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# EFFECTS OF GREEN TURTLE FIBROPAPILLOMATOSIS ON THE REPRODUCTIVE SUCCESS AND EGG COMPOSITION OF GREEN TURTLES (*CHELONIA MYDAS*) NESTING AT FRENCH FRIGATE SHOALS, HAWAIIAN ISLANDS NATIONAL WILDLIFE REFUGE.

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## ABSTRACT

Nesting activity of Hawaiian green turtles was monitored during the 1999 season on Tern Island, French Frigate Shoals, Hawaiian Islands National Wildlife Refuge. Throughout the nesting season, 28 turtles without fibropapillomatosis (FP) and 15 turtles with FP were monitored to determine if reproductive success is affected by FP. Weight (kg) and morphometric measurements (cm) were not significantly different between turtles with and without FP. Although hatch success was not significantly different; clutch sizes of turtles with FP were significantly less ($\alpha < 0.1$) than turtles without FP. A subsample of 8 turtles without FP and 7 with FP were observed to determine the potential effects of FP on egg composition. Average egg weight (g) was greater ($p<0.01$) and the percent crude fat was greater ($p<0.05$) in turtles with FP than in turtles without FP. Percent moisture content, ash and protein were similar for turtles with and without FP. Percent carbohydrate, estimated by difference, and gross energy calculated based on nutrient composition were not significantly different for turtles with and without FP. Nesting turtles with FP did have low overall tumor scores, but appeared to have slightly impaired reproductive success. Turtles with FP may place more energy (e.g., greater egg weight & fat content) into individual eggs versus turtles without FP to compensate for the decreased clutch size.

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## PREFACE

Hawaiian green turtles are listed as threatened by the Endangered Species Act, and of great concern is that the population is affected by the occurrence of fibropapillomatosis (FP). The objective of this study was to determine if reproductive output of nesting turtles is affected by FP. This thesis is divided into three parts. The first part, Chapter 1, contains the literature review and the rationale behind the project. The results are presented in two separate chapters written in journal article format. The first paper titled 'Effects of Fibropapillomatosis on the Nesting Biology of Green Turtles (*Chelonia mydas*) at French Frigate Shoals, Hawaiian Islands National Wildlife Refuge' discusses the difference in reproductive success in turtles with and without FP. The second paper, 'Impact of Turtle Fibropapillomatosis on Egg Production and Composition by Green Turtles (*Chelonia mydas*) at French Frigate Shoals, Hawaiian Islands National Wildlife Refuge' focuses on maternal energy resources and whether the size, volume, and nutrient composition of the eggs and energy expenditures during reproduction differed in turtles with and without FP. These two chapters are followed by a summary, Chapter 4, that present some combined conclusions from the previous chapters.

The methods and results, although discussed in two separate chapters, refer to the same study and the same turtles. Chapter 2 uses the entire sample of turtles, 28 non-FP and 15 FP while Chapter 3 discusses a sub-sample of 8 non-FP and 7 FP.

# CHAPTER ONE

# LITERATURE REVIEW

Sea turtles are long lived reptiles whose history extends back approximately 90 million years (Bustard, 1972). There are seven (and possibly eight) species of sea turtles worldwide: leatherback turtles (*Dermochelys coriacea*), olive ridley turtles (*Lepidochelys olivacea*), Kemp's ridley (*Lepidochelys kempi*), loggerhead turtles (*Caretta caretta*), hawksbill turtles (*Eretmochelys imbricata*), flatback turtles (*Natator depressus*), green turtles (*Chelonia mydas*) and some researchers contend that, the East Pacific stock of green turtles, often called black turtle, is a separate or subspecies. Green turtles are in Class *Reptilia*, Order *Chelonii*, and Family *Chelonidae*. They are listed as threatened except for the populations in Florida and the Pacific coast of Mexico that are listed as endangered under the U.S. Endangered Species Act.

## GREEN TURTLE LIFE HISTORY

Green turtles (*Chelonia mydas*) are omnivorous, leaning to carnivory when in their young pelagic life stage (Bjorndal, 1997). When the young turtles grow to a 20 to 35 cm carapace length (varies with population) they move to shallow waters to feed primarily on seagrasses and algae with an opportunistic diet of jellyfish, salps and sponges (Bjorndal, 1997). Adult green turtles in Hawai'i primarily feed on 56 species of algae (Balazs, 1980; Balazs et al., 1987; Russell and Balazs, 1994). Sea turtles are iteroperous (show repeated cycles of reproduction), with very predictable nesting behaviors, lay a relatively large number of eggs several times during the nesting season,

and show high nest site fidelity (Miller, 1997). The estimated age at first breeding for sea turtles is 30 or more years (Zug et al., 2002); they do not breed at a uniform or minimum size, and size is not a reliable indicator of maturity or breeding status (Miller, 1997). However, it has been observed that all green turtles nesting at French Frigate Shoals had a minimum straight carapace length of 80 cm and that growth does begin to slow as individuals attain sexual maturity (Balazs, 1980; Zug et al., 2002). Most turtle species mature at a species-specific size rather than at a specific age (Moll, 1979). The interval between reproductive periods, or remigration interval, is generally two or more years (Miller, 1997). Sea turtles use beaches with deep, loose sand and typically nest during the warmer months (Miller, 1997). *C. mydas* generally nest at night with an average of three to five clutches per season, a nesting interval of 12 days and a remigration interval of approximately two to four years (Wilbur and Morin, 1988; Miller, 1997; Bustard, 1979). The remigration interval is for building up enough fat for the cost of breeding and the 10-12 months needed for vitellogenesis (Miller, 1997).

Mating occurs at the nesting area one to two months before nesting and females are receptive for about seven to ten days for copulation (Miller, 1997). Sperm collected from each insemination during copulatory events is stored and mixed in the upper portion of the oviduct, therefore eggs in one nest may be fertilized by several different males (Miller, 1997). It usually takes green turtles two to three hours to complete egg laying/nesting (Miller, 1997). Marine turtle produce large clutches of small eggs and a large number of nests per season (Van Buskirk and Crowder, 1994).

*C. mydas* exhibit relatively high egg survival and low reproductive effort relative to body size (Van Buskirk and Crowder, 1994). The mean clutch size of turtles nesting at Tern Island, French Frigate Shoals was 92.4 eggs (Niethammer et al., 1997), whereas clutch sizes were approximately 127.5 eggs on Ascension Island (Hays et al., 1993), 113.1 eggs in Tortuguero, Costa Rica (Bjorndal and Carr, 1989), and 120 eggs on Heron Island, Queensland, Australia (Bustard and Greenham, 1968). Incubation lasts approximately six to thirteen weeks (Miller, 1997).

**HAWAIIAN GREEN TURTLE**

Hawaiian green turtles are a distinct and unique genetic haplotype (Bowen et al., 1992) and are present throughout the entire island chain from Hawai'i to Kure Atoll (Balazs, 1976). The main foraging pastures are on the shallow coastal shelves of the large, inhabited islands (Balazs, 1976), including the Kau and North Kohala districts of Hawai'i Island; the Hana district and Paia of Maui Island; the northern and northeastern coastal areas along the Kalohi and Auau channels of Lanai Island; the southern coastal areas from Kamalo to Halena of Molokai Island; Kailua and Kaneohe Bay, the northwestern coastal areas of Mokuleia to Kawailoa of Oahu Island; and Princeville and northwestern coastal areas of Na Pali and southern coastal areas from Kukuiula and Makahea Point of Kauai Island (Balazs, 1980). Hawaiian green turtles were exploited by humans for various purposes, such as meat and eggs for consumption (Balazs, 1980) and the turtle carapace for personal items (hair combs, food utensils, etc.) and ornamental purposes (NMFS and USFWS, 1998). Due to this human predation, the only remaining colonial nesting site is on the unpopulated, remote atoll of French Frigate

Shoals (FFS), Hawaiian Islands National Wildlife Refuge (HINWR) where 90% of all breeding, both copulation and nesting, occurs (Balazs, 1976). There are records of small numbers of nesting having occurred on beaches on Kauai, Lanai, and Oahu in the 1930s (Balazs, 1976); however, nesting on the main islands by green turtles has largely disappeared. In the past several years, very small numbers of turtles have been confirmed nesting on Kauai at the Pacific Missile Range Facility (Collins, 2001). Before 1997 most of the nesting at FFS occurred on East and Whale-Skate Islands (Balazs, 1976), however, in 1997 Whale-Skate eroded away and most of the nesting appeared to have shifted from Whale-Skate to Tern Island. Tagging studies begun by George Balazs of the National Marine Fisheries Service (NMFS), have shown that turtles observed around the main Hawaiian islands nest at FFS (Balazs, 1976). Additionally, there have been tag recoveries at FFS from turtles that reside farther up the remote islands.

