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Research article

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 CO_2 (PCO_2) in water breathers, which were outside the reportable range in the i-STAT; (iii) differences in O_2-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 O_2-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 PCO_2, 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 (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 PO_2 and PCO_2 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 (O_2, CO_2 and N_2) 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 PO_2 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 PCO_2 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

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 4°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, Ängelholm, Sweden) for the analysis of [Hb].

Control measurements of blood parameters were carried out using established laboratory techniques. Hct was measured in triplicate using microhaematocrit capillary tubes (10 µl) and, after centrifuging at 17 000*g* 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, PO_2 and PCO_2 measurements were performed using a Radiometer BMS 3 Mk2 system, thermostated 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 O_2 content (TO_2) was measured according to Tucker (1967). The Hb-O_2 saturation (sO_2) was calculated from TO_2 after subtracting physically dissolved O_2 according to Boutilier *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: $\delta = (\text{PCA} - \text{control})/\text{control} \times 100$. The δ data were then compared with control measurements by regression analysis ($n = 54$). Normality of distribution was tested with the Shapiro–Wilk test ($P < 0.05$), and homogeneity of variances was tested with Levene's test ($P < 0.05$). The effects of PO_2 and PCO_2 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 PO_2 and PCO_2 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 PO_2 ($P < 0.001$) and PCO_2 ($P = 0.014$) on δpH (Fig. 1C).

Partial pressure of O_2

We found a significant linear relationship between PO_2 measured with the i-STAT and control PO_2 measured with a

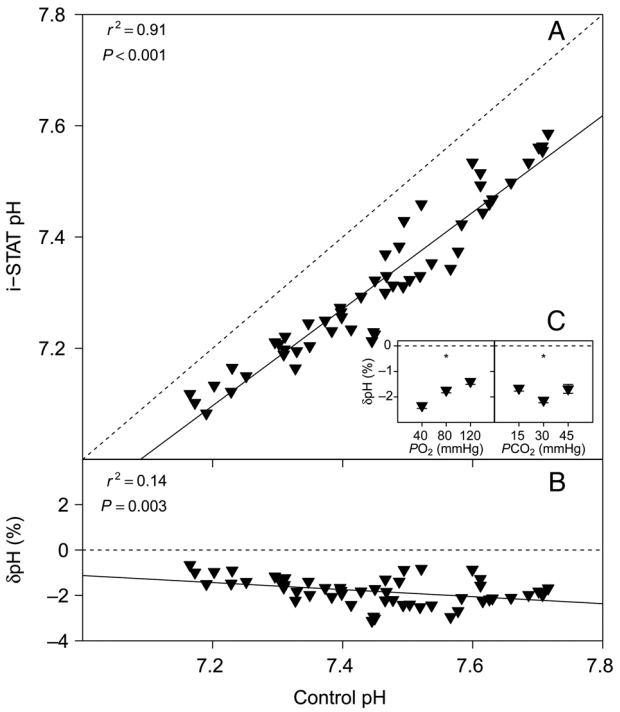

Figure 1: (**A**) Bar-headed goose whole blood pH measured with the i-STAT system (temperature-corrected values) vs. pH measured using a thermostated electrode (control). (**B**) The relative error of i-STAT pH measurements, δpH [in %; calculated as: (i-STAT pH − control pH)/control pH × 100] vs. control pH. Continuous lines represent the fitted linear models (see Table 1 for parameter estimates), and dashed lines represent the lines of identity. (**C**) Effects of partial pressures of O_2 and CO_2 (PO_2 and PCO_2; in mmHg) on δpH. Significant effects within treatments are indicated as * at $P < 0.05$ or NS for non-significant. Data are means ± SEM, and statistical analysis was performed on the absolute δpH values.

Table 1: Parameter estimates (means ± SEM), r^2 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*	r^2	*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
PO_2	−2.152 ± 3.495	0.732 ± 0.040		0.86	<0.001
δPO_2	−32.260 ± 3.792	0.030 ± 0.043		−0.01	0.495
PO_2 (corrected for PCO_2)	−18.203 ± 3.634	0.731 ± 0.030	0.543 ± 0.085	0.92	<0.001
sO_2	−11.959 ± 7.230	1.014 ± 0.091		0.72	<0.001
δsO_2	−34.715 ± 10.847	0.249 ± 0.136		0.046	0.074
PCO_2	1.911 ± 0.327	0.912 ± 0.010		0.99	<0.001
δPCO_2	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(x) = $a + b \times$ control(x) and δ(x) = $a + b \times$ control(x). Multiple linear regression is according to: i-STAT $PO_2 = a + b \times$ control $PO_2 + c \times$ control PCO_2. Abbreviations: [Hb], haemoglobin concentration; Hct, haematocrit; PCA, portable clinical analyser; PCO_2, partial pressure of CO_2; PO_2, partial pressure of O_2; and sO_2, haemoglobin-O_2 saturation.

