J. exp. Biol. 151, 161–173 (1990)
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BLOOD RESPIRATORY PROPERTIES AND THE EFFECT OF SWIMMING ON BLOOD GAS TRANSPORT IN THE LEOPARD SHARK *TRIAKIS SEMIFASCIATA*

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Accepted 8 March 1990

Summary

Changes in vascular pressures, blood respiratory properties and blood gas transport induced by swimming were investigated in the leopard shark *Triakis semifasciata* (Girard). In resting sharks, the mean ventral and dorsal aortic pressures (systolic/diastolic) were 6.8/5.6 kPa and 4.5/3.9 kPa, respectively, and only the former were increased significantly during swimming. Swimming also caused a significant decline in venous P_{O_2} (1.6 to 0.9 kPa), O_2 content (0.9 to 0.4 mmol l^{-1}) and percentage O_2 saturation (S_{O_2}, 39 to 18%) but the arterial variables were not affected. A significant decline in venous pH and an increase in venous P_{CO_2} also occurred during swimming but lactate concentration did not increase during or after swimming. An *in vivo* dissociation curve compiled from blood P_{O_2} and S_{O_2} data for sharks in the resting, swimming and post-swimming recovery phases shows a mean P_{50} of 2.04 kPa, as determined by Hill transformation. The pH–bicarbonate plot for this fish shows a weak blood buffer capacity of 9.3 mmol l^{-1} pH unit^{-1} and during swimming the average blood pH and bicarbonate concentration follow the buffer line which was not compensated in recovery. Neither oxygenated nor deoxygenated blood pH values were affected by CO_2 equilibration, suggesting the absence of a Haldane effect. Thus, at the expense of respiratory acidosis, *Triakis* can aerobically sustain long (up to 60 min) and moderately intense (0.45 L s^{-1}, where L is body length) periods of swimming by increasing cardiac output and tapping its venous reserve.

Introduction

Most vertebrate circulatory systems operate with arterial O_2 tensions at or above that required for haemoglobin (Hb) saturation and, in response to aerobic exercise, elevate O_2 transport to tissues by increasing ventilation and cardiac

Key words: *Triakis semifasciata*, swimming, vascular pressures, blood gas, blood lactate, Haldane effect.

output and lowering venous O_2 reserves (Kiceniuk and Jones, 1977; Jones and Randall, 1978; Haidet, 1989). While this pattern is generally present in fishes, some variations do occur. The lemon shark (*Negaprion brevirostris*), for example, appears not to have Hb-saturating arterial O_2 tensions while resting but does elevate them in response to increased aerobic demand (Bushnell *et al.* 1982). Also, the rainbow trout (*Salmo gairdneri*) apparently has the ability to match ventilation and cardiac output to aerobic demand and sustain an increased O_2 uptake without affecting O_2 reserves (Stevens and Randall, 1967).

The limited data available for sharks indicate that responses to activity may vary among different species. In addition to the above-cited finding for *Negaprion*, work by Taylor (1985, Fig. 18B) suggests that swimming in *Scyliorhinus canicula* is to some extent dominated by a disturbance bradycardia rather than by aerobic demand. Piiper *et al.* (1977) found that *Scyliorhinus stellaris* did not alter vascular pressures during spontaneous swimming but did deplete venous O_2 reserves and accrued an O_2 debt.

Most studies of shark cardiovascular physiology have been limited by the inability to regulate fish activity. We have surmounted this problem by holding and swimming sharks in variously sized water tunnel respirometer systems at the Physiological Research Laboratory, Scripps Institution of Oceanography (SIO). A previous paper (Lai *et al.* 1989) examined the effect of sustained swimming on the pericardial pressure and cardiac output of the California leopard shark, *Triakis semifasciata* (Girard). The present study investigates the effect of steady swimming on the vascular pressures, blood gas variables, and the blood pH and lactate concentrations of this species.

Materials and methods

Experimental animals and conditions of maintenance

Leopard sharks (1.6–2.8 kg, 73–94 cm body length, L) were collected in Elkhorn Slough, Moss Landing, CA, and kept for 9 months in 5000 l holding tanks at La Jolla, CA. They were maintained in running, filtered and aerated sea water at ambient temperature (14–24 °C) and photoperiod and fed chopped mackerel and squid twice weekly, except for experimental fish, which were starved for 7 days prior to study.

