

Table S1: Currently employed methods to construct oxygen equilibrium curves (OEC).

Method	Characteristics of method	Means to record saturation	Type of temperature control	Range of sample volume	pH determination	PO2 determination	Blood treatment	Time per ODC	Pros	Cons	Example applications
Diffusion chamber	Photospectrometric measurement in chamber equilibrated with gas via diffusion capillaries	Monochromatic / continuous spectrum photospectrometry	Thermostated water bath	3 – 15µl	Measurement with pH capillary electrode (BMS radiometer) in separate sub-sample	Injection of known gas mixture via gas mixing pumps	buffered	10-30 min	Small sample volumes	pH measurements in separate blood sample Low data density	(Weber et al., 1976; Morris et al., 1985; Menze et al., 2005; Harnois et al., 2009; Storz et al., 2009; Campbell et al., 2010)
HEMOX-Analyser	Photospectrometric measurement combined with oxygen sensor	Dual wavelength photospectrometry	Cooling loop connect to thermostated water bath	2 – 50µl	none	Clarke electrode	buffered	30 min	Small samples volumes Very accurate High data density	No pH measurement Recording of only few wavelengths	(Guarnone et al., 1995; Stawski et al., 2006; Biolo et al., 2009; Rasmussen et al., 2009; Cook et al., 2012)
Tonometer	Gas equilibration in glass chamber and external measurement of oxygen tension	Measurement of total O ₂ content	Submersion in thermostated water bath	~ 3ml	Measurement of subsample with pH capillary electrode (Radiometer)	Measurement with Clark type electrode	Unbuffered	> 2 hours	Use of native/unbuffered blood	pH measurements in separate blood sample Large sample volumes Low data density	(Pörtner, 1990; Brill et al., 2008)
Thunberg tube / Photometric tonometer	Photospectrometric measurement in cuvette fused with gas equilibrated glass chamber	Monochromatic / continuous spectrum photospectrometry	Immerged into thermostated water bath	~ 3ml	None or in separate blood samples via pH electrodes	Injection of known gas mixture via e.g. gas flow controllers	buffered	> 2 hours	-	No pH measurement or measurement in separate blood sample Large sample volumes Low data density Long equilibration times	(Hill and Wolvekamp, 1936; Olianias et al., 2009; Bonaventura et al., 2010; Seibel, 2012)
Modified cuvette	Photospectrometric measurement in modified cuvette	Continuous spectrum photospectrometry	Temperature controlled cuvette holder	=>400 µl	Simultaneously in same blood sample via immersed micro pH electrode	Injection of known gas mixture via gas mixing pumps	Un-buffered blood	> 4 hours	Direct measurement of pH in same sample Use of native/unbuffered blood	Large sample volume Long lasting measurements	(Zielinski et al., 2001)
Pwee 50 / HemOscan	Photospectrometric measurement of thin blood films enclosed by Teflon-membranes	Dual wavelength photospectrometry	Temperature controlled chamber (Peltier controller)	1-2µl	Estimated via defined PCO ₂ and literature or measurement of subsamples with pH capillary electrode (Radiometer)	Injection of known gas mixture via gas mixing pumps or gas flow controller	unbuffered	30-40 min	Small sample volumes	Indirect pH measurement or measurement in separate blood sample Low data density Recording of only few wavelengths	(Clark et al., 2008; Henriksson et al., 2008; Verhille and Farrell, 2012)
CO-Oximeter	Photospectrometric measurement with modified cuvette	3-6 wavelength photospectrometry	none	35-50µl	None or measurement of subsamples with pH capillary electrode (Radiometer)	None / tonometric equilibration with known gas mixtures	unbuffered	20-60 sec per data point	Measures multiple haemoglobin forms	No pH measurement or measurement in separate blood sample Restricted to haemoglobin Low data density	(Jahr et al., 2001)

