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OXYGEN AND CARBON DIOXIDE TRANSPORT IN ELASMOBRANCHS

PHILLIP R. MORRISON
KATHLEEN M. GILMOUR
COLIN J. BRAUNER

The elasmobranchs are an ecologically diverse subclass of over 1000 species that have evolved to inhabit a wide range of environments and become one of the most speciose groups of vertebrate predators on Earth. This chapter reviews what is known about elasmobranch O_2 uptake, transport, and delivery, as well as CO_2 transport and elimination, and focuses upon two metalloproteins central to these processes, hemoglobin (Hb) and carbonic anhydrase (CA), both of which have undergone distinct functional adaptations. Furthermore, adaptations in relation to life history, which include exercise, hypoxia, salinity, temperature, and, in some species, regional heterothermy, are reviewed. While processes and principles of gas transport and exchange in elasmobranchs are often similar to those of the better described teleosts, there are differences that stand out as clearly worthy of further investigation. Generally, elasmobranch Hbs exhibit a high affinity for O_2 relative to teleosts, which may be associated with a low organic phosphate/Hb ratio and an antagonistic effect of urea on Hb-ATP sensitivity. The Hbs also exhibit a moderate Bohr and Haldane effect, but high buffering by Hb and plasma proteins coupled with the presence of

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plasma accessible CA greatly reduces the interaction between O_2 and CO_2 exchange relative to the situation in teleosts. Moreover, at least in the dogfish, *Squalus suckleyi,* current models of CO_2 excretion suggest similar contributions of the plasma and red blood cell (RBC) to CO_2 excretion, a model that contrasts with the pattern of CO_2 excretion typical of other vertebrates in which near exclusive reliance is placed on the RBC. High plasma buffering and plasma-accessible CA in the gill of dogfish favor HCO_3^- dehydration in the plasma, while HCO_3^- dehydration via the RBC is constrained by low RBC CA activity and the absence of a Haldane effect in this species. In the Hb of the whip stingray, *Dasyatis akajei*, a novel Bohr effect mechanism has been discovered and this same species possesses a novel ATP binding site in Hb. Finally, in the high performing regionally heterothermic sharks, there appears to be a reduction or reversal in Hb temperature sensitivity consistent with regionally heterothermic teleosts, but this remains to be investigated in detail. While gas transport and exchange is a central process associated with the success of elasmobranchs, it has been most thoroughly investigated in just a few species; clearly a great deal remains to be discovered to achieve a more representative understanding of gas transport and exchange in elasmobranch fish.

1. INTRODUCTION

Gas exchange is a prerequisite for aerobic life. In vertebrates the uptake of environmental oxygen (O_2) and the elimination of metabolic carbon dioxide (CO_2) require a gas exchange organ and a system to transport respiratory gases and acid–base equivalents between their sites of consumption or production and the gas exchange organ. Gas transport in vertebrates is achieved through a closed circulatory system; indeed, the basic vertebrate respiratory and circulatory systems were inherited by all extant jawed vertebrates from the most recent common ancestor to both the Osteichthyan and Chondrichthyan fishes, and these systems have been reviewed extensively (e.g., Randall, 1970a,b; Butler and Metcalfe, 1988; Bushnell et al., 1992; Satchell, 1992, 1999; Perry and Tufts, 1998; Brauner and Berenbrink, 2007; Farrell, 2007; see also Chapter 1). Gas exchange in the adults of all known extant Chondrichthyan fishes primarily occurs across five to seven paired filamentous gill arches (Hughes, 1984; Butler and Metcalfe, 1988; Butler, 1999; Wegner, 2015). Respiratory gases are transported in the blood, around the circulatory system, pumped by the heart. Central to the transport and exchange of respiratory gases in vertebrates are two metalloproteins, hemoglobin (Hb) and carbonic anhydrase (CA), which have undergone distinct functional adaptations

within the elasmobranchs to enhance O_2 delivery and accelerate CO_2 elimination respectively.

Elasmobranchs (Selachimorpha and Batoidea) and holocephalans (Chimaeriformes) comprise all extant representatives in the class Chondrichthyes (Janvier and Pradel, 2015). The elasmobranchs are an ecologically diverse subclass of fishes that have evolved to inhabit a wide range of environments and become one of the most speciose groups of vertebrate predators on Earth (Compagno, 1990; Dulvy et al., 2014; Janvier and Pradel, 2015). The osmoconforming strategy of elasmobranchs (see Chapter 4; Ballantyne and Fraser, 2013) and selective forces imposed by the diversity of environments that they inhabit influence gas transport and exchange. Therefore, comparative physiological investigations between the Osteichthyan and Chondrichthyan fishes provide insight into how components of the respiratory cascade have been modified to suit different species that share a similar habitat or lifestyle, but have evolved different osmoregulatory strategies and are separated by over 400 million years of evolutionary history (Janvier, 2007; Janvier and Pradel, 2015). However, there is still much to be learned about gas transport and exchange in the elasmobranchs. Investigations into the respiratory physiology of elasmobranchs date back to the late 19th and early 20th centuries (see Hyde, 1908; Piiper et al., 1970), with much of the knowledge having been collected from experiments on a few small, sedentary species that are relatively easy to catch and maintain in laboratory aquaria (e.g., *Squalus acanthias, Squalus suckleyi, Scyliorhinus canicula, Scyliorhinus stellaris, Heterodontus portusjacksoni, Hemiscyllium ocellatum,* and *Leocoraja erinacea*). Because of their similar morphology and ecology, comparative physiologists tend to lump a few of these and other species into a group referred to as "dogfish," but it is important to remember that these sharks are separated by as much as 300 hundred million years of evolutionary history (Grogan et al., 2012; Sorenson et al., 2014; Janvier and Pradel, 2015). Table 3.1 lists these "dogfish" sharks as well as the most commonly discussed species in this review along with synonyms and misapplied names. Much of the early work in the field has been well reviewed (see Randall, 1970a; Hughes, 1984; Piiper and Scheid, 1984; Randall and Daxboeck, 1984; Butler and Metcalfe, 1988; Nikinmaa and Salama, 1998; Tufts and Perry, 1998; Butler, 1999; Gilmour and Perry, 2010). However, the only reviews dedicated exclusively to elasmobranch respiratory physiology are those by Butler and Metcalfe (1988) and Butler (1999), which primarily covered the cardiorespiratory system with a strong focus on the anatomy of the gill and the cardiovascular system. Here we provide a thorough overview of Hb, CA, and red blood cell (RBC) function in gas transport and exchange in elasmobranchs, along with analyses of O_2 and CO_2 transport in the blood.

Table 3.1
Elasmobranchs species commonly discussed in this review

Species	Synonyms	Environment and distribution
Skates		
Arctic skate, *Amblyraja hyperborea*	*Raja hyperborea*	Bathydemersal, temperate to polar, northern and southern hemispheres
Eaton's skate, *Bathyraja eatonii*	*Raja eatonii*	Demersal, polar, Southern Ocean, southeast Pacific
Little skate, *Leucoraja erinacea*	*Raja erinacea*	Demersal, temperate, west Atlantic
Winter skate, *Leucoraja ocellata*	*Raja ocellata*	Demersal, temperate, west Atlantic
Myliobatid rays		
Bat eagle ray, *Myliobatis californica*		Demersal, temperate to sub-tropical, east Pacific
Cownose ray, *Rhinoptera bonasus*	*R. quadriloba*	Benthopelagic, temperate to tropical, west and east Atlantic
Whip stingray, *Dasyatis akajei*	Japanese stingray, red stingray, *Trygon akajei*	Demersal, temperate to tropical, west Pacific
Atlantic stingray, *Dasyatis sabina*		Demersal, coastal and inshore, euryhaline, temperate to sub-tropical, western Atlantic
South American freshwater stingray, *Potamotrygon motoro*	Amazonian freshwater stingray, *P. circularis*, *P. laticep*	Benthopelagic, tropical, freshwater, South America
Sharks		
Bull shark, *Carcharhinus leucas*	*C. nicaraguensis*, *C. zambezensis*	Coastal and inshore, euryhaline, tropical to sub-tropical, world-wide
Sandbar shark, *Carcharhinus plumbeus*	Brown shark, *C. milberti*, *Eulamia milberti*	Coastal and pelagic, temperate to sub-tropical, cosmopolitan
Lemon shark, *Negaprion brevirostris*	*Hypoprion brevirostris*	Inshore and coastal, tropical to sub-tropical, west Atlantic, northeast Atlantic, east Pacific
Leopard shark, *Triakis semifasciata*		Demersal, temperate to sub-tropical, northeast Pacific

Draughtsboard shark, *Cephaloscyllium isabellum*	Carpet shark, *C. isabella*	Demersal, subtropical, southwest Pacific (New Zealand)
Port Jackson shark, *Heterodontus portusjacksoni*	Bull or horn shark, *Squalus portusjacksoni*	Demersal, temperate to subtropical, west Pacific (Australia)
Epaulette shark, *Hemiscyllium ocellatum*	*Squalus ocellatus*	Demersal, reef-associated, tropical, southwest Pacific
"Dogfish" sharks		
Spiny dogfish, *Squalus acanthias*	Piked or picked dogfish, Pacific dogfish, spurdog, rock salmon, *S. suckleyi*	Benthopelagic, marine and brackish, temperate, north Atlantic and southern hemisphere
Pacific spiny dogfish, *Squalus suckleyi*[a]	Spotted spiny dogfish, piked dogfish, *S. acanthias*	Benthopelagic, marine and brackish, temperate, north Pacific
Small-spotted catshark, *Scyliorhinus canicula*	Lesser-spotted dogfish, *Squalus canicula*	Demersal, temperate to sub-tropical, northeast Atlantic
Nursehound, *Scyliorhinus stellaris*	Greater- or larger-spotted dogfish, European dogfish, *Squalus stellaris*	Demersal, temperate to sub-tropical, northeast Atlantic
Dusky smooth-hound, *Mustelus canis*	Smooth dogfish	Demersal, marine and brackish, temperate to sub-tropical, west Atlantic
Spotless smooth-hound, *Mustelus griseus*	Japanese smooth-hound, smooth dogfish	Demersal, temperate to sub-tropical, northwest Pacific
Lamnid sharks (regional heterotherms)		
Shortfin mako, *Isurus oxyrinchus*	Mako, blue pointer	Pelagic and coastal, temperate to subtropical, cosmopolitan
Salmon shark, *Lamna ditropis*	Mackerel shark, Pacific Porbeagle, *L. ditrotus*	Pelagic and coastal, temperate, north Pacific
Porbeagle shark, *Lamna nasus*	Mackerel shark, *L. cornubica*	Pelagic and coastal, temperate, north Atlantic and southern hemisphere

Current nomenclature, and distribution information were taken from FishBase (Froese and Pauly, 2011), and Compagno et al. (2005).