Oviposition occurs from approximately the end of April to mid-October, peaking between mid-June and early August at FFS (Niethammer et al., 1997). Niethammer et. al. (1997) recorded a mean incubation period of 66 days, a mean clutch size of 92.4 eggs and a mean hatch success of 79% during a six year period on Tern Island, FFS. Additionally, turtles lay up to six clutches per year with a mean of 1.8 clutches (Balazs, 1980). Due to the migration by this population of green turtles to the remote, nesting grounds managed by the U.S. Fish and Wildlife Service which are devoid of land predators, and protection because of the Endangered Species Act, Hawaiian green turtles have increased and have a better chance of recovery.

## PARENTAL INVESTMENT

Parental investment was first defined as ". . .any investment by the parent in an individual offspring that increases the offspring's chance of surviving, hence reproductive success, at the cost of the parent's ability to invest in other offspring" (Trivers, 1972). In other words, when energy expended on an individual offspring is increased, the number of offspring that the parents can produce is decreased and the potential fitness of the individual offspring is increased (Smith and Fretwell, 1974). Smith and Fretwell's ideas were rewritten into four assumptions: 1) parents have a set, limited supply of energy to use for reproduction at any given time, 2) as the number of offspring produced by a parent is increased, each individual offspring's fitness must decrease, 3) a minimum amount of energy is required for offspring to be viable, and 4) the parents will eventually hit a point of diminishing returns where increases in reproductive effort per offspring bring a decrease in the payoff in fitness of the parents (Brockelman, 1975). Additionally, the cost of reproduction is equal to a marginal increase in adult mortality between some initial time and a later time (Stearns, 1976). This would be caused by the parent committing a certain portion of available energy or resources to reproduction at their first reproductive time or during environmental conditions that are less than favorable for reproduction. Another definition of parental investment is simply the product of the number of offspring and subsequent survival to maturity (Lloyd, 1987).

Jönnson and coworkers (1998) used William's (Williams, 1966) definition of reproductive cost to evaluate the negative effects on future fitness arising from resource allocations with positive effects on present reproduction. Total reproductive costs were

further broken into pre-breeding and post-breeding costs. The pre-breeding reproductive cost is any initial physiological or behavioral activity in a life cycle that will lead to the appearance of offspring (Jönnson et al., 1998). Three major pre-breeding aspects of reproduction are: 1) courtship and mating behavior, 2) storage of energy and nutrition, and 3) potential parental care. The costs during this time are increased by the risk of predation, the cost of mate guarding and the eventual physical cost of mating (Jönnson et al., 1998). The post-breeding cost is the adult survival costs after all of the costs incurred from pre-breeding and breeding (Jönnson et al., 1998). The costs during this time have to do with the survival of the adult until the next reproductive event. This hypothesis has a link with the theory of optimal clutch size and why animals may lay fewer eggs than physiologically capable because a reduction in clutch size and parental investment will increase parental survival after breeding (Jönnson et al., 1998). These reproductive costs have been used to discuss reproduction in birds but also apply to turtles.

Marlen and Fischer (1999) defined reproductive effort as the proportion of an animal's resource budget that will be allocated to reproduction, and they defined parental investment as the relative amount of energy allocated to each offspring. In reptiles, the size of the offspring is very small and time to adulthood is reached far less rapidly than compared to warm-blooded animals that have fewer offspring and do not offer parental care after fertilization (Lloyd, 1987). For example, some reptiles maximize lifetime reproductive success by laying less than their maximum potential for each clutch (Congdon et al., 1983a). The clutch size and the frequency of nesting (between season and inter-nesting), therefore, is indicative of the individual's reproductive output (Gibbons et al., 1982). Since there is no parental care in turtles; the eggs represent the

total reproductive investment (Congdon et al., 1983b). Parental investment in reptiles can be divided into two categories: as energy invested in making the embryo and energy in excess of that needed to produce a viable hatchling (Congdon, 1989; Congdon and Gibbons, 1990). Therefore, turtles must do all parental investment *prior* to ovulation, thus the term "pre-ovulatory investment" coined by Marlen and Fischer (1999).

Reproductive effort in reptiles is the total amount of energy available for present reproduction, and parental investment is the quantity of energy to be allocated to each offspring (Congdon, 1989). Reproductive investment involves two major aspects; 1) what determines the total level of reproductive effort and 2) what determines how total reproductive output is apportioned among individual offspring (Congdon and Gibbons, 1987). Most reptiles maximize their lifetime reproductive output by laying many eggs and expending no further energy for their survival (Hays and Speakman, 1991). Fundamental to reproductive investment is the assumption that energy available to the organism is finite. Allocation of more energy to reproduction results in a concomitant reduction in the energy allocated to other elements of the organism's total energy budget (Congdon and Gibbons, 1987).

Parker and Begon (1986) discussed Lack's theories on birds and applied them to reptiles, theorizing that reproductive success is proportional to the rate of production of the surviving progeny. Congdon and Gibbons (1987) further defined parental investment and care in reptiles as the material and energy allocated to behavior associated with mating or defense of breeding territories, the eggs or development of offspring, and the parental care of the young. Maternal investment in turtles is considered to have little or

no competition for nest sites, a large clutch size and reduced or no parental care. Maternal investment, specifically, consists of producing excess yolk to ensure there is enough material needed by the offspring to reach independence (Congdon and Gibbons, 1985).

## EGG DESCRIPTION AND COMPOSITION

Marine turtle eggs are spherical, a creamy white at egg laying turning to pure white throughout the incubation period (Miller, 1985; Bustard, 1972; Ewert, 1979). All egg shells may have evolved to protect the embryos from desiccation, bacteria, fungi (Phillot, 2001) and arthropod predators (Congdon and Gibbons, 1990). Hatching success of marine turtle eggs depends upon such factors as: the salinity, humidity, temperature, erosion, gas flow, rainfall, tidal inundation, and predation (Miller, 1985).

Two unique and major features of reptile eggs are (1) a highly developed calcareous shell and (2) a large proportion of the egg devoted to the yolk (Congdon and Gibbons, 1990). Reptile egg shells are pliable and predominantly made of calcium carbonate as aragonite (Ewert, 1985). The individual aragonite crystals that make up the shell are long and therefore make the egg shell more pliable (Solomon and Baird, 1976). Marine turtles are the only group of reptiles that make their egg shells from a combination of calcite and crystal aragonite (Solomon and Baird, 1976), and this may be an ancestral trait (Congdon and Gibbons, 1990). Female *C. mydas* are able to mobilize 0.14g Ca/day/kg of bodyweight for eggshell production (Miller, 1985). *C. mydas* eggs consist of 4.3% shell, 67.3% yolk and 28.4% albumen (Miller, 1985).

