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Research Article

Blood- and muscle-O_2 storage capacity in North American diving ducks

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Breath-hold diving presents air-breathing vertebrates with the challenge of maintaining aerobic respiration while exercising underwater. Adaptive increases in the oxygen (O_2) storage capacity in the lungs, blood, or muscle tissues can enhance these reserves and greatly extend aerobic foraging time underwater. Here, we report blood- and muscle-O_2 storage parameters (blood hemoglobin concentration ([Hb]), hematocrit, and myoglobin concentration ([Mb]) in the pectoralis and gastrocnemius) for 16 species of diving and dabbling ducks found in North America, and investigate which parameters are correlated with the diving behaviors reported in both the sea ducks (Mergini) and the pochards (Aythini). Both [Hb] in the blood and [Mb] in the gastrocnemius, a major leg muscle used in propulsion for these predominantly leg-propelled divers, were significantly higher in the sea ducks compared to the dabblers (Anatini). The pochards also showed a significant increase in [Hb] and were intermediate between the sea ducks and the dabblers in hematocrit and [Mb] in the gastrocnemius. Among these four variables and total body mass, [Mb] in the gastrocnemius was the most significant predictor of mean species dive time, and these two variables were correlated across the phylogeny. Our results indicate that the observed changes in O_2 storage capacity in the blood and muscles are positively correlated with diving behavior in two clades of ducks, such that larger increases are correlated with longer dive times.

Keywords: breath-hold diving, hematocrit, hemoglobin, myoglobin, oxygen storage

Introduction

Breath-hold diving presents many challenges to air-breathing vertebrates, who must carry sufficient oxygen (O_2) within their tissues to sustain aerobic exercise while underwater. Total O_2 stores for an individual are divided between the respiratory system (inspired air), blood (hemoglobin bound O_2), and muscles (myoglobin bound O_2). Once these onboard O_2 stores are depleted, further underwater activity can be sustained by anaerobic glycolysis, but this leads to the buildup of lactate in the blood

and tissues, which must be processed on return to the surface (Kooyman et al. 1980). Hence, to increase the aerobic dive limit and avoid increased blood and tissue lactate levels, diving animals can manipulate their total O_2 stores, and evolved adaptations exist for increasing O_2 carrying capacity in multiple tissues. Because some diving species generally exhale before diving (Butler and Woakes 1979), and quantification of O_2 stores available in the respiratory system are problematic, especially for animals that are actively diving, we focus here on traits related to O_2 storage in the blood and muscle.

Within each O_2 storage compartment, the total amount of O_2 available depends on both O_2 concentration and the size of the compartment itself. For example, in the blood, O_2 is bound to, and transported by, hemoglobin (Hb) in red blood cells. Thus, the total O_2 available depends on hemoglobin concentration ([Hb]), the amount of red blood cells (packed red cell volume, hematocrit (Hct)), and total blood volume. Using the empirically determined value of 1.34 ml O_2 (g Hb)$^{-1}$, Keijer and Butler (1982) calculated that the total blood O_2 stores in the diving tufted duck *Aythya fuligula* were 33% higher than in the non-diving mallard *Anas platyrhynchos* due in large part to the higher blood volume in the tufted duck. Similarly, muscle O_2 stores depend on the concentration of myoglobin ([Mb]), the monomeric O_2-binding protein found in the muscles, and the mass of the skeletal muscles. [Mb] is not uniform throughout all skeletal muscles, so accurate assessments of the muscle compartment require [Mb] measurements across multiple muscle groups (Arregui et al. 2021).

Increases in [Hb], Hct, and total blood volume can greatly increase the overall carrying capacity of O_2 of the blood, which should increase the amount of O_2 available during a dive (Lenfant et al. 1970, Milsom et al. 1973, Elliott et al. 2010, Ponganis 2015, Stembridge et al. 2019). Concurrent increases in Hb-O_2 binding affinity, as seen in the emperor penguin *Aptenodytes forsteri*, also allow for more efficient O_2 extraction at the low partial pressures of O_2 experienced toward the end of a dive (Ponganis et al. 2007, Meir and Ponganis 2009). A similar pattern holds true in the muscles, with [Mb] in the locomotory muscles of diving mammals and penguins some of the highest seen among animals (Baldwin et al. 1984, Mirceta et al. 2013).

Investigations into diving adaptations thus far have largely focused on marine mammals and penguins due to their exceptional diving abilities (Kooyman and Ponganis 1998). However, the diving ducks represent an underutilized model system that can be used to investigate physiological trait associations related to diving. The 16 species studied here are from three tribes within the Anatinae with variable diving abilities: the deepest diving sea ducks (Mergini, 8 spp.), the mid-tier diving pochards (Aythyini; 3 spp.), and the non-diving dabbling ducks, hereafter referred to as dabblers (Anatini; 5 spp.) (Ingram and Salmon 1941, Schorger 1947, Nilsson 1972, Alexander and Hair 1979). Within these three tribes, diving evolved separately in the sea ducks and the pochards, with sea ducks diverging from the rest of the sub-family Anatinae 3–5 million years before the pochards (McCracken et al. 1999, Gonzalez et al. 2009, Sun et al. 2017). With independent of evolutions of diving in closely related groups, we can investigate whether O_2 storage traits previously associated with diving behavior are altered to a similar degree in two clades of small-bodied diving ducks.

Here, we report blood [Hb] and Hct for pre-breeding, healthy, adult individuals across 16 species of ducks found in North America. We also report [Mb] in two major locomotory muscles: the pectoralis (used in powered flight), and the gastrocnemius (a leg muscle used in swimming in diving). In addition, we assess how changes in traits related to O_2 carrying and storage capacity correlate with the dive capabilities of each species.