[a]The Pacific spotted spiny dogfish, formerly considered *Squaluas acanthias*, has been reclassified as *Squalus suckleyi* based on life history and genetic differences (Ebert et al., 2010; Veríssimo et al., 2010). The separation of these two species is further supported by sequence differences in their Na^+/H^+-exchangers (i.e., NHE2), indicating the possibility of physiological differences between *Squalus acanthias* and *Squalus suckleyi* (Guffey, 2013). Accordingly, any dogfish identified in the literature as *Squalus acanthias*, but caught in the north Pacific we have considered as *Squalus suckleyi*.

2. BLOOD-OXYGEN TRANSPORT

Aspects of the blood-O_2 transport characteristics of elasmobranchs were covered in a previous volume of the *Fish Physiology* series (Brauner and Randall, 1998; Gallaugher and Farrell, 1998; Jensen et al., 1998; Nikinmaa and Salama, 1998). Although elasmobranchs were included in the chapters of that volume, the general focus was on teleosts. The diffusion of environmental O_2 across the gills into the blood of elasmobranchs is reviewed by Wegner (2015). The majority of O_2 that diffuses across the gills into the blood binds reversibly to Hb, encapsulated within the RBCs, and is then convectively transported by the actions of the heart throughout the circulatory system. The cardiovascular and circulatory systems of elasmobranchs are reviewed by Brill and Lai (Chapter 1). In the tissue capillary beds, the partial pressure of O_2 (PO_2) in the blood is higher than the PO_2 of the metabolically active tissues owing to the steady consumption of O_2, and this difference provides the driving force for O_2 diffusion from the blood into the tissues. Hb-O_2 transport can be "fine-tuned" in response to environmental and metabolic demands by interspecific and intraspecific increases in Hb concentration or through modulating Hb-O_2 binding characteristics (Wells, 1999). Thus, the characteristics of Hb and its microenvironment within the RBCs dictate the nature of blood-O_2 transport (e.g., Nikinmaa, 1997; Nikinmaa and Salama, 1998; Brauner and Val, 2005; Wells, 2005; Brauner and Berenbrink, 2007).

2.1. Hemoglobin

Hemoglobin has been superbly shaped by evolution to fulfill the job of binding and transporting O_2 from the gas exchange organ to the metabolically active tissues. Globins or the genes that code for them have been found in all kingdoms, which indicates their importance for physiological function (Weber and Vinogradov, 2001). Within the jawed vertebrates the tetrameric structure of Hb is highly conserved, but primary structural differences underlie functional adaptations to modulate O_2 binding affinity in response to internal and external environmental conditions (Weber and Fago, 2004). Hb structure and function in fishes has been reviewed in previous volumes of the *Fish Physiology* series (Riggs, 1970; Jensen et al., 1998), but much has been learned about elasmobranch Hbs more recently. It has been generally accepted that the Hbs of elasmobranchs lacked the functional adaptations typical of teleosts that allow fine-tuning of Hb-O_2 affinity according to environmental and metabolic demands (Hall and McCutcheon, 1938; Bonaventura et al., 1974a;

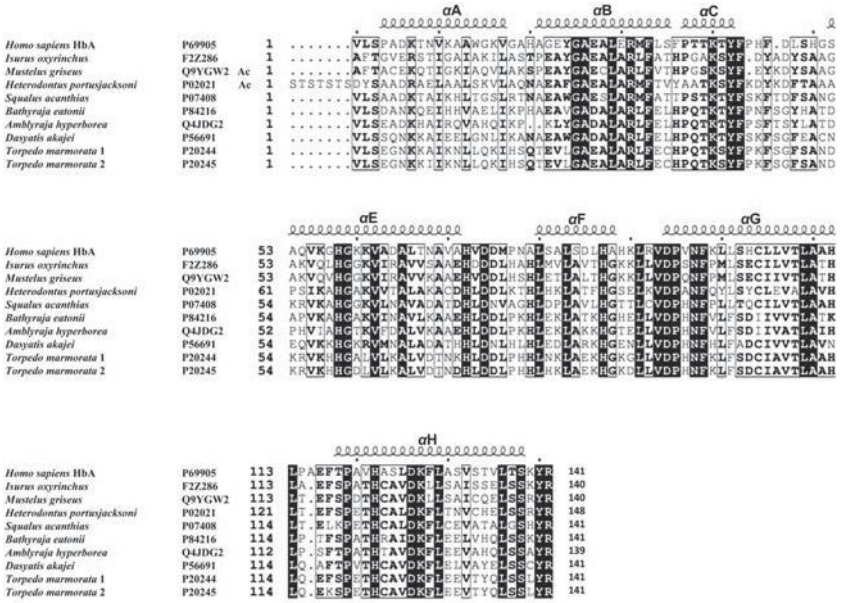
Pennelly et al., 1975; Dickinson and Gibson, 1981; Brittain et al., 1982; Gibson and Carey, 1982; Weber et al., 1983a; Wells et al., 1992; Wells, 1999). Although elasmobranch Hbs do not exhibit the extreme pH sensitivity that is characteristic of teleosts (Pennelly et al., 1975; Farmer et al., 1979; Ingermann and Terwilliger, 1982; Dafré and Wilhelm, 1989; Pelster and Weber, 1990; Berenbrink et al., 2005), there are functional similarities between elasmobranch Hbs and those of other vertebrate lineages that have arisen through distinct mutations that allow fine-tuning of Hb function in some elasmobranch species (Chong et al., 1999; Naoi et al., 2001).

2.1.1. General Principles of Hemoglobin Structure and Function

Jawed vertebrate hemoglobins consist of two α-type and two β-type globins that produce a tetramer formed of two αβ-dimers. Each of the four globin polypeptide chains consists of α-helical segments (named A through H from the N-terminus) that are linked by nonhelical segments (named according to the letters of the flanking helices, i.e., AB through GH), and N- and C- terminal ends are labeled NA and HC, respectively (see Jensen et al., 1998). Between the E and F helices of each chain is the heme pocket that harbors an iron atom bearing heme group that reversibly binds one O_2 molecule; thus, one Hb tetramer can bind up to four O_2 molecules. Although the tetrameric structure of jawed vertebrate Hbs is conserved, the amino acid sequence and length of the polypeptide chains vary among species and vertebrate classes. For example, human HbA, which is the most extensively studied protein, has 141 amino acids in the α-chain and 146 amino acids in the β-chain, whereas the α-chain of elasmobranch Hbs ranges from 140 to 148 residues, and the β-chain, which lacks the D-helix portion that is present in the Hbs of other jawed vertebrates, ranges from 136 to 142 residues (Fig. 3.1). Fig. 3.1 shows the sequences of the major Hb components from eight species of elasmobranchs aligned to human HbA. Hereafter, amino acid residues in elasmobranch Hb will be referenced according to their equivalent numerical position in the α- and β-chains of HbA as shown in Fig. 3.1 (e.g., His F8β = His 92β in HbA).

The oxygen equilibrium curve characterizes the relationship between Hb-O_2 saturation and blood PO_2, and the shape and position of the curve reflect Hb-O_2 binding affinity as well as cooperative homotropic interactions among the heme-binding sites. The tetrameric Hbs of vertebrates may assume two distinct conformations: a low-affinity tense (T-state) conformation that occurs in the tissues and a high-affinity relaxed (R-state) conformation that predominates in the lungs or gills (Monod et al., 1965; Perutz, 1970). The binding and release of allosteric effectors (e.g., H^+, Cl^-, and organic phosphates) to nonheme sites stabilizes one form over the other, functionally altering Hb-O_2 binding affinity between the sites of O_2 uptake

(A) α-chain



(B) β-chain

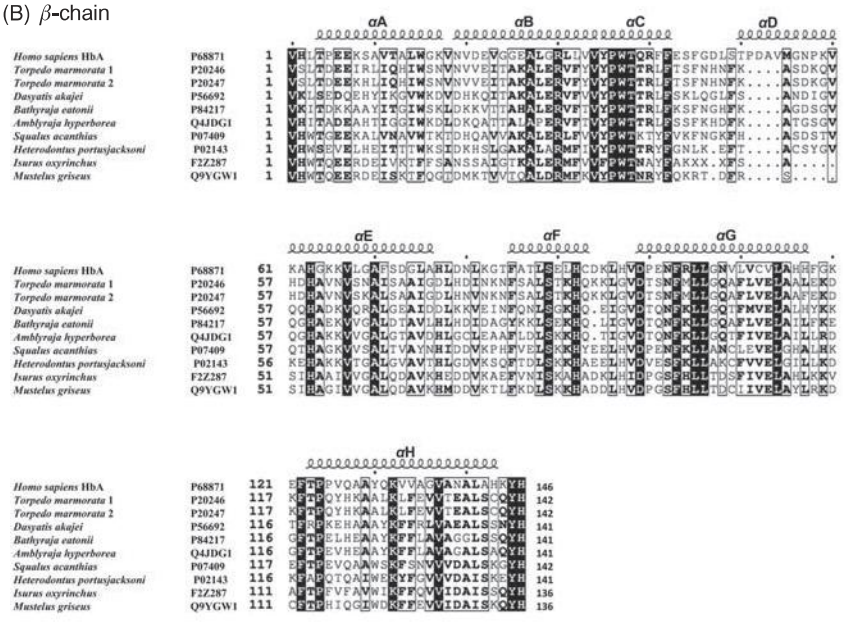


Figure 3.1 (Continued)

and release. The T→R transition is fundamental to the cooperative nature of Hb-O_2 binding, and underlies the sigmoidal shape of the oxygen equilibrium curve because Hb-O_2 affinity increases as successive O_2 molecules are bound. Cooperativity is expressed as the Hill-coefficient (n_H), with values of n_H around unity (~ 1) indicating noncooperative, hyperbolic oxygen equilibrium curves, whereas $n_H > 1$ represents increasing degrees of cooperativity and more sigmoidal curves. Values of n_H for elasmobranch Hb range between 1 and 2 for most species, but reach values as high as 3 (Tables 3.2 and 3.3). A commonly used measure of Hb-O_2 affinity is P_{50}, which is the blood PO_2 that corresponds to 50% Hb-O_2 saturation. The evolution of allosteric modulation of Hb-O_2 affinity through heterotropic interactions with endogenous cofactors (e.g., H^+, Cl^-, and organic phosphates) that bind to nonheme sites permits *in vivo* "fine-tuning" of P_{50} between the sites of O_2 loading and offloading.