thermostated electrode (Fig. 2A). However, no significant relationship was detected between δPO_2 and control PO_2 ($P > 0.05$; Fig. 2B). The PCO_2 had a significant effect on δPO_2 ($P < 0.001$), but no significant effect on δPO_2 was detected for PO_2 ($P = 0.723$; Fig. 2C).

Haemoglobin O_2 saturation

There was a significant linear relationship between sO_2 measured with the i-STAT and control sO_2 determined according to Tucker (1967; Fig. 3A), but we found no significant relationship between δsO_2 and control sO_2 ($P > 0.05$; Fig. 3B). A significant effect of PO_2 on δsO_2 was detected ($P < 0.001$), but not for PCO_2 ($P = 0.875$; Fig. 3C).

Partial pressure of CO_2

There was a highly significant linear relationship between PCO_2 measured with the i-STAT and control PCO_2 measured with a thermostated electrode (Fig. 4A). Also, we detected a significant linear relationship between δPCO_2 and control PCO_2 (Fig. 4B). Both PO_2 ($P = 0.047$) and PCO_2 ($P = 0.017$) had significant effects on δPCO_2 (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 PO_2 ($P = 0.707$) or PCO_2 ($P = 0.442$) 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 PO_2 on δ[Hb] was detected for the i-STAT ($P < 0.001$) and the HemoCue ($P < 0.001$), but PCO_2 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, PO_2, PCO_2 and Hct; however, the i-STAT cannot produce reliable sO_2 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

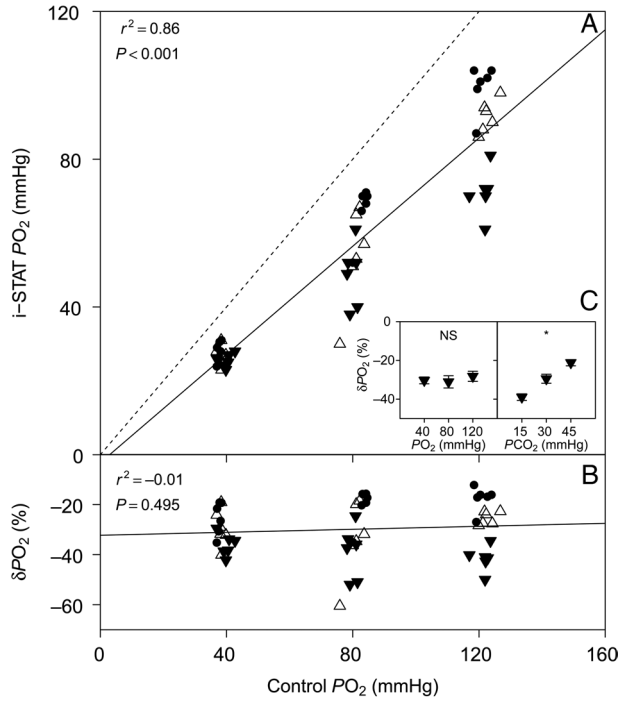

Figure 2: (**A**) Bar-headed goose whole blood PO_2 measured with the i-STAT system vs. PO_2 measured with a thermostated electrode (control) at 15 (inverted filled triangles), 30 (upright open triangles) and 45 mmHg PCO_2 (filled circles). (**B**) The relative error of i-STAT PO_2 measurements, δPO_2 vs. control PO_2. (**C**) Effects of PO_2 and PCO_2 on δPO_2. See legend to Fig. 1 for further information.

