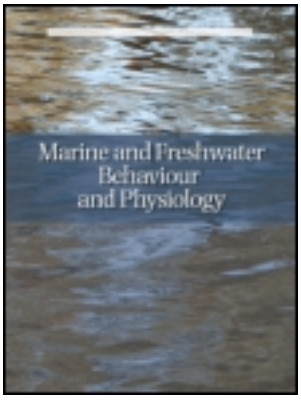

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Temperature effects on blood-oxygen equilibria in relation to movements of the bat ray, Myliobatis Californica in tomales bay, California

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TEMPERATURE EFFECTS ON BLOOD-OXYGEN EQUILIBRIA IN RELATION TO MOVEMENTS OF THE BAT RAY, *MYLIOBATIS CALIFORNICA* IN TOMALES BAY, CALIFORNIA

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In vitro blood-oxygen binding curves for the bat ray, *Myliobatis californica*, were constructed at four temperatures (8, 14, 20, and 26°C) to gain insight into the possible adaptations to ambient temperature regimes in Tomales Bay, California. The curves were hyperbolic (mean n_{50}: 1.06) with high affinities (p_{50}: 0.8–3.2 kPa) and large whole blood buffer values (ß: –14.25 to –16.43 slykes) implying a tolerance to hypoxia and hypercapnia while large O_2 capacities (CBO_2: 3.1–4.1 mmol·L^{-1}) and Bohr factors (Ø: –0.45 to –0.52) indicated high activity levels. The temperature effect (ΔH) was greatest between 14 and 20°C as compared to relatively temperature independent binding between the 8 to 14°C, and 20 to 26°C ranges. The heightened sensitivity between 14 and 20°C parallels a previously-documented large change in respiratory demand for this same temperature range (Q_{10} = 6.81). However, the magnitude of the hematological adjustments were small relative to the large increase in metabolic demand and it is likely that increasing cardiac output plays an important role to safeguard oxygen transport as temperature rises. Thus, the movements of rays between warmer and cooler regions of Tomales Bay are not related to their blood oxygen binding characteristics, but may be due to other factors such as increasing assimilation efficiency, minimizing energy expenditure, or social factors.

KEY WORDS: *Myliobatis*, blood-oxygen equilibria, temperature, behaviour.

INTRODUCTION

The habitat occupied by an organism determines the immediate microclimate an animal experiences and influences its physiological capacities and ultimately its ecological performance (Huey, 1991). Temperature is a key environmental variable which affects the physiology of fish primarily by determining: (1) the rate of chemical reactions, (2) the equilibrium between the formation and disruption of noncovalent forces which stabilize important macromolecular (e.g. biological membranes) and molecular (e.g. proteins) structures and (3) the binding of ligands to proteins (e.g. enzyme-substrate interactions) (Hazel, 1993). Furthermore, because the blood-water barrier in fish gills is only a few microns thick and thermal diffusion is 10 times more rapid than molecular (e.g. oxygen) diffusion, a gill surface area sufficient to oxygenate the blood will cause it to attain the same temperature as the ambient water (Stevens and Sutterlin, 1976).

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Studies of blood-oxygen binding curves can be useful tools for gaining insight into the physiological efficiencies and potential environmental limits of fishes (Krogh and Leitch, 1919; Dobson and Baldwin, 1982a; Cech *et al.*, 1994). The shape and position of the curve represents a compromise between the need to obtain sufficient oxygen from the water and the need to permit unloading at the tissues. For example, active fishes are often characterized by large blood oxygen carrying capacities and Bohr factors (Cech *et al.*, 1984; Dobson *et al.*, 1986) which help maintain oxygen delivery during activity when tissue pH declines and oxygen demand increases. Fishes inhabiting low oxygen environments typically have reduced erythrocytic nucleoside triphosphate (NTP) concentrations which increases hemoglobin-oxygen affinity and safeguards arterial oxygen (Wood and Johansen, 1972). An acute temperature rise decreases oxygen affinity due to the exothermy of hemoglobin oxygenation (Wyman, 1948) and indirectly due to the associated pH decrease (Howell *et al.*, 1970). Furthermore, fish alter erythrocytic NTP levels in response to temperature and oxygen tension changes and the time required to attain a new steady state NTP level can vary from hours to days (Jensen, 1991).