2.1.2. Hemoglobin Multiplicity

Elasmobranch blood, like that of many bony fishes, contains multiple Hb components (Hb isoforms or isoHbs), ranging in number from 2 to as many as 13 isoforms in hemolysates from some species (Manwell, 1963; Andersen et al., 1973; Bonaventura et al., 1974a; Nash and Thompson, 1974; Fyhn and Sullivan, 1975; Martin et al., 1979; Dickinson and Gibson, 1981; Weber et al., 1983a; Dafré and Reischl, 1990; Galderisi et al., 1996; Dafré and Reischl, 1997; Larsen et al., 2003; Verde et al., 2005). Intraspecific phenotypic variation (Hb polymorphism) is also apparent among hemolysates (Bonaventura et al., 1974a; Fyhn and Sullivan, 1975; Martin et al., 1979; Galderisi et al., 1996), however, the functional significance, if any, is not clear. There does not appear to be substantial functional "division of labor" within elasmobranch hemolysates, because all Hb isoforms are functionally similar (Andersen et al., 1973; Pennelly et al., 1975), or Hb isoforms that differ from the bulk of the hemolysate account for only a small portion of the hemolysate (Dickinson and Gibson, 1981). For example, the

Figure 3.1. Sequence alignment of hemoglobin from eight elasmobranchs aligned to human HbA. The spirals and letters above the sequences identify the α-helices, and black dots correspond to every tenth amino acid residue of HbA. The amino acid residues in black boxes are identical in all sequences, and the residues in white boxes are similar in all sequences. Hemoglobin sequences were retrieved from the UniProt database, aligned using Clustal Omega, and edited for print using ESPript 3 (Gouet et al., 2003). UniProtKB accession numbers for both the (A) α- and (B) β-chains of each species Hb are listed after the species name. Ac indicates that the α-amino group of the α-chain is acetylated (i.e., *Mustelus griseus* and *Heterodontus portusjacksoni*). For *Amblyraja hyperborea* the first three residues of α-chain and first residue of the β-chain were filled in according to Verde et al. (2005).

Table 3.2
Hemoglobin characteristics of elasmobranchs

Species	Sample	°C	pH	P_{50}	n_H	Φ $(\frac{\Delta \log P_{50}}{\Delta pH})$	Buffer/Notes	References
Batoidea								
Myliobatiformes								
Dasyatis akajei	Stripped isoHb (dominant component)	24	6.5	27.9	1.9	−0.41	0.06 M heme, 0.05 M Tris/bis-Tris, 0.1 M Cl^-	Chong et al. (1999)
		24	7.4	11.8	2.1			
		24	8.5	4.2	2.1			
	+2 mM ATP	24	6.5	61.4	1.3	−0.58[a]		
	+2 mM ATP	24	7.4	18.7	2.1			
	+2 mM ATP	24	8.5	4.3	2.0			
Dasyatis americana	Hemolysate	25.5	7.4	15.0	1.7		0.033 M phosphate buffer	McCutcheon (1947)
Dasyatis centroura	Hemolysate	25.5	7.4	15.0	1.6		0.033 M phosphate buffer	McCutcheon (1947)
Dasyatis sabina	Hemolysate	25.5	7.4	15.3	1.6		0.033 M phosphate buffer	McCutcheon (1947)
Dasyatis say	Hemolysate	25.5	7.4	13.5–14.5	1.5		0.033 M phosphate buffer	McCutcheon (1947)
Gymnura micrura (=*Pteroplatea micrura*)	Hemolysate	25.5	7.4	13.0–14.5	1.6		0.033 M phosphate buffer	McCutcheon (1947)
Potamotrygon sp.	Stripped hemolysate	20	7.6	1	1–1.6	−0.4	0.06 mM heme, 0.05 M Tris, Φ and n_H from pH 6.0–9.0	Martin et al. (1979)
	+1 mM ATP	20	7.5	3.4				
Rhinoptera bonasus (=*R. quadriloba*)	Hemolysate	25.5	7.4	14.0	1.2		0.033 M phosphate buffer, pH 7.4	McCutcheon(1947)

Rajiformes								
Amblyraja hyperborea (= *Raja hyperborea*)	IsoHb I	2	7.5	12.9		0	0.5–1.0 mM heme, 0.1 M Hepes, 0.1 M NaCl	Verde et al. (2005)
		10	7.5	24.4				
	+3 mM ATP	2	7.5	13.2				
	+3 mM ATP	10	7.5	19.9				
Bathyraja eatonii	IsoHb I	2	7.5	14.5		0	0.5–1.0 mM heme, 0.1 M Hepes, 0.1 M NaCl	Verde et al. (2005)
		10	7.5	28.9				
	+3 mM ATP	2	7.5	13.1		0		
	+3 mM ATP	10	7.5	33.5				
Raja eglanteria	Hemolysate	25	6.8	37–45			0.05 M phosphate buffer	Hall and McCutcheon (1938)
		25	7.1	38				
		25	7.4	26				
Torpediniformes								
Torpedo nobiliana	Stripped hemolysate	20	7.5	15.8	1–1.5	0	0.9 mM heme, 0.05 M Tris. bis-Tris	Bonaventura et al. (1974a)
	+4 M NaCl	20	7.5	2.8	1–1.5			Bonaventura et al. (1974a)
	+1 mM ATP	20	7.5	16.6	1–1.5	0		
	Stripped hemolysate	20	7.5	14.4	1.5		0.05 M Tris, 0.1 M NaCl	Bonaventura et al. (1974b)
	+5 M urea	20	7.5	9.6	1.4			
	+ 4M NaCl	20	7.5	2.9				
Selachimorpha								
Carcharhiniformes								
Carcharhinus leucas	Stripped hemolysate	25	7.4	11			3% Hb, potassium phosphate buffer	Burke (1974)
		25	6.8	17				

(*Continued*)

Table 3.2 (Continued)

Species	Sample	°C	pH	P_{50}	n_H	Φ $(\frac{\Delta \log P_{50}}{\Delta pH})$	Buffer/Notes	References
Carcharhinus plumbeus	Hemolysate	26	7.5	5.6			0.1 M Tris, no difference between unstripped and stripped hemolysates	Brill et al. (2008)
	+133 mM urea	26	7.5	5.7				
	+5.0 mM ATP	26	7.5	26.5				
	+5.0 mM ATP and 133 mM urea	26	7.5	26.2				
Galeorhinus galeus (=*G. australis*)	Stripped hemolysate		6.7	10.9			0.031 mM Hb, 0.05 M bis-Tris, 0.3 M NaCl, 0.36 M urea	Coates et al. (1978)
			7.3	6.9				
	+1.86 mM ATP		6.7	27.5				
	+1.86 mM ATP		7.3	19.5				
	+1.86 mM IMP		6.7	9.8				
	+1.86 mM IMP		7.3	7.3				
Mustelus canis	Hemolysate	25	6.8	12–13			0.05 M Phosphate buffer	Hall and McCutcheon (1938)
		25	7.1	9				
		25	7.4	7				
	Stripped hemolysate	20	7.5	2.2	2.0		0.05 M Tris, 0.1 M NaCl	Bonaventura et al. (1974b)
	+2.5 M urea	20	7.5	2.1	2.0			

Mustelus griseus	Stripped hemolystae	25	6.5	8.1	2.5	−0.19[a]	0.06 mM heme, 0.05 M Tris/bis-Tris, 0.1 M NaCl	Naoi et al. (2001)
		25	7.4	5.9	2.3			
		25	8.5	3.4	1.8			
	+2 mM ATP	25	6.5	20.1	2.4	−0.35		
	+2 mM ATP	25	7.4	10.6	2.6			
	+2 mM ATP	25	8.5	4.0	2.1			
Mustelus mustelus	Hemolysate	25.5	7.4	7.5	1.3		0.033 M phosphate buffer	McCutcheon (1947)
Negaprion brevirostris (=*Hypoprion brevirostris*)	Hemolysate	25.5	7.4	7.6	1.5		0.033 M phosphate buffer	McCutcheon (1947)
Sphyrna tiburo	Hemolysate	25.5	7.4	7.0	1.2		0.033 M phosphate buffer, pH 7.4	McCutcheon (1947)
Triakis scyllium (=*T. scyllia*)	Stripped hemolysate	20	6.8	5.9[a]	1.1		0.1 M Bis-Tris HCl	Kono and Hashimoto (1977)
	+ATP	20	6.8	7.1[a]	1.2			Kono and Hashimoto (1977)
	+GTP	20	6.8	9.7[a]	1.1			
Hexanchiformes								
Notorynchus cepedianus	Stripped hemolysate		6.7	10.4			0.03 mM Hb, 0.05 M bisTris-HCl, 0.3 M NaCl, 0.36 M urea	Coates et al. (1978)
			7.3	8.2				
	+1.86 mM ATP		6.7	12.1				
	+1.86 mM ATP		7.3	10.9				
	+1.86 mM IMP		6.7	10.0				
	+1.86 mM IMP		7.3	8.9				
Lamniformes								
Lamna nasus	IsoHb V	10		1.5	1.9		0.20–0.33 mM heme, 0.1 M Hepes buffer	Larsen et al. (2003)
		26		2.5	1.2			
	+ATP	10		9.7	2.3		$[ATP]/[Hb_4] > 30$	
	+ATP	26		7.4	2.1		$[ATP]/[Hb_4] > 30$	

