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Effect of forced submergence and low seawater temperature on the physiology and behavior of sea turtles

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INTRODUCTION

Marine turtles (Reptilia: Testudines) are distributed widely throughout the tropical and subtropical oceans. Although most marine turtles are confined within the 20° surface isotherms, individuals occasionally stray or are displaced by ocean currents into colder waters. The populations of all five genera have been greatly reduced by man, and all species are now listed as endangered or threatened. With the exception of the leatherback (Dermochelys coriacea), exposure to temperatures much below 20°C may be harmful and at 10-15°C some species may become positively buoyant, unable to dive (Schwartz 1978). There is some evidence that in favorable habitats green (Chelonia mydas) and loggerhead (Caretta caretta) sea turtles may survive prolonged exposure to sea water temperatures less than 15°C by going into a state of winter dormancy or apparent hibernation (Felger et al., 1976 Carr et al., 1980, Ogren and McVea, 1982). The effect of cold temperatures is very important therefore, both for the distribution and survival of sea turtles. It must be completely defined both from the physiological and behavioral standpoints in order to produce practical measures that will protect or rehabilitate sea turtles. This is particularly true of hibernating turtles where disturbing the "hibernaculum" or even dislodging the hibernating turtles may be harmful.

We had found earlier that some features of the blood chemistry of the loggerhead turtle are sensitive to changes in season (Lutz and Dunbar-Cooper, 1979, Dunbar-Cooper and Lutz, 1983). However, it was not known if these effects were due to changes in temperature or to other seasonal factors. It was therefore of particular interest to set up a study that would distiguish between seasonal and temperature dependent factors and to investigate the physiological effects of low temperatures in order to distinguish between cold stunning and hibernation.

Sea turtles are also severely stressed by accidental capture in fishing gear, especially fish and shrimp trawls, where they are trapped submerged and struggling for as much as one half hour. Although it is well established that sea turtles can survive many hours underwater (Lutz and Bentley, in press), the net capture often results in death. It would appear then that the high mortality of net trapped turtles is not submergence per se but is due in some way to the trauma of forced submergence.

Since net drownings may be a major cause of death for U.S. sea turtles it is most important to understand what is happening to the trapped turtle. A knowledge of the primary physiological consequences of forced submergence is essential for the formulation of protocols for treating trapped turtles and enhancing their survival.

In summary, the primary objectives of this study were:

1) To describe the effects of low temperature on sea turtles, particularly those associated with underwater endurance and the effect of premature arousal.

2) To perform analyses on a sample of field caught turtles through one annual cycle in order to compare field events with the laboratory data and distinguish between temperature and other seasonal effects.

3) If encountered, to identify and characterize winter caught dormant and monitor the effects of arousal.

4) To investigate the response of sea turtles to forced submergence.

5) To make recommendations for the resuscitation of turtles trapped in shrimp trawls.

MATERIALS AND METHODS

All temperature related experiments on loggerheads were carried out in a climate controlled laboratory adjusted to the desired experimental temperatures. The turtles were held (two turtles in each run) in separate 1000 liter tanks in sea water. The water was changed routinely, with water at the current experimental temperature.

The turtles ranged in size from 4.3 - 22.7 kg; all turtles could move freely in the tanks, breathing at will during all experiments.

Experiments on the turtles were carried out at five water temperatures:

(°C)	(SD)
10.08	± 0.13 n = 6
14.12	± 1.00 n = 10
20.15	± 0.21 n = 8
25.10	± 0.15 n = 6
30.18	± 0.40 n = 6

The turtles were held at each temperature for at least 1 day before any measurements were made. The time varied with the temperature since at colder (ie less than 15°C) temperatures the animals appeared stressed.

Blood chemistry

Blood was taken from the dorsal cervical sinus as previously described (Bentley and Dunbar-Cooper 1980).

Blood gases (PCO2, P02) and pH were determined immediately on whole blood using a Radiometer BMS Mk2 blood-gas analyzer. The blood gas analyzer was set at the tank temperature, thereby eliminating the need for correction factors.

Plasma bicarbonate was calculated from the pH and PCO_2 data using the temperature dependant CO_2 solubility and dissociation constants of Severinghaus, et al. (1956).

The blood was then centrifuged and the plasma divided into two parts: 1 ml of plasma was added to 2 mls of chilled 8% perchloric acid (PCA), mixed and placed in the refrigerator at least 15 mins. It was then spun again and the supernatant collected and frozen. This treated plasma was analyzed for glucose and lactic acid. The rest of the plasma was frozen and it was later analyzed for osmotic pressure, urea, and the blood electrolytes. The hematocrit was read after centrifugation.

The plasma treated with PCA was analyzed for d-glucose using the Boehringer BMC single vial glucose-HK kit (BMC no. 263826). Lactic acid concentration was determined by the Sigma Chemical procedure no. 826-UV and urea was measured using the Sigma Kit n 640. Readings in both procedures were done on a Beckman DU narrow-bandwidth spectrophotometer.

The untreated plasma was analyzed for osmotic pressure on a Wescor 6100 osmometer. Plasma chloride was measured using an Aminco chloride titrator and sodium, potassium, magnesium and calcium levels were measured using atomic absorption spectrophotometry (Perkin Elmer PE 403).

Respiration

Oxygen consumption rate ($\dot{V}o_2$), minute ventilation ($\dot{V}$), tidal volume (Vt) and breathing rate (Rv) were obtained by placing a plywood cover beneath the surface of the water, forcing the turtle to breathe through the apparatus shown in figure 1.

The turtle breathed by first exhaling into a half-submerged plexiglass collection chamber, forcing a mixture of expired air and chamber air through a one-way valve into a flexible latex collection bag (10 liter weather balloon). Room air was drawn into the chamber through a second one-way valve during inspiration.

Figure 1 Expirate collection apparatus

B - collection bag

C - collection chamber

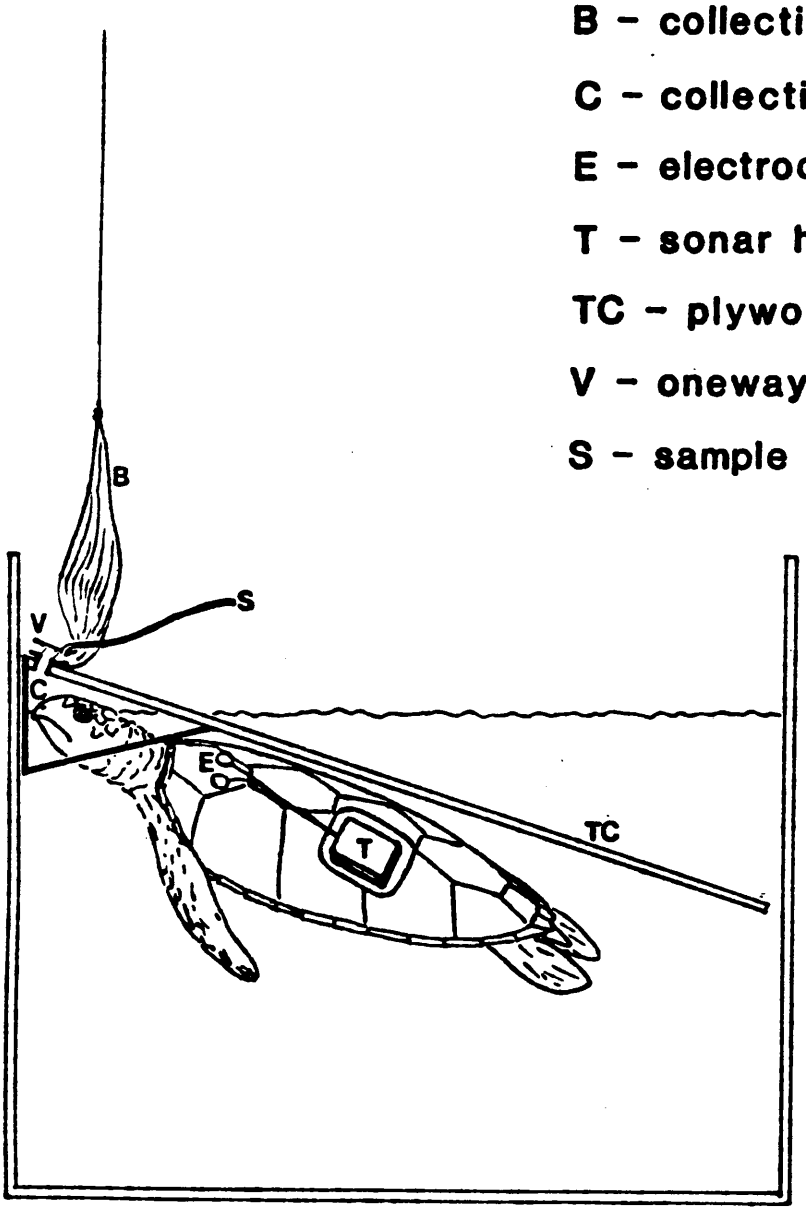
E - electrodes

T - sonar heartbeat transmitter

TC - plywood tank cover

V - oneway anthesia valve

S - sample tube for oxygen analyzer



The collection bag was emptied at a steady rate of 200 ml/min by the pump and flow regulator contained in an Applied Electrochemistry oxygen analyzer while oxygen concentration was recorded on chart paper. Figure 2 shows an idealized example of the resulting plot. Each of the three low-oxygen spikes in figure 2 represents the transiently low oxygen level detected by the oxygen analyzer at the end of an expiration. At this time the part of the collection bag adjacent to the oxygen analyzer sample tube had been flushed with expired air. Within a minute or so, mixing of pure expired air and displaced chamber air took place within the collection bag, and oxygen concentration reached a steady state until the next breath, or exhaustion of collection bag contents.

When the collection bag was completely empty, pump vacuum increased to the opening pressure of the collection chamber exit and entrance valves (0.5 cmH_2O). A mixture of room air and collection chamber air was then sampled and recorded until the beginning of the next breathing bout. During this time sampled oxygen concentrations gradually approached room air levels as expired air trapped in the collection chamber was flushed out.

The amount of oxygen consumed during a breathing bout (V_{bO_2}) was calculated by multiplying the volume of air sampled by the observed drop in oxygen concentration.

$$V_{bO_2} = rt_b (\Delta O_2) \qquad \text{equation 1}$$

where: t_b = duration of bout and subsequent apnea (min)

r = oxygen analyzer sampling flow rate (ml/min)

ΔO_2 = mean difference between oxygen concentration of sample air and room air.

Summing V_{bO_2} over a series of bouts and dividing by time and body weight yields oxygen consumption rate ($\dot{V}_{O_2}$).

FIGURE 2

Oxygen Analyzer Output Plot

t_a = duration of interbout apena

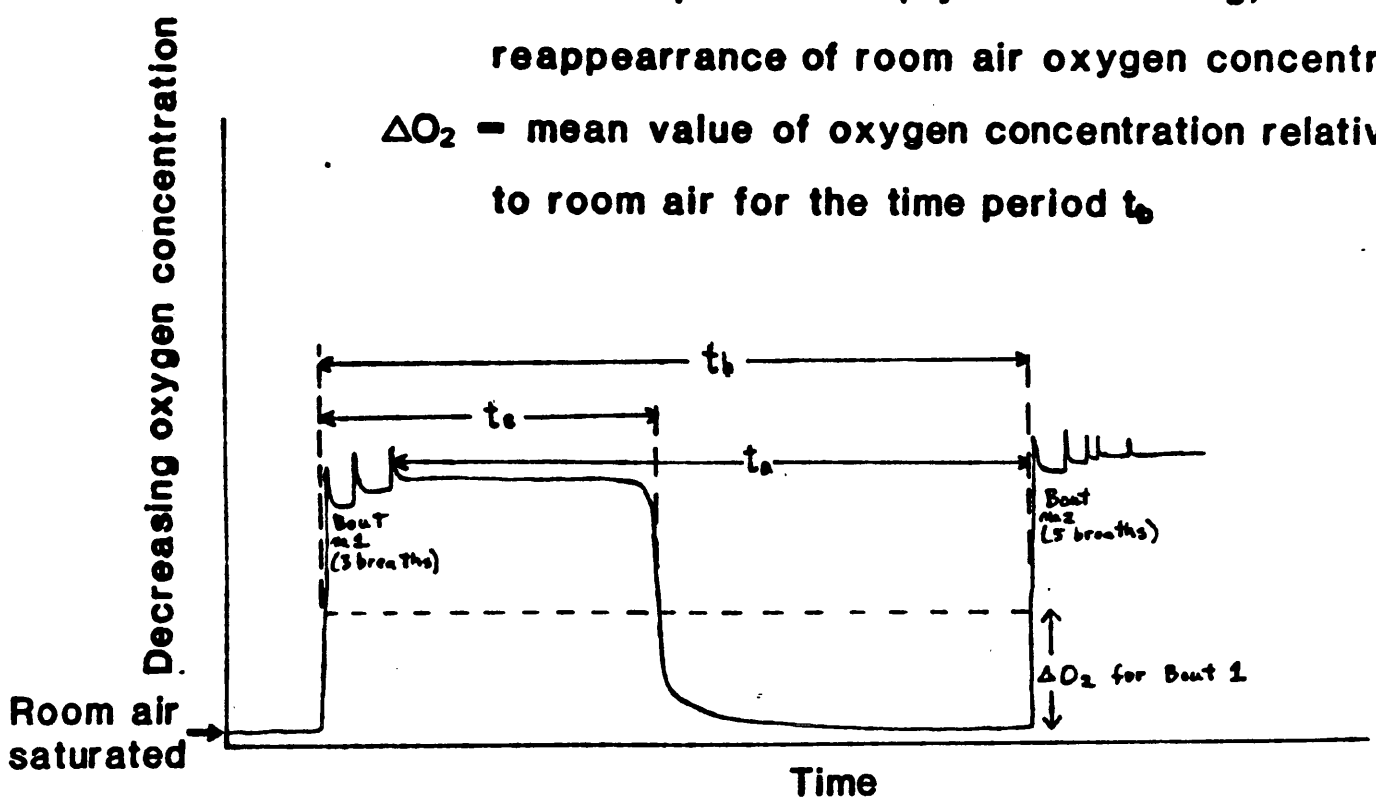
t_b = duration of bout and interbout apnea

t_e = time required to empty collection bag, as shown b

reappearrance of room air oxygen concentration

ΔO_2 = mean value of oxygen concentration relative

to room air for the time period t_b



$$\dot{V}_{O_2} = \frac{\sum V_{bO_2}}{(t_r)(W_b)} \qquad \text{equation 2}$$

where: t_r = time (min) from the beginning of the first bout in the series until the end of the apnea following the last bout summed.
W_b = body weight of turtle (Kg)

The quantity of air exhaled during a breathing bout was proportional to the time necessary to pump the bag empty:

$$V_b = rt_e \qquad \text{equation 3}$$

where: V_b = volume if air exhaled into the collection bag during a bout
r = oxygen analyzer sampling flow rate
t_e = time required to empty collection bag

When V_b is known for several bouts, V, V_t and R_v can be obtained:

$$V = \frac{\sum V_b}{(t_r)(W_b)} \qquad \text{equation 4}$$

$$V_t = \frac{\sum V_b}{\sum n} \qquad \text{equation 5}$$

$$R_v = \frac{n}{t_r} \qquad \text{equation 6}$$

where: R_v = Breathing rate (breaths/min)
V_t = tidal volume (ml)
V = minute ventilation (ml/min. Kg)
$\sum V_b$ = Summation of V_b over a series of bouts
$\sum n$ = Summation of the number of "breath spikes" in each of the above breathing bouts
t_r = time (min) from the beginning of the first bout in the series until the end of the apnea following the last bout
W_b = body weight (Kg)

Due to behavioral variations between runs, we found it desirable to separate respiratory activity into "active" and "quiescent" periods. This reduced the frequency for O_2 consumption avoided by excercise to obscure temperature-related changes in V_{O_2}.