Temperature effects are well studied in acclimated fishes (reviewed in Jensen *et al.*, 1993) but acute effects have received much less attention, especially in relation to behavioral observations. During a telemetry study in Tomales Bay, California, we tracked several bat rays, *Myliobatis californica*, moving from the warm (e.g. 24°C) shallow inner bay to the cooler (e.g. 15°C) deeper outer bay late in the afternoon, and back to the inner bay early the next morning (T.E. Hopkins and S.A. Mattern, unpublished data). Because the resting metabolism of bat rays is highly temperature sensitive between 14 and 20°C (Q_{10} = 6.81), but much less sensitive above and below this range (Q_{10} = 1.85 at 20–26°C and 2.23 at 8–14°C), we hypothesized that the rays were thermoregulating; foraging in the inner bay, and using the outer bay as a temperature refuge (Hopkins and Cech, 1994). The purpose of this study was to determine the effects of acute temperature changes on the *in vitro* blood-oxygen binding characteristics of bat rays in order to gain some physiological insight to their behaviour in the wild.

METHODS

Collection, holding and surgery

Seven bat rays (mean disc width 60.3 cm, range 50.0–80.0 cm, mean weight 5.30 kg, range 4.66–9.09 kg) were captured by hook and line in Tomales Bay at temperatures of 11.8–13.8°C and 33 parts per thousand (ppt) salinity, and transported to the University of California, Bodega Marine Laboratory where they were held in a circular, shaded, 8,000 L outdoor tank, with flowing aerated seawater (11.0 ± 1°C and 33.0 ± 1 ppt) for 2 weeks.

Fish were anesthetized in 300 mg·L^{-1} MS-222 until spiracular ventilation ceased. They were then placed ventral side up on an operating table and the gills ventilated with aerated seawater containing 50 mg·L^{-1} MS-222. A 17 gauge needle was inserted through the skin on the ventral side of the tail, about 3 cm anterior to the origin of the dorsal fin, and a PE-50 cannula with a bevelled tip inserted through the needle and several cm into the dorsal aorta. Cannulae filled with heparinized elasmobranch saline (Hoar and Hickman, 1983) were secured by suturing to the skin. Mean surgery time, including anesthesia, was 22 minutes. Rays were placed individually in covered 210 L circular tanks with aerated, flowing seawater (11 ± 1°C and 33.0 ± 1 ppt) and allowed to recover for a minimum of 2.5 days.

Blood sampling and hematological analyses

One initial 0.8 ml blood sample was taken from the cannula in each individual with a gas-tight heparinized syringe to determine individual blood gas and hematological conditions. PO_2, PCO_2, and pH were measured with a Radiometer PHM 73 analyzer and thermostatted electrodes (E5046, E5036, and G297/K497 respectively). Blood lactate was determined enzymatically (Yellow Springs Instruments (YSI) model 27 analyzer), hematocrit (Hct) by centrifugation, and hemoglobin concentration ([Hb]) by the cyanmethemoglobin method (Sigma kit #525-A). The remaining blood was centrifuged and the plasma aspirated and frozen for later analysis of: glucose (enzymatically, YSI model 27 analyzer), osmolality (vapor pressure, Wescor 1500B), chloride "coulometric" titration, Radiometer CMT 10), sodium and potassium (photometrically, Instrumentation Laboratories 343 flame photometer). The mean cellular hemoglobin concentration was calculated as $Hb \cdot 100 \cdot Hct^{-1}$.

Tonometry

For each pair of curves blood from 4 individuals (78 ml total volume) was pooled with 1,000 international units sodium-heparin in a large flask. Samples of the well-mixed, pooled blood were immediately taken to determine nucleoside triphosphate (NTP) (enzymatically, Sigma kit #366-UV), lactate, Hct, and [Hb]. Blood was loaded into two glass rotating tonometers (Hall, 1960) or kept on ice for 20–40 min before being loaded into a second pair of tonometers. Tonometers were situated in a temperature-controlled water bath at 8, 14, 20 or 26 ± 0.2°C. Tonometer pairs received either humidified air from an air pump, humidified nitrogen from a cylinder (0.03 kPa PCO_2 curves), or humidified gas mixtures (1% CO_2 with balance either air or nitrogen) from Wösthoff gas mixing pumps (1.00 kPa PCO_2 curves). Blood was equilibrated for 20–30 min, after which samples of oxygenated and deoxygenated blood were withdrawn from the tonometers and mixed in a gas-tight, glass syringe with a mixing bead (Edwards and Martin, 1966). A Lex-O_2-Con (Lexington Instruments) was used to determine the oxygen content (CBO_2) of oxygenated (100% saturation) and deoxygenated (0% saturation) pools. PO_2 was measured for each mixture, and pH for each 50% mixture. In three instances (once each at 8, 20, and 26°C) we repeated our PO_2 measurements to assess the repeatability of our mixing tenique. Our second measurements fell within 4–6% of the previous values, indicating good precision of this technique. Several lactate measurements were made on tonometered blood while curves were being constructed to ensure that there was no significant metabolic acidosis, e.g. in the deoxygenated tonometers. In addition, a final blood sample was taken from each tonometer for comparison with initial NTP values. The time from sampling to completion of a pair of oxygen equilibrium curves was less than 2 hr.