Surgical procedures and equipment

Sharks were anaesthetized with tricaine methane sulphonate (MS222, 1:10 000), placed supine on a V-board, and ventilated with aerated water at room temperature containing the same concentration of anaesthetic.

The dorsal and ventral aortas of anaesthetized sharks were cannulated percutaneously using PE 50 tubing. Vascular access was obtained using a 16 gauge thin-walled needle attached to a heparinized syringe that was advanced to the desired vessel from inside the mouth. Local anatomical landmarks on the roof and floor of the mouth were used to locate the point of access for the dorsal and ventral aortas,

respectively. Slight negative pressure applied to the syringe plunger during needle advancement enabled determination of vessel entry by blood aspiration. Once contact with the vessel lumen was confirmed, blood in the syringe was reinjected. The syringe was then removed and a heparinized, elasmobranch-saline-filled PE 50 catheter, long enough (60 cm) to extend from the fish to the outside of the water tunnel, was advanced through the needle and into the vessel a few centimetres beyond the needle tip. Similarity in the diameters of the catheter and the needle prevented leakage through the needle bore.

When it had been determined that blood could be aspirated easily into the catheter and the correctness of the catheter position had been confirmed by blood pressure signals measured with a calibrated Statham pressure transducer (Gould model P23ID), the needle was withdrawn and passed over the end of the tubing. The catheter was sutured in place within the mouth and its free end was passed out of the spiracle, anchored to the body at regular intervals, and routed to, and secured on, the first dorsal fin. Cannulation of each vessel required about 15 min. Because it was necessary to work inside the fish's mouth, ventilation had to be temporarily interrupted. For this reason the surgery was done as rapidly as possible, stopped at regular intervals to ventilate the fish, and a long period of ventilation was allowed between introducing the dorsal and ventral cannulae.

Water tunnel

The experimental water tunnel, similar to the system described by Prange (1976), consists of upper and lower flow channels connected by curved metal vanes at each end to form a closed loop. The fish is placed in the working section (113 cm×30 cm×29 cm) which is contained in the upper channel. Water is driven around the system by a 560 W variable-speed motor (Minarik Blue Chip II) mounted outside the lower channel and coupled to the propeller shaft which enters the unit through sealed bearings. Honeycomb collimator material at the front of the working section confines the shark and reduces working section turbulence. Flow probe measurements at different depths in the working section verified a uniform flow field. The maximum velocity of the system is about 1.25 $m\,s^{-1}$ (calibrated by a General Oceanics Inc., model 2035-mk III flowmeter).

Protocol

Immediately after surgery the shark was placed in a holding tank to recover and then transferred to the water tunnel, which was circulated with fresh aerated sea water. Experiments did not begin until at least 24 h after surgery.

Data were collected at rest, during swimming and during recovery from swimming. All tests were made during the summer at water temperatures ranging from 19 to 22°C and at ambient water O_2 tensions of from 9.2 to 13.6 kPa. Variables measured included ventral and dorsal aortic pressures and the blood arterial (a) and venous (v) tensions (P_{O_2}, P_{CO_2}), total oxygen (Ca_{O_2}, Cv_{O_2}) and total carbon dioxide (Ca_{CO_2}, Cv_{CO_2}), pH and plasma lactate concentration.

During the exercise period, attempts were made to keep all the fish swimming

steadily while vascular pressure and blood gas determinations were made. Periods of swimming ranged from 22 to 60 min and no fish was swum to exhaustion. Relative swimming speeds in these tests ranged from 0.38 to 0.53 $L\,s^{-1}$ (mean 0.45 $L\,s^{-1}$). Blood samples were collected during a recovery period between 14 and 60 min after the fish stopped swimming. Following this, each shark was rested until the next day, when a replicate run was made.

Analytical procedures

Prior to the start of an experiment, the pressure transducers were calibrated manometrically against a static column of water. Blood pressures measured by these transducers were zeroed *via* PE tubing connected to the sea water of the water tunnel. Pressure signals were amplified (Gould, model 13-4615-50) and recorded on a strip chart recorder (Gould, model 2400). The high vascular pressures and the use of the shortest possible catheters permitted recording of high-resolution data even while the fish was swimming. Signals were further improved by placing a slight tension on the catheters to prevent vibration in flowing water.

