex results were unavailable for three of the 19 penguins for which we had TDR and blood data.

2.3 Dive data processing

Lotek TDR tags recorded depth (0.25 m resolution), temperature (0.01°C resolution), and wet/dry state at 1 s intervals. We wanted to focus on the time period that could be linked to the

blood samples. Since the approximate rate of erythropoiesis in birds is 30 – 42 days (Rodnan et al. 1956), we limited the analyses to dives made within 40 days prior to recapture. Given that the last transmission from the Argos tags was August 16, 2018, well before this threshold, and very few tags transmitted locations past May, we do not consider the Argos data further.

To characterise the diving behaviour of foraging individuals, we extracted the dive metrics from the TDR series using the software Divebomb (1.0.7, Nunes 2019) in Python (3.4.10, Python Software Foundation 2019). We first corrected the dive data for drift using a zero offset (Luque & Fried 2011), and we identified the following dive metrics: start of the dive, dive duration, maximum depth, bottom time, and post-dive surface interval. To remove the shallow dives most likely associated with travelling, we discarded all dives shallower than 5 m (Kokubun et al. 2010, Lee et al. 2015, Carpenter-Kling et al. 2017). Following Kokubun et al. (2010) and Lee et al. (2015), we eliminated the inclusion of long surface periods unlikely to be associated with active foraging by only considering dives with post-dive surface interval < 200 s. Through a visual assessment of 100 random dives across all individuals, we quantified that Divebomb gave precise measurements of decent, bottom, accent, and surface phases of dives 94 % of the time. Divebomb produced minor errors distinguishing the bottom phase in cases where undulations in depth occurred over the transition from decent to accent phases.

2.4 Behavioural observations

To assess the reproductive status of penguins, teams of two observers monitored the breeding behaviour of marked birds. Individual presence at the colony was recorded daily at Race Point from September 27, 2018, to October 24, 2018, and Pebble Island from September 29, 2018, to October 24, 2018, through colony scans and monitoring arriving individuals until

dusk (13h00 – 19h00). Focal follows were conducted for > 5 minutes on individuals observed in the colony and lasted until the presence or absence of eggs in an occupied nest could be confirmed. The pair bonding behaviours we monitored included nest building, copulation, pair calling, and pair bowing (Williams 1995). Due to a later than predicted initiation of breeding in 2018, we could not acquire the lay date for most penguins. Thus, we classified penguins into three categories of reproductive status: non-breeding, breeding, and early-laying individuals. Penguins classified as non-breeders were found most often at different locations than nesting penguins, typically towards the edge of colonies, and rarely displayed pair-bonding behaviours. Penguins categorized as breeders were observed conducting pair-bonding behaviours at stable nest site locations. Early laying penguins were observed to be incubating at least one egg prior to October 24, 2018. We note that none of the penguins had laid an egg at Race point at the time of departure of field observers.

2.5 Linking diving efficiency to oxygen storage and carrying capacity indices

We used generalized additive mixed models (GAMM) to determine the relationship between diving efficiency at different maximum depths and our three previously described indices of oxygen storage and carrying capacity (Hb, Hct and body mass). We defined dive efficiency as $\frac{bottom\ time}{dive\ duration + post\ dive\ surface\ interval}$ (Simeone & Wilson 2003, Lescroël & Bost 2005, Lee et al. 2015). Using the R package mgcv (Wood 2011), we used a thin-plate regression spline to model the non-linear relationship between dive efficiency and maximum dive depth. To investigate the potential interactions between maximum depth and the indices of oxygen storage and carrying capacity (Hb, Hct, and mass), we included a tensor product term (with cubic regression spline basis) between maximum depth and each index. To be able to decompose the

interactions with maximum depth from the potential main effects of each index, we included a separate tensor product and a thin-plate regression spline for the relationship with dive efficiency and each index (i.e., used s() + ti() in mgcv). To account for potential biological or behavioural differences in sex and colony, we first included both terms as fixed effects in our GAMM and assessed their importance. As sex was not deemed an important covariate for this analysis (see Supplement 1) and three individuals were missing sex information, our final analysis did not include sex and included these three individuals. We also included a random effect on the intercept to account for the fact that we had multiple dives per penguin.

The diving data were highly temporally autocorrelated, and fitting GAMMs with complex autoregressive-moving-average (ARMA) functions are computationally demanding. For example, running our full GAMMs with ARMA functions took multiple days to run on an iMac 3.6 GHz 10-Core Intel Core i9, and we ran into multiple convergence problems. We found that we had to sub-sample one dive every 70 dives to completely remove the autocorrelation in the residuals of the full model, which resulted in having on average 85 dives per individuals. For model selection, we used the shrinkage method with a double penalty (i.e., select = “TRUE” in mgcv; Marra & Wood 2011), which penalizes both unnecessary wiggliness and linear effect of a covariate. This approach has many advantages over stepwise variable selection and model subset selection, such as exploring a larger model space, and has been previously used to select covariates in diving models (Marra & Wood 2011, Photopoulou et al. 2014).

There were some correlation and concurvity between the covariates. As such, we explore whether, and how, the results change when creating GAMMs with only one of the important oxygen indices at a time. See Supplement 2 for details.

As post-hoc analyses, we further explored how the deep diving behaviour of individuals changed in relation to the oxygen storage and carrying capacity indices deemed important in the GAMM above. As some individuals with low oxygen storage and carrying capacity may reduce, or altogether forgo, doing deep dives (here defined as ≥ 140m), we used simple generalized additive models (GAMs) to assess whether the proportion of deep dives each individual performed changed in relation to each of the selected oxygen index. As high dive efficiency can be achieved by either extending the bottom time or reducing surface recovery between dives, we used new GAMMs to explore how bottom time and post-surface dive intervals for deep dives changed in relation to each of the selected oxygen index. For these new GAMMS, we looked at the oxygen indices separately, as the smaller sample size did not allow to have both in the models at once. We included penguin id as a random effect to account for the fact that we have more than one dive per individual.