It must be stressed that these were long term experiments and the average continuous run was 5 hours and 7 mins.

Heart rate

Heart rates of diving turtles under temperature, forced dive and excercise stress were obtained by using a Grass polygraph and a sonar heartbeat transmission system developed for us by Wyoming biotelemetry

In both systems, stainless steel electrodes were implanted in loose connective tissue just beneath the carapace (Figure 1). These electrodes were continuations of the thin stainless steel cables used as lead wires to the recording devices. One centimeter of insulation was stripped from the cable ends and the wire strands twisted back on themselves. Cable ends were inserted into 1 mm diameter holes drilled through the turtle's carapace so that the un-insulated tip of the cable made contact with soft tissues. The cables were then anchored in place with dental cement, and a 2 cm patch of contact cement was applied over the site.

Heart rates were recorded directly on chart paper by the polygraph while the turtle swam about with flexible wire leads trailing behind it. Excess slack wire was suspended from above by a rubber cord to prevent entanglement.

Free-swimming heart rates were obtained by replacing the polygraph with the Wyoming Biotelemetry transmitter. This device was mounted on the caudal third of the carapace by bolting it to a canvas patch attached to the turtle with contact cement. The transmitter produced a 75 kH_z pulse for each heartbeat detected, which was picked up by a hydrophone and recorded on chart paper.

Stress experiments

1) A 34.6 kg loggerhead was obtained from Miami Seaquarium and fitted with a heart beat transmitter. When the animal appeared recovered from this procedure (described earlier) it was sampled for lactate and glucose. The animal was then forcibly submerged for 35 minutes and resampled. A final sample was taken after another submergence of 3 hours.

2) Three smaller turtles (2.7, 3.2, and 1.9 kg) were obtained from Miami Seaquarium. These turtles were taped to boards and attached to a glass polygraph to record their heart rates. These animals were then forced dived until they showed signs of bradycardia Blood was taken from the turtles four times during these experiments:

1) before emergence, after the onset of bradycardia,
2) 15 mins after emergence
3) 1 hr after emergence,
4) 2.5 hrs after emergenge.

Each turtle was sampled one more time after they had been returned to outside tanks and appeared fully recovered, ie. 24 hours later for the 1.9 and 3.2 kg turtles and 48 hrs for the 2.7 kg turtle.

The blood collected from these turtles was analyzed for hematocrit, glucose and lactic acid as already described.

Field experiments

Six of the NMFS cruises in the Cape Canaveral Channel were attended by researchers from our laboratory. 10 turtles on each cruise were sampled as already described. 5 turtles from each cruise had blood collected once as soon as possible after they were brought aboard and once again 3 - 4.5 hrs later. These blood samples were analyzed for lactic acid and glucose as well as hematocrit, osmotic pressure, urea, Cl, Mg, Na, Na, and Ca.

The second five turtles of each group were only sampled for the latter eight parameters.

The blood was spun, fixed and frozen aboard the trawlers and analyzed back in the laboratory as previously described for the laboratory animals.

RESULTS AND DISCUSSION

1. Behavioral response to low temperature

10°C was determined to be the lowest temperature that we could safely use. At less than 20°C some turtles would lose buoyancy control and float. By 14°C all turtles would lose buoyancy control and float. By 14°C all turtles were floating, with flippers turned out. However, at less than 10°C the flippers would curl under and the animals showed little response to touch. This condition could be reversed by raising the temperature as little as 1°C. The animals generally stopped floating when the water temperature was increased above 20°C. However, one turtle did not regain control even after it had been returned to the outside tanks. After approximately one month the turtle was still floating and not until 3 months was full recovery obtained.

The behavioral response to the temperature regimes used in these experiments did not appear to depend on the time of year that the animals were tested.

2. Blood chemistry

Hematocrit

In the earlier study hematocrit showed no change with season. (Lutz & Dunbar-Cooper 1979). In this study hematocrit also did not change with season (Table 1b) and the average range of values (32-36%) encompasses that found in 1978 (35.4%). The very low values found for the set of turtles sampled in December of 1978 is therefore all the more significant (mean 15%, minimum 5%).

In the temperature experiments the laboratory animals had a substantially lower mean hematocrit (26.7 $\pm$ 3.17 n = 19). However temperature *per se* had no effect on hematocrit, even down to 10°C (Fig. 3). This is further circumstantial

TABLE 1a. Blood chemistry values of loggerhead turtles trapped by shrimp trawl in the Port Canaveral ship channel, November, 1981 - August, 1982.

Date	°C	Na	K	Ca	Mg	Cl
Nov. 1981	24.0	162.2±7.93 (5)	4.18±1.496 (5)	2.18±0.325 (9)	1.49±0.77 (5)	107.0±3.25 (9)
Feb. 1982	19.0	142.07±21.42 (4)	4.145±0.696 (4)	1.863±0.456 (5)	1.976±0.303 (5)	102.7±10.58 (10)
March 1982	18.0	152.1±14.2 (5)	4.17±0.454 (5)	1.40±0.765 (10)	2.31±0.66 (5)	108.85±9.88 (10)
May 1 1982	24.5	165.5±4.41 (3)	4.08±2.04 (3)	2.09±0.41 (8)	2.00±0.62 (4)	110.3±5.62 (9)
May 2 1982	27.0	168.9±4.09 (3)	4.6±0.48 (3)	1.78±0.93 (10)	1.93±0.55 (5)	108.2±13.0 (9)
Aug. 1982	-	156.0±13.5 (4)	5.14±0.83 (5)	1.69±0.96 (8)	2.65±0.69 (5)	117.3±6.57 (9)

Unless otherwise stated, units are mM. L^{-1}. Mean ± S.D., number of samples in parentheses.

TABLE 1b. Blood chemistry values of loggerhead turtles trapped by shrimp trawl in the Port Canaveral ship channel, November, 1981-August, 1982.

Date	°C	Lactate	Glucose	Urea	Osmotic Pressure mOsm	Hematocrit %
Nov. 1981	24.0	-	-	9.43±4.56 (9)	330.1±19.12 (9)	35.3±5.27 (9)
Feb. 1982	19.0	3.51±0.27 (4)	1.17±0.367 (4)	6.78±2.04 (10)	309.0±28.8 (10)	33.7±5.85 (10)
March 1982	18.0	3.42±1.39 (4)	0.98±0.468 (3)	5.55±2.51 (10)	309±9.36 (10)	36.1±5.71 (8)
May 1 1982	24.5	-	-	4.45±2.11 (9)	314.8±10.9 (9)	34.08±5.46 (6)
May 2 1982	27.0	3.58±0.07 (3)	1.31	4.41±3.8 (10)	329.4±20.3 (10)	31.8±3.60
Aug. 1982	-	16.2±8.1 (3)	1.12±0.18 (3)	6.19±4.49 (9)	343.3±23.1 (9)	33.0±2.68 (7)

Unless otherwise stated, units are mM. L^{-1}. Mean ± S.D., number of samples in parentheses.

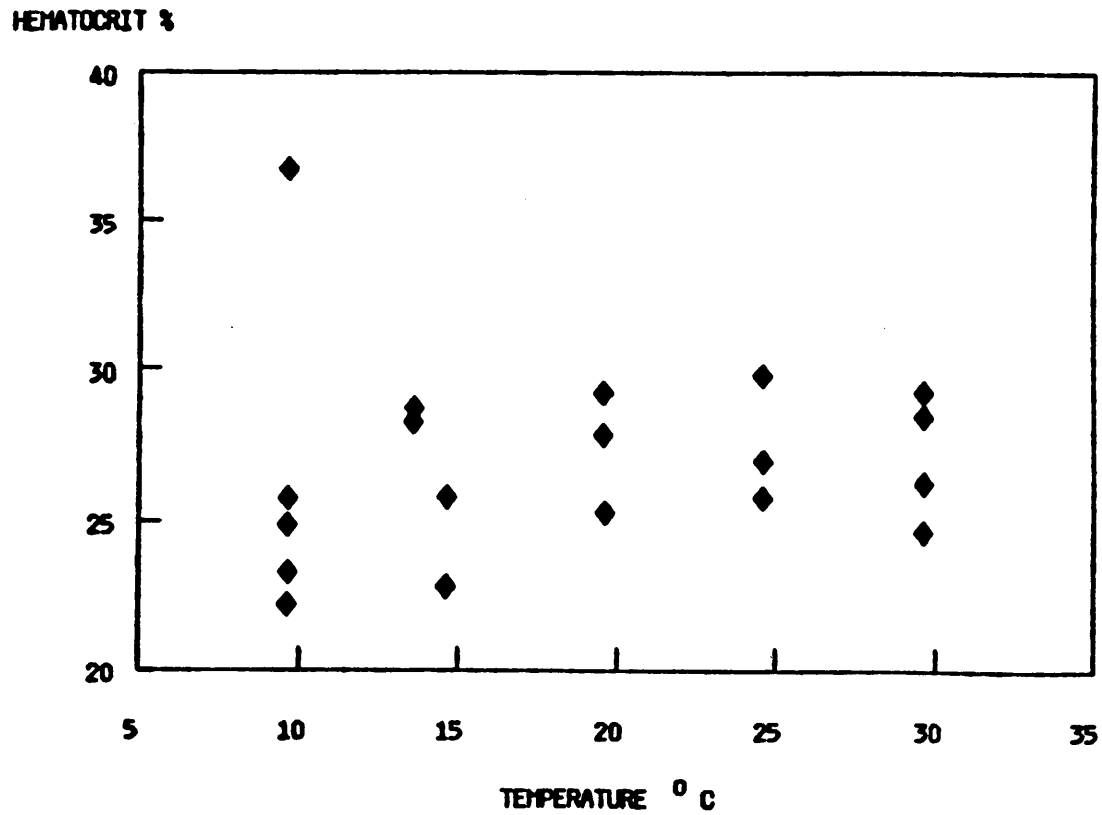


FIGURE 3. The effect of temperature of hematocrit of loggerhead turtle.

evidence to suggest that very low winter hematocrit values formed earlier may be part of a preparatory response for possible hibernation. However, a reduction in hematocrit until temperature has been found for a lizard (Egernia cunninghami) that experiences large diurnal changes in body temperature (Maclean et al. 1975). For some poikilotherms a reduced hematocrit maybe part of the acclimation response to low temperature as it would counteract to some extent the tendency towards increased blood viscosity (Snyder 1971).

Sodium

The 1981/1982 field sampled turtles showed a wide range in sodium values, however no seasonal trend is apparent (table 1). In agreement with the earlier study, it appears that seasonal changes are not a predominant influence on plasma sodium levels (Lutz 1980). Although the laboratory animals appeared to have somewhat elevated sodium levels (table 2) the range of sodium value was less in the temperature experiment than that found in field animals. Possibly the difference in diet between captured and free animal effects blood Na. Lance (1976) has suggested that changes in plasma sodium maybe related to adrenal gland activity.

Potassium

As before the 1981/1982 field data showed little change in potassium levels (Table 1) indicating that potassium is held under tight control. However the earlier study had suggested a general trend of potassium rising as summer progressed (Lutz 1980). Although in this study the highest values were obtained in the warmest months, May and August 1982, a seasonal increase was not apparent. However, in the laboratory temperature studies, plasma potassium showed a marked dependence on temperature over the range of 15 - 30°C (Table 2, Fig 4, $P > 0.99$), the highest temperatures being associated with the highest K^{+}values. An important temperature related feature is apparent here. This trend is, however, reversed at

$10^{o}C$ where a sharp rise in potassium levels is found. The increase in mK^{+} may be one of the signals of metabolic failure at $10^{o}C$ (see below).

Calcium

The calcium values in the second field study were very similar to those found in the first and were approximately 1-2 mM (table 1). No seasonal trend was apparent. Similar values were also found in the temperature studies and it appears, therefore, that there is little or no effect of temperature on plasma calcium levels. This suggests that the high calcium values found in January field animals at Cape Canaveral (Lutz & Dunbar-Cooper 1979) have a physiological significance other than temperature. In many reptiles plasma calcium increases during vitellogenesis (Gilles-Ballien 1974, Lance 1976) though this is not likely to be the case for the January rise in the loggerhead.