Material and methods

Sample overview

Birds were collected over a three-year period from 2019–2021 in Alaska, USA, from ponds and rivers around Fairbanks, the Denali Highway, the Dalton Highway, and Kodiak, during spring migration (mid-May through early June), except in the case of the harlequin ducks *Histrionicus histrionicus* and surf scoters *Melanitta perspicillata*, which were collected from Prince William Sound in March of 2020. While the O_2 transport traits measured here change throughout the year in some species due to plasticity (Fair et al. 2007), the change from March to July in diving ducks is less pronounced than dabblers (Shave and Howard 1976), so we believe that the slight difference in timing for the harlequin ducks and surf scoters should not unduly influence our results. Furthermore, these are the first data of these types collected for many of these species.

All birds collected contributed to a larger project on blood and muscle physiology, and the breadth of muscle samples required for all analyses necessitated that individuals be harvested. All individuals were collected by trained personnel using a 12-gauge shotgun (no. 2, no. 3, or no. 4 non-toxic shot), and when necessary were euthanized by cervical dislocation, following the AVMA Guidelines for the Euthanasia of Animals 2020 (AVMA 2020). All methods were approved prior by the Institutional Animal Care and Use Committee (IACUC) at the University of Miami.

Blood and tissue samples were taken from n = 6–27 individuals from each of the 16 species of diving and non-diving (dabbling) ducks. Upon collection, whole blood was immediately extracted via cardiac puncture using a 3 ml syringe rinsed with 0.5 M EDTA as an anticoagulant. Tissue samples from both the pectoralis and gastrocnemius were then dissected from an intermediate (50%) muscle depth and flash frozen in liquid N_2. Samples were stored at −80 °C until myoglobin assays were completed in the laboratory (below).

Hemoglobin concentration and hematocrit

Immediately after extraction, [Hb] of the whole blood was analyzed using a HemoCue 201+ Analyzer (HemoCue America, Brea, CA, USA), which chemically converts Hb

to azidemethemoglobin before measuring absorbance at 570 and 880 nm (Hudson-Thomas et al. 1994). All values were corrected using an avian correction factor of −1 g dl^{-1} (Simmons and Lill 2006, Qualls et al. 2017). Packed red blood cell volume (Hct) was measured in quadruplicate using 75 mm heparinized capillary tubes. Tubes were spun for 5 min in a ZIPocrit centrifuge (LW Scientific, Lawrenceville, GA, USA), after which Hct was measured. Mean corpuscular hemoglobin concentration (MCHC), which shows the average [Hb] in red blood cells, was calculated using the following equation:

$$\mathrm{MCHC} = \left(\mathrm{Hb}\left[\mathrm{g\ dL^{-1}}\right] \times 100\right) \Big/ \left(\mathrm{Hct}[\%]\right).$$

Myoglobin concentration

Myoglobin concentration was determined using the modified Reynafarje (Reynafarje 1963) method utilized by Dawson et al. (2016, 2020). Frozen tissue was homogenized in 19.25 volumes (1 ml buffer per 1 gram of frozen tissue) of ice-cold homogenization buffer (40 mM potassium phosphate; pH 6.6) and centrifuged for 99 min at 13 700× g and 4 °C (Dawson et al. 2016). The resulting supernatant was transferred to a 25 ml rotating boiling flask and exposed to pure carbon monoxide (CO) for 8 min. Sodium dithionite was added and CO was bubbled in for a further 2 min to ensure complete reduction of Mb. Samples were diluted a further 19.5× with homogenization buffer and transferred to a 1 ml cuvette (10 mm optical path length). The optical density of each sample was measured in triplicate with a homogenization buffer blank at 538 and 568 nm using a VWR V1200 Spectrophotometer (VWR, Radnor, PA, USA). Myoglobin concentration ([Mb]; mg/g) was then determined based on the following equation (Dawson et al. 2016):

$$[\mathrm{Mb}] = \left(\mathrm{OD}_{538} - \mathrm{OD}_{568}\right) \times 112.6.$$

Dive times

Reported mean dive times for each species were taken from the published literature (Table 1). If multiple dive times were published, we prioritized reports taken from wild birds diving in their natural habitat over captive birds. Where possible, the same study was used for multiple species to reduce observer bias. We report both mean and maximum dive times, but only mean dive time was used in our analyses. Although there is evidence that dabbling ducks can dive for food when shallow water is inaccessible (Furilla and Jones 1987), this is not the normal food acquisition behavior of these birds. Here, we limit dive times to those birds who use diving for their main mode of food acquisition, and thus, all dabblers were assigned dive times of 0 s.

Species phylogeny

We reconstructed a phylogenetic species tree as outlined in Schell et al. (2023a). Briefly, previously published autosomal double digest restriction-site associated DNA sequencing (ddRADseq) data (Lavretsky et al. 2021) was aligned using bioinformatics pipelines outlined in Lavretsky et al. (2020). After filtering for missing data and ensuring sufficient coverage (10× per genotype), we used the *SNAPP* (Leaché et al. 2014) function in 'BEAST' ver. 2.5.2 (Bouckaert et al. 2014) to reconstruct the species tree. We dated the phylogeny using *treePL* (Maurin 2020) with divergence times obtained from 'TimeTree' (Kumar et al. 2022).

Statistical analyses – phylogenetic testing

Given that some species studied here are more closely related than others, all analyses were conducted in a phylogenetic

Table 1. Mean and maximum dive times (s) reported in the literature and their sources.

