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PHYSIOLOGICAL CONSEQUENCES OF BASKING, DISEASE AND CAPTIVITY IN THE GREEN TURTLE, CHELONIA MYDAS

by

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of the requirements for the degree of
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To Maya, in hopes that one day, you too will follow your passion.

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CHAPTER I

GENERAL INTRODUCTION

The green turtle (*Chelonia mydas*, Linnaeus, 1758) is unique among marine turtles for its behavior of emerging from the water at times other than nesting and basking on the substrate for extended lengths of time. Although other species of marine turtles are known to bask in the sun while floating on the ocean's surface, termed aquatic basking (see Sato et al. 1995), terrestrial or atmospheric basking occurs only in *C. mydas*, and only in few locations around the world. While solitary basking has been reported to occur in remote areas of the main Hawaiian Islands (Katahira, pers. comm.) and Namibia (Tarr 1987), basking in aggregations currently occurs only on isolated islands in the Pacific Ocean: Northern Australia (Bustard 1974), the Galapagos Islands (Snell and Fritts 1983), and the Northwestern Hawaiian Islands (NWHI: Whittow and Balazs 1982; Fig. 1.1). Historic records of basking on islands off western Mexico (Slevin 1931; cited in Snell and Fritts 1983), and eastern Australia (Limpus, pers. comm.), suggest that the behavior was once more common. Development and human use along these former basking beaches may be at least partially responsible for the absence of this behavior in these areas.

Green turtles are listed as Threatened or Endangered under the U.S. Endangered Species Act of 1973, and population declines throughout the Pacific have been attributed to overexploitation and habitat loss (Eckert 1993). Green turtle populations are also threatened by green turtle fibropapillomatosis (GTFP), which is characterized by the growth of fibroepithelial tumors primarily on the skin, eyes, and cloaca (Herbst 1994). Tumors on an individual *C. mydas* were first described from a turtle originating from Florida and maintained at the New York Aquarium nearly 60 years ago (Smith and Coates 1938). Although GTFP has been described as life-threatening (Eckert 1993), very little is known about the physiological impacts of the disease on individual turtles, or on turtle populations.

Currently, GTFP has been observed in green turtles all around the world: the western Atlantic Ocean, Pacific Ocean, Gulf of Mexico, Caribbean, and the Indian Ocean (see

Herbst et al. 1995; Limpus, pers. comm.). Since the 1980's, incidence of GTFP has risen dramatically, especially in Hawaii (Balazs 1991), Florida (Erhart 1991), and the Caribbean (Williams et al. 1994). In certain foraging areas in the main Hawaiian Islands, 49- 92% of animals sampled have been found to have some degree of tumor growth (Balazs 1991). Although the etiology remains unknown, a herpesvirus is suspected (Herbst et al. 1996).

My research examines numerous aspects of basking, disease, and captivity in captive and wild green turtles from Hawaii and Sea World of California. I chose to work with turtles from Hawaii due to the prevalence of disease and the occurrence of basking in this population. The existence of both of these factors made the Hawaiian green turtles an ideal population to study. In other locations where green turtles bask, incidence of disease is low or non-existent. Turtles from Sea World were especially valuable in order to investigate the long term effects of captivity.

My first set of questions investigate physiological roles that basking serves. In Chapter Two, I describe how basking behavior may be influenced by disease state (specifically GTFP), and how the behavior influences body temperature and oxygen consumption (metabolic rate). Results from these experiments elucidate the roles of thermoregulation, energy conservation, and increased immunity in eliciting this behavior. Furthermore, comparisons of oxygen uptake between diseased and non-diseased turtles help assess the impact of GTFP at both the individual as well as population level.

Patterns of basking and use of the thermal environment for turtles both in captivity and in the wild in the NWHI were also investigated. These findings, described in Chapter Three, improve our understanding of the role of thermoregulation in atmospheric basking. In addition to thermoregulation, this chapter explores biologically-relevant theories that have been proposed to explain the unique basking behavior in green turtles. Theories discussed relate to disease, reproduction, digestion, and predator avoidance.

I investigated the biochemical responses to GTFP and to captivity in turtles from Hawaii and Sea World of California. In Chapter Four, I describe numerous plasma biochemical variables that are compared among turtle groups (diseased and non-diseased,

captive and wild). Such comparisons are useful in establishing diagnostic tools needed to improve our ability to assess the health status of green turtles.

Because all species of marine turtles are currently listed as Threatened or Endangered, information gained on the biology of these animals is valuable in our attempts to reverse this trend toward extinction. If basking is confirmed to serve important roles in the survival of green turtles in the Pacific, then measures must be taken to ensure access to the last few remaining basking beaches. Furthermore, by understanding how disease influences physiological processes at the individual level, impacts of disease at the population level can be better understood. Determination of the cause of GTFP and its long-term implications on population dynamics have been recommended as priorities for the U.S. National Marine Fisheries Service. The survival of green turtle populations in the Pacific is dependent upon the implementation of conservation practices that are based on a thorough understanding of the animals' biology, especially in respect to survivability, fecundity, longevity, and recruitment (Eckert 1993). Hopefully, results from my research will be helpful in establishing scientifically-sound conservation measures.

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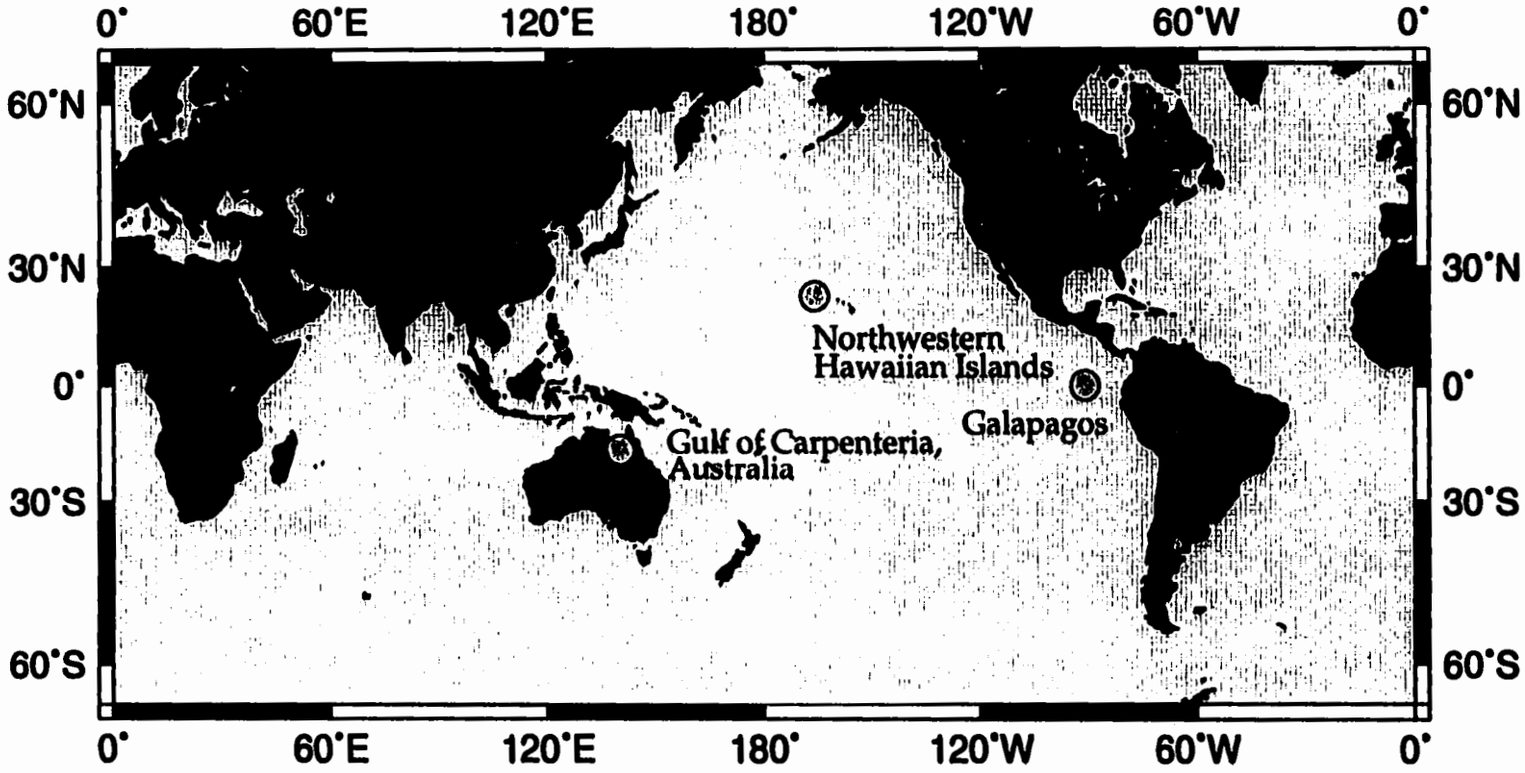

Fig.1. Locations of green sea turtle populations where aggregates of turtles commonly bask.

CHAPTER II

PHYSIOLOGICAL RESPONSES TO ATMOSPHERIC BASKING AND DISEASE IN CAPTIVE GREEN TURTLES

Introduction

The energetic costs of both basking and disease in Hawaiian populations of the green turtle, *Chelonia mydas,* are poorly known, yet important in improving our understanding of physiology of this threatened species. Although a rare behavior among marine turtles, a few populations of green turtles are known to haul out of the water and bask on the substrate for many hours (Bustard 1974; Whittow and Balazs 1982; Snell and Fritts 1983; Garnett 1985). Basking has been shown to increase body temperature in numerous species of turtles (Boyer 1965; Obbard and Brooks 1979; Crawford et al. 1983), including *C. mydas* (Whittow and Balazs 1982; Swimmer et al. 1996). A rise in body temperature ultimately affects numerous physiological properties (Dawson 1975; Bartholomew 1982). Among ectothermic vertebrates, active oxygen consumption rates increase exponentially with increasing body temperature (Bennett 1978, 1982), and a Q_{10} between 2 and 3 is believed to occur in reptiles (Bartholomew 1982; Glass and Wood 1983), including green turtles (Lutz et al. 1989).

Despite increased body temperature, however, oxygen consumption may not necessarily increase if the energetic cost of basking, which requires minimal movement, is lower than the cost of remaining in the water, which could involve strenuous activity. Physical performance (e.g. swimming speed) influences metabolic rates due to increased oxygen demand associated with increasing physical activity (Bennett 1978). In both fish and green turtles, oxygen consumption increases exponentially as swimming speed increases (see Bennett 1978). Rates of metabolism are therefore the combined result of body temperature and activity. If basking results in lower metabolic rate despite the increase in body temperature, then basking would function as a physiological adjustment to minimize energetic cost. Although energy conservation has been suggested as a

possible reason for basking in green turtles (Balazs 1980), this is the first study to test this theory.

In addition to examining the energetic costs of select *C. mydas* behaviors (e.g. basking, swimming), this study also investigates the energetics of disease. Performance measurements, such as metabolic rate, can be useful to determine the physiological impacts of disease on an individual. Improving our understanding of disease is especially valuable for green turtles in the Hawaiian Islands due to the high incidence of disease. In certain foraging areas in the main Hawaiian Islands, turtles affected with green turtle fibropapillomatosis (GTFP) comprise between 49-92% of sampled populations (Balazs 1991). GTFP is characterized by benign internal and external fibroepithelial tumors, primarily on the skin, eyes, and cloaca. Although the etiology remains unknown, GTFP is likely caused by a herpesvirus (Herbst 1994; Herbst et al. 1996). Determination of the cause of GTFP and its long-term implication on population dynamics have been recommended as priorities for the U.S. National Marine Fisheries Service (Eckert 1993). By understanding how GTFP influences physiological properties, such as metabolism, the impact of disease on green turtle populations in the Hawaiian Islands can be more clearly understood.

While both basking behavior and incidence of disease are prevalent in green turtles from Hawaii, it should be noted that incidence of GTFP is not known to be high in the other locations where basking occurs (Galapagos Islands and Carpenteria Islands, Australia).

Numerous vertebrate and invertebrate species respond to disease and parasitic infection with alterations in physiological capacities. One measure of energetic costs is oxygen consumption, or metabolic rate. Most studies report reductions in rates, or metabolic rates in infected individuals (Anderson 1975; Schall et al. 1982; Schall 1983; Hayworth et al. 1987; Kilgore et al. 1988). Other studies, however, have found infected animals have higher metabolic rates than controls (Hurst and Walker 1935 [cited in Anderson 1975]; Lester 1971; Walkey and Meakins 1970). In fish, a reduction in swimming performance (time to exhaustion; Coleman 1993) has been reported due to parasitic infection. In two studies of lizards, however, oxygen uptake was similar in

infected and noninfected lizards (Malvin and Kluger 1979; Christian and Bedford 1995). This will be the first report of the physiological consequences of GTFP on green turtles by comparing the metabolic rates of diseased and non-diseased individuals.

In addition to improving our understanding of the physiological impact of GTFP disease on Hawaiian green turtles, this study seeks to determine the physiological role that basking serves in a species of marine turtle. Comparisons of metabolic rates of basking and non-basking turtles will elucidate the role of basking as an energy-conserving mechanism.

Materials and Methods

Captive Turtles

Nine subadult captive turtles were obtained from the wild in Kaneohe Bay (21°30'N, 157°50'W), island of Oahu, Hawaii, during numerous trips between June and September, 1994. Turtles were captured by hand, brought onto a boat and later transported ca. 15 km to Kewalo Research Facility in Honolulu. Of the nine captive turtles, five had visible signs of GTFP disease and were housed in a separate tank with an independent water supply from the four apparently healthy turtles. Each 8-meter diameter tank received a constant supply of seawater (ranging from 23-27°C) and was scrubbed every third day to reduce algal and diatom growth. Mean size (straight carapace length) for the GTFP-afflicted and non-tumored turtles was 53.6 cm (SE=1.82) and 47.9 cm (SE=1.73), respectively. Each turtle was fed two squid per day six days a week. Wooden ramps, with an incline of approximately 7°, and a basking platform (1m x 2m), were placed in each tank. Basking ramps were exposed to full sunlight during all daylight hours.

Basking of captive turtles was determined either through personal observation or by time-lapse photography. Turtles were considered basking when at least one-half of the body was on the ramp and out of the water. A 35 mm camera (Pentax WR-90) with an interval-timing mechanism was positioned so that both basking ramps were within visible range. Basking data were recorded at 30 and 60-minute intervals for up to 36 consecutive

hours. Data presented in this study are limited to the period between October and December, 1994, during which time all turtles were feeding and were apparently acclimated to their captive situation.

Body temperatures of captive turtles were determined by inserting a thermistor probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) 15 cm depth in the cloaca. This location has been determined to accurately reflect body temperature (Mrosovsky 1980). Temperatures of basking turtles were recorded after the turtles had been basking a minimum of one hour. Body temperatures for non-basking turtles were obtained after the turtle had been in the water for a minimum of two hours.

Metabolic rates were calculated by measuring gas content and volume from expired gases. A funnel-shaped mask made from a plastic container was taped over the animal's head to provide an air-tight seal. One turtle (#Y847) had a large tumor growth covering an eye, thereby preventing an airtight seal for the mask and precluding the individual from this experiment. An acrylic Y-shaped valve connected to the mask above the snout separated inspired and expired gases. The inspiratory side was open to the air, while the expiratory side was connected to a 20L Collins Bag for collection. Total expired volume was determined by compressing the collection bag through a dry gas meter. Samples were drawn out of the bag using Tygon tubing and suctioned through a Drierite drying column. Duplicated dry gas samples were analyzed for concentration of O_2 using a Beckman Oxygen Analyzer. Data presented have been converted to standard temperature, pressure, and water vapor pressure (STPD) values.

Body temperatures were compared for tumored turtles that had been basking and swimming using a two-tailed t-test with a group mean comprised of a mean for each turtle. Oxygen consumption for tumored turtles post-basking and post-swimming were compared for individual turtles and turtle groups. Comparisons between turtle groups (e.g. tumored basking vs. tumored swimming, tumored swimming vs. non-tumored swimming) were compared using a group mean comprised of means for each turtle. Oxygen demand was also compared between groups of tumored and non-tumored turtles. These data were obtained when turtles had similar body temperatures (between 24.5 and

26.3°C). Statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC, V. 6.08). Significance is reported at $p \leq 0.05$.

Results

Only tumored turtles were observed basking, and basking occurred on every observation day (n=47). Non-tumored turtles were never observed on the basking ramp or platform. Tumored turtles (n=5) that had been basking had body temperatures an average of 2.85°C (SE=0.479) above water temperature (Fig. 2.1). These body temperatures ranged from 27.8-32.2°C. Tumored turtles that had been basking had significantly higher body temperatures than when they had remained in the water ($t=4.901$; $p<0.0012$). For turtles that had been in the water prior to body temperature determination, body temperatures averaged 0.49°C (SE=0.045) and 0.53°C (SE=0.065) above water temperature for non-tumored (n=4) and tumored turtles (n=5), respectively (Fig. 2.1). Body temperatures for turtles that had been in the water prior to metabolic determination were in the range of 24.5-26.3°C for non-tumored turtles and 23.3-26.1°C for tumored turtles.

Rates of oxygen consumption plotted by body temperature are presented for tumored turtles after each had been swimming and basking (Figs. 2.2 to 2.5). Most rates of oxygen consumption were lower post-basking as compared to post-swimming. Only one turtle, #J676, had higher metabolic rates after basking compared to after swimming. Comparisons of metabolic rates of basking and non-basking turtles indicate that as a group, turtles that had been swimming had significantly higher rates than when they had been basking ($t=4.005$; $p=0.010$).

Metabolic rates obtained for non-tumored green turtles that had been swimming ranged from 0.006 to 0.026 l O_2 kg^{-1} hr^{-1}, with a mean of 0.145 l O_2 kg^{-1} hr^{-1}, (SE=0.0069).

Comparisons of metabolic rates of tumored and non-tumored turtles at similar body temperatures (between 24.5 and 26.3°C) and activity level indicate that tumored animals

had significantly higher rates of oxygen consumption (mean=0.039 l O_2 kg^{-1} hr^{-1}, SE=0.0094) compared to non-tumored animals (mean=0.015 l O_2 kg^{-1} hr^{-1}, SE=0.0070; t=4.641; p=0.004; Fig. 5).

Discussion

Turtles afflicted with GTFP were observed basking throughout their time in captivity, while non-tumored turtles never basked. Basking resulted in significantly elevated body temperature (by ca. 3°C). Comparisons of metabolic rate of basking and non-basking tumored animals indicate that basking can result in reduced oxygen consumption despite elevated body temperature. The finding that oxygen demand was nearly three times higher in tumored animals as compared to non-tumored animals indicates the severe physiological impact of GTFP on individual turtles. Consequences of these findings are likely to affect the population dynamics of *C. mydas* in Hawaiian waters.

Influence of Disease on Basking Behavior

Turtles infected with GTFP were observed basking frequently, while non-tumored animals never basked. Selecting behaviors that increase body temperature, such as basking, have been observed in other ectothermic organisms infected with disease, and this behavior, known as behavioral fever, has been shown to have adaptive value (Kluger 1978; See Chapter 3). Behavioral fever has been observed to occur in a lizard (Vaughn et al. 1974), freshwater turtles (Monagas and Gatten 1983), a teleost fish (Reynolds et al. 1976), frogs (Kluger 1977), and an amphibian larvae (Casterlin and Reynolds 1977). Animals that attain a behavioral fever showed higher rates of survivorship than when they were maintained at lower temperatures (Kluger 1978).

Water temperature has also been shown to directly influence presence of tumors and incidence of disease in walleyes (*Stizostedion vitreum*; Bowser et al. 1988, 1990). Prevalence of these viral-induced tumors declined from 32% at water temperatures 0-5°C to 5% when water temperature was 20.1-25.0°C (Bowser et al. 1988). Tumor regression

and lower incidence of disease at high temperatures may be due to the suppression of the immune response in low water temperatures as has been observed in numerous species of fish (see Bowser et al. 1988). In another study, temperature was also found to affect the viral load and growth of a renal tumor in leopard frogs (*Rana pipiens*). Low environmental temperatures (4°C) were found to be conducive to viral growth, while elevated temperatures (20-22 °C) were found to enhance lysing of the infected cell, resulting in the release of cellular contents causing tumor growth (Zambernard and Vatter 1966).

Based on information obtained from these aforementioned studies, where ectotherms reduce viral loads by maintaining higher body temperature, diseased green turtles in this study may have used basking as a means to achieve immunological benefits of high body temperature. Unfortunately, determination of viral load was not measured in this study. Many aspects of immunity in marine turtles are poorly understood, and even less so is the effect of temperature on immunity. Once the etiological agent of GTFP is isolated, more research will be needed to determine the effects of temperature on tumor growth and viral load in the green turtle. Such research is needed in order to assess the potential immunological benefits gained by basking turtles.

Energetic Costs of Activity

In this study, metabolic rates determined for non-tumored sub-adult captive green turtles (mean = 0.0145 l O_2 kg^{-1} hr^{-1}) were within the low end of values for standard and resting metabolic rates reported in studies of captive adult green turtles using similar methods (mean = 0.024 l O_2 kg^{-1} hr^{-1}; Prange and Jackson 1976; Jackson and Prange 1979). The higher metabolic rates reported in these previous studies (Prange and Jackson 1976 and Jackson and Prange 1979) may be related in part to differences in turtles' activity level and body temperature, as turtles in those studies had body temperatures approximately 3°C higher than turtles in this study.

Despite increased body temperatures, most values of metabolic rate in this study were lower after turtles had been basking compared to when they had been swimming (Figs. 2.2 to 2.5). Only one turtle, (#J676) had higher metabolic rates after basking compared to

post-swimming. The lower metabolic rates observed in turtles that had been basking (and remaining motionless) compared to when they remained in the water (which includes swimming) suggests that the energetic costs of swimming exceed costs incurred by basking with increased body temperature. Based on predicted Q_{10} of between 2 and 3 in marine turtles (Lutz 1989), one would expect the rate of oxygen consumption to increase with the concomitant increase in body temperature (by 3°C) for basking turtles. The observed results, however, indicate that the energetic costs of swimming must be sufficiently high as to negate the effects of increased temperature coupled with minimal physical exertion.