Magnesium

Plasma magnesium levels appear to be very tightly regulated and the average values only ranged from 1.5 to 2.6 mM L^{-1}(Table 1). This range is almost identical to that found in the earlier study (Lutz & Dunbar-Cooper, 1979) and in the temperature experiment (Table 2). Neither season nor temperature, therefore, had any influence on magnesium concentration indicating that any notable excursion from these values would have the utmost physiological significance.

TABLE 2

The effect of temperature on the osmotic and ionic composition of loggerhead sea turtle blood ($mM.L^{-1}$).

Temperature °C	Na	K	Ca	Mg	Cl	Urea	Osmotic pressure (mosm.)
30°	1.72.8 ±9.631 (6)	4.35 ±0.545 (6)	1.42 0.410 (7)	1.598 ±0.319 (8)	110.9 ±4.42 (8)	11.58* ±4.96 (8)	325.2 ±29.98 (8)
25	1.74	3.81	2.17	1.34 ±0.263 (3)	120.67 ±7.68 (3)	7.37* ±1.28 (3)	304.2
20	170.6 ±10.11 (6)	3.53 ±0.388 (6)	1.48 ± 0.539 (7)	2.13 ±0.681 (7)	109.15 ±3.60 (8)	4.03* ±1.37 (6)	295.27 ±36.38 (7)
15	173.2 ±4.04 (5)	2.86 ±0.939 (5)	1.19 0.134 (5)	1.89 ±0.395	107.64 ±4.52 (5)	3.6* ±1.598 (4)	286.9 ±25.78 (5)
10	184.84 ±14.54 (5)	4.02 ±0.491 (4)	1.28 0.335 (6)	2.03 ±0.570 (7)	108.3 ±6.37 (7)	4.08* ±1.27 (5)	298.9 ±29.06 (7)

* A single turtle with consistently high urea values is not included.

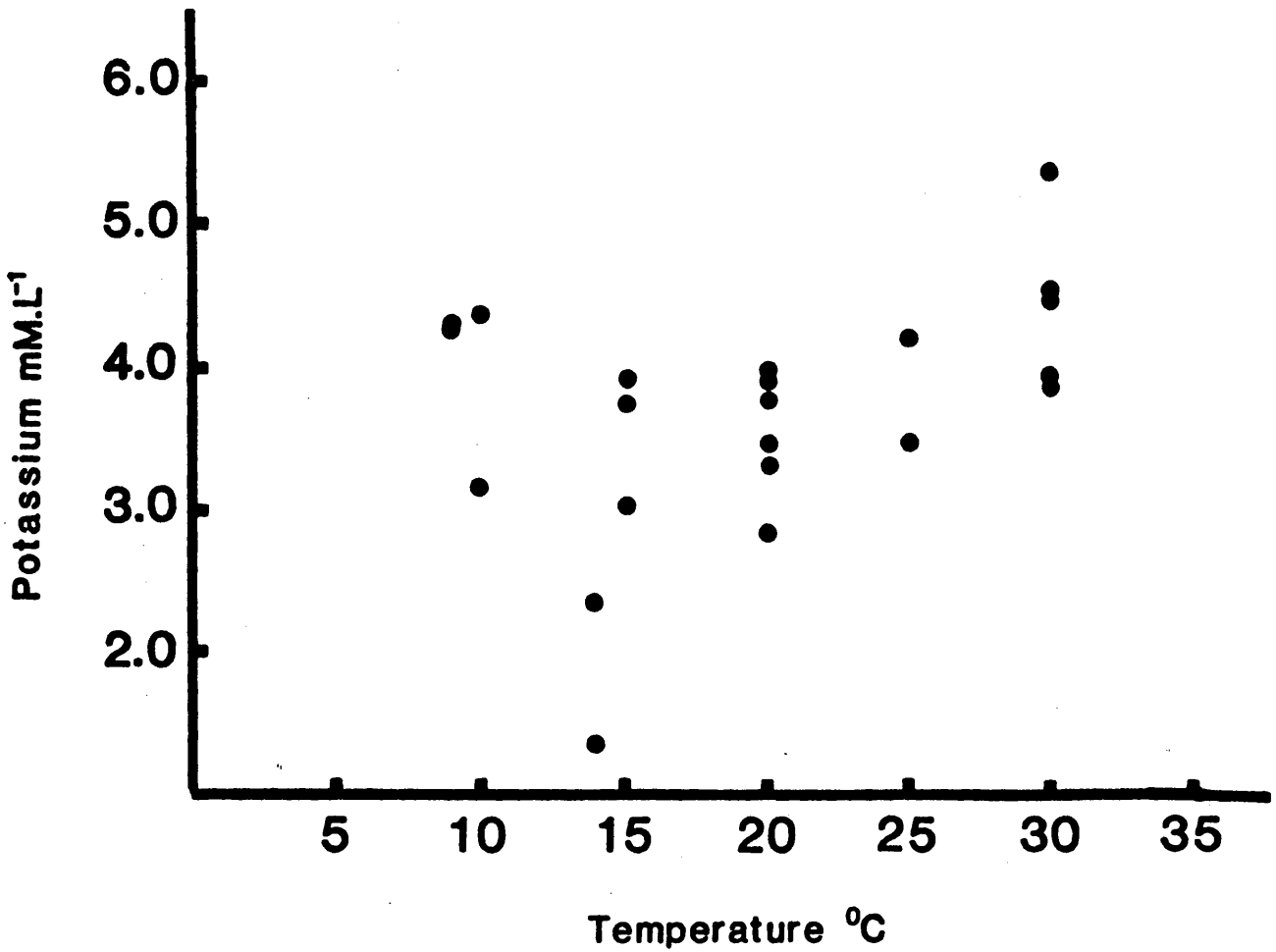


FIGURE 4. The effect of temperature on blood potassium of loggerhead turtle.

Chloride

The chloride values for 1981/82 Cape Canaveral turtles were very similar to those obtained in the earlier study (mean 108.5 mM. L^{-1}) and likewise no seasonal trend is apparent (Table 1). Temperature also appears to have no apparent effect on plasma chloride concentrations (Table 2).

Urea

The plasma urea values ranged widely from 1.4 to 20.9 mM. $^{-1}$ in the field study, and from 0.9 to 15.1 mM. L^{-1} in the laboratory kept animals. As before, no seasonal trend was discernable in this scatter. However some individuals had persistently much higher levels than the others. This was not caused by diet as the laboratory animals were all given the same food. Over the range 30-15°C lower temperatures were associated with lower urea levels (Table 2). But, as with potassium, this pattern was broken at 10°C. Plasma urea concentration is possibly related to the rate of protein catabolism and hence to the metabolic rate of the turtle.

Osmotic pressure

The field study found the lowest plasma osmotic pressure values in February and March 1981, the coldest months, and the highest values in late May, August, and November, the warmer months. A gradual increase as the year progressed had been seen in the earlier study (Lutz 1980). In the temperature studies the osmotic

pressure means show a fall over the range 30 to 15°C, although the fall is not statistically significant (Table 2). This data suggests that temperature may be one of the factors influencing seasonal change, though it is clearly not the sole one.

Lactate

For quiescent turtles kept in the laboratory over the temperature range 15-30°C, blood lactate is very low (0.2-0.4 $mM.L^{-1}$, Table 3). The lactate values found for the turtles were, by contrast ten to forty times higher (3.2 - 16.2 mML^{-1} table 1), an index of the very severe stress of the trawl.

Glucose

In the field, blood glucose was consistently about 1mM L^{-1} (Table 1). Similar values were found in the laboratory experiments over the range 10-25°C (table 3). However, at 30°C there was a slight increase in blood glucose.

3. Blood gases and acid base balance

Blood pH

A highly significant reciprocal relationship was found between blood pH and temperature (Fig 5) which over the range 14-30°C yielded the equation.

$$pH = 7.85 - 0.017\ T \qquad cc = 0.97 \qquad n = 22$$

This finding is consistent with the phenomenon of "constant relative alkalinity" (Reeves 1977) and the slope of $pH/\Delta T$ of - 0.017 is within the range of the neutrality value for 10 - 30°C (-0.018 to - 0.016 Truchot 1981). It is all the more striking, therefore, that at 10°C this system breaks down and the blood becomes severely acidiotic.

TABLE 3

The effect of temperature on glucose, lactate and bicarbonate concentrations of loggerhead sea turtle blood.

($mM.L^{-1}$)

Temperature °C	Glucose	Lactate	Bicarbonate
30	1.560 ±0.31 (9)	0.258 ±0.118 (9)	23.79 +2.58 (8)
25	1.194 ±0.159 (3)	0.207 ±0.004 (3)	23.24
20	1.218 ±0.60 (8)	0.401 ±0.33 (8)	28.64 ±1.573 (8)
15	1.170 ±0.306 (5)	0.502 ±0.194 (4)	31.24 ±1.141 (5)
10	0.939 ±0.51 (7)	1.605 ±1.09 (7)	27.94 ±3.39 (7)

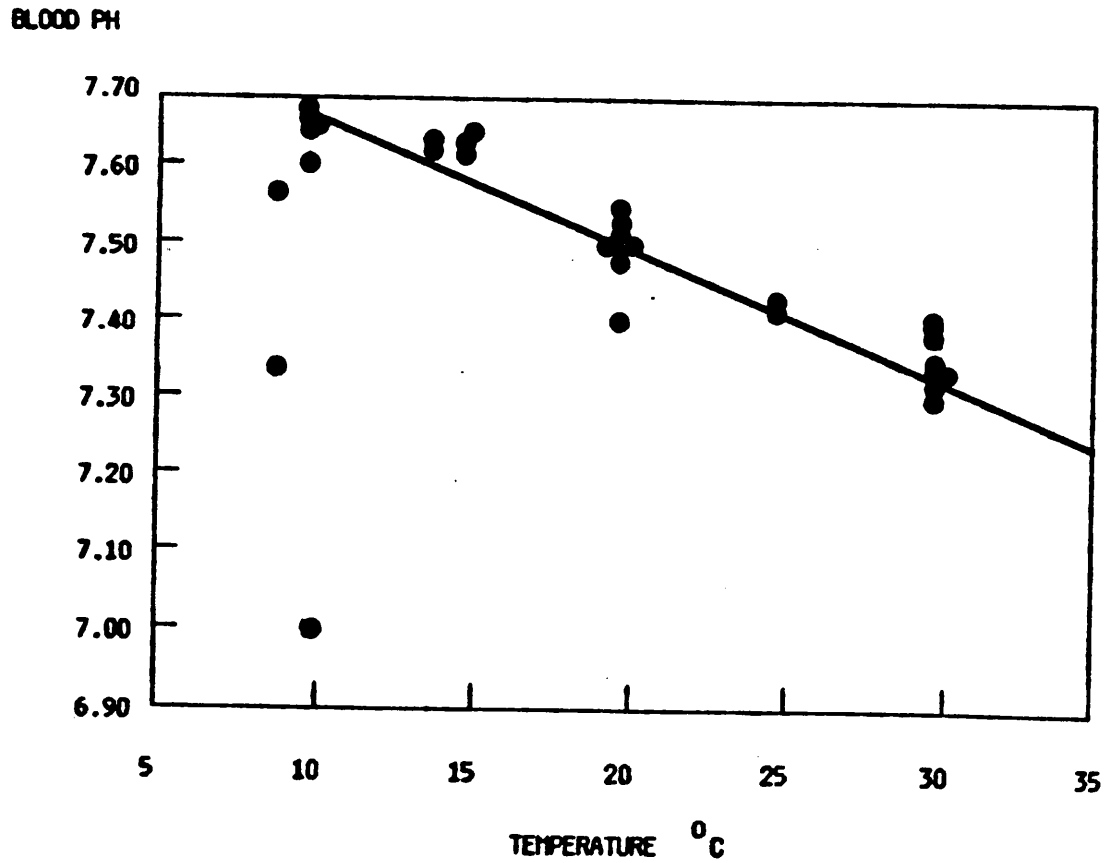


FIGURE 5. The effect of temperature on blood pH of loggerhead turtle.

Carbon Dioxide

Above 10°C increasing temperature is associated with higher blood p CO_2 levels (Fig 6). Under conditions of constant total CO_2, the increased p CO_2 would cause a fall in pH. It is possible therefore that alterations in blood p CO_2 are used to regulate blood pH with respect to temperature. The overall level blood p CO_2 would be controlled by lung ventilation. Below 10°C however there is a large rise in p CO_2 suggesting that this mechanism has broken down.

Oxygen

Over the temperature range 30 to 14°C venous blood oxygen also declined (cc = 0.720, n = 20, Fig. 7). Venous oxygen levels are determined by the difference between oxygen supply and tissue use. Since oxygen demand decreased with temperature (see below) it would appear that supply also decreased but at a somewhat greater pace indicating a reduction in oxygen transport at the cardiovascular or pulmonary levels. The rise at 10°C is very likely due to a sharp reduction in oxygen use.

Bicarbonate

Bicarbonate increases as temperature falls (Fig 8) demonstrating the role of the bicarbonate buffer system in this temperature dependent acid-base balance. However at 10°C bicarbonate appear to decline.

Lactate

Important clues as to what is happening at the critical 10°C can be gathered from Fig. 9. As discussed earlier over the range 15°C-30°C lactate levels are very low (Fig 9, table 2). But by contrast at 10°C there is a large rise in lactate although the animals were quiescent. It would appear that for some reason at 10°C oxygen supply has become insufficient to meet energy demands and that anaerobic glycolysis is being called upon to make a contribution.