Species	Time (s)		Reference
	Mean	Max	
Sea ducks			
Long-tailed duck	39.2	61	Ingram and Salmon (1941)
Harlequin duck	21.7	49	Goudie (1999)
Surf scoter	34.9	83	Goudie (1999)
Red-breasted merganser	22.3	27	Ingram and Salmon (1941)
Common merganser	22.9	34	Nilsson (1972)
Bufflehead	23.9	31.7	Boone and Larue (1998)
Common goldeneye	21.2	39	Ingram and Salmon (1941), Bourget et al. (2007)
Barrow's goldeneye	21.6	25	Beauchamp (1992), Bourget et al. (2007)
Pochards			
Ring-necked duck	18.4	26.5	Alexander and Hair (1979), Jeske and Percival (1995)
Greater scaup	17.2	29	Nilsson (1972)
Lesser scaup	22.9	28.7	Alexander and Hair (1979), Custer et al. (1996)
Dabblers			
Northern shoveler	0		–
American wigeon	0		–
Green-wing teal	0		–
Mallard	0		–
Northern pintail	0		–

framework to account for the non-independence of species (Felsenstein 1985) using R Statistical Software (ver. 4.2.1, www.r-project.org). We tested for outliers within the combined data for all 16 species using Rosner's (1975) test, with the number of suspected outliers derived visually from histograms of trait distribution for each variable. Outliers were only identified in MCHC, and three total outliers were removed from all analyses (two individual sea ducks, one pochard). We also tested for phylogenetic signal in each parameter using Pagel's λ (Pagel 1999), and estimated trait values for parameters showing phylogenetic signal (λ significantly different from 0) were mapped on the phylogeny using the *contMap* function in 'phytools' (Revell, 2012). We did not test for sex differences between species because preliminary power analysis (Cohen's D = 1, significance level = 0.05, power = 0.8) indicated that 17 individuals per sex per species were required, and our sample sizes did not meet that criterion.

Statistical analyses – blood- and muscle-O_2 storage capacity by tribe

We first wanted to determine if there were measurable changes in O_2 storage capacity in the blood ([Hb], Hct, MCHC) and muscle ([Mb] in pectoralis, [Mb] in gastrocnemius) between tribes. Because the tips of our phylogenetic tree are at the species level, not the individual level, we grouped our data by tribe to allow for the replication within groups required for ANOVA. Phylogenetic ANOVA (phytools; Revell 2012) was performed individually for each of the five variables related to blood and muscle O_2 storage capacity listed above, followed by post-hoc Bonferroni tests. We also examined changes in [Mb] between the pectoralis and gastrocnemius using a paired t-test to account for multiple measurements from the same individual.

Statistical analyses – O_2 storage capacity correlates of dive time

We next wanted to investigate whether the observed differences in O_2 storage capacity in the blood or muscles were correlated with dive performance using mean dive time for each species as the dependent variable. Previous work has shown that total body mass is highly correlated with dive time, so we included mass as a model variable as well (Elliott et al. 2010). Using phylogenetic least squares regression (PGLS), we performed model selection on all possible models using the 'AICcmodavg' package (Mazerolle and Mazerolle 2017), which uses AICc to account for small sample sizes when determining the best fit model. Nested models were compared using likelihood ratio tests to check for overfitting of the model.

Results

Species tree phylogeny

The phylogeny used for the phylogenetic analyses here was constructed from 10 731 overlapping autosomal ddRAD-seq loci that met the filtering criteria (Fig. 1). Our tree differs from other published phylogenies most significantly in its placement of sea ducks and pochards sister to each other, rather than sea ducks representing the more basal split from the rest of the Anatinae (Gonzalez et al. 2009, Sun et al. 2017); this is due to the small number of species included here. Another potential artifact from a smaller taxonomic sampling is the placement of the long-tail duck *Clangula hyemalis* sister to the harlequin duck rather than sister to the common eider *Somateria mollissima* as seen in Lavretsky et al. (2021). Finally, the northern shoveler *Anas clypeata* is placed basal to a clade with sea ducks and pochards rather than the rest of the dabblers. For the purposes of phylogenetic testing, preliminary analyses showed that our topology did not lead to different results than one constrained to previously published topologies.

Phylogenetic signal

Pagel's λ indicated that both [Hb] (λ = 1) and Hct (λ = 0.75) showed phylogenetic signal (λ significantly different from 0, $p < 0.05$). Hct was also significantly different from 1 (p = 0. 019), indicating a lesser degree of phylogenetic signal in this trait compared to [Hb] (Fig. 2). Both [Mb] in the pectoralis (λ = 0.55) and gastrocnemius (λ = 0.57) were not significantly different from 0 (Ps = 0.088, 0.056), but were significantly different from 1 (Ps = 0.002, 0.034), indicating less influence of shared history on these two traits.

Blood- and muscle-O_2 storage capacity by tribe

Both the sea ducks and pochards had significantly higher [Hb] compared to the dabblers (p = 0.001; Fig. 3a, Table 2). There was also an overall significant difference in Hct between tribes (p = 0.041), with the diving clades trending higher, but post-hoc tests did not identify significant differences between pairs of tribes (Fig. 3b). As expected, [Hb] and hematocrit were highly correlated ($r^2 = 0.506$, $p < 0.001$; Fig. 4), and as a result, the mean corpuscular hemoglobin concentration (MCHC) was similar across tribes (Fig. 3c). [Mb] in the muscles was highly variable, and significant differences were seen only in the gastrocnemius (p = 0.035), with sea ducks having significantly higher [Mb] compared to the dabblers (Fig. 3d), and the pochards intermediate between the two. [Mb] in the pectoralis did not differ significantly among the three tribes (p = 0.156, Fig. 3e). Only two species had significantly different [Mb] between the two muscle types. [Mb] was significantly higher in the pectoralis of both the northern pintail (*Anas acuta*; p = 0.0001, df = 9) and the harlequin duck (p = 0.048, df = 7).

