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# Assessing Environmental Stress on the Loggerhead Sea Turtle (Caretta caretta) in Virginia Waters

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*College of William and Mary - Virginia Institute of Marine Science*

# ASSESSING ENVIRONMENTAL STRESS ON THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) IN VIRGINIA WATERS

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A Thesis

Presented to

The Faculty of the School of Marine Science

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In Partial Fulfillment

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Master of Arts

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by

Sarah A. Bellmund

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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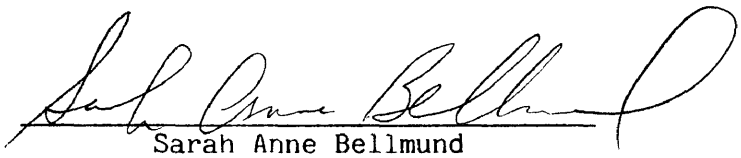

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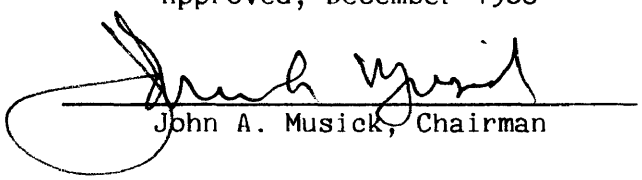

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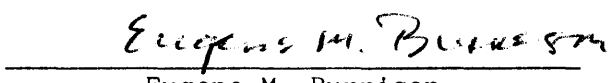

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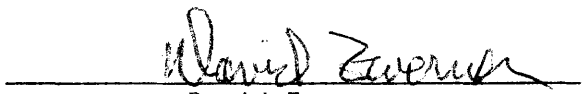
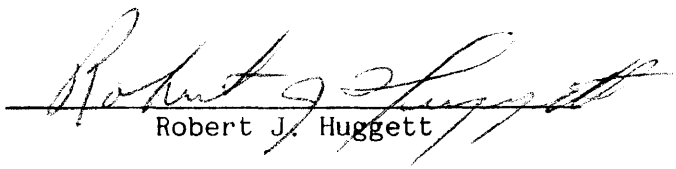
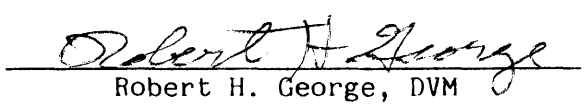


David Zwerner



Robert J. Huggett



Robert H. George, DVM

DEDICATION

This work is dedicated to the Watermen of the Chesapeake Bay for their knowledge, appreciation, and love of the Bay, especially, Otis Carney, Kenny Ethridge, Ed Boyd, Walter Cole Burroughs, and particularly Richard Erdt and Talmadge and Fred Jett for sharing turtles, knowledge of turtle biology, and knowledge of the Bay and especially for making this work so enjoyable.

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ABSTRACT

Blood was taken from wild juvenile loggerhead sea turtles (Caretta caretta) that had been brought to the dock by cooperating fishermen during 1983-1985. Total protein, albumin, calcium, phosphorous, uric acid, creatinine, bilirubin, sodium, potassium, chloride, urea nitrogen, glucose, and corticosterone were measured in serum. Concentrations of these compounds changed in response to environmental changes in salinity, temperature and possibly prey availability. Profiles of juvenile loggerhead serum values were analysed using the multivariate methods of principal component analysis (PCA) and Q-Mode factor analysis. The results of PCA showed changes in sick animal's serum values attributable to impaired osmoregulation. The results of Q-mode factor analysis showed a strong relationship in animal groupings with temperature and salinity as well as stress. In most cases, stress effects were those associated with extreme pathological compromise or heat stress. Some cases showed no obvious correlation to any factor or variable measured and were attributed to underlying causes not detected in the survey methods. The resulting data provides background serum chemistry values for juvenile loggerhead sea turtles and illustrates the usefulness of the multivariate techniques of PCA and factor analysis in describing data relationships that are otherwise not obvious from general observation or univariate statistics.

# ASSESSING ENVIRONMENTAL STRESS ON THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) IN VIRGINIA WATERS

# I. INTRODUCTION

Chesapeake Bay is a major foraging ground for immature Atlantic loggerhead (Caretta caretta) sea turtles (Lutcavage, 1981; Lutcavage and Musick, 1985). During each summer season sea turtle mortality increases drastically, beginning within 2-3 weeks after the arrival of turtles in the spring. Conservation and management personnel who are concerned with the long-term survival of sea turtles require an understanding of the role of environmental stress as a factor that influences the survival of individuals within regional populations of animals. The purpose of this work was to determine the value of serum components as a means to evaluate physiological and environmental stress on juvenile loggerhead turtles (C. caretta) in Chesapeake Bay.

Annual migration of sea turtles into Chesapeake Bay begins in late April and early May (Musick et al., 1984). Turtles reside in the Bay, foraging during the summer, and departing in early fall. Migration from the Bay corresponds with the first severe decline in water temperature, which generally occurs in October. Sea turtle mortality begins within 2-3 weeks after the arrival of turtles in the spring. Although mortality continues throughout the summer, it declines sharply at the end of June. This early summer mortality may comprise up to 78% of the mortalities observed during the entire year

(Lutcavage and Musick, 1985). Over 75% of the documented mortalities in Chesapeake Bay sea turtles since 1979 have occurred during May and June (Musick et al., 1984).

The cause of the mortality observed in Chesapeake Bay sea turtles could not be determined in over 66% of the cases examined for 1979-1984 (Musick et al., 1984; Bellmund et al., 1987). Known causes of mortality in other sea turtle populations stem from a variety of causes including: habitat destruction; predation of eggs, hatchlings, and adults; or interference from commercial fishing vessels (Hildebrand, 1981; Hillstad et al., 1981; Bacon, 1981; Ross, 1981). Possible causes for the mortality observed in Chesapeake Bay during May and June include natural population fluctuations, fishing gear entrapment, disease, and reduced condition due to over-wintering or migration.

Virginia Institute of Marine Science (VIMS) personnel determined the cause of death for 88 of 285 turtles examined during 1979-1983 (Musick et al., 1984, Lutcavage and Musick, 1985). Due to decomposition, most carcasses proved unsuitable for histological examination (Bellmund et al., 1987). Table 1 shows the relative percent for each of the 88 cases where the cause of death was determined. Extensive postmortem changes in beach stranded sea turtles precluded determination of the causes of death in these animals as is the case in most beach strandings of marine animals (Jacobson, personal communication; Stevens, personal communication).

TABLE 1.

Apparent Causes of Death in Virginia Sea Turtles Examined between 1979-1983
(After Bellmund, et al., 1987)

| Category | Number | Percent |
|---|---|---|
| Undetermined | 197 | 69.0 |
| Net Related | 53 | 18.6 |
| Shark Related | 1 | 0.4 |
| Prop Damage | 21 | 7.4 |
| Intentional (Human Induced) | 9 | 3.2 |
| Other Fishing Gear | 4 | 1.4 |
| Total | 285 | 100 |

Many of the turtles that enter the Chesapeake Bay during spring are apparently in worse condition than those that depart in the Fall (Lutcavage and Musick, 1985). A hypothesis explaining this observation is that the first wave of migrants consists of animals that are healthy and animals that are diseased or stressed due to a variety of factors. Stressed or diseased animals die off early in the season and once these animals are removed from the population, mortality declines sharply. If this hypothesis is true then live animals arriving in the earliest portion of the season should constitute two groups with a range between two extremes. One group would survive the season and leave healthy in the fall and the other group would be pathologically compromised and die during the early part of the season.

Sources of stress during migration which may be responsible for mortality include; reduced feeding, energy expenditure, and temperature changes. Other stresses may result from previous capture and associated aspiration of seawater. Animals with these conditions may constitute the stressed or diseased portion of the population. This terminally stressed group would make up the portion of the population that die with no detectable causes in the early part of a summer season in Chesapeake Bay. Early season mortality may represent the death of sub-clinically ill animals.

Reptiles often show no visible signs of disease until actually becoming moribund (Frye, 1981; Lutz, 1972; Jacobson, personal communication). However, disease conditions may be reflected by

shifts in various blood and serum components prior to the animal becoming moribund. Blood has been examined in individuals in various wildlife populations in an attempt to evaluate the effects of environmental stresses (Grunow et al., 1984; Sturbaum and Bergman, 1981; Frye, 1981). These methods have been used to reflect the internal status of individual members of a population with no previous history on the individual. Lutz and Dunbar-Cooper (1987) used these methods to describe the physiological state of sea turtles from Florida waters. Sturbaum and Bergman (1981) used serum chemistry to describe changes induced by heat stress in the box turtle.

All animals that spend the summer in Chesapeake Bay experience the seasonal changes in physical regime associated with a temperate estuary. Resulting physiological changes are then due to factors which include the following: seasonal temperature fluctuations, salinity differences from area to area, and nutritional effects from variations in prey availability. In addition, other factors which may cause more drastic shifts in individual animal measurements include prolonged diving or increased activity during a tidal cycle (Lutcavage et al., 1987; Byles in prep.). These physical cycles affect physiological relationships and pathological conditions that are then reflected in serum chemistry of the general population of C. caretta in Chesapeake Bay.

The purpose of this study was to measure blood components, examine them in context of environmental changes present, and

evaluate them for use in describing the general pathological state of non-captive Virginia populations of juvenile loggerhead sea turtles. The parameters examined included enzymes, proteins, electrolytes, and nitrogenous waste products. Samples from animals were examined by location, broken into early season and late season, and grouped together for multi-variate analyses. Methods chosen to examine this data, beyond examination of mean trends, were principal component analysis (PCA) and Q-mode factor analysis. These procedures allow the examination of variability in blood components resulting from complex physiological and pathological interrelationships.

# II. METHODS AND MATERIALS

## Physical Examination and Location

Sea turtles used in this study were specimens that were incidentally captured alive in pound nets in the lower Chesapeake Bay. They were brought to the dock by pound net fishermen working in cooperation with personnel from VIMS. Pound nets are stationary fishing weirs, which provide free access to air in the head portion of the net and have large openings that allow free movement in and out of the nets for turtles (Lutcavage, 1981; Bellmund et al., 1987). Animals were kept in shade on the boat until their arrival at the dock and subsequent sampling (1-4hrs). Once at the dock, blood was removed using the methods described below, and the animals were examined and measured. After examination and sampling, animals were released from the dock. If the animal appeared stressed or it was not possible to sample blood at the dock, it was taken back to VIMS and allowed to recover in a tank with flowing sea water before blood was drawn. Locations were identified by zone (Figure 1). To facilitate other comparisons zones were combined to represent areas that may comprise homogeneous groups of animals. These areas and their respective zones are combined in Figure 2.

Figure 1. Zones for Virginia Study Site

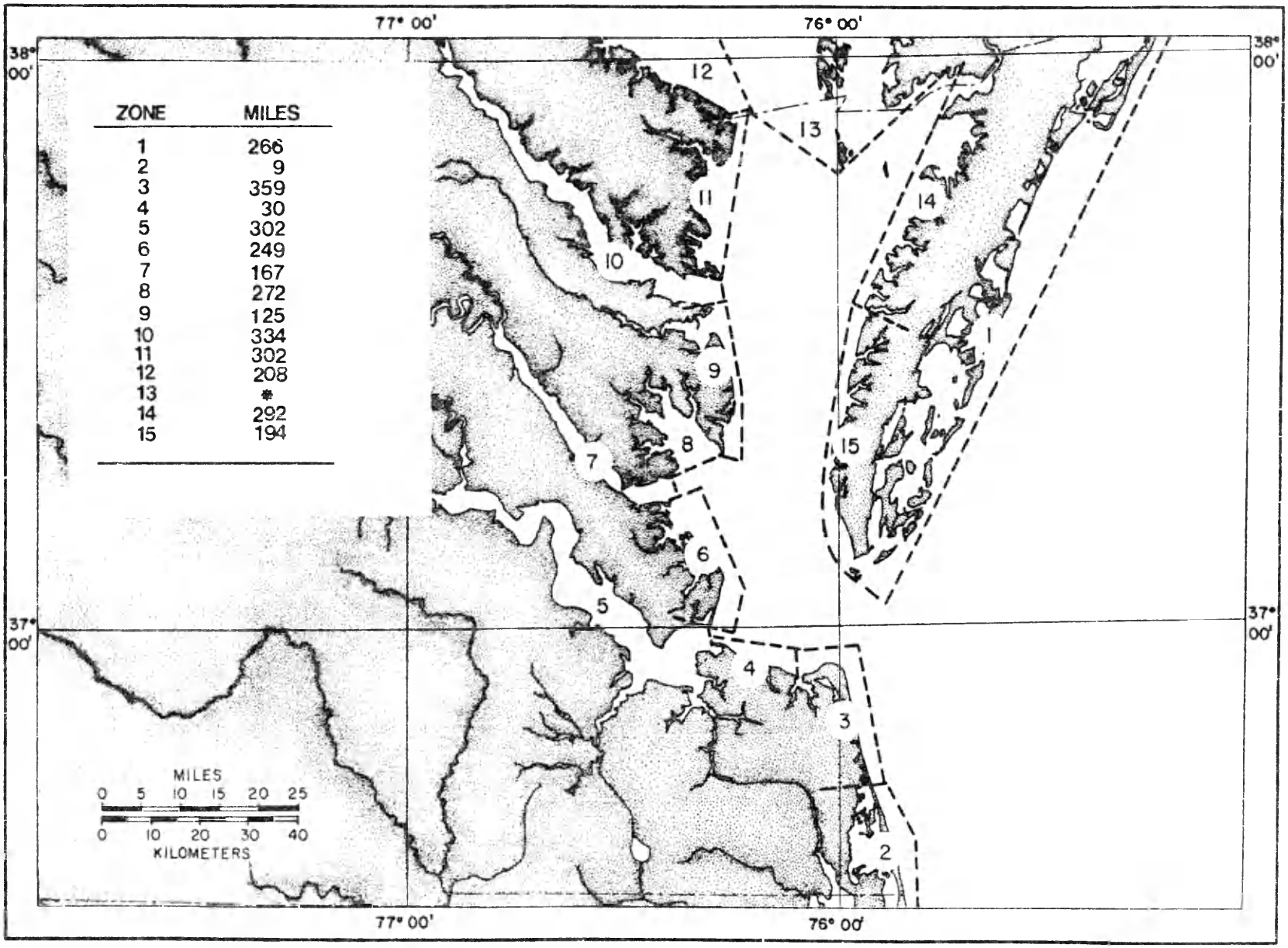
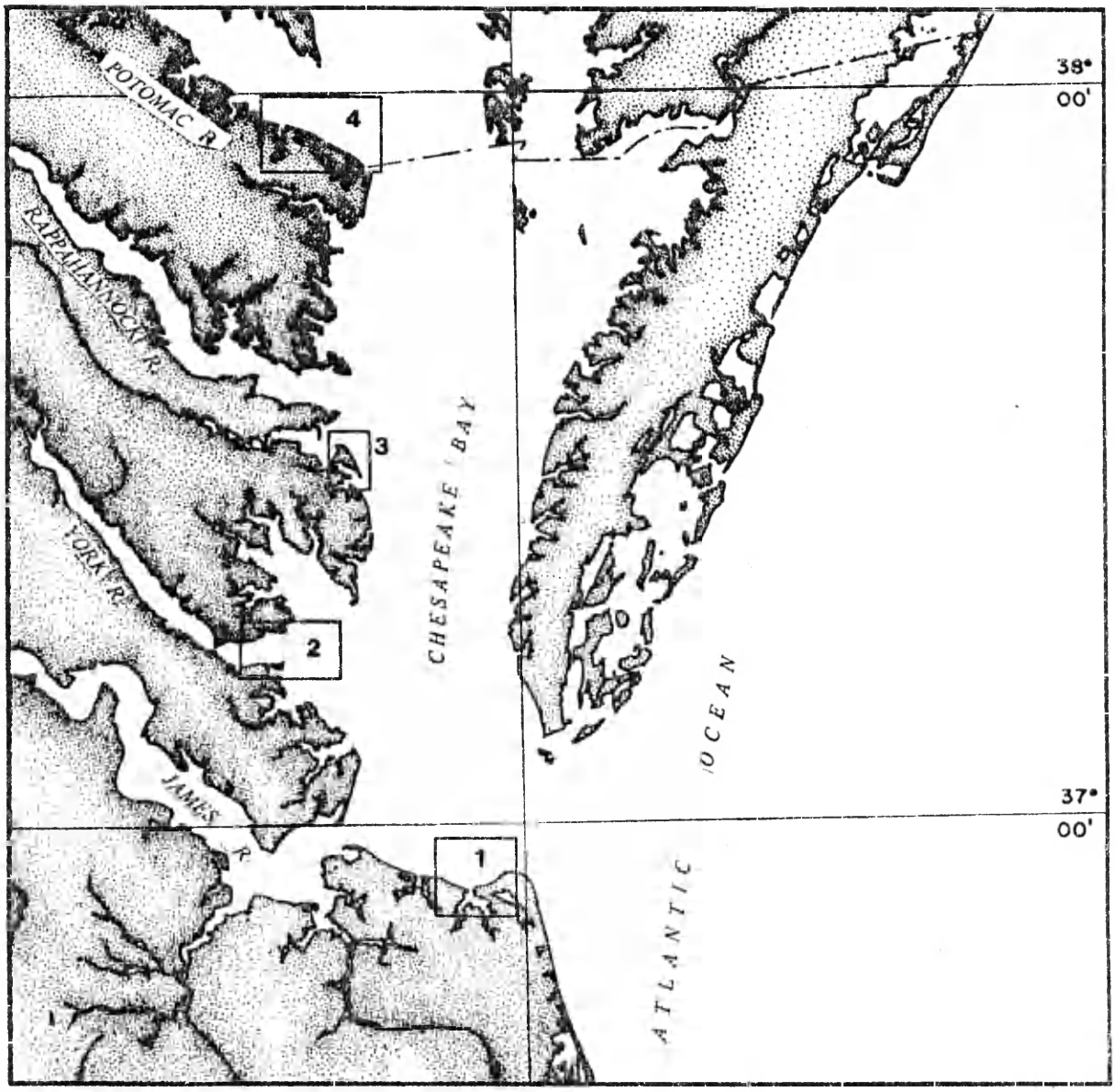

77° 00'
76° 00'
38° 00'
37° 00'
ZONE MILES
1 266
2 9
3 359
4 30
5 302
6 249
7 167
8 272
9 125
10 334
11 302
12 208
13 *
14 292
15 194
MILES
0 5 10 15 20 25
0 10 20 30 40
KILOMETERS

Figure 2. Northern and Southern Areas For Blood comparisons


POTOMAC R.
RAPPAHANNOCK R.
YORK R.
JAMES R.
CHESAPEAKE BAY
ATLANTIC OCEAN
38° 00'
37° 00'
1
2
3
4

STUDY AREA

Weight and straight line length and width measurements were taken as described in Table 2. Dimensions of turtles were taken with one meter tree calipers to the nearest 0.1cm, and a tape measure to the nearest 0.5cm. Weight was taken with a spring balance to the nearest 0.5kg. Cloacal temperatures were taken, when possible, with a laboratory alcohol thermometer or a Schulteiss quick recording thermometer. Water temperatures and average salinities were taken from available Chesapeake Bay data.

