Validation of the i-STAT and HemoCue systems for the analysis of blood parameters in the bar-headed goose, *Anser indicus*

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Every year, bar-headed geese (*Anser indicus*) perform some of the most remarkable trans-Himalayan migrations, and researchers are increasingly interested in understanding the physiology underlying their high-altitude flight performance. A major challenge is generating reliable measurements of blood parameters on wild birds in the field, where established analytical techniques are often not available. Therefore, we validated two commonly used portable clinical analysers (PCAs), the i-STAT and the HemoCue systems, for the analysis of blood parameters in bar-headed geese. The pH, partial pressures of O$_2$ and CO$_2$ (PO$_2$ and PCO$_2$), haemoglobin O$_2$ saturation (sO$_2$), haematocrit (Hct) and haemoglobin concentration ([Hb]) were simultaneously measured with the two PCA systems (i-STAT for all parameters; HemoCue for [Hb]) and with conventional laboratory techniques over a physiological range of PO$_2$, PCO$_2$ and Hct. Our results indicate that the i-STAT system can generate reliable values on bar-headed goose whole blood pH, PO$_2$, PCO$_2$ and Hct, but we recommend correcting the obtained values using the linear equations determined here for higher accuracy. The i-STAT is probably not able to produce meaningful measurements of sO$_2$ and [Hb] over a range of physiologically relevant environmental conditions. However, we can recommend the use of the HemoCue to measure [Hb] in the bar-headed goose, if results are corrected. We emphasize that the equations that we provide to correct PCA results are applicable only to bar-headed goose whole blood under the conditions that we tested. We encourage researchers to validate i-STAT or HemoCue results thoroughly for their specific study conditions and species in order to yield accurate results.

Key words: Bird, carbon dioxide, oxygen, pH, portable clinical analyser

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Introduction

The accurate measurement of blood gases and pH in field studies on animal physiology is not easily accomplished. While proven and established methods are available in the laboratory, these techniques are typically not portable or suited for operation under most field conditions. Consequently, researchers have adopted portable clinical analysers (PCAs), such as the i-STAT system® (Abbot Point of Care Inc., Princeton, NJ, USA), for the analysis of blood parameters on a broad range of animal species, often without previous validation (Stoot et al., 2014). To our knowledge, no previous study has validated the i-STAT for the analysis of blood parameters in an avian species. We have previously validated the i-STAT for its use on a teleost (Harter et al., 2014) and an elasmobranch species (Harter et al., 2015). Results indicated that under the tested...
conditions and for the two species studied, the i-STAT did not report accurate values of blood parameters (except for blood pH). Therefore, and despite the lack of suitable alternatives, the i-STAT system should not be used on fish without additional validation for the particular conditions and species in question. Other researchers have validated the i-STAT system in reptiles and mammals, which are physiologically more similar to birds, compared to fish. McCain et al. (2010) validated the i-STAT system for the parameters Cl−, glucose, K+ and Na+ in a variety of reptile species; however, the reference technique was another automated human blood analyser (Hitachi 911). Wolf et al. (2008) validated the i-STAT for haematocrit (Hct; among other analytes) in various sea turtle species and found significantly lower Hct values in the i-STAT compared with measurements in capillary tubes. Hopper and Cray (2007) validated the i-STAT system for Hct and haemoglobin (Hb) concentration (among other analytes) in cynomolgus macaques and found the i-STAT to overestimate both of these parameters. Similar results were obtained by Larsen et al. (2002), where the i-STAT overestimated Hct in elephant seals, compared with an automated cell counter.

The i-STAT system was developed for the analysis of human blood; therefore, analysis is performed at 37°C (in a heated cartridge) and results are calculated assuming human blood characteristics. Based on our previous results on poikilothermic fish, measurements of blood parameters were inaccurate (Harter et al., 2014, 2015) due to: (i) differences in body temperature between fish and humans and the effects of a closed system temperature increase during analysis (Malte et al., 2014); (ii) the low partial pressures of CO2 (PCO2) in water breathers, which were outside the reportable range in the i-STAT; (iii) differences in O2-binding properties between human and fish Hb; and (iv) the nucleated state of red blood cells (RBCs) in fish compared with non-nucleated cells in humans. While birds also have nucleated RBCs and Hb isoforms with O2-binding characteristics that may differ from those found in humans, birds are homeotherms (~41°C in bar-headed geese) and air breathers with relatively high PCO2, just like humans. Therefore, some of the major constraints for implementing the i-STAT system on fishes are likely to be absent in birds, which may allow for more accurate measurements of blood parameters.