O_2 storage capacity correlates of dive time

The explanatory variables included in our PGLS were [Hb], Hct, [Mb] in the pectoralis, [Mb] in the gastrocnemius, and total body mass. Comparing all models using AICc indicated that [Mb] in the gastrocnemius alone was the best correlate of dive time (AICc = 108.47, Table 3), and this variable was included in 9 of the top 10 models (Supporting information). The top four models based on AICc were nested,

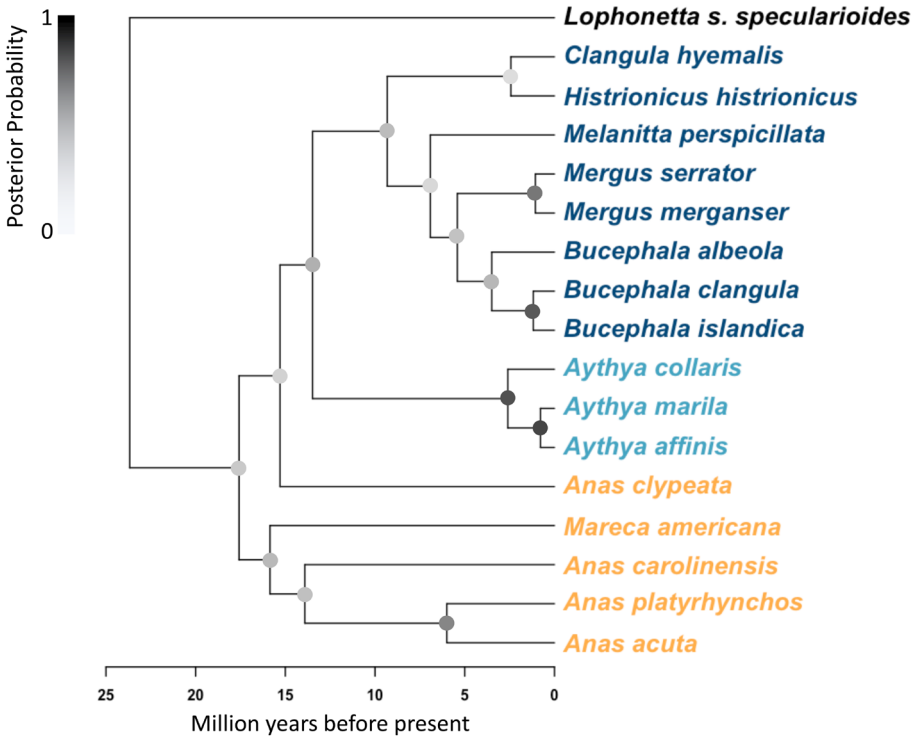

Figure 1. Dated species phylogeny generated in Beast ver. 2.5.2. from 10 731 overlapping autosomal ddRAD-seq loci with branch lengths assigned using *treePL*. Posterior probability support values for each node were generated from 60 million iterations and are presented in gray scale. Tribes are indicated by color: sea ducks (dark blue), pochards (teal), dabblers (gold).

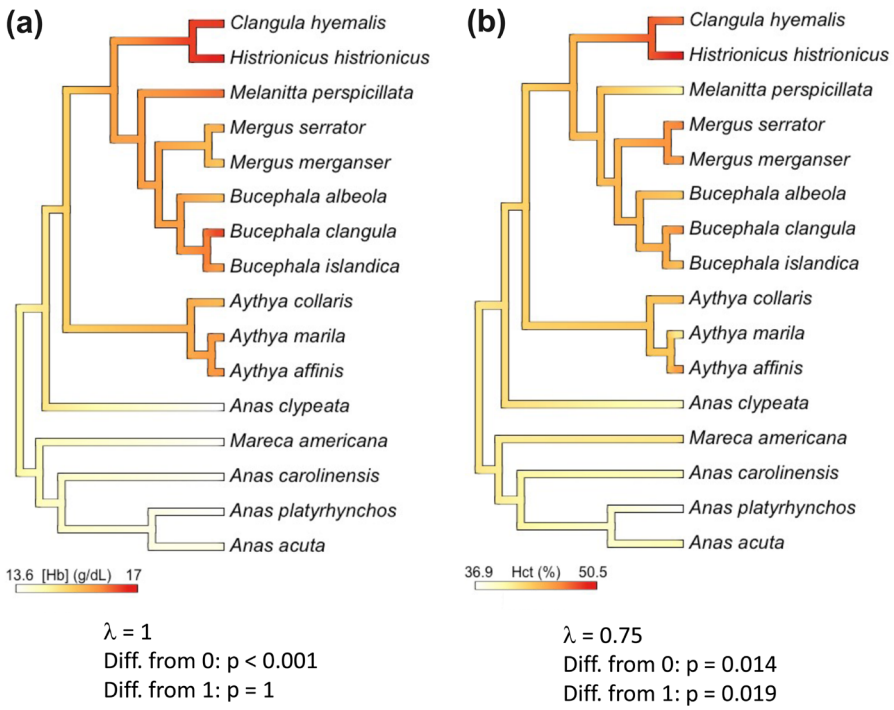

Figure 2. TimeTrees showing our reconstruction of the ancestral states of both (a) [Hb] and (b) Hct mapped onto the phylogeny. Both [Hb] and Hct showed differing degrees of phylogenetic signal according to Pagel's λ. Observed (tip) and estimated trait values are shown by the gradient, and the length of the scale represents 10 million years.

