Long-Term Cold Acclimation Leads to High Q_{10} Effects on Oxygen Consumption of Loggerhead Sea Turtles *Caretta caretta*

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ABSTRACT

We monitored oxygen consumption ($\dot{V}O_2$), body temperatures (T_b), submersion intervals, and circadian rhythms of $\dot{V}O_2$ in nine loggerhead turtles during a 6-mo period. The turtles originated from the Tyrhennian Sea, South Italy (40°51′N, 14°17′E) and were kept in indoor tanks at constant photoperiod while being subject to the seasonal decline in water temperature (T_w = 27.1° to 15.3°C). From summer to winter, all turtles underwent profound reductions in $\dot{V}O_2$ (Q_{10} = 5.4). Simultaneously, their activity was greatly reduced and submergence intervals increased. Over 24-h periods, however, the turtles showed no circadian rhythm in activity or $\dot{V}O_2$. However, there was a significant positive correlation between the proportion of a day spent actively swimming and $\dot{V}O_2$. T_b's were not significantly different from T_w and followed the same seasonal decline. A second experiment was conducted to establish the effect of short-term exposure to various temperatures on $\dot{V}O_2$. T_b equilibrated with the experimental T_w within 3 h. The metabolic responses were again positively correlated with changes in T_w , but this time the corresponding Q_{10} was only 1.3. On the basis of the range of body masses of the turtles used in this study (2–60 kg), the intraspecific scaling exponent for $\dot{V}O_2$ was 0.353.

Introduction

Temperature is one of the most important factors influencing physiological processes. The physiology and behaviour of ectothermic animals, which are not able to maintain a stable high body temperature (T_b) by internal heat production, are predominantly affected by fluctuations in the ambient temperature. Such temperature variations can be either temporary, including differences in microclimates between different locations or changes in solar radiation, or they act over longer timescales due to seasonality or long-lasting displacements. The physiological and behavioural responses of ectotherms to varying environmental temperatures have been studied extensively and are reported in a number of reviews and basic literature on thermal biology (e.g., see Scholander et al. 1953; Huey and Stevenson 1979; Hutchison and Maness 1979; Avery 1982; Cossins and Bowler 1987).

In the sea, daily temperature fluctuations are buffered due to the large specific heat capacity of water, and an organism may stay within a very narrow temperature range unless it travels long distances or into deep waters below the thermocline. In such situations, because of the high thermal conductivity of water, an animal may lose heat quickly when it moves into colder water. To avoid cooling down, bluefin tuna, *Thunnus thynnus*, for example, which repeatedly dive through the thermocline in the search for food, have developed internal heat retention mechanisms to maintain a high muscle temperature (Stevens et al. 2000; Kitagawa et al. 2001). Likewise, great white sharks, *Carcharodon carcharias*, maintain T_b's up to 14°C above the water temperature (T_w), which allows them to hunt agile prey in cold waters (Goldman 1997). It is not only large predatory fish that have thermoconservation capabilities; leatherback turtles, *Dermochelys coriacea*, also penetrate into cold water both at depth and during long-distance movements and may maintain an internal T_b up to 18°C above the T_w (Frair et al. 1972).

The northern and southern distribution of the other species of sea turtles, however, seems to be limited by the annual average 20°C isotherm (Márquez 1990). Water temperatures below 8°C can lead to cold-stunning in sea turtles and eventually to death, so they probably have higher "lower lethal" temperatures (Witherington and Ehrhardt 1989; Morreale et al. 1992; George 1997). The upper lethal temperature is assumed to be near 40°C (Spotila et al. 1997). Ambient temperature regimes differ among sea turtle populations and species. Thus, turtles residing in habitats with seasonality are more exposed to temperature changes than those staying in warm tropical waters. For example, off Cape Hatteras (35°30′N, 75°30′W), Southeastern United States, loggerhead turtles tend to aggregate in waters around 11°C and above during the winter, taking ad-

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Table 1: Change in body mass (M_b [kg]) between September 2000 and February 2001 of the nine loggerhead turtles used in this study

Turtle	Arrival Date	September	October	November	December	January	February
1	March 2, 2000	2.0	…	2.5	2.8	2.8	2.85
2	January 31, 1998	4.55	6.0	6.75	7.5	7.5	7.45
3	January 31, 1998	4.55	5.8	6.15	6.7	6.7	6.7
4	June 30, 2000	14.5	15.7	16.0	16.5	16.5	16.7
5	March 2, 2000	16.8	17.2	17.2	17	16.7	15.8
6	March 2, 2000	21	22.5	22.5	22.5	22.5	22.5
7	July 1, 2000	52.8	53.2	52	51.8	50.2	50.2
8	August 21, 2000	49.0	49.2	50.0	50.5	50.5	50.5
9	July 1995	57.2	58.4	58.8	59.0	59.3	59.5
			Average Changes (%)				
1–3			29.7	14.5	10.7	0	.4
4–6			5.9	.6	.6	.6	−1.4
7–9			1.1	0	.3	−.9	.1

Note. Average decreases in body mass are indicated by negative values.

vantage of the warm Gulf Stream (Epperly et al. 1995). In Shark Bay (25°30′S, 113°30′E), Western Australia, however, green turtles, *Chelonia mydas*, were seen in the winter at much higher T_w's, around 18°C (Preen et al. 1997); whereas in Moreton Bay (27°30′S, 153°18′E), Eastern Australia, green turtles were also found in waters of 15°C during the winter (Read et al. 1996).

Migrations may occur when T_w's drop during the autumn and turtles seek waters with more favourable temperatures. In the Mediterranean, for example, data from satellite tracking of loggerhead turtles indicated that these animals left their feeding grounds in the Tyrrhennian Sea, South Italy, and moved toward the southwest when sea surface temperatures dropped below 20°C (Bentivegna 2002). Even if sea turtles migrate between summer and winter habitats, they are still, at least in the Mediterranean, confronted with an annual temperature range of more than 10°C. Moreover, they are "trapped" within the Mediterranean basin and cannot migrate to warmer regions should winter conditions become particularly severe.

Read et al. (1996) measured T_b's of juvenile green turtles that remain year round in Moreton Bay. These turtles show the same seasonal cycling in T_b as the surrounding seawater, and there was no difference between the temperatures of the turtles and the water. When turtles experience annual variation in T_b, their physiology may be affected. However, metabolic and other physiological responses to changing temperatures are extremely difficult to measure in free-ranging sea turtles, and there are only a few laboratory studies dealing with cold acclimation in sea turtles. Moon et al. (1997) reported decreased feeding and breathing activity in green and Kemp's ridley turtles, *Lepidochelys kempii*, whereas loggerhead turtles showed no change in breathing frequency with decreasing temperatures (Lutz et al. 1989). The work by Lutz et al. (1989) was the only study to characterise the effect of different T_w's on whole-body metabolism, showing that loggerhead turtles responded with a Q_{10} of 2.4 after an acclimation period of 2 d to temperatures between 10° and 30°C. Acute heating and cooling of air in yearling green turtles led to an average Q_{10} value of 2.7 between 15° and 30°C (Davenport et al. 1982).

We monitored body mass (M_b), T_b, food intake, activity, and oxygen consumption ($\dot{V}O_2$) of loggerhead turtles to answer three questions: (1) How does $\dot{V}O_2$ of nonacclimated loggerhead turtles differ from $\dot{V}O_2$ of turtles kept on a real-time seasonal change in T_w? (2) What is the allometric scaling of $\dot{V}O_2$ for loggerhead turtles? (3) Are there circadian rhythms in $\dot{V}O_2$ due to activity or feeding?