Bar-headed geese (Anser indicus) perform bi-annual migrations, in which they ascend the face of the Himalayas in less than a day, climbing to 6000 m, in what has been described as ‘the highest and most iconic trans-mountain migration in the world’ (Hawkes et al., 2011). Not surprisingly, there has been increasing interest among researchers in understanding the physiological adaptations that allow for these exceptional flight performances (Rollemo and Bauer, 1979; Faraci, 1991; Scott and Milsom, 2006; Hawkes et al., 2011; Bishop et al., 2015). For ongoing and future studies, it is pivotal to generate accurate measurements of blood gases, acid–base status, haemoglobin concentration [Hb] and Hct of wild bar-headed geese under field conditions. The aim of the present study, therefore, was to validate the i-STAT system for the analysis of these blood parameters in the bar-headed goose, over a predetermined physiological range of PO2 and PCO2 and taking advantage of the naturally occurring inter-individual variability in Hct. In addition, we validated a second PCA, the HemoCue® (HemoCue AB, Ängelholm, Sweden) for the analysis of [Hb] in bar-headed goose whole blood. Our goal was to identify those parameters that can be measured reliably using the i-STAT and HemoCue systems and, if applicable, provide appropriate correction equations to increase the accuracy of the data produced. We recognize that the i-STAT system can be a powerful tool for measuring blood parameters in the field, if other methods are unavailable, and may greatly contribute to the progress in a given field of research, provided accurate results are obtained. Our results should help researchers to identify the limitations of the i-STAT system and allow them to make an informed decision on whether the i-STAT is the right tool to answer their specific research questions.

Materials and methods

Animals and housing

Bar-headed geese (Anser indicus, Latham 1790) were obtained from Sylvan Heights Waterfowl Park (Scotland Neck, NC, USA) and held at the University of British Columbia animal care facilities for several months before experiments. Twelve animals (2.77 ± 0.18 kg; mean ± SD) were kept in an outside enclosure with free access to shelter, standing water and commercial waterfowl feed. Husbandry conditions and all procedures were approved by and strictly according to the guidelines specified by the Canadian Council on Animal Care (UBC protocol no. A12-0013).

Blood collection

Each experimental day, six unanaesthetized birds were restrained by a technician, and 4 ml of blood was collected from the medial metatarsal vein into a heparinized syringe. Samples were then transferred to glass vials and stored on ice until experiments (typically < 1 h). Aliquots of blood (3 ml) were loaded into each of six Eschweiler tonometers (5 ml total volume), and Hct was measured in triplicate on 30 μl subsamples. Tonometers were incubated in a water bath, thermostated at 41°C, and were continuously flushed with a water-saturated custom-mixed gas (O2, CO2 and N2) from a DIGAMIX 275 6KM 422 Woesthoff pump (Bochum, Germany). Blood samples in tonometers were equilibrated with the respective gas tensions for 1 h before subsamples were taken for analysis.

Experimental design

The 3 ml blood samples in each tonometer were sequentially equilibrated to 40, 80 and 120 mmHg PO2 by changing the composition of the gas mixture; sampling was performed after each equilibration step. This protocol was repeated on three separate days, in which PCO2 was set to 2 (~15 mmHg), 4 (~30 mmHg) or 6% (~45 mmHg). Six replicate samples (a single tonometer containing blood from a single individual; n = 6) were run for each combination of factors, resulting in

Rollema and Bauer, 1979; Faraci, 1991; Scott and Milsom, 2006; Hawkes et al., 2011; Bishop et al., 2015. For ongoing and future studies, it is pivotal to generate accurate measurements of blood gases, acid–base status, haemoglobin concentration [Hb] and Hct of wild bar-headed geese under field conditions. The aim of the present study, therefore, was to validate the i-STAT system for the analysis of these blood parameters in the bar-headed goose, over a predetermined physiological range of PO2 and PCO2 and taking advantage of the naturally occurring inter-individual variability in Hct. In addition, we validated a second PCA, the HemoCue® (HemoCue AB, Ängelholm, Sweden) for the analysis of [Hb] in bar-headed goose whole blood. Our goal was to identify those parameters that can be measured reliably using the i-STAT and HemoCue systems and, if applicable, provide appropriate correction equations to increase the accuracy of the data produced. We recognize that the i-STAT system can be a powerful tool for measuring blood parameters in the field, if other methods are unavailable, and may greatly contribute to the progress in a given field of research, provided accurate results are obtained. Our results should help researchers to identify the limitations of the i-STAT system and allow them to make an informed decision on whether the i-STAT is the right tool to answer their specific research questions.
54 samples overall (18 samples/day). To obtain 18 blood samples from 12 donor birds, six birds were sampled twice, allowing for 2 weeks of recovery between sampling points (there were no significant differences in mean Hct between the first and the second sampling).