2.6 Associating measures of foraging effort with breeding status and oxygen storage and carrying capacity indices

To determine if pre-breeding dive behaviour changes between individuals based on their breeding status and oxygen storage and carrying capacity, we investigated three measures of foraging effort: mean trip duration, time at sea, and diving effort. To estimate mean trip duration and time at sea, we first identified trips at sea using the binary wet-dry data collected with the saltwater sensor of the TDRs. To account for occasional jumps at sea, which results in brief recordings of dry conditions, we averaged the wet-dry data using a 10-minute rolling window. We classified any window value with an average > 0 as wet. We terminated a trip just before a dry period lasting at least one hour, and the subsequent trip began once a new wet value was recorded. The mean trip duration was the average duration of trips for the 40 days prior to

recapture, while the time spent at sea was the sum of all trip durations within that period. To measure the general diving effort, we calculated the total amount of vertical distance travelled for the time spent at sea ($\sum maximum\ \ depth\ (km)\ \times\ 2$; similar to Lescroël & Bost 2005, Booth et al. 2018).

Using multiple linear regressions, we investigated whether each of these measures of foraging effort was associated with breeding status, sex, and the three oxygen storage and carrying capacity indices (Hb, Hct, mass). We only included individuals from Pebble Island since none of the Race Point individuals laid eggs before the end of the field season. To identify the covariates that best explained the data, we fitted models with all possible combinations of these covariates. Given the small sample size (n=13), we compared the models using the Akaike Information Criterion with correction for small sample size (AICc). We considered models that outperformed the null model (model with no covariates) and had ΔAICc < 4 from the best model (Burnham and Anderson 2002). Next, we used Wilcoxon rank sum tests to assess whether there were differences in oxygen indices across sex and breeding status. We used a Bonferroni correction to account for the multiple comparisons.

2.7 Assessing the potential effects of tagging

To limit the potential influence of our tags on the behaviour, energy expenditure, or condition of the animals (e.g., Wilson et al. 1986, Vandenabeele et al. 2015), we minimized drag by using tags with a streamlined design and attaching them in an appropriate caudal position (Bannasch et al. 1994). Furthermore, the overall weight of devices was < 1 % of the average body mass recorded, and similar devices have not affected the diving behaviour of gentoo penguins (Kokubun et al. 2010). However, especially because we deployed tags for an extended period, we wanted to assess whether the data for the tagged penguin were representative of the

untagged animals and look for signs of tagging effects. To do so, we compared the physiology at breeding and breeding status of tagged to untagged penguins. Specifically, we used two-sample t-tests to compare Hb, Hct, and mass at breeding. For the reproductive status, we used χ^2 goodness-of-fit tests, and because of the small sample size we estimated the p-value using a Monte Carlo method (Hope 1968). We note that we were unable to sample sufficient blood for three of the recaptured penguins, as well as unable to measure Hct for one untagged penguin and Hb for another untagged individual. Thus, while the mass comparison included 35 tagged, and 35 untagged individuals, the comparisons for Hct and Hb included only 33 tagged and 34 untagged individuals. In addition, for the comparison of reproductive status, we focussed only on penguins from Pebble Island since none of the penguins at Race Point laid an egg before the end of our field season. Finally, we also quantify the proportion of tagged penguins that increased in mass between capture and recapture and the average weight gain during that period.

All data analyses were performed using R (4.2.1, R Core Team 2022). Values presented in the results are mean ± SD unless otherwise stated, and statistical tests were considered significant at a 0.05 level.

3. Results

3.1 Relationships between diving efficiency and indices of oxygen storage and carrying capacity

The dive efficiency of the penguins in our sample was explained through complex interactions with maximum depth and both Hb and Hct (Table 1, Fig. 2). Our GAMM indicated that only the maximum depth of a dive, and its interaction with Hb and Hct, and a colony effect were important in explaining dive efficiency (Table 1).

Dive efficiency increased at medium depth followed by a decline at deeper depth (Fig. 2A) and the interaction between maximum depth and Hb showed that dive efficiency primarily increased with Hb for deeper dives (Fig. 2B). Post-hoc analyses revealed that this increase in dive efficiency with Hb at deeper depth is primarily explained by an increase in bottom time rather than a decrease in the post-dive surface interval (Fig. 3B-C, Supplement 3). In addition, we found that the proportion of deep dives (≥ 140 m) made by an individual increased with Hb (Fig. 3A, Supplement 3).

The non-linear interaction between maximum depth and Hct was more complex, with multiple and less easily interpretable peaks in dive efficiency across Hct values (Fig. 2C). The post-hoc analyses revealed that neither bottom time, post-dive surface interval, nor the proportion of deep dives showed a significant relationship with Hct (see Supplement 3).

Various aspects beyond dive efficiency varied between individuals. For example, the maximum depth reached by individuals during the 40 days ranged from 74.8 to 217.8 m, and trips at sea ranged from less than one day to 24 days. We provide a general description of dive characteristics, foraging effort measures, and oxygen storage and carrying capacity indices in Supplement 4.

3.2 Foraging effort relationships with oxygen storage and carrying capacity indices, sex, and reproductive status

Two pre-breeding foraging effort measures were associated with oxygen storage and carrying capacity indices, sex, and reproductive status (Fig. 4, Table 2). The best model for diving effort indicated a positive relationship between pre-breeding diving effort and Hb, and the best model for time at sea suggested that early-laying penguins spent less time at sea prior to capture than breeding and non-breeding penguins (Fig 4, Table 2, Supplement 5). However,

many other models for these two measures outperformed the null models and had ΔAICc < 4 (Table 2). The presence of many competitive models is likely explained by the small sample size and some of the relationships we observed between covariates. For example, given that males in our samples have a significantly lower average Hg level than females (W = 34, p-value = 0.045; Fig. 5A), it is not surprising that the second-best model showed that males displayed less diving effort than females. Similarly, early-laying penguins have, on average, a lower Hb level than non-breeding penguins (Fig. 5B). Although this difference is not significant (W = 15, p-value = 0.057), it may contribute to the fact that the second-best model for time at sea did not include breeding status but included a positive relationship with Hb. Average trip duration had the null model as best model (Table 2), and thus was not considered further.

