iStat Blood Gas Methods

*Animal Handling and Phlebotomy*

In March of 2013, Cownose rays (n=10; *R. bonasus*) from the shark and ray touch tank at the New England Aquarium were opportunistically selected for blood sampling during their routine spine clipping. Each blood sample was repeatedly analyzed at fixed intervals in order to 1) monitor the changes in blood acid-base parameters as a function of time, and 2) observe the difference in these values between different syringe types.

*R. bonasus* measured 7.04kg + 0.62 (mean + SE) in weight and 73.9cm + 1.82 in wingspan. Individuals were netted and placed into a 24.5o C bath containing 120 ppm tricaine methanylsulfonate (MS-222) and 240 ppm sodium bicarbonate for approximately 5-10 minutes (until the individual showed no resistance). Upon sedation, *R. bonasus* were placed in supine position and perfused with water from the anesthetic bath. Approximately 3 ml of blood was drawn from the caudal vein into a heparinized plastic syringe using a 22gauge, 1” needle. After sampling, *R. bonasus* were returned to a separate portion of the exhibit and carefully monitored for recovery.

In October 2013, Red-eared sliders (n=10; *T. elegans*) were selected from Rainforest Reptile Shows in Beverly, Massachusetts for blood sampling. *T. elegans* measured 940.4g + 128.30 (mean + SE) in weight, 17.9cm + 0.79 (mean + SE) in length, and 22.42oC + 0.26 (mean + SE) in temperature for *n=10*.

Approximately 2 ml of blood was taken from the subcarapacial sinus of each individual using a 3cc plastic syringe and 22-gauge 1” needle. Syringes were flushed with liquid sodium heparin prior to sampling and plungers within the syringes were lightly coated with mineral oil for lubrication.

*Blood Processing*

Approximately 95 µL aliquots of blood from each sample were analyzed immediately (T0) and at 5, 10, 15, 45, and 90 minutes following T0 (T1, T2, T3, T4, and T5, respectively). Syringes were placed on ice between analyses. Blood was analyzed for pH, *p*CO2, *p*O2, and lactate values on an iStat analyzer with CG4+ cartridges (Abbott Laboratories, Abbott Park, Illinois) thermostatted to 37oC.

Values for the turtle blood samples were temperature corrected to individual internal temperatures using the following equations. Temperature corrections for *p*CO2 and *p*O2 were validated for Kemp’s Ridley turtles by Keller et al. (2012), while pH corrections were validated for *T. elegans,* formerly known as *Pseudemys scripta elegans* at the time of publication by Robin (1962).

pHTC = (0.0144 \* ∆T) + pH

*p*CO2TC = *p*CO2\* 10(–0.019 \* ∆T)

*p*O2TC = *p*O2\* 10(–0.0058 \* ∆T)

CNR blood sample values were temperature corrected to 25°C using the following equations validated for smooth dogfish (*Mustelus canis*) by Gallagher et al. (2010):

pH25°C = 0.795(+ 0.06)pHi-STAT + 1.797(+ 0.42)

*p*CO2 25°C = 0.173(+ 0.01)*p*CO2 i-STAT + 0.775(+ 0.19)

*p*O2 25°C = 0.572(+ 0.03) *p*O2 i-STAT - 1.449(+ 3.56)