**Sampling and analysis protocols**

After equilibration, the six tonometers were sampled sequentially using heparinized, gas-tight Hamilton syringes. A subsample of 90 µl was immediately loaded into an i-STAT cartridge. Measurements were performed using the VetScan i-STAT 1 System (SN:704534-C; software version JAMS 137A/CLEW A28; Abaxis, Union City, CA, USA) with the i-STAT CG8+ cartridge test. Cartridges were stored in their original packaging at −20°C in the dark, and allowed to equilibrate to room temperature overnight prior to experiments. In addition, –10 µl of blood were loaded into a HemoCue 201® microcuvette (HemoCue AB, Angelholm, Sweden) for the analysis of [Hb].

Control measurements of blood parameters were carried out using established laboratory techniques. Hct was measured in triplicate with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the cyanomethaemoglobin method. The [Hb] was measured in triplicate using microhaematocrit capillary tubes (10 µl) and, after centrifuging at 17 000g for 3 min, [Hb] was measured in triplicate with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the cyanomethaemoglobin method. The [Hb] was calculated based on absorption measurements at 540 nm and using an extinction coefficient of 11. Whole blood pH, PO₂, and PCO₂ measurements were performed using a Radiometer BMS 3 Mk2 system, thermostatted at 41°C, with Radiometer acid–base analysers PHM 71 and PHM 84 (Copenhagen, Denmark) and a Cameron Instruments OM200 (Port Aransas, TX, USA). Whole blood total O₂ content (TO₂) was measured according to Tucker (1967). The Hb-O₂ saturation (sO₂) was calculated from TO₂ after subtracting physically dissolved O₂ according to Boultier et al. (1984) and dividing by the theoretical maximal carrying capacity of the rinsed RBCs based upon the tetrameric [Hb] obtained spectrophotometrically.

**Data analysis**

All data were analysed with RStudio 0.98.1049 (RStudio Inc., Boston, MA, USA). Given that i-STAT and HemoCue report [Hb] in different units, we converted both results into millimolar using the molecular weight of human Hb. All i-STAT and HemoCue values were compared with control measurements by regression analysis using the raw data (n = 54). The measurement errors for the i-STAT and HemoCue values relative to control measurements were calculated as follows: δ = (PCA − control)/control × 100. The δ data were then compared with control measurements by regression analysis (n = 54). Normality of distribution was tested with the Shapiro–Wilks test (P < 0.05), and homogeneity of variances was tested with Levene’s test (P < 0.05). The effects of PO₂ and PCO₂ on δ were tested on the squared values of δ (i.e. all values were positive). In some cases, this transformation led to a significant deviation of the distribution from normality, which could not be remediated by data transformation. If such was the case, the effects of PO₂ and PCO₂ on δ were tested with the Wilcoxon rank sum test (P < 0.05, n = 18); otherwise one-way ANOVAs were used (P < 0.05, n = 18). All data are presented as means ± SEM.

**Results**

**pH**

There was a significant linear relationship between pH measurements performed with the i-STAT and control pH measurements with a thermostated electrode (Fig. 1A). The measurement error of i-STAT pH measurements, δpH (expressed as a percentage), relative to control pH is shown in Fig. 1B and is best described by a significant linear relationship, with the equations given in Table 1. There were significant effects of PO₂ (P < 0.001) and PCO₂ (P = 0.014) on δpH (Fig. 1C).

**Partial pressure of O₂**

We found a significant linear relationship between PO₂ measured with the i-STAT and control PO₂ measured with a...
thermostated electrode (Fig. 2A). However, no significant relationship was detected between δPO₂ and control PO₂ (P > 0.05; Fig. 2B). The PCO₂ had a significant effect on δPO₂ (P < 0.001), but no significant effect on δPO₂ was detected for PO₂ (P = 0.723; Fig. 2C).