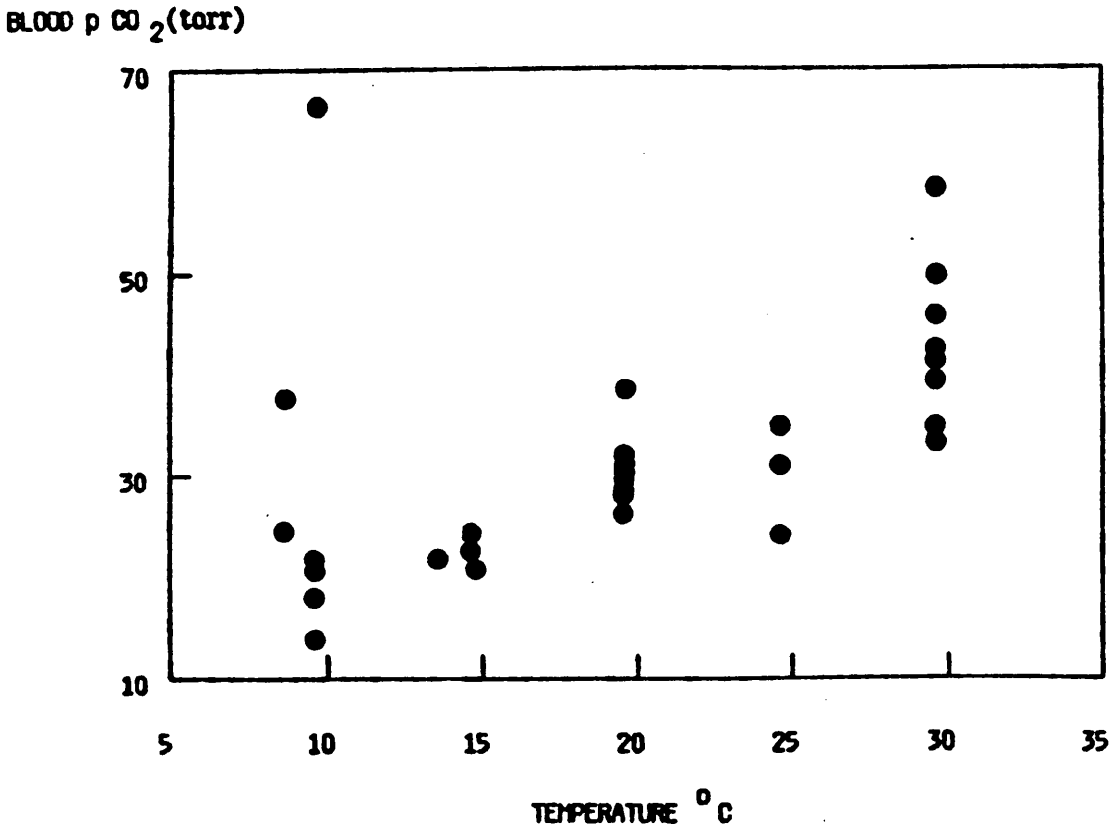


FIGURE 6. The effect of temperature on blood p CO_2 of loggerhead turtle.

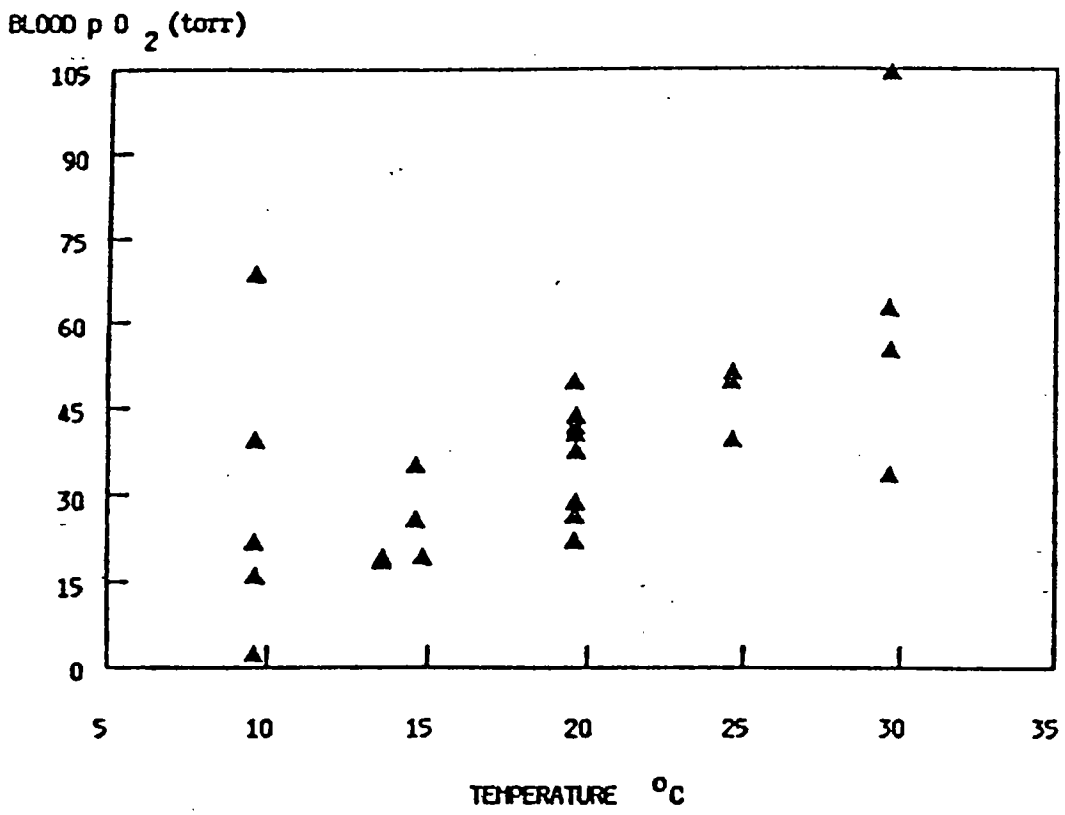


FIGURE 7. The effect of temperature on blood p O_2 of loggerhead turtle.

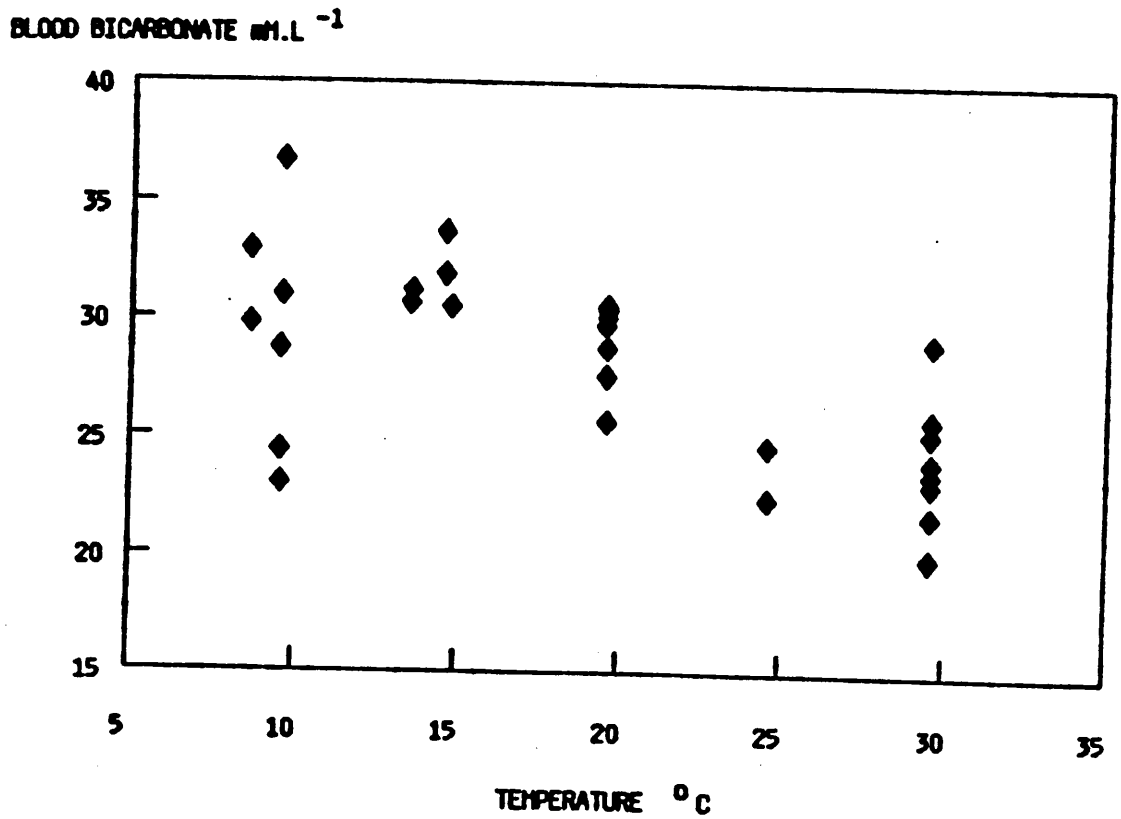


FIGURE 8. The effect of temperature on blood bicarbonate concentration of loggerhead turtle.

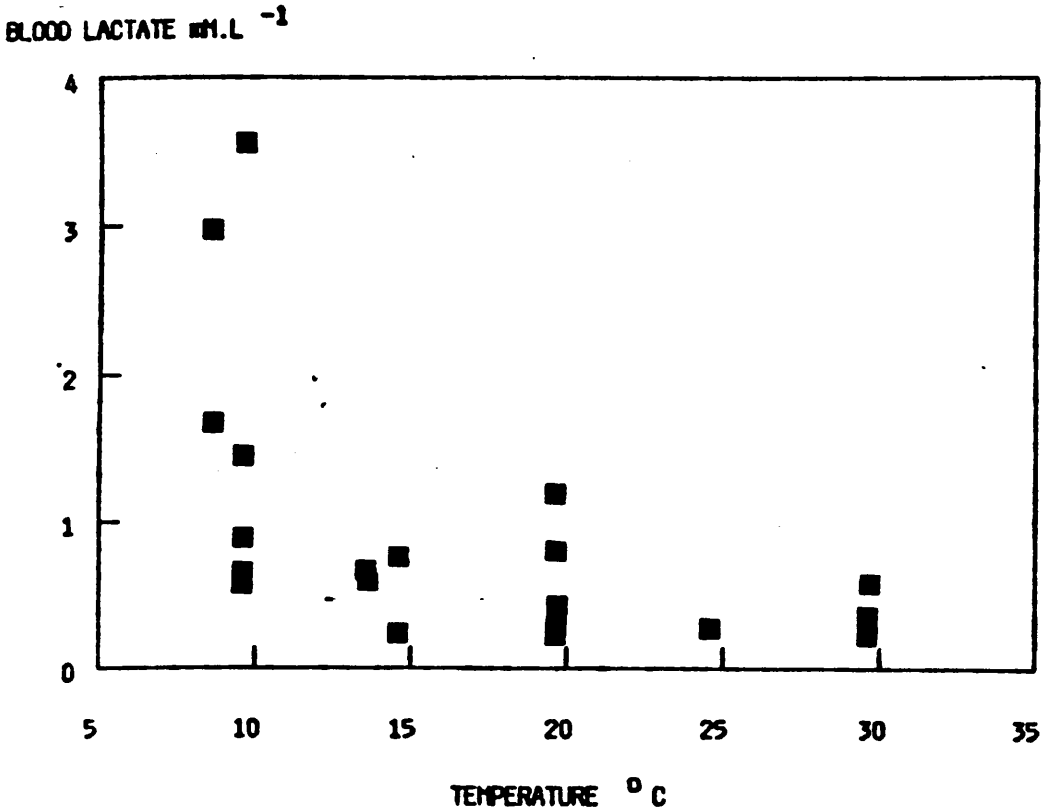


FIGURE 9. The effect of temperature on blood lactate concentration of loggerhead turtle.

It is clear that the acid-base balance failure that occurs at 10°C is not only due to the loss of temperature related control over blood p CO_2, but is also the result of inadequate oxygen supply to some tissues.

It is very likely that the rise in lactate is to some degree buffered by the fall in bicarbonate noted above (Fig 8).

4. Respiration

Oxygen consumption

Long term oxygen consumption measurements (up to 7 hrs) were made in a specially constructed apparatus. We found large variations in oxygen consumption that depended on the amount of spontaneous activity that an individual exhibited. The respiratory data, therefore, was divided into two components, active and quiescent. Table 4 and Fig. 10 show that both components are strongly influenced by temperature, but the active more so. In consequence, the difference between routine active and resting, is greater at higher temperatures. The resting oxygen consumption for these loggerheads (0.4 ml. $kg.^{-1}$ min^{-1}) is very similar to the standard metabolism found by Jackson and Prange (1979) for large nesting green sea turtles (0.35 ml. $kg.^{-1}$ min^{-1}). But their maximal values for animals struggling on the beach are almost five time those found here for the swimming animals (3.85 ml. $kg.^{-1}$ min^{-1}). At 9°C the resting oxygen consumption has fallen to negligible levels (0.05 ml O_2 $\cdot kg^{-1} \cdot min^{-1}$) and the lactate data suggest delivery has been comprised. No diurnal rhythm in oxygen consumption was found other than that resulting from the observation that turtles tended to be quieter at night.

Surprisingly, in two instances, turtles at 30°C became very quiescent for a prolonged time and the oxygen consumption dropped to 0.06 - 0.08 ml $O_2 \cdot kg^{-1} min^{-1}$. This was possibly the result of some heat stress as the respiratory rates in these animals were the highest recorded (0.57 - 0.74 breaths min^{1}).