Blood Sampling and Analysis

Blood was taken from live animals only. It was drawn aseptically from the cervical sinus using a non-heparinized 10cc syringe and a 20G:1 1/2 needle (Owens and Ruiz, 1980; Bentley and Dunbar-Cooper, 1980). Blood was transferred to a siliconized 10cc vacutainer tube and allowed to clot at 4°C for 45 minutes. Blood for serum chemistry was spun at 6000g for 25 mins. Serum was removed with a pipet and transferred into a separate test tube for storage. Serum samples were frozen at -20°C to -40°C until analysis.

Serum was analyzed by several techniques. Testosterone and corticosterone were determined using radioimmune assay (RIA) at Texas A&M University (Morris, 1982). Hormones were measured using RIA techniques according to Owens (1978), Morris (1982), and Morris and Owens (1982).

Table 2.

Sea Turtle Morphometric Measurements

| Measurement | Description |
|---|---|
| Straightline carapace length (CLS) | Length of top shell at longest points, taken with calipers. |
| Straightline carapace width (CWS) | Width taken at widest point with calipers. |
| Curved carapace length (CLC) | Longest length over curve using a tape measure. |
| Curved carapace width (CWC) | Width at widest point, over curve using a tape measure. |
| Plastron length (PL) | Length of plastron at longest point. |
| Plastron width (PW) | Width at widest point of plastron, not including bridge plates, taken with calipers. |
| Plastron with bridge (PWB) | Width at widest point, includes bridge plates, taken with calipers. |
| Weight | Taken with spring balance to nearest 0.5 kg. |

All sera were analyzed using a Hitachi 705 blood analyzer system set to measure reactions within the linear range of sample concentrations (Appendix I). Serum enzyme levels were too low or too variable in concentration to measure due to sample handling effects rather than machine range.

Sera was taken from one individual animal held at two constant temperatures. Blood was sampled before and after feeding, over time, to monitor the change in glucose and urea nitrogen concentrations (Appendix II). Blood was taken once before feeding and sequentially in twenty minute intervals for the first two hours and then approximately every two hours until concentrations decreased to original pre-feeding levels. The first temperature used was 20°C and the animal had been held at that temperature for several months. The animals body temperature was then raised to 25°C over a 4 day period. The animal was not allowed time to physiologically acclimate and the same procedures were then carried out at this temperature. Prior to the first feeding, food was with-held from the animal for seven days. The animal was given 6.6kgs of thawed striped bass (Morone saxitilis) for both feedings. The turtle, was a small male weighting 32 kg with shell length, CLS of 59.4 cm and width, CWS of 48.5 cm.

Blood was taken from live animals only. Serum concentrations of the following constituents were measured; total protein in g/dl (TPRO), albumin in g/dl (ALB), globulins in g/dl (calculated as difference from TPRO - ALB), serum glutamate oxaloacetate

transaminase (SGOT), lactate dehydrogenase (LDH), alkaline phosphatase (ALK), calcium in mg/dl (Ca), inorganic phosphorus in mg/dl (Pi), sodium in MEQ/l (Na), chloride MEQ/l (Cl), potassium in MEQ/l (K), creatinine in mg/dl (Crea), uric acid in mg/dl (UA), blood urea nitrogen (BUN) in mg/dl, total bilirubin, glucose in mg/dl (glu), testosterone in ng/ml, and corticosterone in pg/ml (cort). The condition factor Q (where $Q=\text{weight}/(\text{length})^3$ ), proportional to density of the animal, was calculated and used when appropriate. Turtles that were classified as being apparently healthy were used for all mean calculations unless noted. Means were calculated using a single sample per individual, no double samples were included unless noted.

Initially, results were examined for mean trends with means given as $\pm$ SD. For comparison and statistical analyses, animals were classified by sex, month, size, location and state of health. Sex was determined by testosterone titer in serum. Animals were grouped into two size categories, those $<$ 70.0 cm and those $>$ 75.0 cm, using 75.0 cm CLS as the lower size limit for nesting females (Martin, personal communication; Richardson et al., 1978). Turtles 70-75 cm were used as dividing groups and were not included in this Table only. Samples for individual animals were divided by location as previously described. Turtles were subjectively defined as apparently (or clinically) healthy if they were not emaciated, injured or obviously ill, otherwise they were defined as sick.

## Multivariate Analysis

Multivariate analysis was evaluated to determine effectiveness in relating internal homeostasis to health using blood values from field sampled animals. In this work, the multivariate techniques of principle component analysis (PCA) and Q-mode factor analysis were used to examine relationships in serum components and individual animals. Principle component analysis was run using the 'Factor' routine without iteration on a correlation table in Statistical Package for the Social Sciences (SPSS) Release 7-9 (Nie et al.,1975; Hull and Nie,1981). Homogeneity of variance was tested using Cochran's test and Bartlett-Box F in the 'Manova' routine of SPSS release 7-9 (Nie et al., 1975; Hull and Nie,1981). All multivariate analyses were run on object sets with no missing variables. Q-mode analyses were run using CABFAC, QMODEL, and EXQMODEL, programs developed by Klovan and Miesch (1975). Their modification and adaptation to the PRIME are discussed by Berquist (1986).

### Factor Analysis

Factor analysis is a multivariate technique essentially resulting in a reduction of variable space. Its analytical intent is to find m linear combinations of original n variables to describe real entities without significant information loss (Klovan, 1975). An in-depth discussion of factor analytic techniques can be found in Davis (1986) and Joreskog, et al. (1976). The following discussion is a general description of these methods as they were applied to this data set and relies heavily on these texts.

Factor analytic methods can be divided into two general techniques. These techniques are; R-mode, which includes Principal Component Analysis (PCA), and Q-mode. R-mode techniques place variables in groups and measure the interrelationships between these groups of variables. Q-mode techniques measure interrelationships between objects (individual turtles sampled). Differences between PCA and 'true' R-mode analyses are due to factor determination methods and lack of a statistical model for PCA (Joreskog et al., 1976). In component analysis factors are defined to maximize the percent variance explained. In 'true' R-mode factor analysis factors are chosen to account for the maximum amount of intercorrelation of the variables (Joreskog et al., 1976). Functionally R-mode analyses describe interrelationships within a data matrix of m variables and reflect the correlation of each variable with p mutually uncorrelated underlying factors, assuming $p<m$. (Davis, 1986).

Principal Components

Principal components are the eigenvectors of a variance-covariance or correlation matrix, depending upon the original calculations. In principal component analysis the maximum amount of variance will be resolved by the first principal axis. If a matrix is represented as a series of vectors in multidimensional space and each row of the matrix give the coordinates of the vector represented by that row, then, as in the 2 x 2 example, the vectors define arbitrary axes of a hyperdimensional figure. The eigenvectors now define the orientation of the principal axes of the hyperdimensional

figure and the eigenvalues represent the magnitude of the variance explained by each principle axis. In this way the linear combination of variables that explains the maximum amount of variation in the data can be found. PCA in the SPSS routine bases calculations on the correlation matrix and so resulting groups are unitless (Joreskog et al., 1976).

PCA was then run on groups arranged by time from the apparently healthy animals. Animals were divided into early season (May and June) and late season (July, August, and September). Relationships in this breakdown by time were indeterminant so the data was then analyzed using Q-mode factor analysis.

Q-Mode Analysis

Q-mode analysis was run on all single Caretta blood value sets that had no missing values. The Cosine theta similarity coefficient index was used to assess relatedness. The following serum parameters were used in this analysis;

| | |
|---|---|
| Total Protein | Sodium |
| Albumin | Potassium |
| Calcium | Chloride |
| Phosphorus | Blood Urea Nitrogen |
| Uric Acid | Glucose |
| Creatinine | |

# III. RESULTS

Preliminary evaluation:

Means values of blood serum parameters were calculated by month (Table 3), by location (Table.4), by sex (Table 5), by size (Table 6), and by condition (Table 7). Means are not reported for serum enzymes since they were found to be extremely variable and were affected by differences in handling of samples. Means for uric acid and creatinine are included but the interpretation of these values is uncertain since circulating concentrations of these compounds are at or below the detection levels of the analytical methods used. Means for proteins (Figure 3), serum ions (Figure 4), and organics (Figure 5) are plotted by month for all years combined.

The northern-most site (Potomac River Mouth) represented a discrete location with enough animals to be examined separately (N=43 apparently healthy animals). Mean values for turtles captured from this site were then compared to values derived from the combined data collected from the southern sites. The distance (42.6 km) between the Potomac River site and the most northerly of the combined sites (Cherry Point) is great enough difference to allow this grouping of samples. These sites were divided into zones by Lutcavage (1981) and zones were used to maintain consistency with previous samples from Virginia waters. Zones as shown in Figure 1 and are grouped into

Table 3.
Healthy Caretta caretta Serum Values for Summer Seasons 1983-1985

| Month | Parameter | TPRO | Alb | Ca | P: | UA | Creat. | Na | K | Cl | BUN | GLUC | Na/K | K/BUN | Na/BUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| May | mean | 3.4 | 1.1 | 7.1 | 4.6 | 1.4 | 0.2 | 161 | 3.6 | 112 | 48 | 103 | 46.3 | 8.6 | 38.7 |
| | SD | 0.7 | 0.3 | 3 | 1.3 | 0.6 | 0.1 | 3.6 | 0.6 | 5.6 | 18.9 | 28.3 | 7.9 | 3.8 | 14.5 |
| | N | 12 | 12 | 12 | 12 | 11 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | range | 2.7-4.6 | 0.7-1.9 | 2.2-10.4 | 3.1-7.1 | 0.7-2.2 | 0.1-0.3 | 155-168 | 2.7-4.3 | 103-122 | 26-78 | 72-161 | 39-60 | 3.7-15.5 | 20.1-63.0 |
| June | mean | 3.3 | 1.2 | 4.6 | 5.1 | 1.1 | 0.2 | 159 | 3.9 | 113 | 82 | 159 | 41.5 | 5.0 | 20.7 |
| | SD | 0.7 | 0.4 | 2.7 | 1.4 | 0.4 | 0.1 | 6.4 | 0.6 | 7.5 | 19.3 | 64.9 | 6.0 | 1.4 | 6.4 |
| | N | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 |
| | range | 2.0-4.5 | 0.6-2.0 | 1.4-9.7 | 2.3-7.7 | 0.2-2.2 | 0.1-0.4 | 140-167 | 2.9-4.9 | 90-123 | 40-108 | 22-292 | 33-55 | 3.1-8.8 | 14.6-39.7 |
| July | mean | 3.5 | 1.3 | 5.5 | 6.0 | 0.9 | 0.3 | 158 | 4.7 | 110 | 85 | 170 | 34.5 | 6.2 | 21.3 |
| | SD | 0.7 | 0.4 | 2.2 | 1.4 | 0.4 | 0.1 | 6.3 | 0.8 | 6.7 | 23.2 | 84.6 | 5.5 | 3.2 | 12.3 |
| | N | 16 | 16 | 10 | 10 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| | range | 2.1-5.0 | 0.8-2.5 | 2.3-8.9 | 2.5-8.5 | 0.2-1.5 | 0.2-0.5 | 143-168 | 3.4-6.0 | 97-122 | 24-124 | 81-458 | 26-45 | 4.2-17.5 | 12.6-68.8 |
| August | mean | 3.4 | 1.3 | 5.3 | 5.2 | 1.2 | 0.3 | 153 | 4.8 | 110 | 106 | 184 | 32.3 | 4.7 | 15.2 |
| | SD | 0.6 | 0.4 | 2.4 | 1.2 | 0.3 | 0.1 | 10.7 | 0.7 | 7.9 | 18.6 | 46.3 | 4.9 | 1.2 | 4.6 |
| | N | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | range | 2.7-4.4 | 0.8-2.0 | 3.2-9.6 | 3.7-7.0 | 0.7-1.6 | 0.2-0.4 | 136-166 | 3.9-6.1 | 95-119 | 68-115 | 113-243 | 25.3-38.2 | 3.4-6.8 | 12.3-24.4 |
| September | mean | 3.8 | 1.5 | 5.5 | 6.8 | 0.7 | 0.3 | 153 | 3.5 | 110 | 67 | 99 | 44.5 | 5.9 | 25.8 |
| | SD | 0.5 | 0.2 | 2.3 | 1.6 | 0.1 | 0.1 | 8.4 | 0.6 | 6.5 | 25.1 | 39.7 | 6.9 | 2.6 | 10.9 |
| | N | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | range | 3.0-4.4 | 1.3-1.8 | 2.1-7.7 | 5.2-8.8 | 0.5-0.8 | 0.2-0.3 | 145-164 | 2.6-4.0 | 101-118 | 36-105 | 70-167 | 38-56 | 3.7-10.3 | 15.6-44.2 |

Table 4.

Healthy Caretta caretta Serum Values by Zone 1983-1985

| Zone | Parameter | TPRO | Alb | Ca | P: | UA | Creat. | Na | K | Cl | BUN | GLUC | Na/K | KBUN | Na/BUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zone 3 | mean | 3.1 | 1.0 | 2.9 | 3.9 | 1.0 | 0.2 | 160 | 3.6 | 117 | 73 | 113 | 45.3 | 5.6 | 25 |
| | SD | 0.6 | 0.4 | 1.2 | 1.5 | 0.5 | 0.1 | 4.4 | 0.7 | 4.3 | 30.0 | 54 | 7.0 | 1.9 | 10 |
| | N | 8 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | range | 2.5-4.2 | 0.6-1.6 | 2.0-5.5 | 2.3-5.5 | 0.6-2.2 | .01-0.4 | 155-167 | 3.1-4.9 | 109-121 | 40-108 | 22-197 | 34-51 | 3.1-8.9 | 14.6-38.7 |
| Zone 7 | mean | 3.5 | 1.3 | 4.5 | 5.4 | 0.7 | 0.26 | 158 | 3.8 | 112 | 74 | 134 | 42 | 7.2 | 28.6 |
| | SD | 0.4 | 0.3 | 2.3 | 1.5 | 0.3 | 0.1 | 7.7 | 0.4 | 7.0 | 34 | 64 | 3.1 | 5.9 | 21.3 |
| | N | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | range | 2.9-5.0 | 1.0-1.7 | 1.9-7.9 | 3.8-7.3 | 0.2-1.0 | 0.2-0.3 | 145-164 | 3.3-4.2 | 101-117 | 24-107 | 86-246 | 38-45 | 3.5-17.5 | 15-65.8 |
| Zone 9 | mean | 3.3 | 1.1 | 5.7 | 5.2 | 0.9 | 0.2 | 156 | 3.5 | 114 | 62 | 114 | 46 | 6.0 | 27.6 |
| | SD | 0.5 | 3.3 | 2.8 | 1.8 | 0.6 | 0.1 | 7.5 | 0.6 | 5.6 | 19.4 | 41 | 7.6 | 2.0 | 9.6 |
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | range | 2.7-4.1 | 0.7-1.6 | 1.4-8.7 | 3.1-8.0 | 0.2-2.2 | 0.1-0.4 | 146-168 | 2.6-4.3 | 107-122 | 36-97 | 73-197 | 28-59 | 2.7-10.3 | 16-44.2 |
| Zone 12 | mean | 3.6 | 1.3 | 5.7 | 5.7 | 1.1 | 0.3 | 158.2 | 4.3 | 110.2 | 83 | 176 | 38 | 5.5 | 20.5 |
| | SD | 0.6 | 0.4 | 2.8 | 1.3 | 0.4 | 0.1 | 6.9 | 0.8 | 6.6 | 21 | 66 | 7.4 | 1.4 | 6.8 |
| | N | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 |
| | range | 2.1-5.0 | 0.7-2.5 | 2.1-10.5 | 3.1-8.5 | 0.5-2.0 | 0.1-0.5 | 136-168 | 2.7-6.1 | 90.0-123 | 37-124 | 77-458 | 25-60 | 3.4-9.7 | 12-42 |

Table 5.