The Bohr factor (Ø) was calculated as ΔLog P50/ΔpH. Temperature effect (ΔH, kilocalories per Mole O_2, a measure of temperature sensitivity), was calculated using a form of the van't Hoff equation: $\Delta H = 2.303 \cdot R \cdot (\Delta \text{Log } P_{50}/(\Delta 1/T) \cdot 1000)$, where T is temperature in °K and R is the universal gas constant (Wyman 1964; Powers *et al.*, 1979). Whole blood non-bicarbonate buffer value was calculated as $ß = \Delta[HCO_3^-]/\Delta pH$ mmol $HCO_3^{-1} \cdot$pH unit^{-1} (or slykes), and $[HCO_3^-]$ was calculated using pH and PCO_2 data in the Henderson-Hasselbach equation (Davenport, 1974) using constants for elasmobranchs published by Boutilier *et al.* (1984). Hb subunit cooperativity (n_{50}) was determined from the slope of log (y/100-y) versus log p, where y = percent saturation between 20 and 80%, and p = PO_2 in kPa (Riggs, 1970; Jensen, 1991). Root effect was calculated as the percent loss in CBO_2 between a low and high PCO_2 pair.

RESULTS

Resting, *in vivo*, blood gas and hematological characteristics of bat rays (Table 1) were similar to those of other batoids such as *Dasyatis* (Cameron *et al.*, 1971), *Torpedo* (Hughes and Johnston, 1978), and *Raja* (Graham *et al.*, 1990), with the exception that osmolality was low for an elasmobranch acclimated to full seawater and hemoglobin concentration was high. The low lactate and glucose levels (Table 1) indicated a negligible secondary stress response (Wedemeyer *et al.*, 1990) which we took as an indication that these fish had recovered from surgery.

Bat ray *in vitro* blood oxygen dissociation curves (Figure 1, A–D) were hyperbolic (mean n_{50} = 1.06) with low P50's, large CBO_2, ß, and Ø (Table 2), and no significant Root effects (reviewed in Dafré and Wilhelm, 1989). Lactate measurements of blood during tonometry never exceeded 1.0 and 1.2 mmol·L^{-1} in 0.03 and 1.01 kPa PCO_2

Table 1 Mean (1SE) *in vivo* arterial blood and plasma characteristics from seven cannulated, resting bat rays at 11°C and 33 ppt.

PO_2 (kPa)	11.66 (1.35)
PCO_2 (kPa)	0.08 (1.01)
pH	7.929 (0.04)
Hb (g·dL^{-1})	5.81 (0.35)
Hematocrit (%)	19.31 (1.02)
Sodium (mmol·L^{-1})	239.0 (8.16)
Chloride (mmol·L^{-1})	242.3 (5.61)
Potassium (mmol·L^{-1})	3.61 (0.26)
Lactate (mmol·L^{-1})	0.74 (0.15)
Glucose (mmol·L^{-1})	5.73 (0.13)
Osmolality (mOsm·kg^{-1})	747.0 (23.82)
Plasma Protein (mg·dl)	2.02 (0.09)
Mean cell Hb concentration (g·ml^{-1})	30.09 (0.08)

Table 2 Effects of temperature and CO_2 on *in vitro* bat ray blood-oxygen equilibrium characteristics.

Temp	*PCO_2*	*pH*	*P_{50}*	*CBO_2*	*n_{50}*	*Hct*	*Hb*	*Ø*	*ß*	*ΔH: high PCO_2*	*low PCO_2*
°C	*kPa*		*kPa*	*mmol·L^{-1}*		*%*	*g·dL^{-1}*		*slykes*	*kcal·mol O_2^{-1}*	
8	0.03	8.37	0.8	3.7	0.8						
						23	5.4	–0.45	–16.43		
8	1.01	7.63	1.7	3.5	1.1						
										–5.96	–7.79
14	0.03	8.33	1.0	3.6	0.7						
						23	5.8	–0.47	–16.07		
14	1.01	7.55	2.3	3.5	1.1						
										–16.17	–9.19
20	0.03	7.92	1.8	4.1	1.1						
						23	5.5	–0.52	–16.13		
20	1.01	7.45	3.2	4.1	1.3						
										–3.44	–5.39
26	0.03	7.99	1.6	3.1	1.1						
						20	5.2	–0.47	–14.25		
26	1.01	7.51	2.7	3.1	1.3						

tonometers respectively, which were similar to *in vivo* measurements from resting fish (Table 1). NTP values immediately prior to tonometry ranged from 16.0–17.0 μmol·g Hb^{-1} and were virtually identical following tonometry, ranging from 15.7–16.2 μmol·g Hb^{-1} for all gas mixtures and temperatures.

