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[Article]

Blood Gas, Oxygen Saturation, pH, and Lactate Values in Elasmobranch Blood Measured with a Commercially Available Portable Clinical Analyzer and Standard Laboratory Instruments

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Abstract.—Blood gas, pH, and lactate data are often used to assess the physiological status and health of fish and can often be most valuable when blood samples are analyzed immediately after collection. Portable clinical analyzers allow these measurements to be made easily in the field. However, these instruments are designed for clinical use and thus process samples at 37°C. A few studies have validated the use of portable clinical analyzers for assessing blood gases and acid–base profiles in teleosts, but equivalent data are not available for elasmobranchs. We therefore examined the relationship of blood gas, pH, and lactate values measured with an i-STAT portable clinical analyzer with those measured using standard laboratory blood gas (thermostatted to 25°C) and lactate analyzers in samples taken from three species of carcharhiniform sharks. We found tight correlations ($r^2 > 0.90$) between these methods for pH, pO_2, pCO_2, and lactate level values. We thus developed species-specific equations for converting blood values measured with an i-STAT portable clinical analyzer to those taken at 25°C. Additional studies need to address a wider range of temperatures and elasmobranch species.

Blood gases, acid–base status, and blood lactate can help indicate the condition of fish following stressors such as those associated with capture (e.g., Cliff and Thurman 1984; Wells et al. 1986; Harrenstien et al. 2005; Skomal 2007), including indications of immediate and delayed mortality following release (e.g., Hoffmayer and Parsons 2001; Young et al. 2006; Arlinghaus et al. 2009). Physiological responses to different acute stressors can be relied upon to help inform new or gauge the effectiveness of preexisting management decisions and conservation initiatives for commercially and recreationally important species (Ferguson and Tufts 1992; Wikelski and Cooke 2006; Young et al. 2006). For example, physiological tools can be used to help reveal interspecific responses to a given anthropogenic stressor or to suggest means to attenuate the most detrimental aspects of that stressor in a specific species.

It can be advantageous to assess fish condition prior to release back in the wild (Venn Beecham et al. 2006; Forbert et al. 2009). Portable clinical analyzers (PCAs; Steinmetz et al. 2007) now permit rapid field assessment of blood chemistry values (Erickson and Wilding 1993; Verwaerde et al. 2002) and such devices are now being used in studies of fish (Thompson et al. 2002; Suski et al. 2007; Cooke et al. 2008; Mandelman

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and Skomal 2009). Designed for clinical use, PCAs process samples at 37°C. With samples taken from fish at lower temperatures, use of PCAs results in a closed-system temperature change that will necessarily cause significant changes in blood gases, O_2 saturation, and pH due to temperature-induced changes in hemoglobin O_2 affinity, CO_2 solubility, and reciprocal titration of plasma proteins and plasma bicarbonate (e.g., Brill and Bushnell 1991; Brill et al. 1992). Previous studies using PCAs to assess packed cell volume (PCV) and ions in fish (Kojima et al. 2004; Harrenstien et al. 2005) likewise noted temperature differences as being a confounding factor. To the best of our knowledge only one study has validated PCA data relative to those generated from more traditional laboratory-based instrumentation in teleosts (Harrenstien et al. 2005), and there are no equivalent data for elasmobranchs. We thus sought to assess values derived from a PCA against conventionally accepted laboratory instrumentation using blood from elasmobranch fish.

Methods

Juvenile sandbar sharks *Carcharhinus plumbeus* and adult smooth dogfish *Mustelus canis* were captured from the lagoon system immediately adjacent to the Virginia Institute of Marine Sciences' Eastern Shore Laboratory (Wachapreague, Virginia) via light-weight angling gear. Fight times were minimized and lasted an average of 5 min. Once landed, each shark was placed in a 2-m-diameter polypropylene tank with freshly aerated sweater to allow swim-gliding. After 2 min of acclimation, sandbar sharks were gently restrained and immediately sampled for blood on board. Smooth dogfish were placed in a similar 2-m tank and immediately transported back to the laboratory where they were maintained in shore-side tanks for later experimentation (as described by Brill et al. 2008). Gummy sharks *Mustelus antarcticus* were collected in coastal waters near Mallacoota (Victoria, Australia) by a commercial fisherman using longlines, and were transported to the laboratory facilities in Queenscliff (Victoria, Australia) in a 4,000-L truck-mounted fish transport tank. Sharks were held in circular 19,000-L aquaculture tanks connected to a water flow-through seawater system. Sharks were left to acclimatize for at least 7 d prior to experimentation.