										Recording of only few wavelengths	
Mixing method	Volumetric mixing of known amounts of fully oxygenated and de-oxygenated blood followed by PO ₂ measurement	Setting of O2 saturation by mixing of defined proportions of oxygenated and de-oxygenated blood samples	Immerged into thermostated water bath	Max. 0.8 ml per data point	Measurement with pH capillary electrode (BMS radiometer) or blood gas analyser	Polarographic oxygen electrode via Radiometer (E5046) or Tucker chamber	unbuffered	>50 min per data point	Setting of a desired mixtures allows targeted measurement of e.g. P50 with only a single measurement	Low data density Laborious procedure Time consuming measurement	(Scheid and Meyer, 1978; Meir and Ponganis, 2009; Soegaard et al., 2012)
Tucker chamber	Deoxygenation of blood sample with ferricyanide to release and measure total bound oxygen	Measurement of total bound oxygen	Thermostatically controlled chamber	15-300µl	Measurement with capillary electrode (BMS 2 or PHM 71/73 radiometer)	Polarographic oxygen electrode (Radiometer)	Diluted with saline or unbuffered	>60min	Measurement of total bound oxygen	pH measurements in separate blood sample Low data density	(Tucker, 1967; Herbert et al., 2006; Brill et al., 2008; Petersen and Gamperl, 2011; Leon et al., 2012)

References

- Biolo, A., Greferath, R., Siwik, D. A., Qin, F., Valsky, E., Fylaktakidou, K. C., Pothukanuri, S., Duarte, C. D., Schwarz, R. P., Lehn, J.-M. et al. (2009). Enhanced exercise capacity in mice with severe heart failure treated with an allosteric effector of hemoglobin, myo-inositol trispyrophosphate. *Proc. Natl. Acad. Sci. USA* **106**, 1926-1929.
- Bonaventura, C., Henkens, R., De Jesus-Bonilla, W., Lopez-Garriga, J., Jia, Y., Alayash, A. I., Siburt, C. J. P. and Crumbliss, A. L. (2010). Extreme differences between hemoglobins I and II of the clam *Lucina pectinalis* in their reactions with nitrite. *BBA-Proteins Proteom.* **1804**, 1988-1995.
- Brill, R., Bushnell, P., Schroff, S., Seifert, R. and Galvin, M. (2008). Effects of anaerobic exercise accompanying catch-and-release fishing on blood-oxygen affinity of the sandbar shark (*Carcharhinus plumbeus*, Nardo). *J. Exp. Mar. Biol. Ecol.* **354**, 132-143.
- Campbell, K. L., Roberts, J. E., Watson, L. N., Stetefeld, J., Sloan, A. M., Signore, A. V., Howatt, J. W., Tame, J. R., Rohland, N., Shen, T. J. et al. (2010). Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance. *Nat. Genet.* **42**, 536-540.
- Clark, T. D., Seymour, R. S., Wells, R. M. G. and Frappell, P. B. (2008). Thermal effects on the blood respiratory properties of southern bluefin tuna, *Thunnus maccoyii*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **150**, 239-246.
- Cook, D. G., Iftikar, F. I., Baker, D. W., Hickey, A. J. R. and Herbert, N. A. (2012). Low O₂ acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *J. Exp. Biol.*
- Guarnone, R., Centenara, E. and Barosi, G. (1995). Performance characteristics of Hemox-Analyzer for assessment of the hemoglobin dissociation curve. *Haematologica* **80**, 426-430.
- Harnois, T., Rousselot, M., Rogniaux, H. and Zal, F. (2009). High-level production of recombinant *Arenicola marina* globin chains in *Escherichia coli*: A new generation of blood substitute. *Artif. Cells Blood Substit. Biotechnol.* **37**, 106-116.
- Henriksson, P., Mandic, M. and Richards, J. G. (2008). The osmorepiratory compromise in sculpins: impaired gas exchange is associated with freshwater tolerance. *Physiol. Biochem. Zool.* **81**, 310-319.
- Herbert, Neill A., Skov, Peter V., Wells, Rufus M. G. and Steffensen, John F. (2006). Whole blood–oxygen binding properties of four cold-temperate marine fishes: Blood affinity is independent of pH-dependent binding, routine swimming performance, and environmental hypoxia. *Physiol. Biochem. Zool.* **79**, 909-918.
- Hill, R. and Wolvekamp, H. P. (1936). The oxygen dissociation curve of haemoglobin in dilute solution. *Proc. R. Soc. Lond. B Biol. Sci.* **120**, 484-495.
- Jahr, J. S., Driessen, B., Lurie, F., Tang, Z., Louie, R. F. and Kost, G. (2001). Oxygen saturation measurements in canine blood containing hemoglobin glutamer-200 (bovine): In vitro validation of the NOVA CO-Oximeter. *Vet. Clin. Pathol.* **30**, 39-45.
- Leon, K., Pichavant-Rafini, K., Quemener, E., Sebert, P., Egretau, P. Y., Ollivier, H., Carre, J. L. and L'Her, E. (2012). Oxygen blood transport during experimental sepsis: effect of hypothermia*. *Crit Care Med* **40**, 912-918.
- Meir, J. U. and Ponganis, P. J. (2009). High-affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *J. Exp. Biol.* **212**, 3330-3338.
- Menze, M. A., Hellmann, N., Decker, H. and Grieshaber, M. K. (2005). Allosteric models for multimeric proteins: Oxygen-linked effector binding in hemocyanin†. *Biochemistry* **44**, 10328-10338.
- Morris, S., Taylor, A. C., Bridges, C. R. and Grieshaber, M. K. (1985). Respiratory properties of the haemolymph of the intertidal prawn *Palaemon elegans* (Rathke). *J. Exp. Zool.* **233**, 175-186.
- Olianas, A., Manconi, B., Masia, D., Sanna, M. T., Castagnola, M., Salvadori, S., Messina, I., Giardina, B. and Pellegrini, M. (2009). The oxygen-binding modulation of hemocyanin from the Southern spiny lobster *Palinurus gilchristi*. *J. Comp. Physiol. B* **179**, 193-203.
- Petersen, L. H. and Gamperl, A. K. (2011). Cod (*Gadus morhua*) cardiorespiratory physiology and hypoxia tolerance following acclimation to low-oxygen conditions. *Physiol. Biochem. Zool.* **84**, 18-31.