### Haemoglobin O₂ saturation

There was a significant linear relationship between sO₂ measured with the i-STAT and control sO₂ determined according to Tucker (1967; Fig. 3A), but we found no significant relationship between δsO₂ and control sO₂ (P > 0.05; Fig. 3B). A significant effect of PO₂ on δsO₂ was detected (P < 0.001), but not for PCO₂ (P = 0.875; Fig. 3C).

### Partial pressure of CO₂

There was a highly significant linear relationship between PCO₂ measured with the i-STAT and control PCO₂ measured with a thermostated electrode (Fig. 4A). Also, we detected a significant linear relationship between δPCO₂ and control PCO₂ (Fig. 4B). Both PO₂ (P = 0.047) and PCO₂ (P = 0.017) had significant effects on δPCO₂ (Fig. 4C).

### Haematocrit

The average initial Hct for all sampled birds was 37.8 ± 0.8%, and no significant changes in Hct were detected throughout the tonometry trials (P > 0.05). We found a significant linear relationship between i-STAT Hct and control Hct measured in capillary tubes after centrifugation (Fig. 5A). There was, however, no significant relationship between δHct and control Hct (P > 0.05; Fig. 5B) and no significant effects of PO₂ (P = 0.707) or PCO₂ (P = 0.442) on δHct (Fig. 5C).

### Haemoglobin concentration

Average [Hb] throughout the study was 1.80 ± 0.03 mM. We found significant linear relationships between [Hb] measured with the i-STAT or the HemoCue and control [Hb] measurements with a spectrophotometer (Fig. 6A). In addition, we found a significant linear relationship between i-STAT δ[Hb] and control [Hb], but not between HemoCue δ[Hb] and control [Hb] (Fig. 6B). A significant effect of PO₂ on δ[Hb] was detected for the i-STAT (P < 0.001) and the HemoCue (P < 0.001), but PCO₂ had no significant effect on δ[Hb] in either the i-STAT (P = 0.507) or the HemoCue (P = 0.565; Fig. 6C).

### Discussion

Based on our results, we consider the i-STAT system a useful tool for measuring bar-headed goose whole blood pH, PO₂, PCO₂ and Hct; however, the i-STAT cannot produce reliable sO₂ values, and the HemoCue system seems to provide more robust measurements of [Hb]. We typically found differences between i-STAT and control measurements, but in most cases both measurements scaled linearly and thus the equations presented in Table 1 can be used to correct PCA values. The critical researcher should, however, consider the limitations of the

### Table 1: Parameter estimates (means ± SEM), r² and P-values for the relationships between i-STAT (or HemoCue) vs. control measurements, and between i-STAT (or HemoCue) measurement errors, δ(x) (in %), vs. control measurements (n = 54)

| Measurement | a          | b          | c          | r²     | P-value |
|-------------|------------|------------|------------|--------|---------|
| pH          | 0.839 ± 0.281 | 0.869 ± 0.038 | 0.91       | <0.001|
| δpH         | 9.703 ± 3.768 | −1.547 ± 0.506 | 0.14       | 0.004 |
| PO₂         | −2.152 ± 3.495 | 0.732 ± 0.040 | 0.86       | <0.001|
| δPO₂        | −32.260 ± 3.792 | 0.030 ± 0.043 | −0.01      | 0.495 |
| PO₂ (corrected for PCO₂) | −18.203 ± 3.634 | 0.731 ± 0.030 | 0.92       | <0.001|
| sO₂         | −11.959 ± 7.230 | 1.014 ± 0.091 | 0.72       | <0.001|
| δsO₂        | −34.715 ± 10.847 | 0.249 ± 0.136 | 0.046      | 0.074 |
| PCO₂        | 1.911 ± 0.327 | 0.912 ± 0.010 | 0.99       | <0.001|
| δPCO₂       | 7.663 ± 1.401 | −0.291 ± 0.044 | 0.45       | <0.001|
| Hct         | −1.508 ± 1.268 | 0.906 ± 0.033 | 0.94       | <0.001|
| δHct        | −16.752 ± 3.316 | 0.088 ± 0.087 | 0.00       | 0.318 |
| [Hb]        | −0.442 ± 0.232 | 1.209 ± 0.129 | 0.63       | <0.001|
| δ[Hb]       | −30.917 ± 12.805 | 14.967 ± 7.115 | 0.06       | 0.041 |
| HemoCue [Hb] | −0.272 ± 0.211 | 1.408 ± 0.116 | 0.73       | <0.001|
| HemoCue δ[Hb] | 8.865 ± 11.673 | 9.215 ± 6.440 | 0.02       | 0.159 |