All blood samples from sandbar and smooth dogfish were collected by caudal venipuncture within 30 s of handling. Blood was then left in sealed syringes at room temperature so that the metabolic activity of the red blood cells would reduce O_2, increase CO_2, and decrease pH. Aliquots were periodically removed and assayed side-by-side for pO_2, pCO_2, and pH using an i-STAT PCA with a CG4+ cartridge (Abbott Laboratories, Abbott Park, Illinois), and a Radiometer MKS Mark 2 blood gas analyzer (Radiometer America, Westlake, Ohio) thermostatted to 25°C. Blood oxygen content ($[O_2]$) of each sample was determined by the Tucker (1967) method. Percent saturation was calculated as sample $[O_2]/[O_2]$ max, where $[O_2]$ max (100% saturation) was taken to be the $[O_2]$ of each sample determined after 45 min of equilibration with air. Blood samples for gummy sharks were collected via caudal venipuncture at various points in time during a 72-h recovery period from a 60-min gill-net capture event in a controlled setting, a method developed by Frick et al. (2009). Sharks were quickly restrained by hand in the tank, and blood samples were obtained within 30 s of initial handling of the sharks. Samples were immediately analyzed for lactate ($mmol \cdot L^{-1}$) using an i-STAT PCA with a CG4+ cartridge. Lactate values are not dependent upon temperature. A subsample was used to fill three microhematocrit tubes—subsequently spun at 10,000 × gravity for 5 min—and from which the PCV was determined. The remaining blood was centrifuged (12,000 × gravity for 10 min), and the resulting plasma was removed and frozen at −20°C. These samples were later thawed, and lactate levels were measured using a YSI StatPlus Lactate and Glucose Analyzer (model 2300, Yellow Springs Instrument, Westlake, Ohio). Plasma lactate concentrations were converted to whole-blood equivalents by multiplying by (1 − PCV).

Linear regressions (performed using JMP version 8.0; SAS Institute, Cary, North Carolina) were used to examine the relationship between the blood gases, pH, and lactate levels obtained with an i-STAT PCA and the more conventional laboratory instruments. Two-way nested analysis of variance (ANOVA) was used to determine the presence of species-specific differences in the regression slopes at $\alpha = 0.05$.

In addition, using the equations provided by Mandelman and Skomal (2009), we corrected the data we generated from sandbar shark and smooth dogfish blood via an i-STAT PCA for temperature, namely,

$$\mathrm{pH_{TC}} = \mathrm{pH}_M - 0.011(T - 37) \quad (1)$$

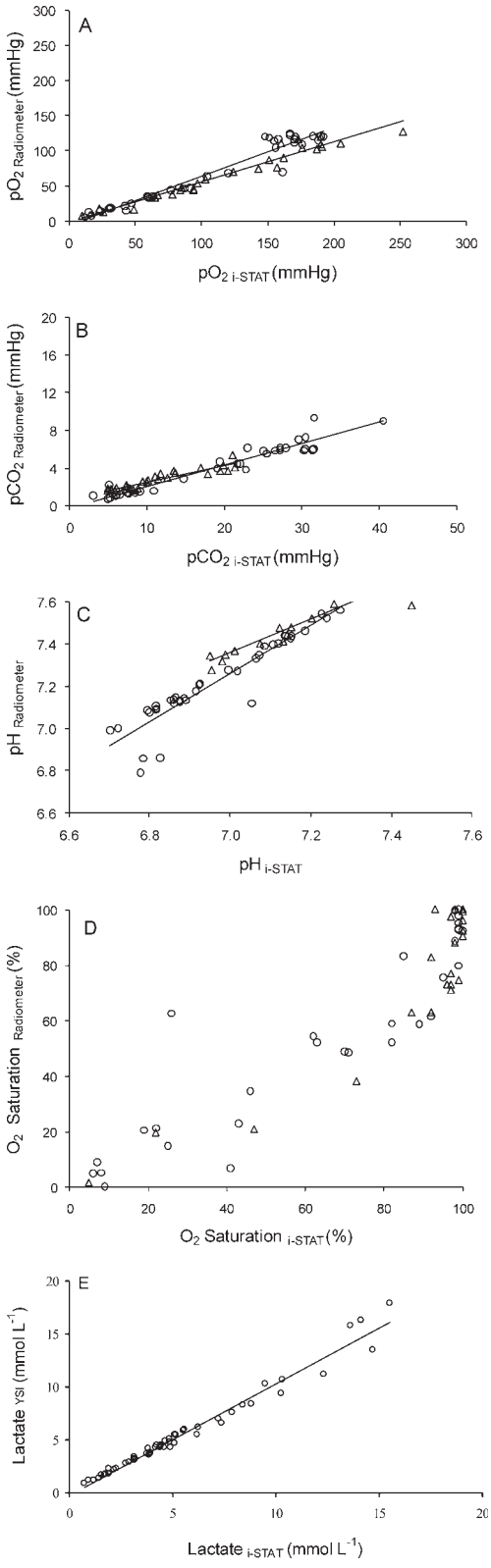
and

$$p\mathrm{CO_{2TC}} = p\mathrm{CO}_{2M}(10^{-0.019\Delta T}), \quad (2)$$

where T is 25°C, M is the measured values from the i-STAT PCA, and TC is the temperature-corrected value.