Metabolic measurements of tumored turtles after they had remained in the water, however, are not necessarily measures of the costs of swimming. Informal observations indicated that swimming was the predominate activity of turtles in the holding tank, but animals were not subjected to any stressor that would force the animal to maintain motions of swimming. Only the last five to ten minutes of the time spent in the water could be considered active swimming as the turtles attempted to avoid being captured for the metabolic experiments by swimming actively. Basking turtles were easily captured while they were basking. In nature, swimming is likely to be more sustained than in the tank, and therefore costs of remaining in the water should be even higher for animals in the wild as compared to those in captivity. If so, basking on land would result in an even larger energy savings as was observed for turtles in captivity.

The energetics of swimming in green turtles is fairly well understood. Although the green turtle has evolved a nearly fusiform body highly adapted to swimming (Lowell 1990), costs of swimming are high due to the animal's need to surface to breath (Bennett 1978, 1982). As swimming speed increases for a green turtle, it's oxygen consumption increases exponentially, and the net cost of swimming for a green turtle is twice that predicted for swimming marine iguanas and five times that of salmonid fishes (Bennett 1978, 1982). Based on values attained for resting and active metabolism in captive green turtles, active metabolism is believed to be 9-10 times the standard level (Jackson and Prange 1979).

Due to the high oxygen demands of activity in green turtle, selecting energy-conserving behaviors, such as basking, could reduce energetic costs and increase available energy without incurring an oxygen debt. It is unfortunate that non-tumored turtles did not bask, as comparisons of energetic costs are therefore only possible with diseased animals. Because I have no reason to suspect otherwise, however, I will assume that healthy animals would gain the same relative energetic benefits as tumored animals by basking.

Increasing net available energy would be especially important in an herbivorous reptile, such as *C. mydas*, whose rates of growth and reproductive output may be nitrogen or energy limited (Wood and Wood 1980). Behaving in ways that minimize oxygen consumption has been observed in numerous vertebrates and it may be advantageous in order to meet energy demands associated with various life history stages (Spotila and Standora 1985). In the Hawaiian green turtle, one such energetically-demanding period is during the breeding season, when animals incur costs associated with migration and reproduction, as well as avoidance of predatory tiger sharks.

Hawaiian green turtles migrate to mate and to nest in the Northwestern Hawaiian Islands (NWHI), which are aproximately 600 km from their foraging grounds in the main Hawaiian Islands. Aggregations, primarily comprised of nesting females (Swimmer, pers. observ.), bask on East Island, a 13-acre atoll that hosts one of the highest numbers of green turtle nests in the NWHI. In the other two populations where basking of *C. mydas* occurs in the wild, incidence of basking is also greatest during the nesting season, and basking animals are predominately nesting females (Bustard 1974; Snell and Fritts 1983). In addition to the high energetic costs expended by both males and females to migrate and to mate, female turtles have added costs incurred by egg production and egg-laying (Shine 1980; Waldschmidt et al. 1987). Assuming that healthy gravid turtles also achieve lower metabolic rates by basking as compared to remaining in the water, the predominance of females basking could be explained as a mechanism to conserve energy during this energetically-costly period.

In addition to reproductive costs, both male and female turtles are subject to high predation pressure by tiger sharks during their time in the NWHI (Swimmer, pers.

observ.), and predator avoidance has been suggested as a theory to explain the basking phenomenon (Balazs 1980). The following observations support this theory: 1) basking occurs at a time of year when density of tiger sharks is especially high in the NWHI (predation risk from sharks is also a threat to green turtles in the main Hawaiian Islands, yet sharks are not as abundant or concentrated as they appear to be around East Island), 2) turtles have no land predators in the NWHI, and therefore basking would be a predator-free refuge, 3) gravid turtles, which comprise a great majority of basking animals, may have reduced agility compared to non-gravid turtles, thereby making them more vulnerable to predators (Schwarzkopf and Shine 1992), and 4) despite incurred heat gain, basking could reduce energy costs that could be especially high due to the turtles' accelerated swim speeds to avoid predation. Basking could therefore offer turtles bioenergetic benefits of reduced energetic expenditure, thereby increasing energy allocated for activities beyond maintenance.

Based on these potential advantages of basking, the behavior could be biologically advantageous for any green turtle population that incurs high energetic expenditures incurred by reproduction and predator avoidance. Due to the observed biological benefits of basking, efforts should be maintained to ensure access to basking beaches for the few remaining populations of *C. mydas* known to bask.

Energetics of Disease

Studies on the metabolic responses to parasitic infection in both endothermic and ectothermic species have shown that infected animals have lower metabolic rates as compared to non-infected controls (Anderson 1975; Schall et al. 1982; Schall 1983; Hayworth et al. 1987; Kilgore et al. 1988). Reduced metabolic rates have been attributed to deficits in hemoglobin levels, which result in decreased oxygen transport capacity (Schall et al. 1982), decreased muscular activity (Coleman 1993), the inability to thermoregulate and supply oxygen to the tissues (Hayworth et al. 1987), and prolonged stress (see Waldschmidt et al. 1987). In two species of reptiles, the green iguana (*Iguana iguana;* Malvin and Kluger 1979) and the frillneck lizard (*Chlamydosaurus kingii;*

(Christian and Bedford 1995), however, oxygen uptake was similar between parasitized and unparasitized lizards.

In contrast to the aforementioned studies, GTFP-afflicted turtles in this study had nearly three times higher metabolic rates than non-tumored turtles after similar activities (swimming) and at similar body temperatures (Fig. 2.6). Increases in metabolic rate in response to infection have been observed in a parasitized snail (Hurst and Walker 1935; cited in Anderson 1975) and fish (Lester 1971; Anderson 1975). Specific mechanisms responsible for increased rates of metabolism due to parasitic infection are unknown, and even less well understood is the influence of a viral infection, such as GTFP. Despite these shortcomings, however, results from this study indicate that disease poses significant physiological demand on an individual, and this information can be useful in understanding the impact of GTFP on green turtle population dynamics.

Additional energy demand imposed on diseased animals could help explain the low number of tumored animals observed in the basking and breeding population of green turtles in the NWHI. Most estimates of GTFP occurrence in the main Hawaiian Islands are approximately 50%, yet in some locations GTFP is virtually non-existent (Balazs pers. comm.). An estimate of the overall incidence of turtles afflicted with GTFP in the main Hawaiian Islands is uncertain, but likely to be higher than the ca. 12% observed in nesting turtles in the NWHI. This could be explained by a regression in tumor growth as the animal grows, and thus tumor size should continue to be monitored over time. Yet, it seems more likely that the additional energy costs imposed upon diseased animals prevents animals from migrating (ca. 600 km) to the breeding grounds. Migration costs (via swimming) alone are quite high, and additional costs incurred by disease and reproduction may preclude tumored animals from migrating and entering the breeding population.

Furthermore, insufficient fat stores in diseased animals could hinder or prevent a turtle from migrating to the breeding grounds. Schall (1983) found that for *Sceloperus* lizards infected with malaria, storing fat was more difficult than for non-infected lizards. Because almost all calories of fat bodies were used in egg production, clutch size of lizards infected with malaria was significantly smaller than for noninfected lizards (Schall

1983). Assuming that green turtles afflicted with GTFP have similar difficulties in storing fat, insufficient fat storage could prevent both the energetically-costly migration to the NWHI as well as a resulting reduction in egg production once in the breeding grounds.

Yet another explanation for the low number of diseased green turtles in the breeding grounds is the possibility that affliction with GTFP results in early death of an animal. Mortality of free-ranging green turtles is difficult to estimate, and thus mortality rates of either healthy or diseased animals are unknown. In laboratory-maintained *Sceloperus* lizards, however, malaria-infected lizards were more likely to die than noninfected . animals, and the same was assumed to occur in wild populations (Schall 1983). Assuming that GTFP also imposes life-threatening physiological constraints on individual green turtles, mortality of diseased-turtles may account for the low number of tumored turtles in the basking and breeding grounds. One way to address this question of differential mortality between diseased and non-diseased animals would be to capture and compare recapture rates. If diseased animals have lower recapture rates than non-diseased turtles, then such findings would lend support to the idea that GTFP results in early death of an animal. This in turn would indicate that GTFP is indeed a life threatening disease.

The observed and hypothesized effects of GTFP on individual turtles suggest that there are likely to be population-level consequences resulting from this disease. Because GTFP has been attributed to added energy demand, and that it may also increase mortality and decrease reproductive output, population size of already-threatened Hawaiian green turtles are likely to be adversely impacted by GTFP.

Several assumptions were made in this study that deserve further inquiry. Because in captivity only tumored turtles basked, data on metabolic rates in response to basking were not available for non-tumored turtles. Determination of metabolic rates for non-tumored turtles that had been basking would strengthen the argument that basking serves to conserve energy. Also, more research on the metabolic response to swimming and increased body temperature would isolate the costs of various activities (e.g. swimming and basking) from increased costs due to elevated body temperature. While I speculate

on the immunological benefits of elevated body temperature (gained via basking), a study that investigates alterations in the host's resistance to disease would lend support to the theory that diseased turtles bask in an attempt to achieve a behavioral fever, which in turn helps fight the disease. Lastly, determining the fate of diseased animals would be especially valuable in clarifying the impact of GTFP on green turtle population dynamics.

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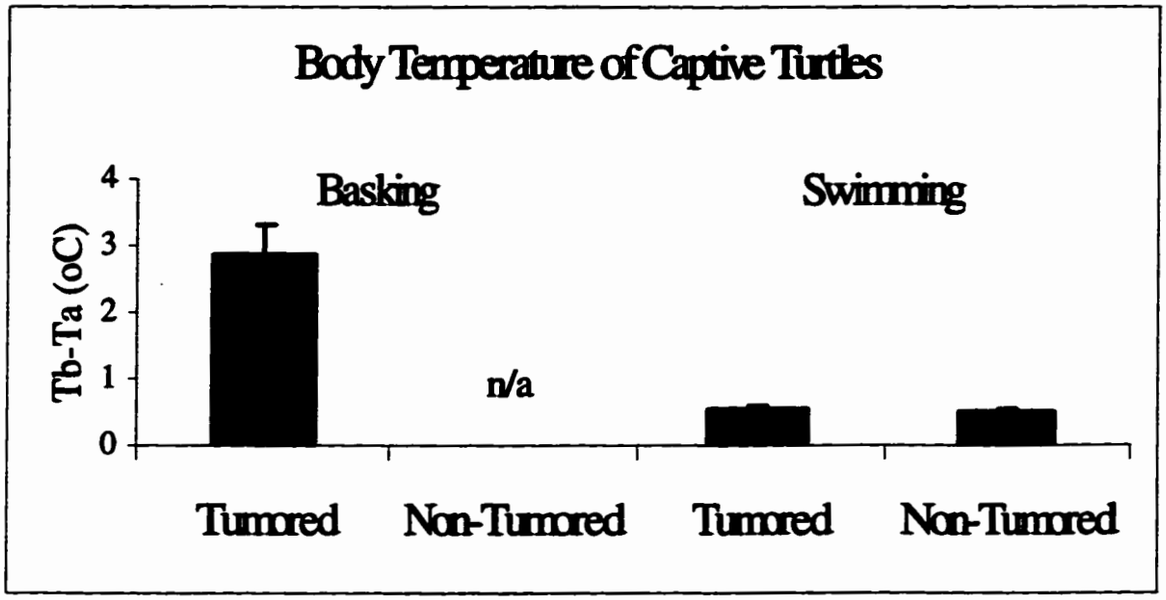

Fig. 2.1. Difference between body temperature and water temperature (±SEM) for captive green turtles post-basking and post-swimming, Honolulu, Hawaii, 1994. Non-tumored turtles in captivity never basked, and thus no data are available for this group.

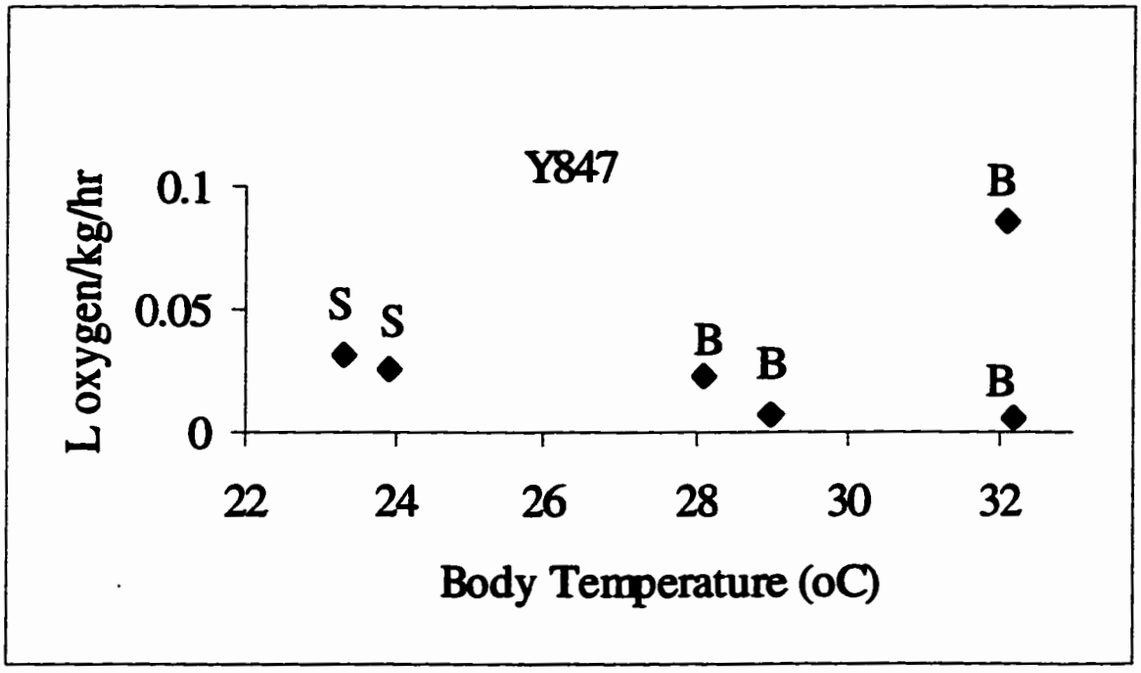

Fig. 2.2. One metabolic rate (l oxygen kg^{-1} hr^{-1}) per recorded body temperature for captive turtle #Y847 after basking (B) and swimming (S). Honolulu, Hawaii, 1994.

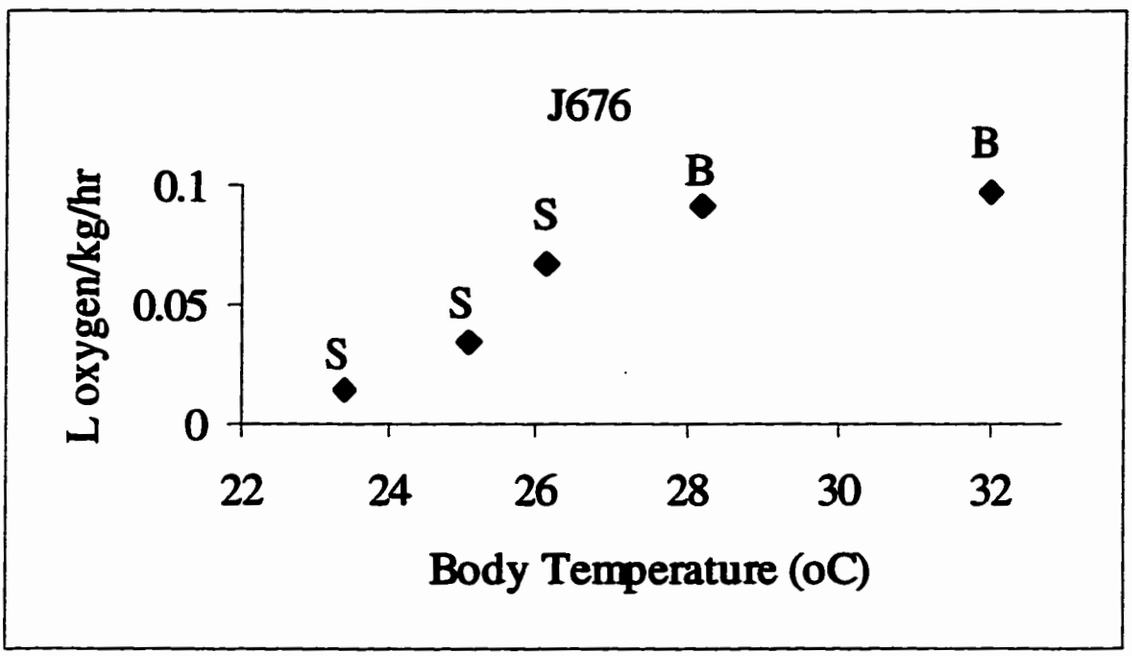

Fig. 2.3. One metabolic rate (l oxygen kg^{-1} hr^{-1}) per recorded body temperature for captive turtle #J676 after basking (B) and swimming (S). Honolulu, Hawaii, 1994.

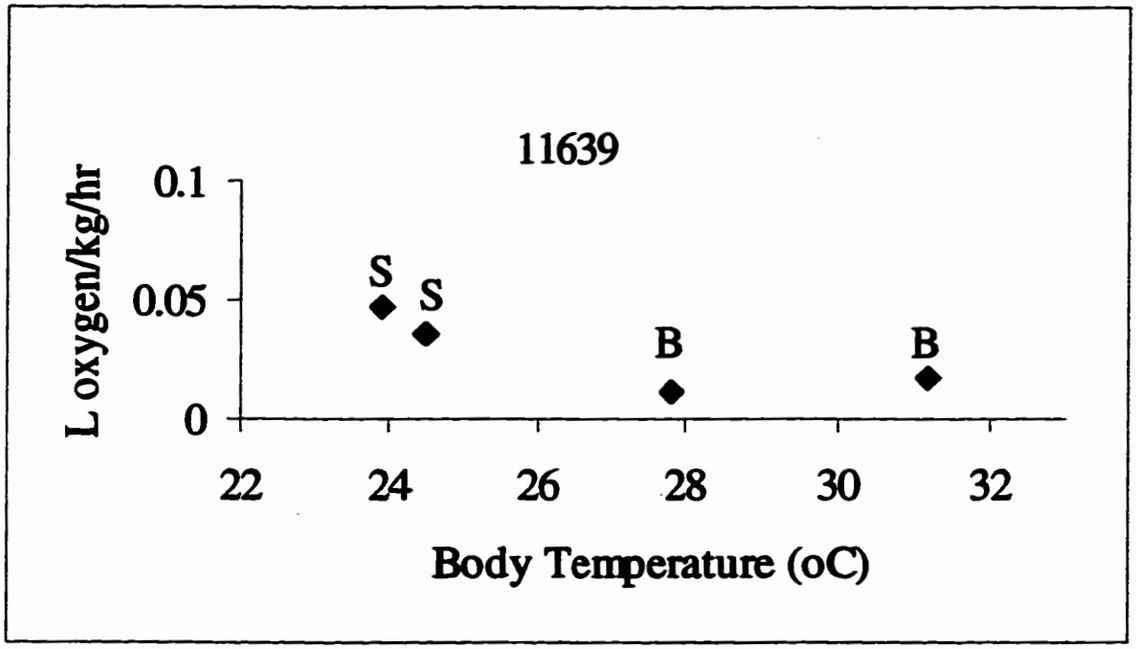

Fig. 2.4. One metabolic rate (l oxygen kg^{-1} hr^{-1}) per recorded body temperature for captive turtle #11639 after basking (B) and swimming (S). Honolulu, Hawaii, 1994.

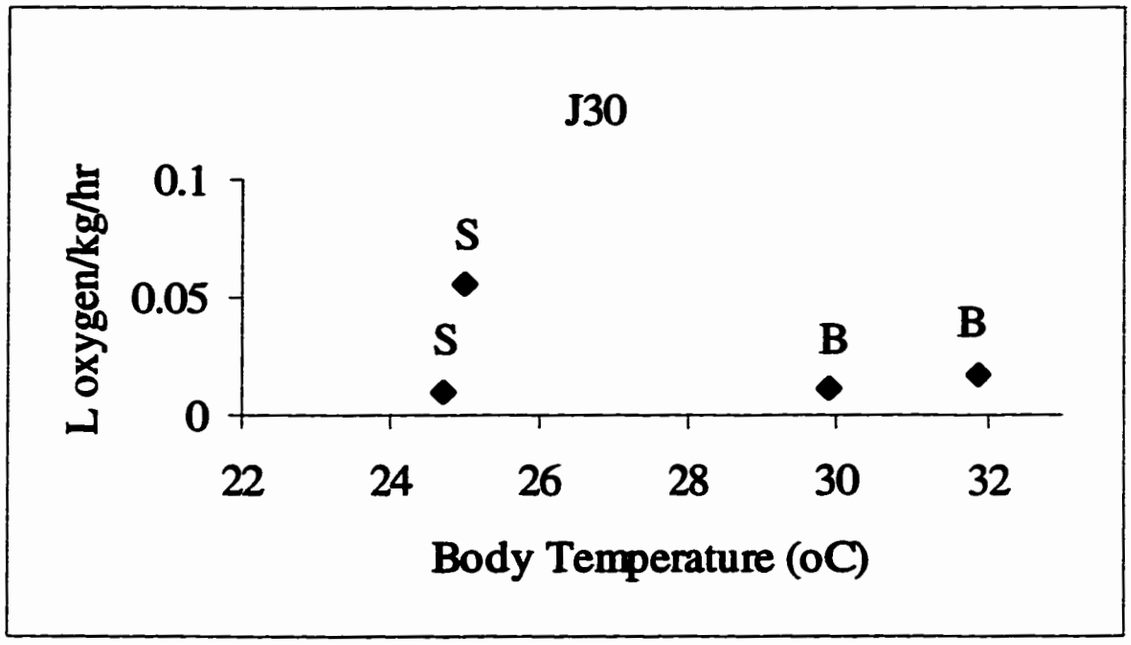

Fig. 2.5. One metabolic rate (l oxygen kg^{-1} hr^{-1}) per recorded body temperature for captive turtle #J30 after basking (B) and swimming (S). Honolulu, Hawaii, 1994.

