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Water-tunnel studies of heat balance in swimming mako sharks

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Summary

The mako shark (*Isurus oxyrinchus*) has specialized vascular networks (retia mirabilia) forming counter-current heat exchangers that allow metabolic heat retention in certain regions of the body, including the aerobic, locomotor red muscle and the viscera. Red muscle, white muscle and stomach temperatures were measured in juvenile (5–13.6 kg) makos swimming steadily in a water tunnel and exposed to stepwise square-wave changes in ambient temperature (T_a) to estimate the rates of heat transfer and to determine their capacity for the activity-independent control of heat balance. The rates of heat gain of red muscle during warming were significantly higher than the rates of heat loss during cooling, and neither the magnitude of the change in T_a nor the direction of change in T_a had a significant effect on red muscle latency time. Our findings for mako red muscle are similar to those recorded for tunas and suggest modulation of retial heat-exchange efficiency as the underlying mechanism controlling heat balance. However, the red muscle temperatures measured in swimming makos (0.3–3 °C above T_a) are cooler than those measured previously in larger decked makos. Also, the finding of non-stable stomach temperatures contrasts with the predicted independence from T_a recorded in telemetry studies of mako and white sharks. Our studies on live makos provide new evidence that, in addition to the unique convergent morphological properties between makos and tunas, there is a strong functional similarity in the mechanisms used to regulate heat transfer.

Key words: lamnid, shark, *Isurus oxyrinchus*, tuna, thermoregulation, endothermy, red muscle, white muscle, stomach, heat balance, water tunnel.

Introduction

This paper reports experiments that tested the capacity of steadily swimming juvenile shortfin mako sharks (*Isurus oxyrinchus*) to control rates of heat gain and heat loss. Juvenile makos inhabit the coastal waters off southern California during the spring and summer of each year and occur sufficiently close to the Scripps Institution of Oceanography to enable their capture and transport to the laboratory. Juvenile makos are most commonly found in the upper mixed water layer (18–22 °C), and telemetry studies have shown that makos undergo frequent dives below the thermocline, subjecting them to changes of up to 10 °C in ambient water temperature (T_a) (Holts and Bedford, 1993). The mako is a member of the shark family Lamnidae, a group convergent with the tunas (*Thunnus* spp.) in specializations for enhanced swimming performance (Bernal et al., 2001). Lamnids are similar to tunas in utilizing a counter-current heat-exchanging retia mirabilia to retain metabolic heat within certain regions of the body, including the aerobic, locomotor red muscle (RM), the eyes and brain and the viscera, thus elevating the temperature of these tissues and adjacent areas above T_a (Bernal et al., 2001; Carey et al., 1985; Carey and Teal, 1969).

Previous studies of lamnid body temperature (T_b), in which electronic thermometers were inserted into the bodies of decked sharks immediately upon capture, documented elevated RM, eye, brain and stomach temperatures (Block and Carey, 1985; Carey and Teal, 1969; Carey et al., 1981; Rhodes and Smith, 1983). That work further showed that RM temperature (T_{RM}) could be as much as 12 °C above T_a (Carey and Teal, 1969; Rhodes and Smith, 1983) and that the T_{RM} excess T_x (T_x=T_{RM}–T_a) was affected both by T_a and by specimen condition (i.e. stressed sharks that required a long time to bring aboard the vessel had a lower T_x).

Telemetry studies have demonstrated a markedly elevated stomach temperature ($T_{STOMACH}$) in large, free-swimming lamnids (e.g. a 160 kg *I. oxyrinchus* and a white shark *Carcharodon carcharias* weighing more than 390 kg). $T_{STOMACH}$ was independent of T_a, suggesting that large lamnids were able to regulate the temperature of their viscera and other body regions within a narrow range (Carey et al., 1978, 1981; Goldman, 1997; McCosker, 1987; Tricas and McCosker, 1984). Previous studies have recorded white muscle temperatures (T_{WM}) in free-swimming lamnids such as white shark (Carey et al., 1982; Tricas and McCosker, 1984), but the T_{RM} of continuously or free-swimming lamnid sharks

has not heretofore been measured, and no studies have determined whether lamnids have the same physiological capacity as tunas to regulate RM heat balance (Bernal et al., 2001; Carey et al., 1985; Dewar et al., 1994; Holland et al., 1992).

Although laboratory studies of thermoregulation are not feasible for either large lamnids or tunas, Dewar et al. (1994) succeeded in documenting heat-balance control in small (0.9–2.1 kg) tunas swimming steadily in a water tunnel. They subjected yellowfin tuna (*Thunnus albacares*), swimming at a controlled velocity, to repeated stepwise rapid changes in T_a (square-wave T_a changes) mimicking the range of temperatures encountered during normal vertical excursions (Block et al., 1997; Dizon and Brill, 1979b). The results showed that yellowfin tuna could regulate their rates of heat gain and heat loss physiologically in order to minimize changes in T_b in a similar manner to free-swimming bigeye tuna (*Thunnus obesus*) (Holland et al., 1992).

The objectives of our study were to use the same experimental model employed by Dewar et al. (1994) to test the capacity of mako sharks to regulate rates of heat gain and loss. This study on steadily swimming juvenile mako sharks used temperature measurements of the RM, white muscle (WM) and stomach as indicators of heat flux following square-wave changes in T_a. Thermoregulatory studies of juvenile makos provide data important for assessing the extent of convergence in the specializations of tunas and lamnids for endothermy (Bernal et al., 2001). Moreover, because large lamnids appear to be capable of precisely regulating regional body temperatures (Goldman, 1997), studies of juvenile makos will offer insight into the relative contributions of physiological mechanisms and body mass to this capability (Brill, 1996; Brill et al., 1994).

Materials and methods

A variable-speed water tunnel was used to impose rapid thermal changes (±1 °C min^{-1}) on steadily swimming mako sharks (*Isurus oxyrinchus*), following the protocol of Dewar et al. (1994). Water tunnel design and operation have been described previously (Graham et al., 1990; Dewar et al., 1994).

Capture and transport

A 6 m skiff was used to capture small mako sharks offshore (within 13 km) of La Jolla, CA, USA. A chum line attracted specimens to the boat. The shark selected for study was either dip-netted or hooked, brought on board, and immediately placed into a specially designed 300 l transport chamber that positioned the fish to allow gill irrigation by continuously flowing oxygenated sea water (19±1 °C) and did not restrain tailbeat frequency and amplitude. Less than 15 s was required to bring a hooked fish aboard the vessel. Transport to the laboratory required approximately 45 min. Specimens were captured with a permit from the California Department of Fish and Game (no. 803058-01), and all experimental and surgical procedures were in accordance with protocols approved by the University of California, San Diego Animal Subjects Program (IACUC).