Results

There were significant positive linear relationships between the i-STAT PCA and radiometer blood gas analyzer values for the pO_2, pCO_2, and pH of blood from both sandbar sharks and smooth dogfish (Figure 1A–C). The following equations relate values obtained



by the i-STAT PCA to those obtained by the Radiometer MKS Mark 2 blood gas analyzer for sandbar shark blood (in all equations, the values in parentheses are standard errors for the regression coefficients, and $P < 0.001$ in all comparisons):

$$p\mathrm{O_{2\,Radiometer}} = 0.697(\pm 0.03)p\mathrm{O_{2\,i-STAT}} - 5.809(\pm 3.28), \quad (3)$$

for which $N = 27$ and $r^2 = 0.95$; and

$$p\mathrm{CO_{2\,Radiometer}} = 0.226(\pm 0.01)p\mathrm{CO_{2\,i-STAT}} - 0.205(\pm 0.24) \quad (4)$$

and

$$\mathrm{pH_{Radiometer}} = 1.144(\pm 0.08)\mathrm{pH_{i-STAT}} - 0.752(\pm 0.53), \quad (5)$$

for which $N = 37$ and $r^2 = 0.87$. The following equations relate values obtained by the i-STAT PCA to those obtained by the Radiometer MKS Mark 2 blood gas analyzer for smooth dogfish blood:

$$p\mathrm{O_{2\,Radiometer}} = 0.572(\pm 0.03)p\mathrm{O_{2\,i-STAT}} - 1.449(\pm 3.56), \quad (6)$$

$$p\mathrm{CO_{2\,Radiometer}} = 0.173(\pm 0.01)p\mathrm{CO_{2\,i-STAT}} + 0.775(\pm 0.19), \quad (7)$$

and

$$\mathrm{pH_{Radiometer}} = 0.795(\pm 0.06)\mathrm{pH_{i-STAT}} + 1.797(\pm 0.42), \quad (8)$$

for which $N = 26$ and $r^2 = 0.88$. The slopes were significantly different between both species for $p\mathrm{O_2}$, $p\mathrm{CO_2}$ and pH (two-way ANOVA: $F_{1,\,62} = 7.62$, $P < 0.01$; $F_{1,\,59} = 11.23$, $P < 0.005$; $F_{1,\,61} = 11.16$, $P < 0.005$, respectively).

In contrast, the relationship of percent oxygen saturation ($\mathrm{sO_2}\%$) values obtained by the i-STAT PCA and Tucker (1976) system for sandbar shark and smooth dogfish blood were nonlinear, especially near 100% saturation (Figure 1D).

There was a significant positive linear relationship

←

Figure 1.—Relationships between **(A)** $p\mathrm{O_2}$, **(B)** $p\mathrm{CO_2}$, **(C)** pH, and **(D)** percent $\mathrm{O_2}$ saturation, as determined by i-STAT PCA (measurement temperature, 37°C) and radiometer blood gas analyzer (measurement temperature, 25°C) using paired blood samples from sandbar sharks (open circles) and smooth dogfish (open triangles). Panel **(E)** shows a comparison between blood lactate values from gummy sharks measured with i-STAT PCA and a Yellow Springs Instrument StatPlus Lactate and Glucose Analyzer.