Turtle eggs have to provide energy for development and maintenance of the embryo and growth of the hatchling (Congdon and Gibbons, 1990). Reptiles create eggs with a greater proportion of egg yolk than those of other egg laying animals, such as birds (Noble et al., 1990). For example, an alligator egg yolk takes up 45% of the weight of the egg while an avian egg may take up approximately 30% (Noble et al., 1990). There are high lipid reserves in the egg yolk to supply the offspring with enough energy to ensure survival after they leave the egg (Marlen and Fischer, 1999; Nagle et al., 1998). As the yolk is the primary nutritional source for the embryo, the yolk has been studied far more than the albumen. The eggs have a thick layer of albumen at oviposition to provide the embryo with water during the first one to two weeks of development (Ewert, 1979; Packard and Packard, 1988). Turtles are not thought to use the albumen later because it is not prominent after the egg is well into development and the parchment-shelled eggs take up water from the environment (Ewert, 1979; Packard and Packard, 1988). In freshwater turtle eggs, nonpolar lipids like, tricylglycerols and cholesteryl esters make up a greater percent of the lipid content than polar lipids in the yolk (Congdon and Gibbons, 1990; Rowe et al., 1995). Tricylglycerols are catabolized into metabolic fuels for activities such as nest emergence and dispersal (Nagle et al., 1998).

The mean egg diameter of *C. mydas* is approximately 44 mm and the mean egg weight is 45g (Miller, 1985). Whole ovipositioned eggs of *C. mydas* have a protein content of 16.5%, fat of 11.6%, fiber of 0.0%, ash of 1.9% and the water content is 66.7% (Miller, 1985). *C. mydas* hatchlings weigh approximately 57.1% of the weight of their egg at laying and the egg shell is a source of calcium for embryo bone development

(Miller, 1985). Additionally, the composition of the eggs is a result of nutrients ingested at the foraging area because the follicles are already set when the female arrives at the nesting grounds (Miller, 1985).

## CLUTCH SIZE

Clutch size is a critical characteristic of a species and there must be an evolutionary explanation for this life history phenomenon (Gibbons et al., 1982). Reproductive output in sea turtles is defined by the number of clutches in a nesting season, the clutch size, the interval between nesting seasons and the length of the reproductive life (Bjorndal and Carr, 1989). Large clutches by large turtles could be due to the fact that bigger turtles can dig deeper pits, however, there is no significant difference in depth of the egg chamber, but perhaps that the chamber might be wider (Hays et al., 1993). There is not an inverse relationship existing between the number of eggs in the clutch and the mean diameter of the eggs (Hays et al., 1993). For sea turtles there seems to be no optimal egg size and these animals are not at the limit of the individual's resource availability (Hays et al., 1993).

Bjorndal and Carr (1989) in Tortuguero, Costa Rica showed a significant positive correlation between clutch size, carapace length, carapace width and plastron length in green turtles. But this relationship accounted for only a small proportion of the variation in clutch sizes. Additionally, the clutches of the first two nests in a given season were smaller than those laid later and the size of the nesting females did not account for the increase in clutch size (Bjorndal and Carr, 1989). However, in Georgia, the clutches laid in the last two nesting events were significantly smaller than the first and second

clutches of loggerheads (Bjorndal and Carr, 1989). The relationship of egg diameter to clutch size was not significant, and egg size was significantly and positively correlated with carapace length; but the relationship between egg size and body mass of female was not significant (Bjorndal and Carr, 1989). Among all nests laid, egg size varied much less within and throughout all clutches than clutch size, and was independent of body size and clutch size.

In nesting loggerhead turtles on Potamaki Beach (Cephalonia, Greece), Hays and Speakman (1991) observed that larger individuals laid larger clutches, but that there was not a significant relationship between egg diameter and carapace length or clutch size. Hawaiian green turtles have the fewest eggs per clutch and produce the fewest number of hatchlings of all of the breeding populations of green turtles (Spotila et al., 1987; Hirth, 1980). The idea has been proposed that there is an evolutionary trade-off between the offspring size and number with the relationship among body size, clutch size, and the shape of the egg (e.g., whether spherical or elliptical) (Elgar and Heaphy, 1989; Congdon and Gibbons, 1985). Parker and Begon (1986) theorized that species that lay several clutches per cycle with no feeding between clutches have no obvious correlation between egg or clutch size and the female's body size – although larger females lay more clutches per nesting season.

There is no significant relationship between the size of the turtle and the number of clutches it lays (Frazer and Richardson, 1986). For example, for animals that lay multiple clutches throughout the nesting season there may be an increased risk to the female for each emergence. Therefore, they may produce the minimum number of hatchlings needed to emerge from the nest, their nest may have an increased potential

for greater nest success, and they hold the maximum number of eggs ready to go in their body (Frazer and Richardson, 1986). Turtles have high variability in some reproductive characteristics, but there are some consistent patterns in their nesting. There is a positive relationship between reproductive output and body size, but the variance may be high in clutch size and in frequency of nesting during the nesting season regardless of body size within a species (Gibbons et al., 1982). Additionally, while the mean clutch size within a species tends to be consistent within a year, the individuals show high variability in clutch size for that year (Gibbons et al., 1982; Ji and Braña, 1999). There may also be a gradual reduction in the clutch size as multiple nests are laid throughout a nesting season (Gibbons et al., 1982). If the clutch size and the frequency of nesting are controlled by resource availability and utilization of those resources by the individual, *and* the maximum limit on the clutch size is set by the maximum body size obtained, then there could be high variability within a population and for an individual because these are determined by seasonal and annual changes in the environment will affect these variables (Gibbons et al., 1982). Simply, the majority of variation in clutch size remains unexplained (Hays and Speakman, 1991).

## OPTIMAL EGG SIZE THEORY

The definition of optimal egg size is the maximum number of individuals that can be produced by the present level of investment in each offspring (Congdon, 1989; Congdon and Gibbons, 1990). Optimal egg size predicts that within a particular

population the amount of variation in the reproductive output among females should chiefly be from the variation in the number of offspring produced followed by the variation in egg size (Congdon, 1989; Congdon and Gibbons, 1990).

Within populations, natural selection has optimized egg size at a point where increased Darwinian fitness associated with increased egg size equals a decrease in fitness caused by a decrease in offspring number (Bjorndal and Carr, 1989; Congdon and Gibbons, 1987). Size of individual offspring should most often be under a strong normalizing selection (in a relatively stable environment) that will reduce variation in egg and offspring size. Therefore, among individuals that produce more than one offspring in a reproductive bout, the main source of variation in total reproductive output will be due to environmental factors such as available resources, age of individuals and changes in body size (Congdon, 1989). These factors will cause changes in the number of eggs produced rather than changes in egg size. Therefore optimal egg size should be observed in animals that have large clutch sizes and no parental care because the investment is in making the egg. Bjorndal and Carr (1989) outlined how green turtles fit predictions of the above model. For example, clutch size varies more than egg size, the amount of variation in the egg size that is accounted for by female carapace length is only one half of that for clutch size, and clutch size varies much more among populations than does egg size (Bjorndal and Carr, 1989). Additionally, among sea turtle populations there is selection for an egg to have a diameter approximately 45mm (Hirth, 1980). There is a positive relationship between egg size and female body and clutch size of turtles that lay elliptical eggs (Rose et al., 1996; Iverson, 1992). However, in turtles that lay spherical

eggs, generally marine species with a large body size, there does not seem to be a relationship because marine species lay proportionally more and smaller eggs (compared to body size) in a single nest (Rose et al., 1996; Wilbur and Morin, 1988; Van Buskirk and Crowder, 1994).

Carapace length of chelonians was positively correlated with egg length and width, and the clutch size was positively correlated with carapace length and egg weight (Elgar and Heaphy, 1989). However, in *Chelonia mydas* the body size and weight were not significantly correlated, but the egg weight and clutch size were not examined (Elgar and Heaphy, 1989). There was a positive correlation between the size of the nesting female and the size of the clutch laid and that the average clutch size and the average curved carapace length of all breeding populations are directly related (Hirth, 1980). In general, terrestrial reptiles lay relatively larger and fewer eggs than freshwater chelonians; and marine chelonians lay the smallest eggs with the largest clutches (Elgar and Heaphy, 1989). Elgar and Heaphy (1989) found that larger turtles may have more resources to invest towards reproduction. Therefore, this could result in an increase in egg size or a larger number of eggs.