Venous and arterial blood samples (0.2 ml) were drawn in sequence and immediately analyzed for P_{O_2}, P_{CO_2} and pH using a Radiometer blood gas microsystem (BMS-3MK2) thermostatted at 20±0.1°C. Electrodes were calibrated using precision gas mixtures or Radiometer precision buffer solutions.

Total O_2 content was determined by the method of Tucker (1967) and total CO_2 by the method of Cameron (1971). Values for bicarbonate ion concentration were calculated by subtracting the concentration of molecular CO_2 from total blood CO_2. Molecular CO_2 concentrations were obtained from the product of P_{CO_2} and the solubility coefficient of CO_2 at 20°C (0.342 mmol $CO_2\,l^{-1}\,kPa^{-1}$; Boutilier *et al.* 1984). Both procedures used 50 μl samples. Blood lactate was measured according to Sigma Technical Bulletin 826-UV on 30 μl samples.

Percentage O_2 saturation of Hb was determined for arterial and venous blood samples (Sa_{O_2}, Sv_{O_2}). First, the oxygen-carrying capacity of the blood was determined by incubating a blood sample with air at 20±0.1°C using a Radiometer BMS-2 and then measuring total O_2 content. Plasma O_2 was also measured by incubation and the resulting solubility coefficient was found to be 14.2 $\mu mol\,l^{-1}\,kPa^{-1}$. The quantity of O_2 bound to the Hb is defined as the O_2-carrying capacity of whole blood minus the amount dissolved in the plasma. O_2 bound to Hb in arterial and venous blood was determined subsequently by measuring both total O_2 content and the P_{O_2} of the sample and subtracting the physically dissolved O_2, which was calculated from sample P_{O_2} and plasma O_2 solubility coefficient. The O_2 bound to Hb was then expressed as a percentage of that bound to Hb in an air-saturated sample.

The presence of a Haldane effect was tested by measuring the pH and total CO_2 content of blood incubated for 25 min at 20±0.1°C at constant P_{CO_2} values of 0.15, 0.30 and 0.51 kPa under fully deoxygenated (CO_2 and N_2) and oxygenated (CO_2 and air) conditions. Blood used for these measurements was obtained directly by puncture of the caudal vein.

Results

Vascular pressures

Mean (±S.E.M.) dorsal and ventral aortic blood pressures of leopard sharks at rest, during swimming and in recovery are shown in Table 1. Ventral aortic pressure is greater and has a larger pulse pressure (systole minus diastole) than that of the dorsal aorta. Both the systolic and diastolic ventral aortic pressures were significantly ($P<0.05$, paired t-test) elevated during swimming and returned to resting levels during recovery. Dorsal aortic blood pressure was unaffected by swimming and remained the same during recovery. The differences between the ventral and dorsal systolic pressures, an index of change in branchial flow or resistance or both during each experimental phase, were: rest, 2.3 kPa; swimming 3.6 kPa; and recovery 3.1 kPa.

Blood respiratory properties

Effects of swimming

Table 2 shows mean values of blood variables measured for *Triakis* at rest, during swimming and in recovery. Values for the percentage O_2 saturation of arterial and venous blood are also shown. Estimates of the O_2 capacity of *Triakis* Hb ranged from 1.5 to 2.8 μmol l^{-1} (mean±S.E.M=2.1±0.1 μmol l^{-1}) and haematocrit values ranged from 14 to 25 % (mean=18.3±2.5 %).

With the exception of lactate concentration and resting pH, significant differences between all arterial and venous variables ($P<0.05$, paired t-test) were found in resting, swimming and recovering sharks (Table 2).

Swimming caused a significant ($P<0.05$) decline in three venous O_2 variables, Pv_{O_2} (44 %), Cv_{O_2} (56 %) and Sv_{O_2} (54 %); the arterial variables, however, were unaffected. Also significantly affected by swimming were venous pH, which declined, and Pv_{CO_2} which increased. Neither the slight drop in arterial pH nor the rise in plasma lactate seen during swimming (Table 2) were statistically significant.