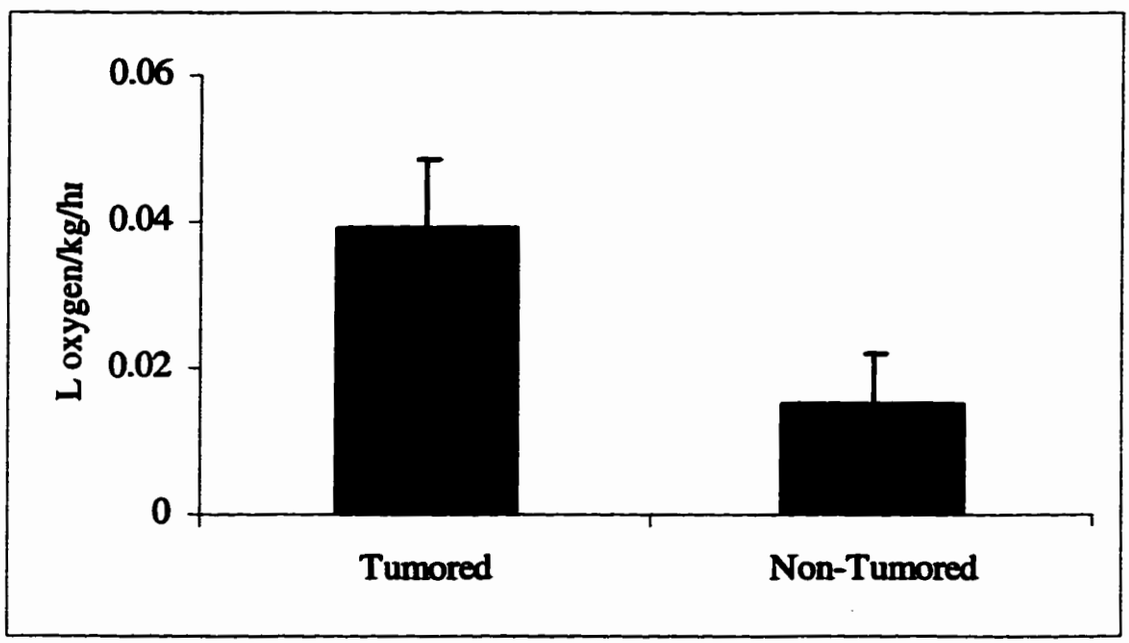

Fig. 2.6. Mean metabolic rates (l oxygen kg^{-1} hr^{-1}) (±SEM) for tumored and non-tumored captive turtles post-swimming. All body temperatures were within 24.5 – 26.1°C (p=0.004). Honolulu, Hawaii, 1994.

CHAPTER III

BASKING BEHAVIOR IN CAPTIVE AND WILD HAWAIIAN GREEN TURTLES

Introduction

In the Northwestern Hawaiian Islands, the Hawaiian green turtle, *Chelonia mydas*, demonstrates a behavior that is uncharacteristic of marine turtles--hauling on shore to bask, both during the day and night. This behavior is termed atmospheric basking and is defined as emerging from water and remaining on the substrate for extended lengths of time during non-nesting periods. Terrestrial basking is a nearly ubiquitous behavior among well-studied freshwater turtles, and is generally considered a means of thermoregulation (Boyer 1965; Obbard and Brooks 1979; Crawford et al. 1983; Spotila et al. 1984). Among marine turtles, however, basking in aggregations is only known to occur in one species, *C. mydas*, and in only a few locations around the world: Australia (Bustard 1974; Garnett 1985), the Galapagos Islands (Snell and Fritts 1983), and the Northwestern Hawaiian Islands (Whittow and Balazs 1982).

This study examines the thermoregulatory function of basking in Hawaiian green turtles. Most studies of basking in freshwater turtles conclude that basking serves primarily as a heat-gaining mechanism whereby turtles bask in order to maximize heat gain while limiting time spent basking. Solar radiation (Boyer 1965; Obbard and Brooks 1979), daily air temperature (Boyer 1965; Obbard and Brooks 1979), operative environmental temperature (see Bakken and Gates 1975; Crawford *et al.* 1983), and temperature differences between air and water (Boyer 1965) have all been shown to be positively correlated with basking in freshwater turtles. Furthermore, basking has been shown to be negatively correlated with precipitation (Obbard and Brooks 1979), and wind (Boyer 1965). For most species of reptiles, basking during the warmest parts of the day allows for maximal heat gain while minimizing the time spent basking, potentially

offsetting costs of behavioral thermoregulation such as reduced foraging time and, if there are land predators, increased vulnerability to predation (see Huey and Slatkin 1976).

Not all studies concur that turtles bask to maximize heat gain by basking during the hottest parts of the day. The painted turtle (*Chrysemys picta*) has been observed basking for long periods of time at relatively cool times of day (Ernst 1971; Schwarzkopf and Brooks 1985). In a preliminary study of green turtles basking in the Northwestern Hawaiian Islands (NWHI), Whittow and Balazs (1982) also found a decline in basking duration as temperatures at the basking beach increased.

Most studies conclude that basking elevates body temperature (see Boyer 1965; Auth 1975; Whittow and Balazs 1982). Theories that have been proposed to explain the benefits of basking or increased body temperatures for marine and freshwater turtles include facilitation in reproductive processes, such as development of egg follicles (Whittow and Balazs 1982; Snell and Fritz 1983), increased digestive efficiency (Boyer 1965; Gatten 1974), enhanced Vitamin D synthesis (Boyer 1965), drying of carapace and integument to remove ectoparasites or algae (Boyer 1965), predator avoidance (Balazs 1980), and energy conservation (Boyer 1965; Balazs 1980). In addition, animals may bask in an attempt to elevate body temperatures to rid the body of pathogens and enhance the immune system. A "febrile state" has been shown to have adaptive value in enhancing host-defense responses to infection, thereby increasing survivorship in numerous vertebrate species (Kluger et al. 1975; Kluger 1991).

One potential function of basking is to elevate body temperature in order to affect physiological processes associated with disease state. In Hawaiian waters, green turtles have a relatively high incidence of green turtle fibropapillomatosis (GTFP; 49-92% of sampled populations; Balazs 1991), a debilitating and disfiguring disease likely caused by a viral agent and characterized by the growth of internal and external benign fibroepithelial tumors (Herbst 1994; Herbst et al. 1996). Given the potential benefits of basking, increasing body temperature to a febrile state might be highly advantageous for diseased green turtles. Although both basking and high incidence of disease are common in the Hawaiian population of green turtles, incidence of GTFP is not known to be prevelant in the other locations where basking occurs.

This study attempts to enhance our understanding of the basking phenomenon in diseased and non-diseased Hawaiian green turtles by examining both captive and wild animals. Specific questions addressed include: 1) Does incidence of disease influence basking? 2) How does basking influence body temperature? and 3) Is basking correlated with environmental variables? Addressing these questions will help clarify the role of basking in the biology of Hawaiian green turtles.

Materials And Methods

Captive Turtles

Nine subadult captive turtles were obtained from the wild in Kaneohe Bay (21°30'N, 157°50'W), island of Oahu, Hawaii, during trips between June and September 1994. Turtles were captured by hand, brought onto a boat, and later transported ca. 15 km to Kewalo Research Facility in Honolulu. Of the nine captive turtles, five had visible signs of GTFP disease and were housed in a separate tank with an independent water supply from the four apparently healthy turtles. Each 8-meter diameter tank received a constant supply of seawater (ranging from 23°C - 27°C) and was scrubbed every third day to reduce algal and diatom growth. Average size (straight carapace length) for the GTFP-afflicted and non-tumored turtles was 53.6 cm (SE=1.82) and 47.9 cm (SE=1.73), respectively. Turtles were each fed two squid per day six days a week. Wooden ramps, with an incline of approximately 7°, and a basking platform (1m x 2m), were placed in each tank. Basking ramps were exposed to full sunlight during all daylight hours.

Basking of captive turtles was determined either through personal observation or by time-lapse photography. Turtles were considered basking when at least one-half of the body was on the ramp and out of the water. A 35 mm camera (Pentax WR-90) with an interval-timing mechanism was positioned so that both basking ramps were within visible range. Basking data were recorded at 30 and 60-minute intervals for up to 36 consecutive hours. Data presented in this study are limited to the period between October and

December 1994, during which time all turtles were feeding and were apparently acclimated to their captive situation.

Body temperatures of captive turtles were determined by inserting a thermister probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) 15 cm into the cloaca. This location has been determined to reflect accurate body temperatures (Mrosovsky 1980). Temperatures of basking turtles were recorded after the turtles had been basking a minimum of one hour. Body temperatures for non-basking turtles were obtained after the turtle had been in the water for a minimum of two hours. Air temperature measurements were obtained from the National Weather Service Honolulu station, located within two air miles of the study site and which is at the same elevation and has a similar topographical profile as Kewalo Research Facility. Air temperatures were recorded in Fahrenheit and later converted to Celsius. Sea water temperatures in the holding tanks were recorded numerous times per day using thermistors. Water temperatures remained between 23-27°C throughout the study period. Substrate temperatures of the basking platform were recorded by drilling a tiny hole and placing a thermocouple under the upper portion of each platform.

Turtles were maintained in captivity until mid December 1994, at which time they were released into Kaneohe Bay.

Wild Turtles

Data on basking behavior of wild turtles were collected on East Island (23°47'N, 166°13'W) at French Frigate Shoals in the NWHI over a six-week period during the peak of the nesting season in June and July 1995. East Island, a 13-acre sand island, is the main site for nesting in the NWHI and during this period was the preferred basking beach for green turtles. Due to potential disturbance of the highly endangered monk seal, I was prohibited from walking around the island during the day when monk seals were often hauled out onto beaches. Consequently, the number of basking turtles was determined by placing a camera with a time-lapse mechanism (Pentax WR-90) at one end of one of the two main basking beaches. Film was changed nightly while patrolling the island for nesting green turtles. Photographs were later analyzed to determine number of turtles

basking. Presence of tumors from GTFP was not discernable from the photographs. Air and sand temperatures throughout the study period were determined with data loggers (VEMCO Minilog-T). Air temperatures were recorded by placing the one data logger in the shade, and a temperature was recorded every three hours. Sand temperatures were determined by placing one data logger 3 cm below the sand surface, both at the northwest beach where basking occurred, and at the southeast beach where basking was virtually non-existent. Sand temperatures were recorded at 15-minute intervals.

Statistics

Statistical interpretation of basking frequency is limited due to the lack of independence among individual turtles. Therefore, only trends of basking and temperatures will be discussed. In some circumstances, correlational analysis was applied to the data using SAS (SAS Institute Inc., Cary NC; V. 6.08 for Windows). These values, however, should be interpreted cautiously. In order to establish independence among captive turtle data, body temperatures were analyzed by obtaining a mean for each individual turtle and applying a two-tailed t-test between turtle groups (tumored-non-basking, tumored-basking, etc.). Sand temperatures at the basking and non-basking beaches in the NWHI were compared using a paired difference t-test. Significance is reported at $p < 0.05$.

Results

Captive Turtles

In captivity, only tumored turtles were observed basking, and basking occurred on every observation day. Non-tumored turtles were never observed on the basking ramp or platform.

Diseased turtles basked throughout the day and night, but with a decline in average number of turtles basking during the early morning hours (0500-0800h; Fig. 3.1). The number of turtles basking increased with increasing air temperatures from 22° to 29°C

(r=0.301; p<0.0001), and then declined with increasing air temperature up to 32°C (r=-0.501; p<0.0001; Fig. 3.2), the highest temperature recorded during the study period. Substrate temperatures and number of turtles basking were negatively correlated (r=-0.514; p<0.0001), yet with a slightly positive slope from 25° to 27°C (r=0.282; p<0.1824), followed by a decline to 35°C (r=-0.540; p<0.0001; Fig. 3.3). There was a slight but significant negative correlation between daily maximum air temperature and average number of turtles basking (r=-0.280; p<0.0001), and it is even stronger for temperatures from 29-32°C (r=-0.343; p=0.0001; Fig.3.4).

Because only tumored animals basked in captivity, data on post-basking body temperatures could be obtained only for tumored turtles. Tumored turtles (n=5) that had been basking had body temperatures an average of 2.85°C (SE=0.479) above water temperature. For turtles that had been in the water, body temperatures averaged 0.49°C (SE=0.045) and 0.53°C (SE=0.065) above water temperature for non-tumored (n=4) and tumored turtles (n=5), respectively. Tumored turtles that had been basking had significantly higher body temperatures than when they remained in the water (t=4.901; p<0.0012).

Wild Turtles

A circadian pattern to basking was evident in adult, wild green turtles in the NWHI (Fig. 3.5) whereby basking frequency was greatest during daylight and relatively rare during night and early morning hours. Basking frequency increased steadily from 0600 until 1400, then decreased until 2300.

Air temperature was positively correlated with average number of turtles basking (r=0.706; p<0.0001), yet there was a decline after 35°C (Fig. 3.6). Sand temperatures at the basking beach were positively correlated with number of turtles basking (r=0.467; p<0.001). Average number of turtles increased with sand temperatures (at the basking beach) until 32°C (r=0.531; p<0.0001), and then declined (r=-0.218; p<0.0001; Fig. 3.7). Throughout the study period, sand temperatures at the basking beach were significantly cooler than sand temperatures on the non-basking beach by an average of 0.41°C (T=-2.306; p<0.022).

Discussion

Comparisons among tumored and non-tumored, as well as captive subadult and wild adult turtles provide a way to better understand the basking phenomenon in *C. mydas*. In captivity, subadult tumored turtles were observed basking both during the day and night, while non-tumored turtles were never observed basking. Diurnal basking of captive turtles resulted in significantly increased body temperatures, thereby confirming the behavior's role in thermoregulation. Basking frequency was positively correlated with air and substrate temperature until a threshold temperature was reached (29°C for air temperatures, 27°C for substrate temperatures), at which point basking frequency decreased. In the wild, basking frequency was highest during early afternoon, corresponding to the hottest time of day. Similar to the captive situation, basking frequency increased with increasing air and substrate (sand) temperature to a threshold temperature (34°C for air temperatures, 32°C for substrate temperatures), at which point basking declined. These results suggest that basking in Hawaiian green turtles can serve numerous functions depending on life history stage and disease-state.

Influence of Disease on Basking

In captivity, only tumored turtles basked, while in the wild, tumored animals comprise only ca. 12% of nesting or basking turtles (Balazs 1991). Two possibilities may explain observations of captive tumored turtles basking. First, tumored turtles may bask in an attempt to reduce energy costs. A previous study found that basking green turtles incur less energetic cost than turtles remaining in the water (see Chapter 2; Swimmer et al. 1996). Furthermore, diseased animals were found to have higher oxygen demand compared to non-tumored turtles when performing similar activities at similar body temperatures (see Chapter 2; Swimmer et al. 1996). Therefore, basking of tumored turtles could be an adaptive behavior that enables diseased animals to conserve valuable energy reserves.

Second, diseased animals may bask in an attempt to elevate body temperatures to both rid the body of pathogens and to enhance their immune system. A rise in the body's

thermoregulatory set point, resulting in a febrile state, has the potential to enhance the survival of an infected organism through the effects of temperature acting directly on the pathogen (i.e., by inhibiting its growth), indirectly by enhancing the immune system, or by a combination of both direct and indirect effects (Kluger 1979).

Numerous pathogens are known to be directly inhibited by heat, thereby providing the rationale for extensive medical therapies involving heat, solar radiation, or both (Gilman and Thilly 1977; Kluger 1979). Elevated body temperatures can enhance the immune response in at least four ways: 1) by increasing mobility and activity of white blood cells, 2) by stimulating interferon production and function, 3) by activating T lymphocytes, and 4) by synergistically influencing reduction in plasma iron concentration that reduces the growth rate of many species of bacteria (Kluger 1979; Kluger 1991). In a population of flattened musk turtles (*Sternotherus depressus*) in Northern Alabama, the majority of basking turtles (61%) was comprised of sick animals (Dodd *et al.* 1988). Development of a fever (via behavioral means) in response to bacterial pathogens has been reported for reptiles (turtles, *Terrapene carolina* and *Chrysemys picta*, Monagas and Gatten 1983), a lizard (*Dipsosaurus dorsalis*, Vaughn et al. 1974), birds (D'Alecy and Kluger 1975, cited in Kluger 1977), amphibians (the frog, *Hyla cinerea*, Kluger 1977), and fishes (*Micropterus salmoides* and *Lepomis macrochirushas*, Reynolds et al. 1976; *Carassius auratus*, Covert and Reynolds 1977). In a laboratory experiment intended to determine how pathogens influence body temperature in two species of freshwater turtles, Monagas and Gatten (1983) injected *Terrepene carolina* and *Chrysemys picta* with live *Aeromanas hydrophila*, a known reptilian pathogen. In response to this bacterial infection, turtles behaviorally thermoregulated and produced fevers. However, responses to viral pathogens, which are most likely responsible for GTFP, are unknown.

Another possible explanation for the preference for basking in tumored turtles as compared to non-tumored turtles is the use of solar radiation to diminish tumor size, as radiation therapy is known to achieve in human medicine. In this study, changes in tumor size were not measured and major differences were not discernible. Assuming that tumored turtles achieve some of the above-mentioned potential advantages of basking, this behavior would be a highly beneficial behavior for green turtles afflicted with GTFP.

The fact that non-tumored subadult captive turtles did not bask in this study suggests that turtle size (age) may also influence this behavior. In all locations where basking behavior occurs in the wild, basking animals consist nearly exclusively of adults (Bustard 1974; Balazs 1980; Snell and Fritz 1983; Garnett 1985; Tarr 1987). Furthermore, at Sea Life Park on Oahu, Hawaii, and Sea World in San Diego, California, non-tumored adult *C. mydas* commonly bask (Swimmer, pers. observ.). Reports of subadult presumably non-tumored turtles basking in captivity (Balazs and Ross 1974) and in the wild (Swimmer, pers. observ.) exist, yet these are rare in comparison to observations for adults. These results suggest that both tumor size and turtle size (age) are likely to influence basking behavior.

Influence of Basking on Body Temperature

Captive tumored turtles that had been basking for a minimum of one hour had mean body temperatures nearly 3°C higher than surrounding water temperature. Unfortunately, body temperature determination for wild turtles was not possible due to limitations of human activity on basking beaches. However, an earlier study of basking green turtles on a different atoll in French Frigate Shoals, NWHI, reported mean cloacal temperatures of basking green turtles as 28.7°C (range: 26.9-31.3°C), which was approximately 5°C higher than ocean temperature (Whittow and Balazs 1982). These findings confirm that basking serves a role in behavioral thermoregulation in *C. mydas*, as has been determined for numerous species of freshwater turtles.

Basking Patterns and Environmental Factors

In captivity, basking of tumored turtles occurred during day and night (Fig. 3.1), while in the wild, nocturnal basking was relatively rare (Fig. 3.5). Other studies have also reported that *C. mydas* in the wild bask primarily during the day (Bustard 1974; Whittow and Balazs 1982; Snell and Fritts 1983). At least two other studies, however, have reported green turtles basking at night (Balazs 1977; Garnett 1985). Because beach and sea water temperatures in the NWHI were similar at night (ca. 25°C), thermoregulation alone is not likely to elicit nocturnal basking.

In both captive and wild sites, basking increased until a certain threshold temperature, at which point there was a negative correlation between basking frequency as well as both substrate and air temperature (Figs. 3.2,3.3,3.6,3.7). Although captive and wild turtles differed in their basking patterns and temperatures at which basking behavior increased or decreased, behaving in ways that prevented the animals from over-heating was evident in both groups.

In order to remain hauled out on the substrate and bask for extended lengths of time without incurring unwanted heat gain, turtles were observed basking in the following ways: 1) in the wild, turtles basked on relatively cool beaches, 2) neither captive nor wild turtles oriented their bodies to maximize heat gain, and 3) turtles in the NWHI were observed to flip sand over their carapace, thereby placing the white, heat-reflective sand over their dark, heat-absorbing carapaces. (Similar observations were made for turtles basking on another atoll in French Frigate Shoals, NWHI; Whittow and Balazs 1982).

To further illustrate this phenomenon in wild turtles, I compared two four-day periods of continuous data of basking and environmental temperatures. During the period of June 16-20, basking frequency was significantly greater than basking frequency during the period of June 29-July 2 ($t=4.35$; $p<0.0001$). During the earlier period, when basking frequency was high, sand temperatures on the basking beach were significantly lower than sand temperatures during the latter period ($t=5.45$; $p<0.0001$). Therefore, it appears that basking behavior declines when environmental temperatures are high.

In the NWHI , the danger of a physiologically damaging heat-stress may influence the behavior of turtles to reduce the amount of available heat gained while basking. Benefits of elevated body temperature impose a trade-off, in which turtles seek to increase benefits associated with basking, while limiting total heat gain to tolerable or unstressful levels. By basking until a certain threshold temperature, turtles could select a preferred body temperature, which, in this study, appeared to differ between smaller subadults as compared to adults.

A preferred, or selected, narrow range of body temperatures is commonly achieved through behavioral means in most ectotherms (Dawson 1975). Given potential costs associated with this form of thermoregulation (e.g. reduced foraging time; see Huey and

Slatkin 1976), the behavior likely offers some adaptive value, such as optimizing certain physiological processes.

Benefits of Basking

In addition to reducing energetic costs (see Chapter 2) and elevating body heat, basking of green turtles may also serve to influence physiological processes such as reproduction and digestion. Furthermore, basking could be a predator-avoidance mechanism, which could serve an important ecological role.

Reproduction

In both oviparous and viviparous ectotherms, basking and elevated body temperatures have been shown to influence females' reproductive physiology and success. Gravid females bask significantly more than either non-gravid females or males in many reptilian species (see review in Shine 1980). Numerous accounts of both marine and freshwater turtles indicate that basking frequency is greater in females than in males, and its occurrence increases during the nesting season (Bustard 1974; Whittow and Balazs 1982; Snell and Fritts 1983; Hammond et al. 1988; Schwarzkopf and Shine 1991). Captive turtles in this study were subadults, and therefore unlikely to be influenced by factors involving reproduction. Observations of wild animals reported in this study, however, were made during the peak of the nesting season on the primary nesting beach in the NWHI, and basking animals were comprised primarily of nesting females (pers. observ.). Although the nature of the relationship between basking and reproduction remains uncertain, the high frequency of *C. mydas* basking during the nesting season suggests the occurrence of a relationship.