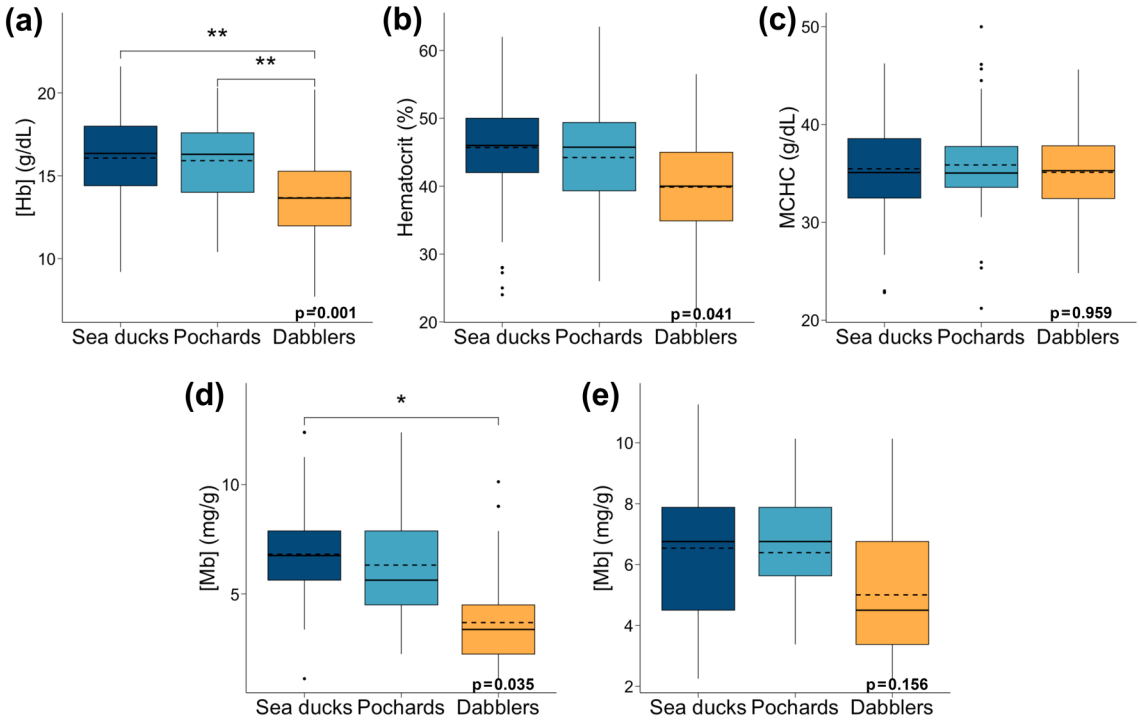

Figure 3. Blood- and muscle-O_2 storage measurements in three tribes of ducks: sea ducks (diving, 8 spp.), pochards (diving, 3 spp), dabblers (non-diving, 5 spp.). In blood O_2 parameters, significant differences between tribes were seen in (a) hemoglobin concentration ([Hb]) and (b) hematocrit (Hct), but (c) mean corpuscular hemoglobin concentration (MCHC) was similar. For [Mb] in the muscle, significant differences between tribes were seen in the (d) gastrocnemius, but not the (e) pectoralis. Box plots show the median and quartile ranges of the data, with the mean indicated by the dashed line. Bonferroni post-hoc tests were used after a phylogenetic ANOVA with the alpha value set as $p < 0.05$ (**$p < 0.01$; *$p < 0.05$).

and likelihood ratio tests indicated that the best model of the four was the most inclusive model, which contained Hct, [Mb] in the pectoralis, [Mb] in the gastrocnemius, and total body mass ($X^2 = 9.11$, $p = 0.028$, $\Delta AICc = 4.22$). In this model, both [Mb] in the gastrocnemius ($p = 0.0002$) and Hct ($p = 0.0442$) were significantly correlated with dive time (Table 3). However, we also note that because inclusion of more parameters did not lead to a net reduction in AIC, these parameters should be considered uninformative (Arnold 2010), such that the model with [Mb] in the gastrocnemius as the only predictor is the most biologically informative model. When comparing individual O_2 storage parameters with mean dive time, only [Mb] in the gastrocnemius was significantly correlated ($p = 0.0005$, Fig. 5).

Discussion

We recognize that in interpreting our results, there is the assumption that the [Hb], Hct and [Mb] measured are representative of each species. All traits studied here can vary with seasonality (Swanson 2010), development (Haggblom 1987), exercise (Butler and Turner 1988), and migration (Palacios et al. 1984). Given that within-species and within-tribe variation is smaller than the among-species or tribe variation, we feel that our conclusions are valid. Furthermore, all individuals were collected during spring migration, which lasts from early March to late May (de la Cruz et al. 2009), so for the comparative aims of this study, variation within individuals and species due to the impact of seasonal and environmental pressures on plasticity should be minimized.

In several cases, the [Hb] values reported here differ from those previously reported in the literature. These observed differences are most likely due to differences in methodology or the time of year that the blood samples were collected, both of which have been found to alter measured blood values (Shave and Howard 1976, Driver 1981, Fair et al. 2007). Multiple methods of [Hb] measurement have been used with waterfowl, including conversion to acid hematin (Bond and Gilbert 1958), conversion to cyanmethemoglobin (i.e. Drabkin's method) (Schalm et al. 1975, Keijer and Butler 1982), and the conversion to azidemethemoglobin used here. While values in the diving ducks are comparable across studies using all three methods (17–18 g dl^{-1}), values differ quite significantly in the dabbling ducks. For example, in the mallard, using the cyanmethemoglobin method yielded 17.5 g dl^{-1} from shot, wild birds (Driver 1981), which is likely too high, vs our measurement of 13.6 g dl^{-1} here, which is more typical of a low-altitude non-diving species (pers. obs.). The HemoCue 201+ used here has been shown to produce comparable readings to other methods in clinical settings (Nkrumah et al. 2011), and can be adjusted for nucleated red

Table 2. Mean ± SE O_2 storage capacity parameters for 16 species of diving and non-diving ducks. Sample size for each measurement is in parentheses. Hemoglobin concentration ([Hb]); hematocrit (Hct); myoglobin concentration ([Mb]). Significant differences between tribes ($p < 0.05$): † Significantly different from dabblers.