Material and Methods

Study Animals

Nine loggerhead turtles (numbered 1–9) housed in the aquarium of the Stazione Zoologica Anton Dohrn (Naples, Italy, 40°50′N, 14°15′E) were studied between September 2000 and February 2001. The turtles were kept in individual tanks with circulating seawater, which was pumped from a location 300 m offshore and 10 m deep in the Gulf of Naples. Consequently, the T_w in the tanks matched the T_w of the Gulf of Naples and underwent the same seasonal temperature variations. The light cycle was kept at 13L : 11D (0700–2000 hours). It is in this way that we intended to isolate the seasonal temperature effect from any photoperiod effect. Initial M_b of the turtles ranged from 2 to 57.2 kg. All turtles were weighed once a month to the nearest 50 g, so that changes in M_b over the experimental period were recorded (Table 1). Tank size varied according to size of the turtle, so that turtles weighing more than 10 kg (turtles 4–9) were kept in 1,000-L round tanks (diameter: 130 cm, height of water: 80 cm) and the three small turtles (turtles 1–3) were

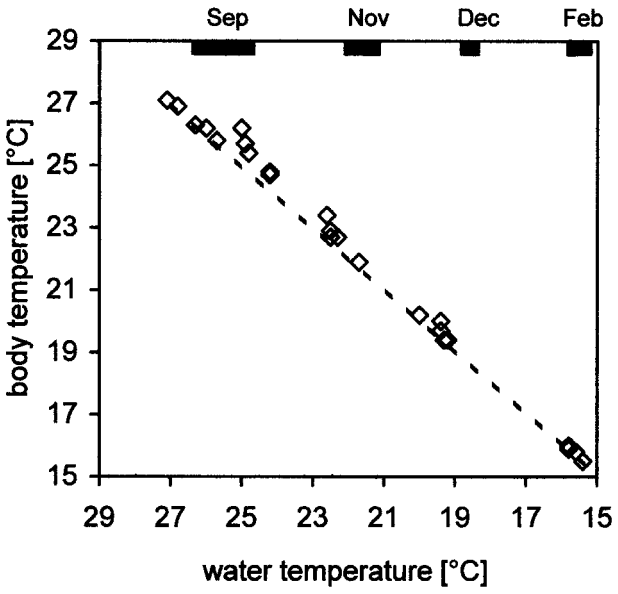

Figure 1. Body temperatures of loggerhead turtles with reference to the prevailing seawater temperature in various seasons. Black bars on top indicate periods during which metabolic rate was measured. Dashed line indicates a regression line with a slope of 1, that is, a slope demonstrating no difference between body temperature and water temperature.

kept in 200-L rectangular tanks. The turtles were fed with individually weighed portions of anchovies, *Engraulis encrasicholus*, 5 d wk^{-1} between 1000 and 1200 hours from September through November. As the T_w decreased toward December, the turtles fed less, and anchovies were provided only 3 d wk^{-1}. Remaining food items were collected from the tank and weighed to the nearest gram, so that the exact amount of ingested food could be recorded.

Respirometric Measurements

Oxygen consumption of individual turtles was measured using an open-flow respirometry system. Exhaled air of the turtle was collected from a hood that was placed over the water surface and had a volume of 4.6 L for turtles between 2 and 6 kg, 19.4 L for turtles between 7 and 30 kg, and 73 L for turtles between 31 and 60 kg. The average through-flow rate ranged from 2.22 to 4.3 L min^{-1} in September, applying the slowest flow to the smallest turtles (turtles 1–3), from 2.35 to 2.92 L min^{-1} in November and from 2.03 to 2.8 L min^{-1} in December; in February the average flow rate was 1.95 L min^{-1} for all turtles. Air samples were passed through silica gel to remove water vapour and then pumped into an Applied Electrochemistry N-37M oxygen sensor and analysed by an Applied Electrochemistry S-3A oxygen analyser (Sunnyvale, Calif.). Oxygen concentrations were recorded by a desktop computer and were averaged over 10-s intervals. The temperature of the air samples was measured, and all oxygen consumption data were STPD corrected.

The tank was covered with a Plexiglas lid into which a breathing hole (area = 0.2 and 0.05 m^2 for round tanks and rectangular tanks, respectively) was cut. The respirometry hood was placed over this hole, so that the turtles could breathe only under the hood. During initial trials with a transparent Plexiglas lid, the turtles had problems finding the breathing hole. Consequently an opaque foil was glued onto the lid, so that the turtles could easily distinguish between hole and covered area. Smaller holes of ca. 1-cm diameter were drilled into the Plexiglas lid with a distance of 10 cm between neighbouring holes. This served two purposes: first, the turtles could be observed from outside; second, this also allowed light to penetrate into the tank, so that the turtles were still subject to the alternating phases of light and darkness.

Seasonal Temperature Effect. Respirometry measurements were first made in September when the mean T_w was 25.4°C and were repeated in early November (mean T_w: 21.8°C), December (mean T_w: 19.4°C), and February (mean T_w: 15.7°C). Oxygen consumption of each turtle was recorded every 10 s for 22/24 h of a single day and night cycle. Usually, the machines were started at 0900 hours (local time) in the morning and run until 1900 hours. The night measurements started at 2000 hours and were continued until 0800 hours the following morning. During each morning and evening break, we established baseline levels of atmospheric O_2 to avoid excessive drift corrections and zeroed and spanned the analyser. In addition to this, the morning break served to finish the experiment with one turtle and start with the next individual.

Repeated measurements of T_w using a digital thermometer (Checktemp, Hanna Instruments) showed that the temperature varied by less than 0.2°C during each day. We therefore took the T_w during respirometry at 0800 hours and 2000 hours as the prevailing temperature for the corresponding $\dot{V}O_2$ of the sea turtles. Body temperatures of the turtles were taken rectally periodically throughout the 6-mo period using the same digital thermometer that was used to measure T_w.

Short-Term Temperature Effect. A series of transfer experiments were made in the second half of November 2000 during which each of the turtles was exposed to experimentally manipulated T_w of 15°, 25°, and 30°C (prevailing acclimation temperature was 19.5°C). Each turtle was transferred into the experimental tank once every 3 d, where it was kept for a mean ± SD period of 2 h 48 min ± 19 min. During this period, $\dot{V}O_2$ was measured using the respirometry equipment as described above. The experimental temperature was maintained by continuously pumping the water of the tank through a combined chiller/heater (model RA/CA 240-680, TECO, Ravenna, Italy).

Six turtles (those numbered 2, 4, 5, 6, 8, and 9) were fed

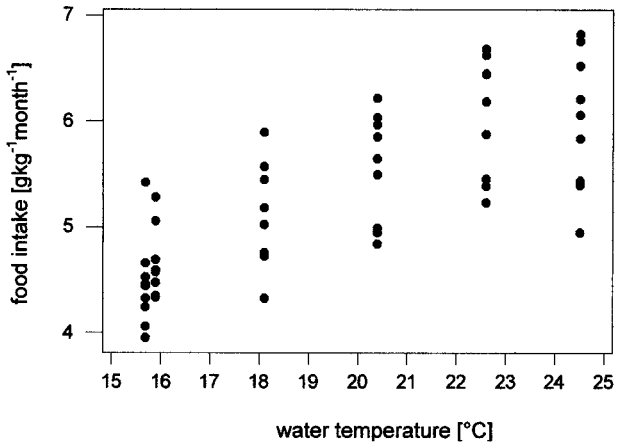

Figure 2. Monthly food intake of the studied loggerhead turtles ($n = 9$) in dependence of the seasonal variation in water temperature.

miniature temperature loggers (DS1921 Thermocron 1-Wire iButton, Dallas Semiconductor, Texas), which recorded T_b at 1-min intervals during the transfer experiments (resolution: 0.5°C, accuracy: ±1°C). Body temperatures of these and all the other turtles were taken rectally before and at the end of a given transfer experiment.