One theory proposed to explain this phenomenon is that heat gain may optimize reproductive processes, such as accleration of egg development (Whittow and Balazs 1982). Additionally, in his discussion on the costs of reproduction in viviparous skinks, Shine (1980) asserts that the increased basking of gravid females serves to elevate body temperatures and accelerate embryogenesis. Because gravid skinks have significantly reduced running speeds compared to non-gravid skinks, they are more vulnerable to

predation. By reducing the gestation period (via basking), one likely advantage of basking is that it decreases predation risk and thereby reduces costs of reproduction (Shine 1980; Schwarzkopf and Shine 1991). In reproductively active Hawaiian green turtles, basking could serve to accelerate egg development, thereby reducing the duration of the nesting process and an earlier migration from the NWHI. This would confer the advantages of reducing turtles' exposure to predatory tiger sharks, as well as enabling turtles to migrate toward the feeding grounds in as short a time window as possible.

Thermoregulation via basking could also serve to increase reproductive fitness by influencing the number and size of viable offspring produced. In a laboratory experiment of gravid *Sceloperus jarrovi*, a viviparous lizard, Beuchat (1988) found that when lizards were allowed to behaviorally thermoregulate and shuttle between different thermal environments, the lizards produced significantly more viable offspring than females that were held at constant temperatures. Furthermore, hatchlings born from the thermoregulating females were significantly larger than those born from non-thermoregulating females. Assuming that larger hatchlings have greater chances of surviving than smaller hatchlings (see Sinervo *et al.* 1992), this behavior could potentially increase offspring fitness as well. If so, behavioral thermoregulation via basking of egg-bearing *C. mydas* could serve to increase maternal fitness.

Predator Avoidance

Green turtles are subject to high predation pressure by tiger sharks during their time in the NWHI, and predator avoidance has been suggested as a theory to explain the basking phenomenon (Balazs 1980). Predatory sharks are also present in the main Hawaiian Islands, where basking of turtles rarely occurs. The shallow depth of the water in the NWHI may add to the risk of predation for turtles as their maneuverability may be limited. Perhaps the high density of tiger sharks in the NWHI is at least partially responsible for basking in this area. Tiger sharks appear to be most abundant around East Island during June and July, a peak time for basking and nesting turtles, as well as a time when albatross chicks commonly fledge. While tiger sharks are known to attack fledgling albatross (pers. observ.), they are also believed to attack marine turtles. On numerous

occasions, I observed nesting green turtles with fresh wounds, strongly indicative of attacks from sharks. Assuming that arriving at and remaining on the basking beach does not increase predation risk, basking on land could serve as a refuge from predators.

While basking of freshwater turtles usually increases an animals' vulnerability to predation, this would not be true for basking of marine turtles in an area absent of land predators. Predator-avoidance strategies of prey species include shifts in habitat use in time or space, and escaping to refuges free from predators. Presence of predators has been shown to alter prey behavior in numerous invertebrate and vertebrate species: planktonic rotifers (*Hexarthra mira* and *Trichocerca chattoni*; Heeg and Payner 1988), mayfly nymphs (*Baetis rhodani*; Tikkanen et al. 1996), snails (Physella sp; Turner 1996), scorpions (*Urodacus armatus*; Quinlan et al. 1994), fishes (*Oncorhynchus nerka*; Bevelhimer and Adams 1993; *Perca fluviatilis*; Eckman and Imbrock 1996; *Lagodon rhomboides*; Jordan et al. 1997), tadpoles (*Hyla arborea*; Chovanec 1992; *Litoria ewingi*; Peterson et al. 1992; *Hyla chrysoscelis*; Bridges and Gutzke 1997), snakes (*Pelamis platurus*; Rubinoff et al. 1988; *Acrochordus arafurae*; Houston and Shine 1994; *Thamnophis elegans* and *T. sirtalis*; Charland and Gregory 1995), hatchling sea turtles (Wyneken and Salmon 1992), and foxes (*Vulpes vulpes*, Penn and MacDonald 1995). While it would be difficult to determine a response to predation risk in a free-ranging turtle, given the abundance of predatory tiger sharks in the NWHI, a behavior that could reduce that risk, such as basking, could be a highly adaptive behavior.

Digestion

Elevated body temperatures have also been shown to influence physiological processes involved with food digestion and assimilation. Digestion efficiency in most ectotherms is positively correlated with body temperatures (see review in Dawson 1975), and numerous studies conclude that fed reptiles behaviorally thermoregulate to maintain higher body temperatures than nonfed reptiles. In freshwater turtles, basking was induced in red-eared turtles (*Trachemys scripta*; Gatten 1974) and in yellow slider turtles (*Pseudemys scripta*; Hammond et al. 1988) soon after ingesting food. By elevating body temperature, the efficiency at which turtles digested food and assimilated energy was

increased. Although basking of marine turtles post-feeding might offer the advantage of optimizing digestive efficiency, I observed that basking frequency of captive turtles study was not influenced by digestive state. In this study, turtles in captivity basked during the day and night, while feeding occurred only in the morning. Furthermore, wild populations of *C. mydas* in the main Hawaiian islands are foraging and not generally basking, while turtles in the NWHI commonly bask and are not generally feeding (G. Balazs, pers. comm.). Taken together, these observations suggest that digestive processes are unlikely to influence basking behavior.

Energy Conservation

In order to determine if basking results in net energy gain (independent of optimizing temperature-dependent physiological processes), metabolic costs of basking must be compared to the costs of remaining in the water. If high energetic demand is required for 1) arriving at a basking site, 2) avoiding predators while at the site, and 3) elevating body temperatures while basking, then basking would presumably not be motivated by energy savings. However, if these costs are lower than the costs of remaining in water (e.g. cost of swimming), then basking could result in net energy benefit.

For green turtles in the NWHI, the majority of individuals have migrated from the main Hawaiian Islands for the primary purpose of reproduction, and do not feed there. Therefore, assuming that migration costs are those associated with reproduction and not basking, the energetic cost of arriving at the basking site is zero. Because there are currently no land predators for a basking turtle, potential energetic costs associated with predation-avoidance should also be zero. The third potential cost, that of energetic demand, however, requires a better understanding of how the costs of basking compare to the costs of remaining in water.

In an earlier study, metabolic rates were determined for captive *C. mydas* that had either been swimming or basking (see Chapter 2; Swimmer et al. 1996). Results of that study indicate that for the majority of turtles studied, basking turtles had significantly lower metabolic rates than turtles that had been swimming, despite elevated body

temperatures. These results suggest that basking of *C. mydas* in the NWHI may be an adaptive means of conserving energy.

Conclusions

Both captive and wild Hawaiian green turtles were observed basking in increasing frequency until a certain threshold temperature, at which point basking frequency declined. Although captive and wild turtles differed in their basking patterns and temperatures at which basking frequency declined, behaving in ways that prevented animals from over-heating was evident in both groups. This study confirms that basking is a behavioral mechanism that enables turtles to regulate body temperatures to a desired range of temperatures. Different temperature preferences for wild and captive turtles may relate to animal size and rate of heating and cooling. The rise in body temperature gained from basking likely serves physiological functions, such as facilitation in reproductive processes and improved immunity. More research is needed to determine how basking and elevated body temperature influence reproductive processes and the immune response. Reproductive benefits from basking can be assessed by comparing clutch size, inter-nesting interval, and hatchling size between basking and non-basking turtles. If green turtles are similar to other reptiles, basking would increase clutch and hatchling size and reduce the inter-nesting interval, thereby serving a beneficial role in reproduction. Effects of basking on immunity can be determined by comparing white blood cells and immunoglobins from basking and non-basking animals. Assuming that marine turtles have similar immunological responses to elevated body temperature as other ectotherms, turtles with elevated body temperatures would have increased immunity. While it would be especially valuable to investigate the effect of basking on survivability, this would be extremely difficult in marine turtles because they are so long-lived.

Although elevated body temperature is known to facilitate physiological processes involved in digestion for many reptiles, it is unlikely that basking serves a role in digestion for Hawaiian green turtles. Independent of the effects of body temperature,

basking may also serve as a way to conserve energy and to escape high predation pressure from tiger sharks. Due to the biological and ecological benefits of basking, efforts to protect basking beaches must be maintained.

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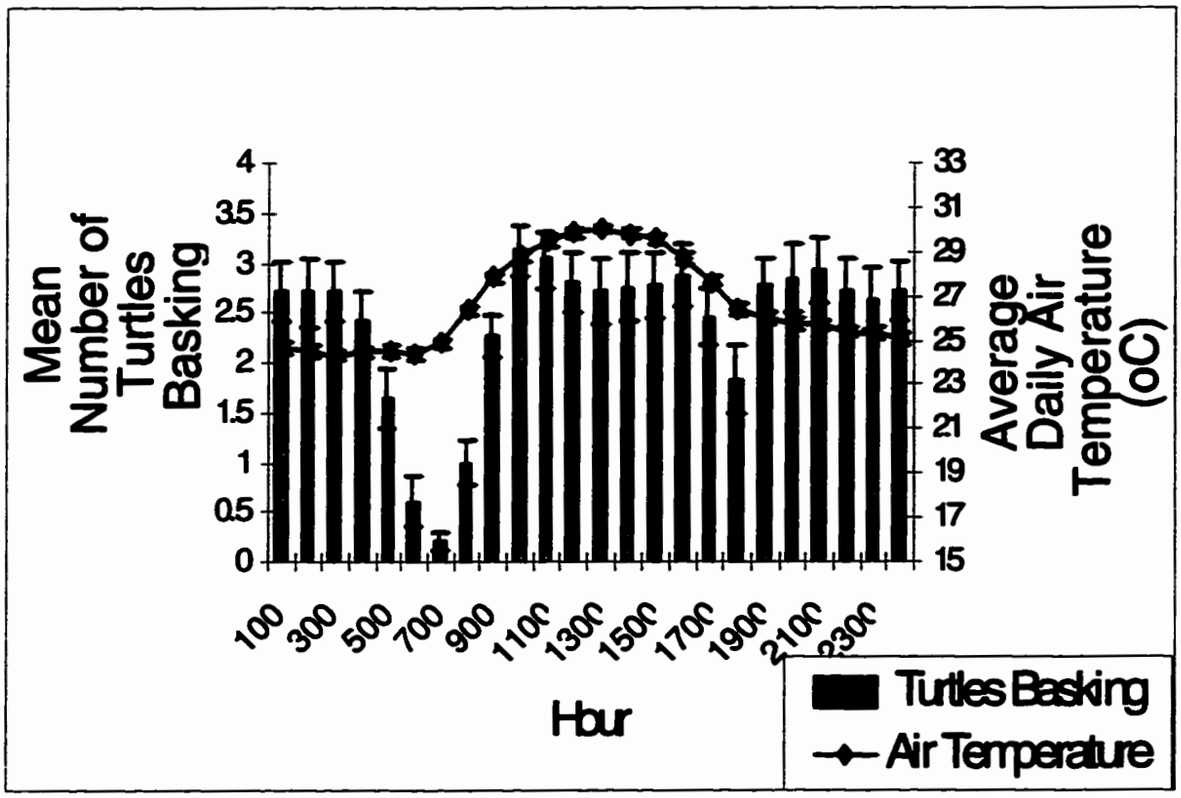

Figure 3.1. Histogram of the mean number of captive subadult green turtles basking (±SEM) and mean air temperatures (±SEM) for each hour recorded at Kewalo Research Facility Honolulu, Hawaii, Fall, 1994.

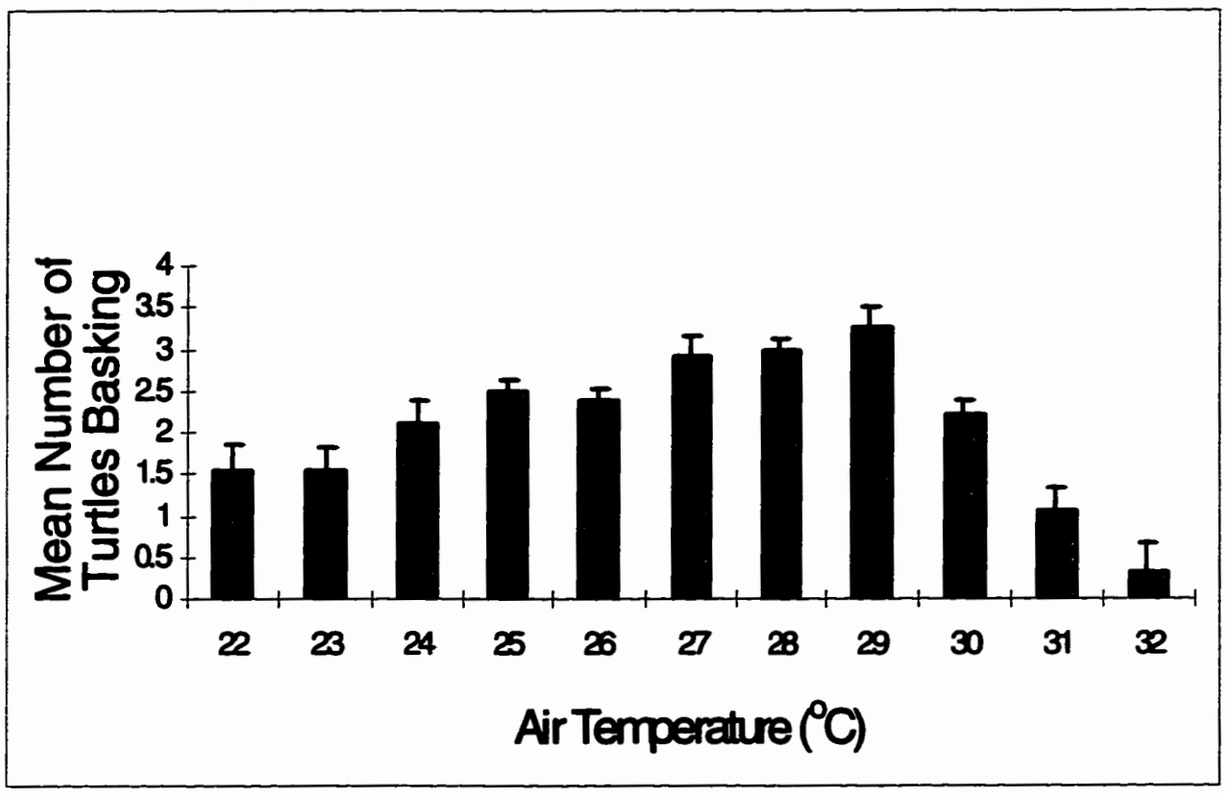

Figure 3.2. Histogram of the mean number of captive subadult green turtles basking (±SEM) per recorded air temperature at Kewalo Research Facility, Honolulu, Hawaii, Fall, 1994. Temperatures are grouped by 1.0°C intervals.

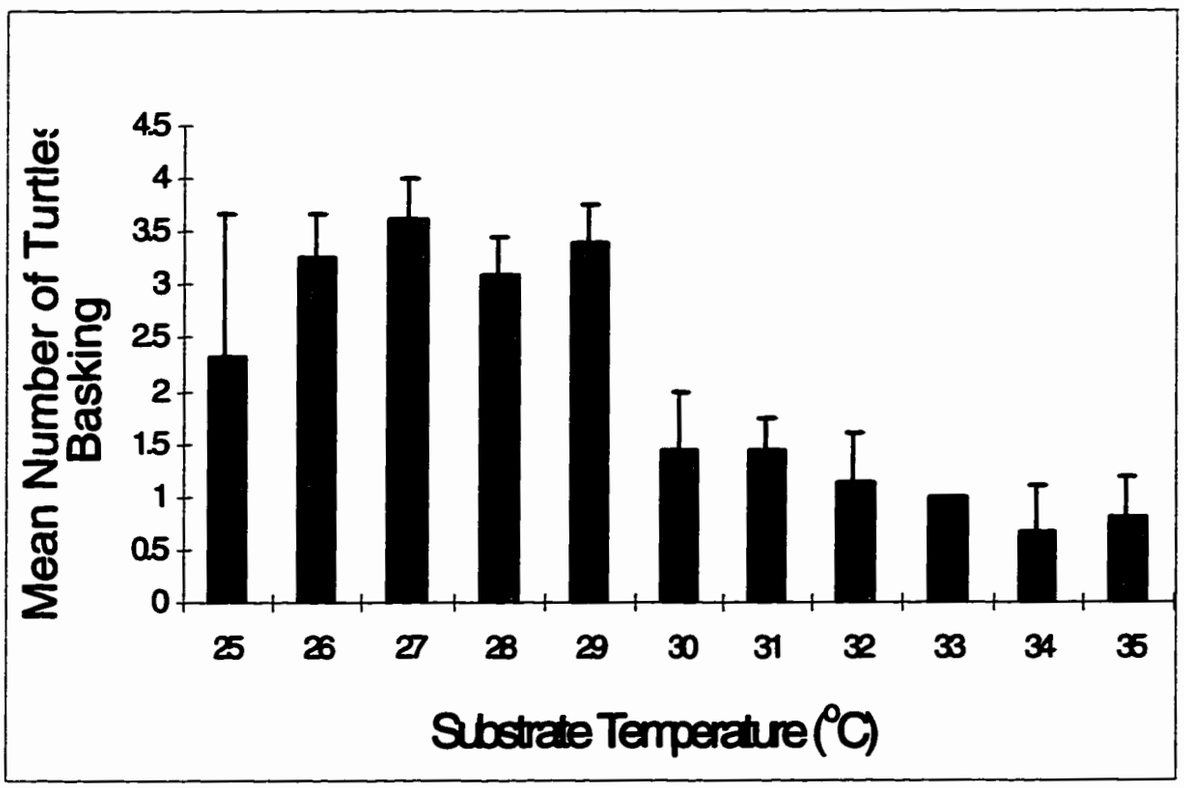

Figure 3.3. Histogram of the mean number of captive subadult green turtles basking (±SEM) per recorded substrate (basking platform) temperature at Kewalo Research Facility, Honolulu, Hawaii, Fall, 1994. Temperatures are grouped by 1.0°C intervals.

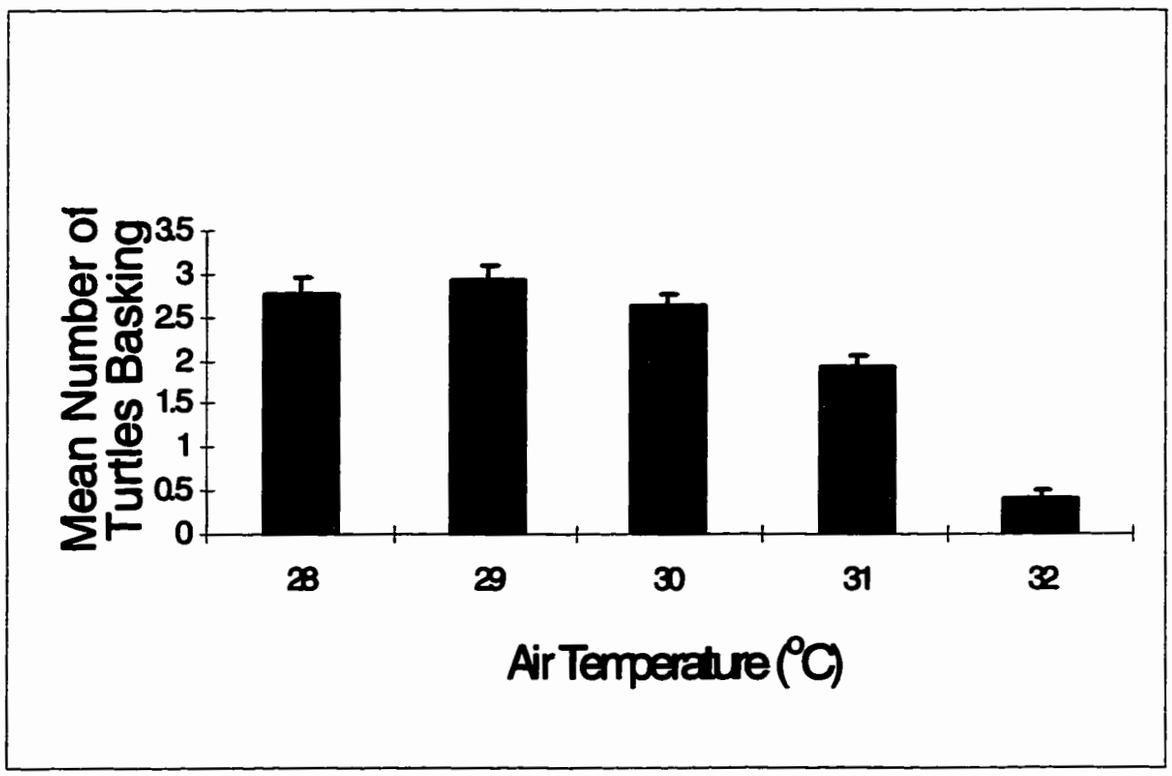

Figure 3.4. Histogram of the mean number of captive subadult green turtles basking (±SEM) per recorded maximum daily air temperature at Kewalo Research Facility, Honolulu, Hawaii, Fall, 1994. Temperatures are grouped by 1.0°C intervals.