Laboratory procedures

Sharks returned to the laboratory were placed inside the water tunnel (19±1 °C) and allowed to adopt a steady swimming velocity (30–50 cm s^{-1}, usually 0.3–0.5 *L* s^{-1}) for approximately

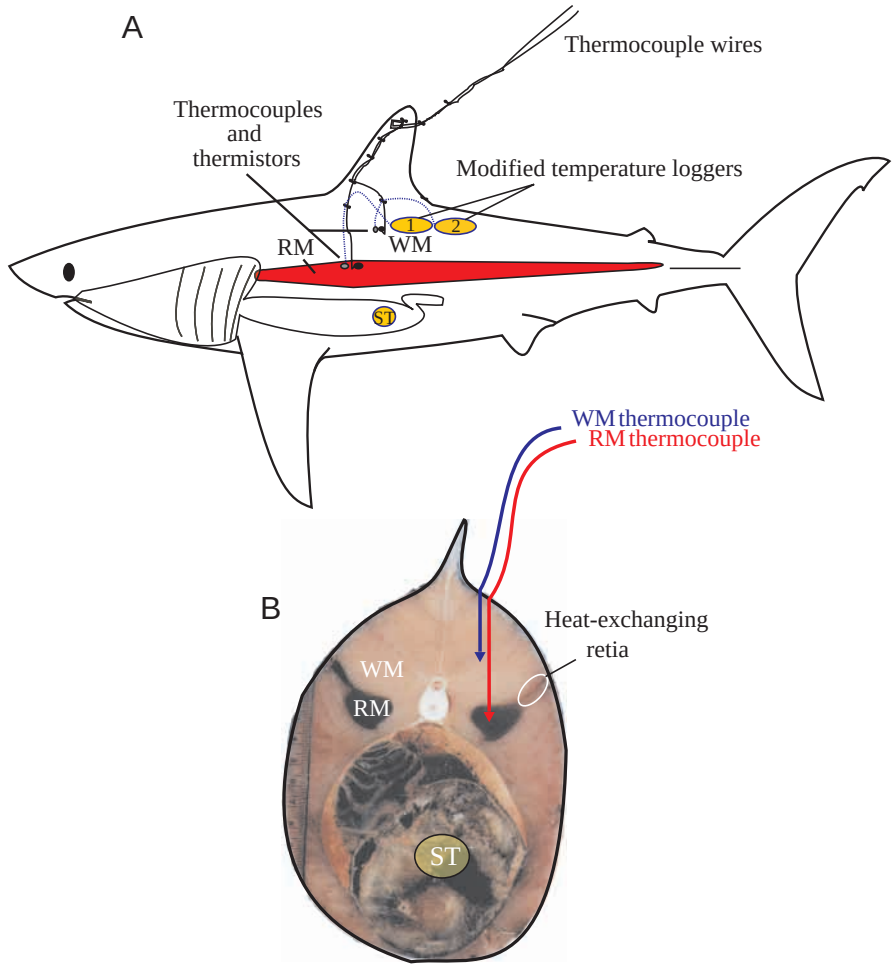

Fig. 1. (A) Diagram of a mako shark showing the positions of the temperature probes. The modified temperature loggers associated with the red (RM) and white (WM) muscle thermistors were attached to the body surface (1 and 2). The position of the stomach temperature logger is indicated by ST. (B) Transverse section (at 45 % *FL*, where *FL* is fork length) of a 10 kg mako shark showing the location of the heat-exchanging retia, the relative vertical positions of the RM and WM thermocouples and thermistors and the temperature logger in the stomach (ST). Note that the WM probes were posterior to the RM probes by 2 % *FL*.

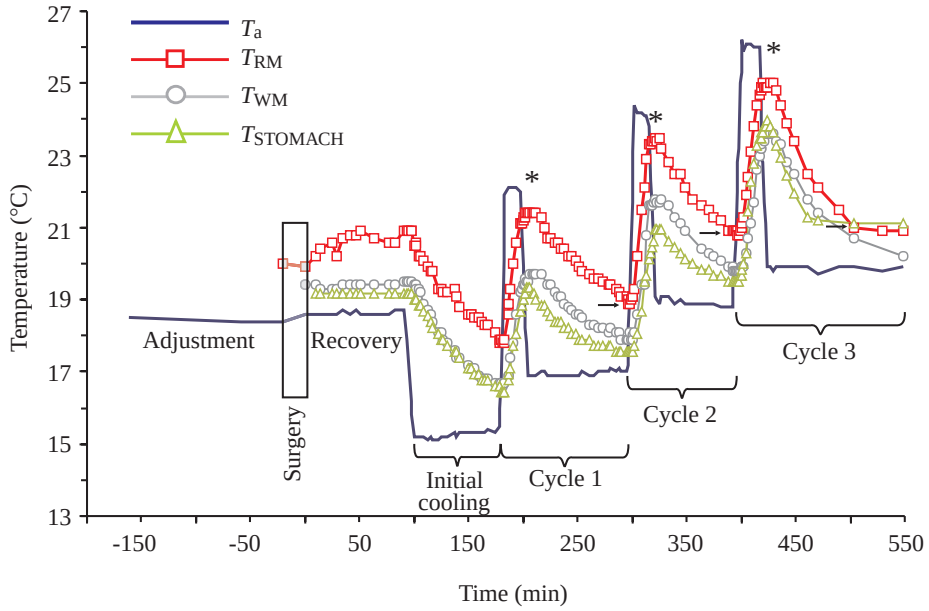

Fig. 2. Complete recordings of red (RM) and white (WM) muscle and stomach temperatures (T) for a 6.9 kg mako shark during its initial adjustment to the water tunnel, the post-surgery recovery period, the initial cooling phase and the three thermal cycles. Note that the time scale reflects pre- and post-surgery periods. The values marked with an asterisk are the estimated values for thermal equilibrium (T_e) in each of the three warming phases, and the horizontal arrows show the T_e for the cooling phases.

200–250 min prior to the surgical implantation of the temperature-monitoring instrumentation. Sharks were then removed from the tunnel and placed on a wooden V-board, where their gills were irrigated with sea water containing tricaine methanosulfonate (MS-222) at a dilution of 1:10 000. Fig. 1 shows the position of the temperature-monitoring instrumentation. Copper–constantan (type T) thermocouples (30 gauge) were inserted into the RM at a position equal to 45 % of fork length (*FL*) and into the WM at 47 % *FL*. The transverse section in Fig. 1B shows the vertical position of the RM and WM thermal probes. Probes inserted in the WM were positioned at approximately half the distance (5–8 cm) between the RM and the skin. Threading the thermocouple wire through a hypodermic needle (10–15 cm, 16 gauge) and bending its tip to form a hook facilitated probe placement at the desired position. The needles were fitted within polyethylene tubing (PE 240, 10 cm), which was left in place to enclose the thermocouple wire following needle withdrawal and to allow the insertion of the thermistor. All thermocouples were connected to a Barnant model 600-1040 meter to verify their integrity during insertion. The indication of a warm temperature was also important for placement of the RM thermocouple. All thermocouple leads were sutured to the skin, then tied into a bundle and attached to the first dorsal fin and led out of the water tunnel (Fig. 1).