Activity Measurements

Five turtles (turtles 4, 5, 6, 7, and 8) were equipped with activity data loggers (Handylog500, Driesen and Kern, Bad Bramstedt, Germany; dimensions: l × w × h, 15 × 2.3 × 2.3 cm; weight in water: 75 g) during the 24-h measurements in September. The data loggers were attached close to the centreline of the posterior part of the carapace and were held in place by strips of Velcro, glued to both the underside of the logger and the carapace. The measurement principal based on a logger-integrated compass system has been described previously (Hochscheid and Wilson 1999). Movements of the turtle resulted in voltage changes of the Hall sensors, which were recorded every 2 s. Data were downloaded via an infrared interface connected to the serial port of a laptop computer (Toshiba, Tecra 730CDT).

Data Analysis

Oxygen Consumption. Oxygen consumption data were averaged over both the whole daytime measurements and the whole nighttime measurements for each turtle. We analysed the influence of T_w on logarithmic transformed $\dot{V}O_2$ using linear regression analysis (Minitab 11, Minitab, State College, Pa.). Both M_b (log-transformed) and individual codes were included as factors, and whenever one of these factors had a significant effect, the $\dot{V}O_2$ was corrected for the factor in question. Comparisons of regression lines were performed using generalised linear modelling (Minitab 11).

Regression equations of $\dot{V}O_2$ on temperature were used to calculate $\dot{V}O_{2^1}$ at the temperature of 15.7°C (T_1) and the $\dot{V}O_{2^2}$ at 25.4°C (T_2), which were used to calculate Q_{10}. This was achieved by inserting the calculated values in the equation

$$Q_{10} = \left(\frac{\dot{V}O_{2^2}}{\dot{V}O_{2^1}}\right)^{10/T_2 - T_1} . \quad (1)$$

T_1 and T_2 were chosen to be 15.7° and 25.4°C, respectively, because these temperatures represented the complete temperature range under which the $\dot{V}O_2$ measurements were made between September and February.

Mean oxygen consumption was also calculated for each hour of the 22-h respirometry run for each turtle. These data were used to test for circadian $\dot{V}O_2$ patterns and to determine minimum $\dot{V}O_2$, which resembled resting $\dot{V}O_2$ ($\dot{V}O_{2R}$).

Surfacing Behaviour. It was possible to estimate the number of surfacing events by examination of the oxygen consumption curve. Typically the curve rose steeply after the turtle breathed at the surface. The response time of the system, that is, the time that elapsed between the breathing event and the visible rise of the curve on the computer screen, was between 50 and 100 s (due to flow rate and volume of the hood and tubing system). The time constant ranged, in accordance with flow rate and hood volume, from 180 to 655 s. During the apnoeic period following a surfacing event, the respirometry system was flushed with fresh air constantly drawn from outside through the hood, and the calculated oxygen concentration approached baseline levels again. However, baseline level was not always reached before the next breathing event, that is, before the next rise of the curve, depending on the duration of the apnoeic period. Direct observation of the breathing behaviour was made on spot checks between 0800 hours and 2000 hours and compared with the O_2 trace.

Activity. The raw data from the three compass channels of the Handylog500 were imported in the Multitrace software (Jensen Software Systems, Laboe, Germany) and visualised as voltage signals over time. No change in the voltage signal in all three channels indicated that the turtle was immobile; voltages changed when the turtle was actively moving. The program calculated the differences in voltage for each data pair consisting of two successive data points (n_1 n_2, n_2 n_3, n_3 n_4, …, etc.) for the complete data file of one channel (e.g., records of one Hall sensor). The maximum difference during a period of immobility was taken as a threshold value between rest and activity. The proportion of data points above the threshold revealed the percentage of time the turtle was actively moving. A previous investigation using this type of data logger has shown that the

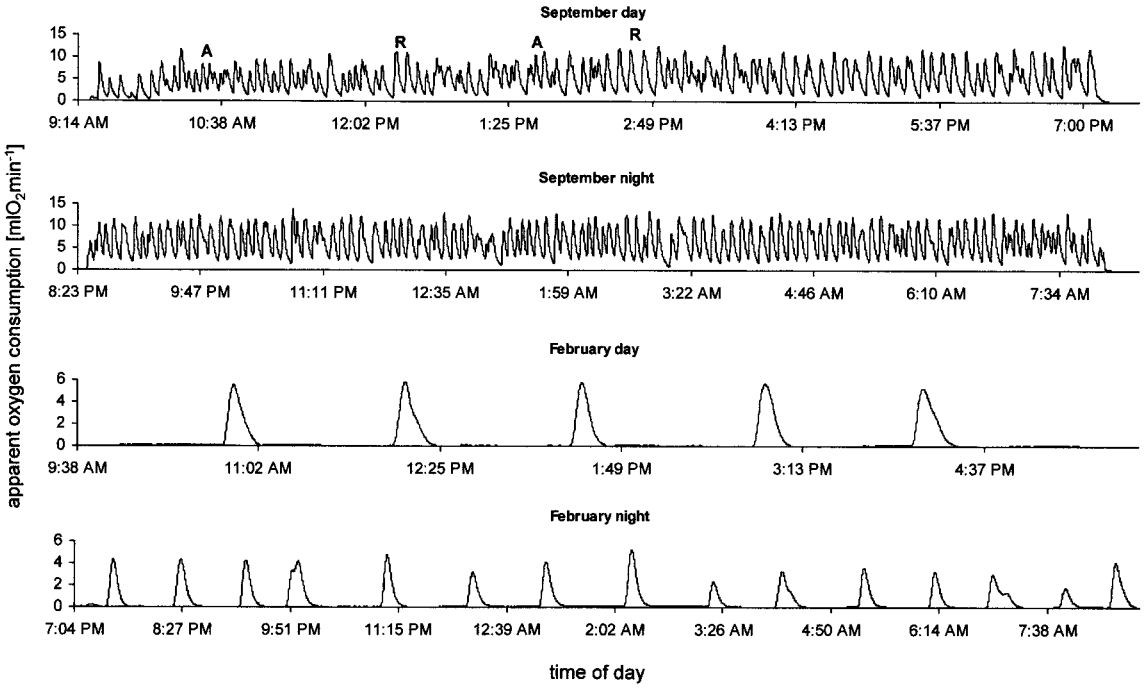

Figure 3. Oxygen consumption of a loggerhead turtle during day and night at the end of the summer (*top two graphs*) and in the winter (*bottom two graphs*). As a result of the intermittent breathing of the turtle, the oxygen analyser measured declining oxygen consumption rates during apnoea, which were thus termed "apparent oxygen consumption" on the value axis. Peaks indicate breathing episodes after periods of apnoea. Different states of activity are indicated by *R* (resting) and *A* (active).

recorded data of a single Hall sensor provides a complete activity profile, so that it is not necessary to analyse the other channels (Hochscheid and Wilson 1999).

Results

Seasonal Temperature Effect

Body Temperature, Food Intake, and Behaviour. Turtle T_b correlated significantly with T_w (linear regression analysis: $F_{2,25} = 3{,}748.26$, $P < 0.001$), showing the same decrease over the experimental period (Fig. 1). Larger individuals tended to have T_b slightly higher than T_w. Body mass was therefore included as a factor in the regression analysis and was also shown to have a significant effect ($t = 4.4$, $P < 0.001$). The regression equation

$$T_b = -0.322 + 1.02T_w + 0.0122M_b \quad (2)$$

(T_b in °C) explained 99.7% of the observed variation in T_b. The intercept of the regression line was not significantly different from 0 ($T = 1.21$, $P > 0.05$). Body temperatures, corrected for M_b, did not differ from T_w ($F_{1,51} = 3.35$, $P = 0.073$) and did not differ from a regression line with a slope of 1 ($F_{1,51} = 0.57$, $P = 0.453$).