A loggerhead turtle study determined that egg size did not increase with body size and varied less than clutch size within a population (Tiwari and Bjorndal, 2000). Van Buskirk and Crowder (1994) determined that clutch size was uncorrelated with body size in loggerheads, but found a significant positive correlation between clutch size and body size, and no significant relationship between size of the turtle and the number of eggs (Frazer and Richardson, 1986). Therefore, clutch size, not egg size, varies with individuals and populations and perhaps, environmental conditions.

## INCUBATION

Incubation time refers to the developmental period from egg laying to hatching (Ewert, 1979); hatching refers to the turtle leaving the shell and the subsequent emergence from the nest. For green turtles, incubation time ranges from 42 to 91 days (in Hawaii 53-97 days (Niethammer et al., 1997)). Hirth (1980) observed that the average incubation time for green turtles is 52.7 days on mainland beaches and 60.8 days on island beaches. He speculated that shorter incubation times on the mainland beaches versus the island beaches might be adaptive in order to reduce predation (Hirth, 1980). As well, incubation periods could be longer under rainy conditions (Hirth, 1980). In general, low incubation temperatures increase duration of the incubation and lower rates of development, while high temperatures decrease the duration of incubation and increase the rate of development (Miller, 1985; Packard and Packard, 1988).

The most important external factors that may affect embryonic development are temperature and moisture. Temperature and moisture influence hatch success, hatch time, hatchling size, behavior, growth, and sex (Ji and Braña, 1999; Packard and Packard, 1988). Most turtles do not have heteromorphic sex chromosomes; instead sexual differentiation depends on the temperature during incubation, called temperature-dependent sex determination (Packard and Packard, 1988; Spotila et al., 1987). Although temperature-dependent incubation outcomes vary among populations and species, incubation for temperatures above 30°C result in primarily females, a mix of males and females at 28-30°C and primarily males from 24-28°C (Packard and Packard, 1988; Ynetma and Mrosovsky, 1980). The middle one third period in incubation is the point at

which the sex of the embryos is determined (Packard and Packard, 1988). In Costa Rica, green turtle females were produced in nests that incubated at greater than 30.0°C and males at less than 28.5°C. Open beach areas have 83.5% of the nests producing females while in the vegetated areas only 7.4% of the nests are female (Spotila et al., 1987). In Michoacan, Mexico, temperatures less than 27°C in the middle trimester produced 100% males, and temperatures greater than 30°C resulted in 100% females (Alvarado and Figueroa, 1987).

**INTERNESTING**

The interval of years between reproductive periods (remigration interval) is thought to be adaptive to allow for enough energy and fat to be stored to afford the high cost of the extensive migration and breeding (Van Buskirk and Crowder, 1994). The interval between nesting events in one reproductive period, or internesting, allows for the production of the next clutch within that season. Ovulation for the subsequent clutch typically occurs within 36 hours of the previous nesting event (Miller, 1985). Internesting periods in green turtles vary among populations; for example, in Sarawak an average of 10 days, in Costa Rica 12-14 days (Moll, 1979) and off Ascension Island, 14 days (Mortimer and Portier, 1989; Carr et al., 1974). Between nesting events, turtles appeared to travel slowly (Mortimer and Portier, 1989; Carr et al., 1974).

## NUTRITION

Green turtles are not known to feed extensively at the nesting grounds; therefore, they must arrive with enough energy stored for the entire nesting effort (Kwan, 1994). Green turtles spend at least one year of feeding in order to deposit enough fat for vitellogenesis (Kwan, 1994). For example, in non-breeding and post-breeding turtles sub-carapace depot fat was approximately less than 1mm of fat, and animals undergoing vitellogenesis had fatty tissue of up to 2 cm (Kwan, 1994). Additionally, turtles that had nested the previous season had low levels of depot fat compared to females observed to be in active vitellogenesis (Kwan, 1994).

Adult green turtles are primarily herbivorous and display a hindgut-fermenting digestive system. Hawaiian green turtles forage primarily on 56 species of both red and green algae (Russell and Balazs, 1994; Balazs et al., 1987). Green turtles are at least as efficient as ruminants in fiber digestion (Bjorndal, 1979), because there are microbial populations within the colon that help break down algae or seagrasses (Bjorndal et al., 1991). The typical retention time within the intestinal tract is 156-325 hours (Brand et al., 1999), and the major outcome of fermentation are volatile fatty acids which provide an important energy source to the turtle (Bjorndal, 1979). In Australia, red algae (*Hypnea* and *Gracilaria*) were selected most often out of the possible forages (Brand-Gardner et al., 1999). Surprisingly, these algae had the lowest gross energy, low fiber and the highest nitrogen content of the other possible forages such as sea grasses and green algae (Brand-Gardner et al., 1999).

At breeding beaches, good forage is typically not available in the area, so it has been speculated that most turtles fast during breeding. (Mortimer and Portier, 1989; Carr et al., 1974). However, about 54% of the females nesting at Raine Island, on the northern Great Barrier Reef, had evidence of supplemental forage in their gut (Tucker and Read, 2001). The turtles ate mostly a calcareous algae that perhaps was helpful to the turtle by providing calcium for the production of eggshells or to neutralize stomach acid (Tucker and Read, 2001). This study concluded that nesting female turtles do forage on small quantities of the low quality food available.

## ENERGETICS

Energy available to an animal is finite, determined by the resource availability, foraging, handling costs and the level of risk for obtaining those resources (Congdon et al., 1982). Reproductive energetics is the reproductive effort, or proportion of total energy, procured over a specified and biologically meaningful time interval that an organism devotes to reproduction (Congdon et al., 1982). Additionally, there is an energetic trade-off between growth and reproduction.

Marine turtles are unusual in Reptilia because of the pelagic lifestyle of hatchlings and the migrations that adults engage in between the foraging and nesting areas (West et al., 1992; Wyneken, 1997). Marine turtles, in order to sustain energetically expensive activities such as migration and repeated nesting during a season, must have a large body size (Hendrickson, 1980). Due to their large body size the maximum $O_2$ consumption does not increase to the same degree as in smaller reptiles and mammals (Bennett, 1982). One way to look at how much energy a task takes is to look at the difference between

maximal and resting oxygen consumption or aerobic scope. In general, the maximal aerobic power input scale is a fractional power of body mass that approximates 0.75 or $m^{0.75}$ (where m = mass) for most animals. However, for large reptiles, the maximal oxygen consumption does not increase to the same degree, and the value is $m^{0.06}$ (Bennett, 1982). The resting and active energetic requirements of large reptiles are met largely by aerobic catabolism rather than anaerobic catabolism (Bennett, 1982). A green turtle's energy metabolism during the two to three hours involved in nesting is approximately ten times their standard resting level (Jackson and Prange, 1979). Turtles are not necessarily maximally active during this process although they have metabolic rates twice those predicted by the 30°C regression equation for reptiles (Jackson and Prange, 1979). The resting rate of marine turtles is 0.0697 $O_2 \cdot kg^{-1} \cdot h^{-1}$ (Wyneken, 1997) and the nesting rate is 0.27 $O_2 \cdot kg^{-1} \cdot h^{-1}$ (Jackson and Prange, 1979). Nesting activity and the energetic expense for egg production (Kwan, 1994) involve considerable exertion and energy expense by the individual turtle. Egg production in turtles nesting in Totuguero absorbed 39.3 – 42.6% of the energy needed for the total reproductive effort (Bjorndal, 1982). The energy expended for the nesting season in Surinam was 304,850 kj (1523kj/egg) and 71,970 kj (641 kj/egg) in Tortuguero (Bjorndal, 1985). *C. mydas* deposits approximately 17% of its body weight in eggs in one nesting season (Hirth, 1971).

## STUDY SITE

French Frigate Shoals (FFS) is located at 23°45'N x 166°10'W and was discovered on 6 November 1786 by La Perouse when he almost ran aground on the reef of the atoll. FFS is a volcanic, crescent-shaped reef; the long axis is 19 nautical miles in a NW to SW direction, the outer width 31 nautical miles and the inner, 18 nautical miles. There are 12-14 sand islands in the entire atoll; some are transient and disappear depending upon weather conditions. The United States (U.S.) gained formal possession on 4 January 1859 in accordance with the U.S. Guano Act of 1856. On 3 February 1909, President Theodore Roosevelt signed an executive order setting aside the Northwestern Hawaiian Islands, except Midway, as bird preserves (Amerson, 1971).