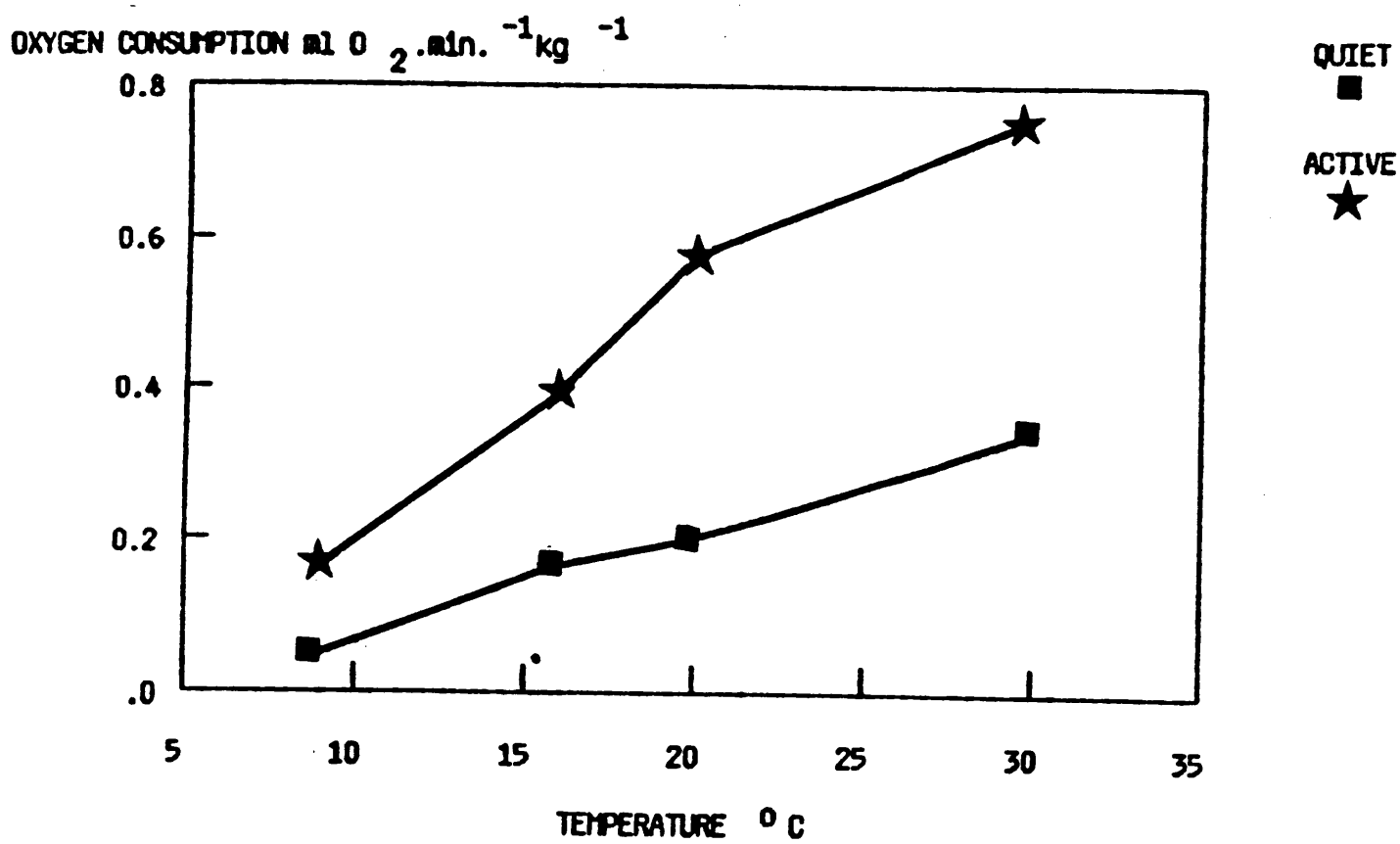


FIGURE 10. The effect of temperature on oxygen consumption of loggerhead turtle.

Ventilation

Little consistent change was seen in tidal volume or ventilation rate over the range 9°C - 30°C (table 4). However the air convection requirement $\dot{V}e/\dot{V}o_2$ increased markedly as temperature fell (Table 4, Fig. 11). The increase was particularly noticable in the "quiet" turtles where a fall from 30°C to 15°C resulted in a four fold increase in the Ve/Vo_2 ratio. Remarkably an almost identical change has been found for the fresh water turtle Pseudemy scripta in changes in blood P CO_2 (Jackson 1971) and might therefore be the ventilatory mechanism for turtles by which blood pH is adjusted to the appropriate value for body temperature. Significantly the process breaks down abruptly at 10°C. The air convection requirement at 30°C (23.6) is very similar to that found for nesting greens by (31.2) Jackson and Prange (1979).

With activity there is a higher extraction efficiency over the temperature range of 10-30°C. Under these circumstances oxygen requirements are probably the major determinants of ventilation.

5. Heart rate

The heart rate varied so much and was so affected by activity, submergence, length of submergence and breathing pattern the whole concept of a standard heart rate must be called into question. For example Fig. 12 is a record of the changes in heart rate over a 2-hour period in a free swimming loggerhead fully acclimated to the holding tank. The record can be divided into several phases. (a) The turtle rests on the bottom of the tank for approximately ½ hr with a heart rate of 4-6 beats per min (bpm) and transistory 8 bpm. (b) When the turtle rises to breathe the heart rate rises to 18bpm and then fluctuates around 8-11 bpm while the turtle slowly swims, rising to 13 bpm just after a breath. While quietly resting on the bottom (c) the heart rate slowly drifts down to 5 bpm, quickly rises to 11 bpm after a breath and fluctuates between 8-12 while slowly swimming (d).

TABLE 4

Effect of temperature on respiration of loggerhead turtle.

Temperature °C		Tidal volume (ml)	Respiration rate breaths min^{-1}	Ventilation rate $\dot{V}e$ ml $kg^{-1}min.^{-1}$	Oxygen consumption $\dot{V}o_2$ ml $kg^{-1}min.^{-1}$	$\dot{V}e/\dot{V}o_2$
30	a	482	0.54	16.5	0.75	21.8
	r	444 ±127	0.27 ±0.10	7.88 ±2.46	0.34± 0.084	23.6±4.1
20	a	263±63.1	0.83±0.21	15.18±2.15	0.57±0.137	28.1±7.0
	r	285±82.3	0.29±0.09	6.20±2.08	0.20±0.086	34.5±12.6
16	a	216±72.3	0.92±0.16	14.56±2.36	0.39±0.055	38.8±12.4
	r	395	0.48	14.85	0.17	94.9
9	a	239±124	0.46±0.21	7.24±2.58	0.17±0.063	48.8±26.06
	r	204±90.2	0.12±0.06	1.62±1.32	0.05±0.013	38.7±18.2

a = active
r = resting

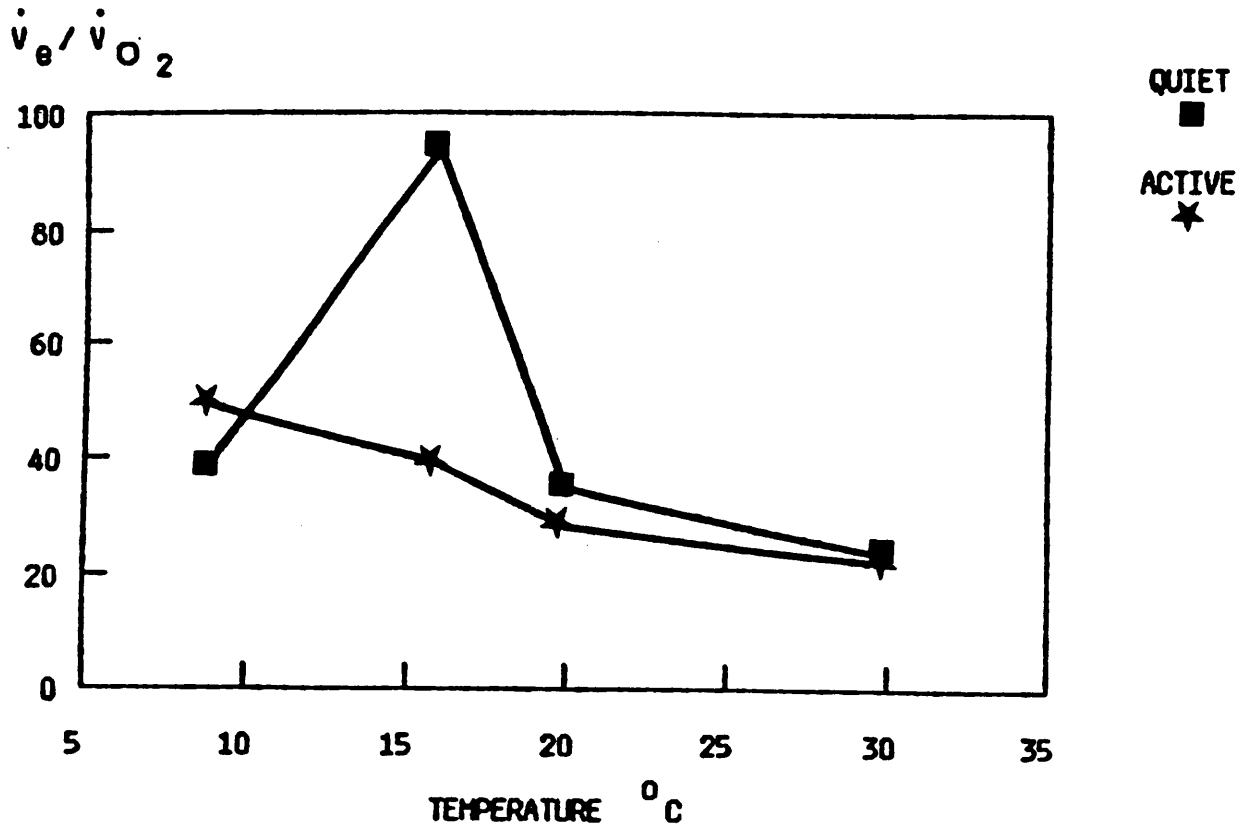


FIGURE 11. Oxygen extraction efficiency of the loggerhead turtle.

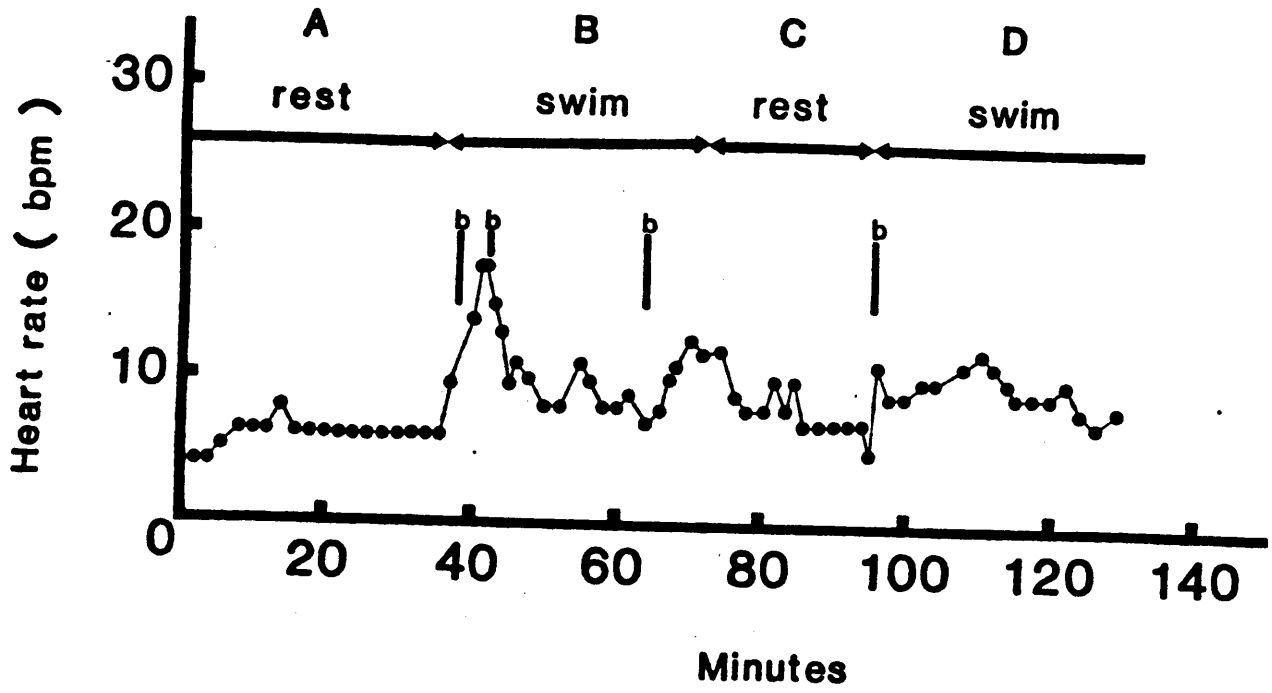


FIGURE 12. Variation in heart rate in free swimming turtle (b=breath taken). See text for further details.

In the temperature study no clear relationship was found between heart rate during activity and temperature Fig. 13 but interestingly the highest values were found at 10°C a probable result of the stress of this temperature. In this case heart rate would not be an index of metabolic rate as oxygen consumption is severely depressed at 10°C. Davenport (1982) likewise found no clear relationship between heart rate and temperature in hatching green turtles.

6. Forced submergence and recovery

1. Lactate

As mentioned above very high blood lactate values were recorded from turtles just taken from trawls. Although the rate of recovery was highest in the most stressed group (group C, Fig 14) the actual rate of recovery was slow, and animals on deck 3-5 hrs after release from the trawl nets still showed a considerable lactemia (Table 1). It is possible that disturbance of the activity on deck was a continuing stress to these animals, retarding the rate of recovery. In the simulated forced dive experiments, strapped turtles held underwater showed a similar increase in blood lactate (Fig 15).

The lactate levels remain elevated for at least 2.5 hrs after the forced dive indicating that the sea turtle has a very limited capacity for quick recovery after such an "insult." Commonly, in the laboratory and on deck, the turtles were lethargic and passive during this period. Clearly turtles that have been held underwater forcably (including trapped and dragged in a shrimp trawl) are physiologically disturbed for some considerable time after their release. Full recovery however had taken place within 24 hrs. (Fig 15).