3.3 Potential effects of tagging

Almost all (32/35) tagged penguins recaptured gained weight during the winter, with an average gain of 1.1 kg ± 0.7. However, on average the tagged penguins were slightly, but significantly, lighter at recapture than the untagged penguins (tagged penguins: 6.9 kg ± 0.6, untagged penguins: 7.3 kg ± 0.5; $t_{66.498}$ = 2.812, p-value = 0.006). We did not find any other significant differences between tagged and untagged penguins. Their Hb and Hct values were similar (Hb: $t_{62.863}$ = -0.524, p-value = 0.774; Hct: $t_{63.633}$ = 1.480, p-value = 0.144), and their breeding status did not differ significantly (χ^2 = 0.866, simulated p-value = 0.712). However, given the small sample size for the breeding status analysis (tagged =18, untagged = 19), we note that, compared to the untagged penguins, there were more tagged penguins that did not participate in breeding and fewer tagged penguins that laid early (Supplement 2). Overall, our results suggest that it is unlikely the relationships we found with blood oxygen storage and

carrying capacity indices (i.e., Hb and Hct) are affected by tagging, but that tagging can reduce the mass of the penguins and may affect their behaviour. See Supplement 6 for more details.

4. Discussion

Our results show that the diving efficiency and pre-breeding diving effort of gentoo penguin were related with Hb and Hct. The relationships with Hb were amongst the strongest, with efficiency of deep dives (≥ 140m) increasing with Hb level. This increase in efficiency can be explained by the fact that Hb limits the total amount of oxygen able to be carried by the blood (Minias 2015, Roncon et al. 2018). Of the factors that could have affected dive efficiency, higher Hb was primarily associated with an increase in the bottom time of the dive rather than reduced recovery time (i.e., no decrease in post-dive surface interval with increased Hb levels were observed). Gains in dive efficiency, and associated increased time at the bottom, are likely important to gentoo penguins, as they increase the time available to search for and capture prey during foraging dives. As prey are almost always encountered during the bottom phase of dives (Simeone & Wilson 2003, Takahashi et al. 2004, 2008, Kokubun et al. 2010), increased bottom time translates into time spent where prey is most likely to be encountered. For dives below 140 m, penguins with high Hb (20-31 g/dL) increased their mean bottom phase by 22 % compared with penguins with low Hb (10-20 g/dL). Such an increase could impact foraging success.

Proportion of deep dives performed by penguins increased with Hb levels, with only individuals with high Hb levels regularly diving 140 m or deeper. Pre-breeding diving effort also increased with Hb levels, suggesting that individuals with high Hb levels may use a deep diving strategy that consequently requires longer horizontal travel time in order to reach deep water further from shore. Such individuals are accessing resources only available at deep depths,

giving them access to a wider variety of prey items and potentially decreasing intraspecific competition (e.g., Cimino et al. 2016). Similar findings have been documented in other species. For example, macaroni penguins (*Eudyptes chrysolophus*) forage in habitats within their aerobic capacity (Crossin et al. 2015), and Hb is correlated with higher-quality diets (reviewed in Minias 2015).

Dive efficiency displayed a complex non-linear relationship with Hct levels and Hct was a covariate in some highly ranked models associated with diving effort and time at sea. The complexity of this non-linear relationship, and the fact that Hct was not in the best linear model of any of the foraging effort measures, could be associated with the counteracting effects of high Hct levels. While a high Hct level is associated with a higher number of oxygen-carrying red blood cells, it is also linked to increased blood viscosity, and thus increased cardiac workload or decreased blood flow (Birchard 1997, Schuler et al. 2010, Reinhart 2016). In addition, optimum Hct levels can be affected by factors such as age and disease (Reinhart 2016, Johnstone et al. 2017). In research employing Hct as an index of body condition, higher values are often assumed to indicate better physiological performance (reviewed in Fair et al. 2007, Minias 2015). In contrast, our results suggest that Hct has a complex non-linear relationship with dive efficiency and that higher Hct may not always result in higher dive efficiency. The use of Hct as a physiological indicator has been criticized due to inconsistent relationships with performance and fitness-related traits in many species (reviewed in Fair et al. 2007, Minias 2015, Johnstone et al. 2017). For example, intermediate, rather than high Hct levels were linked to maximum longevity and recruitment rate in house wrens (*Troglodytes aedon*, Bowers et al. 2014). Our results suggest that, while Hct likely relates to diving ability, linear relationships are unlikely to

capture the complex relationship between these variables, and that non-linear models should be used whenever the sample size allows.

While values of both Hb and Hct aligned with previously reported values for gentoo penguins (20.3 ± 4.9 g/dL and 49.1 ± 4.5 % in our study; 20.7 ± 1.6 g/dL and 50 ± 4 % in Ibañez et al. 2015), they were not closely correlated to one another (see Supplements 2-4). Because mature red blood cells have similar amounts of Hb, we expected a correlation between Hct and Hb. However, ecological stressors can affect these indices differently (Bańbura et al. 2007, Wagner et al. 2008, Johnstone et al. 2017), and weak correlations have been documented in other seabird species (e.g., Kaliński et al. 2011). While some anemic birds with low Hb concentrations may be unable to improve their condition, some will actively regenerate red blood cells (Jaensch & Clark 2004, Fair et al. 2007, Campbell et al. 2010). In such cases, whether Hb and Hct will remain correlated will be affected by erythropoiesis. Erythropoiesis increases the number of immature red blood cells, which are larger and initially have lower Hb content, disproportionately increasing Hct but not Hb (Wagner et al. 2008, Campbell et al. 2010, Johnstone et al. 2017). We identified one individual that may have demonstrated evidence of compromised health. This non-breeding penguin had the lowest Hb and yet the fourth highest Hct. Anemia can be associated with additional health conditions, such as heavy parasite loads or severe injury (Vleck et al. 2000, Jaensch & Clark 2004, Fair et al. 2007, Clark 2015). Despite showing no apparent signs of injury at the time of re-capture, this anemic individual was one of the only individuals that lost mass between April and October. Such an example supports experimental designs that measure both Hb and Hct when assessing individual health (Johnstone et al. 2017).