Table 1. *Ventral and dorsal aortic pressures of leopard sharks at rest, during swimming and in recovery*

	Rest	Swimming	Recovery
Ventral aorta			
Systole (kPa)	6.8±0.8	8.3±0.5*	7.9±0.5
Diastole (kPa)	5.6±0.5	6.8±0.3*	6.4±0.3
Dorsal aorta			
Systole (kPa)	4.5±0.4	4.7±0.3	4.8±0.5
Diastole (kPa)	3.9±0.4	4.1±0.3	4.4±0.4

Values are mean±S.E.M. ($N=6$).
* Significant differences between rest and swimming ($P<0.05$, paired t-test).
L, body length.
Swimming velocity was 0.38–0.51 $L\,s^{-1}$.

Table 2. *Mean arterial and venous blood P_{O_2}, P_{CO_2}, percent O_2 saturation, total O_2, total CO_2, pH and lactate levels in the leopard shark during rest, swimming and recovery*

	Rest	Swimming	Recovery
Pa_{O_2} (kPa)	8.8 (±0.7, 7)	8.4 (±0.6, 7)	9.1 (±0.4, 6)
Pv_{O_2} (kPa)	1.6 (±0.2, 7)	0.9* (±0.1, 7)	1.6 (±0.2, 6)
Sa_{O_2} (%)	95 (±1, 5)	91 (±3, 5)	94 (±3, 5)
Sv_{O_2} (%)	39 (±4, 5)	18* (±1, 5)	42 (±5, 5)
Ca_{O_2} (mmol l^{-1})	2.0 (±0.1, 7)	1.9 (±0.1, 7)	2.0 (±0.1, 6)
Cv_{O_2} (mmol l^{-1})	0.9 (±0.1, 7)	0.4* (±0.1, 7)	0.8 (±0.1, 6)
Pa_{CO_2} (kPa)	0.24 (±0.01, 7)	0.31* (±0.02, 7)	0.27 (±0.01, 6)
Pv_{CO_2} (kPa)	0.34 (±0.02, 7)	0.46* (±0.02, 7)	0.36 (±0.02, 6)
Ca_{CO_2} (mmol l^{-1})	3.7 (±0.6, 5)	4.4 (±0.3, 5)	3.5 (±0.2, 5)
Cv_{CO_2} (mmol l^{-1})	5.2 (±0.6, 5)	6.4 (±0.4, 5)	4.9 (±0.2, 5)
pHa	7.775 (±0.022, 7)	7.709 (±0.021, 7)	7.766 (±0.014, 6)
pHv	7.745 (±0.017, 7)	7.647* (±0.018, 7)	7.710 (±0.019, 6)
Lactate			
Arterial (mmol l^{-1})	1.79 (±0.25, 6)	3.74 (±0.73, 6)	2.72 (±0.49, 6)
Venous (mmol l^{-1})	1.88 (±0.19, 7)	3.18 (±0.43, 7)	2.47 (±0.18, 6)

Numbers in parenthesis are ±S.E.M., *N*.

P_{CO_2} measurements are accurate to 0.02 kPa.

All blood gas measurements were made at 20 °C.

* Significant differences ($P<0.05$, paired *t*-test) between the mean for a treatment and the mean value in the resting fish.

Mean ambient water conditions: P_{O_2} 15.86±0.4 kPa; P_{CO_2} 0.16±0.01 kPa; 19–22 °C.

During recovery, blood variables that had changed significantly during swimming returned towards the levels recorded at rest and did not differ significantly from them. Recovery thus led to significant increases in Pv_{O_2}, Sv_{O_2} and Cv_{O_2} and significant decreases in Pa_{CO_2}, Pv_{CO_2}, Ca_{CO_2} and Cv_{CO_2}. Blood lactate and pHa did not change significantly from swimming to recovery; however, pHv did return to its pre-exercise level. Also, lactate concentrations during the first 15 min of