Stomach temperatures, recorded over intervals of ca. 22 h, were obtained for three turtles (turtles 5, 6, and 9). Turtles 5 and 9 maintained constant T_b's (turtle 5: $T_b = 20$°C, $T_w = 20.0$°C, 0119–0017 hours; turtle 9: $T_b = 21.0$°C, $T_w = 20.1$°C, 0405–0037 hours); whereas in turtle 6, T_b changed between 19.0° and 19.5°C from 1608 to 1439 hours (the iButton temperature loggers measured only in 0.5°C increments). During these periods, turtles 5, 6, and 9 consumed 806, 495, and 966 g anchovies, respectively.

At the beginning of the study period, turtles were fed on average 233 g anchovies kg M_b^{-1} mo^{-1} (range: 24–547 g kg^{-1} mo^{-1}). They continued feeding during the cold winter period, but food intake decreased significantly with decreasing T_w (average food intake for February: 37 g kg^{-1} mo^{-1}; range: 8–117 g kg^{-1} mo^{-1}, Fig. 2). The relationship between food intake and T_w was described by the equation

$$\ln \mathrm{FI} = 2.95 + 0.177T_w - 0.398 \ln M_b, \quad (3)$$

where FI (in g kg^{-1} mo^{-1}) was food intake ($r^2 = 0.88$, $F_{2,53} = 179.76$, $P < 0.001$). A Q_{10} of 5.87 was calculated for food intake using Equations (1) and (3), substituting $\dot{V}o_2$ with FI.

Table 1 shows that all turtles, except turtles 5 and 7, gained M_b during this 6-mo study. Increases in M_b were generally higher in the autumn (September–November = $\Delta M_{b,autumn}$) than in the winter (December–February = $\Delta M_{b,winter}$; paired *t*-

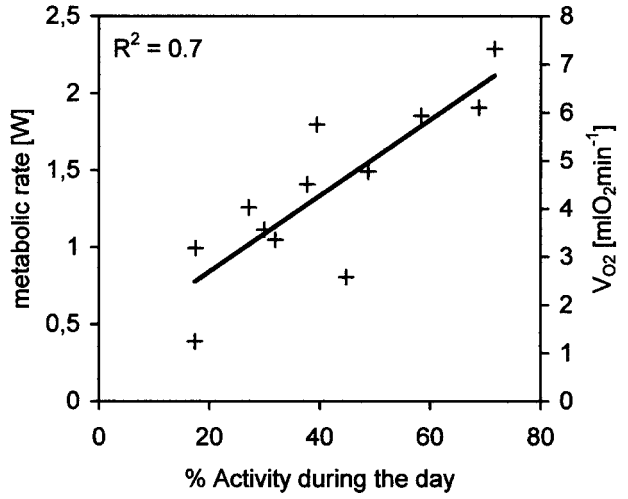

Figure 4. Metabolic rates and oxygen consumption rates ($\dot{V}O_2$) of sea turtles as a function of time per day (or night, respectively) spent in activity (measured during the same period of $\dot{V}O_2$ measurements); for example, 0% of time = turtle resting the whole day (except when surfacing to breathe), and 100% of time = turtle swimming in tank all day.

test of the differences between $\Delta M_{b,autumn}$ and $\Delta M_{b,winter}$: $t = 3.87$, $P < 0.01$, $n = 9$). The lower increases or stability in M_b coincided with the period of low food intake during the winter months.

There was a decrease in the turtles' overall activity in the second half of the experimental period (November–February). While the turtles alternated between actively swimming and resting on the bottom of the tank during the warmer months (September–November), periods of activity became rare in the colder months (December–February), when the turtles were mainly engaged in long periods of submergence during which they rested on the bottom of the tank. This behaviour pattern was also revealed in the oxygen consumption data, examples of which are plotted against time in Figure 3. Each peak in the oxygen consumption curve represents a surfacing event after a period of submergence. When the oxygen consumption curve returned to the baseline level before it rose once more, it indicates the turtle is resting (e.g., *R* in Fig. 3), whereas peaks in short succession without return to baseline level between them indicates levels of higher activity (e.g., *A* in Fig. 3). The time elapsed between two successive peaks in the oxygen consumption curve represented an estimate of the time spent in apnoea. The comparison of late summer and winter oxygen consumption patterns clearly demonstrates that surfacing was more frequent in September (mean ± SD surfacing frequency = 12.8 ± 5.5 h^{-1}) while apnoeic intervals were much longer in February (mean ± SD surfacing frequency = 1.5 ± 0.7 h^{-1}; two-sample *t*-test: $t = 6.18$, $P < 0.001$, $n = 9$; Fig. 3).

The activity data obtained from the data loggers revealed a wide range of activity levels (expressed as the percent of time of the corresponding respirometry file) during both day (27.2%–71.6%) and night (17.4%–68.6%). Daily or nightly mean $\dot{V}O_2$ increased significantly with increasing activity level (Fig. 4).

Using the regression equation

$$\dot{V}O_2 = 0.0737(\%\ \text{activity}) + 1.04 \quad (4)$$

($\dot{V}O_2$ in mL O_2 min^{-1}; linear regression analysis: $r^2 = 0.7$, $F_{1,11} = 22.35$, $P < 0.01$, data for day and night were combined in the regression analysis, $\dot{V}O_2$ were corrected for M_b), we calculated the $\dot{V}O_2$ for 0% and for 100% activity. Oxygen consumption rates at 0% activity averaged 1.04 mL O_2 min^{-1} and was assumed to represent $\dot{V}O_{2R}$. Oxygen consumption rates at 100% activity (8.41 mL O_2 min^{-1}) was eight times $\dot{V}O_{2R}$. Metabolic rates estimated from these values were 0.35 W during resting and 2.82 W for 100% activity.

Oxygen Consumption. We obtained eight sets of respirometry data, four per night and four per day, for each turtle, except for two missing sets for turtle 2 and one missing set for turtle 3 due to electrical failure. Oxygen consumption rates during the day did not differ significantly from $\dot{V}O_2$ during the night (Wilcoxon's signed rank test, Wilcoxon's statistic = 319.0, $P = 0.308$, $n = 32$). The overall relationship between $\dot{V}O_2$, seasonal changes in T_w, and M_b was best described by the equation

$$\ln \dot{V}O_2 = -2.87 + 0.168T_w + 0.353 \ln M_b \quad (5)$$

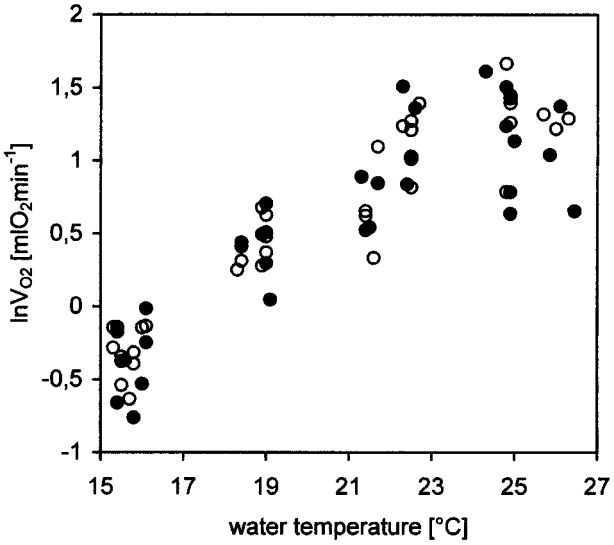

Figure 5. Oxygen consumption ($\dot{V}O_2$) of loggerhead turtles ($n = 9$) at seasonally different water temperatures. Open circles are $\dot{V}O_2$ obtained during daytime, filled circles are $\dot{V}O_2$ at night.