Redundant T_{RM} and T_{WM} data were obtained by placing a thermistor adjacent to each thermocouple. For this application, a temperature logger (ONSET Tidbit, 1 Hz sampling rate) was modified to expose its thermistor wire (protected in PE 90 tubing) for insertion into the muscle. The logger was also modified for attachment (Prism P/N 40140 adhesive) to the body surface (Fig. 1). An additional unmodified temperature logger was inserted into the stomach *via* the esophagus. Placement of the temperature-monitoring instruments required less than 20 min, at which time sharks were returned to the water tunnel and allowed to recover from surgery and handling for an additional 120–200 min. Thermocouple, thermistor and stomach logger positions were verified *post mortem*.

Square-wave temperature changes

The duration of the entire experimental protocol ranged from 800 to 1200 min, and data were collected for mako swimming velocity and RM, WM and stomach temperature and T_a. O_2 was monitored using a dissolved oxygen meter (YSI-52) and maintained above 80 % by adding oxygen to the water.

Fig. 2 details the experimental protocol followed for all makos. The magnitude of temperature changes for all the thermal cycles in this study mimics the temperature changes observed in telemetered free-swimming makos during repeated dives (Holts and Bedford, 1993). Because the initial T_a differed in each of the studies, the experimental design was normalized by first cooling all makos from ambient (18.5–20.5 °C) temperature to 15 °C. This was carried out following recovery from anesthesia, and makos were kept at 15 °C for sufficient time to allow a new RM equilibrium temperature (T_e, the temperature at which there is a change in T_{RM} of less than 0.1 °C over 10 min) (Neill et al., 1976) to be reached. The makos were then subjected to three cycles of rapidly imposed (±1 °C min^{-1}) changes in T_a that increased by 2 °C in each cycle. In all the cooling phases of these cycles, T_a was decreased by 5 °C and maintained until T_{RM} reached T_e. For the heating cycles, T_a was increased by 7 °C and maintained until the new T_{RM} reached 0.5 °C below T_a, at which point the next cooling cycle was initiated. The three thermal cycles are designated as follows: cycle 1, 15→22 °C and 22→17 °C; cycle 2, 17→24 °C and 24→19 °C; cycle 3, 19→26 °C and 26→21 °C.

Controls

For control studies, the magnitude, sequence and direction of temperature changes carried out on the swimming makos were

repeated using dead sharks. The bodies of two makos (5 and 10.7 kg) previously used in the swimming studies were fitted with a new set of temperature-monitoring instrumentation (in the same longitudinal position as in the live studies) (see Fig. 1) so that T_{RM}, T_{WM}, $T_{STOMACH}$ and T_a could be monitored continuously. The duration of the cooling phases was maintained until the control T_{RM} reached values equal to the T_e of swimming makos. The duration of the warming phases was maintained until T_{RM} reached 0.5 °C below T_a. The thermal rate coefficients (k) were then calculated from these thermal cycles (see below), and T_e was considered to be equal to T_a.

Calculation of the thermal rate coefficient

From Newton's law of excess temperature, the change in the tissue temperature of a fish over time is a function of T_a (Brill et al., 1994; Dewar et al., 1994; Graham, 1983; Neill and Stevens, 1974):

$$\frac{dT_{tissue}(t)}{dt} = k[T_a(t) - T_{tissue}(t)] + Hp\,, \quad (1)$$

where T_{tissue} is the tissue temperature of the fish (°C) at any time t (min), k is the thermal rate coefficient (°C min^{-1} °C^{-1} thermal gradient; min^{-1}), T_a is the ambient water temperature (°C) and Hp is the metabolic heat production (°C min^{-1}) (because mako swimming velocity was nearly constant in all tests, Hp is assumed to be equivalent to RM metabolic heat production). Integrating equation 1 and substituting T_e for T_a because T_{tissue} has a T_x and will not reach T_a (Brill et al., 1994; Neill et al., 1976) results in:

$$\frac{T_e - T_{tissue}(t)}{T_e - T_{tissue}(0)} = e^{-k(t-l)}\,, \quad (2)$$

where $T_{tissue}(0)$ is the value immediately following the latency period (i.e. when tissue temperature begins to change), $T_{tissue}(t)$ is the tissue temperature t min after the initial time, T_e is the equilibrium temperature (Neill et al., 1976) and l is the latency time (min) for the change in T_{tissue} following the change in T_a (Brill et al., 1994). (Note: our model assumed that there was no change in the heat stored within the RM and that Hp is constant, and this term can be eliminated from equation 2.)

Transformation and rearrangement of equation 2 enables the instantaneous thermal rate coefficient, k, for any given time to be determined:

$$k = -\frac{1}{t-l}\ln\frac{T_e - T_{tissue}(t)}{T_e - T_{tissue}(0)}\,. \quad (3)$$

Rearranging this expression into a linear equation yields:

$$\ln|T_e - T_{tissue}(t)| = \ln|T_e - T_{tissue}(0)| - k(t-l)\,, \quad (4)$$

and the least-squares linear regression of $\ln|T_e\text{-}T_{tissue}(t)|$ against time t (after the removal of the latency period) yields a function with a slope of $-k$ (Fechhelm and Neill, 1982; Brill et al., 1994). The use of a general exponential decay model results in negative values of k; however, we have reported only absolute values of k to simplify the comparisons of the rate constants between the thermal phases and among the different sharks. In addition, it is possible to use the reciprocal of the rate constant (k^{-1}) to calculate the time constant, which is the time required for T_{tissue} to reach e^{-1} (63 %) of the value of the new T_e (Riggs, 1963).

Determination of T_e

Because lamnid sharks are endothermic, a square-wave change in T_a could be expected to result in a new T_e (T_e=T_a+T_x). In these studies, the duration of the lowered T_a during the cooling phases was sufficiently long to allow mako T_{RM} to reach T_e. However, because our experimental objective was to determine whether makos could make rapid changes in thermal conductivity similar to those described for yellowfin tuna (Dewar et al., 1994), we used warming phases that were too short to allow T_{RM} to reach T_e. Therefore, the estimation of T_e for the warming phases was achieved by finding the value at which T_e resulted in the best fit of equation 4, as defined by a minimum residual means square (RMS) error.

Plasma [lactate]

Plasma lactate levels were measured using the Sigma (735-10) lactate kit.

Results

Recordings of tissue temperature in relation to T_a were obtained for five steadily swimming makos that swam slowly (30–60 cm s^{-1}, 0.3–0.6 L s^{-1}, where L is body length) with tailbeat frequencies ranging from 0.9 to 1.1 Hz. Body size did not correlate with either swimming speed or tailbeat frequency, and none of the makos exhibited a capacity for sustained swimming over a range of velocities. Fig. 2 and Fig. 3 show the T_{RM}, T_{WM} and $T_{STOMACH}$ data for these five sharks, and Tables 1–3 summarize the estimated thermal rate coefficients during the three thermal cycles.