(*Continued*)

Table 3.2 (Continued)

Species	Sample	°C	pH	P_{50}	n_H	Φ $(\frac{\Delta \log P_{50}}{\Delta pH})$	Buffer/Notes	References
	IsoHb III	10		0.9	1.8	+ 0.5	Φ from pH 7.5 to 8.3	
						−0.6	Φ pH <7.5	
		26		2.2	1.2	0		
	+ATP	10		9.9	2.4	−0.76	Φ from pH 7.0 to 7.3, [ATP]/[Hb_4] >30	
	+ATP	26		8.4	2.1	−0.3	Φ from pH 7.0 to 7.3, [ATP]/[Hb_4] >30	
Squaliformes								
Squalus acanthias	Stripped hemolysate	10	7.85	2.3	1.1	−0.21	0.3–0.4 mM heme, 0.05 M Tris/bis-Tris	Weber et al. (1983a)
Squalus suckleyi	Purified hemolysate (crystalline)	20	6.7	28	1.0	−0.34	Potassium phosphate buffer	Manwell (1963)

P_{50} refers to the PO_2 (mmHg) at which hemoglobin is 50% saturated with O_2, n_H refers to the Hill coefficient at 50% hemoglobin saturation, and Φ refers to the Bohr coefficient ($\Delta \log P_{50}/\Delta pH$). °C and pH refer to the conditions under which P_{50} was determined. The "Sample" column describes the type of hemoglobin solution and any added cofactors. "Buffers/Notes" refers to the buffers included in the hemoglobin solution and conditions under which n_H, and Φ were made.

[a]Indicates respective parameter was estimated from data or figure in reference.

Table 3.3
Whole-blood characteristics of chondrichthyan fishes.

Species	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	[Hb] (g dl^{-1})	Hct (%)	MCHC (g l^{-1})	P_{50}	n_H	Φ	pHa	pHv	Comments	References
HOLOCEPHALI														
Chimaeriformes														
Chimaera monstrosa					2.7	15.7	170							Larsson et al. (1976)
Hydrolagus colliei					2.9–3.4			16	1.1[a]	Absent			P_{CO_2} 2.5–28 mmHg, 11°C	Hanson (1967)
ELASMOBRANCHII														
Batoidea														
Myliobatiformes														
Dasyatis guttata					8.2	21.7	389							Filho et al. (1992b)
Dasyatis sabina	90	14.2				14.6							23°C, cannulated	Cameron et al. (1971)
					3.6	24.3								Dabruzzi and Bennett, 2014
Dasyatis say					3.6	14.3	235							Filho et al. (1992b)
Myliobatis californica	87.4		0.6		5.8	19.3	301				7.93		11°C, cannulated, resting	Hopkins and Cech (1994a)
					5.4	23		6.0	0.8	−0.45			pH 8.37, P_{CO_2} 0.2 mmHg, 8°C	
					5.4	23		12.8	1.1	−0.45			pH 7.63, P_{CO_2} 7.6 mmHg, 8°C	
					5.8	23		7.5	0.7	−0.47			pH 8.33, P_{CO_2} 0.2 mmHg, 14°C	
					5.8	23		17.3	1.1	−0.47			pH 7.55, P_{CO_2} 7.6 mmHg, 14°C	
					5.5	23		13.5	1.1	−0.52			pH 7.92, P_{CO_2} 0.2 mmHg, 20°C	
					5.5	23		24	1.3	−0.52			pH 7.45, P_{CO_2} 7.6 mmHg, 20°C	
					5.2	20		12	1.1	−0.47			pH 7.99, P_{CO_2} 0.2 mmHg, 26°C	
					5.2	20		20.3	1.3	−0.47			pH 7.51, P_{CO_2} 7.6 mmHg, 26°C	

(*Continued*)

Table 3.3 (Continued)

Species	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	[Hb] (g dl^{-1})	Hct (%)	MCHC (g l^{-1})	P_{50}	n_H	Φ	pHa	pHv	Comments	References
Myliobatis goodie					4.9	18.0	298							Filho et al. (1992b)
Potamotrygon motoro					3.1	13.5	230	4.6	1.2–1.4	−0.05			Washed RBCs, pH 7.4, Φ calculated from pH 6.5–8.0, 29°C	Johansen et al. (1978)
Potamotrygon motoro (=*P. circularis*)					5.2	23.0	226	6.7	1.3	−0.26			Washed RBCs, pH 7.4, Φ calculated from pH 6.5-8.0, 29°C	Johansen et al. (1978)
(=*P. laticeps*)					3.8	8.0	475	8.1	1.3–1.7	−0.25			Washed RBCs, pH 7.4, Φ calculated from pH 6.5-8.0, 29°C	Johansen et al. (1978)
Potamotrygon sp.						24		12.3		Present			No CO_2, pH 7.7, 30°C	Martin et al. (1979)
								17.0					P_{CO_2} 42.6 mmHg, 30°C	Martin et al. (1979)
Potamotrygon spp.								11.8–12.7 16.6–19.7		−0.19– −0.26			No CO_2, 30°C P_{CO_2} 43 mmHg, 30°C	Powers et al. (1979b)
Rhinoptera bonasus										−0.41			Washed RBCs, Φ calculated from pH 6.9-8.0, 20°C	Scholnick and Mangum (1991)
Rhinoptera bonasus					6.7	25.1	286							Filho et al. (1992b)
Taeniura lymna					4.8	14.8	318							Baldwin and Wells (1990)
Trygonoptera testacea					3.7	21	173						19°C	Cooper and Morris (1998)
Rajiformes														
Amblyraja radiate (=*Raja radiata*)					3.8	16.7	216							Larsson et al. (1976)

Atlantoraja castelnaui (=*Raja castelnaui*)					2.6	15.4	205							Filho et al. (1992b)
Atlantoraja cyclophora (=*Raja cyclophora*)					5.9	17.9	366							Filho et al. (1992b)
Atlantoraja platana (=*Raja platana*)					4.1	16.3	234							Filho et al. (1992a)
Aptychotrema rostrata	82		1.9		3.0[b]	12.2	242[b]	47.6	2.1		7.8		28°C, P_{50} *in vivo*, cannulated, respirometer	Speers-Roesch et al. (2012a)
Dipturus batis (=*Raja batis*)					2.9	19.0	154							Larsson et al. (1976)
Leucoraja naevus (=*Raja naevus*)					2.4	23.0	106							Leray (1982)
Leucoraja ocellata (=*Raia oscillata*)	70	14	1.3	2.6		20.0					7.82	7.67		Dill et al. (1932)
								11[a]					P_{CO_2} 1 mmHg, 0.2°C	
								20[a]	2.0				P_{CO_2} 1 mmHg, 10°C	
								45[a]					P_{CO_2} 1 mmHg, 25°C	
								95[a]					P_{CO_2} 1 mmHg, 38°C	Dill et al. (1932)
Leucoraja ocellata (=*Raja ocellata*)	100		0.8		2.8	12.5	239	27.6	1.8	−0.29	7.83 pHi 7.4		pH 7.82, P_{CO_2} 0.75 mmHg, 12°C, cannulated, flow-through chamber	Graham et al. (1990)
								34.6	2.0	−0.29			pH 7.40, P_{CO_2} 7.50 mmHg, 12°C	
Glaucostegus typus (=*Rhinobatos batillum*)					3.8	10.5	373							Baldwin and Wells (1990)
					3.9	13.8	281	14.8	2.2	−0.08			Washed RBCs, pH 7.8, no CO_2, 25°C	Wells et al. (1992)
								16.1	1.7				Washed RBCs, pH 7.4, no CO_2, 25°C	
Glaucostegus typus (=*Rhinobatos typus*)					3.2	14.4	220						22–24°C	Lowe et al. (1995)

(*Continued*)

Table 3.3 (Continued)

Species	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	[Hb] (g dl^{-1})	Hct (%)	MCHC (g l^{-1})	P_{50}	n_H	Φ	pHa	pHv	Comments	References
Raja clavata (=*Raia clavata*)	58							30.2	2.5	−0.25	7.7		pH 7.7, 15°C, cannulated	Hughes and Wood (1974)
Raja microocellata					2.9	21.1	113							Leray (1982)
Rajella lintea (=*Raja lintea*)					3.4	19.3	172							Larsson et al. (1976)
Rhinobatos horkelii					6.1	18.9	260							Filho et al. (1992b)
Rhinobatos percellens					4.4	13.3	329							Filho et al. (1992b)
Rioraja agassizii (=*Raja agassizi*)					4.5	17.7	231							Filho et al. (1992b)
Sympterygia acuta					4.7	21.6	226							Filho et al. (1992b)
Sympterygia bonapartii (=*S. bonapartei*)					3.2	13.8	241							Filho et al. (1992b)
Torpediniformes														
Narcine brasiliensis					4.0	17.1	227							Filho et al. (1992b)
Torpedo marmorata	70							20.2		−0.32	7.82		pH 7.8, 15°C, cannulated, respirometer	Hughes (1978)
								28[a]					20°C	
Zapteryx brevirostris					4.9	19.0	247							Filho et al. (1992b)
Selachimorpha														
Carcharhiniformes														
Carcharhinus brevipinna (=*C. maculipinnis*)					7.2	30.1	265							Filho et al. (1992b)
Carcharhinus limbatus					8.4	22.3	278							Filho et al. (1992b)
Carcharhinus melanopterus					4.14	17.1	243							Baldwin and Wells (1990)
					4.11	17.0	242.9	11.1	1.7	−0.35			Washed RBCs, pH 7.8, no CO_2, 25°C	Wells et al. (1992)
								17.9	2.2	−0.35			pH 7.4	