Linear regressions are according to: PCA(α) = a + b × control(α) and δα) = a + b × control(α). Multiple linear regression is according to: i-STAT PO₂ = a + b × control PO₂ + c × control PCO₂. Abbreviations: [Hb], haemoglobin concentration; Hct, haematocrit; PCA, portable clinical analyser; PCO₂, partial pressure of CO₂; PO₂, partial pressure of O₂; and sO₂, haemoglobin-O₂ saturation.
The accuracy of i-STAT pH measurements and therefore, it cannot be recommended to extrapolate these linear corrections beyond the tested conditions.
PCO₂ (as in Fig. 2), a clear trend emerges, indicating that δPCO₂ will be smallest at 45 mmHg PCO₂ and will increase at lower PCO₂ tensions (Fig. 2C). The arterial PCO₂ of bar-headed goose blood has been shown to fall below 10 mmHg in hyperventilating birds in severe hypoxia (Scott and Milsom, 2007). Human arterial PCO₂ (~40 mmHg; Crosby and Robbins, 2003) closely matches our highest tested PCO₂ (45 mmHg), and given that the i-STAT is optimized for the analysis of human blood, it is not surprising that highest accuracy of PCO₂ measurements is achieved in these conditions. To correct i-STAT PCO₂ measurements on bar-headed goose blood by taking into account both PO₂ and PCO₂, a multiple linear regression model was fitted to the data in Fig. 2A, and the resulting equation is presented in Table 1 (see PO₂ corrected for PCO₂). In fact, the addition of PCO₂ as a predictor of i-STAT PO₂ significantly increased r² from 0.86 to 0.92 (One-way ANOVA, P < 0.001). The mechanism by which PCO₂ affects the accuracy of i-STAT PO₂ measurements, however, is not known. It seems possible that in fact it is changes in blood pH (that are intrinsically associated with changes in PCO₂) and therefore changes in the redox potential of the sample that may affect the reading of the amperometric PO₂ sensor within the i-STAT cartridge. This, however, remains to be tested thoroughly. As a result of these uncertainties, we emphasize that the linear equations presented here are likely to be applicable only to bar-headed goose whole blood under the tested conditions. Correction of i-STAT results should always be performed with caution, and additional validations for the specific study conditions are recommended.

Measurements of sO₂ with the i-STAT system showed a significant linear relationship with control sO₂ measurements made using the method of Tucker (1967). On average, the i-STAT underestimated control sO₂ by ~40% at low Hb-O₂ saturations, but by <10% at full Hb saturation (Fig. 3B). The variability of sO₂ measurements was large, however, and several replicate samples would be required to obtain a mean value that could be corrected into a more accurate measurement using our linear equations. A significant effect of PO₂ on δsO₂ indicates that changes in this factor will influence the accuracy of i-STAT sO₂ measurements, and it seems that this will predominantly be the case at low PO₂ tensions (Fig. 3C). The i-STAT system calculates sO₂ from measured PO₂ and pH and calculated HCO₃⁻ values, by assuming a constant Hct and a ‘normal’ Hb-O₂ affinity of human blood. Thus, this system cannot account for inter-specific differences in Hb-O₂ affinity (or its modulation by allosteric effectors), fluctuations in Hct or the presence of dysfunctional Hb species (e.g. met-, sulf- and carboxyHb; i-STAT Technical Bulletin, 2013b). Therefore, we cannot recommend the i-STAT system for the analysis of sO₂ in birds (including the bar-headed goose) or any other non-human species (Harter et al., 2014, 2015).
A validated the i-STAT for the low blood cant effects of conditions that we tested is not recommended, owing to significant variations. The accuracy of these results was smaller in the i-STAT compared with the HemoCue, which measures [Hb] on whole blood samples. HP was within 15% (Fig. 5B). Measurements of Hct performed with the i-STAT system were consistently lower than control measurements in capillary tubes, on average by ~15% (Fig. 5B). However, there was a highly significant linear relationship between i-STAT Hct and control Hct, which accounted for 94% of the observed variation ($r^2 = 0.94$). We found no significant effects of PO$_2$, PCO$_2$ or Hct on δHct (Fig. 5B and C); therefore, we can recommend correcting i-STAT Hct measurements on bar-headed goose blood with the linear equation provided in Table 1. The i-STAT system measures Hct by means of whole blood conductometry, where a higher fraction of RBCs will decrease sample conductivity. This measurement is corrected for temperature, sample volume and plasma ion levels (albeit only Na$^+$ and K$^+$). In fish, the i-STAT generally underestimated Hct by 30–45% (Harrenstien et al., 2005; DiMaggio et al., 2010; Harter et al., 2014, 2015), suggesting that the conductive properties of a whole blood sample from bar-headed goose resembles the characteristics of human blood more closely than that of fish. However, in all of the above studies, Hct was underestimated by the i-STAT, and this may be a consequence of the nucleated state of RBCs in both fish and birds. Mammals (including humans) have non-nucleated RBCs. Therefore, researchers using the i-STAT system on any non-mammalian species can expect an underestimation of Hct and need to validate these results appropriately.