Changes in T_{RM}

Fig. 3 shows how T_{RM} varied during the initial post-anesthesia recovery period in response to the 15 °C exposure period and as a result of the three thermal cycles. During the recovery period of 120–200 min, mako T_{RM} was 2–3 °C above T_a and this difference was maintained throughout the 15 °C exposure period.

Analyses of the effects of the three thermal cycles on T_{RM} focus on k values, on latency times and on the time for RM to reach T_e during the cooling phase. No significant difference [non-parametric, single-factor analysis of variance (Sokal and Rohlf, 1998)] exists among the k values for the different cooling and warming phases (Table 1). However, the overall k values during cooling are significantly lower than those during warming. No significant difference was found between the latency times for cooling (1.5–20 min) and warming (1–10 min) (Table 1), and latency was not affected by T_a.

The times to reach RM T_e during the cooling phase were up to 100 min in cycle 1, 75 min in cycle 2 and 90 min in cycle 3,

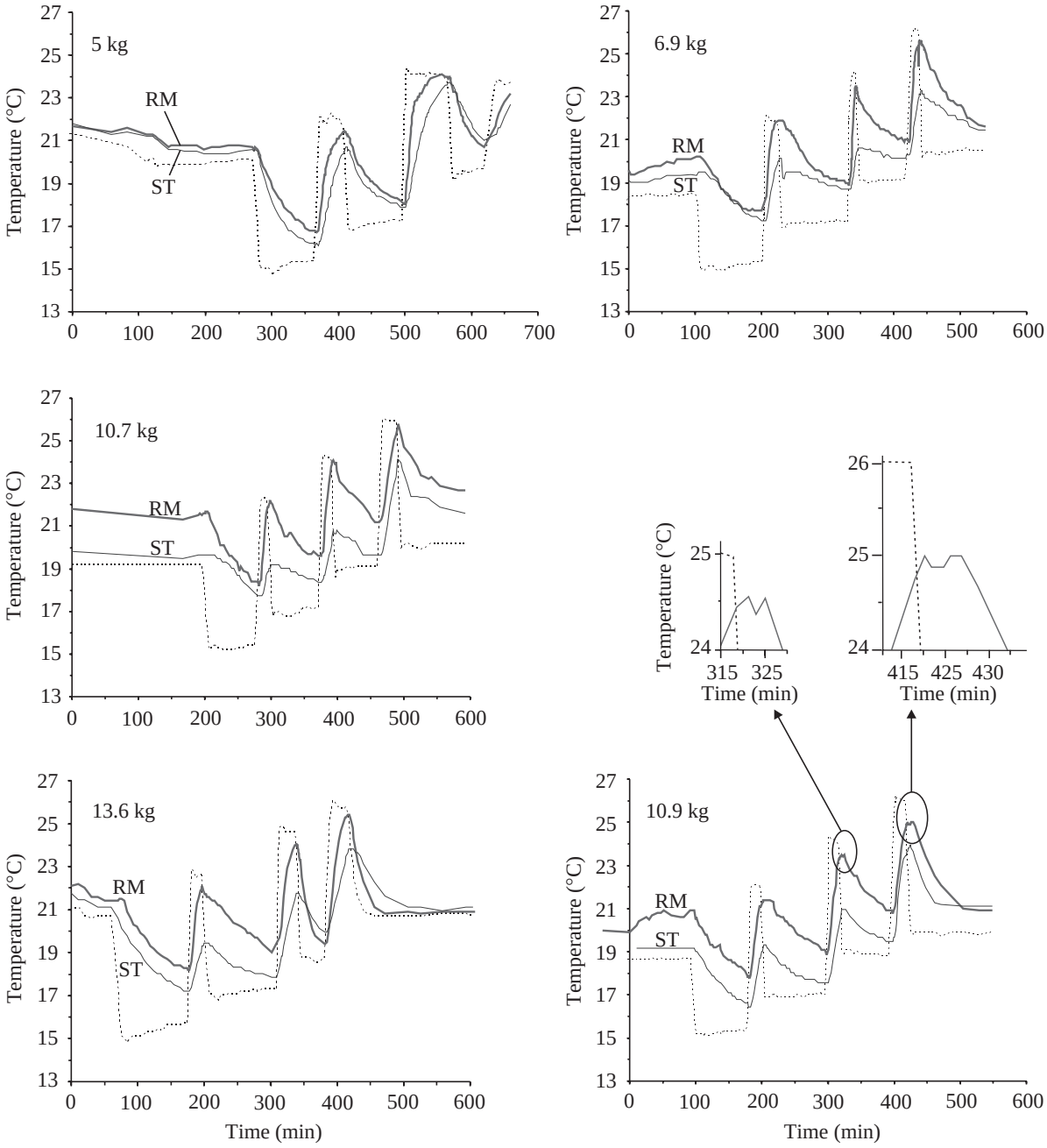

Fig. 3. Temperature changes in the red muscle (RM; bold tracing) and stomach (ST; light tracing) of five mako sharks. The dotted line represents the ambient water temperature. Insets for the 10.9 kg mako recording show RM temperature detail and the possible thermal notch during the onset of cooling cycles 2 (left-hand inset) and 3 (right-hand inset) (see Discussion).

with T_x values between 1 and 3 °C (Fig. 3). The mean time constant (k^{-1}) for cooling (27.1±2.8 min, mean ± S.E.M., N=14) was significantly higher than that for warming (9.1±0.6 min, mean ± S.E.M., N=14). However, for the 13.6 kg mako, the times to reach T_e were markedly shorter in cycles 2 (45 min) and 3 (55 min) (Fig. 3). Relative to the cooling phases, the durations of the warming phases were shorter by a factor of approximately 10 (range 10–16 min), with the exception of the 5 kg individual, which took 35 min in cycle 1 and 40 min in cycle 2 (Fig. 3).

Changes in $T_{STOMACH}$

Fig. 3 also shows the $T_{STOMACH}$ data. During the 120–200 min recovery period, mako $T_{STOMACH}$ was 1–3 °C above T_a, and this difference was maintained throughout the 15 °C exposure period. The k values for the stomach were similar to those calculated for RM during cooling. In contrast to RM, however, no difference was detected for the warming and cooling k values (Table 2). Latency times for $T_{STOMACH}$ (cooling, 5–27 min; warming, 5–13 min) were similar to those observed for RM.