The temperature effect (ΔH) for both the low and high PCO_2 curves was greatest between 14 and 20°C (largest absolute value), indicating a greater sensitivity to hemoglobin-oxygen loading and unloading in this range compared to the relative temperature independent binding at 8–14°C and 20–26°C ranges (Table 2). Increasing temperature generally decreased blood oxygen affinity and pH except at 26°C (Table 2), but had little effect on n_{50} and hemoglobin concentration. CBO_2 increased from 8 to 20°C and then decreased at 26°C.

Increasing PCO_2 from 0.03 to 1.01 kPa decreased blood pH and increased P_{50} at each temperature (Table 2, Figure 1, A–D) and had little effect on CBO_2 or n_{50}. Bohr factors (Ø) were large for an elasmobranch and indicated a heightened sensitivity to PCO_2 and decreased pH especially at 20°C (Table 2).

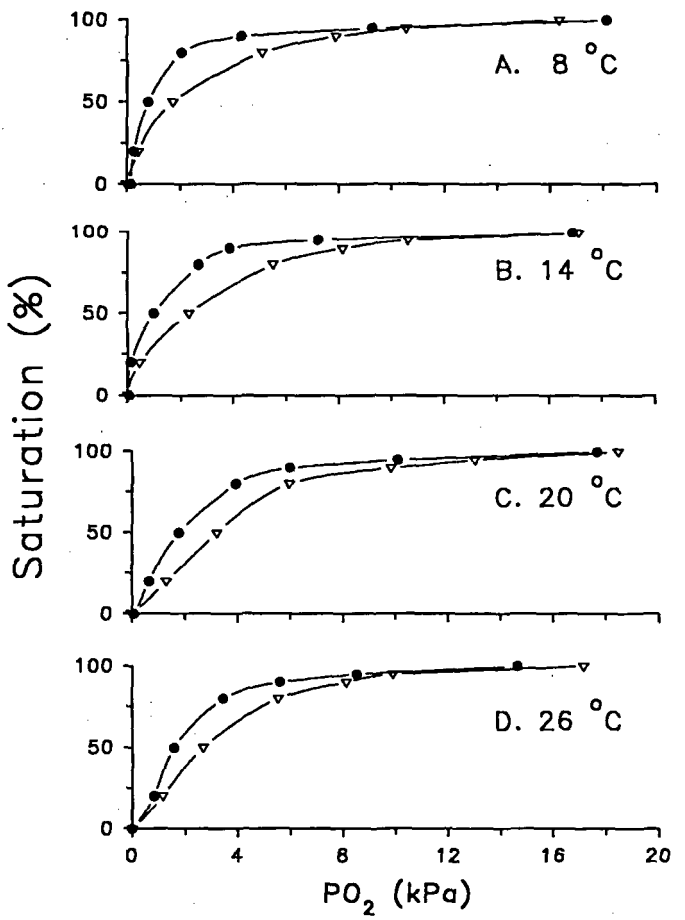

Figure 1 *In vitro* whole blood oxygen equilibrium curves for the bat ray at 8, 14, 20, and 26°C with 0.03 kPa PCO_2 (solid circles), and 1.01 kPa PCO_2 (open triangles).

DISCUSSION

The bat ray is a large (up to 90 kg), benthic predator that forages in shallow bays and estuaries (MacGintie, 1935; Talent, 1985) where the possibility of encountering elevated temperatures and hypoxia is high. Rays are common in Tomales Bay, California, from spring to fall when temperatures range between 14 and 24°C, but are absent during the winter months (December-February) when salinities fluctuate around 20 ppt and water temperatures remain below 12°C (Ridge, 1963; Hopkins, 1993; Smith et al. 1991). In a telemetry study in Morro Bay, California, a bat ray was tracked moving an average of 0.74 km·hr–1 and frequenting the shallow parts of the bay, where hovering and short bursts of speed were observed (Dubsky, 1974). This behaviour pattern and movement rate is similar to that of telemetered bat rays in Tomales Bay which have been tracked moving from the warmer (e.g. 24°C) inner bay to the cooler (e.g. 14°C), deeper outer bay in the late afternoon and back to the inner bay early the following morning (T.E. Hopkins, and S.A. Mattern, unpublished data). This pattern of moving between warm and cool areas may represent an attempt to minimize metabolic expenditures or increase assimilation efficiency (Brett, 1971; Carey and Scharold, 1990).