Table 2: Oxygen consumption of *Caretta caretta* measured under natural varying water temperature (T_w) from September 2000 through February 2001

Month	Mean ± SD	Minimum	Maximum	n	T_w (°C)	Q_{10} (ΔT_w)
September:						
$\dot{V}O_2$	3.974 ± 1.49	2.382	6.732	9	25.4	2.44 (3.6)
Metabolic rate	1.33 ± .5	.797	2.253			
November:						
$\dot{V}O_2$	2.882 ± 1.23	1.353	4.826	9	21.8	12.84 (2.4)
Metabolic rate	.964 ± .41	.453	1.615			
December:						
$\dot{V}O_2$	1.562 ± .53	.999	2.46	7	19.4	6.93 (3.7)
Metabolic rate	.523 ± .18	.334	.823			
February:						
$\dot{V}O_2$	.763 ± .24	.423	1.102	9	15.7	
Metabolic rate	.255 ± .24	.142	.369			

Note. $\dot{V}O_2$ is given in mL O_2 min^{-1}, metabolic rate in W. The corresponding Q_{10} values were calculated for the intervals September–November, November–December, and December–February.

($\dot{V}O_2$ in mL O_2 min^{-1}; $r^2 = 0.75$, $F_{1,69} = 102.6$, $P < 0.001$; Fig. 5). Using Equations (1) and (5), we calculated a Q_{10} value of 5.36 for the seasonal effect of T_w on oxygen consumption. Minimum $\dot{V}O_2$ measured during a day and night cycle were assumed to represent resting $\dot{V}O_2$ ($\dot{V}O_{2R}$) and were also correlated to seasonal temperature changes:

$$\ln \dot{V}O_{2R} = -3.94 + 0.195T_w + 0.303 \ln M_b \quad (6)$$

($\dot{V}O_2$ in mL O_2 min^{-1}; $r^2 = 0.67$, $F_{2,32} = 29.79$, $P < 0.001$). The Q_{10} for $\dot{V}O_{2R}$ derived from Equation (5) was 7. We also calculated Q_{10} values for shorter periods between September and November, November and December, and December and February, during which T_w dropped on average 3.2°C (Table 2).

Short-Term Temperature Effect

Body Temperature. Body temperatures, recorded by the ingested data loggers, were successfully obtained for turtles 5 and 9 for experimental temperatures of 25° and 30°C and for turtle 6 for an experimental temperature of 15°C. All the T_b's of these turtles are listed in Table 3 together with T_b's taken rectally of the other turtles. Temperature readings of the data loggers deviated somewhat from those made with the digital thermometer, due to the relatively low resolution and accuracy of the iButton loggers. The continuous records of the data loggers showed, however, that all turtles reached a stable new T_b at least 24 min before being retransferred into their home tank. Pairwise comparison of temperature differentials between turtle and water revealed no differences before and after the transfer (paired t-test: $t = 0.1$, $P = 0.92$, $n = 18$).

Oxygen Consumption. Oxygen consumption of all turtles increased when they were transferred into water that was warmer than their acclimation temperature. Transfer into experimental water that was colder than the acclimation temperature resulted in decreased $\dot{V}O_2$ (Fig. 6). The relationship between $\dot{V}O_2$ (corrected for M_b) and experimental T_w was described by the linear regression equation

$$\ln \dot{V}O_2 = 0.0286T_w + 0.169 \quad (7)$$

($\dot{V}O_2$ in mL O_2 min^{-1}). This increase in $\dot{V}O_2$ with increasing experimental temperatures was significant (least squares regression analysis: $r^2 = 0.532$, $F_{1,26} = 28.43$, $P < 0.001$). The resulting Q_{10} value was 1.33.

The relationship described by Equation (7) was compared to the relationship between $\dot{V}O_2$ and seasonal T_w (Eq. [5]). Both slope and intercept of the two regression lines were significantly different from each other (generalised linear modelling: different slopes: $F_{1,96} = 76.17$, $P < 0.001$; Fig. 7). The response to short-term transfer was much less profound than the effect of seasonal changes (e.g., the slope of the seasonal regression line was approximately six times the slope of the transfer regression line).

Circadian Variation in Oxygen Consumption Rate

Oxygen consumption rates of each hour of a 22-h measurement were established for each turtle and each of the four different study months. There was substantial variation in individual circadian $\dot{V}O_2$ patterns. Individual turtles did not have consistent circadian $\dot{V}O_2$ patterns through the study period. For example, turtle 5 tended to have higher $\dot{V}O_2$ at nighttime in September (Fig. 8). The same turtle showed a decrease in $\dot{V}O_2$

toward the evening and had slightly lower $\dot{V}O_2$ at night than during the day in December (Fig. 8). Mean hourly $\dot{V}O_2$ (corrected for M_b) from all turtles were combined to establish a general trend in circadian $\dot{V}O_2$. There was no clear pattern in diurnal or nocturnal $\dot{V}O_2$ variation (Fig. 9). At no time of the year was there a significant difference between $\dot{V}O_2$ at different hours of the day (repeated-measures ANOVA: September: $F_{21,174} = 1.31$, $P = 0.178$; October/November: $F_{20,177} = 1.51$, $P = 0.086$; December: $F_{21,141} = 0.4$, $P = 0.991$; February: $F_{22,170} = 1.12$, $P = 0.329$).

Discussion

Effect of Water Temperature on Physiology and Behaviour of Acclimated and Nonacclimated Sea Turtles

Little is known about the physiological changes involved in long-term exposure of sea turtles to cold temperatures, mainly because of the difficulties studying these animals underwater in the field. Controlled laboratory studies are a first step toward the understanding of the adaptations of otherwise difficult-to-access animals. With respect to sea turtles, there are reports of temperature-induced migration as well as hibernation, but both are poorly characterised and have yet to be verified (Ogren and McVea 1995). Moon et al. (1997), in an attempt to simulate hibernation in the laboratory, showed that cold-acclimated Kemp's ridley turtles and green turtles had a lower food consumption and longer submergence times. We observed the same behaviour in the loggerhead turtles used in this study.

There was no evidence that our sea turtles controlled their T_b in the course of the seasons either behaviourally or physiologically. To an extent, this may have been because opportunities to behaviourally thermoregulate by, for example, basking at the water surface, were unavailable in the captive setting of our study. Nevertheless, the same effect was measured in free-ranging green turtles (Read et al. 1996), suggesting our data may reflect the free-ranging thermoregulatory behaviour. A short-term increase in regional T_b may occur during periods of activity (Standora et al. 1982). Sato et al. (1994) monitored inter-nesting loggerhead turtles (M_b: 68.9–88.2 kg) at sea and showed that their median T_b was 1.1° to 1.7°C higher than median T_w. We concluded that this temperature difference was due to metabolic heating. However, the turtles in our study did not show any capacity to increase their T_b above T_w or maintain a significantly elevated T_b by metabolic means alone. This could again reflect the captive situation where opportunities for extended swimming bouts were denied to the animals because of the restricted space. Nevertheless, though some turtles did engage in activity bouts, alternation between resting and routine activity did not affect stomach temperatures. It appears that T_w alone dictated the internal temperature in our animals. An example of this can be seen in the results of the heating and cooling experiments: although sea turtles seem to possess the physiological capacity to control heat exchange (Smith et al. 1986; Hochscheid et al. 2002), their T_b equilibrated with the experimental T_w within 3 h (Table 3); hence, these heat exchange mechanisms may only be used to modulate the rate of equilibration of T_b to T_w rather than sustaining an elevated T_b.