Serum Values for Healthy Caretta caretta by Sex 1983-1985

| | Parameter | TPRO | Alb | Ca | P: | UA | Creat. | Na | K | Cl | BUN | GLUC | Na/K | K/BUN | Na/BUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Females | mean | 3.2 | 1.0 | 3.2 | 4.7 | 1.0 | 0.3 | 157 | 4.2 | 113 | 82 | 146 | 39 | 5.5 | 21.7 |
| | SD | 0.5 | 0.3 | 1.1 | 1.4 | 0.4 | 0.1 | 7.3 | 0.8 | 7.1 | 26 | 55 | 8.0 | 1.6 | 8.4 |
| | N | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| | range | 2.5-4.5 | 0.6-2.0 | 1.9-5.5 | 2.3-7.0 | 0.2-2.2 | 0.1-0.4 | 136-167 | 2.7-5.8 | 95-123 | 41-114 | 22-246 | 27-58 | 3.1-8.8 | 12.3-38.3 |
| Males | mean | 4.0 | 1.4 | 3.9 | 6.2 | 1.0 | 0.3 | 159 | 4.3 | 113 | 76 | 149 | 38 | 6.0 | 23.5 |
| | SD | 0.5 | 0.3 | 1.3 | 1.7 | 0.3 | 0.1 | 6.9 | 0.9 | 4.9 | 27 | 49 | 8.4 | 1.3 | 8.9 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | range | 3.5-5.0 | 0.9-1.6 | 2.7-5.4 | 3.2-8.2 | 0.6-1.4 | 0.1-0.4 | 149-168 | 3.1-5.2 | 108-122 | 40-114 | 70-207 | 365-50.0 | 4.1-7.7 | 13.4-38.7 |

## Table 6.
## Serum Values for Healthy Caretta caretta by Size 1983-1985

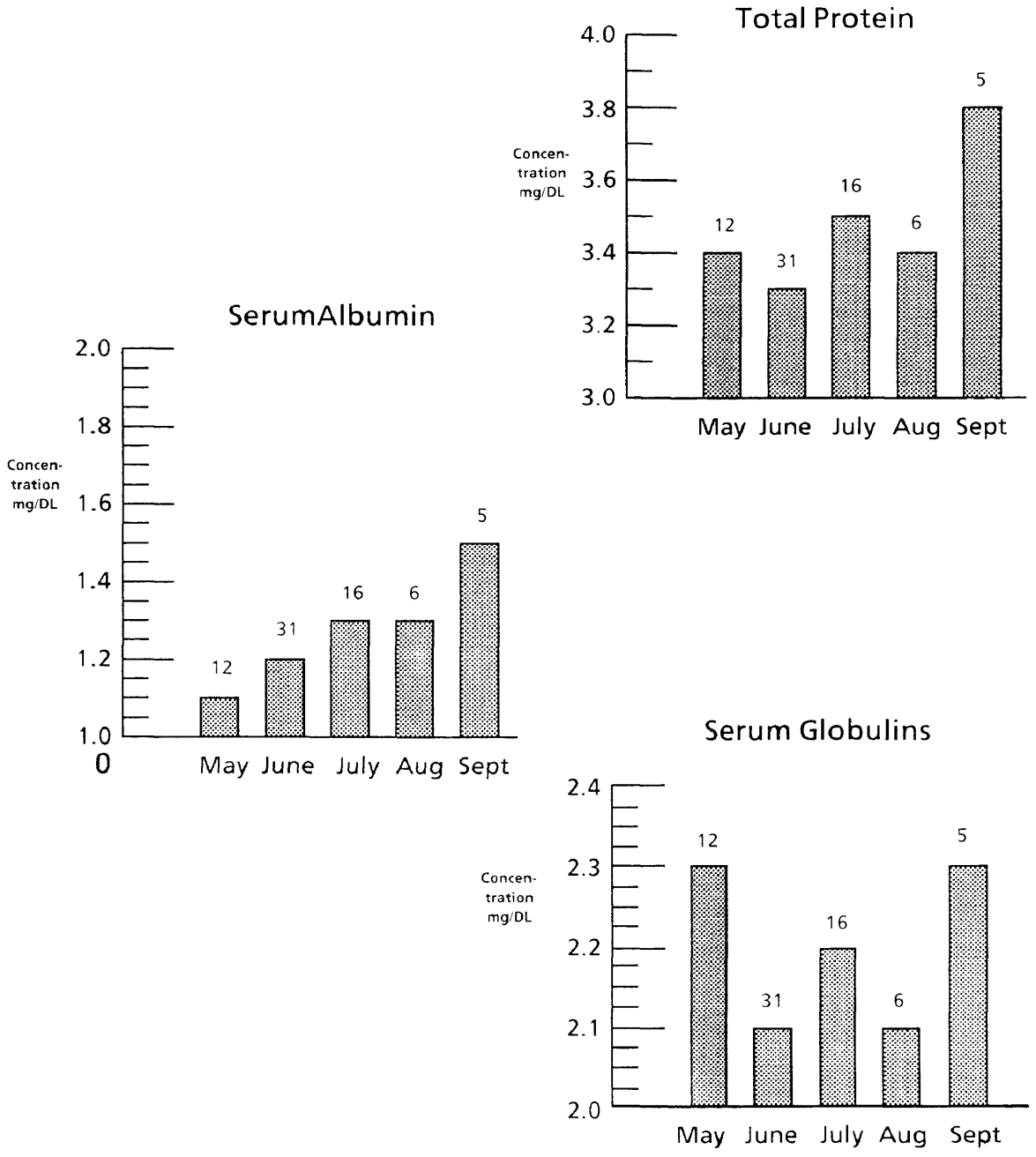
| Size | Parameter | TPRO | Alb | Ca | P: | UA | Creat. | Na | K | Cl | BUN | GLUC | Na/K | K/BUN | Na/BUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLS | mean | 3.3 | 1.2 | 4.8 | 5.3 | 1.0 | 0.2 | 157 | 4.0 | 112 | 80 | 143 | 40.5 | 5.6 | 22.7 |
| <70.0 cm | SD | 0.6 | 0.3 | 2.5 | 1.5 | 0.4 | 0.1 | 7.3 | 0.8 | 7.5 | 26 | 52 | 8.1 | 2.7 | 11.3 |
| | N | 49 | 19 | 45 | 49 | 19 | 48 | 49 | 49 | 49 | 49 | 49 | 49 | 49 | 49 |
| | range | 2.0-4.6 | 0.6-2.0 | 1.4-10.4 | 2.3-8.2 | 0.2-2.2 | 0.1-0.4 | 136-168 | 2.6-6.0 | 90-123 | 24-124 | 22-246 | 26.2-60.4 | 3.1-17.5 | 12.2-65.8 |
| CLS | mean | 3.9 | 1.4 | 7.3 | 5.6 | 1.3 | 0.2 | 161 | 4.2 | 113 | 68 | 167 | 40.3 | 7.2 | 30.3 |
| >75.0 cm | SD | 0.5 | 0.4 | 2.6 | 1.4 | 0.6 | 0.1 | 4.4 | 0.9 | 5.7 | 28 | 100 | 9.7 | 3.1 | 18.7 |
| | N | 18 | 18 | 16 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | range | 2.9-5.0 | 0.7-2.5 | 2.1-10.3 | 3.2-8.8 | 0.5-2.7 | 0.1-0.5 | 150-168 | 2.3-6.1 | 97-122 | 20-115 | 57-458 | 25.2-70.8 | 3.8-13.8 | 13.4-81.5 |

Table 7.

Serum Values for Caretta caretta 1983-1985

| | Parameter | TPRO | Alb | Ca | P: | UA | Creat. | Na | K | Cl | BUN | GLUC | Na/K | K/BUN | Na/BUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Apparently Healthy | mean | 3.5 | 1.2 | 5.5 | 5.4 | 1.2 | 0.2 | 158 | 4.0 | 112 | 76 | 147 | 40 | 6.0 | 24.6 |
| | SD | 0.7 | 0.4 | 2.7 | 1.5 | 1.2 | 0.1 | 6.8 | 0.8 | 6.9 | 27 | 68 | 8.3 | 2.8 | 13.5 |
| | N | 75 | 75 | 69 | 75 | 75 | 73 | 74 | 74 | 74 | 75 | 75 | 74 | 74 | 74 |
| | range | 2.0-5.0 | 0.6-1.9 | 1.4-10.4 | 2.3-8.8 | 2.0-11.0 | 0.1-0.5 | 136-168 | 2.3-6.1 | 90-123 | 9-124 | 17-458 | 25-71 | 3.1-17.5 | 12.3-81.5 |
| Sick or Injured | mean | 3.9 | 1.8 | 6.5 | 6.3 | 1.2 | 0.3 | 150 | 3.5 | 108 | 54. | 138 | 47.4 | 8.4 | 37.9 |
| | SD | 1.2 | 0.6 | 2.0 | 2.0 | 1.0 | 0.1 | 16.2 | 1.3 | 14.9 | 24 | 67 | 14.2 | 4.8 | 22.7 |
| | N | 14 | 14 | 14 | 14 | 14 | 14 | 12 | 12 | 12 | 14 | 13 | 12 | 12 | 12 |
| | range | 1.6-6.1 | 0.3-2.7 | 2.4-8.9 | 3.3-11.5 | 0.3-4.3 | 0.1-0.4 | 105-168 | 2.1-6.7 | 64-124 | 19-87 | 67-239 | 15.6-70.5 | 3.7-15.5 | 17.5-77.9 |

Figure 3.
Mean Seurm Protein Values for All Healthy Animals



# Figure 4.
## Mean Serum Electrolytes for All Healthy Turtles

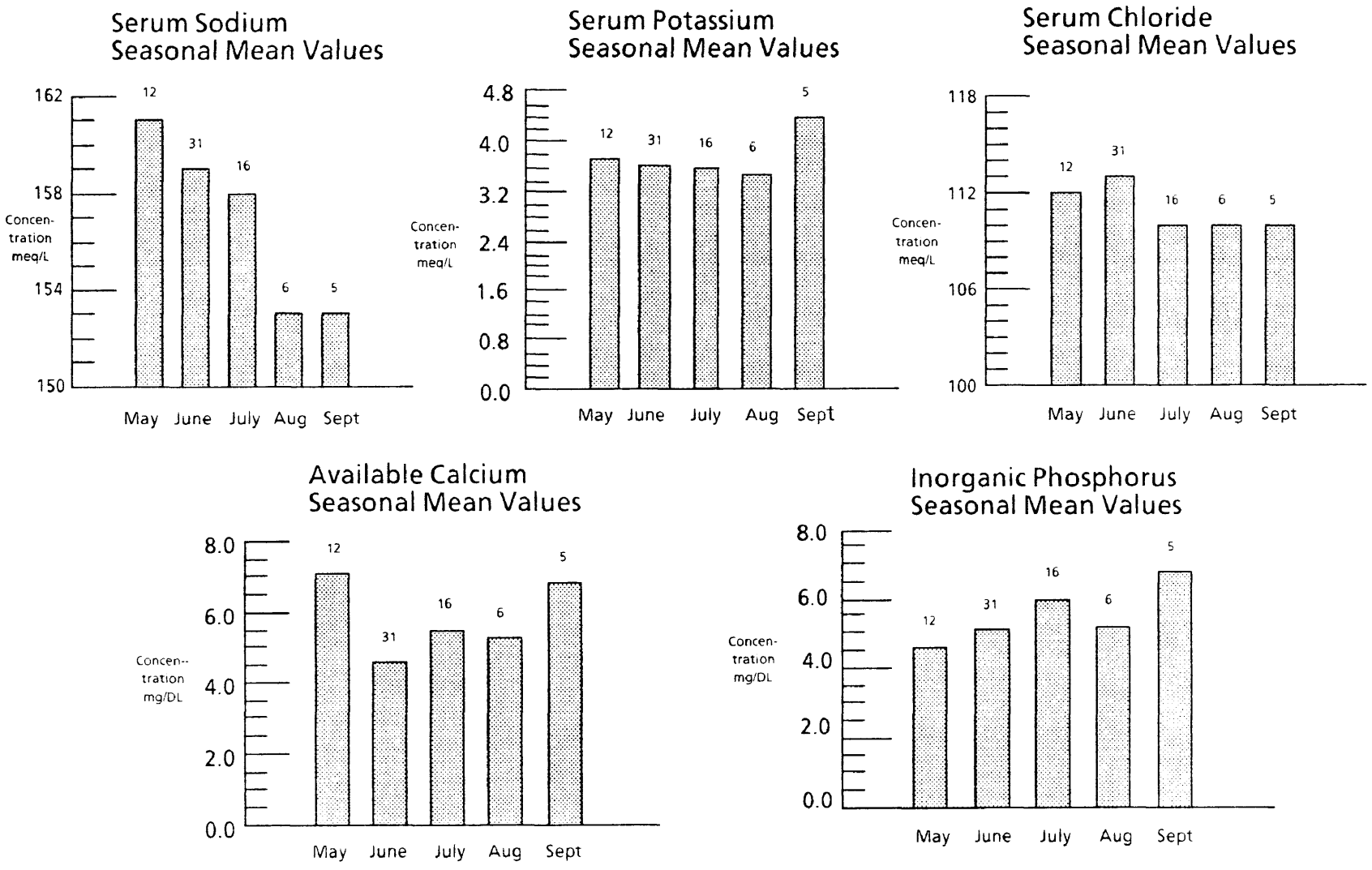
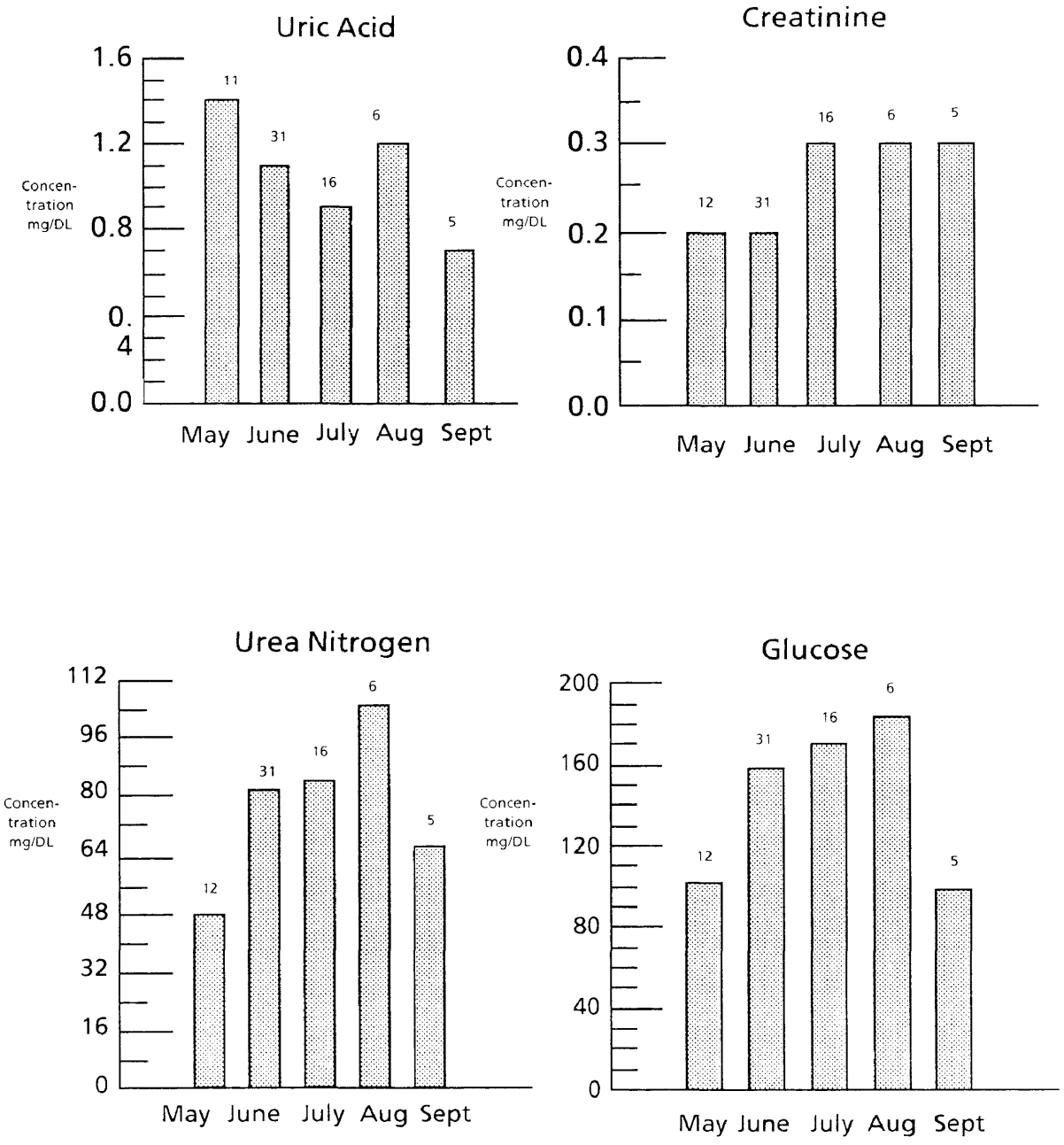


Figure 5.
Serum Organics for all Healthy Turtles



areas in Figure 2. Mean concentrations were plotted for the Potomac River site (Area 4) and compared with southern sites (Area 1,2,3: Zones 3, 5, 6, 7, and 9) for proteins (Figure 6), serum ions (Figure 7), and organic components (Figure 8).

Total Protein

Mean values for total protein ranged from 3.3 - 3.8 g/dl over the season. Mean total protein values for males were 4.0 ± 0.5 g/dl while those of females were 3.2 ± 0.5 g/dl. Mean total protein values were 3.3 ± 0.6 g/dl for animals less than 70.0cm CLS and 3.9 ± 0.5 g/dl for turtles greater than 75.0cm CLS. Apparently healthy turtles had a mean total protein of 3.5 ± 0.7 g/dl, while sick or injured animals had a mean value of 3.9 ± 1.2 g/dl.