Compared to most elasmobranchs, bat ray blood has comparatively high affinity (low P_{50}), a large Bohr factor (Ø), blood oxygen capacity (CBO_2), and a low cooperativity (n_{50}) (Table 2, Figure 1). In addition, the non-bicarbonate buffering capacity (ß) of bat rays (Table 2) is greater than in other elasmobranchs (e.g. ß = 9.0 in *Squalus suckleyi*, Lenfant and Johansen; 1966, ß = 9.3 in *Triakis semifasciata*, Lai *et al.*, 1990; ß = 11.0 in *Raja ocellata*, Graham *et al.*, 1990). Among teleosts, high affinity, hyperbolic binding curves and large ß values are associated with hypoxia and hypercapnia tolerance (Johansen and Weber, 1976; Wood *et al.*, 1977; Powers, 1980). Conversely, reduced affinity, large Bohr factor, and high blood-oxygen carrying capacity are typical of active fishes (Cech *et al.*, 1984). The situation is not as clear in elasmobranchs however, as species with widely differing activity levels and aerobic scopes show similar blood oxygen characteristics (Butler and Metcalfe, 1988). By teleostean standards then, bat ray blood appears to be well adapted to hypoxia and hypercapnia (low P_{50}, n_{50}, and high ß), and activity (high CBO_2 and large (Ø).

Bat rays are morphologically specialized for active swimming (McEachran, 1990) and the large ß and Ø values lend support to the hypothesis of Dobson *et al.* (1986), that a high blood buffering capacity may be important for extending muscle performance in active fishes. However, a scaled-up blood buffering capacity requires an increased pH sensitivity of hemoglobin for releasing oxygen, otherwise oxygen delivery to the tissues during exercise would be compromised. Thus, the bat rays' large Bohr factor would facilitate oxygen delivery from the blood to the muscles for a small change in blood pH during exercise.

Bat ray blood-oxygen binding was relatively temperature-independent (low absolute value of ΔH) in the 8–14 and 20–26°C ranges, but showed an increased temperature sensitivity between 14–20°C (Table 2). These ΔH values parallel changes in bat ray resting metabolism measured over the identical temperature ranges (Hopkins and Cech, 1994). A temperature increase from 14 to 20°C decreased affinity while Ø (a measure of CO_2 and pH sensitivity) and CBO_2 increased. An acute temperature rise will decrease blood-oxygen affinity directly due to the exothermic nature of Hb-oxygen binding, and indirectly due to the associated pH decrease (Howell *et al.*, 1970). Increasing Ø and CBO_2 would safeguard oxygen unloading at the tissues in response to an increased respiratory demand assuming environmental oxygen was not limiting. The large (25%) drop in

CBO_2 between 20 and 26°C may be due to oxidation of a component or part of the hemoglobin *in vitro*.

Between 14–20°C bat rays may be sacrificing oxygen uptake ability (by decreasing affinity) to increase oxygen off loading to compensate for a 311% increase in respiratory demand over this same temperature range (Hopkins and Cech, 1994). Because changes in environmental temperature can influence both oxygen availability and oxygen-dependent metabolic rate functions (Fry, 1957, 1971), *in vivo* oxygen binding properties can be augmented during acclimation by changes in hemoglobin concentration (e.g. via splenic contraction, Yamamoto *et al.*, 1985), water shifts to lactate loaded muscles (Wood, 1991), or changes in allosteric phosphate cofactors (Dobson and Baldwin, 1982b), all of which may assist oxygen transport at higher temperatures. While these changes can take from hours to days to become fully effective (Jensen *et al.*, 1993), the difference between the immediate (short-term) respiratory demands and (long-term) hematological acclimation could be met almost instantly by simply increasing cardiac output so as to safeguard blood oxygen transport (Heath and Hughes, 1973).

While our data are an instantaneous sample of a system which can be altered over several time scales, we feel our *in vitro* results are indicative of the direction, but perhaps not the actual magnitude of the changes which occur in a bat ray moving between water masses of different temperature. Thus, we conclude that movements of rays out of the warmer portions of Tomales Bay are not related to their blood oxygen binding characteristics but may be due to other factors such as minimizing energy expenditure (Hopkins and Cech, 1994), maximizing assimilation efficiency (Brett, 1971), or daily social aggregations (Klimley *et al.*, 1988; Tricas, 1980).

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