Carcharhinus obscurus			6.2	18.2	345							Emery (1986)
			4.8	15.0	324							Filho et al. (1992b)
Carcharhinus plumbeus			5.1	14.9	350							Emery (1986)
Carcharhinus plumbeus (=*C. milberti*)			4.4	16.1	311							Filho et al. (1992b)
			4.01	17.7	228	20.3	2.4	−0.56			pH 7.92, P_{CO_2} 1.5 mmHg, 25°C	Brill et al. (2008)
			4.40	21.4	206		2.4	−0.37			Exercise stressed, pH 7.64, P_{CO_2} 1.5 mmHg, 25°C	
Carcharhinus porosus			5.8	29.9	248							Filho et al. (1992b)
Cephaloscyllium isabellum (=*C. isabella*)			3.5[b]	16.8	209[b]	4.83		−0.49			pH 7.674,5°C	Tetens and Wells (1984)
			3.0[b]	15.0	208[b]	8.3	1.5	−0.32			pH 7.7, 15°C	
Cephaloscyllium ventriosum		0.5 – 14.5	2.7	13.5						7.60–8.04	15°C, caudal venepuncture	King (1995)
Galeocerdo cuvier			6.5	19.8	338							Emery (1986)
								−0.38			Washed RBCs, Φ calculated from ~ pH 6.9–7.9, 20°C	Scholnick and Mangum (1991)
Mustelus fasciatus			4.9	23.5	222							Filho et al. (1992b)
Mustelus schmitti			4.2	20.4	221							Filho et al. (1992b)
Negaprion acutidens			5.5	18.2	300							Baldwin and Wells (1990)
			3.6	13.0	277	9.9	1.7	−0.24			Washed RBCs, pH 7.8, no CO_2, 25°C	Wells et al. (1992)
						12.3	2.0	−0.24			Washed RBCs, pH 7.4, no CO_2, 25°C	
Negaprion brevirostris	32.5	7.1	3.6	14.9		11.8		−0.36	7.72	7.54	pH 7.62, P_{CO_2} 0 mmHg, 24°C, 15% Tris buffer, cannulated, rest/ free swimming	Bushnell et al. (1982)
Prionace glauca			5.0	15.2	332							Emery (1986)
			2.9	11	264						Exercise stressed	Wells et al. (1986)

(*Continued*)

Table 3.3 (Continued)

Species	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	[Hb] (g dl^{-1})	Hct (%)	MCHC (g l^{-1})	P_{50}	n_H	Φ	pHa	pHv	Comments	References
Prionace glauca (continued)	105.4	28.1				8					7.66	7.50	20–22°C, cannulated, swimming 0.45 BL/s $^{-1}$, N=1	Lai et al. (1997)
Scyliorhinus canicula					5.62	21.8		21.5	1.7	−0.43			pH 7.58, P_{CO_2} 2.2 mmHg, 17°C	Pleschka et al. (1970)
								25.9					pH 7.38, P_{CO_2} 7.3 mmHg, 17°C	
Scyliorhinus canicula	90.9	21.3									7.88	7.83	7°C, PiO_2 140 mmHg, cannulated, restrained	Butler and Taylor (1975)
	114.4	34.5									7.81	7.77	12°C, PiO_2 140 mmHg	
	97.6	32.9									7.74	7.68	17°C, PiO_2 131 mmHg	
	13.8	6.6									7.91	7.84	7°C, PiO_2 43 mmHg	
	16.5	7.0									7.74	7.66	12°C, PiO_2 42 mmHg	
	16.8	6.0									7.68	7.64	17°C, PiO_2 39 mmHg	
	95	23									7.76	7.71	15°C, PiO_2 148 mmHg, cannulated, respirometer	Short et al. (1979)
	31	11									7.68	7.59	PiO_2 77 mmHg	
	91.2		0.55								7.84		15°C, spinalectomized, cannulated	Truchot et al. (1980)
	92.9		1.3		7.3	12	616				7.59		21°C, cannulated, respirometer	Duthie and Tort (1985)
	98.1		1.0		4.8	16.9	295				7.78 pHi7.3		15°C, cannulated, flow-through chambers	Wood et al. (1994)
	93		1.1		4[b]	16.7					7.78 pHi7.3		15°C, cannulated, flow-through chambers	Perry et al. (1996)

Scyliorhinus stellaris	81	11	1.9	3.3		16		12			7.76	7.66	pH 7.79, P_{CO_2} 1.4 mmHg, 17°C, anaesthetized, cannulated	Piiper and Schumann (1967)
	49	10	2.0	2.6							7.78	7.71	16°C, cannulated, free-swimming	Baumgarten-Schumann and Piiper (1968)
								16	1.8				P_{CO_2} 1.5 mmHg, 17°C, *in vivo* and *in vitro*	Piiper and Baumgarten-Schumann (1968)
	64		2.1										17.8–19.2°C, cannulated, resting	Piiper et al. (1977)
Sphyrna lewini					10.0	26.5	370							Emery (1986)
					8.4	27.3	343							Filho et al. (1992b)
Sphyrna tiburo						19.9–22.2							28°C	Carlson and Parsons (2003)
Sphyrna zygaena					6.6	25.4	281							Filho et al. (1992b)
Triakis semifasciata	66	12	1.8	2.55		18.3		15.3	1.1–1.5		7.78	7.75	P_{50} and n_H *in vivo* at 15°C, blood gases and pH at 19–22°C, cannulated	Lai et al. (1990)
Heterodontiformes														
Heterodontus portusjacksoni	82	22.5	2.6–3.2	3.5–4.1	4.4	20		19		0			P_{CO_2} 0–1 mmHg, 20°C	Grigg (1974)
	97.5	24.8	2.4	1.9	3.7	20	188				7.82	7.82	19°C, caudal puncture	Cooper and Morris (1998a,b)
	105	33.8	2.1	2.3		19		14.4	2.0	−0.11	7.82	7.76	19°C, cannulated	Cooper and Morris (2004a,b)
Lamniformes														
Alopias vulpinus					13.6	37.4	360							Emery (1986)
					11.9	33.0	360							Filho et al. (1992b)
Carcharias taurus (=*Odontaspis taurus*)					5.9	21.9	283							Filho et al. (1992b)
Carcharodon carcharias					13.5	36.0	379							Emery (1986)
Isurus oxyrinchus					12.1[b]	32.4	383[b]	10.6	≈ 1.5	+0.16			Exercise stressed, pH 7.6, no CO_2, 25°C	Wells and Davie (1985)
					14.3	40.8	369							Emery (1986)

(*Continued*)

Table 3.3 (Continued)

Species	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	[Hb] (g dl^{-1})	Hct (%)	MCHC (g l^{-1})	P_{50}	n_H	Φ	pHa	pHv	Comments	References
					10.5	34	318						Exercise stressed	Wells et al. (1986)
					14.0	28.7	359							Filho et al. (1992b)
	82.5	30.4				24					7.44	7.29	20–22°C, cannulated, swimming	Lai et al. (1997)
Orectolobiformes														
Chiloscyllium punctatum					6.3[c]	20.9[c]	278						24°C	Chapman and Renshaw (2009)
					7.5[d]	27.3[d]								
Hemiscyllium ocellatum					3.64	13.4	272							Baldwin and Wells (1990)
Hemiscyllium ocellatum (=*H. oscellatum*)					5.59	19.7	286.0	10.7	1.9	−0.29			Washed RBCs, pH 7.8, no CO_2, 25°C	Wells et al. (1992)
								14.2	2.2	−0.29			Washed RBCs, pH 7.4, no CO_2, 25°C	
					5.3[c]	19.0[c]	274						24°C	Chapman and Renshaw (2009)
					6.2[d]	22.8[d]								
	97.5		1.35		2.9[b]	13.4	219[b]	32.0	1.3		7.87		28°C, P_{50} *in vivo*, cannulated, respirometer	Speers-Roesch et al. (2012a)
Squaliformes														
Etmopterus spinax					3.0	18.9	168							Larsson et al. (1976)
Somniosus microcephalus					3.2	20.5	156							Larsson et al. (1976)
Squalus acanthias					2.9	15.3	188							Larsson et al. (1976)
					3.3	20.9	158							Leray (1982)
	111		1.7								7.85		14–15°C, cannulated, resting	Swenson and Maren (1987)
					2.7[b]	11.6	235[b]	13.2	1.7	−0.28			pH 7.85, P_{CO_2} 2.2 mmHg, 15°C	Wells and Weber (1983)

Squalus cubensis					7.3	31.0	234							Filho et al. (1992b)
Squalus suckleyi	68.8	8.1	2.3	3.4	2.6–3.4	13–26		17	1.2[a]	Absent	7.47	7.36	pH 7.6, P_{CO_2} 0.5 mmHg, 11°C, cannulated, free-swimming	Lenfant and Johansen (1966)
	77	13											9–10°C, cannulated, restrained	Hanson and Johansen (1970)
	104	14											9°C, cannulated, restrained	Cameron et al. (1971)
(=*S. acanthias*)					3.0	14.8	208						12°C	Perry and Gilmour (1996)
(=*S. acanthias*)	102		1.3								7.8		11°C, cannulated, flow-through chamber	Richards et al. (2003)
(=*S. acanthias*)	117.4	6.3	1.24	1.6							7.87	7.81	13°C, cannulated, flow-through chamber	Gilmour and Perry (2004)
Squantiniformes														
Squatina argentina					4.3	23.1	246							Filho et al. (1992b)

PaO_2, PvO_2, $PaCO_2$, and $PvCO_2$ refer to the *in vivo* partial pressure (mmHg) of O_2 and CO_2 in arterial and venous blood, respectively, P_{50} refers to the PO_2 (mmHg) at which hemoglobin is 50% saturated with O_2, n_H refers to the Hill coefficient at 50% hemoglobin saturation, Φ refers to the Bohr coefficient ($\Delta \log P_{50}/\Delta pHe$)., and pHa and pHv refer to the *in vivo* arterial and venous pH, respectively. Comments refer to the conditions under which P_{50}, n_H, and Φ were made, and/or the conditions under which *in vivo* measurements were made.

[a]Indicates respective parameter was estimated from data or figure in reference.