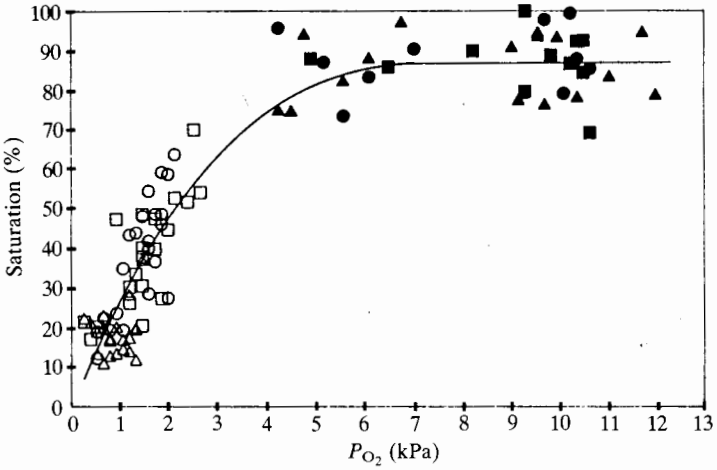

Fig. 1. Functional *in vivo* oxygen dissociation curve for arterial (closed symbols) and venous (open symbols) blood of *Triakis semifasciata* during rest (circles), swimming (triangles) and recovery (squares).

recovery (arterial: $1.8\pm0.4\,mmol\,l^{-1}$, $N=5$; venous: $2.2\pm0.2\,mmol\,l^{-1}$, $N=4$) did not differ significantly from those measured later (60 min, arterial: $1.9\pm0.5\,mmol\,l^{-1}$; venous: $3.2\pm0.5\,mmol\,l^{-1}$).

In vivo *oxygen affinity*

Blood P_{O_2} and percentage O_2 saturation data compiled for *Triakis* during each experimental phase form an *in vivo* O_2 equilibrium curve (Fig. 1). A double logarithmic Hill transformation for blood P_{O_2} and S_{O_2} data taken at rest, during swimming and during recovery periods indicates that oxygen binding is weakly cooperative (rest $n=1.5$; swimming $n=1.4$; recovery $n=1.1$). The mean P_{50} (2.04 kPa) was averaged from the resting (1.87 kPa), swimming (2.26 kPa) and recovery (2.00 kPa) Hill plots.

Blood acid–base status

Table 3 shows that there were no differences in bicarbonate concentration between oxygenated and deoxygenated blood following *in vitro* incubation with 0.2, 0.4 and 0.7 kPa of CO_2. Oxygenated and deoxygenated blood pH values were also not affected by CO_2 equilibration. The pH–bicarbonate diagram (Fig. 2) for *Triakis* is based on a whole-blood mean pK value of 5.96 (±0.02, $N=18$), determined to be the same ($P>0.05$) for both oxygenated and deoxygenated blood incubated at different P_{CO_2} values.

The *in vitro* buffer line in Fig. 2 was generated from data reported in Table 3. The blood buffering capacity, indicated by the slope of the buffer line (i.e. the change in bicarbonate concentration per unit change in pH) is $9.3\,mmol\,l^{-1}$ pH unit^{-1}. Fig. 2 reveals that shifts in average blood pH and bicarbonate during

Table 3. *Mean* in vitro *pH and bicarbonate data for leopard shark blood (N=5) equilibrated at 0.2, 0.4, 0.7 kPa CO_2 under oxygenated and deoxygenated conditions at 20°C*

	Deoxygenated		Oxygenated	
P_{CO_2} (kPa)	pH	$[HCO_3^-]$ ($mmol\,l^{-1}$)	pH	$[HCO_3^-]$ ($mmol\,l^{-1}$)
0.2	7.745 (±0.021)	3.86 (±0.18)	7.663 (±0.046)	4.49 (±0.24)
0.4	7.577 (±0.014)	5.06 (±0.32)	7.572 (±0.017)	4.79 (±0.31)
0.7	7.456 (±0.009)	6.35 (±0.24)	7.461 (±0.021)	6.69 (±0.64)

There is no significant difference in pH and bicarbonate values between the oxygenated and deoxygenated blood.

Parenthetic values are ±S.E.M.