				Pectoralis		Gastrocnemius	
Species	Body mass (g)	[Hb] (g dl^{-1})	Hct (%)	Mass (g) (% total)	Myoglobin (mg g^{-1})	Mass (g) (% total)	Myoglobin (mg g^{-1})
Sea ducks	**877 ± 32 (118)**	**16.1 ± 0.3† (118)**	**45.7 ± 0.8 (118)**	**128.2 ± 4.6 (14.6%)**	**6.54 ± 0.33 (77)**	**5.9 ± 0.4 (0.67%)**	**6.82 ± 0.34† (71)**
Long-tailed duck *Clangula hyemalis*	812 ± 20 (16)	16.8 ± 0.8 (16)	47.8 ± 1.3 (15)	124.8 ± 4.0 (15.4%)	7.99 ± 0.70 (10)	3.4 ± 0.1 (0.42%)	9.80 ± 0.63 (10)
Harlequin duck *Histrionicus histrionicus*	698 ± 24 (8)	17.0 ± 0.7 (8)	50.5 ± 2.0 (8)	85.5 ± 4.0 (12.3%)	7.04 ± 0.51 (8)	4.5 ± 0.3 (0.64%)	5.35 ± 0.73 (8)
Surf scoter *Melanitta perspicillata*	1220 ± 12 (6)	16.4 ± 1.3 (6)	40.7 ± 2.7 (6)	151.2 ± 2.3 (12.4%)	7.32 ± 1.00 (6)	8.4 ± 0.2 (0.68%)	8.07 ± 0.74 (6)
Red-breasted merganser *Mergus serrator*	1036 ± 35 (8)	15.7 ± 0.8 (8)	47.3 ± 3.4 (8)	144.5 ± 6.1 (13.9%)	5.49 ± 0.75 (8)	10.1 ± 0.8 (0.98%)	4.79 ± 0.51 (8)
Common merganser *Mergus merganser*	1429 ± 64 (16)	15.4 ± 0.6 (16)	46.4 ± 1.5 (16)	196.9 ± 9.1 (13.8%)	7.57 ± 0.73 (11)	13.8 ± 0.9 (0.97%)	6.24 ± 0.38 (11)
Bufflehead *Bucephala albeola*	443 ± 10 (27)	15.5 ± 0.5 (27)	43.9 ± 1.4 (27)	60.3 ± 2.3 (13.6%)	6.24 ± 0.69 (11)	2.2 ± 0.1 (0.50%)	6.76 ± 0.85 (8)
Common goldeneye *Bucephala clangula*	906 ± 45 (17)	16.7 ± 0.6 (17)	46.7 ± 2.0 (17)	147.6 ± 7.1 (16.3%)	6.19 ± 0.79 (12)	4.7 ± 0.3 (0.51%)	7.09 ± 0.34 (10)
Barrow's goldeneye *Bucephala islandica*	1000 ± 37 (21)	16.0 ± 0.6 (20)	44.3 ± 1.9 (21)	153.9 ± 6.5 (15.4%)	4.81 ± 0.34 (11)	5.1 ± 0.4 (0.52%)	6.31 ± 0.45 (10)
Pochards	**846 ± 16 (58)**	**15.9 ± 0.4† (57)**	**44.2 ± 1.0 (58)**	**118.8 ± 2.9 (14.0%)**	**6.39 ± 0.44 (34)**	**3.9 ± 0.1 (0.46%)**	**6.32 ± 0.48 (31)**
Ring-necked duck *Aythya collaris*	790 ± 15 (11)	15.6 ± 0.8 (11)	44.4 ± 2.0 (11)	117.3 ± 2.1 (14.8%)	6.53 ± 0.47 (10)	3.7 ± 0.1 (0.46%)	6.53 ± 0.84 (10)
Greater scaup *Aythya marila*	966 ± 20 (20)	16.0 ± 0.7 (19)	41.5 ± 1.9 (20)	126.6 ± 6.2 (13.1%)	6.53 ± 0.33 (10)	4.4 ± 0.2 (0.45%)	6.19 ± 0.74 (10)
Lesser scaup *Aythya affinis*	780 ± 16 (27)	16.0 ± 0.4 (27)	46.2 ± 1.3 (27)	113.6 ± 3.8 (14.6%)	6.19 ± 0.50 (14)	3.7 ± 0.1 (0.47%)	6.24 ± 0.84 (11)
Dabblers	**716 ± 24 (96)**	**13.7 ± 0.3 (96)**	**39.9 ± 1.1 (92)**	**118.6 ± 4.1 (16.6%)**	**5.0 ± 0.29 (52)**	**3.4 ± 0.1 (0.47%)**	**3.69 ± 0.24 (50)**
Northern shoveler *Anas clypeata*	620 ± 12 (22)	13.6 ± 0.5 (22)	39.5 ± 1.5 (21)	102.5 ± 2.2 (16.6%)	6.04 ± 0.38 (11)	2.8 ± 0.2 (0.46%)	4.50 ± 0.81 (10)
American wigeon *Mareca americana*	783 ± 14 (23)	13.7 ± 0.7 (23)	41.8 ± 1.7 (21)	132.7 ± 2.6 (16.9%)	5.52 ± 0.68 (10)	3.7 ± 0.1 (0.47%)	3.94 ± 0.56 (10)
Green-winged teal *Anas carolinensis*	385 ± 7 (20)	13.7 ± 0.5 (20)	39.8 ± 1.3 (19)	60.0 ± 1.8 (15.6%)	4.73 ± 0.55 (10)	1.7 ± 0.2 (0.45%)	3.49 ± 0.74 (10)
Mallard *Anas platyrhynchos*	1105 ± 41 (11)	13.6 ± 0.7 (11)	36.9 ± 2.3 (11)	173.8 ± 12.1 (15.7%)	4.09 ± 0.63 (11)	6.0 ± 0.4 (0.54%)	3.94 ± 0.81 (10)
Northern pintail *Anas acuta*	869 ± 21 (20)	13.9 ± 0.7 (20)	40.0 ± 1.5 (20)	149.2 ± 3.6 (17.2%)	4.62 ± 0.46 (10)	3.8 ± 0.1 (0.44%)	2.59 ± 0.29 (10)