The very large increase in lactate before the end of the forced dive contrasts to the very slow rise seen in marine mammals that have been forced to dive (Davis 1983). In marine mammals the major increase in blood lactate takes place after the dive, the results of reestablishing full circulation to all the body organs. This

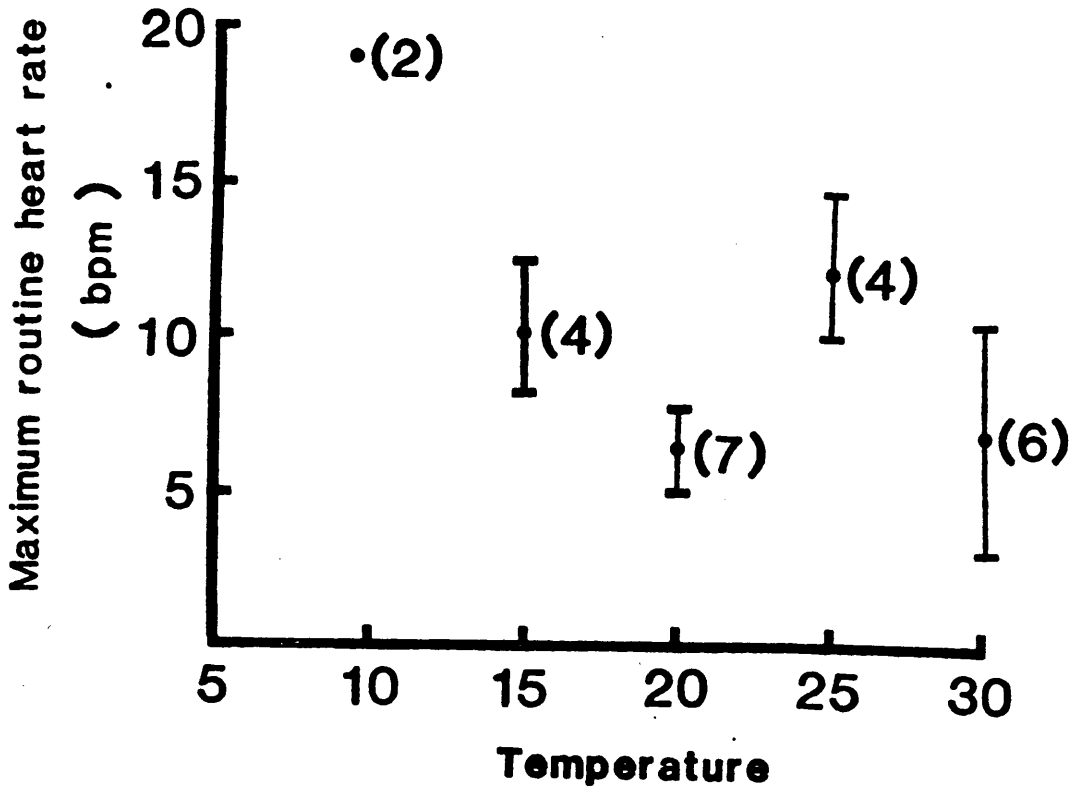


FIGURE 13. Effect of temperature on heart rate. Bar is standard error, number of animals in parenthesis.

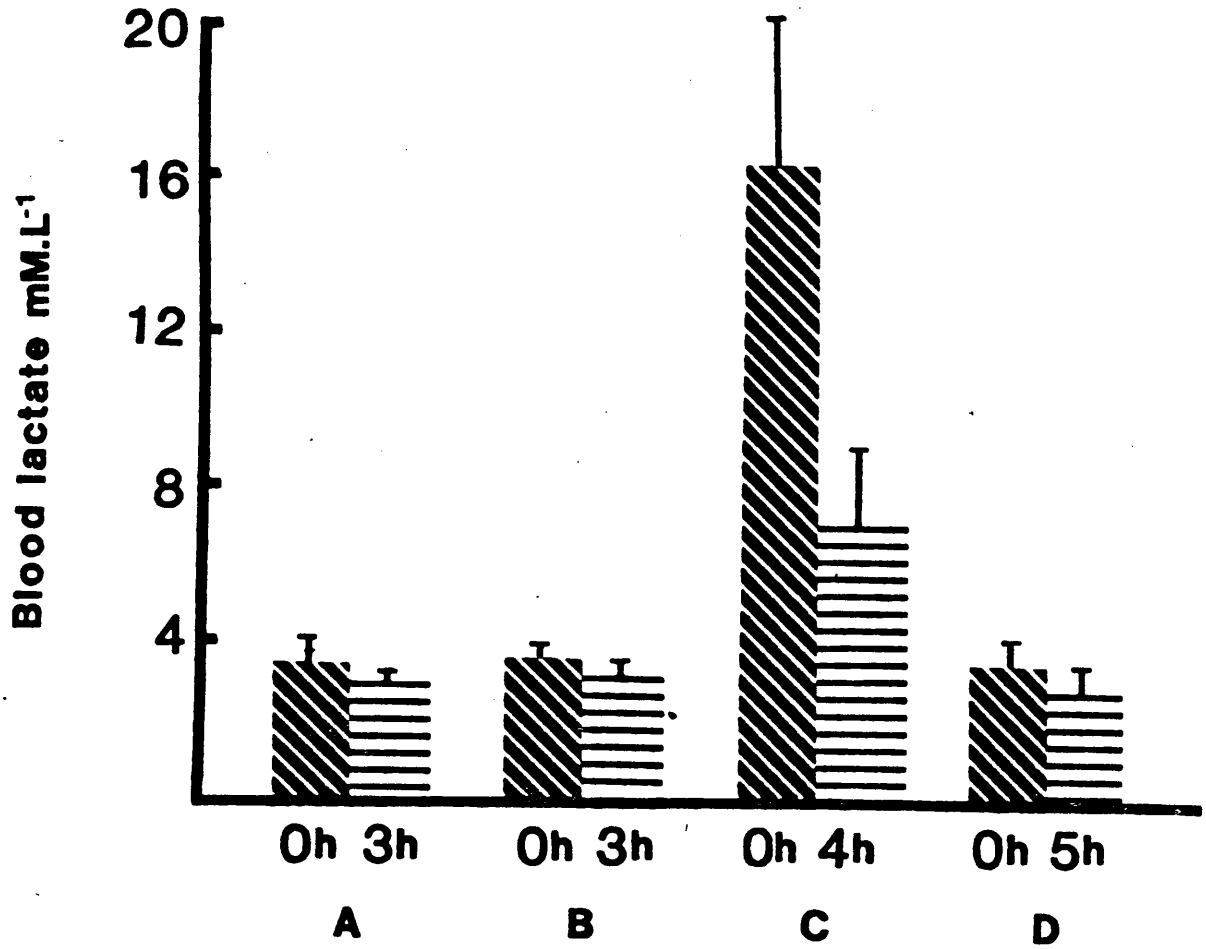


FIGURE 14. Blood lactate levels in loggerhead sea turtles, freed from shrimp trawl nets (0 h) and 3 and 5 hours later. Standard error bar indicated.

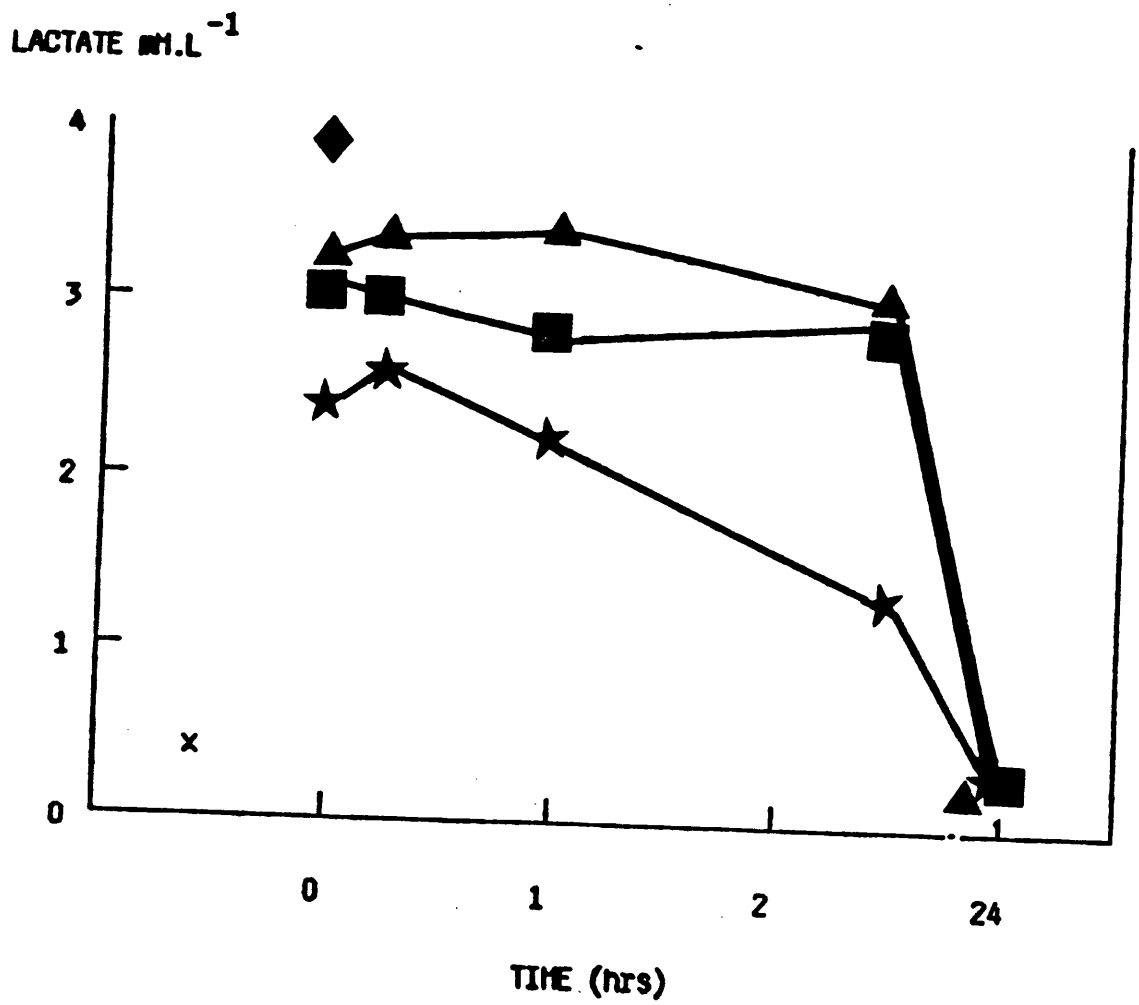


FIGURE 15. The changes in blood lactate concentration following forced dives in loggerhead turtle.

suggests that either the cardiovascular adjustments to diving made by the mammal are not found in the sea turtle or that all tissues, including brain, go anaerobic in the forced dive sea turtle. These are reasons to believe that the latter may be the most important response (Lutz in press).

2. Glucose

The predive glucose levels were 1.22 ± 0.6 mM and there was no indication that these levels fell through out the forced dive period (Fig 16). In the mammal by contrast blood glucose stores are depleted on forced dive (Davis 1983).

Presumably in the turtle the rate of glucose removal from the central pool is balanced by replenishment from the liver, an advantage of maintaining a relatively open circulation. Interestingly there was an increase in blood glucose within 10-15 min after the dive. Hyperglycemia has also seen in the turtles recovering on the deck of the shrimp trawlers and in some cases this persisted up to 4 hrs (Fig. 17). This suggests a temporary increase in hepatic glycogenolysis had occured. A very similar response in seen in the mammal under conditions of hypoxia. The temporary post dive hyperglycemia may serve to facilitate the rapid restoration of some key organs, eg brain, to energy balance.

3. Hematocrit

Subsequent sampling showed changes in hematocrit, and there was a slight, but statistically insignificant, increase in hematocrit 3 hours after the forced dive. (Fig 18). A temporary increase in hematocrit had been noted earlier on turtles recovering on deck after being trawled in shrimp nets (Lutz and Dunbar-Cooper 1979). There is evidence that in some species hematocrit may change to meet different circulatory requirements (Lutz 1982).

4. Blood chemistry

Interestingly, there was a fall in osmotic pressure after 3 hr on deck, from 322.8 ± 12.79 mOsm (n = 4) to 309 ± 8.09 mOsm (n = 4) that was statistically

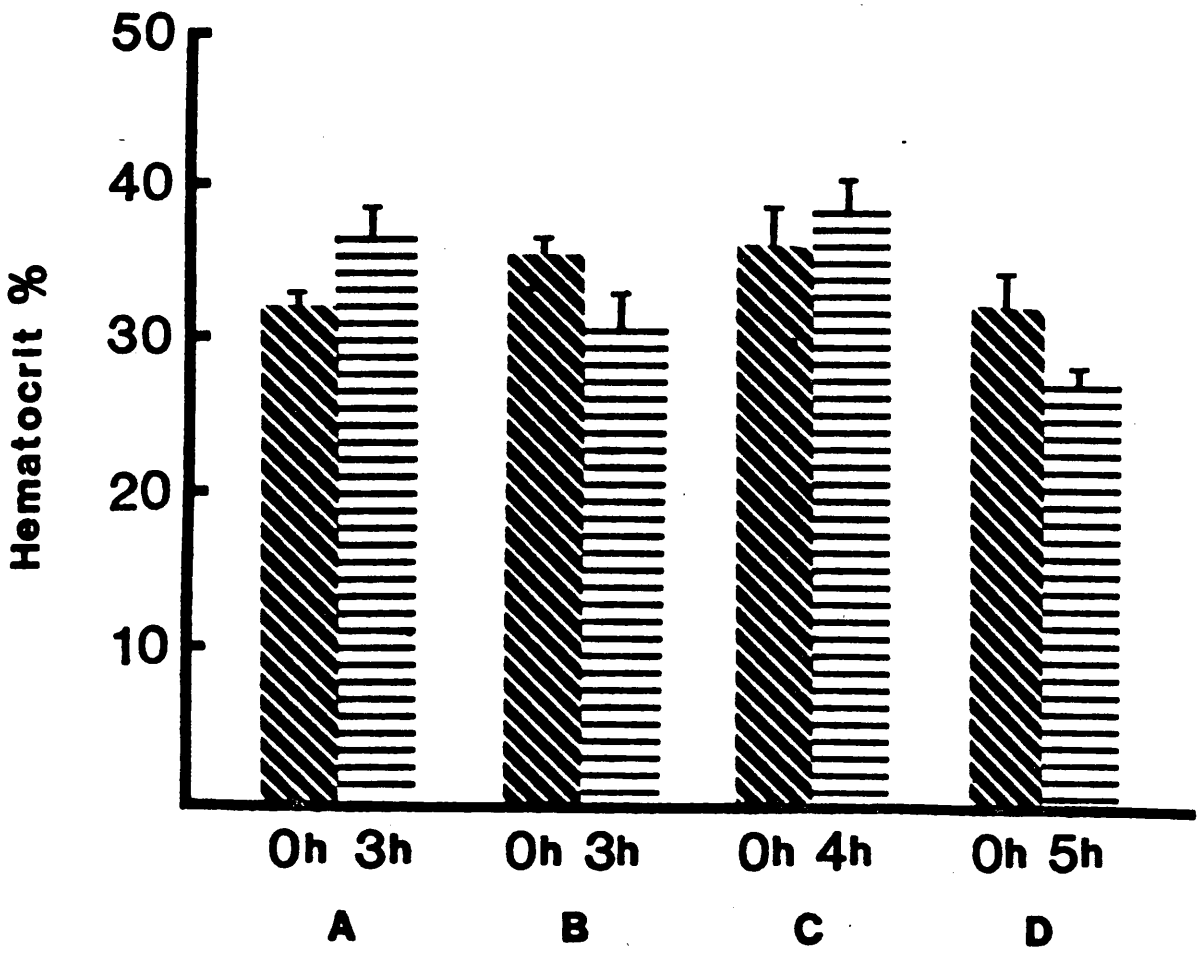


Figure 18. Hematocrit levels in loggerhead sea turtles, freed from shrimp trawl (0 h) and 3, 4 and 5 hours later. Standard error bar indicated.