According to optimal foraging theory, penguins maximize their bottom time and minimize energy spent travelling down to where prey is located (Stephens & Krebs 1986, Wilson et al. 2010, Zimmer et al. 2010). As such, shallow dives should be preferred, requiring less travel time and energy to swim down to depth (Shepard et al. 2009, Zimmer et al. 2010). Deeper dives are only profitable when prey capture rates, size, or caloric value outweigh the energetic costs of diving (Sala et al. 2014b). In our data, peak dive efficiency occurred in shallow foraging habitat at approximately 60 m deep (Fig. 2A), not far from previously reported values (25-30 m; Lee et al. 2015). In line with theory, this putative optimal depth (60 m) was repeatedly visited by penguins (see Supplement 4). However, likely to reach energy-rich prey, many penguins foraged far deeper than this putative optimal depth (maximum depth reached by individuals: 166 m ± 29).

Diet studies suggest that deeper dives may provide greater access to larger prey that may be more valuable to gentoo penguins than the smaller prey in shallower habitat. Clausen et al. (2005) compared the diet of gentoo penguins in colonies around the Falkland Islands with surveys of prey abundance during the 2000 pre-breeding season. They highlight preferential foraging of squid and fish over krill, which has also been highlighted in the historical diet of gentoo penguins (McMahon et al. 2019). Prey likely to be essential for Pebble Island and Race Point penguins includes bentho-demersal lobster krill (*Munida gregaria*), found at depths 26-116m, and Patagonian squid (*Doryteuthis gahi*), found at depths 60-96m, both of which were in high abundance close to the colonies in 2000 (Clausen et al. 2005). A deeper squid species (*Moroteuthopsis Ingens*), found at depths 100-166m, and various rock cod species (*Patagontothen spp.*), found at depths 40m-205m, are abundant further offshore and were found in their diet in 2000 (Clausen et al. 2005). In a more recent assessment of prey, rock cod were

found to be a principal prey item for gentoo penguins in the Falkland Islands (Handley et al. 2016). Generally, for marine species, prey size increases with depth (Masello et al. 2010, Miller et al. 2010).

Using dive efficiency as a measure of diving abilities implicitly assumes that animals typically dive to their aerobic dive limit and does not account for the numerous factors influencing dive time and efficiency. For example, predators adapt foraging strategies based on prey conditions (e.g., Magellanic Penguins, *Spheniscus magellanicus*; Sala et al. 2014a) and may abandon a dive early in poor prey conditions (e.g., Thums et al. 2013, Viviant et al. 2016). Such behavioural factors likely explain the low dive efficiency at shallow depths. However, deep diving is more energetically costly to undertake (Shepard et al. 2009, Zimmer et al. 2010). As such, oxygen storage and carrying capacity are more likely to influence the behaviour of animals foraging at deeper depths (Thompson & Fedak 2001, Viviant et al. 2016) and may explain why the relationship with Hb is more apparent at depth.

Gentoo penguins that laid early spent significantly less time at sea than non-breeding penguins, a difference potentially indicative of having high breeding obligations and higher foraging efficiency. Participating in breeding compels individuals to defend nest locations and partake in pair-bonding behaviours, requiring many species of penguins to fast during this time (Williams 1995). While gentoo penguins do not fast during breeding, they increase their time on land to maintain a nest site for approximately two weeks before egg-laying (Black 2016). All monitored gentoo penguins, except one non-breeder, were captured at least 11 days (14.5 ± 2.2 days, September 30 to October 07) before eggs were first seen in the colony (October 18). Thus, while early-laying penguins may have been maintaining nest sites for a few days at the end of the

TDR observation period, most of the 40-day data would be prior to nesting and may be associated with early-laying penguins requiring less time to meet energetic demands.

Acquisition of food resources during the pre-breeding period is essential, as breeding birds must expend additional energy to defend their nest and produce eggs (Williams 2012). Successful acquisition of high quality resources relates to earlier laying, which can influence reproductive success (Sorensen et al. 2009). For example, early breeding European shag (*Phalacrocorax aristotelis*) spend less time foraging in winter than other individuals (Daunt et al. 2006), successfully breeding Adélie penguins (*Pygoscelis adeliae*) are more efficient foragers (Lescroël et al. 2010), and breeding Manx shearwater (*Puffinus puffinus*) spend less time in winter foraging and flying than those that defer breeding (Shoji et al. 2015). In species such as the Adélie penguin, non-breeding individuals were 90 % of the mass of breeders (Vleck & Vleck 2002). We found no significant differences in mass between non-breeding, breeding, and early-laying penguins, but the non-breeding penguins were 93 % the weight of early-laying penguins. As such, the decrease in time at sea for early-laying gentoo penguins could indicate that these individuals may achieve food requirements in less time during the pre-breeding period.

While none of the indices of oxygen storage and carrying capacity were in the best model for the time spent at sea, Hb, Hct, and mass were included in comparable models (AICc < 4), and there were relationships between breeding status and these indices. In many bird species, Hb levels in females decline during the egg production and laying period (reviewed in Minias 2015). Though the differences were not significant, our data show analogous patterns, where early-laying penguins have lower Hb levels than breeding and non-breeding penguins. Given the observed increase in diving efficiency with Hb levels, such reduction in Hb could create important trade-offs between foraging capacity and reproduction. Other penguin species, such as

macaroni penguins, have evolved extreme size dimorphism due to similar trade-offs between aerobic condition and egg production (Jubinville et al. 2020). As the physiology of each sex is affected differently by breeding (Williams 2012, Desprez et al. 2018), future studies with a larger sample size could explore sex-specific associations between Hb levels, foraging capacity, and breeding, and further disentangle these potential trade-offs.