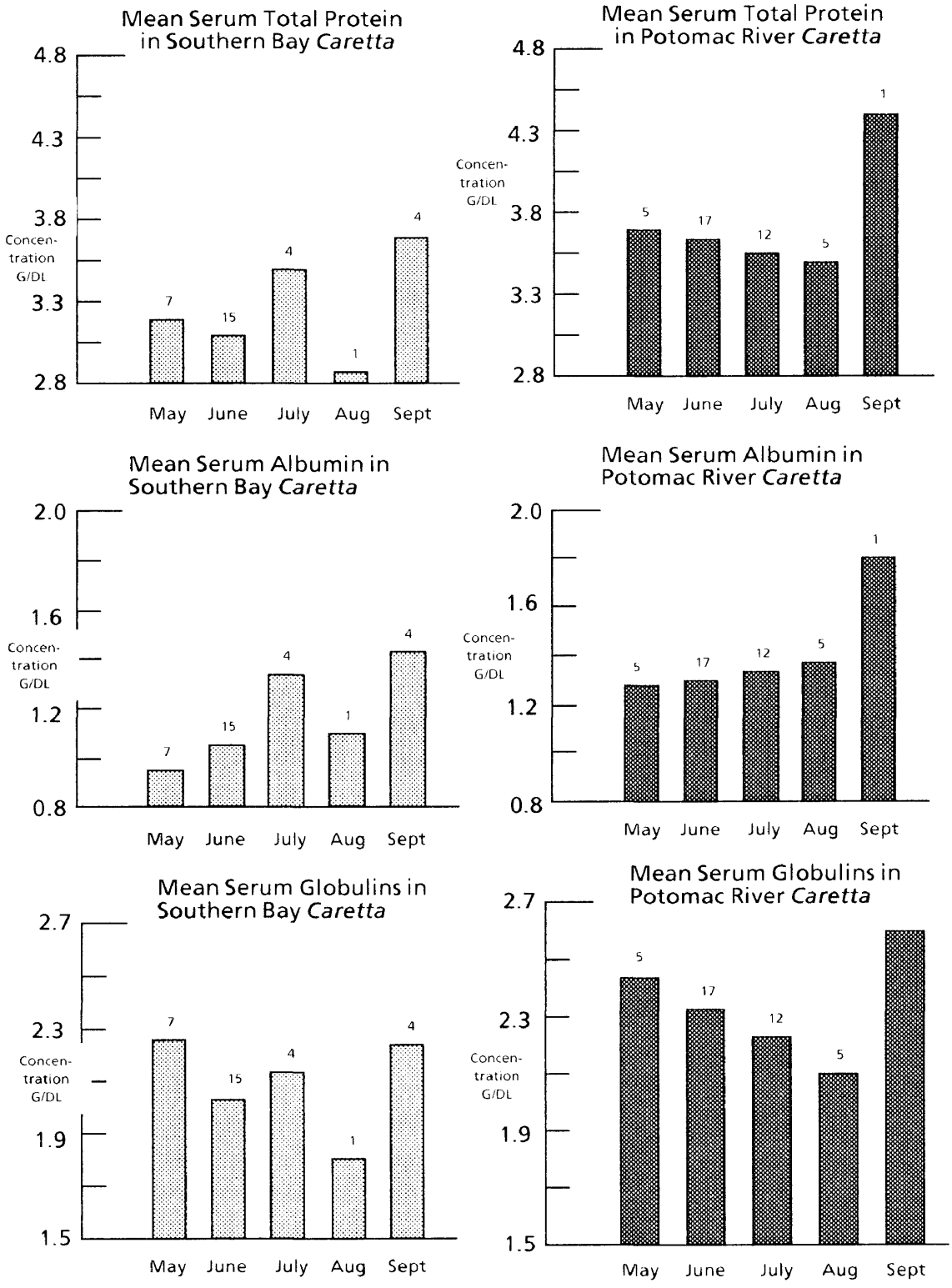
Albumin

Although, mean albumin values increased steadily from 1.1 ± 0.3 g/dl to 1.5 ± 0.2 g/dl from May to September their was no pattern to mean values from different locations. Mean albumin values were 1.0 ± 0.3 g/dl for females and 1.4 ± 0.3 g/dl for males. The mean albumin concentration was 1.2 ± 0.3 g/dl for animals less than 70.0cm CLS and 1.4 ± 0.4 g/dl for animals greater than 75.0cm CLS. The mean value for apparently healthy animals was 1.2 ± 0.4 g/dl while the mean value for this constituent was 1.8 ± 0.6 g/dl for sick or injured animals.

Calcium

Calcium values were highest in May (7.1 ± 3.0 mg/dl) and October (7.3 ± 2.1 mg/dl), while June through September ranged from 4.6 (±

Figure 6.
Seasonal Differences in Proteins of *Caretta* from Different Regions 1983-1985



# Figure 7.
# Seasonal Differences in Electrolytes of *Caretta* from Different Regions 1983-1985

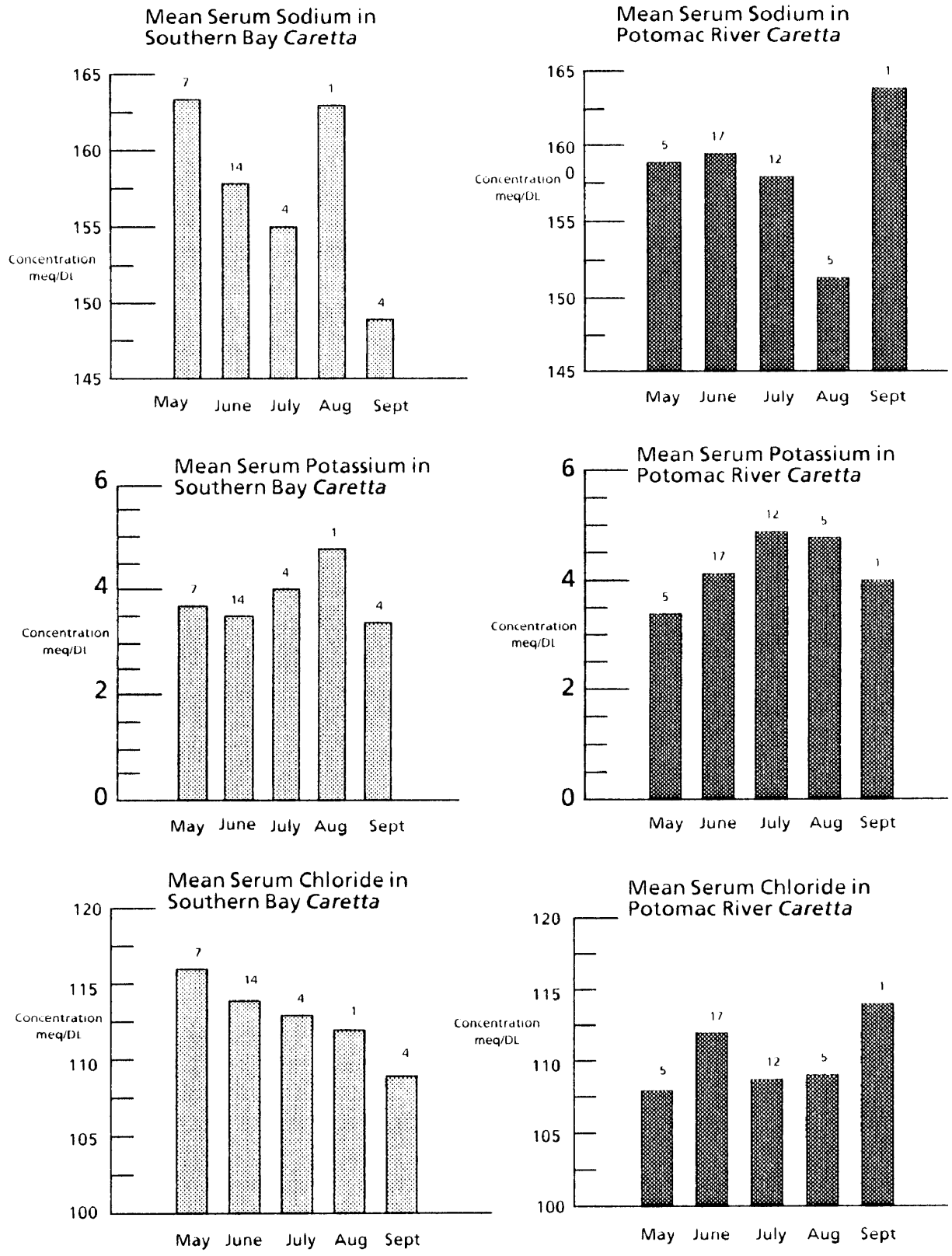
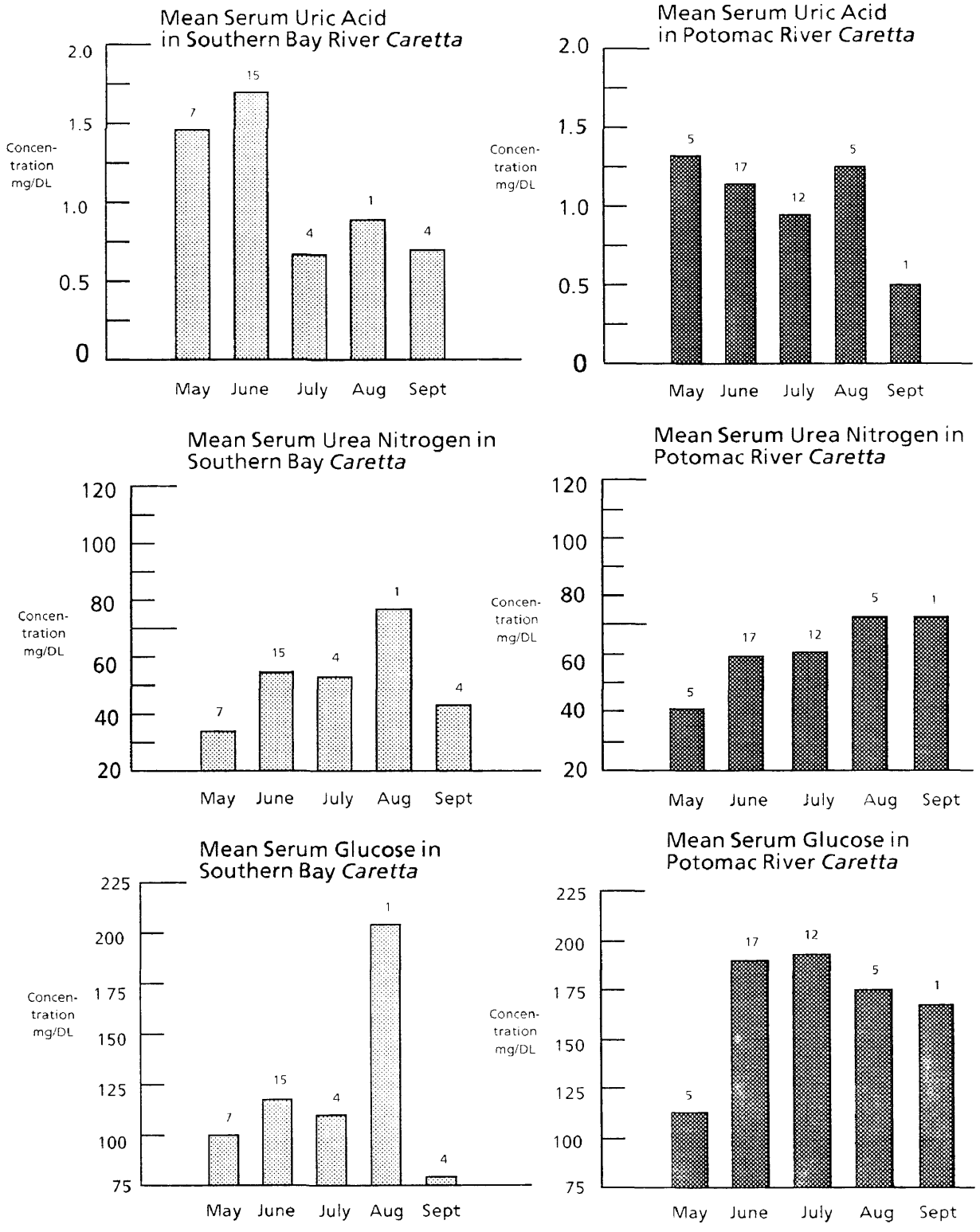


Figure 8.
Seasonal Differences in Organic Components
from *Caretta* of Different Regions 1983-1985



2.7) to 5.5 (± 2.3) mg/dl, respectively. Calcium levels were 3.2 ± 1.1 mg/dl in females and 3.9 ± 1.3 mg/dl in males. Serum calcium was 4.8 ± 2.5 mg/dl in animals less 70.0 cm and 7.3 ± 2.6 mg/dl in animals greater than 75cm.

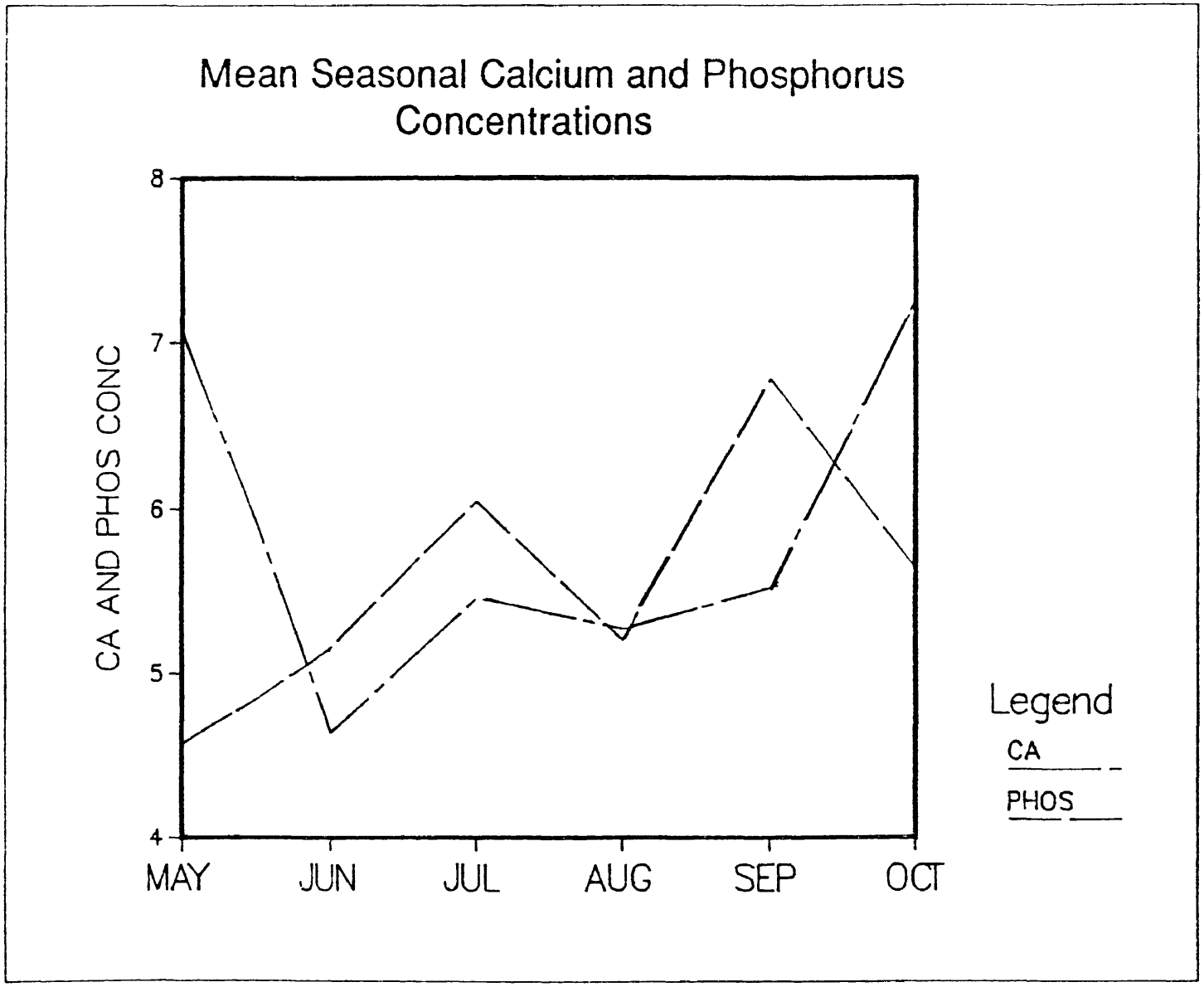
Phosphorus

When plotted over the season, there was a trend towards increasing serum inorganic phosphorus concentration during the summer. When plotted together phosphorus values followed calcium values closely in healthy animals (Figure 9). Phosphorus values were 6.2 ± 1.7 mg/dl in male turtles and 4.7 ± 1.4 mg/dl in females. Mean phosphorous was 5.4 ± 1.5 mg/dl in apparently healthy animals and 6.3 ± 2.0 mg/dl in sick and injured turtles.

Sodium

This was the most variable electrolyte from area to area. Electrolytic differences were most apparent in this parameter. Differences were most visible when this parameter was examined separately for the northern-most site and this data compared with the most extreme southern site. Although mean values were within 2 meq/l for all areas, standard deviations were less (sd=4.4, n=8) in the high salinity Bay mouth site (Zone 3) than in the lower salinity northern River site (sd=6.9, n=43). Animals over the season only ranged from 151 to 164 in the northern river site (Figure 7), while animals in the southern (Zone 3) site ranged from 149 to 163 (Figure 7). Mean sodium values were more variable in smaller animals (less than 70.0 cm), sd=7.3, n=49, while larger animals (greater than 75.0cms), sd=4.4, n=18 showed less variation in this parameter. Mean

Figure 9. Seasonal Calcium and Phosphorus Concentrations


Mean Seasonal Calcium and Phosphorus Concentrations
CA AND PHOS CONC
8
7
6
5
4
MAY
JUN
JUL
AUG
SEP
OCT
Legend
CA
PHOS

sodium values showed an inverse relationship with albumin(Figure 10). Mean values for apparently healthy animals was 158 ± 6.8 meq/l and 150 ± 16.2 meq/l in sick or injured turtles.

Chloride

Mean chloride concentration decreased from a high value of 117 ± 4.3 meq/l in the Bay mouth site, to a low of 110 ± 6.6 meq/l in the Potomac River site. Chloride values changed with sodium over the season (Figure 11). Chloride and sodium were tightly regulated in C. caretta. Ranges for this parameter were greatest in animals from the low salinity Potomac River site, Cl ranged from 90-123 meq/l in this area (Zone 12). Sick turtles showed the greatest variation in this component. Apparently healthy turtles had chloride values ranging from 90-123 ± meq/l with a mean of 108 ± 6.9 meq/l, while sick turtles had a range of 64-124 meq/l with a mean of 108 ± 14.9 meq/l.