In 1932 and 1933 FFS was used for naval seaplane maneuvers. The original sand island was approximately 600 yards long by 150 yards wide (Amerson, 1971). In 1942, FFS was increased by dredging and designated as an airbase. Today Tern Island resembles an aircraft carrier with a runway that extends down the center and is made of finely crushed coral that was dredged from the nearby coral reefs. Tern Island is a man-augmented island that is approximately 3,100 ft long with an E-NE to W-SW orientation (Amerson, 1971). After the war the area was used as a fishery and then as a Coast Guard LORAN station (Amerson, 1971). Military occupation of FFS ended in 1979, when the U. S. Fish and Wildlife Service resumed control and management as a field station for the Hawaiian Islands National Wildlife Refuge.

Sea turtles were first recorded by Lt. John M. Brooke of the USS Fenimore Cooper, on a voyage to chart the atoll in 1859. In 1888 the schooner *Wandering Minstrel* observed large numbers of turtles at FFS; and then in 1891, the first biological survey

was conducted by the Hawaiian bark *Kaaloakai*, and it was noted that there were large numbers of turtles within the atoll. A commercial fishing base was established at FFS in 1946. The two fishing companies captured turtles for the crew's diet and for the Honolulu market. Eventually, the venture became too expensive; the number of turtles had dwindled due to disturbance, and with the resumption of refuge status, the fishing companies left. Turtles may have used the original Tern Island for basking and nesting prior to the Navy construction of the airstrip and the seaplane fueling station. During military occupation a few turtles were observed to crawl and nest on the southeast island edge (Amerson, 1971).

## FIBROPAPILLOMA

### *Description and Distribution of Fibropapilloma*

Marine turtle fibropapilloma (FP) is an epizootic disease of marine turtles (Herbst and Klein, 1995; Quackenbush et al., 1998; Herbst, 1994) that has a world-wide circumtropical distribution and has been recorded in all major oceans (Herbst, 1994). FP is a neoplastic disease consisting of benign fibromas, cutaneous papillomas and fibropapillomas (Aguirre et al., 1998; Jacobson et al., 1989). The turtles in Hawai'i develop ". . . lobulated tumors (fibropapillomas) on their skin, scales, scutes, eyes and surrounding tissues, oral cavities and viscera" (Balazs, 1991) The size of the tumors range from 0.1 cm to greater than 30 cm (Aguirre, 1998). Small tumors are pigmented brown to dark grey or black with a rough and papillary surface, while the large tumors

have a cauliflower-like appearance and a fibromatous surface. Tumors may also be necrotic, hemorrhagic and infested with piscicolid leeches (*Ozobranchus branchiatus*) and their eggs (Aguirre et al., 1998).

Tumors on green turtles were first observed and described in the Tortugas (Lucke, 1938) and in Key West, Florida (Smith and Coates, 1938). The first firmly documented case of FP in the Pacific was in Kaneohe Bay, Oahu, Hawai'i in January 1958 (Balazs, 1991). Incidences of FP generally occur in near-shore areas with high human activity (Herbst and Klein, 1995; Herbst, 1994). FP is reaching epidemic proportions in areas of Florida and Hawai'i. Before 1982 there were not many sightings of turtles with FP, but accounts increased greatly during the 1980s and 1990s (Herbst and Klein, 1995; Balazs, 1991). By 1991, some populations of turtles within the main Hawaiian Islands had 92% of green turtles exhibiting FP (Balazs, 1991). The cause of FP is unknown, but many hypotheses have been put forward. In a 1998 workshop at the 18$^{th}$ Annual Symposium on Biology and Conservation of Sea Turtles, the workshop members discussed several hypotheses for the cause of FP, including parasites, ultra violet rays, toxicants, and viruses.

Although FP tumors are considered benign, they can be extremely debilitating to turtles. Herbst & Klein (1995) suggested that FP presence was associated with areas impacted by agriculture, industry, or urban development. Additionally, in 1941 the majority of the tumors in the gall bladder of marine turtles had blood flukes or ova, and perhaps these parasites contributed to tumorigenesis (Smith et al., 1941). They hypothesized that eating algae and sea grass containing encysted larvae could infect the turtles, as has been observed with other herbivorous animals. In studies of Hawaiian

turtles, there are numerous spirochid trematode ova within the tumors, while turtles from Florida had no observed trematode eggs in tumors (Aguirre et al., 1998; Jacobson et al., 1989). Turtles with FP in Hawai'i and Florida were also found to have viral DNA for herpesvirus in the tissues with tumors, while turtles without tumors had no viral DNA (Aguirre et al., 1998; Quackenbush et al., 1998). Furthermore, the herpesvirus was different between turtles from Florida and Hawai'i (Quackenbush et al., 1998). The herpesvirus etiology is still unproven; however, the virus is associated with FP and has been documented in green (Herbst and Klein, 1995; Quackenbush et al., 1998) and loggerhead turtles (Lackovich et al., 1999). The herpesvirus still has not been successfully isolated, cultured and maintained in the lab (Lackovich et al., 1999; Curry et al., 2000). The chelonian herpesvirus may also be infective for a period of time within the marine environment (Curry et al., 2000). There is an association between the distribution of fibropapilloma and the distribution of toxic benthic dwelling *Prorocentrum* species (dinoflagellates that dwell on macroalgae and seagrasses) that are known to produce okadaic acid, a known tumor promoter (Landsberg et al., 1999). In Hawai'i, it has been shown that turtles with more advanced and numerous tumors had significantly impaired cell mediated immune status (Work et al., 2001). On the other hand, a moderate amount of tumors had no obvious effect on movement patterns or habitat use (Brill et al., 1995).

## RECOVERY PLAN

The current goals of the U.S. Pacific Green Turtle Recovery Plan are to monitor, assess, and conduct biological investigations at turtle breeding sites, develop computer population modeling programs for green turtles, assess post-hooking survival and ecology of pelagic habitats in areas that are fished, investigate green turtle fibropapilloma, conduct cooperative research and provide assistance to Pacific Island and Rim Nations (NMFS and USFWS, 1998).

## OBJECTIVES

French Frigate Shoals is a remote atoll and the population of threatened Hawaiian green turtles is fortunate in having abundant nesting habitat compared to other populations. Adult turtles migrate from throughout the Hawaiian Archipelago to FFS (Niethammer et al., 1997). While many studies have been conducted on the effects and etiology of FP there have been no studies on whether the reproduction in green turtles is impaired due to FP. The objectives of this study were to see if there are differences between turtles with FP and turtles without FP in morphometrics, clutch size, nest success, incubation periods, time of nesting, composition of eggs and egg morphometrics.

# CHAPTER TWO

# EFFECTS OF FIBROPAPILLOMA ON THE NESTING BIOLOGY OF GREEN TURTLES (*CHELONIA MYDAS*) AT FRENCH FRIGATE SHOALS, HAWAIIAN ISLANDS NATIONAL WILDLIFE REFUGE


## ABSTRACT

Hawaiian green turtle nesting activity was monitored on Tern Island, French Frigate Shoals during the 1999 season. Throughout the nesting season, 28 turtles without fibropapilloma (FP) and 15 turtles with FP were monitored. Clutch size of turtles with FP was significantly less ($\alpha < 0.1$) than turtles without FP. Hatch success and morphometric measurements were not significantly different between turtles with and without FP. However, nesting turtles with FP had low overall tumor scores and appeared to have slightly impaired reproductive success.