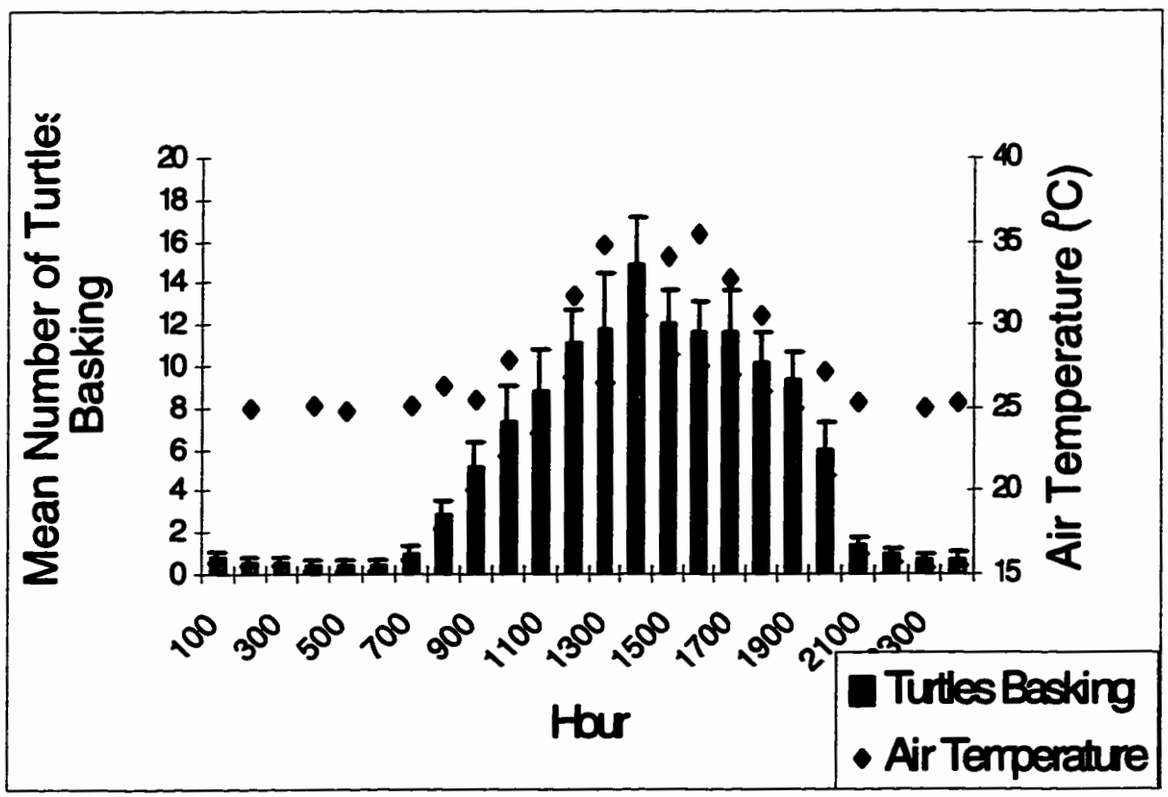

Figure 3.5. Histogram of the mean number of wild adult green turtles basking (±SEM) and mean air temperatures for each hour recorded at East Island, French Frigate Shoals, Northwestern Hawaiian Islands, June and July, 1995. Missing data of air temperatures are due to the fact that air temperatures were only recorded at 3-hr intervals.

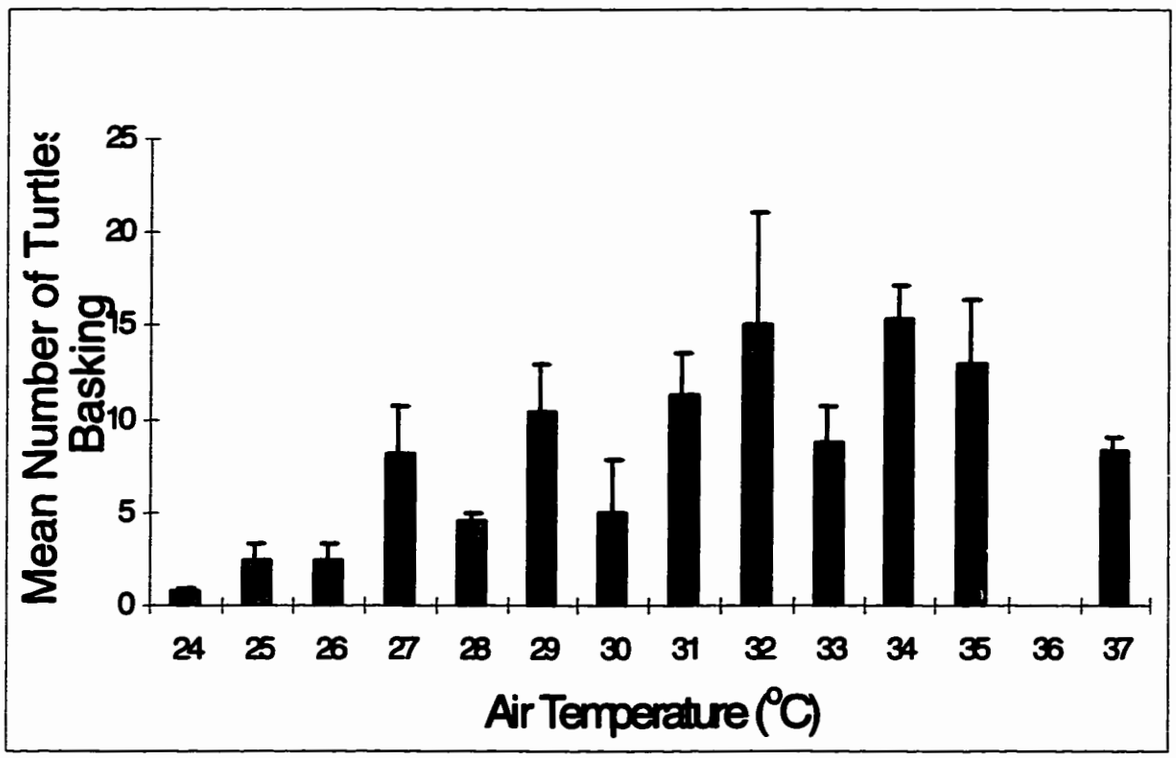

Figure 3.6. Histogram of the mean number of wild adult green turtles basking (±SEM) per recorded air temperature at East Island, French Frigate Shoals, Northwestern Hawaiian Islands, June and July, 1995. Temperatures are grouped by 1.0°C intervals. No data were recorded within 36.0-36.9 °C.

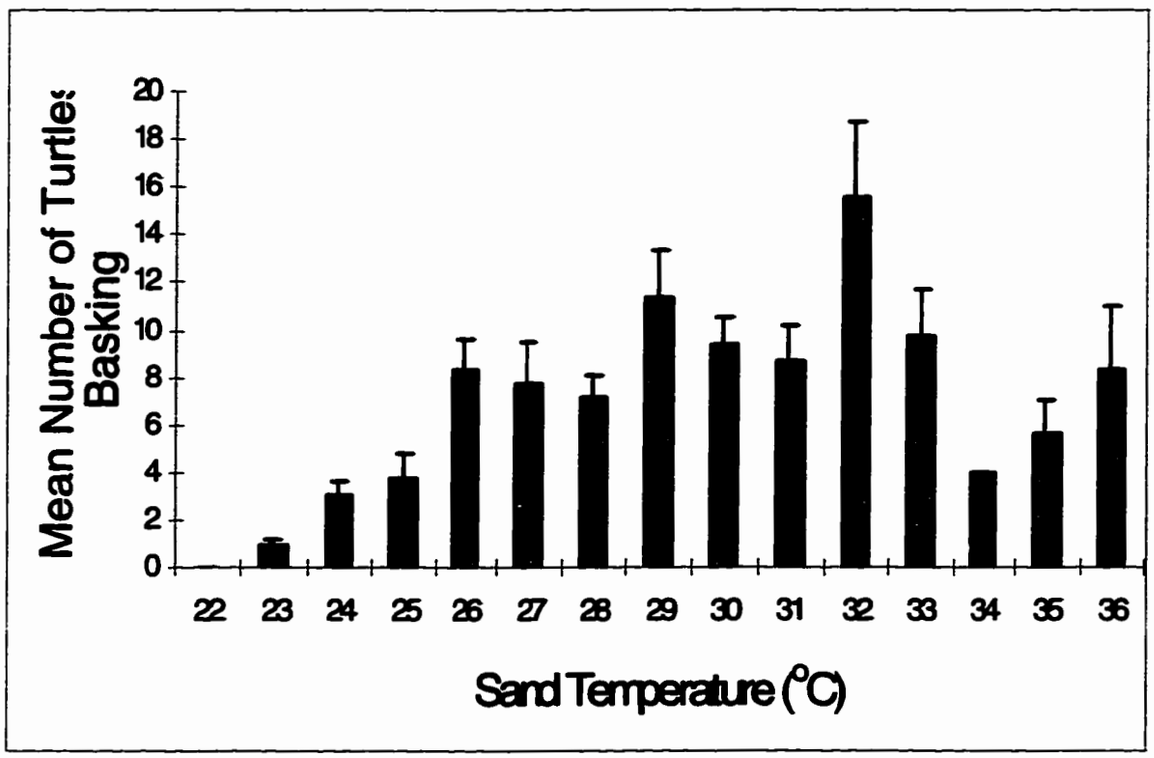

Figure 3.7. Histogram of the mean number of wild adult green turtles basking (±SEM) per recorded sand temperature at East Island, French Frigate Shoals, Northwestern Hawaiian Islands, June and July, 1995. Temperatures are grouped by 1.0°C intervals.

CHAPTER IV

BIOCHEMICAL RESPONSES TO DISEASE AND CAPTIVITY IN THE GREEN TURTLE

Introduction

Throughout their tropical and sub-tropical ranges, green turtles (*Chelonia mydas*) are currently listed as threatened or endangered under the U.S. Endangered Species Act of 1973. In some areas such as Hawaiian waters, conservation and legislative efforts have helped to increase population sizes (Balazs 1991; Eckert 1993). In certain areas, however, a high percentage of turtle populations are afflicted with green turtle fibropapilloma disease (GTFP). Both the Florida and Hawaiian populations seem to be the most severly affected with GTFP, and diseased turtles comprise approximately 33-61% (between 1986-1990; Erhart 1991) and 49-92% (1989-1990; Balazs 1991) in densely populated areas in Florida and Hawaii, respectively.

GTFP is characterized by the growth of benign internal and external fibroepithelial tumors (Herbst 1994). The etiology of this disease remains unknown, yet a herpesvirus is suspected (Herbst 1994; Herbst et al. 1996). Because every species of marine turtle has now been observed with fibropapilloma tumors, understanding the impacts of disease at both the individual and population level has been recommended as priorities by the U.S. National Marine Fisheries Service (Eckert 1993). Furthermore, as captive maintenance and captive breeding of marine turtles increases, more information is needed so that managers of captive populations can maintain environmental conditions that optimize turtles' health and minimize stress while in captivity.

In an attempt to determine physiological responses of green turtles to GTFP disease and to captivity, I compared various plasma biochemical parameters between groups of diseased, non-diseased, wild, and captive turtles. These variables were also correlated with time in captivity in order to determine the effect of captivity on turtles' biochemical composition. Many of these variables are commonly used as a diagnostic technique to

assess health status of individuals or populations among mammalian species. In most lower vertebrates, however, biochemical data are relatively scarce, and use of these diagnostic variables has been limited. This study is intended to provide baseline data for healthy and diseased green turtles, which is the first step in improving our diagnostic capabilities of the health status of threatened or endangered marine turtles. Animals with visible signs of diseases, such as GTFP, will provide especially valuable reference data.

In this study, the following plasma biochemistry parameters and their associated conditions were evaluated in healthy and diseased green turtles: Corticosterone levels for stress; lactate levels for differential reliance upon anaerobic metabolism; glucose for metabolic function; aspartate aminotransferase (glutamic oxalacetic transaminase;AST) and alkaline phosphatase (ALP) for cellular damage or tissue necrosis; alanine aminotransferase (ALT) for liver damage; proteins for dehydration, insufficient protein production, or protein loss; calcium for endocrine or metabolic problems; cholesterol and triglyceride for potential lipid metabolism disorders; and uric acid for kidney damage.

The objective of this research is to provide a comparison of plasma chemistry values for captive and wild, healthy and diseased green turtles. Study animals were comprised of captive and wild subadult turtles with and without GTFP, and healthy captive adult turtles. All study animals originated from the main Hawaiian Islands, except for the group of adult captive turtles from Sea World of California (SWC), which originated from various locations in the Pacific Ocean. Because turtles from SWC have been in captivity for many years, they are especially valuable in order to evaluate the long-term effects of time in captivity. Comparisons among these groups offers the opportunity to better understand the physiological responses of disease and captivity on a species of marine turtle. By establishing baseline data for both tumored and non-tumored, captive and wild animals, future assessment of an animal's well-being may be more easily determined. Managers could use such information to better assess health status of both wild and captive populations of green turtles.

Materials and Methods

Field Methods

Nine subadult captive turtles were obtained from the wild in Kaneohe Bay (21°30'N, 157°50'W), island of Oahu, Hawaii, during numerous trips between June and September 1994. Turtles were captured by hand, brought onto a boat, and later transported ca. 15 km to Kewalo Research Facility in Honolulu. Of the nine captive turtles, five had visible signs of GTFP disease and were housed in a separate tank with an independent water supply from the four apparently healthy turtles. Each 8-meter diameter tank received a constant supply of seawater (ranging from 23- 27°C) and was scrubbed every third day to reduce algal and diatom growth. Average size (straight carapace length) for the GTFP-afflicted and non-tumored turtles was 53.6 cm (SE=1.82) and 47.9 cm (SE=1.72), respectively. Turtles were each fed two squid per day six days a week. Throughout the study period, turtle weights remained within 1 kg of original weights for all study animals. At time of capture, blood was obtained according to methods described in Owens and Ruiz (1980) between 45-70 minutes after each turtle was brought onto the boat. Data from this first sampling date were included in calculating means of values while in captivity. When obtaining blood samples during the turtles' stay in captivity, blood was drawn within five minutes of being handled in the tank. These blood samples were collected in the late afternoon or early evening, on eight separate days from early November to mid December. Blood was placed in heparinzed Vacutainers and stored on ice for approximately 1-2 hours before being spun in a centrifuge for 10 minutes. All plasma samples were then stored at -70°C until analyzed. Turtles were maintained in captivity until mid December 1994, at which time they were released back into Kaneohe Bay. All data used in analyses of biochemical responses to captivity were obtained from these nine captive Hawaiian turtles.

Blood samples from 13 wild Hawaiian green turtles were obtained between 0900-1200h on 18 August 1994, in Kaneohe Bay after turtles had been captured in the same way as described above. All data used in analysis of plasma from wild turtles are from this one sampling day. Six of these turtles were apparently non-diseased (herein referred

to as non-tumored) because they had no visible exterior tumor growth, while seven were considered diseased with GTFP (herein referred to as tumored) due to the presence of visible tumors. Seawater temperature (ca. 25 cm depth) was recorded using a thermister probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio), and water temeperatures remained within 28.0-29.0°C throughout the three hour sampling period. Average sizes (straight carapace length) for tumored and non-tumored turtles were 55.2 cm (SE=2.08) and 50.2 cm (SE=4.54), respectively. Approximately 8 cc of blood was collected and placed in heparinized vacutainers from each turtle between 45-70 minutes after their removal from the water. Turtles remained on the boat covered with wet towels and placed on their plastrons until blood was collected. Blood was kept on ice for no longer than 2 hours until spun for 10 minutes in a centrifuge, and plasma samples were stored at -70°C until analyzed. Because Kaneohe Bay is a common feeding ground for green turtles around Oahu, these turtles had most likely been feeding prior to their capture.

Eight captive turtles residing at Sea World of California (SWC) were removed from the water in the "Turtle Lagoon" between 0830-0930 on 19 September, 1996. Using a thermistor probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio), ambient water temperature in the lagoon was 23.3°C. All animals were considered clinically healthy and did not show signs of GTFP. Many of these turtles had been in captivity for over ten years, and were originally obtained from the wild in the Pacific Ocean. Average turtle size (curved carapace length) was 80.3 cm (SD=6.83). Approximately 8 cc of blood was collected, and samples were placed in heparinized Vacutainer tubes. All blood was stored on ice for approximately three hours until being spun in a centrifuge for 10 minutes. Plasma samples were stored at -70°C until analyzed. Turtles at SWC had a diet consisting of sardines, smelt, squid and shrimp and were fed three times per week (T. Dirksen, pers. comm.). Turtles had not eaten for 48 hours prior to blood collection.

Laboratory Methods

Plasma corticosterone levels of all turtle groups except SWC turtles were determined using standard radioimmunoassay (RIA) techniques at the University of Florida,

Gainesville, where assays specific to green turtle plasma have been developed. Samples were extracted with 5 mL diethyl ether prior to RIA analysis. Each sample was analyzed in duplicate, and results were corrected for the extraction efficiency of 83%±6.1%. Standard curves were prepared in phosphate-buffered saline (PBS) with known amounts of radioinert corticosterone (10, 25, 50, 100, 250, 500, 1,000 and 2,000 pg/mL) purchased from Amersham Corporation (Arlington Heights, Ill.). The minimum concentration per tube, which was distinguishable from zero, was 18.3 pg/mL. Cross-reactivities of the corticosterone antiserum (no. 07-189016, ICN Biomedicals, Costa Mesa, Calif.) with other steroids were 6.1% for desoxycorticosterone, 0.29% for progesterone, 0.19% for cortisol, and <0.1% for all other steroids examined.

Plasma samples (approximately 740 pg/mL) were assayed serially in volumes of 10, 25, 50, 75, and 100 uL (final volume of 100 uL with charcoal stripped plasma). The inhibition curve for corticosterone was parallel to the standard curve, and the test of homogeneity of regression indicating that the curves did not differ. Further characterization of the assay involved measurements of known amounts (5, 10, 25, 50, 100, 250, and 500 pg) of the radioinert hormone in 100 uL charcoal-stripped plasma. For corticosterone ($Y=10.1 + 0.947X$, where Y is the amount of corticosterone measured [pg] and X is the amount of corticosterone added [pg]; $R^2=0.8793$), interassay and intrassay coefficients of variation were 7.9% and 10.3%, respectively. Aliquots from each sample (100uL) were extracted with 5 mL diethyl ether. After vortexing for one minute, the aqueous phase was frozen in a dry-ice methanol bath and the ether phase poured off into 12 x 75-mm glass tubes and evaporated. PBS buffer was added to standards (250 uL) and dried samples (300uL). The tritiated hormone (100uL) and each antiserum (100 uL) were added to all standard and sample tubes. All tubes were incubated overnight at 4°C. After incubation, 250 uL of dextran-coated charcoal (0.5% charcoal and 0.05% dextran) was added to each tube to separate the unbound hormone from the antibody-bound fraction. All tubes were centrifuged (1,200 rpm) for 10 minutes. A sample (0.5 mL) of the supernatant was drawn from each tube and mixed with 3.5 mL of scintillation cocktail (Scintiverse BD, Fisher Scientific) in plastic scintillation vials. All vials were counted for

1 minute, and hormone titers in ng/mL were calculated with the standard curve generated in the assays (Gregory 1994).

Plasma samples from all turtle groups were analyzed for biochemical profiles using a Du Pont Analyst® Benchtop Chemistry System in the Physiology Laboratory at Hubbs-Sea World Research Institute in San Diego, California. Parameters analyzed include various blood enzymes, calcium, cholesterol, triglyceride, glucose, uric acid, and total protein. Plasma lactate levels were determined using a 2300 STAT YSI Glucose-L-Lactate Analyzer at the Physiological Research Laboratory at Scripps Institute of Oceanography, University of California, San Diego. Lactate samples were analyzed in duplicate, and the mean was recorded.

Statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC, V. 6.08). In order to determine differences in mean values of biochemical parameters among turtle groups while accounting for repeated sampling of individual turtles, a repeated measures analysis of variance followed by a Tukey multiple comparison post-hoc test was used. This type of profile analysis was used in order to account for differences in dates of blood collection. Biochemical responses to captivity were analyzed for captive Hawaiian turtles using a repeated measures test with an interaction term for time in captivity. This type of correlation analysis was used to analyze potential changes in mean biochemical values for each sampling period during the turtles time in captivity, which extended from the summer months until mid December. Effects of time in captivity were also compared between tumored and non-tumored turtles Hawaiian turtles. Significance is reported at $p \leq 0.05$. Due to the numerous comparisons conducted, only p-values of significant findings will be reported.

To determine if there was a biochemical signature that could be used to identifly turtles into either a tumored or non-tumored group, I applied discriminant function analysis to the data. Complete data sets were available for 18 non-tumored and 26 tumored turtles from both captive and wild populations of Hawaiian green turtles. Differences in sample size were taken into account by weighing parameters by number of cases per group.

Results

Corticosterone levels of Hawaiian green turtles varied significantly between captive and wild turtle groups (Fig. 4.1). Tumored wild turtles had significantly higher levels of corticosterone than tumored captive turtles ($p=0.0001$), and non-tumored wild turtles had higher levels than non-tumored captive turtles ($p=0.0006$). Levels of corticosterone were similar for turtles within captive and wild groups. Corticosterone levels were not determined for SWC turtles.

Levels of lactate differed significantly among turtle groups (Fig. 4.2), with significantly higher levels observed in tumored wild turtles compared to tumored and non-tumored captive turtles ($p=0.0001$, $p=0.0025$, respectively). Non-tumored wild turtles also had significantly higher levels of lactate compared to tumored and non-tumored captive turtles ($p=0.0001$, $p= 0.0009$, respectively). Levels of lactate were significantly lower in SWC turtles compared to tumored ($p=0.0001$) and non-tumored wild turtles ($p=0.0001$), and tumored and non-tumored captive turtles ($p=0.0001$, $p=0.0001$, respectively). Lactate levels were significantly higher for non-tumored as compared to tumored captive Hawaiian green turtles ($p=0.0051$), but they were similar between groups of wild turtles.

Glucose levels were similar among all turtle groups except for higher levels reported for tumored turtles in the wild as compared to tumored turtles in captivity ($p=0.0313$; Fig. 4.3).

Aspartate aminotransferase (GOT or AST) levels significantly differed among turtle groups (Fig.4.4). SWC turtles had significantly higher levels of AST than captive non-tumored, captive tumored, wild non-tumored, and wild tumored ($p=0.0.0001$, $p=0.0001$, $p=0.0102$, $p=0.0001$, respectively). Levels of AST were significantly higher in non-tumored wild turtles as compared to non-tumored captive animals ($p=0.0085$), though levels within captive and wild groups were similar.

All groups had similar Alanine aminotransferase (ALT) levels except for SWC turtles that had significantly higher levels than captive non-tumored, captive tumored, wild non-

tumored, and wild tumored turtles (p=0.0082, p=0.0100, p=0.0029, p=0.0043, respectively; Fig. 4.5).

Alkaline phosphatase (ALP) levels differed among groups (Fig. 4.6). In captivity, tumored turtles had significantly higher values than non-tumored turtles (p=0.0148), though differences between tumored and non-tumored animals were not apparent in wild turtles. Levels of ALP were similar between wild tumored turtles and SWC turtles, and each group had significantly higher average levels than non-tumored captive Hawaiian turtles (p=0.0301, p=0.0180, respectively). All other groups had similar levels of ALP.

SWC turtles were observed to have significantly higher levels of total protein than tumored and non-tumored captive Hawaiian turtles (p=0.0012, p=0.0055, respectively), but similar levels to wild tumored Hawaiin turtles (Fig. 4.7). Although protein levels did not differ between tumored and non-tumored groups either in captivity or in the wild, tumored wild turtles showed significantly higher levels of protein than tumored turtles in captivity (p=0.0204).