5. Conclusion

Seabirds are long-lived and favour conservative breeding strategies prioritizing their survival when conditions are sub-optimal for breeding (Storey et al. 2017). Here, we show that a higher Hb level was associated with greater dive efficiency at depth, increased tendency to make deeper dives, and additional foraging effort. The non-linear relationship between Hct and diving efficiency helps to inform some of the key limitations involved in using Hct as an indicator of blood oxygen storage and carrying capacity and individual conditions (Johnstone et al. 2017). Pre-breeding behaviour was related to the eventual reproductive status of gentoo penguins, where deferred breeders spent more time at sea.

Foraging is an essential activity for survival, and understanding how an individual's physiology and health affect dive performance, selection of foraging habitat, and breeding participation is essential to understanding the effects natural and anthropogenic ecosystem changes can have on populations. In the Falkland Islands, the continental shelf is a popular fishing ground, and gentoo penguins are occasionally caught as bycatch and potentially compete for prey with fishing industries (Clausen & Pütz 2003, Trathan et al. 2015). Their extended winter range could overlap with exploration for hydrocarbons, and there is a consideration of further developing fisheries inshore (Augé et al. 2015). Given the associations between an

individual's Hb and Hct levels and their diving and foraging patterns, these human activities could affect portions of the population differently. For example, reducing prey availability nearshore could be especially detrimental for penguins with low oxygen storage and carrying capacity.

According to the island-wide census of 14 colonies, nesting pair numbers and breeding success in 2018 were above the long-term annual average recorded since 2003 (Stanworth and Crofts 2019), suggesting that the penguins in our study experienced relatively prosperous environmental conditions. However, in a year of low environmental conditions, the costs of an inferior strategy could be more prevalent (Fronstin et al. 2016, Storey et al. 2017). Therefore, further understanding the relationship between body conditions and diving efficiency, and its impact on foraging success and breeding participation across a range of environmental conditions, may be crucial in understanding breeding participation and population dynamics in the future.

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7. Animal care

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Table 1. Results from the final GAMM for dive efficiency. We present the estimate, standard error of the parametric coefficient (intercept and fixed effect), as well as their associated test statistic, and p-value. We also present the estimated degrees of freedom (EDF), test statistic, and p-value associated with the smooth terms of GAMM. For model selection, we used the shrinkage method with double penalty, which allows non-important terms to be shrunk to the null.

Term	Parametric coefficient				Significance of smooth terms		
	Estimate	SE	*t*	p-value	EDF	F	p-value
Intercept	0.295	0.006	47.606	< 0.001			
Colony (Race point)	0.034	0.014	2.474	0.014			
s(Depth)					7.856	33.369	< 0.001
s(Mass)					1.000	0.005	0.943
s(Hct)					1.000	2.302	0.129
s(Hb)					1.000	0.140	0.708
ti(Depth, Mass)					1.000	2.613	0.106
ti(Depth, Hct)					10.675	3.985	< 0.001
ti(Depth, Hb)					9.683	5.110	< 0.001

Table 2. Model selection results for the multiple linear regressions relating three measures of foraging effort to oxygen store indices, sex, and breeding status. For each measure, only the null models (no covariates), and the models that outperformed them, are shown in the table. We present the number of parameters estimated (k), the Akaike Information Criterion with correction for small sample size (AICc), and the difference in AICc from the best model (ΔAICc).

Response	Covariates	k	AICc	ΔAICc
Foraging effort	Hb	3	171.0	--
	Sex	3	171.4	0.34
	Hct	3	172.6	1.56
	(Null)	2	172.7	1.69
Time at sea	Breeding status	4	66.0	--
	Hb + Hct + Mass	5	67.1	1.17
	Hb + Mass	4	67.9	1.91
	Hb	3	68.3	2.32
	Breeding status + Mass	5	69.8	3.82
	Breeding status + Sex	5	70.3	4.36
	Breeding status + Hb	5	70.4	4.47
	(Null)	2	70.5	4.51
Trip duration	(Null)	2	59.0	--