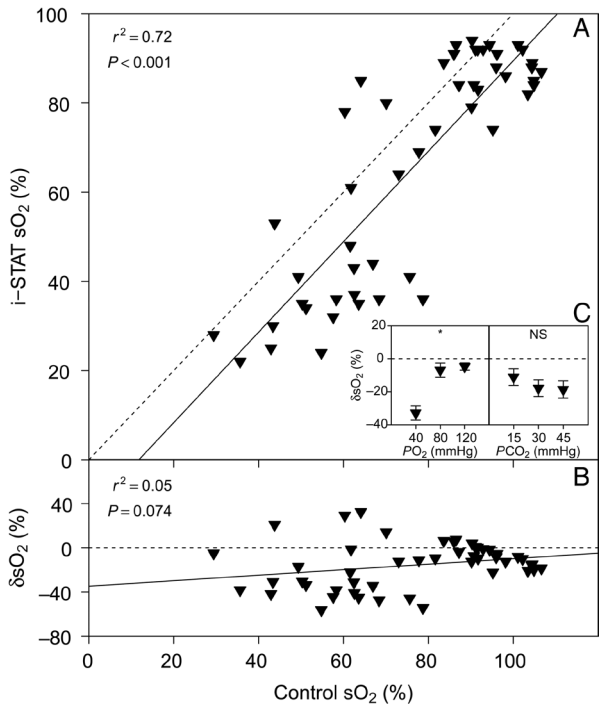

Figure 3: (**A**) Bar-headed goose haemoglobin-O_2 saturation (sO_2) measured with the i-STAT system vs. control sO_2 measured according to Tucker (1967). (**B**) The relative error of i-STAT sO_2 measurements, δsO_2 vs. control sO_2. (**C**) Effects of PO_2 and PCO_2 on δsO_2. See legend to Fig. 1 for further information.

i-STAT system and their potential implications for the validity of the generated results, which are discussed in more detail in the following paragraphs. For more information on the methodology used by the i-STAT system, we refer to a summary published by Harter *et al.* (2014; Table 2) or to the i-STAT procedure manual for more detail (i-STAT Procedure Manual, 2014).

Our results indicate that pH measurements with the i-STAT system on bar-headed goose whole blood scaled linearly with control pH measurements performed with a thermostated electrode. The variation of i-STAT pH measurements was ~0.1 pH units and, on average, the i-STAT underestimated control pH by ~2%. These results are consistent with previous validation studies of the i-STAT system on fish (Harrenstien *et al.*, 2005; Gallagher *et al.*, 2010; Harter *et al.*, 2014, 2015); however, it remains unclear why the i-STAT underestimates blood pH across the different species that have been tested. Given the small measurement error of i-STAT pH measurements (δpH) in the bar-headed goose, it seems possible to correct these values with the linear equations reported in Table 1, if greater accuracy is required. The significant effects of PO_2 and PCO_2 on δpH indicate that changes in these factors will affect the accuracy of i-STAT pH measurements and therefore, it cannot be recommended to extrapolate these linear corrections beyond the tested conditions.

The PO_2 measurements performed with the i-STAT displayed a large variability compared with control PO_2 measurements with a thermostated electrode. This variability in PO_2 measurements increased at higher PO_2 tensions. As a consequence, δPO_2 was also highly variable and ranged from –10 to –50% at 120 mmHg PO_2. This is in line with the results of a previous i-STAT validation study on sandbar shark (*Carcharhinus plumbeus*), which described increasing variability of PO_2 measurements at higher PO_2 tensions (Harter *et al.*, 2015). In the sandbar shark, this heterogeneity of variances was explained largely by differences in treatment temperature (tested at 15, 20 and 25°C) and the effects of a closed system temperature increase during measurements (Malte *et al.*, 2014). Given that the i-STAT system was developed for the analysis of human blood, samples in cartridges are heated to 37°C. However, the i-STAT does not have a cooling function, and if blood is loaded at 41°C (such as for the bar-headed goose), the small volume of just 90 µl is likely to cool, to some unknown degree (between 41 and 37°C), during analysis (~2 min). However, we do not expect this relatively small temperature gradient (~4°C) to have a major impact on blood PO_2 due to the effects of closed system cooling.