Significantly higher levels of calcium were observed for tumored wild Hawaiian turtles as compared to both tumored and non-tumored captive Hawaiian turtles (p=0.0001, p=0.0038, respectively; Fig. 4.8) and SWC turtles (p=0.0092). Wild non-tumored turtles were also shown to have calcium levels significantly higher than levels in tumored captive turtles (p=0.0091). All groups of non-tumored turtles had similar levels.

Significantly higher levels of cholesterol were observed for turtles from SWC compared to captive non-tumored, captive tumored, wild non-tumored, and wild tumored turtles (p=0.0001, p=0.0002, p=0.0001, p=0.0001, respectively; Fig. 4.9). All groups of Hawaiian turtles had similar cholesterol levels.

Turtles from SWC had significantly higher levels of triglyceride than captive non-tumored, captive tumored, wild non-tumored, and wild tumored turtles (p=0.0001, p=0.0001, p=0.0001, p=0.0001, respectively; Fig. 4.10). Additionally, tumored wild turtles had significantly higher levels than tumored and non-tumored captive turtles (p=0.0130, p=0.0478, respectively).

Tumored captive animals had significantly higher uric acid levels than both tumored and non-tumored wild animals (p=0.0014, p=0.0015, respectively; Fig. 4.11). Similarly,

levels for non-tumored captive turtles were significantly higher than levels for tumored and non-tumored wild animals ($p=0.0178$, $p=0.0166$, respectively). Uric acid levels was similar for all groups of captive turtles.

All data used in the above analyses are presented in Table 4.1.

Of the biochemical parameters investigated in captive Hawaiian turtles, only AST and cholesterol significantly changed throughout time in captivity, and both parameters increased during this time ($p=0.0127$, $p=0.0495$, respectively; Figs. 4.12 and 4.13). Furthermore, cholesterol levels in tumored turtles increased at a higher rate than in non-tumored turtles ($p=0.026$).

Results from discriminant function analysis (DFA) indicate that biochemical variables can be used to predict the probability that a turtle originated from either the tumored or non-tumored group. In non-tumored turtles, 88.9% of the predictions of biochemistry values based on DFA classifications were correct, and 88.5% of the tumored cases were correctly predicted ($p=0.002$; Fig. 4.14).

Discussion

Measurements of blood parameters indicate numerous differences between captive and wild Hawaiian green turtles. Levels of corticosterone, lactate, triglyceride, glucose, and calcium were higher in wild green turtles as compared to captive turtles, while uric acid levels were lower in wild turtles as compared to captive turtles. Additionally, turtles from SWC, which had been in captivity the longest, had higher levels of ALT and triglycerides as compared to nearly all other groups. Statistical analysis did not detect an influence of disease in any of the blood parameters for turtles in the wild. Tumored Hawaiian turtles in captivity, however, had higher levels of ALP and lower levels of lactate compared to non-tumored captive turtles. Predictions of turtle group (tumored or non-tumored) based on discriminant function analysis classification were correct 89% for both tumored and non-tumored turtles, suggesting that diseased animals had a distinct signature of plasma biochemistries compared to non-tumored turtles. In captive

Hawaiian green turtles, time in captivity also influenced levels of plasma AST and cholesterol, which increased throughout time in captivity. Furthermore, rates of increase of cholesterol were higher in tumored turtles compared to non-tumored turtles, suggesting an influence of disease. Because levels AST and cholesterol were associated with time in captivity, results of multiple comparisons among turtle groups are not valid and thus only trends will be discussed. Differences in diet, environmental conditions, and turtle size, help to interpret these results.

Corticosterone

Although a precise definition remains unclear, Moberg (1985, p.29) states that stress can be viewed as "any external stimulus that challenges homeostasis". Adrenal glucocorticoids, such as cortisol and corticosterone, have been used as indices of stress among the vertebrate classes (see Harvey et al. 1984). Activity of the hypothalamo-pituitary-adrenal axis in marine turtles is typical of most vertebrates (Morris 1982; cited in Owens and Morris 1985), whereby the organisms' response to stress influences glucose utilization and other metabolic activities stimulated by the adrenal or interrenal gland (Norris 1980). The subsequent increase in glucocorticoids mobilizes energy stores, mainly from the liver, which help the animal overcome an immediate threat by maintaining homeostatic balance (Harvey et al. 1984; Moberg 1985). The sole use of glucocorticoids as an indicator of stress, however, is not recommended due to the array of effects that a stressor may evoke (Valverde 1996). Recent studies with loggerhead (*Caretta caretta*) and green turtles, however, report a positive relationship between corticosterone levels and acute stress, which typically is attributed to capture, restraint, and bleeding of an animal (Wibbels et al. 1990; Gregory 1994; Aguirre et al. 1995).

Based on the assumption that levels of corticosterone are positively associated with stress experienced by turtles, results from this study indicate that both tumored and non-tumored Hawaiian turtles were more stressed in the wild compared to captivity (Fig. 4.1). Life in captivity is usually assumed to impose additional stresses to an animal due to confinement, over-crowding, etc. These results are therefore unexpected. In an attempt to explain these findings, the following factors should be considered: First, turtles from

the wild were likely to have been captured in a more stressful way than turtles in captivity. Second, the time delay once animals were captured until blood was collected (45-70 mins for wild turtles, <5 min in captivity) may have resulted in elevated corticosterone levels. (Aguirre and co-authors [1995] report a two-fold increase in corticosterone one hour after capture as compared to values recorded immediately upon capture). Lastly, circadian variation in corticosterone levels also may have biased data reported here. Daily corticosterone cyclicity is not known specifically for green turtles. In wild olive Ridley sea turtles (*Lepidochelys olivacea*), corticosterone levels did not vary throughout a 24-hr period (Valverde 1996). In captive loggerhead sea turtles, however, corticosterone levels peaked in the early morning (Schwantes 1986, cited in Valverde 1996). One explanation for corticosterone cyclicity is that levels vary in response to marked activities, such as feeding regimes (Thurmond et al. 1986). In this study, wild animals were captured and bled before noon, a period of active feeding for green turtles in Kaneohe Bay as indicated by the presence of food particles (sea grasses) in turtles' mouths. The majority of plasma samples from captive animals, however, were obtained after 1400h, many hours after feeding. Until more is known about corticosterone cyclicity in green turtles, such sampling biases interfere with the ability to conclude that corticosterone levels were consistently higher in wild turtles as compared to captive animals. If no cycle is found and corticosterone levels are confirmed to be a reliable indicator of stress in green turtles, then my results conclude that wild animals experience more stress than captive turtles. If corticosterone does experience clear cyclicity in green turtles, then results from this study are biased and should not be used as a measure of stress.

Season also influences corticosterone levels. Employing similar sampling and laboratory methods to those reported in this paper, Aguirre and co-authors (1995) report a nearly two fold increase in corticosterone levels for non-tumored wild Hawaiian green turtles as compared values reported here. In this study, wild turtles were sampled in summer, while Aguirre and colleagues report values for turtles sampled in early autumn. Similar differences potentially due to season were reported for loggerhead sea turtles in Florida. Small loggerhead turtles caught by trawling in summer had significantly higher

levels of corticosterone than those captured in winter (Gregory 1994). Therefore, corticosterone levels reported in this study may differ from levels reported elsewhere due to the influence of season on corticosterone levels.

In an earlier study of Hawaiian green turtles, elevated corticosterone levels were found to be associated with disease (GTFP), which the authors attribute to a chronic, or long term stress (Aguirre et al. 1995). They found turtles sampled at 1 hr post-capture had mean plasma corticosterone levels significantly higher in tumored (5.5 ng/mL) vs. non-tumored animals (2.29 ng/mL). In this study, however, corticosterone levels were similar between tumored and non-tumored turtles both in captivity and in the wild. Due to variation among study design and results, more research is needed in order to determine the glucocorticoid response as a result of GTFP. In order to draw conclusive statements on factors eliciting a corticosteroid response in green turtles, experiments that isolate the effects of disease, captivity, and the acute stress involved in obtaining blood must be performed.

Lactate

Measures of blood or tissue lactate can be useful to determine oxygen debt and reliance upon anaerobic metabolism. After periods of physical activity and depletion of oxygen stores, lactic acid concentrations rise in response to the reduction of pyruvic acid to lactic acid to fuel activity. Concentrations of lactate can therefore help elucidate the relative energy demand on a given animal. In reptiles, elevated lactate levels have been reported post-activity in freshwater turtles (Gatten 1974), lizards (Pough and Alexander 1985; Gleeson and Dalessio 1989, 1990) and snakes (Ruben 1976). Other studies in lizards (Wilson and Gatten 1989) and marine iguanas (Gleeson 1979, 1980), however, report no change in blood lactate levels despite changes in metabolic rate during various locomotor activities (eg. walking, basking, swimming, foraging).

Given the high aerobic capacity of marine turtles (Seymour 1982), levels of lactate may not accurately reflect physical exertion, but rather may be indicative of the stress involved in breath-holding, or apnea. In green turtles, Butler and colleagues (1984) found no change in lactate levels associated with varying metabolic rates during swimming.

Other studies of lactate in marine turtles, however, suggest that lactate is produced in response to an acute stressor that causes periods of apnea. In green turtles, Berkson (1966) found that blood lactate concentrations increased in response to dives and forced submergence. Similarly, Lutz and Dunbar-Cooper (1981) found a ten to forty times increase in blood lactate for trawled turtles compared to quiescent turtles, and Wood and Ebanks (1984) observed a nearly 20-fold increase in blood lactate level for a green turtle forcibly submerged underwater for 15 min. The authors attribute this rise in lactate to severe stress experienced by the turtle.

In this study, elevated lactate levels observed in wild turtles (Fig. 4.2) may be indicative of high levels of physical exertion or apnea-induced stress experienced during their capture. Lower levels of lactate observed in captive turtles likely results from reduced swimming activity, either due to lethargy, crowding, or both. Lactate values reported here suggest that wild Hawaiian turtles exert the highest levels of excercise, while captive turtles in Sea World of California are the least physically-active group.

Blood Enzymes

In numerous species, damage to the cell membrane causes release of enzymes from the cell into the blood, resulting in elevated levels of enzymes in plasma or serum (Bush 1991; Meyer et al. 1992). Elevations in levels of enzymes, such as AST and ALT, are indicative of cellular damage or tissue necrosis occurring in skeletal muscle, cardiac muscle, or in the liver (Bush 1991; Meyer et al. 1992).

Levels of AST significantly increased during turtles' time in captivity, and both tumored and non-tumored turtles showed similar rates of AST increase (Fig. 4.12). In the wild, AST levels were also similar between tumored and non-tumored animals (Fig. 4.4). The finding of similar AST levels between diseased and non-diseased turtles was unexpected due to higher levels of AST reported for one GTFP-debilitated captive turtle as compared to three clinically healthy captive green turtles (756 U/L vs. 155 U/L for diseased and healthy turtles, respectively; Norton et al. 1990). Furthermore, in a study of desert tortoises (*Xerobates agassizii*) afflicted with chronic upper respiratory tract disease (URTD), elevated levels of AST were found in diseased animals as compared to controls,

which the authors attribute to tissue damage (Jacobson et al. 1991). Aguirre and colleagues (1995) also found significant increases in AST in wild tumored turtles as compared to wild non-tumored turtles, yet these differences were only evident at 24h post-capture. Such a delayed response is common in mammals with skeletal muscle damage, whereby AST levels usually peak after 12-24h post insult to skeletal muscle (Bush 1991. In order to better understand the role of AST in reptilian disease, levels of AST should be measured from a sufficient number of animals with varying degrees of debilitation.

The increase of AST levels during the turtles' maintenance in captivity suggests that, diet, stress, confined space, or a combination of these conditions results in cellular or tissue damage, likely of cardiac or skeletal muscle. The high levels of AST observed for SWC turtles are likely to reflect extensive tissue damage due to the turtles' prolonged maintenance in captivity.

Levels of ALT were similar among all groups except for SWC turtles (Fig. 4.5), which had levels two-to-four fold higher than for any other group examined, which suggests liver damage for SWC turtles. In their study of captive green turtles, Norton et al. (1990) report substantial increases in ALT levels in a debilitated animal as compared to healthy ones (26.0 U/L vs. 7.0 U/L for diseased and healthy turtles, respectively), suggesting the enzyme's role in disease. In this study, however, no association was detected between disease state and levels of ALT in turtle plasma. Due to differential response of ALT to disease, the usefulness of measuring this blood parameter is limited to extreme cases until more information is known about the enzymes' specific role in reptiles.

Alkaline phosphatase (ALP) is a membrane-associated enzyme found in numerous tissues. Increased production of ALP, and subsequently higher plasma ALP values, result when membrane fragments are released directly into the plasma due to cell damage, likely as a result of an acute injury (Meyer et al. 1992). Due to considerable variation of ALP values among and within species, its usefulness as a diagnostic parameter is highly species-specific. In reptiles, increased ALP levels have been linked to

hyperparathyroidism, numerous bone diseases (Paget's disease, rickets), and renal insufficiency (Frye 1981).

In this study, ALP levels were significantly higher in SWC turtles and tumored captive Hawaiian turtles as compared to non-tumored captive turtles, yet no differences were found in relation to disease state for wild animals (Fig. 4.6). Because obvious deformities in bone structure were not apparent in tumored turtles, damage to the kidneys associated with GTFP seems most likely to have caused the observed elevation of ALP. Aguirre and colleagues (1995), however, found ALP levels consistently higher in wild healthy turtles as compared to tumored turtles. Norton and colleagues (1990) also report higher ALP levels for healthy captive animals as compared to the one captive diseased animal (25.7 vs 6.0 U/L., for healthy and diseased turtles, respectively). In desert tortoises, however, ALP levels were similar for diseased and non-diseased animals (Norton et al. 1991). Until a greater understanding on the role of ALP in reptiles is established, the high variation in ALP data from the above-mentioned studies preclude this parameter as being useful in diagnosing health status of green turtles.

Protein

In mammals and in at least two species of amphibians, increases in plasma protein result as a mechanism for maintaining plasma volume when an animal is dehydrated (Horowitz 1984; Hillman et al. 1987). Although plasma proteins are believed to play a role in the distribution of water between blood and tissues in reptiles (Dessaur 1970), specific changes in plasma proteins in response to hydration state are not clearly understood in reptiles (Christian et al. 1996). Reduced levels of protein (hypoproteinemia) result when the body is unable to produce enough protein or when there is an increased loss of proteins. Potential causes of insufficient protein production include protein starvation, intestinal malabsorption, pancreatic insufficiency, malnutrition (parasitic or dietary), and liver disorders (Bush 1991; Meyer et al. 1992). Loss of protein can be caused by renal disease, skin lesions, and external hemorrhaging (Bush 1991; Meyer et al. 1992).

The observed hypoproteinemia of tumored captive turtles as compared to tumored wild turtles (Fig. 4.7) is unexpected due to the diets of captive turtles consisting primarily of protein (squid), thereby suggesting a source of protein loss or reduced protein production. For SWC captive turtles, however, protein levels were relatively high, possibly caused by dehydration, a diet high in protein, or both. Aguirre and colleagues (1995) observed a slight hypoproteinemia in GTFP turtles as compared to healthy ones, but only after 24 hr post capture. Norton and colleagues (1990) also report lower levels of total protein for the debilitated turtle as compared to healthy green turtles. In this study, disease did not influence plasma protein levels.

Calcium

In the present study, levels of calcium were lower in captive turtle groups as compared to wild turtles (Fig. 4.8), yet no differences were observed in relation to disease state. Although calcium levels were also similar for diseased and healthy desert tortoises (Jacobsen et al. 1991), hypocalcemia was observed in both a captive diseased green turtle (Norton et al. 1990) as well as in wild diseased green turtles (Aguirre et al. 1995). This could be due to different diets or holding conditions. Consequently, more research is needed to isolate the effects of captivity, diet, and disease on plasma calcium levels in green turtles.

Disorders likely to cause hypocalcemia in diseased and/or captive animals include conditions such as necrotizing pancreatitis, and dietary imbalance (insufficient Vitamin D, excess phosphorous; Meyer et al. 1992). When the ratio of calcium and phosphorous is imbalanced in the diet, the parathyroid gland(s) become hyperactive, often resulting in skeletal deformities due to calcium deficiencies in bony tissues. These conditions are common in captive turtles and iguanas fed diets high in lean meats or lettuce. Therapy includes supplements of calcium and oral Vitamin D_3, or simply changing the diet to the more natural diet for that particular species (Frye 1981). Managers of captive green turtle populations can reverse symptoms of hypocalcemia with dietary supplements or by maintaining green turtles on their naturally herbivorous diet.

Cholesterol

Major plasma lipids, such as cholesterol and triglyceride, are bound to proteins and transported throughout the body. Disturbances in lipid metabolism can be measured via changes in serum cholesterol, which is secreted from the liver in the form of bile acids. Some causes of increased plasma cholesterol include high fat diets, severe trauma, liver damage, renal loss of protein, and starvation (Bush 1991; Jacobsen et al. 1991; Meyer et al. 1992).

In this study, levels of cholesterol significantly increased in Hawaiian turtles throughout their time in captivity. Furthermore, diseased animals had significantly higher rates of cholesterol increase while in captivity compared to non-tumored captive turtles (Fig. 4.9). Hypercholesterolemia has been observed in diseased desert tortoises, and the authors attribute the increase to the response of diseased turtles to starvation and an attempt to meet energy needs through lipid metabolism (Jacobsen et al. 1991). In this study, both tumored and non-tumored turtles in captivity were fed the same diet. Given the nearly three-fold higher energy needs of tumored turtles (Swimmer et al. 1996; see Chapter 2), however, this diet may not have been sufficient to meet the animals' energy needs. Similar levels of plasma cholesterol for tumored and non-tumored turtles in the wild may be due to tumored animals' acquisition of ample food sources to meet its' energy needs. Alternatively, the higher rate of cholesterol increase for captive tumored turtles compared to non-tumored turtles (Fig. 4.13) may be due to stress, liver damage, or renal loss of protein not encountered by non-tumored animals.

The high levels of cholesterol for turtles from SWC may be due to a prolonged fatty diet, or perhaps to a bias in animal size of sampled animals. Bolten and Bjorndal (1992) found a positive correlation between cholesterol levels and body size (straight carapace length), as well as significantly higher cholesterol levels in female green turtles as compared to males. Although the sex ratio of all captive turtles was approximately equal, high levels of cholesterol in SWC turtles may be at least partly attributed to their larger adult body sizes as compared to sub-adults study animals in Hawaii.

Triglyceride

In the liver, triglycerides are enzymatically hydrolyzed to fatty acids, which combine with protein before being released into plasma (Frye 1981). Triglyceride is the most common form in which lipid is stored (in adipose tissue), and serves as a primary energy source (Bush 1991). In captive reptiles, the most common cause for elevations in plasma triglyceride is a metabolic defect in lipid transport or synthesis of proteins, resulting in obesity of the animal caused by overfeeding (Frye 1981). Elevated levels of triglyceride in SWC turtles (Fig. 4.10) are likely due to a prolonged fatty diet, insufficient exercise, or both. Low levels of triglycerides in captive Hawaiian turtles may be due to an insufficient diet.

Uric Acid

In reptiles, uric acid is a major product of protein catabolism and is filtered from the blood by kidney tubules. In numerous reptilian species, including freshwater turtles, lower winter temperatures reduce tubule function, resulting in a rise in uric acid levels (see review in Dessauer 1970). When levels of uric acid rise, crystal deposition of the acid occurs in the tissues, leading to a condition similar to gout. Visceral gout and renal retention are commonly observed in captive reptiles, often as a result of a diet high in protein or organ meat (Frye 1981). Chelonians in particular appear to be susceptible to articular gout, which has clinical signs similar to humans and other mammals--swollen, firm, and painful joints (Frye 1981).

In this study, uric acid levels were markedly different among the groups, with captive Hawaiian turtles showing the highest levels and wild animals with the lowest levels, while no differences were observed between tumored and non-tumored animals (Fig. 4.11). The exclusive protein diet (squid) fed to captive Hawaiian turtles is very likely to have caused this two fold increase over wild turtles. Cool autumn temperatures may also contribute to elevations in uric acid for the captive group. Low values of uric acid in SWC turtles may be due to a mixed diet of protein and fat. Consequently, a more natural and mixed diet is suggested so that gout-like conditions can be avoided in captive green turtles.

Discriminant Function Analysis

Results from discriminant function analysis (fig. 4.14) indicate that turtles could be classified into groups (tumored and non-tumored) by a multivariate analysis of the plasma biochemical parameters tested. In both groups, turtles were correctly identified as tumored or non-tumored in 89% of the cases, indicating that turtles affected with GTFP had distinct combinations of plasma biochemistries from non-tumored turtles. While the nature and cause of these differences remains uncertain, the high predictive ability of multivariate analysis suggests that GTFP results in significant alterations in an animals' biochemistry that warrant further inquiry.

Conclusions

This study identified numerous differences in the plasma biochemistries between captive and wild turtles. Variables affected by captivity include corticosterone, lactate, AST, glucose, calcium, triglyceride, and uric acid. Diet, crowding, and exercise regime are likely to be responsible for these differences. This research provides information on the diagnostic capabilities of certain blood parameters, as well on the influences of diet and exercise regimes on the health status of green turtles. Such information can be valuable for managers of captive green turtles seeking to maintain turtles in a manner most similar to natural conditions.

Additionally, I found that disease (specifically GTFP) influenced levels of various blood parameters in turtles. While single-variable analysis revealed effects of GTFP for animals only in captivity (ALP and lactate), multivariate analysis (DFA) found that plasma biochemistries from both captive and wild turtles correctly predicted if turtles were tumored or tumor-free in 89% of cases. These results suggest GTFP influences turtle biochemical composition, yet the nature of these effects may be difficult to assess on an individual biochemical parameter basis.

More information is needed on the specific roles of biochemical parameters in marine turtles. As this paper confirms, interpretation of biochemical profiles is difficult to assess

in most lower vertebrates, including marine turtles. Of particular interest has been the diagnostic ability of plasma corticosterone to reflect the level of stress experienced by a marine turtle. More data on diseased, non-diseased, and clearly stressed animals are needed to improve our ability to diagnose the health status of green turtles using plasma biochemistries.