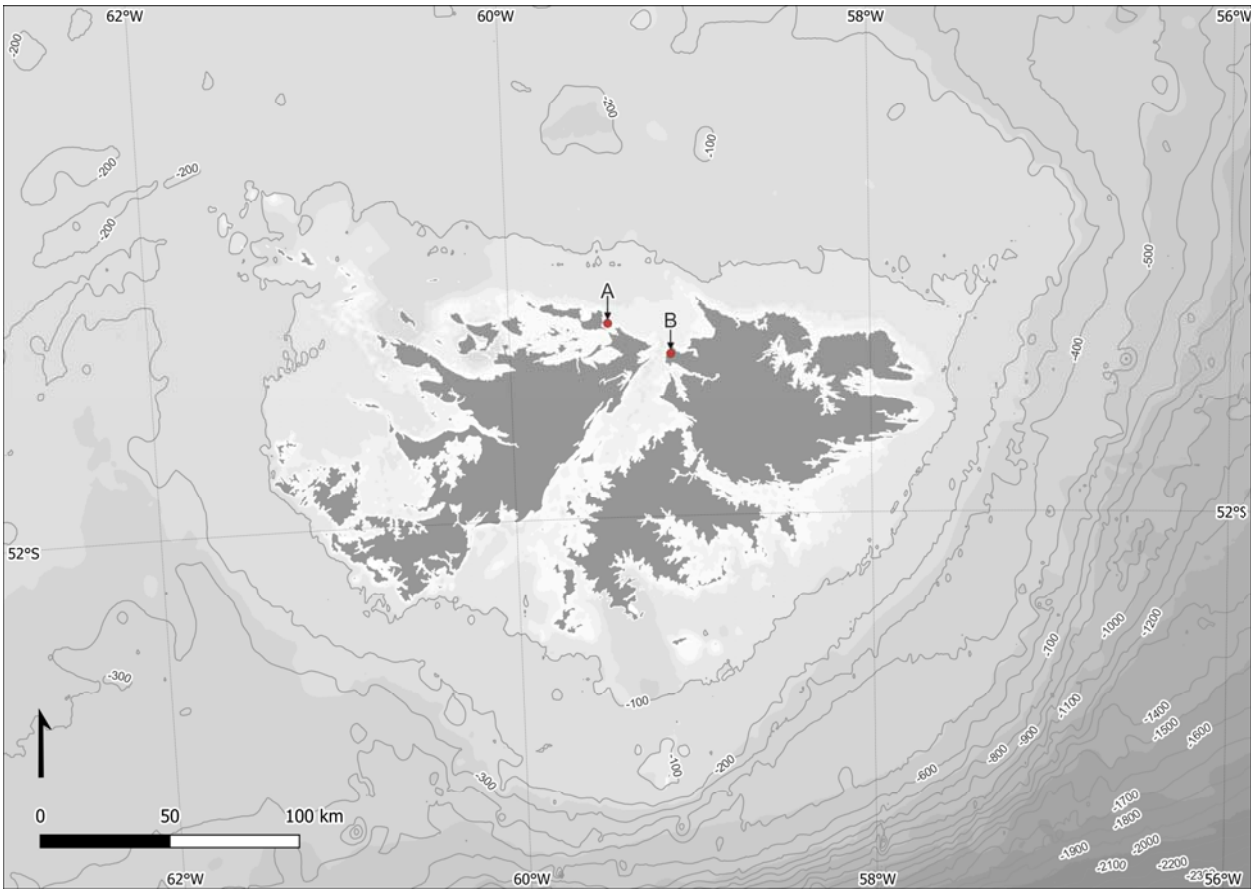

Figure 1. Map of the Falkland Islands showing both study sites: A) Tamar Point, Pebble Island (51.32°S, 59.45°W), B) Rookery Sands, Race Point (51.44°S, 59.11°W).

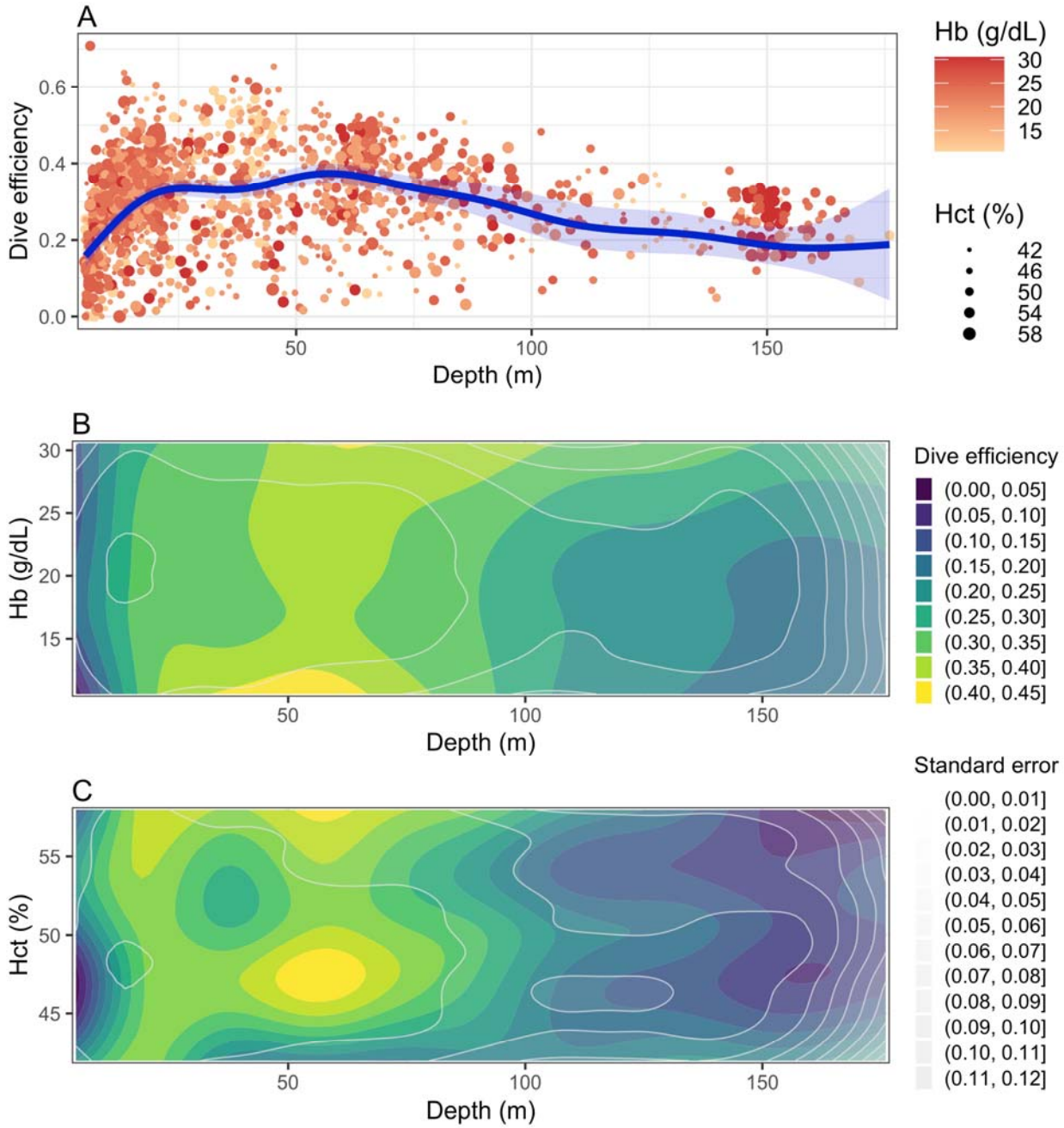

Figure 2. Slices of predictions from the final GAMM. Panel A displays how the predicted dive efficiency changes in response to varying maximum depth values of a dive. The blue band represents the 95% confidence intervals. The points show the observed values, with the color representing the Hb levels and the size representing the Hct level. Panel B shows how dive

efficiency is predicted to change with both depth and Hb, while panel C shows how dive efficiency is predicted to change with both depth and Hct. For both these panels, the color represents the dive efficiency value (yellow representing highest efficiency) and the grey bands represent the standard error (more opaque grey representing higher standard error).