Table 1. *Rates of heat gain and loss determined for the red muscle of swimming mako sharks during three thermal cycles*

			Red muscle: cooling phase ($T_{RM}>T_a$)										Red muscle: warming phase ($T_{RM}<T_a$)									
			Cycle 1				Cycle 2				Cycle 3		Cycle 1				Cycle 2				Cycle 3	
			ΔT_a 22→17 °C				ΔT_a 24→19 °C				ΔT_a 26→21 °C		ΔT_a 15→22 °C				ΔT_a 17→24 °C				ΔT_a 19→26 °C	
Mass	*L*	Swimming velocity	*k*	r^2	k^{-1}	*l*	*k*	r^2	k^{-1}	*l*	*k*	r^2	k^{-1}	*l*	*k*	r^2	k^{-1}	*l*	*k*	r^2	k^{-1}	*l*
(kg)	(cm)	($L\,s^{-1}$)	(min^{-1})		(min)	(min)	(min^{-1})		(min)	(min)	(min^{-1})		(min)	(min)	(min^{-1})		(min)	(min)	(min^{-1})		(min)	(min)
4.99	87	0.5–0.6	0.044	0.97	23	6	0.044	0.98	23	12	–	–	–	0.099	0.99	10	7	0.100	0.97	10	4	–	–	–
6.88	89	0.4–0.5	0.034	0.98	29	12	0.038	0.99	26	1.5	0.033	0.99	30	5	0.209	1.00	5	5	0.120	0.98	8	3.5	0.175	0.98	6	6
10.66	101	0.4	0.036	0.91	28	6	0.032	0.94	31	6	0.040	0.96	25	8	0.185	0.99	5	10	0.096	0.99	10	5	0.084	0.99	12	5
10.89	101	0.3–0.4	0.032	0.97	31	20	0.035	0.98	29	14	0.030	1.00	33	12	0.095	0.99	11	5	0.130	0.97	8	4	0.093	0.98	11	8
13.62	121	0.5	0.019	0.98	53	7	0.096	0.99	10	4.5	0.109	0.95	9	7	0.114	0.98	9	7	0.091	0.99	11	1	0.091	1.00	11	4
Mean ± S.E.M.			0.033±0.004				0.049±0.010				0.053±0.020				0.140±0.020				0.107±0.007				0.111±0.020			

k, thermal rate coefficient; T_a, ambient temperature; T_{RM}, red muscle temperature; *L*, total body length.
Equation 4 was used to calculate *k* values (see Materials and methods for details).
Also shown are the time constants (k^{-1}, the time required for T_{RM} to reach approximately 63 % of the value at thermal equilibrium) and latency times (*l*) for each thermal change phase.

The estimated times for the stomach to reach T_e (T_x=1–2 °C) during cooling are as follows: cycle 1, 70–100 min; cycle 2, 65 min; cycle 3, 95 min. As was the case for RM, the experimental protocol precluded measurement of T_e during warming. Only in the 5 kg mako did $T_{STOMACH}$ increase to a value similar to that of RM (Fig. 3).

Changes in T_{WM}

Comparison of the WM temperature recording for the 6.9 kg mako (Fig. 2) with its RM data (Fig. 3) indicates similar warming and cooling patterns for both tissues and, for this reason, the WM data for the other sharks are not illustrated. The rates of heat gain and heat loss for the WM of all makos are shown in Table 3. The rates of WM heat loss during the cooling phases did not differ from the RM *k* values; however, the rates of WM heat gain during the warming phases were significantly lower than those of the RM (paired *t*-test) (Sokal and Rohlf, 1998).

Control fish studies

No tissue differences were observed for the rates of heat gain or loss in two control (dead) makos (5.0 and 10.7 kg), and only the T_{RM} values are shown in Fig. 4. Table 4 presents the combined mean *k* values for the two specimens. Rates of heat gain and loss for the live 10.7 kg mako differ markedly from the control data (Fig. 4); cooling *k* values for RM in the dead

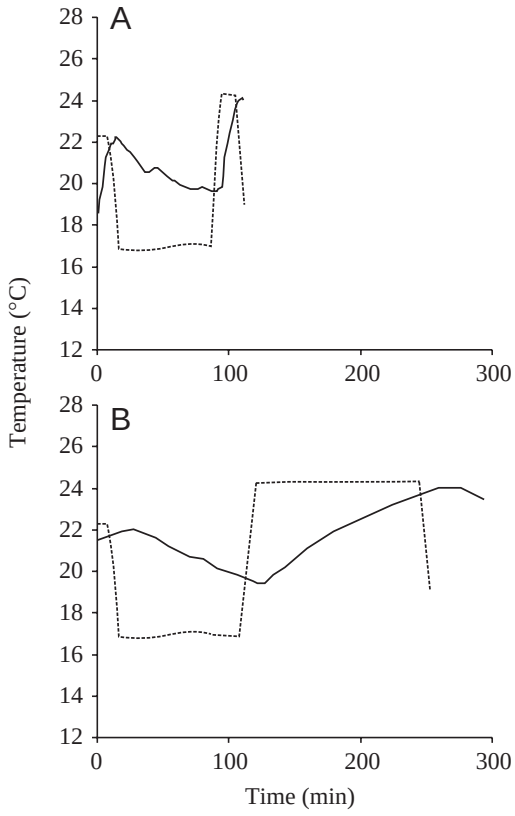

Fig. 4. Comparison of the red muscle cooling and warming rates of a 10.7 kg swimming mako shark (A) and of its *post-mortem* body (B). The dashed line represents ambient water temperature and the solid line is red muscle temperature.

Table 2. *Rates of heat gain and loss determined for the stomach of swimming mako sharks during three thermal cycles*

			Stomach: cooling phase						Stomach: warming phase					
			Cycle 1		Cycle 2		Cycle 3		Cycle 1		Cycle 2		Cycle 3	
Mass (kg)	L (cm)	Swimming velocity ($L\,s^{-1}$)	ΔT_a 22→17 °C k (min^{-1})	r^2	ΔT_a 24→19 °C k (min^{-1})	r^2	ΔT_a 26→21 °C k (min^{-1})	r^2	ΔT_a 15→22 °C k (min^{-1})	r^2	ΔT_a 17→24 °C k (min^{-1})	r^2	ΔT_a 19→26 °C k (min^{-1})	r^2
4.99	87	0.5–0.6	0.042	0.99	0.087	0.94	–	–	0.035	0.99	0.039	1.00	–	–
6.88	89	0.4–0.5	0.033	0.93	0.024	0.87	0.025	0.96	0.059	1.00	0.045	0.99	0.051	1.00
10.66	101	0.4	0.040	0.94	0.020	0.93	0.033	0.96	0.030	0.98	0.029	0.99	0.043	1.00
10.89	101	0.3–0.4	0.037	0.97	0.041	0.97	0.044	0.98	0.044	1.00	0.035	0.99	0.062	0.98
13.62	121	0.5	0.031	0.97	0.067	0.94	0.037	0.97	0.037	0.98	0.031	1.00	0.032	0.98
Mean ± S.E.M.			0.036±0.002		0.048±0.013		0.045±0.016		0.040±0.005		0.035±0.003		0.047±0.006	

k, thermal rate coefficient; T_a, ambient temperature; L, total body length.
Equation 4 was used to calculate k values (see Materials and methods for details).