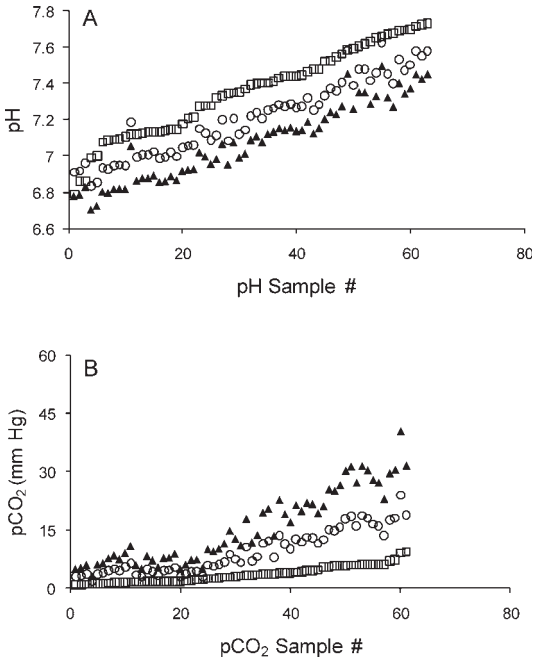


FIGURE 2.—Values for **(A)** pH and **(B)** (pCO_2) in full distribution (using pooled data from both sandbar sharks and smooth dogfish) in ascending order for the radiometer blood gas analyzer at 25°C (open squares); i-STAT PCA values manually converted to 25°C using equations from Mandelman and Skomal (2009; open circles); and raw i-STAT PCA values at 37°C (triangles).

between the lactate values generated by the i-STAT PCA values and those from the YSI analyzer (Figure 1E):

$$\text{Lactate}_{\text{YSI}} = 1.04(\pm 0.02) \times \text{Lactate}_{\text{i-STAT}} - 0.168(\pm 0.15), \quad (9)$$

for which $N = 54$, $r^2 = 0.97$ and $P > 0.05$.

The slope was not different from 1 (paired-t test: df = 53, $t = 0.96$, $P > 0.05$). On average, lactate values from the i-STAT and YSI analyzer differed by 0.11 mm/L (i.e., about 3%).

The equations provided by Mandelman and Skomal (2009) for the temperature correcting pH and pCO_2 data did not correctly predict for values measured by the radiometer blood gas analyzer (at 25°C) from the data provided by an i-STAT PCA (at 37°C; Figure 2A, B).

Discussion

The Radiometer MKS Mark 2 blood gas analyzer has a long and accepted history of measuring pH and blood gas values in fish (e.g., Evans 1982; Gilmour et al. 1997; Brill et al. 2008). As expected, due to differences in measurement temperatures, values generated from the i-STAT PCA differed significantly from those obtained from the laboratory blood gas analyzer. However, the relationships between pH, pO_2, and pCO_2 data, between the i-STAT PCA and conventional laboratory instrumentation were highly linear for blood from both sandbar sharks and smooth dogfish. For pH and blood gas tensions, the relationships appear to vary slightly by species, possibly reflecting interspecific differences in plasma proteins, blood buffering capacity, etc. Thus, raw i-STAT PCA values for pH, pO_2, and pCO_2 can be confidently converted to 25°C for sandbar and smooth dogfish based on the respective equations provided above, assuming similar temperature conditions. The application of these equations to other elasmobranch species, however, should be carried out with caution. The species-specific differences in how these values change by temperature clearly implies that a broader range of species must be investigated. A broader range of temperatures should also be investigated, ideally utilizing tonometers for the most precise blood gas partial pressures in which to compare values generated by the i-STAT PCA. Importantly, the tight linear fits observed within species suggests that the i-STAT is a reliable instrument for generating raw (non-temperature-corrected) values for these blood variables in elasmobranches, when applicable (e.g., in those studies evaluating relative changes induced by a particular stressor.

The i-STAT PCA does not appear to be a reliable tool for deriving $sO_2\%$ values in the species assessed in this study. The likely basis for this goes beyond temperature differences. Unlike the direct sensing for pH and blood gases, the i-STAT PCA calculates $sO_2\%$ post hoc by way of the bicarbonate ion (HCO_3^-) concentration, which in itself is calculated by the i-STAT PCA, using mammalian based constants in the Henderson Hasselbalch equation.

The YSI StatPlus Lactate and Glucose analyzer has a long and accepted history of measuring plasma lactate concentrations in fish (e.g., Farrell et al. 2001; Danley et al. 2002; Cooke et al. 2008). There was an essentially 1:1 relationship of blood lactate values measured by the i-STAT PCA and conventional laboratory instrumentation. The i-STAT PCA can thus be used as a reliable analytical tool for measuring blood lactate concentration of elasmobranchs in the field without the necessity of correction.

Our results also show that previously published temperature conversions (Mandelman and Skomal 2009) of pH and pCO_2 from i-STAT values (at 37°C) to 25°C underestimate values more than those determined using a Radiometer blood gas analyzer thermostatted to the appropriate temperature. Thus,

caution should be exercised when utilizing previously published equations, at least at the 25°C point. As stated, this study also suggests that the i-STAT PCA could be a reliable tool for generating pH, pO_2, pCO_2, and lactate values if the appropriate species-specific equations are employed for correcting the data to the animal's temperatures.

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