Table 3: Body temperatures (T_b) of various-sized loggerhead turtles

Turtle and T_b (accl.)	T_{ac}	ΔT	T_b (transf.)	T_{ex}	ΔT
1:					
19.4	19.4	0	15.7	15.2	.5
19.5	19.4	.1	25.6	25.4	.2
19.4	19.3	.1	30.1	29.5	.6
2:					
19.7	19.4	.3	30.3	30.3	0
3:					
19.4	19.4	0	16.0	15.9	.1
19.6	19.4	.2	24.8	24.8	0
19.4	19.3	.1	30.1	29.8	.3
4:					
19.4	19.2	.2	15.7	15.1	.6
20.2	20.0	.2	24.0	24.1	−.1
5[a]:					
18.5	20.0	1.5	23.5	24.1	−.6
19.0	19.4	.4	28.5	29.0	−.5
6[a]:					
19.5	19.4	.1	16.5	14.9	1.6
7:					
19.8	19.4	.4	17.0	15.0	2.0
20.0	19.4	.6	28.7	28.9	−.2
8:					
19.7	19.5	.2	16.1	16.0	.1
19.7	19.4	.3	28.4	18.4	0
9[a]:					
21.5	20.1	1.4	23.5	24.0	−.5
20.0	19.3	.7	27.5	29.0	−1.5

Note. T_b was measured in the acclimation tank (T_b [accl.]) and after a period of about 3 h spent in a tank with experimentally manipulated water temperature (T_b [transf.]). All temperatures are in °C. T_{ac} = acclimation temperature; T_{ex} = experimental temperature; ΔT = difference between T_b and T_{ac} or T_{ex}, respectively. (A negative sign indicates that T_b was lower than water temperature.)

[a] T_b was recorded by a data logger (see "Material and Methods").

Oxygen consumption rates of sea turtles have been measured in only a few previous studies, and they are in general comparable to the $\dot{V}O_2$ presented here (Table 4). Of the studies reported in Table 4, only Lutz et al. (1989) measured $\dot{V}O_2$ in relation to T_w, and they also found a significant positive correlation. However, according to Lutz et al. (1989), the Q_{10} of loggerhead turtles subject to a temperature range of 10° to 30°C was 2.4, whereas we calculated an overall Q_{10} of 5.4. This discrepancy is probably explained by the shorter acclimation time

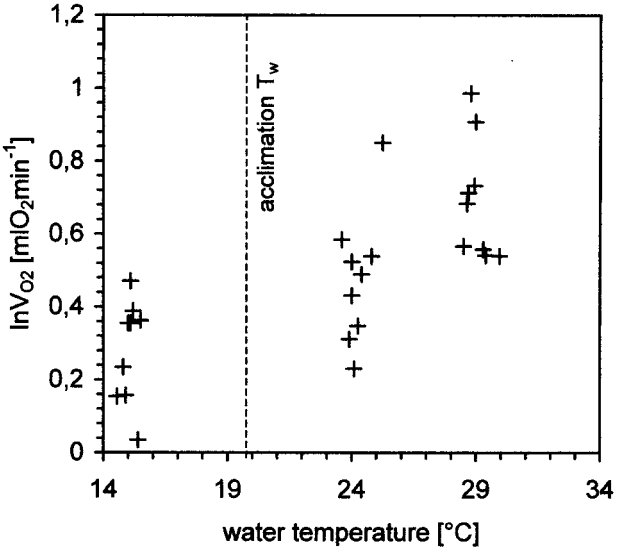

Figure 6. Oxygen consumption ($\dot{V}o_2$) of loggerhead turtles subject to a sudden change in water temperature (T_w). Dashed vertical line indicates T_w to which the turtles were acclimated.

of the turtles in Lutz et al.'s (1989) study; in Lutz et al. 1989, animals were kept at each experimental temperature for only 2 d before measurements. We demonstrated that, by transferring turtles in various temperature baths, nonacclimated sea turtles also have elevated or reduced $\dot{V}o_2$ at warmer or colder T_w, respectively, but the Q_{10} of the response was only 1.3. Acclimation time is therefore a critical factor in establishing the metabolic responses of sea turtles to changing T_w. The metabolic basis of this effect remains uncertain; however, it is particularly important when inferring the physiological changes involved in the response to seasonal temperature changes.

The high overall Q_{10} value was probably not solely a result of metabolic depression. Despite being provided with abundant food throughout the year, turtles fed much less during the colder months and as a consequence gained less or even lost M_b (Table 1). The Q_{10} effect therefore includes a contribution from the differences in food intake in the different seasons. An interesting question is whether the change in ambient temperature resulted directly in the changes in metabolic rate, thus driving the changes in food intake, or whether a component of the change in metabolism was a consequence of a direct effect of temperature on food intake. Separating these explanations is not possible in our data set since temperature and food intake covaried with season. Experimentally separating the effects in future studies will also not be easy. Cold-acclimated turtles in February would not elevate their intake of food even if large amounts were provided, and we attempted to feed summer turtles at the winter ration level, but in the short term this resulted in elevated activity, presumably as the animals searched for food. It appears that temperature, food intake, and metabolic rate are intimately linked together in these sea turtles and that changes in food intake may be a cause or consequence of the changes in metabolic rate, with both being heavily dependent on ambient temperature and period of acclimation.

In our experiment, we maintained a constant light cycle. Free-ranging turtles, however, may get important cues from changing day length. Owens et al. (1980) found a diurnal rhythm of melatonin in green turtles and proposed that these animals possess a functional "clock" to monitor day-length changes. The significance of photoperiod for overwintering in sea turtles needs further investigation, but it is striking that seasonal variation of temperature alone evoked such a strong metabolic response in the loggerhead turtles in this study. This observation supports the review by Ultsch (1989), who stated "It is clear that temperature is the major cue for the entrance into hibernation among turtles."

The temperature effect becomes even more remarkable when looking at the metabolic responses to small temperature changes between different months (Table 2). Q_{10} calculations based on such small temperature differences are problematic, but in this case it was useful to emphasise the data in Table 2: between September and November the Q_{10} was 2.4, which was in a normal range compared to others reported for reptiles (Bennett and Dawson 1976; Ultsch 1989). However, the Q_{10} between November and December was 12.8 for a temperature drop of only 2.4°C. It is intriguing that such evident metabolic suppression occurs so abruptly in the turtles that had no other environmental clue than temperature. This period of strong

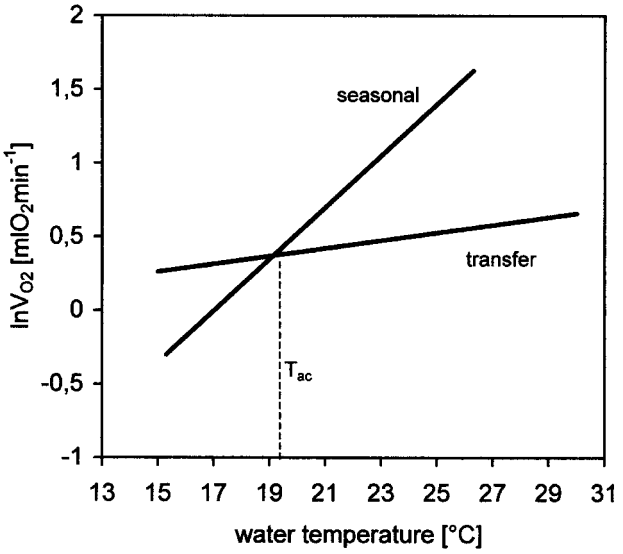

Figure 7. Effect of temperature on oxygen consumption ($\dot{V}o_2$) of loggerhead turtles. Comparison of two situations: seasonal change in temperature and short-term exposure to different temperatures during transfer experiments. T_{ac} is the water temperature to which the turtles were acclimated during the transfer experiments.