Potassium

Potassium values for animals from the Potomac River (Zone 12) had a wide range (2.7-6.1meq/l) while the range for the Bay mouth animals was 3.1-4.9 meq/l. When values from zone 12 were plotted by month and compared to those values from all other areas it showed a steady increase through most of the summer, while other areas plotted separately showed a more random pattern (Figure 7). Mean values for this electrolyte increased during the summer (Figure 7). Mean values for this electrolyte were similar in both male (4.3 ± 0.9 meq/l) and female turtles (4.2 ± 0.8 meq/l) (Table 5). Apparently healthy animals had a mean value of 4.0 ± 0.8 meq/l, while sick and injured turtles had a mean value of 3.5 ± 1.3 meq/l. This parameter

Figure 10. Seasonal Sodium and Albumin Concentrations

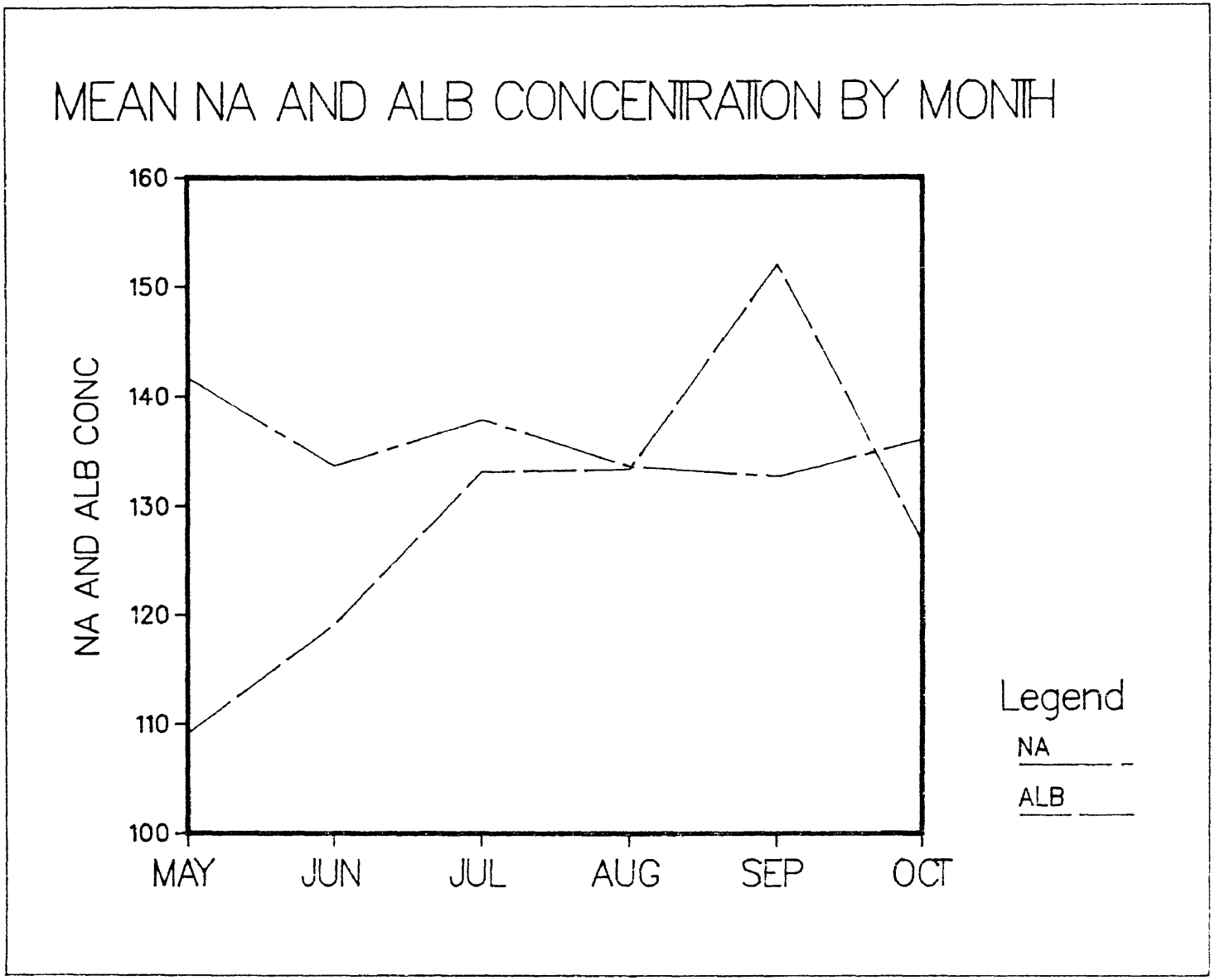
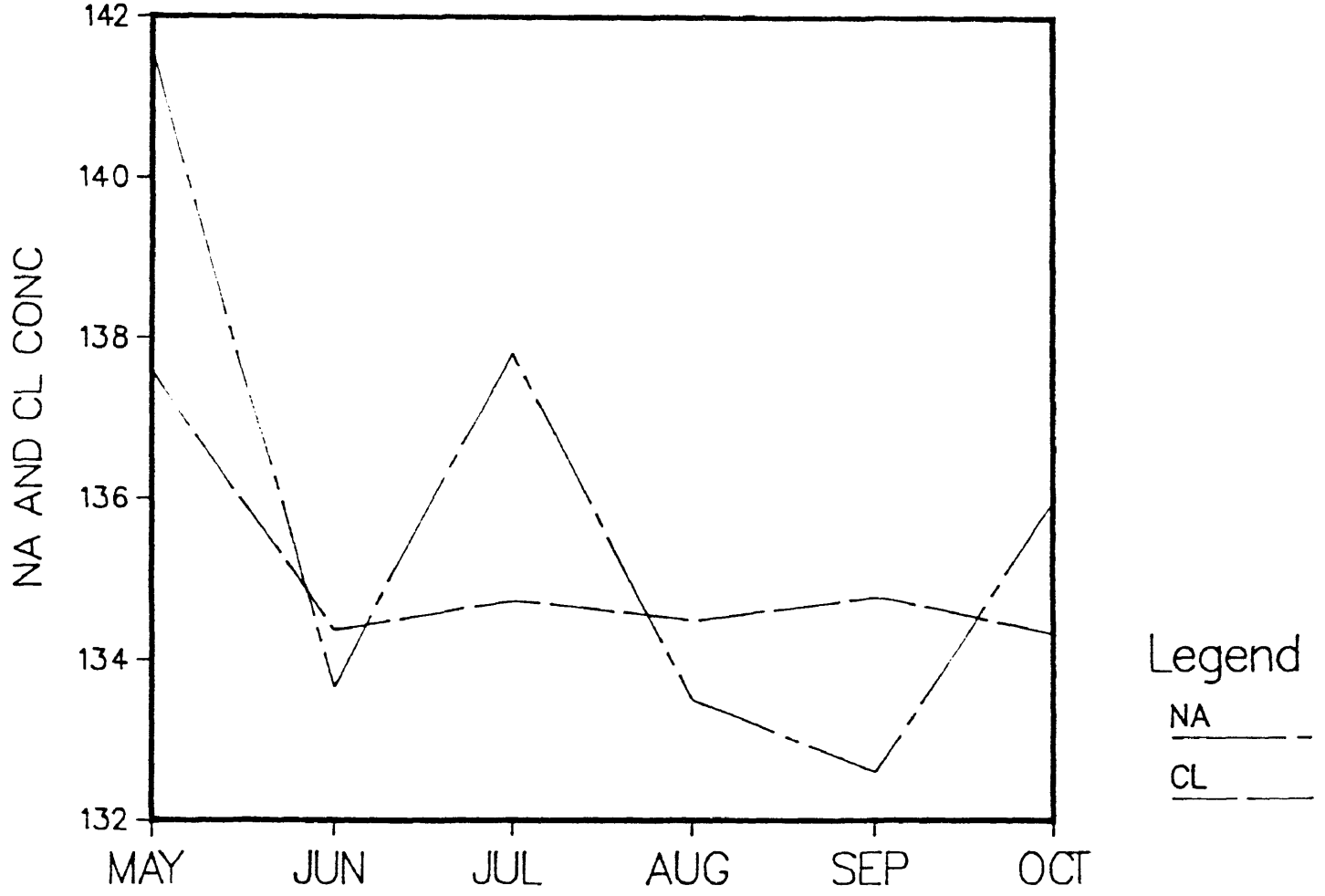

MEAN NA AND ALB CONCENTRATION BY MONTH
NA AND ALB CONC
160
150
140
130
120
110
100
MAY
JUN
JUL
AUG
SEP
OCT
Legend
NA
ALB

Figure 11. Mean Sodium and Chloride Concentration by Month

# MEAN NA AND CL CONCENTRATION BY MONTH



exhibited a direct relationship with urea nitrogen in healthy animals (Figure 12).

Blood Urea Nitrogen

Blood urea nitrogen increased from a low mean value of 48 mg/dl in May to a peak mean value of 106 mg/dl in August. Apparently healthy animals had mean values of 76 $\pm$ 27 mg/dl, while sick or injured animals had a mean value of 54 $\pm$ 24 mg/dl. This parameter was variable in all cases and had consistently wide ranges.

Glucose

Potomac River turtles showed the widest range and had higher mean levels of glucose than turtles from any of the other sites. Glucose increased over the summer through August, and declined in September and October in both sick and healthy animals. The mean and standard deviations were greater in large turtles, but were not affected by sex. Levels of urea nitrogen and potassium mirrored this temporal and spatial increase in mean glucose values (Figure 13). Glucose was variable and showed large deviations in healthy animals, large animals,animals collected during July, and animals collected from Zone 12.

Glucose and Urea Nitrogen Analysis

Results of preliminary examination of the changes in glucose-urea nitrogen as a function of temperature are shown in Figure 14. Urea nitrogen rose at about the same time at both temperatures. Glucose remained elevated longer at 26$^{o}$ C than at 20$^{o}$ C. Since the animal was not allowed time to acclimate physiologically, this can be

Figure 12. Mean Seasonal Potassium and Urea Nitrogen Concentrations

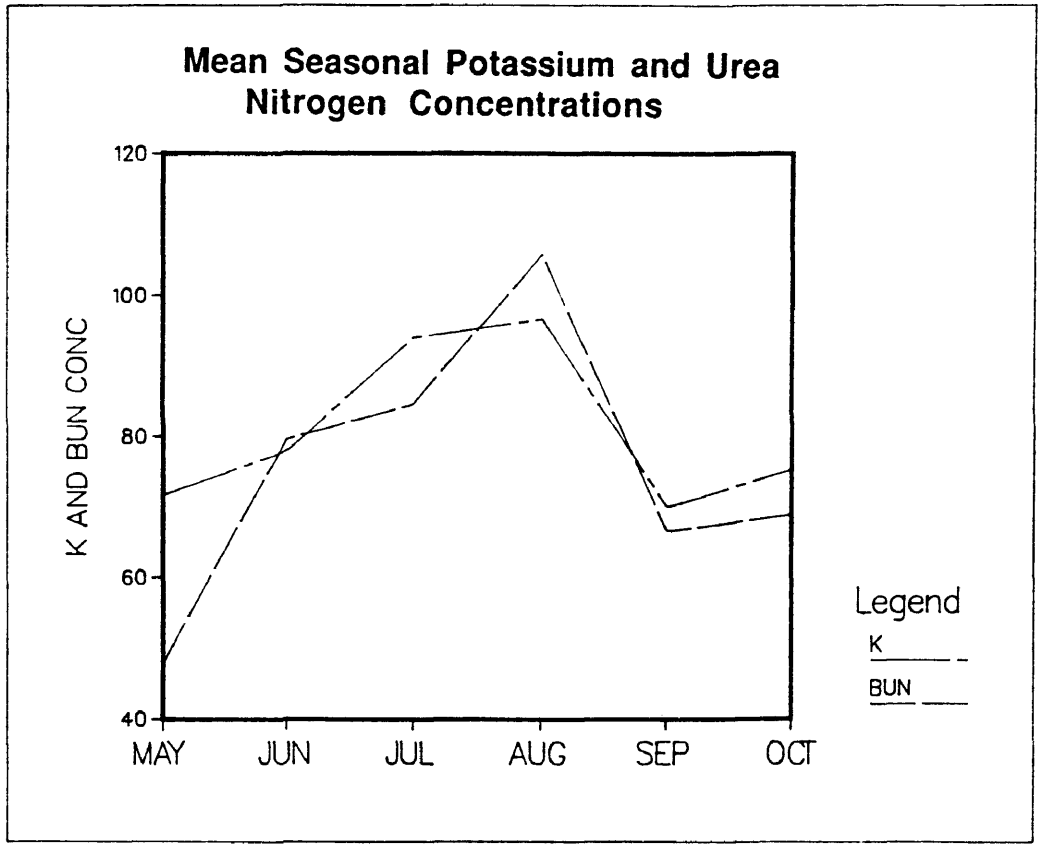

Mean Seasonal Potassium and Urea Nitrogen Concentrations
120
100
80
60
40
K AND BUN CONC
MAY
JUN
JUL
AUG
SEP
OCT
Legend
K
BUN

Figure 13. Mean Potassium Blood Urea Nitrogen and Glucose Concentration by Month

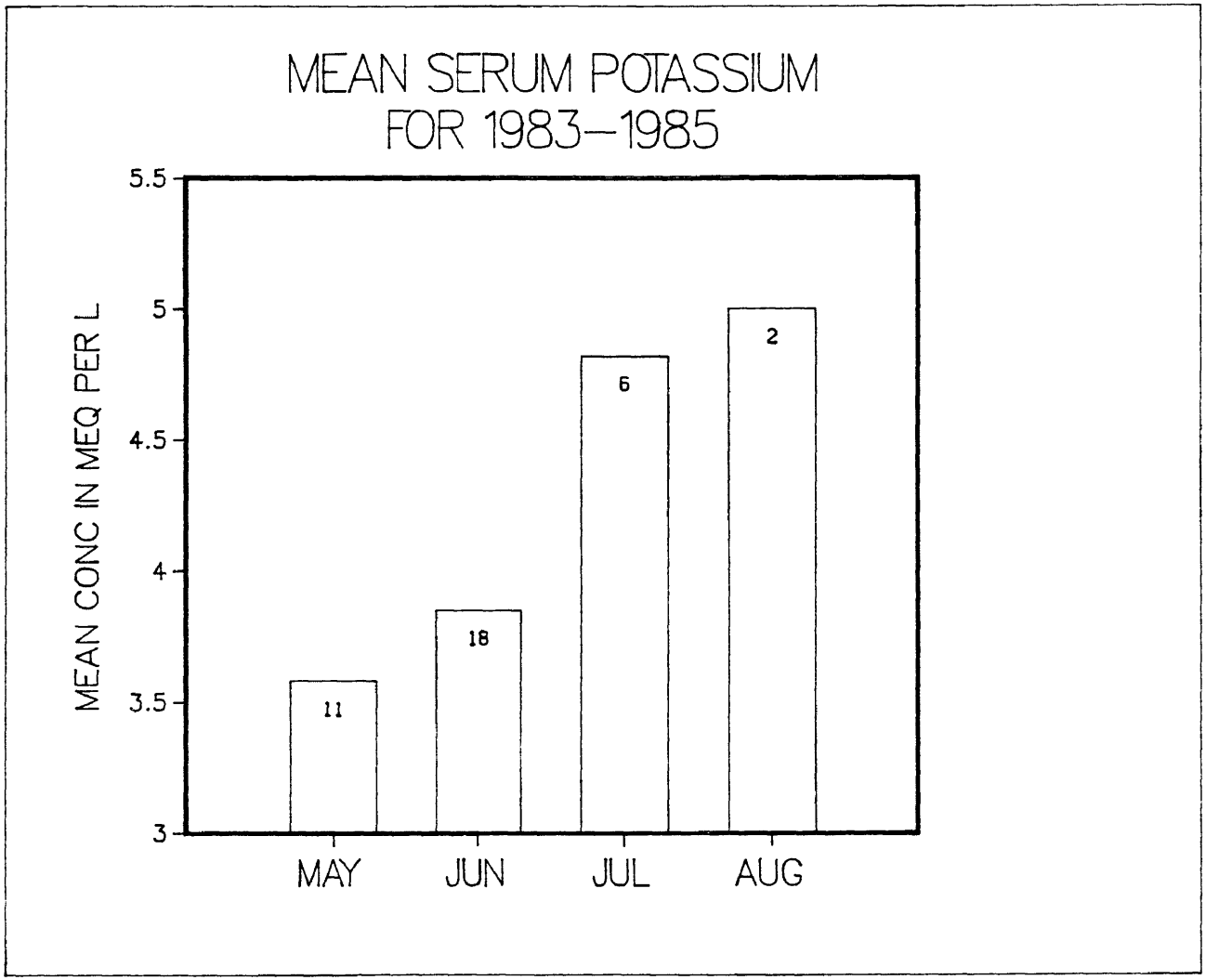
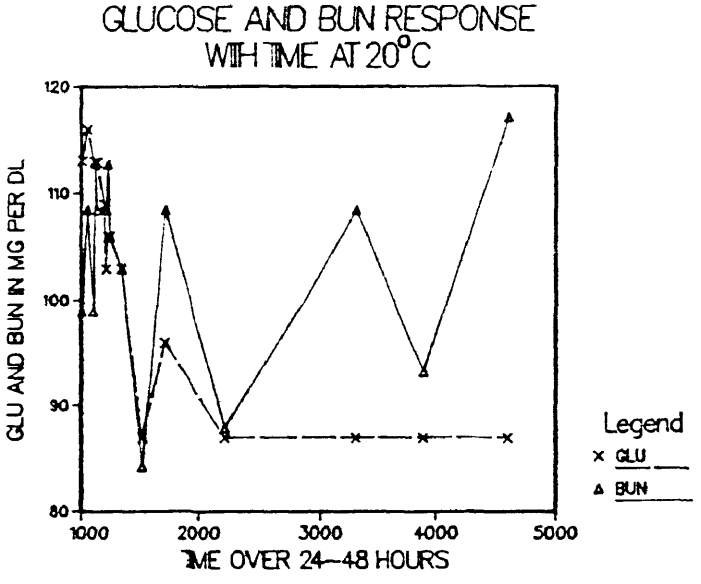
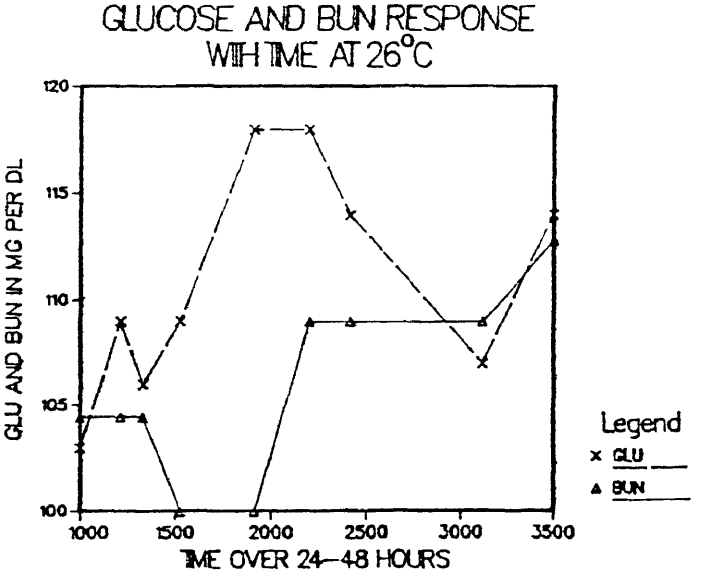

MEAN SERUM POTASSIUM
FOR 1983–1985
MEAN CONC IN MEQ PER L
5.5
5
4.5
4
3.5
3
11
18
6
2
MAY
JUN
JUL
AUG

Figure 14. Glucose and Urea Nitrogen
Response to Temperature

# Glucose and Urea Nitrogen Response to Temperature



assumed to be a physical response to temperature only. Glucose may have had a more uniform response at the higher temperature but more analyses on more animals must be done to make any conclusive statements about these relationships.