## INTRODUCTION

Green turtles (*Chelonia mydas*) are threatened except in Florida and on the Pacific coast of Mexico where they are listed as endangered under the U.S. Endangered Species Act. Hawaiian green turtles are a distinct and unique genetic haplotype (Bowen et al., 1992) and are present throughout the entire island chain from Hawai'i to Kure Atoll (Balazs, 1976). The main foraging pastures are on the shallow coastal shelves of the large, inhabited islands (Balazs, 1976). The only remaining colonial nesting site is on the remote atoll of French Frigate Shoals (FFS) located at 23°45'N X 166°10'W (Fig. 2.1). FFS is part of the Hawaiian Islands National Wildlife Refuge managed by the U.S. Fish and Wildlife Service. Additionally, FFS is the location of more than 90% of all green

turtle breeding occurring in the Hawaiian Islands, occasional nesting occurs at other islands along the island chain. Oviposition occurs from approximately the end of April to mid-October and nesting peaks between mid-June and early August at FFS (Niethammer et al., 1997).

Turtle fibropapilloma (FP) is an epizootic disease that may threaten the survival of green turtles (Balazs, 1991; Herbst and Klein, 1995; Quackenbush et al., 1998). By 1991, populations of green turtles within the main Hawaiian Islands had records of 92% of the population exhibiting FP (Balazs, 1991). The viral etiology is still unproven; however, FP is linked to a chelonian herpesvirus (Herbst and Klein, 1995; Quackenbush et al., 1998) that is associated with FP in green turtles and loggerhead turtles (*Caretta caretta*) (Lackovich et al., 1999; Quackenbush et al., 1998). An additional hypothesis is that turtles with tumors are immunosuppressed (Aguirre et al., 1995; Work et al., 2000; Work et al., 2001). A scoring index was developed where turtles exhibiting a tumor score of 2 or 3 have more than five 1cm tumors, more than five 1-4 cm tumors and at least 1 tumor greater than 4 cm in diameter (Work and Balazs, 1999). Turtles with tumors score of 2 and 3 had significantly impaired cell mediated immune status and thus, immunosuppression may come about from the growth of tumors (Work et al., 2001).

The objective of this study was to determine if there is a difference in reproductive success between turtles with and without FP. The variables used to define reproductive success in this study were tumor score, morphometrics, clutch size, nest success, incubation period, and frequency and time of nesting during the season.

## METHODS

Data were collected on Tern Island, French Frigate Shoals during the 1999 nesting season. The data collection consisted of four and a half months of monitoring turtle nesting activity. There were 157 nesting green turtles identified on Tern Island between May 13, 1999 and September 29, 1999. A sample of twenty-eight non-FP and fifteen FP turtles were monitored throughout the entire nesting season for this study. Turtles were captured before the first nest and after the third nest laid. At the time of the first capture the turtles were given a tumor score classification described by Work and Balazs (1999). The turtles were weighed (kg); the straight carapace length, curved carapace length, straight and curved carapace width on the $6^{th}$ scute, and head width and girth were measured (cm). Turtles were tagged using Passive Integrated Transponder (PIT) tags supplied by the National Marine Fisheries Service, Marine Turtle Research Program (brand Destron). We tried to witness and mark each nest laid by the study turtles. Four methods were used to keep track of and positively identify all nests laid by the study turtles: 1) a piece of flagging tape with the nest and turtle number written on it was dropped in among the eggs, 2) a nest marker ball (nest number & turtle number written on it) attached to a long piece of coated wire was also placed in the egg chamber (the long wire was visible above the sand), 3) a stake was placed 1 meter inland from nest marker ball wire and 4) and the location was drawn into a field notebook.

Nests were excavated three days after signs of hatching or on the $102^{nd}$ day, whichever came first, after the nest was laid. When a nest was positively identified as a

study nest by finding the nest marker ball or flagging tape within it, the number of empty eggs shells, live hatchlings remaining in nest, dead hatchlings, unhatched eggs with embryos, and infertile eggs were recorded (Niethammer et al., 1997).

Statistical analysis to compare morphometrics was a general linear model (SAS v. 8, 1999). Some nests were possible outliers, and it was difficult to tell whether it was a single nest or whether there was one nest laid on top of the other. Therefore, as criteria for outliers we used 2 times the standard deviation on either side of the mean; thus, we excluded nests with recorded clutch sizes lower than 33 eggs and greater than 153 eggs. One FP turtle was entirely excluded from the analysis of clutch size and nest success because both nests she had laid were infertile and two other outliers were eliminated. A general linear model (SAS v. 8, 1999) was run to compare overall FP versus non-FP turtles within clutches. An $\alpha$ level of 0.1 was used to determine significance in this study based on the small sample size of FP turtles.

## RESULTS

Turtle nesting activity was monitored for 140 nights. During the nesting season, eggs were witnessed for 161 nests, turtles covering eggs were witnessed for 112 nests and backfilling was witnessed for 200 nests leading to a total of 473 possible nests. Of the 157 turtles observed, 24 (15.3%) had FP. The majority of the nesting occurred on South Beach of Tern Island (Figure 2.2). Shell and Crab beaches were used occasionally by nesting turtles. Morphometric data were recorded for 44 turtles, and 43 of these were observed throughout the nesting season. There was no significant difference between turtles without and with FP in any of the morphometric measurements and weights (Table 2.1).

Of the 43 turtles observed, 15 turtles with FP were observed throughout the nesting season. Of the 15 turtles, 12 had an overall tumor score of 1 (where turtles had tumors less than 4 cm and the majority had tumors less than 1 cm) and the remaining three turtles had an overall tumor score of 2 (where turtles had at least one tumor greater than 4 cm). There was a significant difference in clutch size between non-FP ($92.9 \pm 2.8$) and FP ($80.9 \pm 5.7$) turtles ($p = 0.07$). There was no significant difference in hatch success, incubation period, nesting interval and mean nesting date between FP and non-FP turtles (Table. 2.2). Additionally, there was no significant difference in the number of nests laid by turtles with FP and turtles without FP ($\chi^2 = 4.69$, probability = 0.32 and $\chi^2 = 1.81$, probability = 0.87, respectively) (Table 2.3).

The average rainfall from May 13, 1999 through November 30, 1999 was 0.08 inches/day, with a range of 0 to 3.4 inches/day (Table. 2.4). The maximum average temperature was 29°C and the minimum average temperature was 23°C (Table. 2.4). The average incubation periods, rainfall, and maximum and minimum temperatures for nests laid during the individual months of May, June, July, August (& 1 September) are listed in Table 2.5.

## DISCUSSION

Oviposition at Tern Island, FFS started on May 13, 1999 and ended September 29, 1999, approximately the same times as observed in previous studies (Balazs, 1980; Niethammer et al., 1997). A previous study at FFS determined that the length of time between oviposition of Hawaiian green turtles ranged from 11 to 18 days with a mean of 13 days (Balazs, 1980). Turtles in this study had similar results with a range from 10 to 20 days and a mean of $13.8 \pm 0.14$ days. Additionally, turtles in this study also showed

high site fidelity described by Hirth (1971). This was evidenced in one monitored turtle who was observed digging up her previous nest in order to lay another in its place. All of the incubation periods, temperatures and average rainfall were similar throughout the nesting season except for a high rain event at the end of May. Within a 24-hour period Tern Island received over 3 inches of rain and, on average, incubation times for the nests laid fifteen days before and for several days after the rain event were slightly longer (Table 2.6 and Figure 2.3). This observation follows other studies postulating that changes in environmental conditions can affect length of incubation (Hirth, 1971; Hirth, 1980; Miller, 1985; Packard and Packard, 1988; Ji and Braña, 1999).