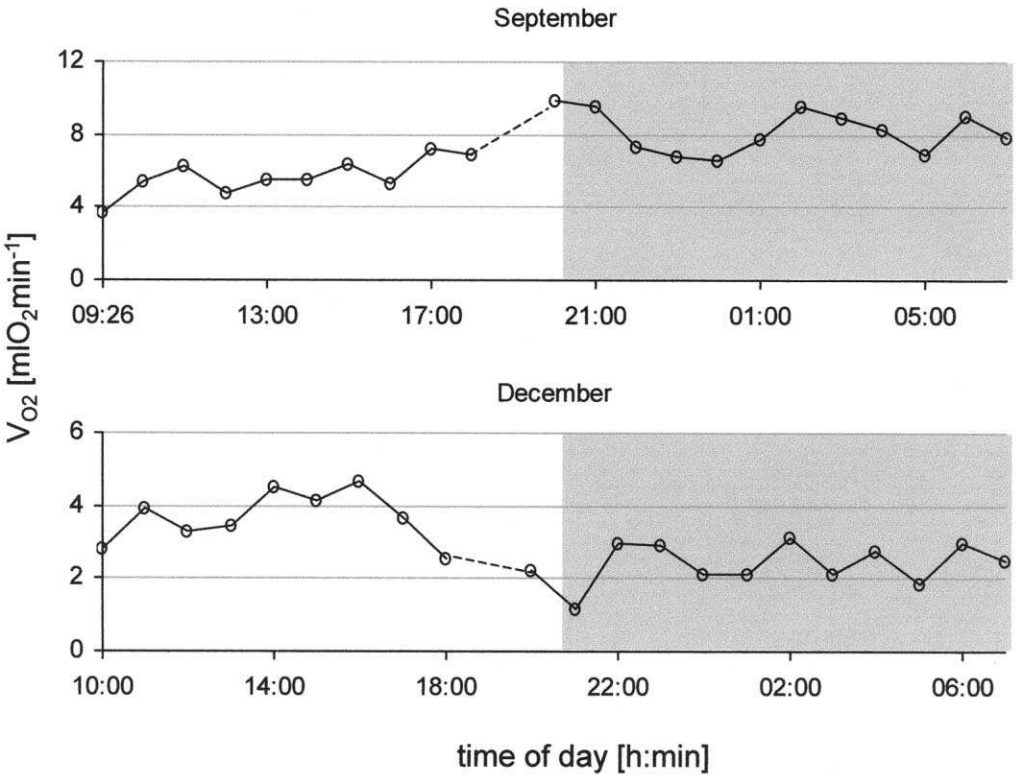

Figure 8. Variation of oxygen consumption ($\dot{V}O_2$) of loggerhead turtle 5 during a 24-h period in September 2000 and December 2000. Shown are mean hourly values for each hour of the day. Missing values are due to routine checkup of the respirometry system (see "Material and Methods"). Shaded areas indicate hours of darkness.

metabolic suppression coincides with the time of year when surface temperatures of the Tyrrhenian Sea drop below 20°C and turtles tracked by satellite have been shown to leave the region and move southeastward (Bentivegna 2002). Another recent satellite study on green turtles in the Mediterranean has also shown that these turtles initiated a distinct overwintering behaviour in coincidence with declining T_w's (Godley et al. 2002). Further studies are required to establish if a temperature threshold exists that signals the onset of the winter and to find other signals that lead the turtles to reduce their metabolism.

Allometry of Oxygen Consumption Rate

The size range of turtles used in this study (2–60 kg) made it possible to establish an intraspecific scaling exponent of 0.353 for $\dot{V}O_2$. These exponents are useful because they allow predictions of the energy requirements of animals as they grow. However, the estimate derived in our study is much lower than the only previously derived intraspecific scaling exponent reported for sea turtles (Prange and Jackson 1976), which was between 0.826 and 0.944 (for minimal and maximal metabolic rate, respectively) in green turtles. Clearly, the large differences between these exponents would have large impacts on the estimated energy demands of growing turtles.

The interspecific scaling exponent within classes for a wide range of organisms approximates 0.75 (Schmidt-Nielsen 1990), but for thermoregulatory reasons it has been argued that interspecific scaling exponents for ectotherms should be closer to 1 (Kooijman 1993). In line with this theory, an extensive study on teleost fish revealed an interspecific scaling exponent of 0.8 (Clarke and Johnston 1999), and values for various reptiles groups tended to be 0.8 and higher (Bennett and Dawson 1976). The intraspecific scaling exponent derived by Prange and Jackson (1976) in green turtles seems to fit the interspecific exponents closely, while our derived exponent in loggerheads does not. However, there is no reason to expect intraspecific scaling exponents to conform to interspecific exponents and, indeed, valid reasons to expect them not to (McMahon 1973). A closer look at Prange and Jackson's (1976) data reveals that mean $\dot{V}O_2$ from five hatchlings and nine immature turtles weighing less than 1 kg were combined with measurements of a single adult turtle weighing 127 kg (the authors stated that $\dot{V}O_2$ of two adult turtles were available, but only data from one turtle were utilised). Clearly a scaling exponent derived from