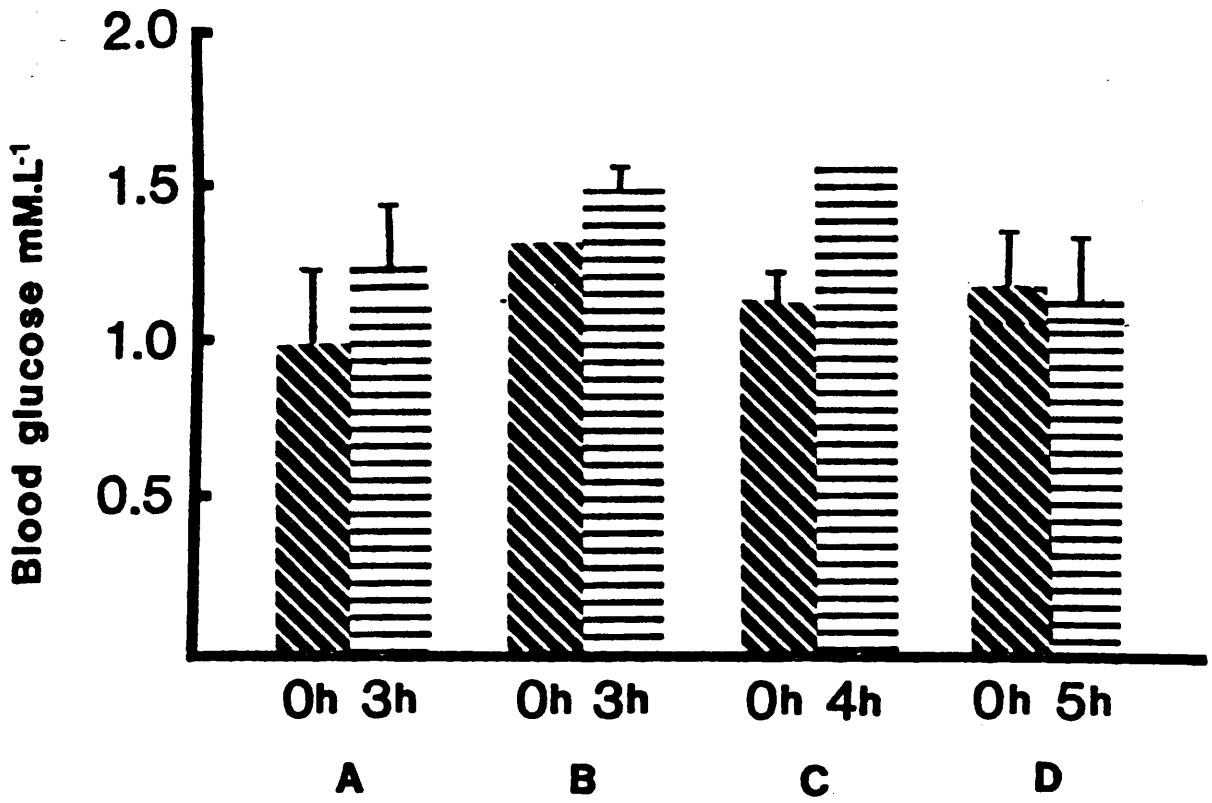


FIGURE 16. Blood glucose levels in loggerhead sea turtles, freed from shrimp trawl (0 h) and 3 and 5 hours later. Standard error bar indicated.

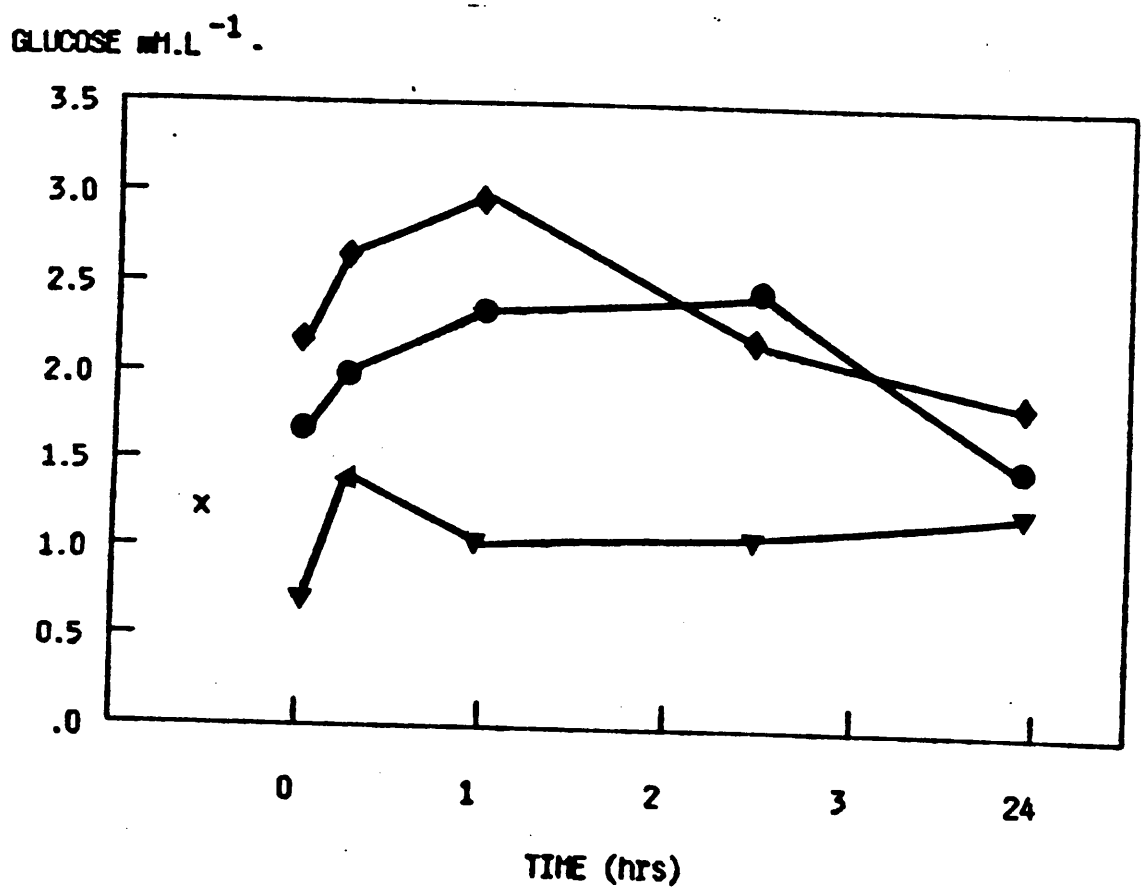


FIGURE 17. Changes in blood glucose concentration following forced dives in loggerhead turtle.

significant. However, no significant change took place in blood urea or sodium and chloride levels over this period, which remained similar to control values.

5. Heart rate

While for free swimming turtles the average heart rate ranged from 10-15 bpm, during struggling the heart beat of telemetered turtles reached as high as 30 bpm, i.e.,there appears to be an excitement tachycardia.

The forced dive experiments showed some very interesting similarities and differences. The initial heart rates were also high (27 -32 bpm) indicating that the stress of strapping had caused a significant tachycardia (Fig 19 a, b,c). Since these heart rates were all so similar, it is possible that about 30 bpm is the maximal heart rate for turtles of this size. This restraint tachycardia has been mistaken by some authors to be the normal heart beat (Berkson 1966). Forced submergence produced severe brachycardia with heart rates going down to as low as 1.5 bpm. But the times for the establishment of brachycardia were widely divergent, a 1 min, b 16 mins and C 70 min! Continued tachycardia was clearly related to the persistence of struggling and, in most cases, struggling while submerged resulted in transient increase in heart rate (Fig 19 a b c). Taking blood proved to be a particularly severe stress. In the case of the turtle that showed the most rapid onset of brachycardia, the low heart rate persisted for 17 mins after the animal had been taken out of the water (Fig 19 a). During this period the turtle was in a comatose state showing no external sign of life. We have observed such a death-like unresponsive state in laboratory and field animals after long forced submergence, and it can take as much as 3/4 hr before the first breath is drawn. However, maximal tachycardia was found as soon as regular breathing was established following a gradual decline in heart rate to the original (unstressed) values (Fig 14b).

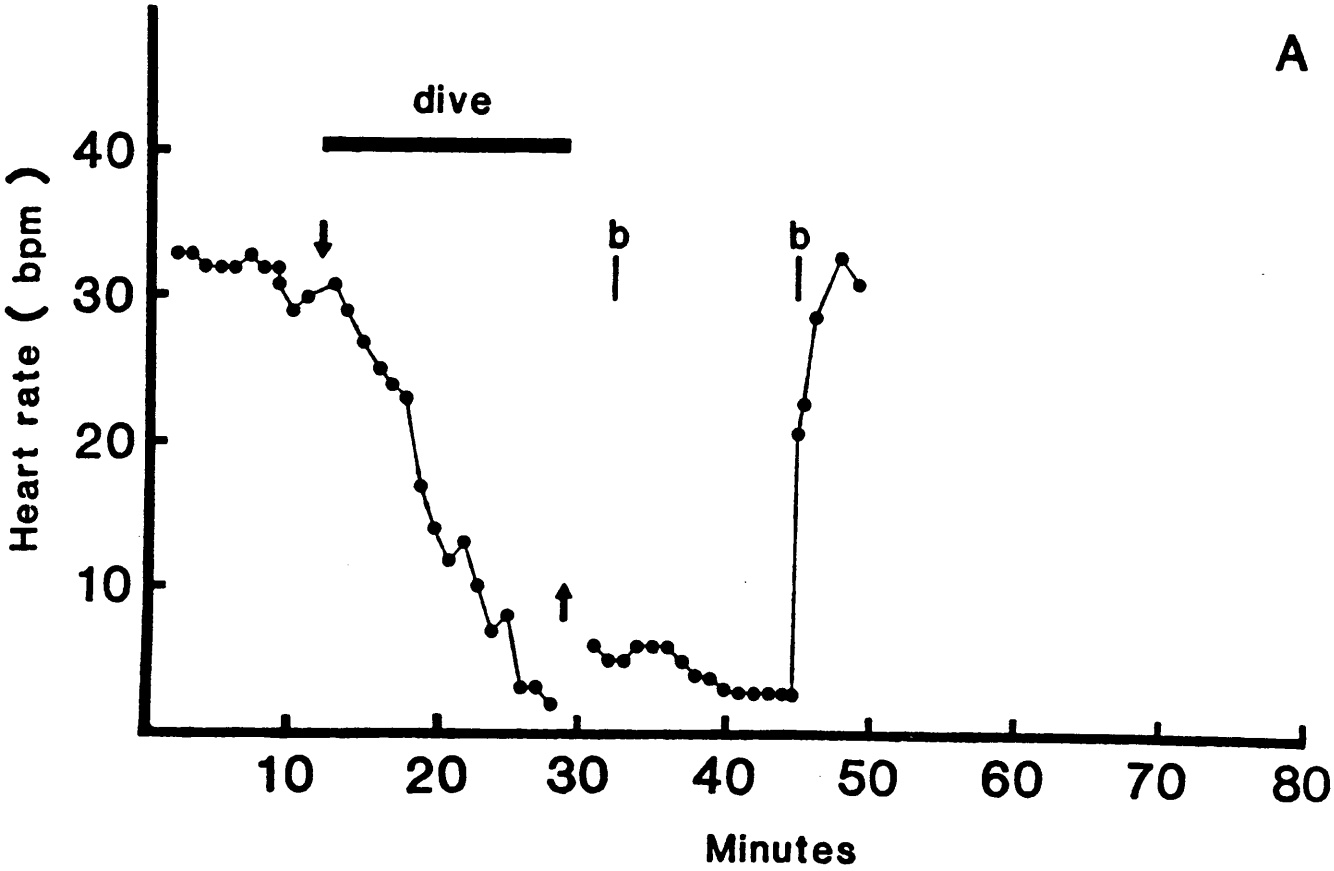


FIGURE 19A. Effect of forced dive on heart beat in turtle.
Bar indicates dive duration, s = struggle, b = breath, bl = blood taken.