### Corticosterone

Mean corticosterone values were plotted seasonally for 1984-1985 (Figure 15). Values were not available for animals from 1983. Multiple sample runs for this analyses were not consistent, probably due to the presence of an interfering substance. However, this compound was not found in all samples, was not indicated in the standards, or the recovery for the analysis and was never identified. Values for this hormone are therefore used as a general indicator only.

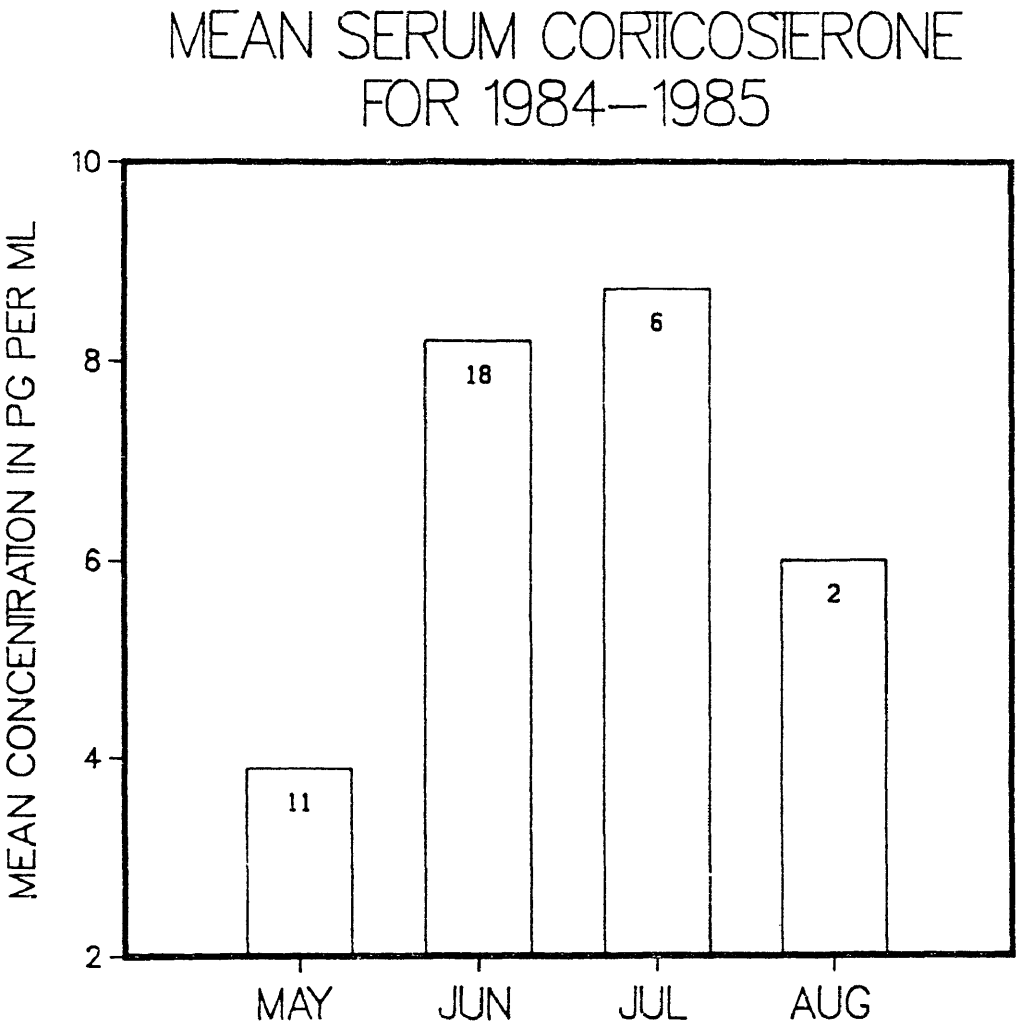
## Multivariate Analysis

### Principal Components Analysis

Principal component analysis (PCA) of serum components yielded general relationships which differed between healthy and sick turtles. The three major factor groups determined for healthy animals were:

| Group 1 | Group 2 | Group 3 |
|---|---|---|
| Albumin | Urea Nitrogen | Sodium |
| Total Protein | Potassium | Chloride |
| Calcium | Glucose | |
| Phosphorus | | |

Figure 15. Mean Serum Corticosterone for 1984-1985


MEAN SERUM CORTICOSTERONE
FOR 1984–1985
MEAN CONCENTRATION IN PG PER ML
10
8
6
4
2
11
18
6
2
MAY
JUN
JUL
AUG

When serum values from the sick and injured group of animals were then analyzed, variable groupings changed. Mean values for those animals classified as sick or injured are found in Table 7. Variable groups for sick animals were:

| Group 1 | Group 2 | Group 3 |
|---|---|---|
| Albumin | Sodium | Urea Nitrogen |
| Total Protein | Chloride | Glucose |
| Calcium | Potassium | |
| Phosphorous | | |

The first group was composed of the same factors in both sick and healthy animals. Factors in the second group changed so that this grouping for sick animals included potassium, sodium, and chloride. The third group for sick animals was then composed of urea nitrogen and glucose. Potassium moved from the urea nitrogen/glucose group in healthy animals to the electrolyte group in apparently sick animals. The entire electrolyte group moved from third most important factor in healthy animals to second in sick and injured animals.

Q-Mode Analysis

General statistics for all turtles included in this analysis are found in Table 8. The analysis was run on 11 variables and 74 samples for 99% error explanation. Using the varimax solution, two factors explained 99.01% variance (dissimilarity) of the data with the relative input from each factor being 51.638% and 47.371%, respectively. The varimax factor loadings are listed in Table 9. The data best fit a two factor model. Best fit to a factor model was

Table 8.
Mean Sera Values for Samples Included
in Multi-Variate Analysis

| Variable | No. | Mean | Standard Deviation | Minimum Value | Maximum Value |
|---|---|---|---|---|---|
| TPRO | 1 | 3.5 | 0.78 | 1.6 | 6.1 |
| ALB | 2 | 1.3 | 0.47 | 0.3 | 2.7 |
| CA | 3 | 5.6 | 2.66 | 1.4 | 10.4 |
| PHOS | 4 | 5.5 | 1.53 | 2.3 | 11.5 |
| UA | 5 | 1.1 | 0.6 | 0.2 | 4.3 |
| CRE | 6 | 0.2 | 0.1 | 0.1 | 0.5 |
| NA | 7 | 157 | 9.4 | 105 | 168 |
| K | 8 | 3.9 | 0.89 | 2.1 | 6.7 |
| CL | 9 | 111 | 8.6 | 64 | 123 |
| BUN | 10 | 72 | 27 | 19 | 124 |
| GLUC | 11 | 150 | 68.4 | 22 | 458 |

Table 9.
Program CABFAC Results of the VARIMAX
Factor Score Matrix

| Variables | No. | Factor 1 | Factor 2 |
|---|---|---|---|
| Total Protein | 1 | 0.016 | 0.004 |
| Albumin | 2 | 0.004 | 0.003 |
| Calcium | 3 | 0.027 | 0.004 |
| Phosphorus | 4 | 0.021 | 0.010 |
| Uric Acid | 5 | 0.005 | 0.001 |
| Creatinine | 6 | 0.000 | 0.001 |
| Sodium | 7 | 0.776 | 0.101 |
| Potassium | 8 | 0.013 | 0.009 |
| Chloride | 9 | 0.566 | 0.054 |
| Blood Urea Nitrogen | 10 | 0.230 | 0.173 |
| Glucose | 11 | -0.152 | 0.978 |

based on evenness of the variance explained. The addition of factors did not evenly divide variance, further factors only accounted for 3-4% of variance. Factor one loaded heavily on sodium, chloride, urea nitrogen, and negatively on glucose.

Solutions showed factors to be made up of two groups. Endmembers chosen using Imbries solution were MT-21-84 for Factor 1 and MT-88-83 for Factor 2. Using Imbries solutions all turtles were plotted and are shown in Figure 16. Animals grouped at either extreme (endmember turtle groups) are marked in Figure 17. Collective data for endmember turtles from Factor Group 1 are listed in Table 10. Collective data for endmember turtles from Factor Group 2 are found in Table 11. In addition to endmember animals, other animals grouped together with factor scores of .7900-.8100. Animals falling into this intermediate group collective data is provided in Table 12. Although this group shows no obvious direct trends, it contains two animals that were sub-clinically ill, (MT-12-84) and (MT-124-84). This group contains several stressed animals (MT-36-83, MT-32-84, and MT-62-83).

The compositions of the Factor groups are controlled by the variable loadings. Factor one loaded heavily on sodium, chloride, and urea nitrogen. Factor two loaded on glucose, urea nitrogen, and sodium, however input from glucose was much higher than that of the other two variables. The composition of Factor group one was controlled by sodium and chloride with some input from urea nitrogen while the composition of factor group two was controlled by glucose

Figure 16. Turtle Grouped by Serum Variables: Varimax Factor Loadings for a Two Factor Solution

**Turtles Grouped by Serum Variables:**
**Varimax Factor Loadings for a Two Factor Solution**

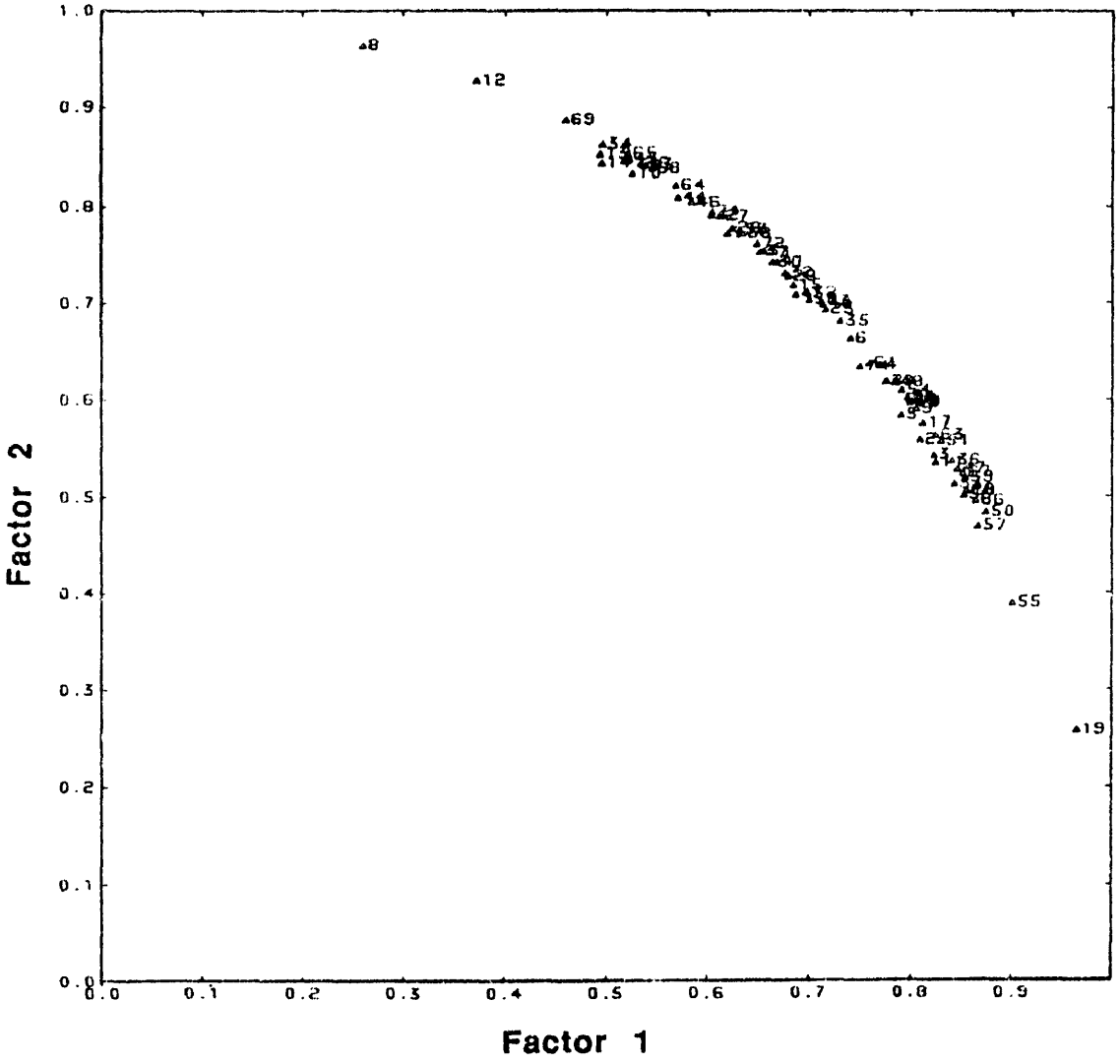


Figure 17. Turtles Grouped by Serum Variables: Varimax Factor Loadings for a Two Factor Solution