Table 3. *Rates of heat gain and loss determined for the white muscle of swimming mako sharks during three thermal cycles*

			White muscle: cooling phase						White muscle: warming phase					
			Cycle 1		Cycle 2		Cycle 3		Cycle 1		Cycle 2		Cycle 3	
Mass (kg)	L (cm)	Swimming velocity ($L\,s^{-1}$)	ΔT_a 22→17 °C k (min^{-1})	r^2	ΔT_a 24→19 °C k (min^{-1})	r^2	ΔT_a 26→21 °C k (min^{-1})	r^2	ΔT_a 15→22 °C k (min^{-1})	r^2	ΔT_a 17→24 °C k (min^{-1})	r^2	ΔT_a 19→26 °C k (min^{-1})	r^2
4.99	87	0.5–0.6	0.042	0.99	0.055	0.95	–	–	0.042	1.00	0.046	0.99	–	–
6.88	89	0.4–0.5	0.037	0.97	0.081	0.91	0.034	0.94	0.131	0.99	0.121	0.88	0.108	0.98
10.66	101	0.4	0.046	0.96	0.061	0.97	0.047	0.97	0.072	0.98	0.080	0.97	0.060	0.97
10.89	101	0.3–0.4	0.037	0.99	0.036	0.98	0.026	1.00	0.051	0.98	0.065	0.98	0.050	0.98
13.62	121	0.5	0.027	0.97	0.074	0.97	0.043	0.98	0.059	0.98	0.056	0.99	0.063	0.98
Mean ± S.E.M.			0.038±0.003		0.062±0.008		0.038±0.004		0.071±0.016		0.074±0.013		0.070±0.012	

k, thermal rate coefficient; T_a, ambient temperature; L, total body length.
Equation 4 was used to calculate k values (see Materials and methods for details).

makos are one third of than for the live sharks, which also warmed 10 times faster than the dead sharks.

Red muscle temperature measurements

Fig. 5 shows the relationship between T_a and the stable T_{RM} values (defined operationally as the estimated T_e) determined for the five swimming makos. The regression equation for this line yields a slope that is significantly different from 1.0 (P<0.01), and this line intercepts the line for T_{RM}=T_a at approximately 26 °C. Also shown in Fig. 5 is the T_a/T_{RM} regression determined by Carey et al. (1985) for decked makos (5–200 kg, T_a=12–27.5 °C) in which T_{RM} was elevated by up to 11 °C above T_a. Comparison of these regression equations indicates that the small makos in our study had a significantly lower intercept (6±2 °C, mean ± 95 % confidence interval, N=26) but that the slope (0.76±0.11, mean ± 95 % confidence interval, N=26) did not differ from that of the decked makos (Carey et al., 1985).

Discussion

Laboratory studies with lamnids

This study examined the capacity of steadily swimming juvenile mako sharks to regulate rates of heat gain and loss in their RM, WM and stomach during a series of controlled, square-wave changes in T_a. Lamnid sharks are commonly classified as ‘high-performance’ fishes, but little is known about their physiological performance during aerobically powered sustained swimming. Investigations of lamnids are important because of this group’s convergence with tunas for specializations that increase metabolic performance, including endothermy (Bernal et al., 2001), and because they are considered to be the most active swimming sharks. However, because lamnids are rarely encountered, usually occur in remote locations and are of large size, most studies of their capacity for endothermy have been restricted to decked fish or specimens to which telemetry monitors could be attached (Block and Carey, 1985; Carey et al., 1978, 1982, 1985; Carey

Table 4. *Rates of heat flux in the locomotor muscle of mako shark, white shark, blue shark and three species of tuna*

	Experimental protocol	Tissue	Sample size	Number of thermal cycles	Mass (kg)	Range ΔT_a test (ºC)	Cooling k (min^{-1})		Range ΔT_a test (ºC)	Warming k (min^{-1})		Reference
							Range	Mean		Range	Mean	
Mako shark	Laboratory study in water tunnel, velocity controlled, hardwired	RM	5	3	5–13	Upper: 26→21 Lower: 22→17	0.019–0.109	0.044	Upper: 19→26 Lower: 15→22	0.084–0.209	0.120	This study
Mako shark	Laboratory study in water tunnel, dead fish, hardwired	RM	2	3	5–10.6	Upper: 26→21 Lower: 22→17	0.010–0.015	0.013	Upper: 19→26 Lower: 15→22	0.009–0.010	0.010	This study
White shark	Field study, velocity uncontrolled, free-swimming telemetry	Deep WM	1	1	≈940*				14.7→16.7		0.004	Carey et al. (1982)
Blue shark	Field study, velocity uncontrolled, free-swimming telemetry	Deep WM	1	5	≈99*	26.5→8	0.003–0.006	0.005	8→26.5	0.015–0.028	0.020	Carey and Scharold (1990)
Yellowfin tuna	Laboratory study in water tunnel, velocity controlled, hardwired	RM	9	3	1–2	Upper: 32→27 Lower: 26→21	0.015–0.099†	0.050†	Upper: 25→32 Lower: 19→26	0.029–0.156	0.094	Dewar et al. (1994)
Bigeye tuna	Field study, velocity uncontrolled, free-swimming telemetry	RM	2	9	7–12	Upper: 26→12 Lower: 22→11	0.0002–0.0005	0.00035	Upper: 14→26 Lower: 12→22	0.04–0.37	0.205	Holland et al. (1992); Holland and Sibert (1994)
Skipjack tuna	Laboratory study, velocity uncontrolled, hardwired	RM	2	1	1.2–1.8	23.5→18.3	0.023–0.044	0.033	19→24	0.028–0.067	0.047	Neill et al. (1976)

*Derived from the fork length *versus* body mass relationship of Kohler et al. (1994).
Fork lengths: white shark 428 cm (460 cm total length); blue shark 247 cm.
No data available to estimate heat loss during cooling in the white shark.
†Only the first 2 cooling phases were used.
T_a, ambient temperature; k, thermal temperature coefficient; RM, red muscle; WM, white muscle.

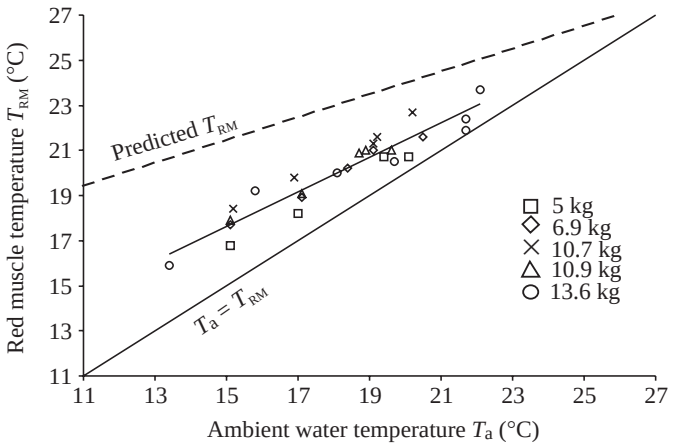

Fig. 5. Relationship between ambient water temperature (T_a) and red muscle temperature (T_{RM}) determined for mako sharks weighing 5–13.6 kg and swimming steadily at 0.3–0.6 $L\,s^{-1}$: T_{RM}=6.18+(0.76±0.11)T_a (mean ± 95 % confidence interval; r^2=0.90, N=26). The dashed line represents the T_a/T_{RM} regression determined for decked mako sharks: T_{RM}=13.8+(0.513±0.22)T_a [mean ± 95 % confidence interval; r^2=0.36, N=38) (Carey et al., 1985)].

and Teal, 1969; Goldman, 1997; Lowe and Goldman, 2001; McCosker, 1987; Rhodes and Smith, 1983; Tricas and McCosker, 1984).