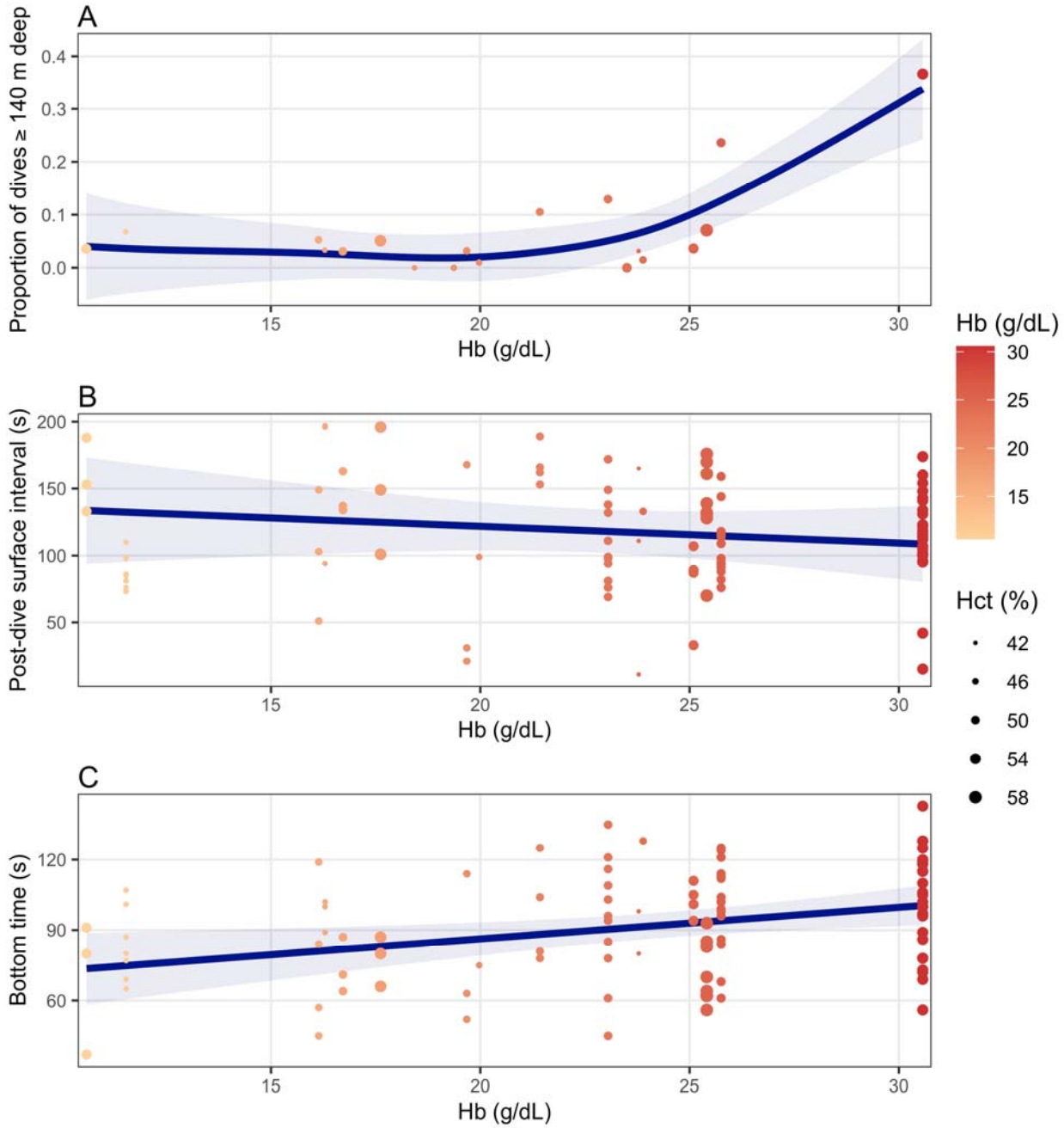

Figure 3. Association between Hb and the proportion of dives an individual made at deeper depth (≥ 140m, panel A), as well as changes in deep dives' post-dive surface intervals (panel B) and bottom times (panel C). The blue lines represent the predicted value, and the blue bands

represent the 95% confidence intervals. The points represent the observed values, with darker reds representing higher Hb levels, and larger points representing higher Hct levels.