**Turtles Grouped by Serum Variables:**
**Varimax Factor Loadings for a Two Factor Solution**

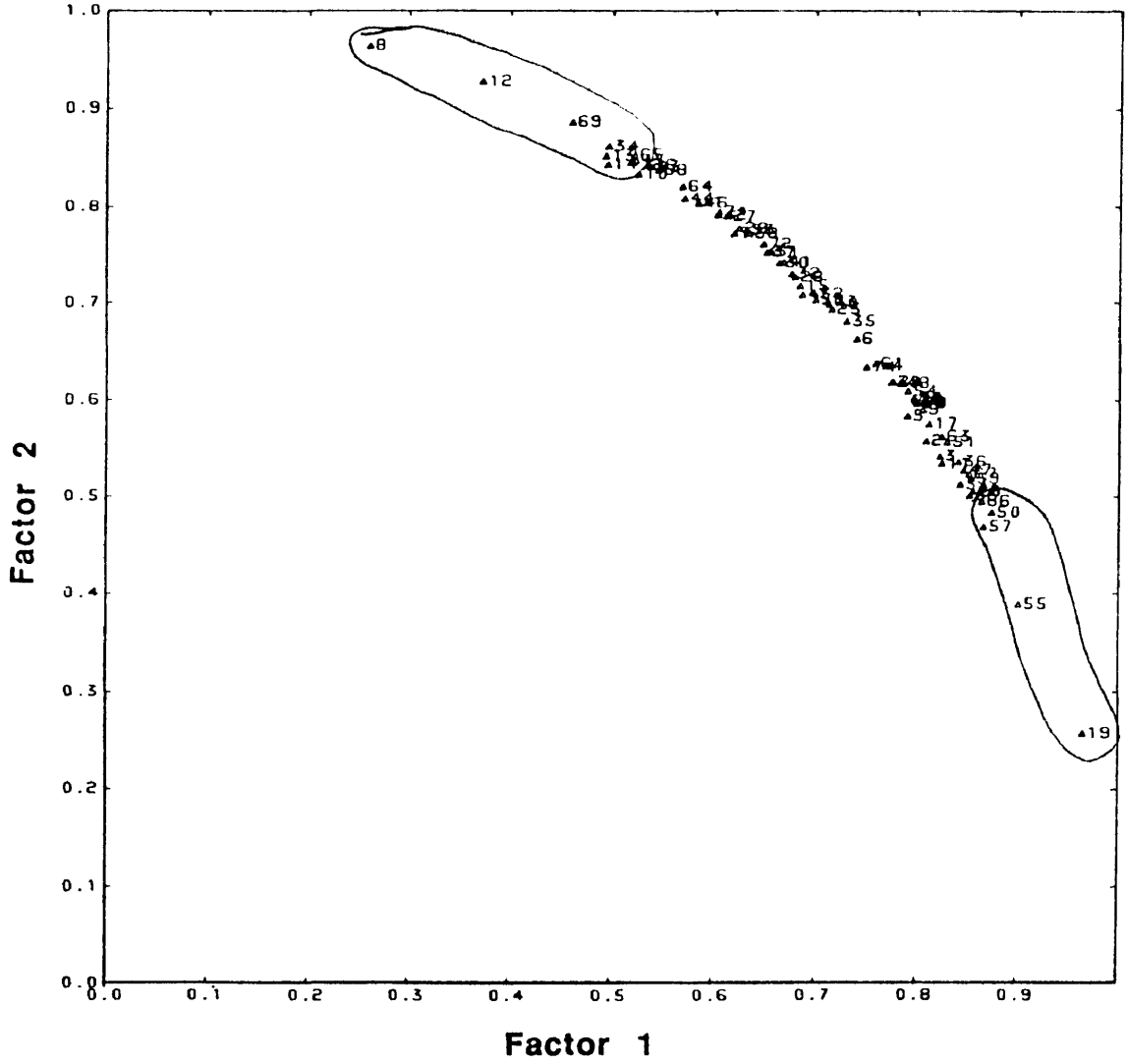


Table 10. Endmember Groups and Serum Profiles for Factor Group 1

| ID no. | Marine Turtle No. | Sample No. | Factor Score | Date | Tpro (g/dl) | alb (g/dl) | Glob (g/dl) | Ca (mg/dl) | Phos (mg/dl) | Uric acid (mg/dl) | Cre (mg/dl) | Na (meq/l) | K (meq/l) | Na/K | Cl (meq/l) | BUN (mg/dl) | Glu (mg/dl) | Wt (kg) | CLS (cm) | CWS (cm) | Zone | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | MT-21-84 | 5757 | .9655 | 1-VI-84 | 2.6 | 0.6 | 2.0 | 2.3 | 3.4 | 2.2 | 0.1 | 159 | 3.2 | 49.7 | 121 | 57 | 22 | 22 | 53.7 | 45.6 | 3 | From Lynnhaven, high salinity apparently healthy. early season cool water animal |
| 55 | MT-140-84 | 5809 | .9014 | 12-XII-84 | 4.2 | 1.2 | 3.0 | 8.0 | 4.6 | 2.7 | 0.1 | 163 | 2.3 | 70.9 | 119 | 20 | 57 | 57.5 | 75.7 | 61.4 | | Cold stunned, from Eastern Shore of VA. From 3°C water, 8°C after 4hrs, 14°C when sampled 12hrs after arrival, apparently healthy |
| 57 | MT-04-85 | 5853 | .8665 | 9-V-85 | 4.4 | 1.4 | 3.0 | 10.3 | 5.2 | 1.4 | 0.2 | 164 | 3.6 | 45.5 | 116 | 26 | 76 | 120.0 | 89.9 | 72.9 | n/a | Taken offshore of VA BCH during trawl trip, apparently healthy, cloacal temp. 17°C |
| 50 | MT-129-84 | 5800 | .8743 | 22-IX-84 | 4.2 | 1.5 | 2.7 | 5.4 | 8.2 | 0.8 | 0.2 | 149 | 3.9 | 38.2 | 109 | 61 | 70 | 47.0 | 67.6 | 55.4 | n/a | Taken in Norfolk Canyon during longline cruise Cloacal temp. 20°C |
| 66 | MT-48-85 | 5866 | .8648 | 9-VI-85 | 4.4 | 1.5 | 2.9 | 7.7 | 5.6 | 0.7 | 0.2 | 162 | 2.9 | 55.8 | 109 | 80 | 77 | 45.0 | 66.4 | 54.9 | 12 | Turtle from Potomac River, held at VIMS, this sample from one month after arrival. Apparently healthy |
| 56 | MT-03-85 | 5852 | .8522 | 9-V-85 | 3.3 | 1.0 | 2.3 | 8.5 | 4.3 | 2.1 | 0.1 | 164 | 4.2 | 39 | 116 | 31 | 84 | 101.0 | 89.3 | 70.7 | n/a | Taken on Offshore trawl trip, off VA BCH. Cloacal temp. 18°C. Apparently healthy. |

Table 11. Endmember Groups and Serum Profiles for Factor Group 2

| ID no. | Marine Turtle No. | Sample No. | Factor Score | Date | Tpro (g/dl) | alb (g/dl) | Glob (g/dl) | Ca (mg/dl) | Phos (mg/dl) | Uric acid (mg/dl) | Cre (mg/dl) | Na (meq/l) | K (meq/l) | Na/K | Cl (meq/l) | BUN (mg/dl) | Glu (mg/dl) | Wt (kg) | CLS (cm) | CWS (cm) | Zone | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | MT-88-83 | 5568 | .9635 | 2-VIII-83 | 4.2 | 2.5 | 1.7 | 8.9 | 8.5 | 1.0 | 0.5 | 162 | 5.9 | 27 | 97 | 85 | 458 | 68.0 | 72.2 | 62.3 | 12 | Stressed, fought prior to sample difficult to get blood, hot |
| 12 | MT-113-83 | 5578 | .9271 | 7-X-83 | 4.0 | 1.9 | 2.1 | 8.3 | 6.0 | 4.4 | 0.4 | 106 | 7.0 | 15 | 59 | 57 | 244 | 36.0 | 65.1 | 55.1 | 15 | Sick turtle from the Eastern shore of VA (Nassawaddox Creek), died shortly after sampling, severely emaciated |
| 69 | MT-54-85 | 5872 | .8868 | 19-VI-85 | 3.7 | 1.5 | 2.2 | 9.7 | 7.0 | 1.3 | 0.3 | 164 | 4.5 | 36 | 116 | 81 | 292 | 86.5 | 83.7 | 66.9 | 12 | Recapture from 1984, apparently healthy when sampled |
| 34 | MT-83-84 | 5773 | 8623 | 22-VI-84 | 4.5 | 2.0 | 2.5 | 4.9 | 4.9 | 1.0 | 0.3 | 154 | 4.3 | 35.8 | 109 | 59 | 246 | 31.0 | 55.6 | 50.0 | 12 | Apparently healthy, from Potomac River, sampled at dock and released |
| 13 | MT-119-83 | 5577 | .8525 | 29-X-83 | 3.5 | 1.6 | 1.9 | 6.6 | 4.9 | 1.1 | 0.2 | 150 | 2.4 | 62.5 | 112 | 29 | 239 | 16.0 | 49.1 | 43.7 | 7 | Turtle with respiratory problems that died in captivity after apparent recovery. Histo. and necropsy showed extensive lung damage with systemic infection. Taken from Poquoson VA |
| 14 | MT-120-83 | 5572 | .8438 | 2-XI-83 | 3.5 | 2.0 | 1.5 | 8.3 | 5.2 | 0.9 | 0.2 | 148 | 2.1 | 70.4 | 113 | 19 | 233 | 31.0 | 62.7 | 52.6 | 3 | Taken from Portsmouth VA, emaciated severely lethargic- leeches on neck and head. Died 5-VIII-84. |
| 65 | MT-43-85 | 5868 | .8534 | 6-VI-85 | 4.1 | 1.5 | 2.6 | 9.7 | 6.4 | 0.9 | 0.2 | 161 | 4.4 | 36.6 | 112 | 80 | 247 | 49.0 | 70.0 | 59.3 | 12 | Apparently health heat stressed cloacal temp. 30°C taken from Potomac River |
| 45 | MT-120-84 | 5793 | .8481 | 13-VIII-84 | 3.9 | 1.5 | 2.4 | 6.5 | 5.5 | 1.6 | 0.4 | 154 | 6.1 | 25.2 | 111 | 115 | 243 | 87.0 | 82.1 | 67.0 | 12 | Potomac River turtle, sampled at the dock, apparently healthy, cloacal temp. 33.5°C |

Table 12. Intermediate Turtles Described by Factor Analysis

| ID no. | Marine Turtle No. | Sample No. | Date | Factor Score | Tpro (g/dl) | alb (g/dl) | Glob (g/dl) | Ca (mg/dl) | Phos (mg/dl) | Uric acid (mg/dl) | Cre (mg/dl) | Na (meq/l) | K (meq/l) | Na/K | Cl (meq/l) | BUN (mg/dl) | Glu (mg/dl) | Wt (kg) | CLS (cm) | CWS (cm) | Zone | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | MT-36-83 | 5591 | 6-VI-83 | .8093 | 2.4 | 0.8 | 1.6 | 6.2 | 3.0 | 1.7 | 0.2 | 144 | 3.4 | 42.4 | 91.0 | 96 | 85 | 28 | 58.0 | 50.0 | 5 | Taken 6-IV-83 in pound net off Grandview apparently healthy, heat stressed. |
| 5 | MT-78-83 | 5597 | 12-VII-83 | .7906 | 3.6 | 1.7 | 1.9 | 7.9 | 7.3 | 1.0 | 0.2 | 158 | 4.2 | 37.6 | 109 | 24 | 105 | 37 | 64.5 | 53.2 | 7 | Taken from pound nets, York River mouth, held at lab, apparently healthy. Taken 22-VI-83, sampled 12-VII-83 |
| 9 | MT-91-83 | 5564 | 26-IX-83 | .8058 | 3.0 | 1.3 | 1.7 | 7.7 | 5.2 | 0.8 | 0.2 | 146 | 2.6 | 56.2 | 107 | 70 | 98 | 25 | 55.4 | 49.2 | 9 | Taken in poundnet off Cherry point, in Chesapeake Bay. Apparently healthy, held at Lab prior to sampling |
| 16 | MT-12-84 | 5752 | 29-V-84 | .7970 | 1.6 | 0.3 | 1.3 | 3.2 | 5.9 | 1.1 | 0.1 | 149 | 3.0 | 49.6 | 110 | 71 | 104 | 65 | 78.8 | 64.4 | 3 | Taken in Lynnhaven Inlet at the Bay Mouth, high salinity. Unhealthy, covered in Barnacles and emaciated, held at VIMS for treatment. Low hematocrit. |
| 22 | MT-32-84 | 5761 | 6-IV-84 | .7980 | 2.8 | 0.9 | 1.9 | 2.0 | 3.9 | 0.6 | 0.1 | 158 | 3.1 | 51 | 109 | 77 | 107 | 30 | 59.5 | 49.1 | 3 | Taken from Lynnhaven Inlet, high salinity area. apparently healthy, slight heat stress. active |
| 24 | MT-62-84 | 5763 | 14-VI-84 | .7966 | 2.9 | 1.3 | 1.6 | 4.8 | 5.3 | 1.0 | 0.3 | 164 | 3.6 | 45.5 | 116 | 78 | 113 | 53 | 71.8 | 58.4 | 7 | Apparently healthy, from poundnets on York Spit some heat stress, |
| 49 | MT-124-84 | 5805 | 22-VII-85 | .7985 | 4.1 | 1.9 | 2.2 | 8.7 | 5.9 | 0.6 | 0.1 | 164 | 3.8 | 43.2 | 113 | 79 | 111 | 32 | 63.5 | 50.4 | 10 | Sick turtle from Deltaville, emaciated respiratory compromised. A later sample taken after holding this animal for 10mos. 22-VIII-85 |
| 54 | MT-137-84 | 5808 | 25-X-84 | .7911 | 3.6 | 1.3 | 2.3 | 8.6 | 5.0 | 0.6 | 0.2 | 163 | 4.0 | 40.7 | 116 | 80 | 115 | 39 | 63.7 | 52.1 | 12 | Apparently healthy, taken from Potomac River, cloacal temp. 21°C, |

Table 12. Intermediate Turtles Described by Factor Analysis

| ID no. | Marine Turtle No. | Sample No. | Date | Factor Score | Tpro (g/dl) | alb (g/dl) | Glob (g/dl) | Ca (mg/dl) | Phos (mg/dl) | Uric acid (mg/dl) | Cre (mg/dl) | Na (meq/l) | K (meq/l) | Na/K | Cl (meq/l) | BUN (mg/dl) | Glu (mg/dl) | Wt (kg) | CLS (cm) | CWS (cm) | Zone | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 60 | MT-16-85 | 5859 | 16-V-85 | .7999 | 3.9 | 1.2 | 2.7 | 7.7 | 3.4 | 0.7 | 0.1 | 157 | 2.9 | 54.1 | 107 | 78 | 105 | 52 | 73.7 | 58.5 | 12 | Apparently healthy,taken in the Potomac River, cloacal temp. 24°C |
| 62 | MT-19-85 | 5863 | 22-V-85 | .7999 | 4.6 | 1.9 | 2.7 | 10.4 | 6.0 | 1.0 | 0.2 | 161 | 4.1 | 39.3 | 107 | 75 | 107 | 22 | 51.5 | 45.9 | 12 | From Potomac River,Fat apparently healthy, no epibiota. |

with some input from sodium and urea nitrogen. Turtle MT-65-84 was recaptured three separate times over the summer of 1984. Its blood samples were included as separate samples. When these values were examined in the analysis they progressed from one group to another with each subsequent sample taken later in the season. Animals from endmember group one were characterized as coming from high salinity (>30 ppt) and having low cloacal temperatures (<22$^{o}$C). Sera partial profiles for turtles with low body temperatures are found in Table 13. Animals found in endmember group 2 were generally (except MT-54-85) listed as stressed, sick or had abnormally high cloacal temperatures.

Table 13.

Sera Profiles for Caretta with Low Body Temperatures

(<18oC)

| ID No. | Turtle Number | Date | total protein | albumin | calcium | phos-phorus | sodium | potas-sium | chloride | urea nitrogen | glucose | Temp. °C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5851 | MT-02-85 | 09-V-85 | 2.9 | 1.1 | 8.2 | 5.5 | 164 | 4.2 | 118 | 27 | 72 | 16.0 |
| 5898 | NC-CC | 19-II-85 | 3.3 | 1.3 | 6.1 | 5.9 | 158 | 2.9 | 109 | 70 | 118 | |
| 5852 | MT-03-85 | 09-V-85 | 3.3 | 1.0 | 8.5 | 4.3 | 164 | 4.2 | 116 | 31 | 84 | 18.3 |
| 5853 | MT-04-85 | 09-V-85 | 4.4 | 1.4 | 10.3 | 5.2 | 164 | 3.6 | 116 | 26 | 76 | 17.0 |
| 5809 | MT-140-84 | 13-XII-84 | 4.2 | 1.2 | 8.0 | 4.6 | 163 | 2.3 | 119 | 20 | 57 | 15.9 (8.2 °C on 12-XII-84) |
| 5810 | MT-140-84 | 20-XII-84 | 4.0 | 1.2 | 7.6 | 5.8 | 163 | 2.5 | 118 | 36 | 120 | 18.0 |

# V. DISCUSSION

## Underlying General Trends

Trends for serum values of turtles from Chesapeake Bay can be broken into several related categories. Serum measurements were affected by geographic location (due to physical differences in sites), seasonal changes, and pathological differences. Trends in serum samples primarily indicated effects on two major systems. The systems most reflecting changes were osmoregulation and metabolism (or nutritional differences). Results of a multi-variate analysis of the data also support these observations.

## Geographic Variations

Animals from the northern site were most distinctly different from animals form the southern site. These sites were the high salinity (30-32 ppt) southern Bay mouth site at Lynnhaven Inlet (Zn 3, Figure 1) and the more northern lower salinity (8-18 ppt) site at the Potomac River mouth (Zn 12) (Chen *et al*. 1984; Kuo *et al*. 1982). Sites located between these areas were more closely related to the southern site. Animals from southern sites were exposed to higher salinity and most samples were taken earlier in the season than samples from animals at the northern site. Because of geographic and physical differences in location, behavior of turtles differed between sites (Bellmund *et al*., 1987). Animals in the northern site were more representative of resident feeding populations that move into the Bay

early during the season and remain until cold weather forces migration out of the Bay (Lutcavage and Musick, 1985; Keinath et al , 1988). This behavior exposes these animals to longer periods of lower salinity.

Animals from the northern area were taken from nets that use fishing methods different than those of the southern Bay. These nets have a large by-catch of the Blue crab, Calinectes sapidus. Sea turtles taken from these nets had feces primarily composed of this species of crab. Caretta taken from more southern sections of the Bay are documented to be primarily feeding on the horseshoe crab, Limulus polyphemus (Lutcavage and Musick, 1985; Lutcavage, 1981). The choice of prey items may impose variable nutritional differences between locations.

Osmoregulation

Physiologically, sea turtles, as regulating poikilothermic animals, must compensate for a wide range of environmental changes. Ionic regulation affects acid-base balance, water regulation, and internal homeostatic control of many systems (Lutz, 1972; Stewart, 1978; Dunson and Weymouth, 1965; Dunson, 1979; Jackson and Ultsch, 1982). Data taken from the northern site show that animals from this area have lower but more variable electrolyte levels than animals taken from the higher salinity site (Table 4). This may reflect lower activity of the salt gland in response to decreased osmotic challenge. Evans (1973) found that sodium efflux from Caretta was 300uM/100g/hr in sea water. After transfer from sea water (SW) to

fresh water (FW) gland function decreased and sodium efflux decreased by 90%-99% within 6 hr and attained a rate of 0.7-1.4uM/100g/hr or 0.2-0.5% of the sodium loss of seawater acclimated animals after 1-2 days (Evans, 1973). Salt gland function in Chelonia mydas shut down after transfer from sea water to fresh water within 25 hours (Kooistra and Evans, 1976). Salinity in zone 12 ranges from 8 ppt to 18 ppt from April to Dec depending upon rainfall (Chen et al., 1984).

Animals foraging over the season in lower salinity can be expected to have lower electrolyte values and different sodium:potassium ratios than animals from higher salinity waters. Although salt gland function shuts down in freshwater to retain electrolytes, sodium efflux continues via the cloaca and integument (Kooistra and Evans,1976; Robinson and Dunson,1976; Evans, 1973). Grigg (1981) found that Crocodylus porosus collected from the the wild over a salinity gradient from fresh water to sea water had, in freshwater, decreased serum sodium, increased serum potassium, and decreased urinary sodium. In salt water these animals had increased serum sodium, decreased serum potassium, and increased urinary potassium. Turtles from the Potomac River (low salinity) showed lower serum sodium and chloride averages than the group for Virginia animals but their average potassium concentration increased over the summer. Turtles in Lynnhaven (Zone 3, the mouth of the Bay) showed higher and more constant levels of serum sodium and chloride but lower levels of potassium, reflecting the trends reported by Grigg (1981) in C. porosus. When serum values for all animals were grouped

together sodium and chloride declined with time over the summer. This decline probably reflects extended periods of time in lower salinity.