Although these approaches have yielded important findings about lamnid body temperature, the experimental design used in this study enabled the direct testing of mako heat balance under controlled swimming velocity and T_a. This study and previous work at sea (Graham et al., 1990) demonstrate the feasibility of conducting long-term water-tunnel studies on juvenile makos and, thus, open the possibility of additional laboratory research with this species. Even though we had success in keeping makos alive in a large laboratory tank for up to 4 days, lamnids do not survive in captivity for very long, so our studies were conducted on sharks immediately after capture and transport.

We recognize the potential limitations of confined water-tunnel studies on lamnid sharks. The existing experimental apparatus limits our work to juvenile makos and restricts the shark's capacity to select swimming speed and depth (temperature), both behavioral components of thermal balance that affect RM heat production (Hp_{RM}) during descents and ascents (Brill et al., 1994). Nonetheless, this is the only method currently available in which both T_a and swimming velocity can be controlled. Future studies of heat balance in free-swimming makos using physiological telemetry are needed to validate our laboratory findings and to test hypotheses about the capacity for physiological thermoregulation in larger makos.

T*STOMACH*

Although the makos that we studied had elevated stomach temperatures, we found that $T_{STOMACH}$ varied directly with T_a. This was unexpected because telemetry studies indicate that $T_{STOMACH}$ in mako and white sharks is fairly constant (Carey et al., 1981; McCosker, 1987) and, in some cases, independent of T_a (Goldman, 1997). Although the makos we studied had food in their stomachs, capture and handling stress may have adversely affected digestion and assimilation, the major physiological sources of endogenous heat production (Bernal et al., 2001; Carey et al., 1981, 1984; Goldman, 1997; Stevens and McLeese, 1984). It is also possible that thermal balance was impaired in fishes swimming in the water tunnel by their inability to control either swimming velocity or T_a behaviorally. Another important factor probably affecting this comparison is the marked size differences between the juvenile makos in our study (5–13.6 kg) and the estimated size range of the telemetered lamnids (160–2000 kg), which raises the possibility that the capacity to elevate and control $T_{STOMACH}$ is not fully developed in small makos and is dependent upon a large body size (i.e. thermal inertia effect) or both (Carey et al., 1981, 1985; Goldman, 1997; Neill and Stevens, 1974).

T*a and* T*RM*

The lower RM T_x values obtained for swimming makos in this study relative to the T_{RM} data reported for decked makos by Carey et al. (1985) (Fig. 5) may be attributable to several factors. As stated above, it is possible that T_x was affected by handling stress and by not allowing the shark to select swimming speed and T_a. Several studies have noted that individuals that appeared to be more stressed had a lower T_x (Carey et al., 1981, 1985; Goldman, 1997). In contrast, the makos used in our study appeared to be in good physical condition. This was determined from a combination of steady swimming throughout the experimental protocol, the finding that the T_{RM} of each shark increased and reached T_e during the post-anesthesia recovery period and the measurement of post-experiment plasma lactate concentrations lower than 3 mmol l^{-1} [lactate values for highly stressed captured makos generally exceed 16 mmol l^{-1} (Wells et al., 1986) (D. Bernal, unpublished observations)]. Thus, it appears that another factor contributed to the T_x differences shown in Fig. 5.

Carey et al. (1985) did not report the body size of the makos they studied, but morphological data in their paper indicate that these were much larger (up to 200 kg) than those in our study. Assuming this to be the case, the differences shown in Fig. 5 suggest a lower endothermic capacity in smaller makos and, with the $T_{STOMACH}$ data above, indicate an ontogenetic component for heat balance. Although comparative data for tuna indicate the general absence of a mass effect on RM T_x (Linthicum and Carey, 1972), the capacity for regional endothermy does vary ontogenetically (Dickson, 1994; Dickson et al., 2000; Graham and Dickson, 2001).

Red muscle heat flux

Several variables may affect the RM k values determined for a fish during thermal cycle studies, and these need to be evaluated prior to concluding whether any observed changes in k reflect a physiological control mechanism or are the results

of other indirect thermal effects on physiology (Brill et al., 1994; Graham, 1983; Stevens and Neill, 1978).

A general problem in thermal cycle studies is how *k* might be affected by the instantaneous change in T_x caused by the abrupt shift in T_a (Neill et al., 1976; Neill and Stevens, 1974). For example, even when physiological effects are not considered, *k* is likely to be affected more by a large than by a small change in T_a (Fechhelm and Neill, 1982; Neill and Stevens, 1974). This complication was minimized in our studies by using the same temperature-change protocol on all makos and by exposing each mako to the same series of thermal cycles that, although similar in their magnitude of T_a change, shifted the thermal range by 2 °C. Also, estimates of *k* for control (dead) makos in the same thermal cycles showed that, while the effects of T_x were markedly different from those in live sharks (Fig. 4), *k* was largely unaffected by either the magnitude or direction of T_a change.

The two major physiological processes affecting heat balance that would also be affected by thermal cycling are Hp_{RM} and cardiovascular functions (i.e. cardiac output and vascular resistance) (Brill et al., 1994; Graham and Dickson, 2001). Because swimming speed was controlled, the effects of Hp_{RM} on *k* were not a factor in our studies.

There are no data for thermal effects on mako cardiovascular function but, because the temperature of the heart is equal to that of its surroundings, from first principles (Schmidt-Nielsen, 1993) cooling would be expected to reduce heart rate and lower cardiac output (the product of heart rate and stroke volume) and this, combined with the greater viscosity of cool blood, would slow blood flow, thus decreasing *k* (Brill et al., 1994; Graham, 1975; Graham and Dickson, 2001; Holland and Sibert, 1994). The opposite effect of warming on these same parameters would be expected to increase *k*. In addition, because of the general exponential effect of temperature on functions such as heart rate (Schmidt-Nielsen, 1993), during both cooling and warming, the magnitude of *k* would be expected to vary with T_a.

The present study shows that the combined mean value of *k* for mako RM during warming is significantly greater, by a factor of 2.7, than that for RM cooling and, thus, conforms to the expectations described above. However, if the observed changes in *k* resulted solely from alterations in cardiovascular performance (i.e. heart rate and stroke volume), then a trend towards lower *k* values at lower T_a values and higher *k* values at higher T_a values should have been found. Instead, the data indicate no effect of the magnitude of T_a on *k*, either during heating or during cooling, suggesting that the observed *k* values may result from modulation of heat flux by the sharks and are thus not solely the consequence of thermal effects on the cardiovascular system. Future studies featuring simultaneous recordings of T_{RM}, heart rate, stroke volume and retial blood flow are needed to test this.