The significant effect of PCO_2 on δPO_2, however, indicates that changes in PCO_2 affect the accuracy of i-STAT PO_2 measurements and therefore may partly explain the observed variability. In fact, if i-STAT PO_2 measurements are grouped by

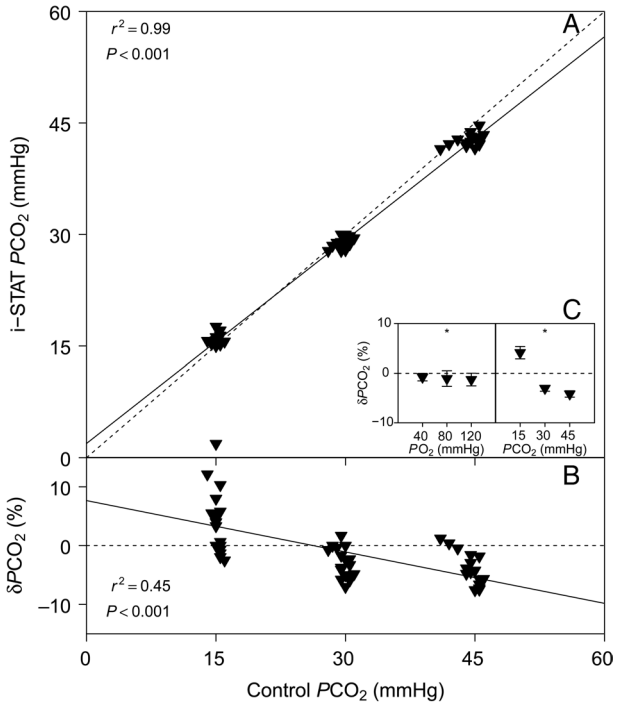

Figure 4: (**A**) Bar-headed goose whole blood PCO_2 measured with the i-STAT system vs. PCO_2 measured with a thermostated electrode (control). (**B**) The relative error of i-STAT PCO_2 measurements, δPCO_2 vs. control PCO_2. (**C**) Effects of PO_2 and PCO_2 on δPCO_2. See legend to Fig. 1 for further information.

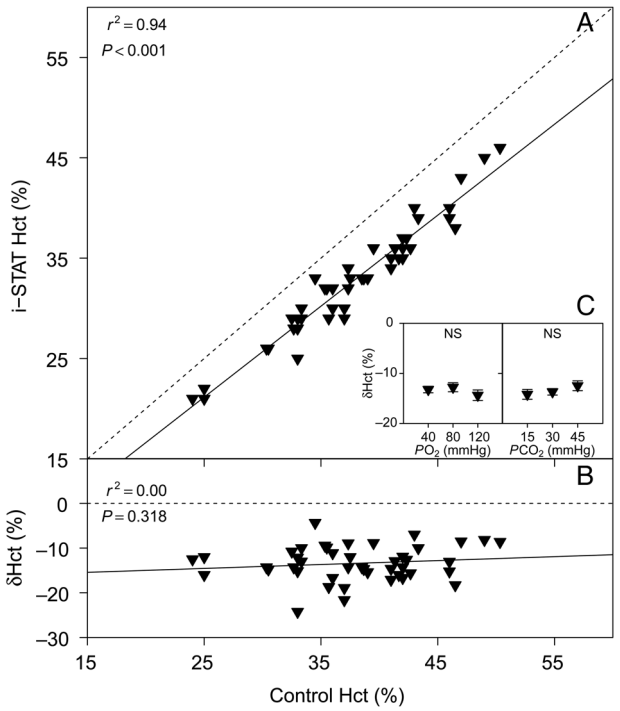

Figure 5: (**A**) Bar-headed goose haematocrit (Hct) measured with the i-STAT system vs. Hct measured in microcapillary tubes (control). (**B**) The relative error of i-STAT Hct measurements, δHct vs. control Hct. (**C**) Effects of PO_2 and PCO_2 on δHct. See legend to Fig. 1 for further information.