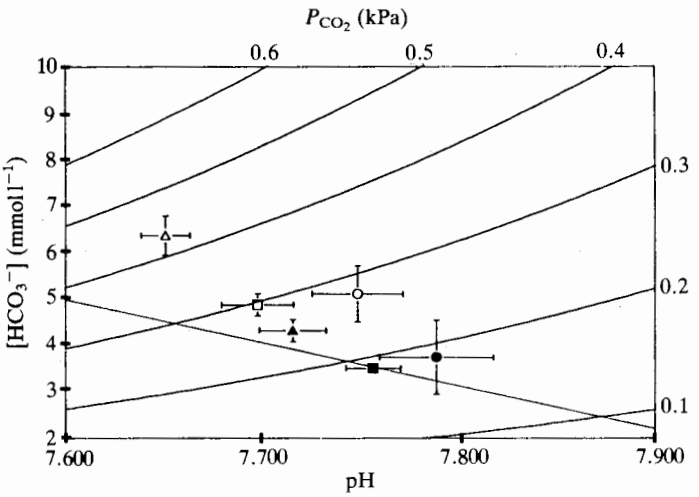

Fig. 2. Bicarbonate concentrations in relation to pH and P_{CO_2} (isobars) of arterial and venous blood of *Triakis semifasciata* during rest, swimming and recovery. Solid diagonal line is the *in vivo* buffer regression for oxygenated and deoxygenated whole-blood samples equilibrated with 0.2, 0.4 and 0.7 kPa CO_2 gas mixtures. Symbols as in Fig. 1. Error bars are ±S.E.M.

swimming follow the buffer line and that recovery did not involve pH compensation through an increase in bicarbonate level.

Discussion

Shark swimming performance

The relatively large size of sharks has limited most studies of their swimming and

respiration. Only a few investigators have examined shark cardiovascular and respiratory adjustments during swimming (Piiper *et al.* 1977; Brett and Blackburn, 1978; Bushnell *et al.* 1982). Some sharks (*Squalus*) have proved unsatisfactory for controlled swimming (Brett and Blackburn, 1978) and others (*Scyliorhinus*) could only be swum sporadically (Piiper *et al.* 1977). The data obtained for *Scyliorhinus* were based largely on resting and spontaneously swimming fish and thus little is known about respiratory adjustments during sustained activity (Piiper *et al.* 1977). Also, *Negaprion*, which lives in tropical waters and can cruise steadily, had to be prodded to swim in an annular tank at uncontrolled velocities in the studies of Bushnell *et al.* (1982, 1989). Previous studies by our group (Scharold *et al.* 1989; Lai *et al.* 1989) and the present investigation are the first to control shark swimming speed and duration for the time required to determine quantitative changes in respiration and cardiovascular function. Several observations verify that the sharks used in this study were swimming within the scope of normal aerobic activity. First, the relatively long swimming durations and moderate swimming speeds ($0.45\,L\,s^{-1}$) did not cause lactate concentrations to change significantly from those at rest, nor did a pulse of lactate appear during recovery. Second, recent findings by Graham *et al.* (1990) show that *Triakis* similar in body size to those used in this study have a critical swimming speed of $1.27\pm0.12\,L\,s^{-1}$, which indicates that the sharks in the present study were swimming well within their aerobic limit.

Effects of swimming on vascular pressures

The increase in ventral aortic pressure measured in swimming *Triakis* is consistent with observations of an elevated cardiac stroke volume (Lai *et al.* 1989). The larger systolic transbranchial blood pressure gradient (from 2.3 to 3.6 kPa, Table 1) during swimming results from this elevated cardiac output and could, in turn, be expected to increase the respiratory exchange area (Farrell *et al.* 1980). Blood pressure data (Table 1) and data on the cardiac output of *Triakis* (Lai *et al.* 1989), allow an estimation of the relative branchial resistance during rest and swimming to be made. Lai *et al.* (1989) found the mean cardiac output of *Triakis* to be 76 ml min^{-1} at rest and 108 ml min^{-1} during swimming at speeds similar to those used in the present study (Table 4). Assuming that blood shunting around the gills is minimal, the ratio of the drop in transbranchial blood pressure (by convention measured in Torr) to cardiac output (ml min^{-1}) estimates relative branchial resistance. As seen in Table 4, this resistance ($\Delta P/\dot{Q}$) remains about the same during rest and swimming, showing that the increased cardiac output is nearly matched by the increased branchial capacitance.