- Pörtner, H. O.** (1990). An analysis of the effects of pH on oxygen binding by squid (*Illex illecebrosus*, *Loligo pealei*) haemocyanin. *J. Exp. Biol.* **150**, 407.
- Rasmussen, J. R., Wells, R. M. G., Henty, K., Clark, T. D. and Brittain, T.** (2009). Characterization of the hemoglobins of the Australian lungfish *Neoceratodus forsteri* (Krefft). *Comp. Biochem. Physiol., A: Comp. Physiol.* **152**, 162-167.
- Scheid, P. and Meyer, M.** (1978). Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. *J. Appl. Physiol.* **45**, 818-822.
- Seibel, B. A.** (2012). The jumbo squid, *Dosidicus gigas* (Ommastrephidae), living in oxygen minimum zones II: Blood-oxygen binding. *Deep-Sea Res. Pt. II.*
- Soegaard, L. B., Hansen, M. N., van Elk, C., Brahm, J. and Jensen, F. B.** (2012). Respiratory properties of blood in the harbor porpoise, *Phocoena phocoena*. *J. Exp. Biol.* **215**, 1938-1943.
- Stawski, C. Y., Grigg, G. C., Booth, D. T. and Beard, L. A.** (2006). Temperature and the respiratory properties of whole blood in two reptiles, *Pogona barbata* and *Emydura signata*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **143**, 173-183.
- Storz, J. F., Runck, A. M., Sabatino, S. J., Kelly, J. K., Ferrand, N., Moriyama, H., Weber, R. E. and Fago, A.** (2009). Evolutionary and functional insights into the mechanism underlying high-altitude adaptation of deer mouse hemoglobin. *Proc. Natl. Acad. Sci. USA* **106**, 14450-14455.
- Tucker, V. A.** (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.
- Verhille, C. and Farrell, A. P.** (2012). The in vitro blood-O₂ affinity of triploid rainbow trout *Oncorhynchus mykiss* at different temperatures and CO₂ tensions. *J. Fish Biol.* **81**, 1124-1132.
- Weber, R. E., Lykkeboe, G. and Johansen, K.** (1976). Physiological properties of eel haemoglobin: hypoxic acclimation, phosphate effects and multiplicity. *J. Exp. Biol.* **64**, 75-88.
- Zielinski, S., Sartoris, F. J. and Pörtner, H. O.** (2001). Temperature effects on hemocyanin oxygen binding in an Antarctic cephalopod. *Biol. Bull. (Woods Hole)* **200**, 67-76.