[b]Converted using a constant for human HbA: 1 g $dL^{-1} = 0.1551$ mmol Hb_4 L^{-1}

[c]Captive sharks

[d]Wild sharks

hemolysate of the salmon shark, *Lamna ditropis* (=*Lamna ditrotus*), eluted into four distinct fractions, one of which accounted for only 5% of the total heme but was functionally distinct from the two predominant and functionally similar fractions that comprised 80% of the total heme (Dickinson and Gibson, 1981). Some elasmobranch hemolysates are comprised of electrophoretically anodal Hbs that have "normal" sensitivities to pH, phosphates, and temperature (e.g., Andersen et al., 1973; Fyhn and Sullivan, 1975; Weber et al., 1983a), which is similar to class I teleost, except that the anodal Hbs of teleosts tend to express a marked pH sensitivity (see Jensen et al., 1998, for a classification of teleost Hbs). For example, electrophoresis revealed that the six Hbs that comprise the hemolysate of the spiny dogfish separated into three distinct anodal bands (fractions I+II, III+IV, and V+VI) with isoelectric points (at 10°C) near pH values of 7.7, 7.4, and 6.9, respectively (Weber et al., 1983a). The fraction containing Hbs III+IV comprised the largest proportion of the hemolysate and exhibited an O_2 affinity similar to the whole hemolysate, but lower than the two less predominat fractions. The main component (III+IV) of *Squalus acanthias* hemolysate also displayed higher heterotropic interactions (i.e., sensitivity to pH and ATP) than the other two components (I+II, and V +VI), and a higher pH sensitivity (i.e., Bohr effect; see Section 2.1.4) than the intact hemolysate (Weber et al., 1983a). However, this situation is unlike the marked heterogeneity typical of class II teleosts that possess a labor force of both cathodal and anodal Hbs with very different sensitivities to pH, phosphates, and temperature (Jensen et al., 1998; Fago et al., 2002; Brauner and Val, 2005). Blood from the regionally heterothermic porbeagle shark, *Lamna nasus*, contains seven distinct Hbs, three of which (Hbs V, IV, and III) account for most of the hemolysate, and are functionally similar with isoelectric points (at 16°C) of 7.58, 7.62, and 7.68, respectively (Larsen et al., 2003). In the presence of ATP the Hbs of *Lamna nasus* exhibit a reverse temperature dependency, whereby increasing temperature increased O_2 affinity (see Section 2.1.7), which is similar to class III teleosts (e.g., Atlantic bluefin tuna, *Thunnus thynnus*; Rossi-Fanelli and Antonini, 1960), although class III teleost Hbs display higher pH sensitivities than the ATP dependent pH sensitivity of *Lamna nasus* Hbs (Larsen et al., 2003).

It is likely that the functional heterogeneity observed in some elasmobranch hemolysates results from different reactivity rates between the α- and β-chains (Andersen et al., 1973; Bonaventura et al., 1974a; Brittain et al., 1982) and from Hb multiplicity that may arise from the formation of tetramers that contain more than two types of globins (Galderisi et al., 1996). These Hb hybrids have been proposed as one possible explanation for the high number of Hb isoforms in the hemolysate of the marbled electric ray, *Torpedo marmorata* (Galderisi et al., 1996). *In vitro*, *Squalus acanthias* Hbs exist in equilibrium between oxygenated dimeric and deoxygenated tetrameric Hb

(Fyhn and Sullivan, 1975), and although the Hbs of other elasmobranchs appear to exist as tetramers in both the oxy and deoxy states, an equilibrium between tetrameric and dimeric Hb may contribute to the formation of Hb hybrids, and thus Hb multiplicity (Galderisi et al., 1996). The functional significance of Hb multiplicity, if any exists, has not been thoroughly investigated in the elasmobranchs.

2.1.3. Ontogenetic Changes to Hemoglobin

Distinct fetal Hb isoforms that have higher intrinsic O_2 affinities than adult Hbs are present in a number of egg-laying and live-bearing elasmobranchs (Manwell, 1958; Manwell, 1963; Pennelly et al., 1975; Scholnick and Mangum, 1991; King, 1994). The ancestral reproductive mode in elasmobranchs was likely egg-laying (oviparity), but live-bearing (viviparity) and different forms of maternal input including placental viviparity have evolved independently in a number of lineages (Dulvy and Reynolds, 1997; Awruch, 2015). Juvenile (2 week old) *Squalus acanthias* also have higher Hb-O_2 affinities than adult sharks, and fetal isoHbs persist for at least 10 days posthatch in *Cephaloscyllium ventriosum*, which may ensure adequate O_2 extraction from environmental water until Hb concentration and hematocrit (Hct; the percentage of RBCs in blood) increase to adult levels (Weber et al., 1983a; Wells and Weber, 1983; King, 1994). A high Hb-O_2 affinity (see Section 2.3) should benefit prehatch and embryonic individuals by enhancing O_2 extraction in the egg case microenvironment or in fetal circulatory systems (Manwell, 1958; Pennelly et al., 1975; King, 1994).

2.1.4. pH and the Evolution of the Bohr Effect

A decrease in blood pH lowers Hb-O_2 affinity (increases P_{50}) in many vertebrates, permitting a relatively rapid rightward shift of the oxygen equilibrium curve associated with CO_2 production during blood capillary transit. This pH dependency of Hb-O_2 affinity is known as the Bohr effect, named for one of its co-discoverers (Bohr et al., 1904). The alkaline Bohr effect refers to a decreased Hb-O_2 affinity that accompanies declining pH, typically between pH values of 9 and 6, whereas the acid or reverse Bohr effect refers to an increase in Hb-O_2 affinity with declining pH at values typically outside the physiological range (below $pH \approx 6$); the latter is present in some elasmobranchs Hbs (e.g., Larsen et al., 2003; Verde et al., 2005). Various aspects of the Bohr effect have been well reviewed (e.g., Riggs, 1988; Giardina et al., 2004; Jensen, 2004; Berenbrink, 2006), so here discussion will be limited to an evolutionary comparison of the magnitude and mechanism of the Bohr effect in elasmobranch Hbs.

The magnitude of the Bohr effect is quantified as either the Bohr coefficient (Φ) or the Haldane coefficient ($\Delta z H^+$), the latter of which describes the number of Bohr protons that are bound per mole of O_2

released from Hb upon deoxygenation at constant pH. If the shape of the oxygen equilibrium curve is symmetrical, and if other allosteric effectors that differentially bind to the T- and R-state Hb conformations are absent, then the Bohr and Haldane coefficients are thermodynamically equivalent (Wyman, 1964) as is shown in the following relationship:

$$\Phi = \frac{\Delta \log P_{50}}{\Delta \mathrm{pH}} = \frac{1}{4}\Delta z\mathrm{H}^{+} \tag{3.1}$$

where Φ values are equal to one quarter $\Delta z\mathrm{H}^{+}$ values that are determined by acid–base titrations of Hb (e.g., Jensen, 1989; Berenbrink et al., 2005; Regan and Brauner, 2010a). According to Eq. (3.1), if $\Delta z\mathrm{H}^{+}$ is high then a greater number of H^{+} ions will be bound upon a shift from the R- to T-state (oxy to deoxy Hb), which corresponds to a larger change in log P_{50} per unit change in pH (rightward shift of the oxygen equilibrium curve). The magnitude of the Bohr–Haldane effect is likely the product of a species' physiological demands and its evolutionary history, in that large Φ values may be optimal for acid–base homeostasis and lower values for blood-O_2 transport (Lapennas, 1983; Brauner and Randall, 1998; Berenbrink, 2006). Furthermore, the magnitude of the Bohr effect is additionally influenced by Cl^{-}, organic phosphates, CO_2, temperature, and the experimental pH range (e.g., Weber et al., 1983a), all of which have species-specific influences on Hb-O_2 affinity.

The molecular mechanism of the Bohr effect reflects deoxygenation-linked proton binding at several amino acid residues that stabilise the T-state conformation of the α- and β-chains of jawed vertebrate Hbs. In human HbA, the amino acid residues attributed to the alkaline Bohr effect include Val 1α (NA1), His 122α (H5), His 2β (NA2), Lys 82β (EF6), His 143β (H21), and His 146β (HC3) (Perutz, 1983; Berenbrink, 2006; Mairbäurl and Weber, 2012). Because the pK_a values of many histidine imidazole groups are within physiological pH values (pH 6 to 8) it is likely that the majority of Bohr protons bind to histidine side chains, which is thought to account for about 90% of the alkaline Bohr effect in human HbA measured in the presence of 0.1 M Cl^{-} (Lukin and Ho, 2004; Berenbrink, 2006). In the T-state conformation, the C-terminal histidine of HbA (His 146β) accounts for over 60% of the alkaline Bohr effect (in the presence of 0.1 M Cl^{-}) owing to a salt bridge that forms with Asp94β in the same subunit (see Berenbrink, 2006). This salt bridge does not form in any of the sequenced elasmobranch Hbs (see below). In some elasmobranch Hbs the Bohr effect persists over a wide pH range (Mumm et al., 1978; Pennelly et al., 1975; Martin et al., 1979; Weber et al., 1983a), and because elasmobranch Hbs have high specific buffer values (Table 3.4) that correlate with an increased number of physiological buffer groups (i.e., titratable histidine residues) (Jensen, 1989;

Table 3.4
Buffer values

Species	β Whole blood	β Separated plasma	β Hb	ΔzH^+	References
Teleostei					
Ameiurus punctatus	−14.3 (25)	−5.8			Cameron and Kormanik (1982)
A. nebulosus	−15.5 (24)	−5.7	−0.12 mmol/g Hb/pH unit		Szebedinszky and Gilmour (2002)
Anguilla rostrata	−10.1 (20)	−2.7			Hyde et al. (1987)
Catostomus commersoni	−8.8 (28)	−2.3			Wilkes et al. (1981)
Hippoglossoides elassodon	−6.6 (14)	−2.1			Turner et al. (1983)
Katsuwonus pelamis	−8.0 (25)	−3.1		4.1	Tufts and Perry (1998) and Jensen (2001)
	−11.2 (41)	−3.1			Perry et al. (1985)
Oncorhynchus mykiss	−9.7 (25)	−2.6	−6.7	3.0	Tufts and Perry (1998) and Berenbrink et al. (2005)
	−10.5 (24)	−2.4			Wood et al. (1982) and Gilmour et al. (2002)
Platichthys stellatus	−7.0 (25)	−2.9			Wood et al. (1982)
Salvelinus fontinalis	−7.5 (35)	−3.3			Packer and Sunkin (1979)
Elasmobranchii					
Leucoraja ocellata	−11.0 (13)	−6.6			Tufts and Perry (1998) and Graham et al. (1990)
Raja clavata	−10				Hughes and Wood (1974)
Raja rhina	−6.11 (13.5)	−2.83			Gilmour et al. (2002)
Myliobatis californica	−14.3 (20) to −16.4 (23)				Hopkins and Cech (1974a)