A different pattern of branchial resistance apparently exists in *Scyliorhinus*. During swimming this fish increased its cardiac output by a factor of 1.7 (Piiper *et al.* 1977). This increase is similar to that in *Triakis*. However, changes in dorsal and ventral aortic pressures during swimming in *Scyliorhinus* were small or nonexistent (rest to swimming: dorsal, 3.4 to 3.2 kPa; ventral, 4.6 to 4.6 kPa). Assuming minimal gill blood shunting, the estimated branchial resistances for

Table 4. *Estimation of branchial peripheral resistances and blood O_2 and convection variables for leopard sharks at rest and during swimming*

	Resting	Swimming
Blood flow properties		
Heart rate (beats min^{-1})	51	55
Stroke volume (ml $beat^{-1}$)	1.48	1.97
$\dot{Q}$ (l min^{-1})	0.075	0.108
Branchial resistances		
ΔP (systolic pressure difference ventral–dorsal aorta, Torr)	17.2	26.8
$\Delta P/\dot{Q}$ (PRU, Torr min^{-1} ml^{-1})	0.23	0.25
Blood convection requirement		
Arterial–venous O_2 difference (mmol ml^{-1})	0.0011	0.0015
$\dot{M}_{O_2}$ (mmol min^{-1})	0.083	0.163
($\Delta\dot{M}_{O_2}$=0.163−0.083=0.08 mmol min^{-1})		
$\dot{Q}/\dot{M}_{O_2}$ (l $mmol^{-1}$)	0.904	0.663
β (mmol l^{-1} kPa^{-1})	0.153	0.200

Resting and swimming cardiac output ($\dot{Q}$) values are estimated from heart rate and cardiac stroke volume data reported in Lai *et al.* 1989.

Resistance estimates are based on the difference in transbranchial systolic pressures (Table 1) divided by cardiac output (by convention expressed as PRU, where pressure units are Torr and $\dot{Q}$ values are ml min^{-1}).

Tissue oxygen consumption ($\dot{M}_{O_2}$) calculations are the product of mean arteriovenous O_2 differences (Table 2) and $\dot{Q}$ (Lai *et al.* 1989).

A mean haematocrit of 18 is assumed. $\dot{Q}/\dot{M}_{O_2}$, blood convection requirement; β, blood O_2 capacitance coefficient (the difference between arterial and venous contents divided by the difference between arterial and venous partial pressures).

Scyliorhinus are 0.48 PRU during rest and 0.33 PRU during swimming. Reasons for the different patterns of branchial resistance in these sharks are unclear. Large and persistent branchial resistances changes due to exercise are atypical in fishes (Randall, 1982).

Blood respiratory properties and the effect of swimming on blood gas transport

Our study of *Triakis* provides additional comparative data on the respiratory properties of elasmobranch blood. The mean P_{50} (2.04 kPa) estimated for this fish is similar to that of other elasmobranchs (Lenfant and Johansen, 1966; Piiper and Baumgarten-Schuman, 1968; Pleschka *et al.* 1970; Cameron *et al.* 1971; Bushnell *et al.* 1982; Wells and Weber, 1983). Furthermore, the whole-blood buffering capacity of *Triakis* (9.3 mmol pH $unit^{-1}$) is similar to that of *Squalus suckleyi* (9.0 mmol pH $unit^{-1}$, Lenfant and Johansen, 1966). The absence of a Haldane effect in *Triakis* (Table 3) also agrees with most studies (*Raja oscillata*: Dill *et al.* 1932; *Mustelus canis*: Ferguson *et al.* 1938; *Squalus suckleyi*: Lenfant and Johansen, 1966; *Scyliorhinus canicula*: Albers and Pleschka, 1967), although there have

been a few contrary reports (*Raja clavata*, Hughes and Wood, 1974; *Squalus acanthias*, Wells and Weber, 1983).