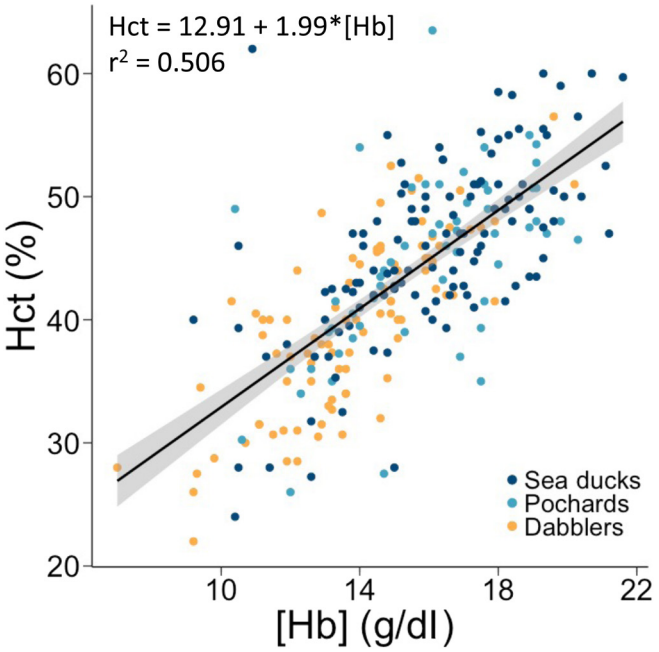

Figure 4. Correlation analysis of hemoglobin concentration ([Hb]) and hematocrit (Hct) in 16 species of ducks, colored by tribe. Best fit line determined by ordinary least squares regression (OLS), with 95% confidence interval shown by gray shading. ($p < 0.001$). OLS was used over PGLS because it allows for inclusion of multiple individuals per species.

blood cells found in birds using the avian correction factor of −1 g dl^{-1} (Simmons and Lill 2006), so we are confident that the [Hb] readings reported here represent valid baseline measurements for these species. Furthermore, we are confident that values reported here represent high-quality blood samples that generally only can be obtained by cardiac puncture immediately after a bird has been harvested.

Total O_2 storage capacity is an important factor in determining the aerobic dive limit for air-breathing birds and mammals. The longer diverged sea ducks appear to have increased their total O_2 storage capacity via concurrent increases in both the blood and muscle tissues, as shown by the observed increases in [Hb], Hct, and [Mb] in the gastrocnemius. The more recently diverged pochards show similar increases in [Hb] and are intermediate between the sea ducks and dabblers for Hct and [Mb] in the muscles. The observed increase in [Hb] in the pochards is contrary to work done comparing [Hb] between pochards (redhead *Aythya americana*, canvasback *Aythya valisineria*, greater scaup *Aythya marila*) and dabblers (mallard, American black duck *Anas rubripes*), where previous authors concluded that there was no difference between the two groups (Gilbert and Bond 1949, Bond and Gilbert 1958). The most likely explanation is a difference in methodology, quality of blood sample, or small sample size in the original studies. In both comparisons (Gilbert and Bond (1949), Bond and Gilbert (1958)), no more than 19 birds were used per group. More recent work comparing the diving tufted duck to the mallard showed a relative increase in

Table 3. Top five models of hematological parameters on dive time using phylogenetic least squares regression (PGLS) and determined by AICc values. All parameter estimates are given as value ± SE, with the associated p-value in parentheses. All possible models are presented in the Supporting information.

Model	K	AICc	ΔAICc	−lnL	Intercept	[Hb]	Hct	[Mb] (p)	[Mb] (g)	Mass
~ [Mb] (g)	3	108.47	0	−50.23	−8.95 ± 5.64 (0.14)	–	–	–	3.67 ± 0.82 (0.0005)	–
~ [Mb] (g) + mass	4	110.98	2.513	−49.67	−6.47 ± 6.20 (0.32)	–	–	–	3.92 ± 0.86 (0.0005)	−0.005 ± 0.005 (0.35)
~ Hct + [Mb] (g) + mass	5	111.75	3.284	−47.88	−38.33 ± 19.2 (0.07)	–	0.70 ± 0.40 (0.11)	–	3.98 ± 0.80 (0.0003)	−0.002 ± 0.005 (0.70)
~ Hct + [Mb] (p) + [Mb] (g) + mass	6	112.69	4.219	−45.68	−38.42 ± 17.49 (0.05)	–	0.86 ± 0.38 (0.04)	−2.49 ± 1.34 (0.09)	5.05 ± 0.93 (0.0002)	0.0015 ± 0.005 (0.76)
~ [Hb] + [Mb] (g) + mass	5	113.06	4.597	−48.53	−46.36 ± 30.04 (0.15)	2.80 ± 2.06 (0.20)	–	–	3.64 ± 0.86 (0.001)	−0.004 ± 0.005 (0.41)

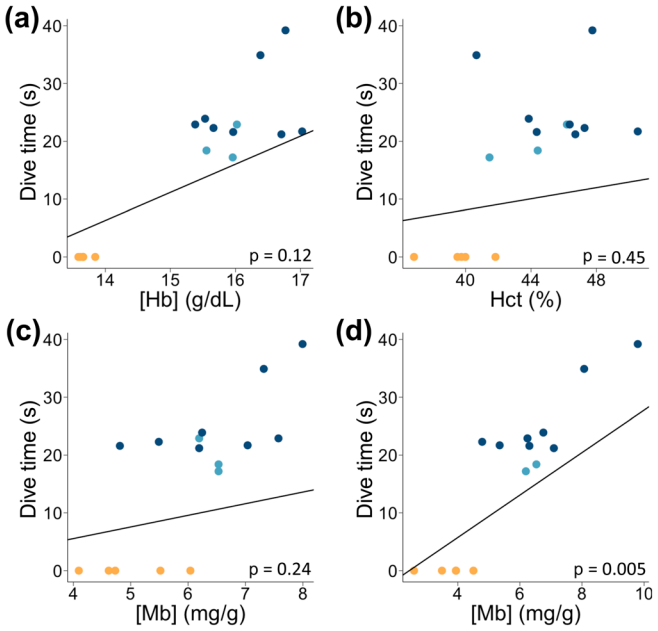

Figure 5. Myoglobin concentration in the gastrocnemius is the only O_2 storage parameter correlated with dive time based on PGLS using a single predictor variable. (a) Hemoglobin concentration [Hb], (b) hematocrit (Hct), (c) myoglobin [Mb] concentration in the pectoralis, (d) myoglobin concentration [Mb] in the gastrocnemius. In panel (d), the common merganser and lesser scaup had identical dive times and [Mb] values, which is why it appears that one pochard is missing. Points are colored by tribe: sea ducks (dark blue), pochards (teal), dabblers (gold). Trend line was fit using PGLS, and reported p-value is for the explanatory variable from PGLS.