(*Continued*)

Table 3.4 (Continued)

Species	β Whole blood	β Separated plasma	β Hb	ΔzH^+	References
Mustelus asterias			−11.5	1.1	Berenbrink et al. (2005)
Scyliorhinus stellaris	−8.0	−10 (true plasma) −4.2			Albers and Pleschka (1967) and Piiper et al. (1972)
	−8.8 (18)	−2.6	−11.4	0.3	Tufts and Perry (1998) and Berenbrink et al. (2005)
Squalus acanthias	10–12 (pH 7.85)		−11.7	1.0	Weber et al. (1983a) and Berenbrink et al. (2005)
Squalus suckleyi	−9.0 (13–26)	−6.5			Tufts and Perry (1998) and Lenfant and Johansen (1966)
Triakis semifasciata	−9.3				Lai et al. (1990)

β Whole blood and β separated plasma refers to buffer values in Slykes (mmol HCO_3^- pH $unit^{-1}$ L^{-1}), and are taken from Tufts and Perry (1998) and the listed references. β Hb refers to the hemoglobin buffer value (mol H^+ per mol Hb_4 and pH) in organic phosphate-free, deoxygenated hemolysates at physiological pH and Cl^-, and are from Jensen (2001) and Berenbrink et al. (2005). ΔzH^+ refers to Haldane coefficients (mol H^+ per mol Hb_4) measured by acid–base titrations on stripped hemolysates and are taken from Berenbrink et al. (2005). Unless otherwise stated, values in parenthesis are hematocrit values from Tufts and Perry (1998).

Berenbrink et al., 2005; Berenbrink, 2006), the manifestation of the Bohr effect over a wide pH range may result from a high number of histidine residues that have different pK_a values (Mumm et al., 1978; Aschauer et al., 1985; Weber et al., 1983a). In contrast, teleost fish Hbs generally have low buffer values (Jensen, 1989, 2001; Berenbrink et al., 2005), a characteristic that appears to be strongly associated with a reduction in the number of titratable histidine residues and the evolution of a large Bohr effect and the Root effect (i.e., a decrease in cooperativity and blood oxygen-carrying capacity caused by low pH, even at high PO_2) (Root, 1931; Pelster and Randall, 1998; Berenbrink et al., 2005; Regan and Brauner, 2010a,b). Recent structure–function analyses of species Hb representing all major lineages of jawed vertebrates provide evidence that a low Bohr effect was the ancestral state (i.e., ≤ 1 Bohr proton per Hb_4), and increases in the magnitude of the Bohr effect evolved independently in the amniotes and early actinopterygiians, with further increases that evolved in the teleosts, avians, and reptilians due to a reduction in histidine content, and thus specific Hb buffer value (Berenbrink et al., 2005; Berenbrink, 2006). Since the Bohr effect in HbA, and also possibly elasmobranch Hbs, depends on contributions from numerous histidine sites, a reduction in histidine content may result in a decreased Bohr effect (see Berenbrink, 2006). Thus, in HbA the magnitude of the Bohr effect is largely dependent on the salt bridge that forms between His 146β and Asp94β in the T-state conformation (see reviews by Kilmartin and Rossi-Bernardi, 1973; Berenbrink, 2006; Mairbäurl and Weber, 2012). In elasmobranchs, the presence of a Bohr effect in many selachian Hbs likely reflects oxygenation-dependent influences from histidine residues, but by different amino acid arrangements than HbA (Aschauer et al., 1985; Chong et al., 1999; Naoi et al., 2001); however, an increase in the magnitude of the Bohr effect caused by a novel mechanism that includes the terminal histidine (His 146β) appears to have occurred in the myliobatid stingrays (Chong et al., 1999).

Stingrays in the order Myliobatiformes have some of the largest Bohr effects measured in elasmobranchs (Tables 3.2 and 3.3). Of all the sequenced batoidean Hbs, only *Dasyatis akajei* Hb exhibits a marked Bohr effect ($\Phi = -0.41$, and -0.58 in the presence of ATP), which results from amino acid interactions different than that of human HbA (Chong et al., 1999). The salt bridge that forms between His 146β and Asp 94β in HbA and contributes to the majority of the Bohr effect cannot form in *Dasyatis akajei* Hb, possibly due a glutamine residue that replaces aspartate at position FG1 in the β-chain (Asp 94β in HbA) (Chong et al., 1999). Remarkably, pH sensitivity evolved in *Dasyatis akajei* Hb through a hydrogen bond that forms in the T-state between the C-terminal histidine and an asparagine residue at position HC1β (Lys 144β in HbA), which is responsible for a large

part of the Bohr effect in this stingray (Chong et al., 1999). Some close relatives of *Dasyatis akajei* also possess Hbs with a Bohr effect (e.g., *Dasyatis sabina*, $\Phi \approx -0.3$ to -0.4; Mumm et al., 1978), but the Hbs of other genera of batoideans exhibit little to no Bohr effect (Table 3.2), which indicates that the "stingray Bohr effect" may have evolved within the order Myliobatiformes, possibly representing a further independent evolution of the magnitude of the Bohr effect within jawed vertebrates.

Selachian Hbs exhibit small to moderate Bohr effects (Table 3.2). The small Bohr effects in *Mustelus griseus* Hb ($\Phi = -0.19$) and *Heterodontus portusjacksoni* Hb were attributed to the lack of any considerable interaction between residues that would additionally stabilize the T-state conformation, and acetylation of the free α-amino groups of the α-chain in *Heterodontus portusjacksoni* Hb (Fisher et al., 1977; Nash et al., 1976; Naoi et al., 2001). The slightly larger Bohr effect in *Squalus acanthias* Hb ($\Phi = -0.21$) is consistent with the Haldane coefficient ($\Delta z\mathrm{H}^{+} = 1.0$) (Weber et al., 1983a; Berenbrink et al., 2005), and likely arises from proton binding to the high number of titratable histidine side chains and the non-acetylated α-chains (Aschauer et al., 1985; Jensen, 1989). The latter is proposed because the conformation of *Squalus acanthias* Hb limits any interaction or bonding between the terminal histidine on the β-chain and other amino acid residues that would additionally stabilise the T-state, similar to *Mustelus griseus* and *Heterodontus portusjacksoni* Hb (Aschauer et al., 1985). Unfortunately, no functional studies were coupled to the structural study of *Isurus oxyrinchus* Hb, but as Aschauer et al. (1985) proposed for *Squalus acanthias* Hb, a nonpolar alanine residue at F6β (Leu 91β in HbA) may inhibit salt-bridge formation between AspFG1β and the terminal histidine, which would prevent any contribution to the overall Bohr effect. This is in line with the observations of Andersen et al. (1973) that Hb-O_2 dissociation rates (carbon monoxide replacement reaction) were independent of pH in *Isurus oxyrinchus* hemolysate. In the isoHbs of the closely related porbeagle shark, *Lamna nasus*, the Bohr effect is also very small or reverse (i.e., acid Bohr effect), but is intensified in the presence of ATP ($\Phi \approx -0.76$ in the presence of ATP). In contrast to these two lamnid sharks, a moderate Bohr effect is present in the stripped hemolysate of the carcharhinid blue shark, *Prionace glauca* ($\Phi \approx -0.4$; Pennelly et al., 1975), but structural studies are lacking for this species' Hbs. Thus, within the elasmobranchs there appears to be unique oxygenation-dependent interactions between histidine residues that contribute to the Bohr effect, and an increase in the magnitude of the Bohr effect by a novel mechanism appears to have evolved in the myliobatid stingrays. Given the paucity of studies conducted to date on the large number of species that exist, there remains a great deal to be learned about the evolution of the Bohr effect in this group.

2.1.5. Organic Phosphate Binding to Hemoglobin

The presence of organic phosphates in the RBCs of jawed vertebrates has an additional modulatory effect on the allosteric interaction between O_2 and proton binding sites that increases the magnitude of the Bohr effect of most tetrameric Hbs (Jensen et al., 1998; Val, 2000; Jensen, 2004). The RBCs of fishes are nucleated and thus contain mitochondria that produce the nucleoside triphosphates (NTPs) ATP and GTP, which are the principle allosteric effectors of Hb in most fishes. In contrast, avian RBCs contain inositol pentaphosphate (IPP), and the anucleate RBCs of mammals contains 2,3-bisphosphoglycerate (2,3-BPG). In most elasmobranch RBCs, ATP is the predominant organic phosphate (Leray, 1979; Johansen et al., 1978; Leray, 1982; Weber et al., 1983a; Filho et al., 1992a); however, GTP is the more potent allosteric effector of Hb-O_2 affinity (Kono and Hashimoto, 1977; Weber et al., 1983a), and in some elasmobranch RBCs the concentration of GTP is equal to or greater than that of ATP (Kono and Hashimoto, 1977; Borgese et al., 1978; Bartlett, 1982; Filho et al., 1992a; Wells et al., 1992). Additionally, inosine monophosphate (IMP) has been reported from the RBCs of a number of elasmobranchs, and IPP from the RBCs of *Squalus acanthias* and the electric ray, *Torpedo nobiliana* (Borgese and Nagel, 1978; Coates et al., 1978; Wells et al., 1992). However, IMP does not appreciably decrease Hb-O_2 affinity in either *Notorynchus cepedianus* or *Galeorhinus galeus* (Table 3.2; Coates et al., 1978), and ATP is clearly the predominant NTP in *Squalus acanthias* RBCs (Bartlett, 1982; Weber et al., 1983a). In general, the total NTP concentration and the NTP/Hb ratio are lower in elasmobranchs than teleosts; within the elasmobranchs, selachian RBCs generally contain a greater absolute concentration of NTPs than batoidean RBCs (Filho et al., 1992a). The lower NTP/Hb ratio in elasmobranchs RBCs compared to that of teleosts, and the antagonistic effect of urea on Hb-ATP sensitivity (see below; Weber et al., 1983b), may be the basis for the generally lower whole blood P_{50} values reported for elasmobranchs, but to our knowledge this has not been investigated.