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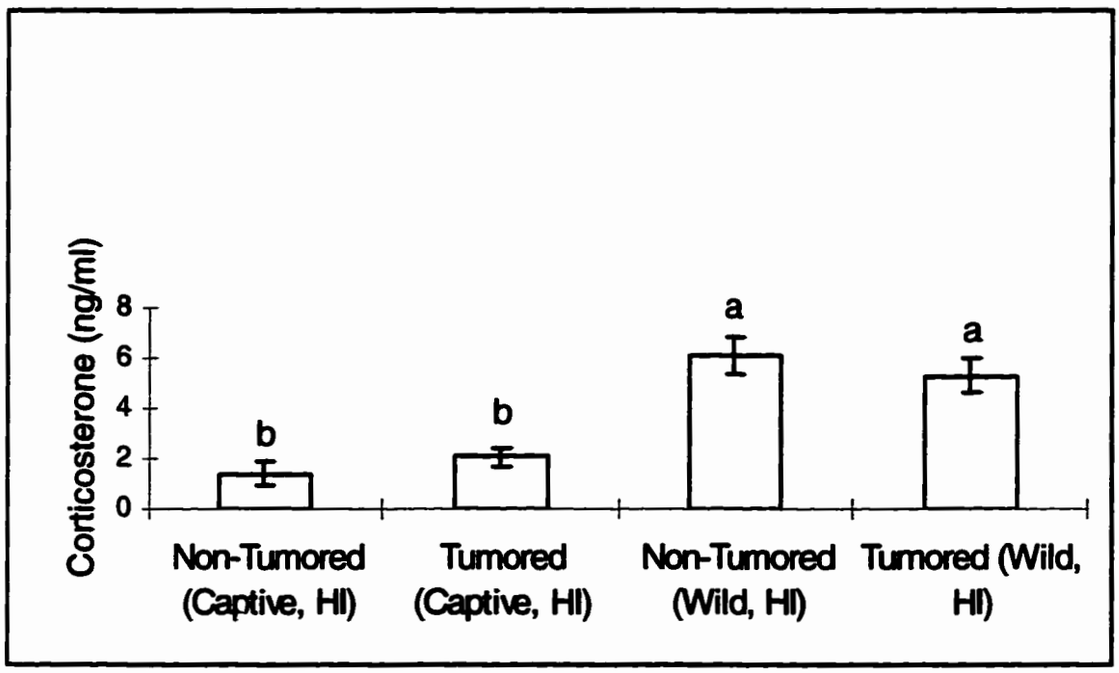

Figure 4.1. Mean plasma corticosterone levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

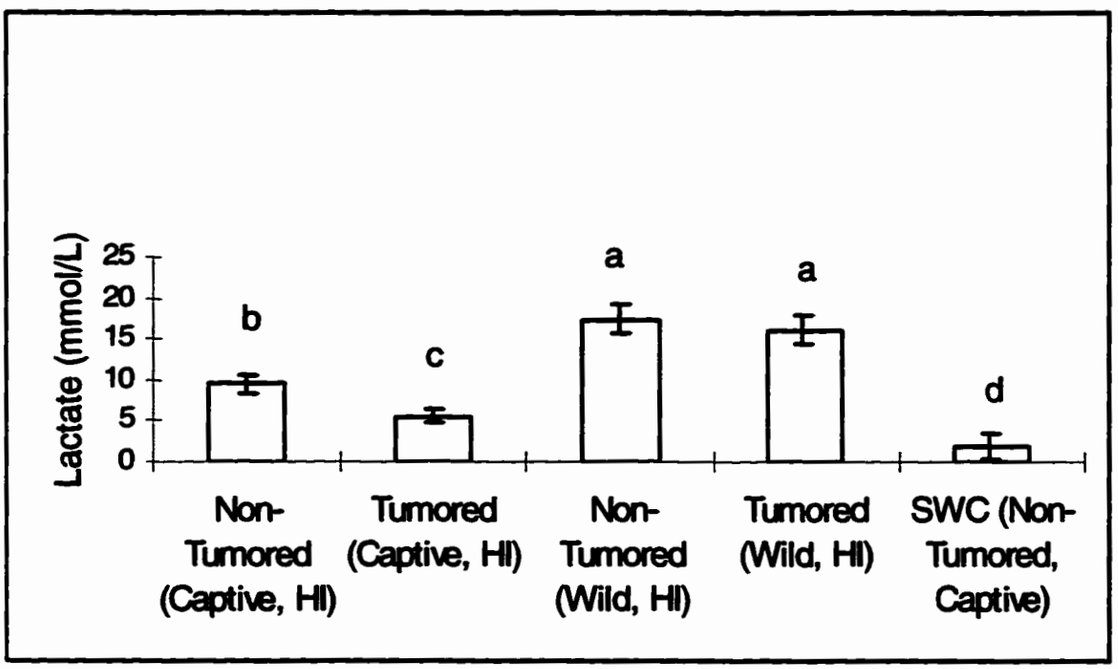

Figure 4.2. Mean plasma lactate levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

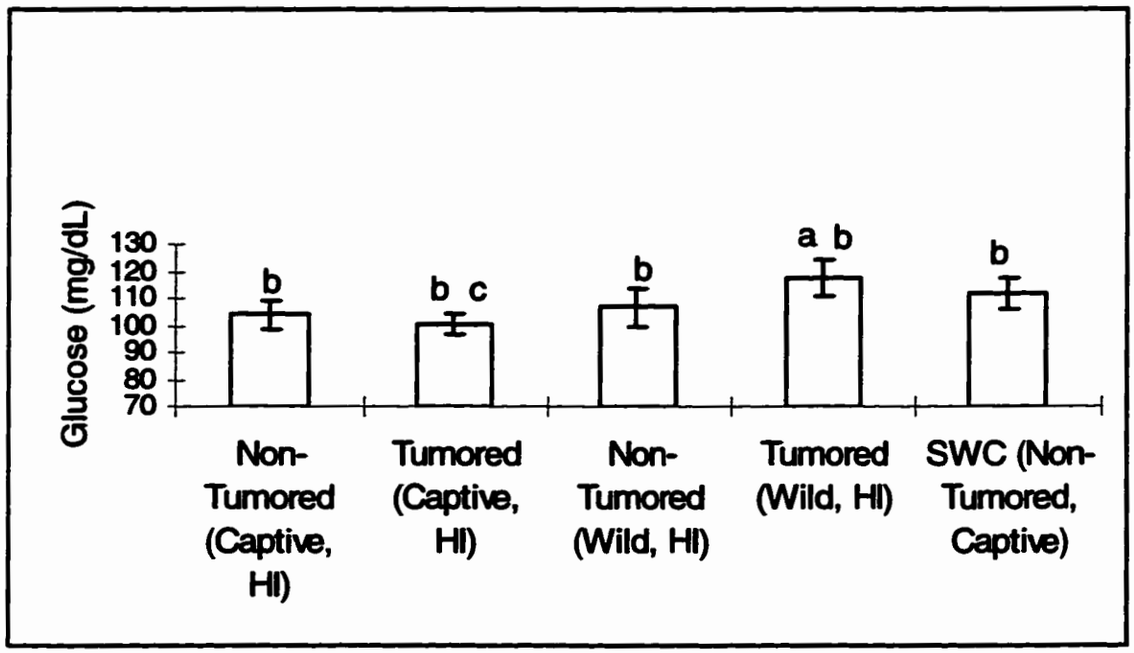

Figure 4.3. Mean plasma glucose levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

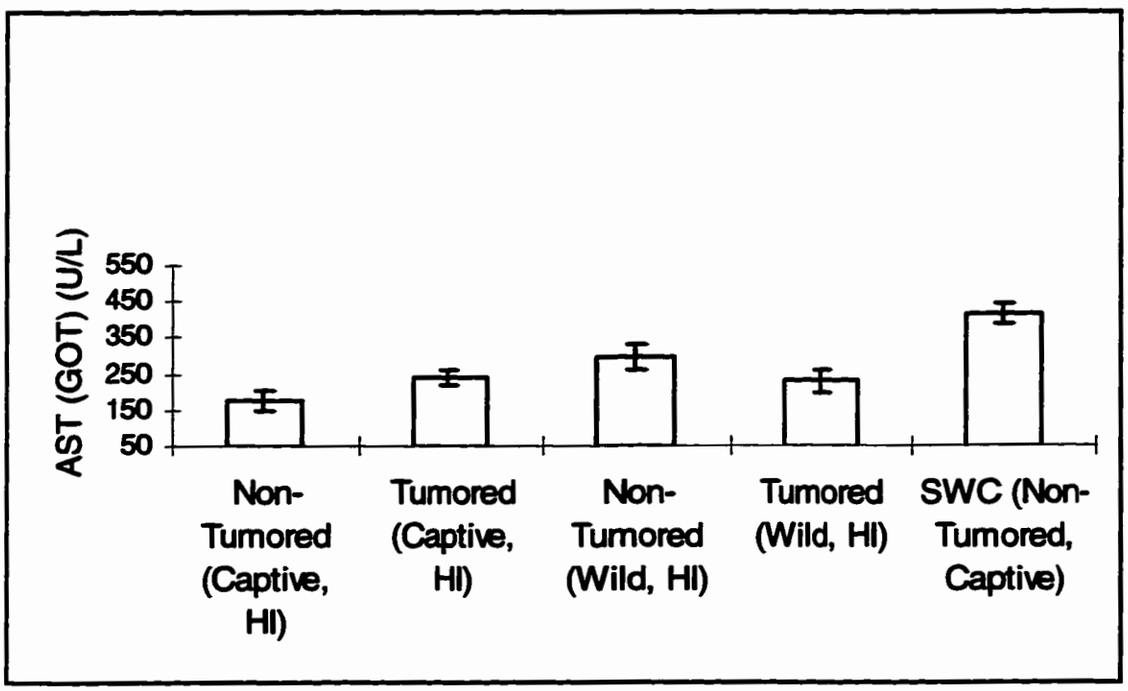

Figure 4.4. Mean plasma aspartate aminotransferase (AST or GOT) levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Significance among values is not reported due to the significant effect of time in captivity on values.

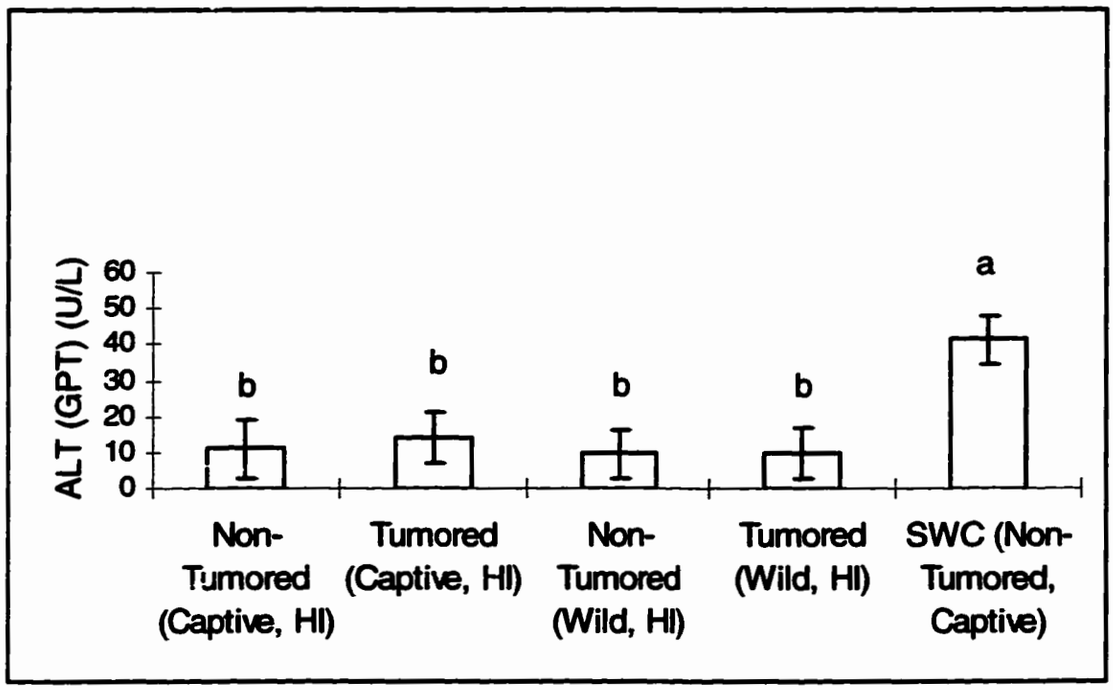

Figure 4.5. Mean plasma alanine aminotransferase (ALT) levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

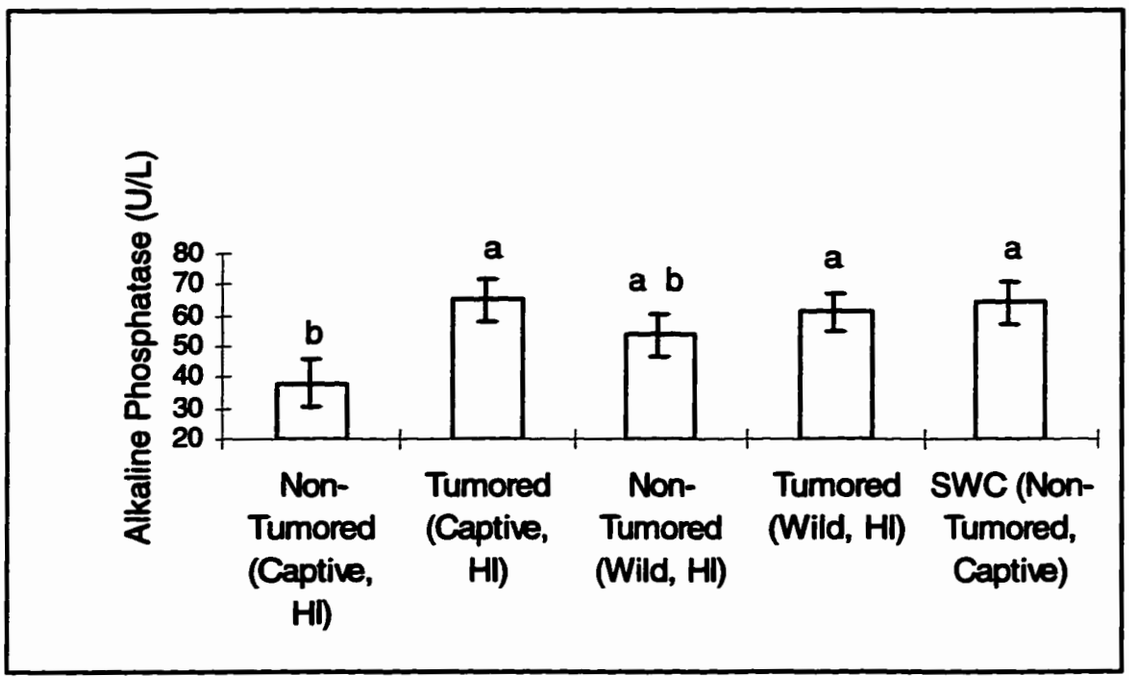

Figure 4.6. Mean plasma alkaline phosphatase (ALP) levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

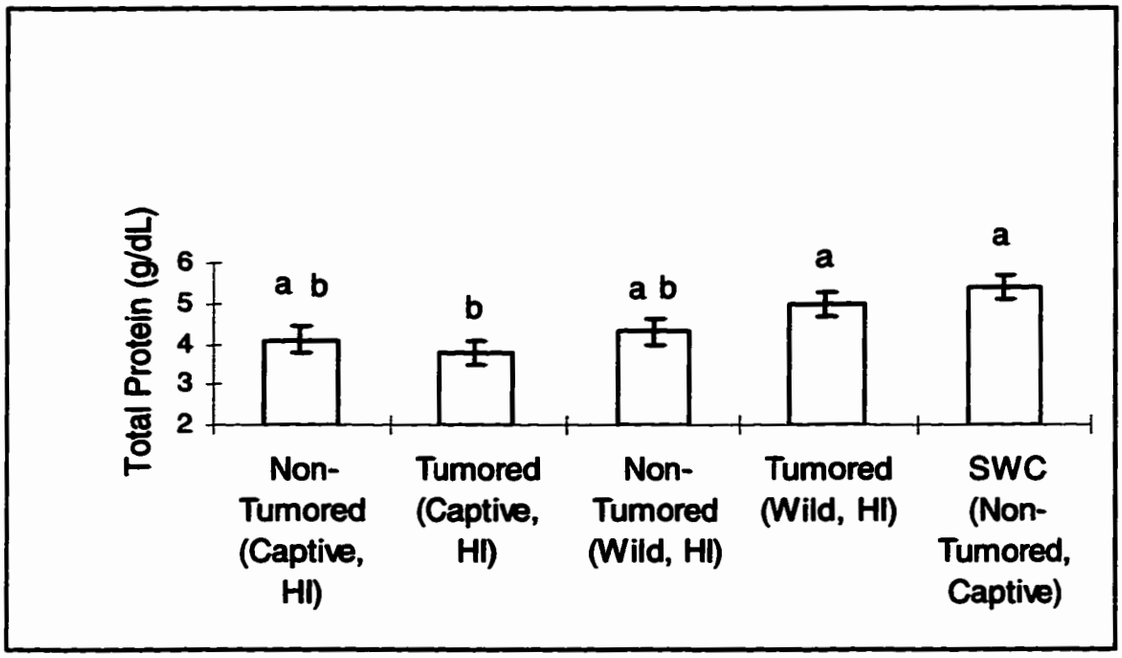

Figure 4.7. Mean plasma protein levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

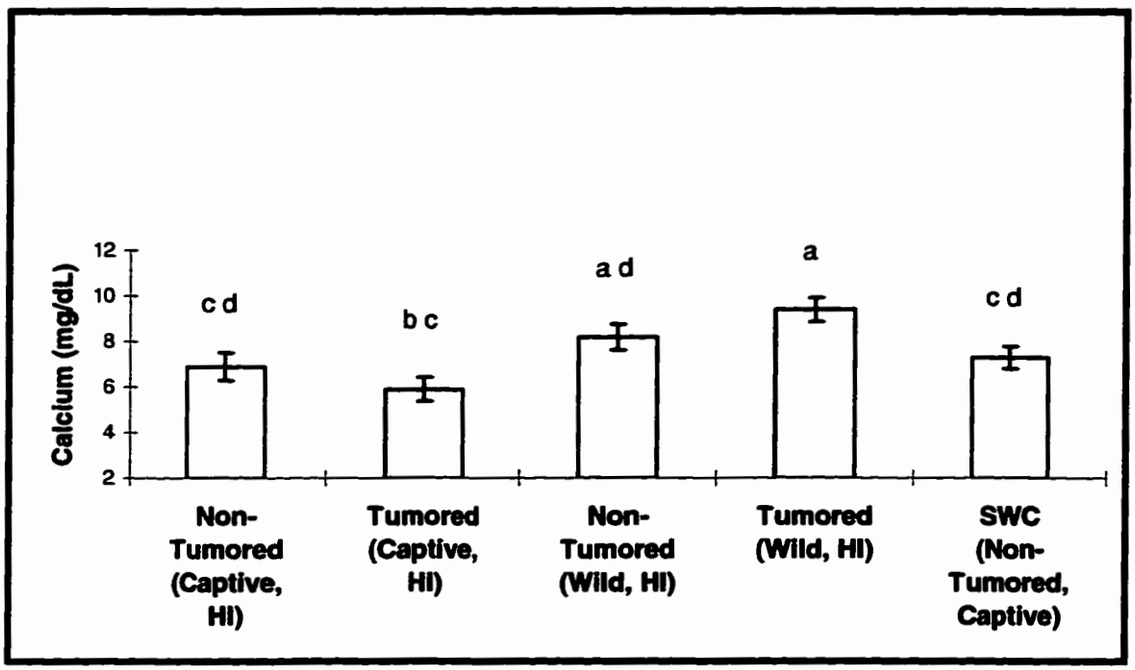

Figure 4.8. Mean plasma calcium levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

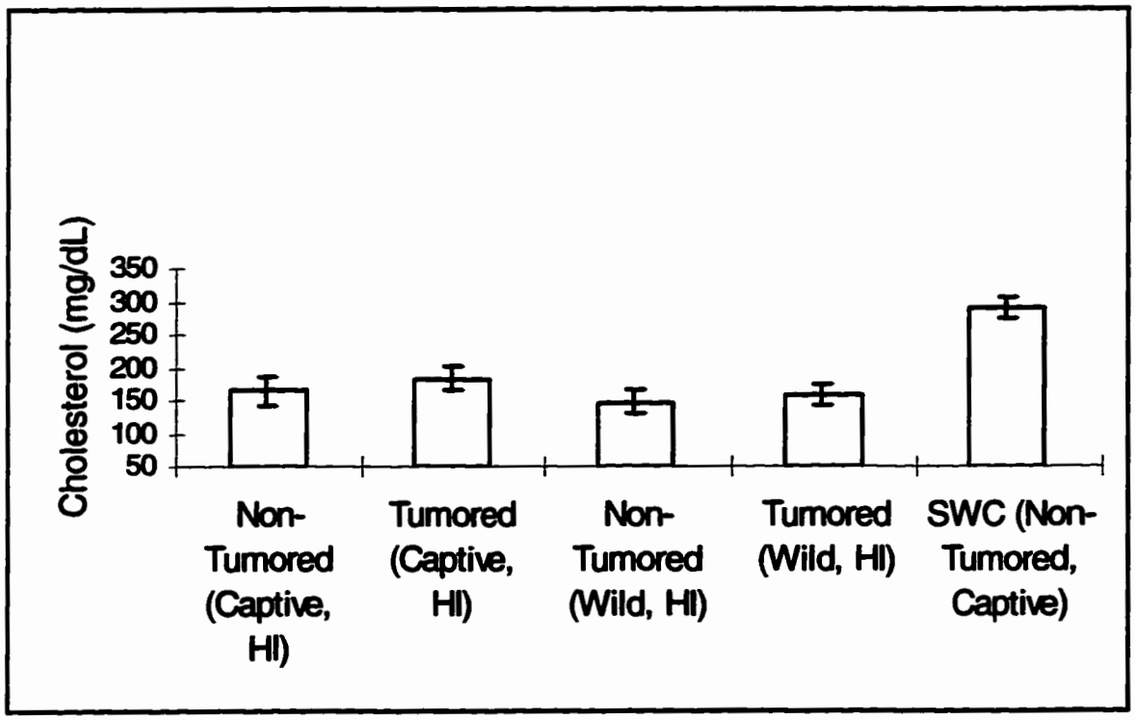

Figure 4.9. Mean plasma cholesterol levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Significance among values is not reported due to the significant effect of time in captivity on values.