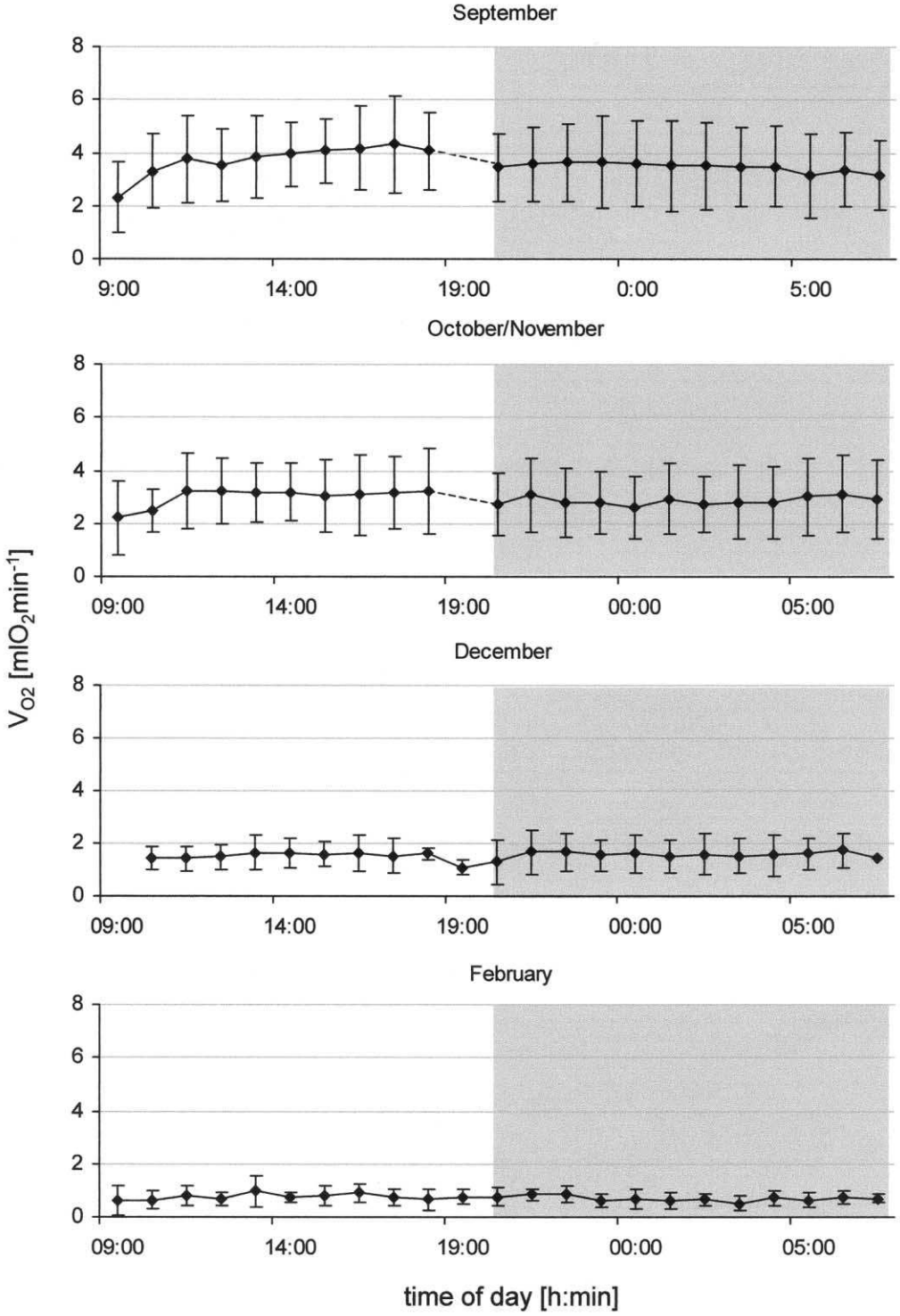

Figure 9. Mean hourly $\dot{V}O_2$ (and SD) of nine loggerhead turtles over a 24-h period for September, November, December, and February. Shaded areas indicate hours of darkness.

Table 4: Standard oxygen consumption rates ($\dot{V}O_2$) of diverse sea turtle species

Species	$\dot{V}O_2$	T_a (°C)	Reference
Chelonia mydas	.023	23–27	Prange and Jackson 1976
C. mydas	.07	25	Prange 1976
C. mydas	.06	26–30	Jackson and Prange 1979
C. mydas	.075	25	Davenport et al. 1982
C. mydas	.119	28	Butler et al. 1984
C. mydas	.099	25	Prange and Ackerman 1974
Dermochelys coriacea	.387	n.a.	Paladino et al. 1990
D. coriacea	.065	22–27	Lutcavage et al. 1992
Caretta caretta	.045	25	Lutz et al. 1989
C. caretta	.03	25.4	This study

Note. $\dot{V}O_2$ given in mL O_2 kg^{-1} h^{-1}. Minimum $\dot{V}O_2$'s were selected in cases where standard $\dot{V}O_2$ was not specified. T_a = ambient temperature; n.a. = not available.

such nonnormally distributed data may be unreliable. However, it is also possible that there are real species differences in the metabolic consequences of growth and body size for $\dot{V}O_2$. This discrepancy highlights the paucity of information on the metabolic rates of sea turtles generally and the need for more studies.

Circadian Rhythm of Oxygen Consumption Rate and Activity

Daily fluctuations in $\dot{V}O_2$ are common in reptiles as in many other groups, such as insects, birds, and mammals, and may, among other functions, serve energy conservation during the inactive phase of the day (reviewed in Bennett and Dawson 1976; for a more recent study, see, e.g., Kirsch and Vivien-Roels 1984). Despite the distinct dark and light phases, there was no overall difference between day and night $\dot{V}O_2$ in the loggerhead turtles we studied. We recorded marked amplitudes in individual turtles, but peak $\dot{V}O_2$ occurred at different times of the day. In our study there were two major external factors that could have induced a physiological response: feeding of turtles and the light cycle. The absence of a corresponding daily oscillation of $\dot{V}O_2$ in all turtles indicates that neither of these factors had a significant effect. This was somewhat unexpected since $\dot{V}O_2$ is generally known to increase after feeding, a phenomenon termed "specific dynamic action" (SDA; Kleiber 1961; Garrow 1974). SDA has been shown to comprise a significant portion of the daily energy requirements of exothermic animals (reviewed in Bennett and Dawson 1976; Jobling 1981). In three freshwater turtle species studied by Secor and Diamond (1999), $\dot{V}O_2$ peaked 24–36 h after feeding, and elevated $\dot{V}O_2$ was maintained for several days. Therefore, our turtles, which fed every day (every second day in the winter), may have experienced an overall elevation of $\dot{V}O_2$ where the SDA from one day overlaid the SDA response from the previous meal. A 24-h measurement of $\dot{V}O_2$ was probably not sufficient, therefore, to isolate SDAs of single feeding events when feeding occurred as frequently as in our study. However, 24 h should have been sufficient to record a diurnal rhythm in oxygen consumption. Kirsch and Vivien-Roels (1984), using a similar experimental approach, showed a marked circadian rhythm of $\dot{V}O_2$ in two tortoises, *Testudo hermanni*, at warm temperatures (28°C), though this rhythmicity disappeared at low temperatures (8°C).

Here, activity was the only obvious variation in the turtles' daily cycle that was evidently correlated with metabolic differences (Fig. 4). Consequently, maximum $\dot{V}O_2$ rose to two to three times the resting $\dot{V}O_2$ when the turtles performed routine swimming movements. Based on the current data set, we suggest that circadian changes in activity underpin the circadian variations in $\dot{V}O_2$, but peak activity may occur at any time of day or night. It is clear that the confined space of a tank does not allow sea turtles, which normally use a much larger three-dimensional space, to perform naturally. Activity of free-ranging turtles (including, e.g., commuting between depth and surface, foraging movements) may occur in more regular patterns as it has been shown for inter-nesting leatherback turtles (Eckert et al. 1989) and for immature hawksbill turtles (van Dam and Diez 1996). It would be interesting to see whether metabolic rates in the field also oscillate with the prevailing activity patterns.

The elevated costs of activity for a turtle swimming in a confined space emphasise that free-living sea turtles probably face considerable energetic demands during their long-distance migrations (e.g., our data suggest that $\dot{V}O_2$ of a turtle actively swimming most of the day would be ca. eightfold elevated with respect to resting $\dot{V}O_2$; see Fig. 4). Similar increases in $\dot{V}O_2$ were also measured in small juvenile green turtles while swimming at speeds between 0.35 m s^{-1} (Prange 1976) and 0.6 m s^{-1} (Butler et al. 1984). These findings imply major energetic consequences for migrating turtles, such as the green turtles travelling from Brazil to nest at Ascension Island, which apparently do not feed during the long migration or during the inter-nesting period (Hays et al. 2002).

Housing and Handling Effect

Handling of the study animals during the transfer experiments may have elevated their metabolic rates because of handling stress, but such an effect would not be apparent in the seasonally acclimating animals that were not touched during the routine respirometry measurements. We are confident, however, that the differences between the short-term and long-term measurements do not reflect a stress artefact because the responses of the animals during the transfer experiments involved a bi-directional change in $\dot{V}O_2$, indicating that they were predominantly affected by the temperature difference rather than by

stress, which would be expected to generate a uniform increase independent of temperature.

Conclusions

Low temperatures slow down circulation, oxygen uptake, metabolic processes, and enzyme activities. Thus, an ectotherm like the loggerhead turtle, whose internal temperature follows closely the ambient temperature, experiences marked physiological changes as ambient temperatures decline. These changes differ, however, depending on the state of acclimation of the animals. Responses to seasonal temperature changes were considerably more profound than short-term changes in temperature during tank transfer experiments. The seasonal change in $\dot{V}O_2$ was matched by a parallel change in food intake. At present it is not clear to what extent reduced food intake was a consequence of the reduced $\dot{V}O_2$, whether lowered temperature independently affected the two traits, or whether lowered temperature caused both the $\dot{V}O_2$ and food intake to decline, with the lowered food intake stimulating a further reduction in $\dot{V}O_2$. Separating between these alternative patterns of causality will form the focus of future work. Despite the profound reductions in $\dot{V}O_2$ with season, none of the loggerhead turtles in this study performed prolonged submergences (in the order of weeks). The intraspecific scaling exponent for loggerhead turtles at 0.353 was much lower than the only other intraspecific exponent available for this group. This highlights a paucity of data on sea turtle metabolic rates.

Acknowledgments

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