Organic phosphate binding to elasmobranch Hbs reduces Hb-O_2 affinity in most selachians and at least one batoidean species (Table 3.2). In human HbA and most teleost fish Hbs, organic phosphates bind to specific amino acid residues at positions NA1, NA2, EF6, and H21 in the central cavity between the two β-chains, which reduces Hb-O_2 affinity by stabilizing the T-state conformation (Perutz and Brunori, 1982; Gronenborn et al., 1984; Jensen et al., 1998). The amino acid residues in the phosphate binding region of HbA are not conserved in batoidean Hbs, which may explain why most of the skates and rays lack any significant allosteric effect of ATP on Hb-O_2 binding (Bonaventura et al., 1974a; Verde et al., 2005). In contrast, the

β-chains of Hb from the selachians *Squalus acanthias*, *Heterodontus portusjacksoni*, and *Mustelus griseus* possess the same amino acids as human HbA at positions NA1, NA2, and EF6, and a positively charged lysine residue at H21 where HbA has a positively charged histidine (Fig. 3.1). Consequently, the site that binds organic phosphates in HbA and teleost Hbs also has been implicated in binding ATP in the T-state conformation of *Squalus acanthias* and *Mustelus griseus* Hb, which concomitantly decreases Hb-O_2 affinity and increases the magnitude of the Bohr effect for both of these species (Aschauer et al., 1985; Weber et al., 1983a; Naoi et al., 2001). Both ATP and inositol hexaphosphate (IHP) markedly decreased Hb-O_2 affinity in a number of sharks in the orders Carcharhiniformes and Lamniformes (Table 3.2, and see Pennelly et al., 1975), and IHP also reduced Hb-carbon monoxide (CO) affinity in the salmon shark, *Lamna ditropis* (=*Lamna ditrotus*) (Dickinson and Gibson, 1981). It is not clear whether the site of 2, 3-BPG binding in human HbA similarly binds NTPs in the Hbs of lamnid sharks because a serine substitution at H21β (His 143β in HbA) of *Isurus oxyrinchus* Hb may inhibit NTP binding in the central cavity between the β chains (Fig. 3.1). However, novel phosphate binding sites may be present in lamnid shark Hbs because in *Lamna nasus* Hbs the oxygenation-dependent release of ATP causes the overall heat that is normally released during Hb-oxygenation to be retained even though heme-oxygenation is intrinsically exothermic (see Section 2.1.7; Larsen et al., 2003).

In at least one stingray, *Dasyatis akajei*, the magnitude of the Bohr effect is increased in the presence of ATP. Remarkably, not only does *Dasyatis akajei* Hb exhibit a novel Bohr effect mechanism, but it also possesses a novel ATP binding site that is located within the central cavity between the two β-chains just inside the 2, 3-BPG binding site of human HbA. In this region, Arg 104β and Ala 135β of human HbA are substituted for two positively charged amino acid residues, lysine (G6β) and arginine (H13β), respectively, that favor binding of ATP in the T-state conformation (Fig. 3.1; Chong et al., 1999; Verde et al., 2005). Except for *Torpedo marmorata* Hb, all other sequenced elasmobranch Hbs possess positively charged residues at position G6β (Arg 104β), but lack a substitution for a positively charged residue at position H13β (Ala 135β). Additionally, the presence of ATP does not have a substantial effect on Hb-O_2 affinity for *Torpedo nobiliana*, either of the polar skates, *Amblyraja hyperborea* and *Bathyraja eatonii*, or a freshwater stingray, *Potamotrygon sp.* (Bonaventura et al., 1974a; Martin et al., 1979; Verde et al., 2005). Therefore, the presence of the novel ATP binding site described for *Dasyatis akajei* Hb may have evolved within the family Dasyatidae, although clearly further structure–function studies of Hb from myliobatid rays are required to investigate this hypothesis.

2.1.6. Interactions of Hemoglobin with Urea and TMAO

Some elasmobranchs possess urea insensitive globin proteins, a trait that may be crucial for dealing with their high blood urea levels (see Chapter 4; Ballantyne and Fraser, 2013). This trait, however, is also present in some bony fishes and invertebrate lineages and was thus likely inherited by the elasmobranchs (Edelstein et al., 1976; Weber et al., 1977; Scholnick and Mangum, 1991). The concentration of urea in the blood plasma of marine elasmobranchs held or captured in seawater ranges from 290 to 490 mM, but RBC intracellular values are higher owing to the fraction of urea bound to Hb (Browning, 1978; Yancey and Somero, 1980; Tetens and Wells, 1984; Wells et al., 1992; Wood et al., 1994; Brill et al., 2008; Ballantyne and Fraser, 2013; see also Chapter 4). Urea concentrations ranging from physiological to pharmacological levels only slightly increased Hb-O_2 affinity or had very little influence on Hb-O_2 affinity for a number of batoideans (Bonaventura et al., 1974b; Martin et al., 1979; Scholnick and Mangum, 1991) and selachians (Bonaventura et al., 1974b; Scholnick and Mangum, 1991; Wells et al., 1992; Cooper and Morris, 2004; Brill et al., 2008). However, urea increased Hb-O_2 affinity for the draughtsboard shark, *Cephaloscyllium isabellum* (=*Cephaloscyllium isabella*), and North Sea spiny dogfish, *Squalus acanthias* (Weber, 1983; Weber et al., 1983a,b; Tetens and Wells, 1984). Curiously, for *Squalus acanthias* captured in the western Atlantic Ocean, Hb-O_2 affinity was almost insensitive to urea (Scholnick and Mangum, 1991). These reported differences for *Squalus acanthias* Hb may be due to the experimental methods and parameters employed by each group of researchers (i.e., stripped hemolysates used by Weber and colleagues vs. washed and re-suspended RBCs by Scholnick and Mangum), or may reflect real variation that exists among *Squalus acanthias* populations (Scholnick and Mangum, 1991). The mechanism of Hb-urea binding and the relative sensitivity or insensitivity of elasmobranch Hb to urea has been discussed in some detail and may be at least partially related to the integrity of the tetrameric molecular structure of Hb (Bonaventura et al., 1974b; Weber, 1983; Weber et al., 1983a,b; Aschauer et al., 1985).

Urea and TMAO differently affect the O_2 affinity and the oxygenation-linked binding of ATP to elasmobranch Hbs. The urea-induced increase in Hb-O_2 affinity of *Squalus acanthias* (North Sea) is more pronounced at pH 7.7 than at pH 7.3, values that are in the range of elasmobranch arterial and venous blood pH values (Table 3.3), and urea also decreased cooperativity and reduced the ATP sensitivity of *Squalus acanthias* Hb (Weber et al., 1983a,b). The cooperativity of Hb-O_2 binding was relatively unaffected by urea in other elasmobranch Hbs (Bonaventura et al., 1974b; Tetens and

Wells, 1984; Scholnick and Mangum, 1991). However, physiologically relevant concentrations of urea also decreased the effect of ATP on Hb-O_2 affinity in *Cephaloscyllium isabellum*, but not in *Carcharhinus plumbeus* (Tetens and Wells, 1984; Brill et al., 2008). While urea influences ligand binding to Hb for some species, TMAO had no discernable effect on the Hb-O_2 equilibria for *Squalus acanthias*, *Heterodontus portusjacksoni*, or *Rhinoptera bonasus* (Weber, 1983; Scholnick and Mangum, 1991; Cooper and Morris, 2004; Kolhatkar et al., 2014). Since urea reduced the O_2 affinity and ATP sensitivity of *Squalus acanthias* Hb (Weber et al., 1983a,b), and urea-TMAO counteraction appears to be absent in *Squalus acanthias* RBCs (Weber, 1983; Kolhatkar et al., 2014), Weber (1983) proposed that ATP subsumes the role of TMAO in counteracting the effects of urea in the RBCs of *Squalus acanthias*. However, urea did not eliminate the effect of ATP on Hb-O_2 affinity in *Squalus acanthias* and *Cephaloscyllium isabellum*. Therefore, Hb-O_2 equilibria studies conducted in the absence of urea may overestimate the allosteric effect that NTPs have on elasmobranchs Hbs (Weber et al., 1983a). Furthermore, since TMAO tends to increase the rigidity of proteins, the lack of a TMAO effect on *Squalus acanthias* Hb function may preserve the conformational changes responsible for the Bohr effect (Weber, 1983), and recent findings indicate that TMAO may play a thermoprotective role in *Squalus acanthias* RBCs (Kolhatkar et al., 2014). Why some selachian species possess urea sensitive Hbs and others do not is unclear. More research is needed to understand this trait and its relevant importance to the urea osmoconforming strategy of elasmobranchs.

2.1.7. Enthalpy of Hemoglobin-Oxygenation

Due to the exothermic nature of heme-oxygenation, rising temperature will usually decrease Hb-O_2 affinity directly. In most fishes, Hb can be considered to carry heat because endothermic deoxygenation in the tissue capillaries and exothermic oxygenation in the gill lamellae result in outward conductive heat transport (Jensen et al., 1998). The temperature sensitivity of Hb-O_2 binding can be quantified by the overall enthalpy (or apparent heat) of oxygenation ($\Delta H'$), which is calculated according to the van't Hoff isochore:

$$\Delta H = 2.303 \cdot R \cdot \frac{\Delta \log P_{50}}{\Delta \frac{1}{T}} \tag{3.2}$$

where R is the gas constant and T is the absolute temperature (Wyman, 1964). Calculation of $\Delta H'$ according to Eq. (3.2) assumes linearity of the van't Hoff plot [$\Delta \log P_{50}/\Delta(1/T)$], but $\Delta H'$ itself can be temperature dependent (Fago et al., 1997; P.R. Morrison, T.S. Harter, R.W. Brill, and C.J. Brauner,