### Nutritional Differences

The sodium changes in these animals may also reflect the decreased input of sodium through nutritional pathways (Dunson and Mazzotti, 1988). The cutaneous uptake of sodium by the diamond-backed terrapin (Malaclemys terrapin) was found to be negligible and the major route of sodium uptake in seawater occurred orally, through the buccal cavity (Robinson and Dunson, 1976). Evans (1973) noted that in the loggerhead reduced salt load was followed by a reduction in nasal salt gland secretions. The reduction in salt load acts as a feedback mechanism reducing efflux of salt from the system, however continued integumental and cloacal efflux occurs. If this occurs in Chesapeake Bay turtles then animals not actively taking in salt would still excrete it at some level. Although the salt gland ceases to pump ions, there is still ionic flux via the integument and the cloaca (Kooistra and Evans, 1976; Robinson and Dunson, 1976; Evans, 1973). In support of this theory, turtles from the Potomac River and hibernating turtles examined by Lutz and Dunbar-Cooper (1987) from the Florida coast also had lower sodium values than animals actively eating in high salinity waters.

Animals taken from the northern site had consistently higher values for serum total protein, albumin, urea nitrogen, and glucose than animals from the southern sites (Figures 6 & 8). In addition

these animals were generally larger and heavier with average Q [(Q=CLS3/wt)x 10**5] values that were higher than animals from the southern sites (Bellmund et al. ,1986). General observation of the pectoral and pelvic areas of these animals indicated that they were fatter and apparently healthier than animals from more southern sites. This may be affected by the difference in diet with the northern animals feeding more extensively on Callinectes while southern animals fed primarily on Limulus (Lutcavage, 1981; Bellmund et al., 1987).

## Seasonal Variations

### Osmoregulation

Seasonal affects are related to length of time animals are exposed to salinities less than that of full strength sea water. This is one reason for differences observed in apparently healthy animals from the northern Potomac River site and other southern sites examined. Explanations for these observed trends include an increase in fluid output. In fresh water cloacal fluid has a flow rate of 2-4 times that in SW (Kooistra and Evans, 1976). In low salinity water animals may increase their intake of water, which in conjunction with salt gland shut down, help maintain sodium and electrolyte levels.

Caretta in the Potomac River show an inverse relationship between sodium and potassium over time (Figure 7). Animals in lower salinity water continue to exchange sodium over an entire summer season even though the osmoregulatory challenge is severely reduced. The seasonal results of this adaptation are that animals remaining in

relatively high salinity waters (>18ppt) for the summer have higher serum electrolyte levels than animals foraging in lower salinity waters for the entire season. This is the pattern found during the summer season in Chesapeake Bay animals

This interpretation is supported by Bennett et al. (1986) finding that hatchling C. caretta drink sea water to compensate for osmotic and excretory water loss. In Crocodylus porosus plasma potassium was different (4.1-3.5mM/L) over a salinity gradient from fresh water to sea water (Grigg, 1981). In this same study potassium in cloacal urine of C. porosus showed a steady increase proportional to the salinity gradient. If C. caretta follow the same strategy as Malaclemys and C. porosus they then would excrete potassium, which is taken up as a counter ion to sodium, via the kidney (Robinson and Dunson, 1976; Gilles-Baillien, 1973; Grigg,1981). If this were true then one would expect the sodium:potassium ratio to change over environmental salinity ranges. The Na:K ratio for these animals by location is found in Table 4. This ratio demonstrates compensatory regulation of sodium and potassium for C. caretta from Chesapeake Bay, in C. caretta studied by Evans (1973), and in Caretta from Florida (Lutz and Dunbar-Cooper, 1987)

Metabolism and Excretion

In addition to salinity differences, hibernation, decreased feeding, activity, and temperature also have an important affect on osmoregulation and blood chemistry of sea turtles. Lutz and Dunbar-Cooper (1987) have defined the biochemical plasma profile of an

apparently hibernating sea turtle. The most definitive of these parameters were believed to be sodium, magnesium, and calcium (Lutz and Dunbar-Cooper,1987). Seasonal changes are also related to variations in temperature from early season low temperatures during migration to higher temperatures in late summer.

Changes seen by Haggag et al (1966) indicate that protein, glucose, and non-protein nitrogen change during hibernation in the lizard Varanus greseus. They found that there was a decrease in protein and glucose and an increase in non-protein nitrogen in hibernating animals. These trends mirror trends seen in early season animals in Chesapeake Bay and also reflect changes seen by Lutz and Dunbar-Cooper (1987). Animals in Chesapeake Bay were lower in total protein, inorganic phosphorus, and urea nitrogen early in the season. Haggag et al. (1966) attribute the rise in non-protein nitrogen to a build up of excretory end products due to decreased excretion during hibernation. Animals in Chesapeake Bay forage for the summer season and would be expected to increase all of these parameters to some extent in response to food availability and seasonal temperature. Temperature affects digestive turnover rate and food intake in reptiles and so an animals digestive patterns and efficiency are directly affected by seasonal temperature fluctuations (Parmenter, 1981). Animals migrating into Chesapeake Bay early in the season have moved through water that is from 14-16oC (Musick unpubl observation). This means that animals moving into the Bay early in the season are physiologically acclimatized to temperatures that are much lower than the temperature of the water they were taken from.

Turtles would then be expected to exhibit serum profiles more characteristic of cold reptiles, that is animals with a lowered metabolic rate. Figure 14 shows the results of work on one Caretta which was sampled after feeding to find the affects of lowered temperature on glucose and urea nitrogen metabolism. This work showed that decreased temperature changed relative circulating levels of urea nitrogen and glucose after feeding. This same relationship was also visible in animals arriving early in the season in Chesapeake Bay and in cold stunned animals from this region. Cold stunned animals have low urea nitrogen and glucose values relative to mean values of these parameters calculated for apparently healthy animals (Tables 7 & 14). Jackson and Ultsch (1982) found that at 3°C potassium increased from 2.4 meq/l to 9.0 meq/l in anoxic freshwater turtles (Chrysemys picta belii). They also found dramatic increase in total calcium and magnesium.

Gilles-Baillien (1973) reports that Malaclemys acclimatized to seawater had higher plasma osmotic pressure than animals acclimatized to fresh water. This increase in osmotic pressure is attributed to an increase in sodium, chloride and urea in sea water adapted animals (Gilles-Baillien, 1970). Urea values were found to peak in September, just prior to hibernation, in seawater adapted Malaclemys and this effect was described as an osmoregulatory adaptation to compensate for hibernation decreases in other electrolytes (Gilles-Baillien, 1973). This pattern was also seen in loggerhead sea turtles from Chesapeake Bay whose glucose and urea nitrogen increased during the summer until September. The patterns in Malaclemys for

sodium, chloride, and potassium were similar for both fresh and salt water adapted animals, these values were highest from May to September but were progressively lowered during hibernation (Gilles-Baillien, 1973). Loggerheads in Chesapeake Bay showed an increase over the summer in total protein, albumin, and phosphorus.

Pathological Changes

Sick and injured animals had a lower mean sodium level than apparently healthy animals. If sodium, chloride, and potassium are plotted together in apparently healthy animals and in sick animals, sodium and chloride change together in healthy animals but all three electrolytes change together in sick or injured animals. Sick and injured animals generally had serum components that were more variable for electrolytes and total protein/albumin values. This group also had higher mean globulins and lower mean urea nitrogen and glucose than apparently healthy animals.

Pathologic changes in these animals can be related to temperature effects, disease affects, and changes related to severe emaciation (Sturbaum and Bergman, 1981; Austin et al., 1927; Bonnet, 1979; Frye, 1981). Temperature changes affect the rate of uptake and excretion involved in renal processing, and digestive or metabolic function. Disease affects vary from animal to animal. Severely emaciated animals often had high serum total protein and low or extremely variable electrolyte values. Emaciation may, simply be a manifestation of internal disease effects and result in high globulin values and severely altered osmoregulatory capacity. Lutz et al

(1986) found that the main system compromised by application of crude oil to sea turtles was osmoregulation. However complex physiological interrelationships function to control metabolic processes and are often not detectable by examination of individual mean values in blood of lower vertebrates (Lutz,1972)

## Multi-Variate Analyses

### Principal Component Analysis

Principal component analysis (PCA) of the data was run on animals classified as apparently healthy or sick and injured. Three factors groups were defined by PCA for both groups of animals. The first factor in both groups was composed of the variables albumin, total protein, calcium, and phosphorus. The second factor group in healthy animals was composed of urea nitrogen, potassium, and glucose. Factor group 3 was composed of sodium and chloride. PCA of sick and injured animals defined factor groups for factors two and three differently than they were defined for healthy animals. Group 2 was composed of sodium, chloride and potassium. Group 3 was composed of urea nitrogen and glucose.

The first factor in PCA was defined by the same variables in both healthy and sick groups. This result indicates that this is a fundamentally important group and is stable whether the animals are healthy or not. Calcium and phosphorus interact with all ions and charged species to affect ionic regulation and acid-base balance (Stewart, 1978; Jackson and Ultsch, 1982). These compounds can be present in blood, in part bound to proteins, particularly albumin.

They are affected in juvenile animals by variations in ionic regulation, acid base balance, growth requirements, temperature, metabolism, and various pathologies (Guyton, 1982; Frye, 1981;Sturbaum, 1982).

Since all animals in this work were juvenile, growth would also be expected to be an important component of serum chemistry. Growth regulates the circulating concentrations of calcium, and phosphorus. As juvenile animals grow bone is laid down at a rapid rate. In chelonians the massive amount of bone laid down makes the circulating concentration of calcium very important in the total changes in circulating biochemical parameters examined. Calcium is carried by plasma proteins such as albumin. The calcium/phosphorus ratio in bone is between 1.3 and 2.0 (Guyton, 1982). The circulating and incorporated concentration of calcium is regulated by a variety of mechanisms and is dependent on phosphate as a balancing compound. Hence, as expected, calcium and phosphorus in Chesapeake Bay animals were related to total protein and albumin in PCA. In addition the ontogenic changes seen in the shells of developing juvenile chelonians is responsible for changing availability in the exchangeable ion pool (Dunson and Heatwole, 1986). This is important for calcium and phosphorus regulation as well as for sodium and potassium.

Changes in serum concentrations of calcium or phosphorus may come from disruption of internal regulatory mechanisms, physiological charges due to a varying physical environment (ie. temperature and

salinity), or from nutritional input of these compounds. The presence and circulating concentration of ionized calcium is determined by the nutritional availability of calcium, the availability and activity of vitamin D3 (cholecalciferol), temperature, the ionic balance of the plasma, the colloidal osmotic pressure from calcium/albumin complexing, and the absorption or removal of calcium by the body (Frye, 1981; Jackson and Ultsch,1982; Guyton,1981; Jackson et al ,1984; Stewart,1978). Many of these conditions combine to result in synergistic or concurrent effects and can often be interrelated. Calcium in reptiles is involved in bone growth, membrane function, and the strong ion difference (SID) (Jackson et al ,1984; Stewart, 1981; Ultsch et al ,1984). Calcium is used as part of the buffering system within turtles (Jackson et al , 1984; Ultsch et al , 1984) and can be mobilized from bone.

Changes in the composition of variables in the second factor group in both data sets (for apparently healthy and sick or injured turtles) were important in examining internal homeostatic changes and differences between these two groups. In apparently healthy animals the second Factor group was composed of urea nitrogen, potassium, and glucose. In sick and injured animals, the second Factor group was composed of sodium, chloride, and potassium. The composition of Factor group 2 for apparently healthy animals was made up of parameters that are controlled primarily by excretion and metabolism. Factor group 3, for apparently healthy animals, was composed of sodium and chloride, a group obviously related to osmoregulation.

Factor group 2 on sick and injured animals was made up of sodium, chloride, and potassium. Factor group 3 was made up of urea nitrogen and glucose. The switch of importance of potassium in this group of animals from the group which apparently describes renal activity and includes urea nitrogen and glucose to the osmoregulatory group with sodium and chloride indicates an important internal change. The shift in these variable groupings indicates that osmoregulatory ability was affected in the sick group. The change in electrolyte relationships could be due to a loss of function in the salt gland and the loss of the Na-K pump. The same effect was seen in the application of crude oil to sea turtles, this resulted in the loss of osmoregulatory capacity (Lutz et al.,1986). Due to these effects it appears that the most important system to examine in sea turtles to determine the overall status of an animal is to examine the electrolyte balance and the Na+, Cl-, and K+ concentrations

Q-Mode Analysis

Turtle groupings in Q-mode analysis were based primarily on salinity, temperature, and physiological stress. The variables delineating these groups were the same. In both cases sodium, urea nitrogen, and glucose (chloride may also be included, depending upon cut-off values for Varimax factor loadings) were included. Osmoregulation and temperature were of primary importance. Gilles-Baillien (1973a&b) and Haggag et al. (1965,1966), among others, report the use of non-protein nitrogen in reptiles as an osmoeffector. In addition, they report that all components picked in this analysis change with hibernation and seasonality. Increased

glucose in the endmember group for Factor II can be considered as a response to heat stress or disease state and the resulting physiological changes that occur in moribund or stressed animals (Sturbaum, 1982; Sturbaum and Bergman, 1981; Frye, 1981; Paulson and Hutchinson, 1987). Wedemeyer (1970) describes Selye's general adaptive syndrome in response to stress as including increases in blood glucose level and nitrogen metabolism. The general adaptive syndrome may be characterized by non-specific metabolic changes such as " The morphological, biochemical, and physiological changes which occur as a result of stress " (Wedemeyer, 1970). Changes accompanying this syndrome include increased nitrogen metabolism and increased blood glucose level. Physical response is manifested by alarm reaction, resistance and exhaustion. These changes could result in the grouping of apparently stressed animals with elevated internal temperatures and high serum glucose into Factor group II.

Three dying animals were found in the Factor group II list of end members. Turtle MT-113-83 died shortly (within a week) after blood was drawn for analysis. This animal was severely emaciated yet had the second highest blood glucose level of all animals examined. All sick turtles in this factor group had low blood urea nitrogen levels. Stress hormones in these animals were not assessed due to the uncertainty of the results and because 1983 analyses were not available. Animals included in Factor group II due to increased temperatures were generally characterized by fighting and $>30^{o}C$ temperatures. It is possible that animals were included due to

nutritional status but it was not possible to determine the magnitude of this effect

Animals from Factor group I were characterized by low cloacal temperatures (<22$^{o}$C) and being found in high salinity (generally off-shore) waters. This group of animals included two severely cold stunned animals (< 10$^{o}$C) that plotted slightly off the factor line. All animals, except those that were cold stunned, had high urea nitrogen and low glucose. No obvious trend was found for absolute values of sodium and chloride, however they were included as the two most important variables in this Factor group. When serial urea nitrogen and glucose measurements were made on a single animal held at different temperatures their relationships changed. At 20$^{o}$C, urea nitrogen was higher than glucose but at 26$^{o}$C this relationship was reversed. These changes in these components with temperature nicely reflect relationships seen in Factor group I animals from the field.

Factor scores were tightly grouped for a third set of animals. Factor scores in this group ranged from .7900-.8100 . This group of animals include two sub-clinically ill turtles and other animals that had no obvious reason to be included. It may be that this group represents the sub-clinically ill group of animals that have no other obvious symptoms other than complex internal changes.

# VI. CONCLUSIONS

This work provides a data base of blood serum values for healthy and sick loggerhead sea turtles from the wild. It illustrated the difficult nature of assessing condition from absolute serum values in wild populations with no previous history. It was not possible to divide turtles into sick and healthy categories based upon individual serum profiles. Indeed, it was not possible to divide turtles into these categories using multivariate analyses. Using multivariate analyses it was only possible to look at relative relationships within serum variables and develop categories based upon variable relationships. Profile groups using PCA were based on metabolism and growth, ionic environment, and metabolic excretory state. Factor analysis divided groups based upon their physical environment and in part on their health (internal environment). It showed the importance of external physical environment to poikilothermic animals and the inherent difficulties in describing animals closely tied to their external environment.

Relationships and general relative differences in various serum compositions were reflected in all analyses and the literature. Multivariate factor analysis divided turtles along a gradient best defined by salinity, temperature, and physiological stress. Data and

analyses suggest that osmoregulation is an important system to examine when evaluating internal homeostasis in marine turtles. Marine animals must have an effective functional osmoregulatory system to continue to maintain a favorable osmotic gradient. Once this system is compromised the entire well being of the animal is in jeopardy. As such this system is both conservative in its physiological range and responsive to environmental fluctuations in temperature, nutritional availability, and particularly salinity. The result of this dual nature is a system that is critical in maintaining internal homeostasis and valuable in determining the status of the animal.

Appendix I.
Serum Analytical Methods

| Test Name | Catalog Number | Application Sheet Reference Number |
|---|---|---|
| Total Protein | 749117 | 6-417-0285 |
| Albumin | 620174 | 8-50414501-1282 |
| Calcium,GABA | 857825 | 6-689-0887 |
| Phosphorus | 836281 | 6-443-0186 |
| BUN/Glucose Twin Reagent | 842273 | 6-951-0787 |
| Uric Acid | 704156 | 6-477-0684 |
| Creatinine | 704130 | 6-494-0684 |
| Total Bilirubin, DPD | 977179 | 6-495-0687 |
| GOT/GPT Twin Pack | 842265 | 6-592-0486 |
| Alkaline Phosphatase | 704024 | 6-455-0587 |
| LDH, TRIS | 620099 | 8-50415001-1282 |
| Na, K, Cl, by Ion Selective Electrode | 749044, 749052, 836105, 836113, 836121, 646911, 646938, 608250 | 6-457-0187 |

Reference Book Title: Boehringer Mannheim Diagnostics, Inc. Hitachi 705, Operator's Reference Manual, Section 3. REAGENTS

Published by ;
Boerhringer Mannheim Diagnostics, Inc.
Hitachi 705 Clinical Support
9115 Hague Road
Indianapolis, Indianna 46250

Appendix II

| Test | Method | Reference |
|---|---|---|
| Blood Urea Nitrogen (BUN) | Quantitative colormetric determination of BUN - Direct method | Crocker, C.L., A. J. Med. Tech., 33,361 (1967) |
| Glucose | Quantitative colormateric determination of glucose. | Hultman, E. Nature 183,108. 1959.<br><br>Dubowski, K.M. Clinical Chem. 8, 215 ,1962. |

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