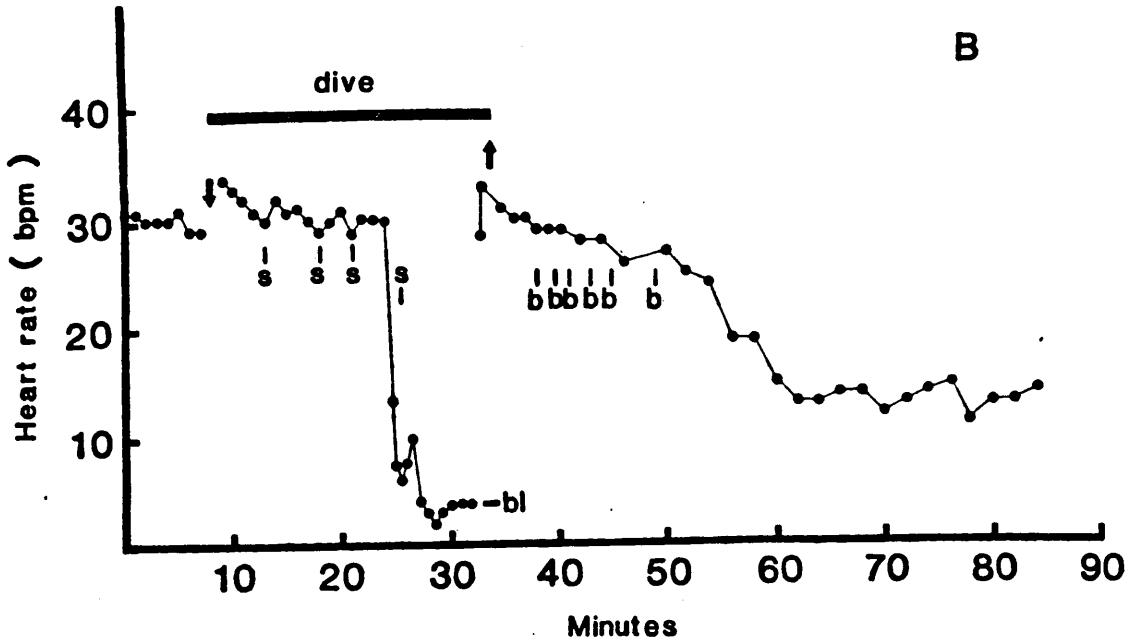


FIGURE 19B. Effect of forced dive on heart beat in turtle. Bar indicates dive duration, s= struggle, b = breath, bl = blood taken.

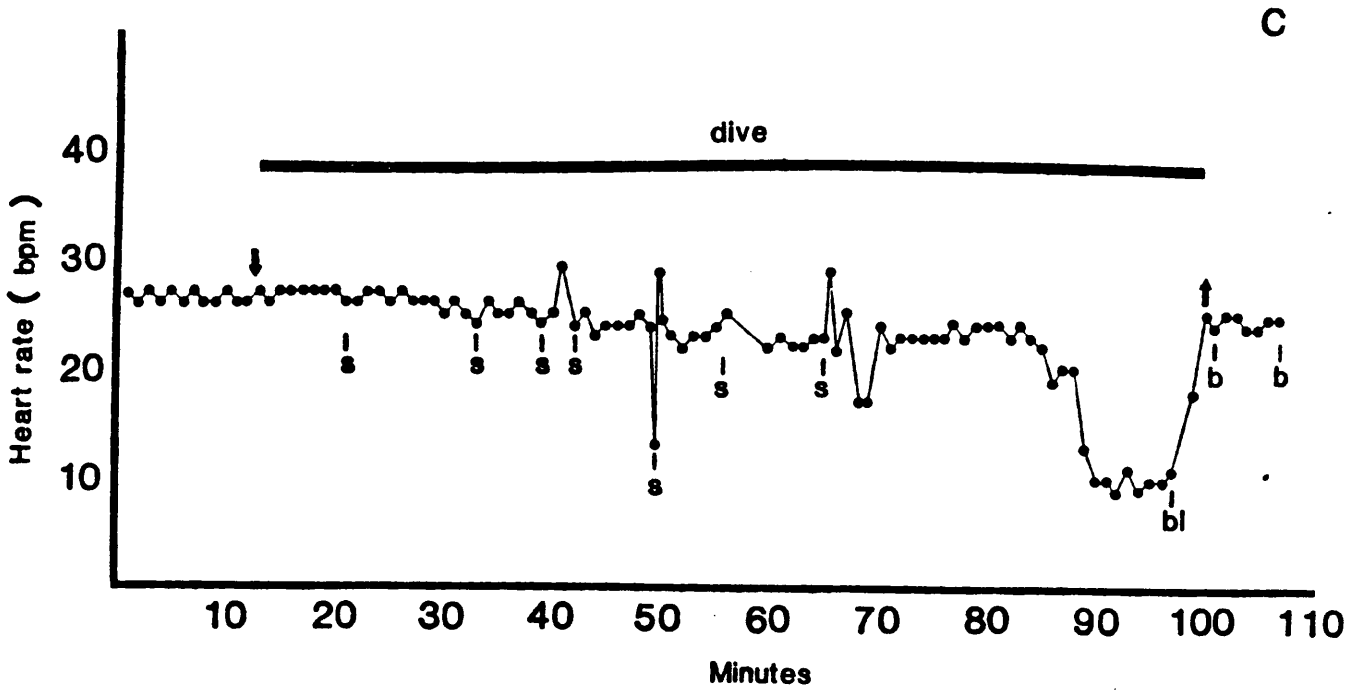


FIGURE 19C. Effect of forced dive on heart beat in turtle.
Bar indicates dive duration, s = struggle, b = breath, bl = blood taken.

SUMMARY AND CONCLUSIONS

Seasonal and temperature effects

Without doubt low temperature has a very marked effect on the biology of sea turtles. Behavioral disturbances were noted in some animals at temperatures less than 20°C and were manifested in most at 15°C. Severe physiological malfunctioning occurred at 10°C. However, between 10°C and 30°C there is evidence of a rather complex system of physiological adjustment.

The seasonal changes in blood potassium and urea that had been suggested in the earlier field studies appear to be mainly due to changes in temperature. Blood osmotic pressure likewise appears to be influenced by temperature. On the other hand, blood sodium and chloride values varied widely with no apparent relationship to either season or temperature. Magnesium and glucose values were very stable under all conditions and blood hematocrit showed little excursion. The acid-base balance of the loggerhead showed a highly significant and important dependency on temperature, in keeping with the "relative alkalinity" concept. The lower pH values at higher temperatures appeared to be caused by increased blood pCO_2 and lowered blood bicarbonate. The blood pCO_2, in turn, appeared to be regulated by an increase in the air convection requirement at higher temperatures.

Oxygen consumption was also higher at higher temperatures but activity was an overriding factor here.

Critical temperatures

The respiratory data suggested some stress effect at 30°C in that several individuals showed hyperventilation at this temperature with a depressed oxygen consumption. However, all animals showed a very sharp breakdown in physiological mechanisms at 10°C. Oxygen consumption was severely reduced while blood pO_2 increased pointing to a failure of oxygen uptake at the tissue level. The increase in blood potassium suggests that cell ion pump mechanisms are becoming less

efficient. The large fall in blood pH and rise in blood pCO_2 points to a collapse in the acid-base regulatory process and the large increase in blood lactate is evidence that anaerobic processes are being called upon to supply energy. In this context the increase in heart rate is paradoxical.

Temperature as a limiting factor in distribution.

It appears that between 10°C and 15°C there is a failure of important metabolic processes in the loggerhead one consequence of which is acidosis. Interestingly, Kraus and Jackson (1980) report a change in pH control occurs between 15 and 25°C in the more tropical green sea turtle, (Ackerman and White 1980) found that the Galapagos iguana becomes acidotic at temperatures less than 15°C, and Bickler (1982) reports that the tropical lizard Dipsosaurus dorsalis is acidotic at temperatures less than 18°C. They note that this lizard does not normally encounter temperatures less than 25°C. Although the evidence is circumstantial and meager, it suggests that perhaps the metabolic (or pH) temperature syndrome of a species is matched to its operating temperature range, and that the failure of the system is the physiological determinant of the lowest temperature that the species can tolerate. If this is true then it is a finding of some considerable importance in physiological ecology and it will be of great interest to describe the T/pH curves for other species of sea turtle.

Hibernation

It is now clearly apparent that for a loggerhead to survive in temperatures of around 10-15°C for any considerable time it must be in a quite different physiological state from normal, i.e. hibernation. We now have sufficient information on the normal state of loggerheads and the effect of cold stunning to be able to unambiguously recognize hibernation, but unfortunately since the Cape Canaveral study was started in 1979 a sufficiently cold winter has not yet appeared. Prime candidates for indicators are, however, a decrease hematocrit, and large increases in the tightly regulated plasma magnesium and calcium levels.

Forced submergence stress

Undoubtedly, forced submergence is a severe trauma for sea turtles. Mere confinement or handling can elicit maximal tachycardia. This is compounded by forced submergence whose effect depends very much on the degree of struggling. Struggling causes a rapid consumption of energy reserves and a high output of lactic acid. Sooner or later, submerged sea turtles lapse into a death-like comatose condition with a severe bradycardia. Under these conditions, a turtle brought on deck may show no outward sign of life including response to stimuli or breathing for up to 1 hr. Recovery appears to be very slow, the heart rate does not return to normal for as much as ½ hr. after the normal breathing pattern is established and lactic acid may still be very high after 5 hrs.

Resuscitation recommendations for trapped turtles released from shrimp trawls

In view of the above finding of the very severe physiological disturbances brought about by forced submergence, it is recommended that under no circumstances should a released turtle showing no outward sign of life, be declared dead until at least 3 hrs. have passed. Sharp agitation or pressing on the plastron after 15-30 mins. on board may stimulate breathing. Comatose or sluggish turtles held on deck should be placed belly side down with the posterior end elevated to assist sea water drainage from the lungs. Although ideally turtles should be held for 24 hrs. to allow for full recovery from the severe acidosis, it is probably sufficient to hold active turtles for 2-5 hrs. It might be possible to alleviate the acidosis by adding bicarbonate but it would be necessary to find out first of all if such a treatment was effective, the quantity necessary, and the best mode of application. Special precautions are necessary at temperatures less than 20°C and turtles must be sheltered from temperatures greater than 30°C.

LITERATURE CITED

Ackerman, R.A. and F.N. White. The effects of temperature on acid-base balance and ventilation of the marine iguana. Respir. Physiol. 39: 133-147. 1980.

Bentley, Timothy B. and Ann Dunbar-Cooper. A Blood Sampling Technique for Sea Turtles. Contract No. Na-80-GE-A-00082 for the National Marine Fisheries Service. September 1980.

Berkson, Harold. Physiological adjustments to prolonged diving in the Pacific green turtle (Chelonia Mydas Agassizii). Comp. Biochem. Physiol. 18: 101-119. 1966.

Bickler, Philip E. Intracellular pH in lizard Dipsosaurus dorsalis in relation to changing body temperature. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 53(6): 1466-1472. 1982.

Carr, A., L. Ogren, and C. McVea. Apparent hibernation by the Atlantic loggerhead turtle off Cape Canaveral, Florida. Biological Conservation. 19: 7-14. 1980.

Davenport, J., G. Ingle, and A.K. Hughes. Oxygen uptake and heart rate in young green turtles (Chelonia mydas). J. Zool., Lond. 198: 399-412. 1982.

Davis, Randall, W. Lactate and glucose metabolism in the resting and diving harbor sea (Phoca vitulina). J. Comp. Physiol. 153: 275-288. 1983.

Dunbar-Cooper, A., and P.L. Lutz. Changes in the blood chemistry of loggerhead sea turtles (Caretta caretta) trawled from the Cape Canaveral ship channel (Abstract). Am. Soc. Ichth. and Herp. 63 Ann. Meet. 1983.

Felger, R.S., K. Cliffton, and R. Regal. Winter dormancy in sea turtles: independent discovery and exploitation in the Gulf of California by two local cultures. Science 191: 283-285. 1976.

Gilles-Baillien, M. Seasonal variations in reptiles. In: Florkin, M. and Scheer, B.T. (Ed.) Chemical Zoology. IX: 353-376. 1974.

Jackson, Donald C., and Henry D. Prange. Ventilation and gas exchange during rest and exercise in adult green sea turtles. J. Comp. Physiol. 134: 315-319. 1979.

Jackson, D.C. The effects of temperature on ventilation in the turtle, Pseudemys scripta elegans. Respir. Physiol. 12: 131-140, 1971.

Kraus, David, R., and Donald C. Jackson. Temperature effects on ventilation and acid-base balance of the green turtle. Am. J. Physiol. 239 (Regulatory Integrative Comp. Physiol. 8): R254-R258. 1980.

Lance, Valentine. Studies on the annual reproductive cycle of the female cobra, Naja naja. Seasonal variation in plasma inorganic ions. Comp. Biochem. Physiol. 53A: 285-289. 1976.

Lutz, P.L. and A. Dunbar-Cooper. Physiological studies on loggerhead sea turtles caught on shrimp trawl surveys in the Cape Canaveral ship channel, Florida, December 1978 to April 1979. Final Report to NMFS, 21 pp. Contract FSE 43-9-12-40. 1979.

Lutz, P.L. Report on the physiology of sea turtles for July, August, and September 1979 of surveys of Cape Canaveral, Florida. FSE 43-9-12-40. National Marine Fisheries Service. 1980.

Lutz, P.L. Oxygen transport in vertebrate blood. Challenges. In: K. Johansen and C.R. Taylor (Eds.), A Companion to Animal Physiology, Cambridge University Press. 1982.

Lutz, P.L. and T.B. Bentley. Adaptions to diving in the sea turtle. Copeia. In press.

Maclean, Graeme S., Anthony K. Lee, and Philip C. Withers. Haematological adjustments with diurnal changes in body temperature in a lizard and a mouse. Comp. Biochem. Physiol. 51A: 241-249. 1975.

Ogren, Larry and Charles McVea, Jr., Apparent hibernation by sea turtles in North American waters. In: Bjorndal, Karen A. (Ed.) Biology and Conservation of Sea Turtles. Conserv. Wash., D.C. Smithsonian Inst. Press. 583 pp. 1982.

Reeves, R.B. The interaction of body temperature acid-base balance in ectothermic vertebrates. Annu. Rev. Physiol. 39: 559-586. 1977.

Schwartz, F.J. Behavioral and tolerance responses to cold water temperatures by three species of sea turtles (Reptilia, Cheloniidae) in North Carolina. Florida Marine Research Publication 33: 16-18. 1978.

Severinghaus, J.W., M. Stupfel, and A.F. Bradley. Variations of serum carbonic acid pK' with Ph and temperature. J. Appl. Physiol. 9: 197-200. 1956.

Snyder, G.K. Influence of temperature and hematocrit on blood viscosity. Am. J. Physiol. 220: 1667-1672. 1971.

Truchot, J.-P. L' equilibre acido-basique extracellulaire et sa regulation dans divers groupes animaux. J. Physiol., Paris. 77: 529-580. 1981.