We performed simultaneous measurements of [Hb] with two commonly used PCAs, the i-STAT and the HemoCue, and compared these values with [Hb] measured with a spectrophotometer using the cyanomethaemoglobin method. Values generated with both PCAs yielded significant linear relationships with control measurements (Fig. 6A); however, [Hb] was smaller in the i-STAT compared with the HemoCue, which underestimated control [Hb] by ~20% (Fig. 6B). While the average [Hb] measured by the i-STAT was consistent with control measurements, there was a substantial amount of variation, which may require increasing the number of replicate samples to obtain an accurate mean [Hb]; the same applies for the HemoCue. Considering that the i-STAT does not measure [Hb], but calculates it from Hct (simply by multiplying Hct values by 0.34; i-STAT Technical Bulletin, 2013a), it is surprising that i-STAT measurements were more accurate than those obtained from the HemoCue, which measures [Hb] photometrically after conversion to azide-methaemoglobin (HemoCue Manual, 2015). Given that in the i-STAT, Hct was underestimated by ~15% and [Hb] was not, it seems that the high accuracy of [Hb] measurements was rather coincidental, which could be verified using broader ranges of Hct and [Hb] than those used here. The simple algorithm used by the i-STAT to convert Hct measurements into [Hb] values is highly susceptible to changes in mean corpuscular [Hb], which may occur with changes in the physiological status of the bird (e.g. stress, exercise or osmotic disturbances; Riddick et al., 1971; Nikinmaa and Huestis, 1984; Prats et al., 1996) or changes in
environmental conditions (e.g. hypoxia; Black and Tenney, 1980). Given that the HemoCue genuinely measures [Hb], the values obtained are likely to be more robust to inter-specific differences and other confounding factors associated with the physiological status of the animal or environmental conditions. Therefore, and despite the lower accuracy compared with the i-STAT system, we consider the HemoCue the better instrument for measuring [Hb] in bar-headed goose whole blood. The highly significant linear relationship between HemoCue [Hb] and control values (Fig. 6A) indicates that a correction of HemoCue results is possible with the linear equation provided in Table 1. However, changes in PO$_2$ (Fig. 6C) may affect the accuracy of HemoCue (and i-STAT) [Hb] measurements and likewise the validity of the correction equation. We also emphasize that the obtained results may vary with species, even among birds.

**Conclusion**

The i-STAT system is a reliable tool for measuring blood parameters in the bar-headed goose. For whole blood pH, PO$_2$, PCO$_2$ and Hct, we found significant differences between i-STAT and control measurements; however, in general these results can be corrected by using the linear equations provided in Table 1, thereby allowing researchers to increase the accuracy of i-STAT results. The SO$_2$ measured with the i-STAT displayed a substantial variability and is likely not a robust measurement over a broader range of study conditions or species. While the i-STAT system yielded satisfactory measurements for [Hb] in bar-headed goose whole blood, we consider the HemoCue a more reliable tool to assess this blood parameter, as long as the results are corrected. We emphasize that the linear equations presented here are valid only for the range of conditions that we tested and that extrapolation beyond these values or their application to other species (including other birds) will require appropriate validation.

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