PCO_2 (as in Fig. 2), a clear trend emerges, indicating that δPO_2 will be smallest at 45 mmHg PCO_2 and will increase at lower PCO_2 tensions (Fig. 2C). The arterial PCO_2 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 PCO_2 (~40 mmHg; Crosby and Robbins, 2003) closely matches our highest tested PCO_2 (45 mmHg), and given that the i-STAT is optimized for the analysis of human blood, it is not surprising that highest accuracy of PO_2 measurements is achieved in these conditions. To correct i-STAT PO_2 measurements on bar-headed goose blood by taking into account both PO_2 and PCO_2, a multiple linear regression model was fitted to the data in Fig. 2A, and the resulting equation is presented in Table 1 (see PO_2 corrected for PCO_2). In fact, the addition of PCO_2 as a predictor of i-STAT PO_2 significantly increased r^2 from 0.86 to 0.92 (One-way ANOVA, $P < 0.001$). The mechanism by which PCO_2 affects the accuracy of i-STAT PO_2 measurements, however, is not known. It seems possible that in fact it is changes in blood pH (that are intrinsically associated with changes in PCO_2) and therefore changes in the redox potential of the sample that may affect the reading of the amperometric PO_2 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 sO_2 with the i-STAT system showed a significant linear relationship with control sO_2 measurements made using the method of Tucker (1967). On average, the i-STAT underestimated control sO_2 by ~40% at low Hb-O_2 saturations, but by <10% at full Hb saturation (Fig. 3B). The variability of sO_2 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 PO_2 on δsO_2 indicates that changes in this factor will influence the accuracy of i-STAT sO_2 measurements, and it seems that this will predominantly be the case at low PO_2 tensions (Fig. 3C). The i-STAT system calculates sO_2 from measured PO_2 and pH and calculated HCO_3^- values, by assuming a constant Hct and a 'normal' Hb-O_2 affinity of human blood. Thus, this system cannot account for inter-specific differences in Hb-O_2 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 sO_2 in birds (including the bar-headed goose) or any other non-human species (Harter *et al.*, 2014, 2015)

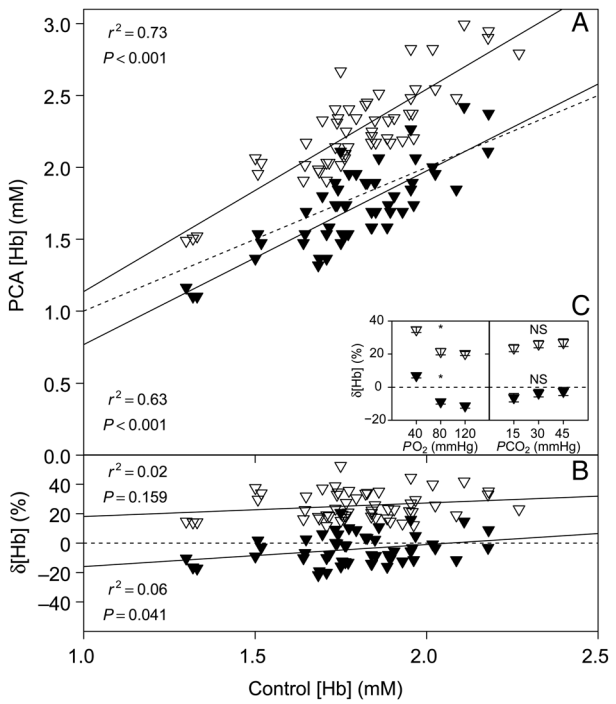

Figure 6: (**A**) Bar-headed goose haemoglobin concentration [Hb] measured with the i-STAT system (filled symbols) and the HemoCue (open symbols) vs. [Hb] measured with a spectrophotometer (control). (**B**) The relative error of i-STAT or HemoCue [Hb] measurements, δ[Hb] vs. control [Hb]. (**C**) Effects of PO_2 and PCO_2 on δ[Hb]. See legend to Fig. 1 for further information.

without thoroughly validating the results for the specific study conditions.

Measurements of blood PCO_2 with the i-STAT system corresponded well with control measurements using a thermostated electrode. We detected a highly significant linear relationship between values generated with both methods, which accounts for 99% of the observed variation ($r^2 = 0.99$; Fig. 4A). Over the range of tested PCO_2, δPCO_2 was within 10% of control measurements. The accuracy of these results can be improved further by using the linear equation described in Table 1. However, extrapolation of results beyond the conditions that we tested is not recommended, owing to significant effects of PO_2 and PCO_2 on δPCO_2. Especially low PCO_2 tensions will decrease the accuracy of i-STAT PCO_2 measurements, and this result is consistent with previous studies that validated the i-STAT for the low blood PCO_2 tensions observed in fish (Harter *et al.*, 2014, 2015). As previously discussed, we found that PO_2 and PCO_2 measurements in the i-STAT do influence one another, even though they are measured separately. Overall, we consider the i-STAT system a reliable tool to assess PCO_2 in bar-headed goose whole blood, under the conditions that we tested. Users should bear in mind, however, that in severe hypoxia the arterial PCO_2 of bar-headed goose blood can fall below 10 mmHg (Scott and Milsom, 2007), which may bode poorly for an accurate measurement of PCO_2 (and PO_2) with the i-STAT system.

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 geese 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, δ[Hb] was smaller in the i-STAT compared with the HemoCue, which overestimated 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 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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