In general, for turtles the source of variation in clutch size remains unexplained (Hays and Speakman, 1991). There could be high variability within a population and within an individual because seasonal and annual changes in the environment may affect their reproductive effort (Gibbons et al., 1982). For example, mean clutch size within a turtle species tends to be consistent across the year; however, individuals may show a high variability in individual clutch sizes (Gibbons et al., 1982; Ji and Braña, 1999). The average clutch sizes in this study showed a high variability (overall sample variance mean = 573 eggs). Gradual reduction in the clutch size during multiple nests laid throughout a nesting season has been suggested (Gibbons et al., 1982). However, the results of this study did not exactly show this pattern (Table 2.6 & Figure 2-5). The clutch sizes did decrease by clutch six; but, with the large sample variation and small sample size for clutch six, it is difficult to determine the pattern. On the other hand, there was a significant difference in clutch size between turtles with FP and turtles without FP; FP turtles tended to have smaller clutches ($p = 0.07$) than non-FP turtles. The FP nest

success also tended to be less than non-FP turtles, but not significantly different ($p = 0.2$). Additionally, both treatments of turtles fell within the ranges of previous studies (Niethammer et al., 1997; Hirth, 1971; Balazs, 1980)

*Chelonia mydas* body size and weight were not significantly correlated (Elgar and Heaphy, 1989). The turtles appeared to not show differences between the initial weights at the beginning of the season and the weight lost during the nesting, but the sample size in this study was too small to conduct any statistics (Fig. 2.6).

FFS is one of the most northern green turtle nesting colonies in the world (Hirth, 1971) and the only main nesting colony for the Hawaiian green turtle. Even though the numbers of Hawaiian green turtles has been increasing since being listed under the Endangered Species Act, the occurrence of FP is also increasing. Therefore, it is important to know how FP may be affecting the overall health of the population. The results of this study show that the reproductive success of turtles with FP is somewhat impaired due to the lower clutch size and slightly reduced hatch success. Turtles that are heavily tumored (having a tumor score greater than 2) have not been observed nesting at FFS. Perhaps, as Work et. al. (2001) postulates, turtles are immunosuppressed at a certain overall tumor score, the overall reproductive success is less than turtles without FP.

## TABLES

Table 2.1. Morphometrics of green turtles nesting at FFS. Variables are curved carapace length (CCL), curved carapace width (CCW), head length (HL), head width (HW), head girth (HG), and weight (Wt.).

| (cm) | All<br>(n) mean ± SE<br>range | Non-FP<br>(n) mean ± SE<br>range | FP<br>(n) mean ± SE<br>range |
|---|---|---|---|
| CCL | (44) 97.6 ± 0.6<br>88–104 | (28) 97.2 ± 0.8<br>88–105 | (15) 98.4 ± 1.1<br>88.2–105 |
| CCW | (38) 90.9 ± 0.7<br>82–102 | (25) 90.8 ± 0.9<br>82–102 | (13) 91.1 ± 1.0<br>87–100 |
| HL | (37) 17.4 ± 0.3<br>13–21 | (24) 17.4 ± 0.4<br>14–20 | (13) 17.6 ± 0.6<br>13–21 |
| HW | (38) 13.0 ± 0.8<br>12–15 | (25) 12.9 ± 0.1<br>12–14 | (13) 13.1 ± 0.2<br>12–15 |
| HG | (38) 43.8 ± 0.8<br>41–49 | (25) 44.6 ± 0.3<br>42– 48 | (13) 42.3 ± 2.2<br>41– 49 |
| Wt.(kg) | (64) 102.6 ± 2.1<br>61–139 | (44) 101.1 ± 2.6<br>61–136 | (20) 105.8 ± 3.6,<br>74–139 |

Table 2.2. Nesting information of green turtles nesting at FFS. Variables are clutch size (CS), hatch success (HS), incubation (Incub.), nesting interval (Nesting Int.), and nesting date. (** p = 0.07, * p = 0.2.)

| | All<br>(n) mean ± SE<br>range | Non-FP<br>(n) mean ± SE<br>range | FP<br>(n) mean ± SE<br>range |
|---|---|---|---|
| CS (# eggs)** | (97) 89.7 ± 2.6<br>17–143 | (71) 92.9 ± 2.8<br>17–143 | (26) 80.9 ± 5.7<br>24–138 |
| HS (% hatched)* | (97) 80.6 ± 2.1<br>2–100 | (71) 82.2 ± 2.3<br>2–100 | (26) 76.3 ± 4.9<br>4–100 |
| Incub.(days) | (73) 67.0 ± 1.25<br>54–102 | (53) 66.8 ± 1.4<br>54–102 | (19) 68 ± 2.7<br>55–99 |
| Nesting Int. | (151)13.8 ± 0.14<br>10–20 | (105)13.8 ± 0.16<br>10–20 | (46)13.9 ± 0.3<br>11–20 |
| Nesting Date | (113) 10 Jul 99 ± 2 | (80) 7 Jul 99 ± 3 | (33) 13 Jul 99 ± 4 |

Table 2-3. Percentage of the number of nests laid and average number of nests laid throughout the season. ($p>0.05$)

| # of Nests Laid | % Overall n = 43 | % non-FP n = 28 | % FP n = 15 |
|---|---|---|---|
| 1 Nest | 27.9 | 25.0 | 33.3 |
| 2 Nests | 16.3 | 10.7 | 26.7 |
| 3 Nests | 18.6 | 21.4 | 13.3 |
| 4 Nests | 23.3 | 25.0 | 20.0 |
| 5 Nests | 9.3 | 10.7 | 6.7 |
| 6 Nests | 4.7 | 7.1 | 0.0 |
| Average # Laid | 2.8 ± 3.5 | 3.1 ± 3.3 | 2.4 ± 5.1 |

Table 2.4. Average rainfall and temperature from 13 May – 30 November, 1999 at FFS.

| Month | n | Mean Rainfall/Day (cm) ± SE | Mean Max. Temp. (°C) ± SE | Mean Min. Temp. (°C) ± SE |
|---|---|---|---|---|
| May | 18 | 0.60 ± 0.5 | 26 ± 0.3 | 22 ± 0.3 |
| June | 29 | 0.23 ± 0.1 | 27 ± 0.2 | 23 ± 0.2 |
| July | 30 | 0.13 ± 0.02 | 28 ± 0.1 | 24 ± 0.2 |
| August | 31 | 0.14 ± 0.05 | 29 ± 0.1 | 23 ± 0.1 |
| September | 30 | 0.09 ± 0.04 | 29 ± 0.1 | 24 ± 0.1 |
| October | 31 | 0.26 ± 0.1 | 28 ± 0.2 | 23 ± 0.2 |
| November | 30 | 0.17 ± 0.07 | 27 ± 0.1 | 23 ± 0.2 |
| Overall Mean | 199 | 0.21 ± 0.05 | 28 ± 0.1 | 23 ± 0.1 |
| | Range | 0 – 8.7 | 23 – 30 | 18 – 26 |

Table 2.5. Mean incubation period for green turtles, rainfall and temperatures by month at FFS.

| Month Eggs Were Laid | n | Average Incubation Period (days) inches ± SE | Average Rainfall (cm) ± SE | Max. Temp. (°C) ± SE | Min. Temp. (°C) ± SE |
|---|---|---|---|---|---|
| May | 7 | 82 ± 7 (3) | 0.26 ± 0.005 | 28 ± 0.03 | 24 ± 0.01 |
| June | 28 | 66 ± 1.5 (15) | 0.13 ± 0.002 | 28 ± 0.03 | 23 ± 0.01 |
| July | 42 | 67 ± 2 (33) | 0.13 ± 0.002 | 28 ± 0.02 | 24 ± 0.01 |
| August & September 1 | 26 | 66 ± 2 (22) | 0.16 ± 0.01 | 28 ± 0.1 | 23 ± 0.02 |

Table 2.6. Mean clutch sizes and nest successes of both FP and non-FP nesting green turtles clutches 1 – 6 (± SE).

| Clutch Number | n | Clutch Size | Nest Success |
|---|---|---|---|
| 1 | 26 | 90.7 ± 5.1 | 76.5 ± 4.2 |
| 2 | 26 | 90.3 ± 5.8 | 81.9 ± 4.4 |
| 3 | 21 | 90.9 ± 4.6 | 85.5 ± 4.6 |
| 4 | 15 | 85.5 ± 6.6 | 83.4 ± 2.9 |
| 5 | 6 | 93.0 ±12.2 | 70.9 ± 15.4 |
| 6 | 2 | 72.0 ± 24.0 | 46.5 ± 3.5 |