Comparison of T_{RM} *control mechanisms in tuna, mako and other sharks*

The makos in the present study demonstrated some capacity to modulate heat transfer and, in a manner similar to tunas (Holland and Sibert, 1994), to switch from a mode that 'minimized *k* in order to defend RM and other tissue temperatures in cool water' to a mode that 'elevated *k* in order to rapidly restore tissue temperature once the fish returned to warmer (i.e. $T_a > T_{RM}$) water'. The objective of this section is to compare the apparent heat-balance capacity of the mako with that of tunas and to compare their rates of heat gain and loss with those of the white shark and the blue shark (*Prionace glauca*).

The estimated *k* values for yellowfin and bigeye tunas during cooling and warming (Table 4) are consistent with changes in heat flux attributable to alterations in heat-exchanger efficiency (i.e. maximum efficiency during cooling results in a low *k* and minimum efficiency during warming results in a high *k*) (Brill et al., 1994; Dewar et al., 1994; Holland et al., 1992; Holland and Sibert, 1994) and are similar to our results from makos. As in the case of makos, the heat-transfer data for yellowfin tuna were obtained using a water tunnel (Dewar et al., 1994). Because the fish were swimming at a constant velocity, the magnitude of thermal-cycle-induced changes in *k* could not be accounted for either by Hp_{RM} or by thermal inertia (Neill and Stevens, 1974), suggesting modulation of retial efficiency (Brill et al., 1994; Dewar et al., 1994). Telemetry data for bigeye tuna demonstrated a similar mechanism (Holland and Sibert, 1994), but the estimated *k* values differed from those for makos by nearly two orders of magnitude (Table 4). Both species-specific differences in retial structure and control and the behavioral options for controlling swimming velocity (Hp_{RM}) that can be used by free-swimming tunas may account for these differences.

Although our data show similar *k* values for the mako and yellowfin (Table 4), we were unable to demonstrate other 'fine-scale' T_{RM} modulation mechanisms seen in yellowfin. Specifically, a thermal notch [an abrupt reduction and then rise in T_{RM} followed by a more gradual decline during the onset of the cooling phase (Dewar et al., 1994)] was not observed in the makos. [The only suggestion of a thermal notch is seen in the second and third cooling phases of the 10.9 kg mako (Fig. 3); however, the magnitude of the notch (0.2 °C) is less than that measured in yellowfin (0.5 °C).] We were also unable to observe more rapid cooling rates or reduced latency times in makos exposed to warmer ambient temperatures, and swimming speeds could not be varied sufficiently to determine how Hp_{RM} might potentially have affected T_{RM} and heat loss rates.

What factors might account for the absence of the fine-scale heat balance in makos, attributes found in water-tunnel studies with yellowfin tuna? First, the capacity of any fish to maintain a T_x requires very high heat-exchanger efficiency (Brill et al., 1994; Carey et al., 1981; Dizon and Brill, 1979a; Graham, 1983; Stevens and Neill, 1978). Retia in both tunas and lamnids are similar in having smooth muscle layers in the vessels (Carey et al., 1985; Dickson, 1988; Graham and Dickson, 2001; Stevens and Neill, 1978) and the potential for nervous system control of blood flow (Brill et al., 1994; Dizon

and Brill, 1979a). A principal difference in the retial blood flow pattern of tunas and mako relating to heat flux is the presence, in most tunas, of two prominent routes for blood flow into the RM compared with one in makos (Bernal et al., 2001). Another tuna/mako difference important for heat balance is the larger relative RM mass in tunas (Bernal et al., 2001). Unlike the yellowfin, which had higher heat-loss rates in warmer water (i.e. highest heat loss rates were observed in fish warmed to 32 °C), makos did not demonstrate higher cooling *k* values following exposure to the warmest conditions in our studies (26–21 °C). Two possible reasons for this were that we did not warm the makos sufficiently to elicit a rapid heat loss and the absence of the same degree of control over heat-exchanger efficiency.

A paucity of data precludes comparison of our findings for the thermoregulatory capacity of juvenile makos with data for other shark species. The only comparative data available are telemetered T_{WM} recordings obtained for a single blue shark and a single great white shark (Carey and Scharold, 1990; Carey et al., 1982). Both these fish are much larger than the makos we studied (makos, 5–13.6 kg; blue shark, approximately 99 kg; white shark, approximately 940 kg), and neither swimming velocity nor T_{RM} was recorded. We have, nevertheless, estimated the thermal rate coefficients for these two fish by digitizing the T_a and T_{WM} data and incorporating them into equation 4.

Table 4 compares the heat-flux estimates for blue and white shark deep WM with those for mako RM. In the case of the blue shark, the warming and cooling *k* values are similar to that of the control (dead) mako RM but lower than that of swimming makos. Blue sharks are not endothermic, and their RM is not positioned deep within the body. Thus, even though the T_{WM} recordings obtained by Carey and Scharold (1990) were from deep within the blue shark's body, the *k* estimates are consistent with the relatively low perfusion rate of this tissue compared with RM. This suggests that heat retention in the deep WM of the blue shark is the result of a low thermal conductance (i.e. thermal inertia) (Neill et al., 1976).

White sharks are endothermic and have a RM position similar to that of makos. Thus, the deep T_{WM} telemetry data obtained by Carey et al. (1982) were probably from a region in close proximity to the warm RM of this fish. The data set of Carey et al. (1982) was used to estimate the rate of deep WM heat gain during an abrupt 2 °C increase in T_a (from 14.7 to 16.7 °C) (Carey et al., 1982). Table 4 shows that this small increase in T_a resulted in a much lower rate of heat gain than was observed in both the RM and WM of swimming and control makos. This difference is attributable to the small (2 °C) magnitude of the change in T_a and the vastly larger size of the white shark (100 times larger than our makos).

Future understanding of the control of heat balance in pelagic sharks will depend upon telemetry studies that utilize developing technology (Lowe and Goldman, 2001) to monitor T_a, T_{RM}, T_{WM}, $T_{STOMACH}$ and swimming speed of free-swimming juvenile mako sharks, other larger lamnids and other sharks.

In summary, juvenile mako sharks appear to be functionally similar to yellowfin tuna in their capacity to alter RM heat balance in response to changes in T_a, presumably by the mechanism of regulating retial heat-exchange efficiency. However, both T_{RM} and $T_{STOMACH}$ recorded for juvenile makos are lower than those recorded for larger makos (Carey et al., 1985), and we found no evidence indicating that juvenile makos could regulate $T_{STOMACH}$ with the same precision as large makos (Carey et al., 1981) and white sharks (Goldman, 1997). These differences suggest that a large body size in conjunction with the ability to alter retial efficiency may be essential for the maintenance of warmer T_{RM} and $T_{STOMACH}$ values.

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