[Hb] in the diver as compared to the dabbler, similar to our results (Keijer and Butler 1982). Although not included here, changes in blood volume could also greatly impact blood O_2 stores, and a study by Stephenson et al. (1989) showed that blood volume was the only circulatory O_2 parameter increased in the tufted duck after dive training, hinting at its importance in altering blood-O_2 stores.

[Hb] can be increased via changes in red blood cell size, number, or both, and recent work on Andean hummingbirds shows that the adjustment mechanism employed varies with elevation (and thus ambient partial pressure of O_2), with lowland species on the higher end of their elevational range and high-altitude species increasing cell size, while mid-elevation species increase cell number (Williamson et al. 2023). While constant exposure to low ambient O_2 pressure at altitude presents organisms with a different physiological challenge, diving ducks could also be expected to manipulate Hb mass in a similar fashion. Since smaller cells increase the speed at which O_2 can diffuse in and out of the cell (Hawkey et al. 1991), diving ducks should exhibit more, smaller cells which would favor rapid use and replenishment of O_2 during a diving bout, but this remains to be investigated.

Increased muscle [Mb] in the locomotory muscles is extremely common in diving birds and marine mammals, and [Mb] correlates with dive performance in multiple groups (Weber et al. 1974, Noren and Williams 2000, Helbo and Fago 2012). We report a similar correlation here, with PGLS showing that [Mb] in the gastrocnemius, an important leg muscle for these leg-propelled divers, is the only single variable significantly correlated with dive time across the phylogeny (Fig. 5). Mb has a high O_2 affinity (Wright and Davis, 2015), so Mb-bound O_2 stores only become available when O_2 tensions in the muscle are lower toward the end of the dive, or if blood flow is restricted to the muscle as is seen in marine mammals. Because diving ducks such as the tufted duck increase blood flow to the legs while vigorously swimming or diving (Bevan and Butler 1992a), Mb-bound O_2 stores need not make up such a large portion of the overall body O_2 stores. However, given the correlation between [Mb] in the gastrocnemius and mean dive time, our results suggest that a higher [Mb] confers an advantage during longer dives by increasing available O_2 from that stored in the muscle late in the dive. Higher [Mb] could also increase Mb-facilitated O_2 diffusion within the cell, which would speed up O_2 transport from the capillaries to its end use at the mitochondria (Merx et al. 2001, Wittenberg and Wittenberg 2003). This facilitative action could thus ensure that the large blood-O_2 stores are fully utilized throughout a dive. This dual function of O_2 storage and intracellular transport would explain the association with high levels of Mb and longer mean dive times of these species.

Taken together, the observed increases in both blood and muscle O_2 carrying and storage capacity in the sea ducks, and to a lesser extent the pochards, prepares them well for underwater foraging by extending their aerobic dive limit. Previous work in the tufted duck concluded that their dives remain well within their calculated aerobic dive limit (Woakes and Butler 1983), so the O_2 stores reported here are likely in excess of what is needed for a standard diving bout. O_2 consumption also decreased during longer dives, suggesting that there may be a reduction in aerobic metabolism through reduced effort to remain submerged during longer or deeper dives (Bevan et al. 1992b). Indeed, even the deep diving long-tailed duck, which has been caught in nets at depths of 55 m and has an average dive time of ~ 40 s (Ingram and Salmon 1941, Schorger 1947), appears to remain within its aerobic dive limit. Weighing less than 1 kg, dives of these lengths or depths would require either large O_2 stores or a heavy reliance on anaerobic glycolysis with long surface intervals to process the resultant buildup of lactate. During diving bouts, the dive to pause ratio varied between 2.9–3.9 (Nilsson 1972), but was not correlated with depth, indicating that this species also has O_2 stores in excess of what is required. The long-tail duck may also exhibit a reduction in aerobic metabolism during longer dives as was seen in the tufted duck. Furthermore, the high intra- and interspecific variation seen here could indicate that increasing O_2 storage capacity is under less intense natural selection towards an optimum, as is seen in Andean hummingbird species at high absolute altitudes and near the top of their elevational range limits (Linck et al. 2023).

Conclusion

O_2 storage capacity is a crucial determinant of how long aerobic metabolism can be sustained during a single breath-hold dive. Here, we report various hematological and muscular O_2 storage parameters across 16 species of North American ducks. We also show that duration of breath-hold diving is correlated with an increase in O_2 storage and carrying capacity in both the blood and locomotory muscles. This increase in available O_2 while diving would function to extend the aerobic dive limit in these species, allowing them to successfully inhabit their underwater foraging niche without shifting to anaerobic glycolysis. Further investigations into respiratory O_2 storage capacity and blood volume measurements would allow for calculations of the total amount of onboard O_2 stores these animals have, adding to our growing understanding of avian dive physiology.

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Author contributions

Elizabeth R. Schell: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (lead); Writing – original draft (lead). **Jeff White**: Investigation (supporting); Methodology (equal) Writing – review and editing (equal). **Kevin G. McCracken**: Conceptualization (equal); Funding acquisition (lead); Resources (lead); Supervision (lead); Writing – review and editing (equal).

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.p5hqbzkvz (Schell et al. 2023b).

Supporting information

The Supporting information associated with this article is available with the online version.

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