The significant drop in Pv_{O_2} and Cv_{O_2} during swimming (Table 2) indicates that *Triakis* taps its venous O_2 reserve to compensate for the elevated metabolic needs. This observation is in agreement with Piiper *et al.* (1977), who saw a 55 % reduction in Cv_{O_2} during spontaneous swimming by *Scyliorhinus*. The high levels of venous O_2 present in both *Scyliorhinus* and *Triakis* at rest contrasts markedly with the condition found for resting *Negaprion*, which had practically no venous reserve (Bushnell *et al.* 1982). Bushnell *et al.* (1982) also measured significant increases in Pa_{O_2} (+40 %), Ca_{O_2} (+31 %) and haematocrit (+10 %) during activity in *Negaprion*. Other than obvious species and methodological differences we have no explanation for why a markedly different physiological pattern is seen in resting and swimming *Negaprion* as opposed to *Triakis* and *Scyliorhinus*, in which Ca_{O_2} and Pa_{O_2} did not change. Stevens and Randall (1967) suggested that an active species such as the rainbow trout may not alter its arteriovenous O_2 difference if both ventilation and cardiac output can be adjusted to meet swimming O_2 requirement. The significant decline in the Pv_{O_2} seen for swimming *Triakis* demonstrates that this mechanism is not present.

When combined with earlier data (Lai *et al.* 1989), our findings for *Triakis* enable us to examine the effects of activity on various aspects of cardiac performance and blood gas transport (Table 4). Lai *et al.* (1989) showed that the measured cardiac output ($\dot{Q}$) of *Triakis* during swimming (Table 4) was the result of increases in both mean cardiac stroke volume (1.48 to 1.97 ml $beat^{-1}$) and mean heart rate (51 to 55 beats min^{-1}). The product of $\dot{Q}$ and the mean arteriovenous O_2 difference provides an estimate of total tissue O_2 consumption ($\dot{M}_{O_2}$, Table 4); the values are 0.083 mmol min^{-1} at rest (i.e. $51\times1.48\times0.0011$) and 0.163 mmol min^{-1} during swimming, the rest–swimming increment ($\Delta\dot{M}_{O_2}$) is 0.08 mmol min^{-1}.

The ratio $\dot{Q}/\dot{M}_{O_2}$, termed the blood convection requirement, is an index of the mechanical energy needed to supply metabolic demand and Table 4 shows that, as a result of greater O_2 extraction, this value declines in swimming sharks. Similarly, the shift in β, the blood O_2 capacitance coefficient, to a higher value during swimming (Table 4) demonstrates that, owing to the curvilinear nature of the Hb dissociation function, a slight decline in venous P_{O_2} during swimming translates into a large incremental change in $\dot{M}_{O_2}$.

While these observations document the role for O_2 reserve utilisation, data in Table 4 also permit us to calculate the relative contributions of the three variables (heart rate, cardiac stroke volume and arteriovenous O_2 difference) to swimming $\dot{M}_{O_2}$. This is done by determining the percentage reduction in the active $\dot{M}_{O_2}$ increment resulting when $\dot{M}_{O_2}$ is calculated with two of the three variables at swimming level and the remaining one at its rest level. First, combining the resting arteriovenous O_2 difference with the swimming heart rate and stroke volume results in an $\dot{M}_{O_2}$ of 0.119 mmol min^{-1} ($55\times1.97\times0.011=0.119$), an increase of 0.036 mmol min^{-1} above resting $\dot{M}_{O_2}$. This value is 45 % of the rest–swimming $\dot{M}_{O_2}$ increment [$(0.036/0.08)\times100$] and, by difference, means that the arterio-

venous O_2 contribution to the active $\dot{M}_{O_2}$ amounts to about 55 %. Using the same calculations but rearranging the variable kept at rest level, the contribution of cardiac stroke volume is estimated to be about 51 % of the active $\dot{M}_{O_2}$, whereas heart rate increase provides only about 15 %. Because these percentages are relative and calculated independently they do not combine to 100 %. They do, nevertheless, indicate that increased stroke volume and the increased use of venous reserves during swimming each contribute equally to the active $\dot{M}_{O_2}$ of *Triakis semifasciata*.

This work was supported by the National Science Foundation (DCB84–16852, DCB87–18449 and DCB88–02291). NCL was supported by these grants and by a dissertation fellowship from the University of California, San Diego, and this paper constitutes part of his doctoral dissertation at the University of California, San Diego. We wish to thank T. Camarillo, W. R. Lowell, J. Jacobson and S. Stutton for technical assistance, C. Lennert for statistical assistance, and Drs R. H. Rosenblatt, G. N. Somero, G. L. Kooyman and W. H. Berger for reviewing the manuscript.

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