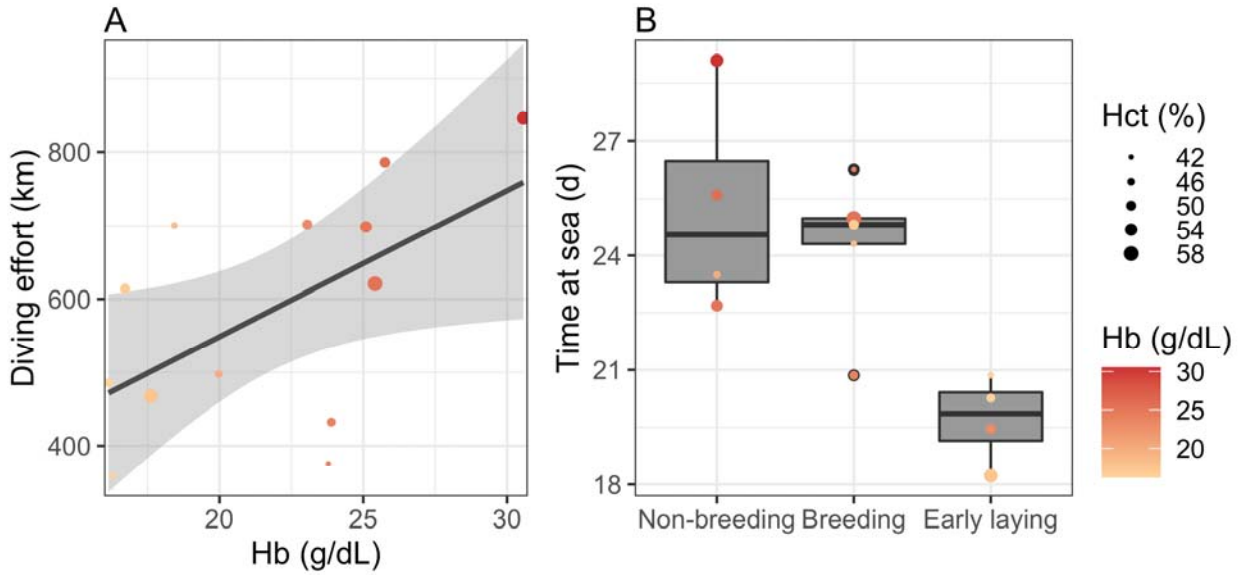

Figure 4. Relationship between Hb and reproductive status and two of the pre-breeding foraging effort measures for the penguins from Pebble Island. Panel A shows the relationship between diving effort (sum of the vertical distance while at sea) and Hb. The line shows the linear regression line, the band is the 95% confidence interval. Panel B shows the relationship between the time spent at sea and the reproductive status. In both panels the points are the observations, with the colour representing the Hb level and the size representing the Hct level.

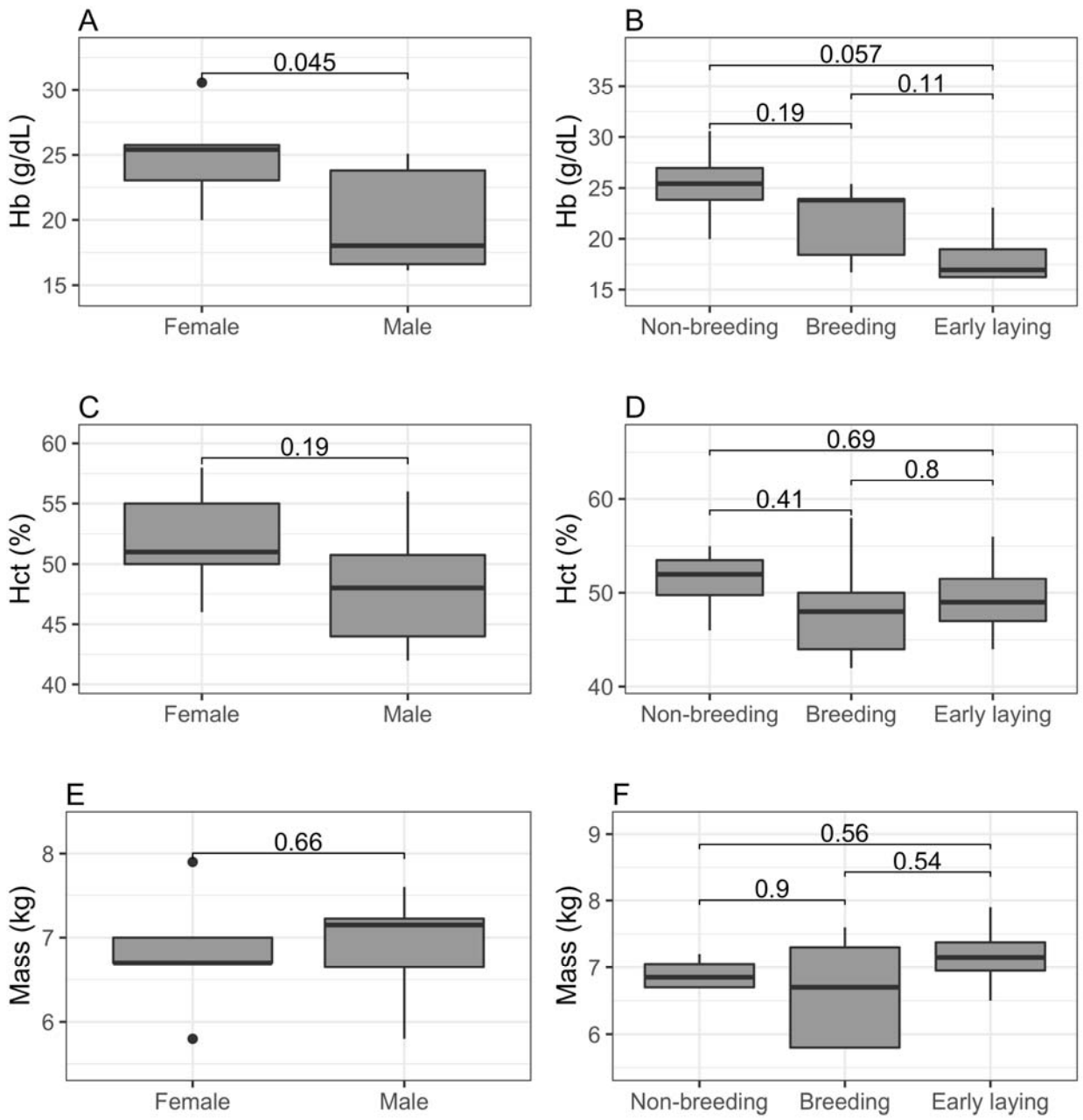

Figure 5. Variation in the three oxygen storage and carrying capacity indices across sex (panels A, C, E) and breeding status (panels B, D, F). Panels A and B show the boxplots for Hb, panels C and D for Hct, and panels E and F for mass. The values presented are the p-values associated with the Wilcoxon rank sum tests associated with the pairwise comparisons.