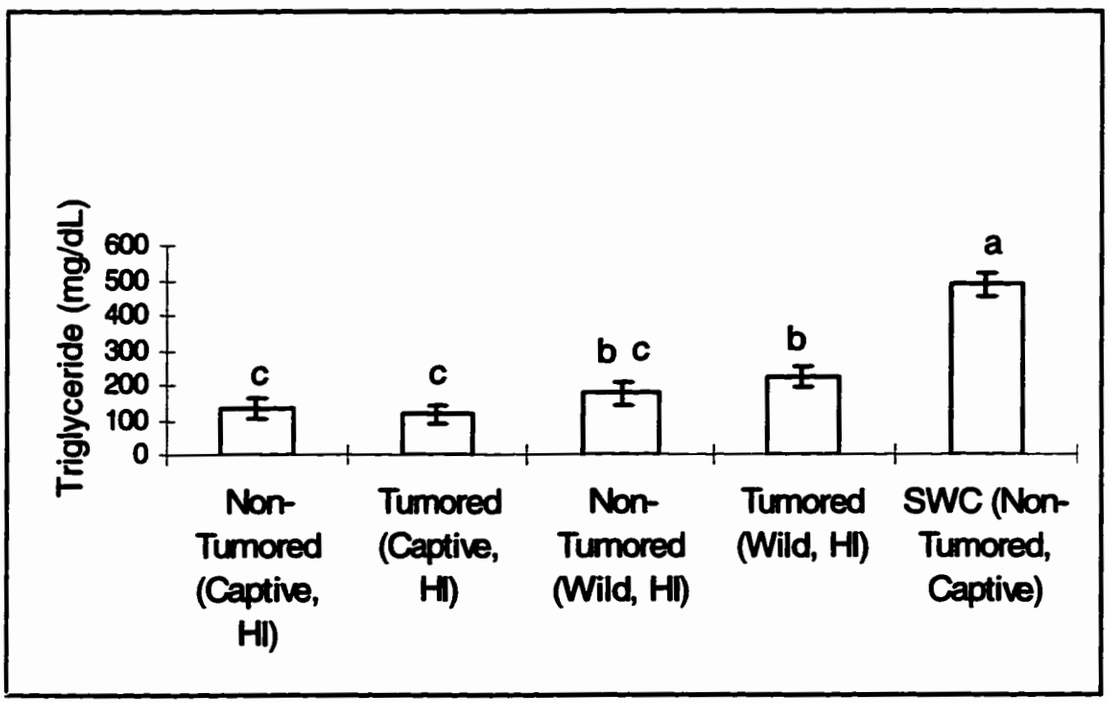

Figure 4.10. Mean plasma triglyceride levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

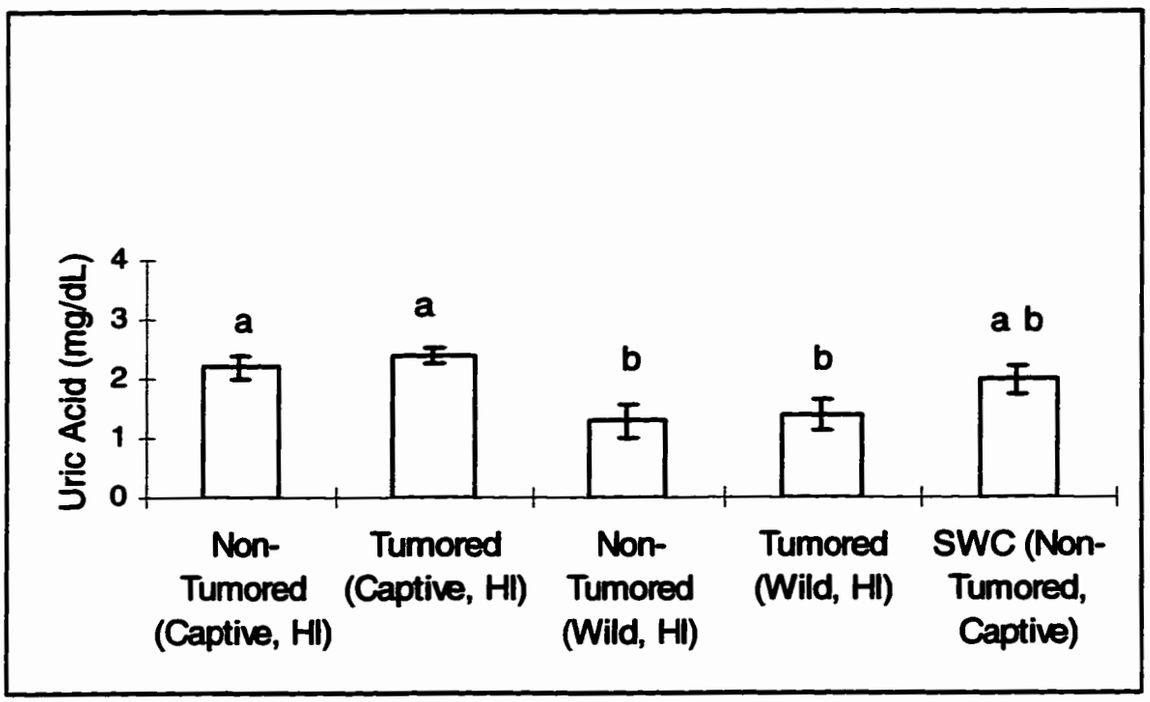

Figure 4.11. Mean plasma uric acid levels (± SEM) measured among captive and wild, tumored and non-tumored turtle groups. Values with different letters represent significant differences ($p<0.05$).

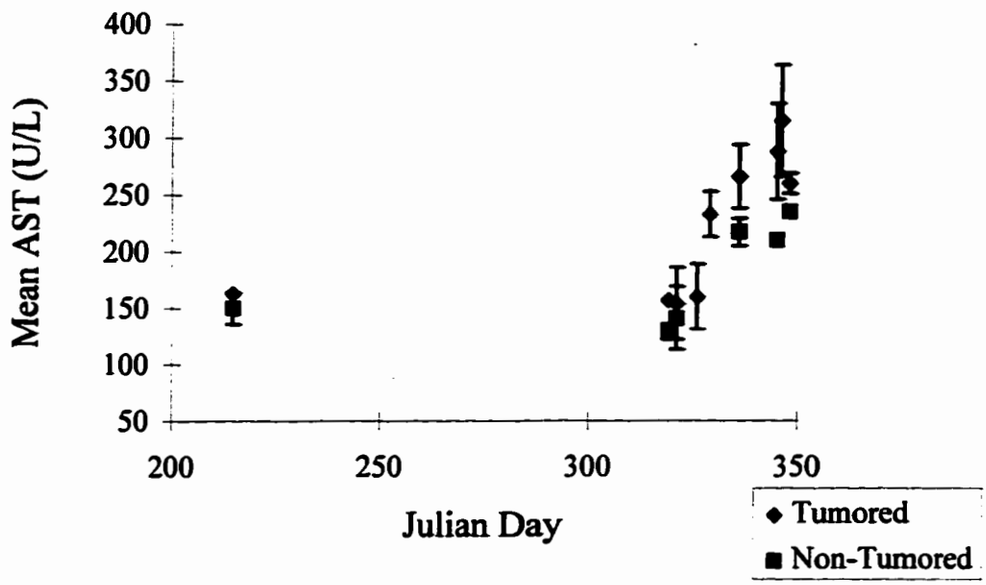

Fig. 4.12. Mean values of aspartate aminotransferase (AST) (+SEM) for tumored and non-tumored captive Hawaiian green turtles throughout time in captivity. Day of capture was Julian Day 214. Levels of AST increased significantly ($p<0.05$) during this captive period in Honolulu, Hawaii, 1994.

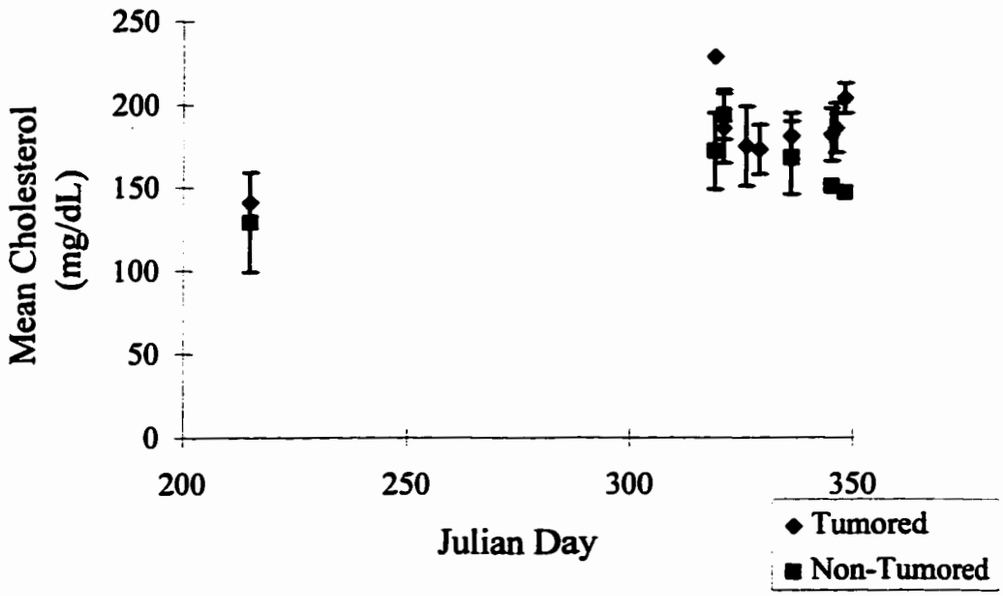

Fig. 4.13. Mean values of cholesterol (+SEM) for tumored and non-tumored captive Hawaiian green turtles throughout time in captivity. Day of capture was Julian Day 214. Levels of cholesterol increased significantly ($p<0.05$) during this captive period in Honolulu, Hawaii, 1994. Furthermore, the rate of cholesterol increase was greater in tumored turtles compared to non-tumored turtles ($p<0.05$).

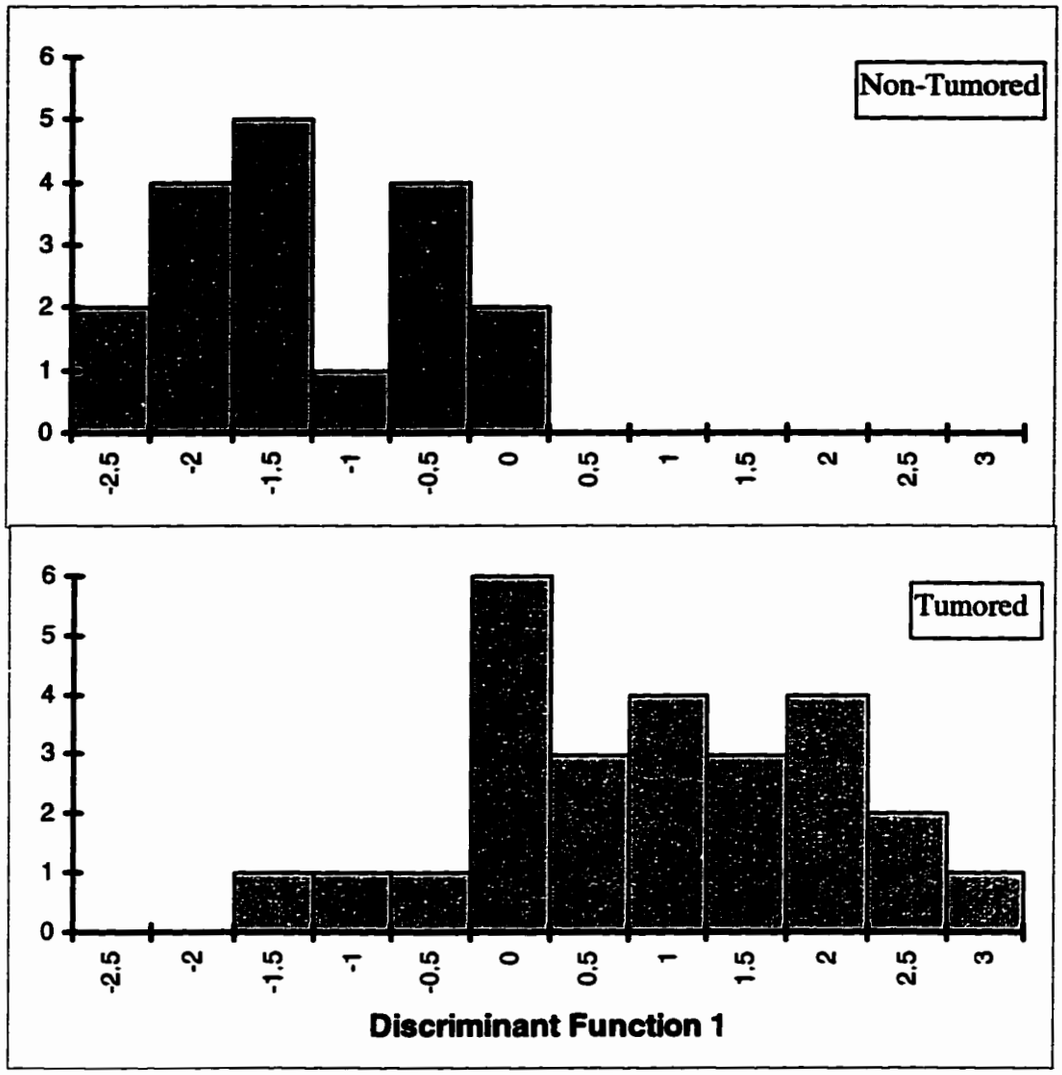

Fig. 4.14. Distribution of non-tumored (top) and tumored turtles (bottom) on the first axis of a discriminant function based on all biochemical parameters. Discriminant function analysis correctly predicted turtle groups (tumored and non-tumored) in 89% of cases for both tumored and non-tumored turtles. (Non Tumored: Mean = -1.32, Std Dev = .75, n = 18; Tumored: Mean = .92, Std Dev = 1.14, n = 26).

Biochemical Parameter	Non-Tumored (Captive, HI)	SE	Tumored (Captive, HI)	SE
Corticosterone (ng/ml)	1.38	0.48	2.08	0.37
Lactate (mmol/L)	9.53	1.07	5.52	0.75
Glucose (mg/dL)	103.9	4.99	100.54	3.96
AST (GOT) (U/L)	176.56	26.50	238.42	21.92
ALT (GPT) (U/L)	11.11	7.95	13.88	7.07
Alkaline Phosphatase (U/L)	38.07	7.68	65.11	6.81
Total Protein (g/dL)	4.07	0.33	3.92	0.29
Calcium (mg/dL)	6.81	0.61	5.95	0.53
Cholesterol (mg/dL)	163.35	21.59	180.4	19.11
Triglyceride (mg/dL)	134.88	29.92	117.46	25.70
Uric Acid (mg/dL)	2.19	0.19	2.44	0.14

	Non-Tumored (Wild, HI)	SE	Tumored (Wild, HI)	SE
Corticosterone (ng/ml)	6.072	0.736	5.3	0.68
Lactate (mmol/L)	17.37	1.79	16.16	1.66
Glucose (mg/dL)	106.67	6.91	117.71	6.40
AST (GOT) (U/L)	297	32.95	230.14	30.50
ALT (GPT) (U/L)	9.7	6.64	9.8	7.27
Alkaline Phosphatase (U/L)	53.8	6.78	61	6.28
Total Protein (g/dL)	4.26	0.34	4.97	0.31
Calcium (mg/dL)	8.15	0.57	9.37	0.53
Cholesterol (mg/dL)	147.67	19.14	157.86	17.72
Triglyceride (mg/dL)	175.33	31.66	222.43	29.31
Uric Acid (mg/dL)	1.32	0.29	1.37	0.26

	Sea World of California	SE
Corticosterone (ng/ml)	n/a	n/a
Lactate (mmol/L)	1.84	1.552
Glucose (mg/dL)	111.88	5.987
AST (GOT) (U/L)	418.38	28.531
ALT (GPT) (U/L)	41.33	6.64
Alkaline Phosphatase (U/L)	64.2	6.783
Total Protein (g/dL)	5.41	0.293
Calcium (mg/dL)	7.34	0.492
Cholesterol (mg/dL)	290.13	16.571
Triglyceride (mg/dL)	486.67	31.662
Uric Acid (mg/dL)	1.98	0.247

Table 4.1. Mean values (+SEM) of biochemical parameters from subadult green turtles with and without fibropapilloma disease (GTFP) in captivity and in the wild in Hawaii, as well as from captive adult turtles without GTFP from Sea World of California.

CHAPTER V

CONCLUSIONS

This study identified numerous biological consequences of both basking behavior and fibropapillomatosis (GTFP) disease in the green turtle, *Chelonia mydas*. The rare behavior of atmospheric basking exhibited by a few populations of green turtles in the Pacific Ocean is likely to be both physiologically and ecologically adaptive. In the first set of experiments described in Chapter 2, only turtles afflicted with GTFP were observed basking, and basking occurred on every observation day. These results are consistent with similar studies of ectotherms that elevate body temperatures via behavioral means in an attempt to enhance their immune response. Even though turtles in the Hawaiian Islands may be the only population where both basking and GTFP occur, these results suggest that basking for turtles in this area may act to increase resistance to GTFP. Basking in regions without high incidence of GTFP may be used by turtles to increase resistance to other diseases. Further study is needed to determine the immunological benefits of basking and elevated body temperature in marine turtles.

For tumored turtles, basking resulted in elevated body temperatures by a mean of nearly 3°C. Despite this elevation in body temperature, however, basking turtles had lower metabolic rates than they did after they had remained in the water. This suggests that basking may be a behavioral mechanism that conserves energy. More studies are needed to isolate the effects of body temperature and activity (eg. swimming, basking) on metabolic rate. These studies should also be done on animals without GTFP so that energy conservation can be confirmed for non-diseased animals.

Presence of GTFP was also found to influence metabolic rates among turtles. Turtles with GTFP were reported to have significantly higher metabolic costs than non-diseased turtles at similar activities and body temperature. These findings may help explain the lower number of diseased turtles in the breeding and basking grounds in the Northwestern Hawaiian Islands (NWHI) as compared to their foraging grounds in the main Hawaiian Islands. The disease exerts an energetic cost on physiological processes, which could

adversely affect already threatened populations of green turtles in Hawaii. If possible, mortality rates of diseased turtles need to be determined. Perhaps this could be accomplished by investigating differential mortality between tumored and non-tumored turtles by comparing capture and recapture rates between turtle groups.

Patterns of basking and use of the thermal environment for turtles both in captivity and in the wild in the NWHI are described in Chapter 3. In captivity, diseased subadult turtles basked both during the day and night, while in the wild, primarily tumor-free adult turtles basked diurnally. The thermoregulatory function of basking was confirmed due to the observed increase in body temperature after basking. In both captive and wild turtle groups, turtles basked until a certain threshold temperature, at which point frequency of basking declined, suggesting that turtles did not bask in ways that maximize potential heat gain. Temperature-dependent processes involved in reproduction may benefit from basking. Studies comparing clutch size, hatchling size, and inter-nesting intervals between basking and non-basking green turtles would help confirm the beneficial role of basking in reproduction.

Independent of heat gain, basking on land may also serve as a refuge from predatory tiger sharks. Due to the high prevalence of tiger sharks during the breeding and basking season in the NWHI, predator avoidance is a likely factor in eliciting this behavior.

In an attempt to improve our diagnostic capabilities of the health status of captive and wild green turtles, I compared numerous plasma biochemical values for captive and wild, healthy and diseased green turtles. Results from this study, discussed in Chapter 4, indicate that maintaining turtles in captivity altered their biochemical composition. Levels of corticosterone, lactate, aspartate aminotransferase (AST), glucose, calcium, triglyceride, and uric acid differed in turtles maintained in captivity as compared to turtles in the wild. Two variables, AST and cholesterol, significantly increased throughout time in captivity for Hawaiian green turtles. Marked elevations of numerous variables were observed in turtles from Sea World of California. Data from these animals are especially valuable as they represent biochemical values from turtles exposed to prolonged captive conditions. Reasons for the observed biochemical responses to captivity are likely a result of diet, crowding, and exercise regime. For some variables, recommendations are

made that can be useful to managers of captive green turtles who seek to maintain turtles in a manner most similar to natural conditions.

Biochemical responses to GTFP were not detected for individual parameters for turtles in the wild. In captivity, however, levels of lactate, alkaline phosphatase (ALP), and cholesterol were each affected by presence of GTFP. Discriminant function analysis (DFA) of biochemical parameters was used to determine if biochemical composition could be used to classify turtles into tumored or non-tumored animals. For both groups, DFA correctly predicted turtle group (tumored or non-tumored) in 89% of cases, suggesting that animals with GTFP had a distinct signature of plasma biochemistries. Because of the high variation among biochemical responses to disease in other studies of reptiles, including green turtles, more research is needed before the diagnostic capabilities of plasma biochemical values can be confirmed. Data presented in this study are therefore especially valuable in order to achieve this goal.

In sum, results from this comprehensive study illustrate the numerous ways in which basking results in biological and ecological benefits to green turtles. For diseased animals, basking could serve to increase the animal's immune response, which ultimately may affect its survival. For reproductively active turtles that have migrated ca. 600km to the breeding grounds, conserving energy while in the breeding grounds may be especially beneficial as it could offer increased energy to allocate for reproductive processes. For females, basking and elevated heat gain may increase physiological properties that could enable eggs to be laid in the shortest time window as possible, thereby enabling turtles to return to the foraging grounds. For all animals in the NWHI, where the water is shallow presumably making animals more vulnerable to tiger sharks, basking may serve as a refuge from predation.

It is my hope that these findings will be useful in ensuring that access to the last few remaining basking beaches be maintained. Furthermore, this study reports the significant physiological impact of GTFP that are likely to have long term implications on green turtle population dynamics in Hawaii. Lastly, biochemical data presented here may be instrumental in establishing ways to assess and improve the health status of threatened green turtles.