## FIGURES

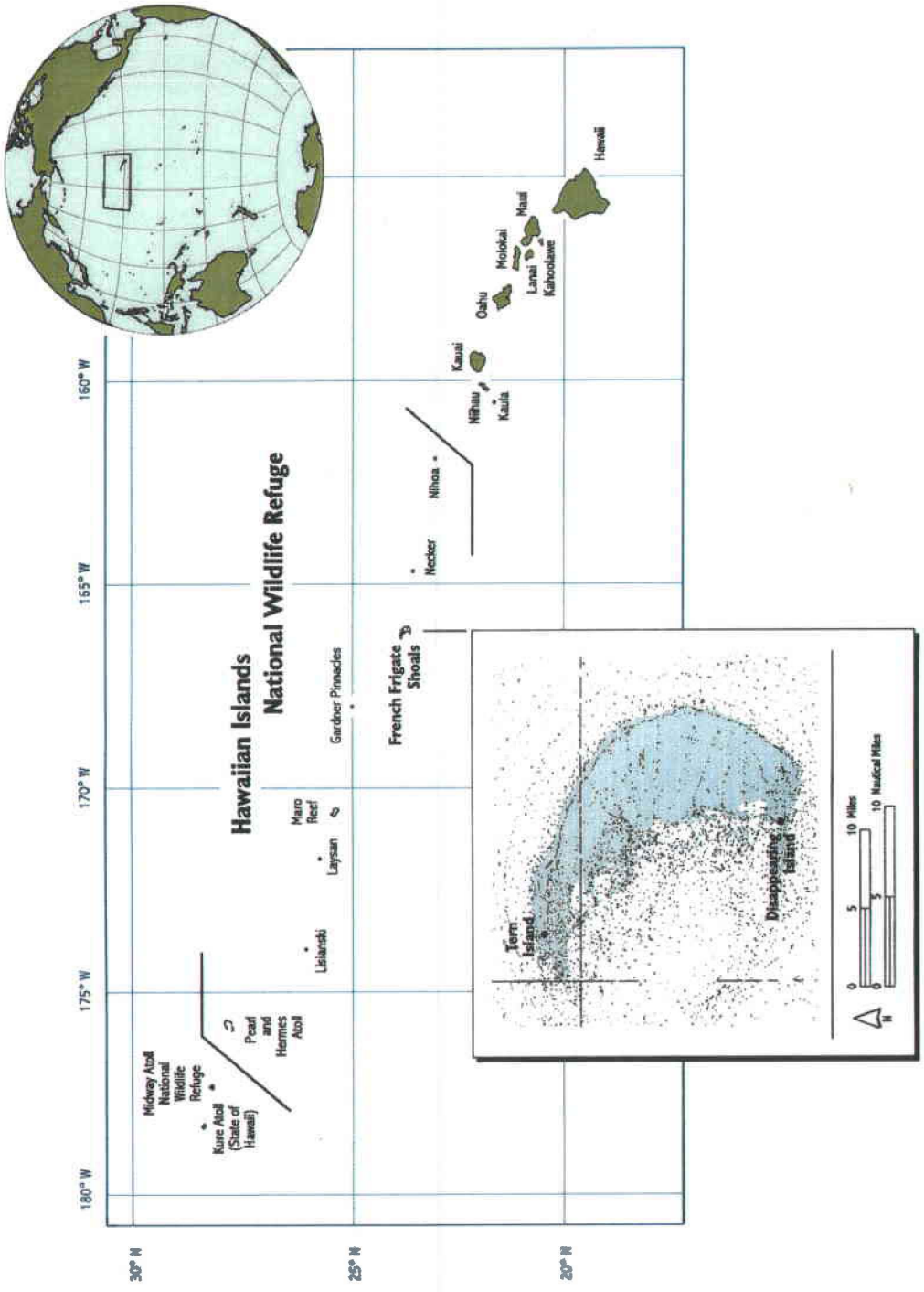


**Figure 2.1. Location of French Frigate Shoals, Hawaiian Islands National Wildlife Refuge (Courtesy of Chris Swenson, USFWS).**

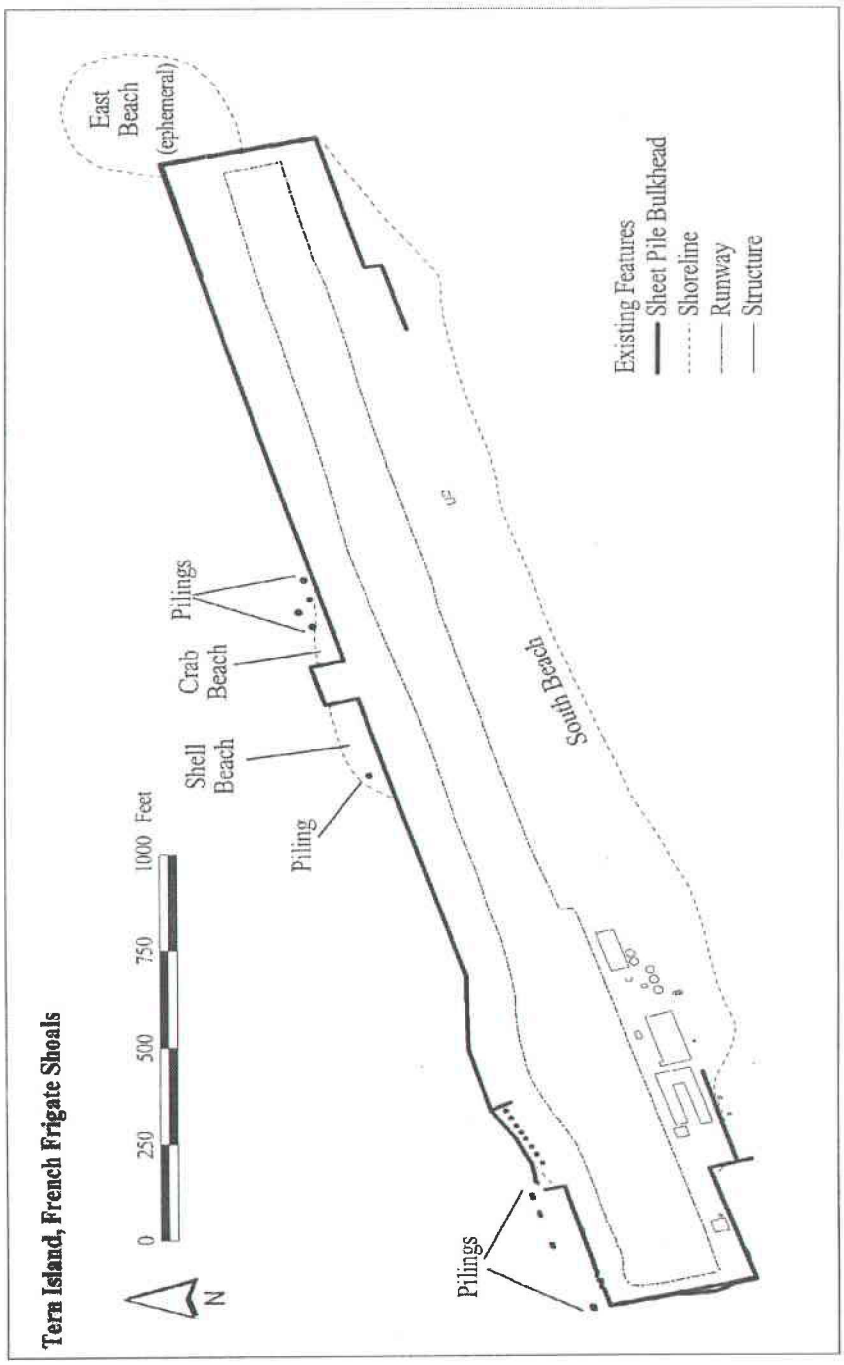


Figure 2.2. Map of Tern Island (Courtesy of Chris Swenson, USFWS).

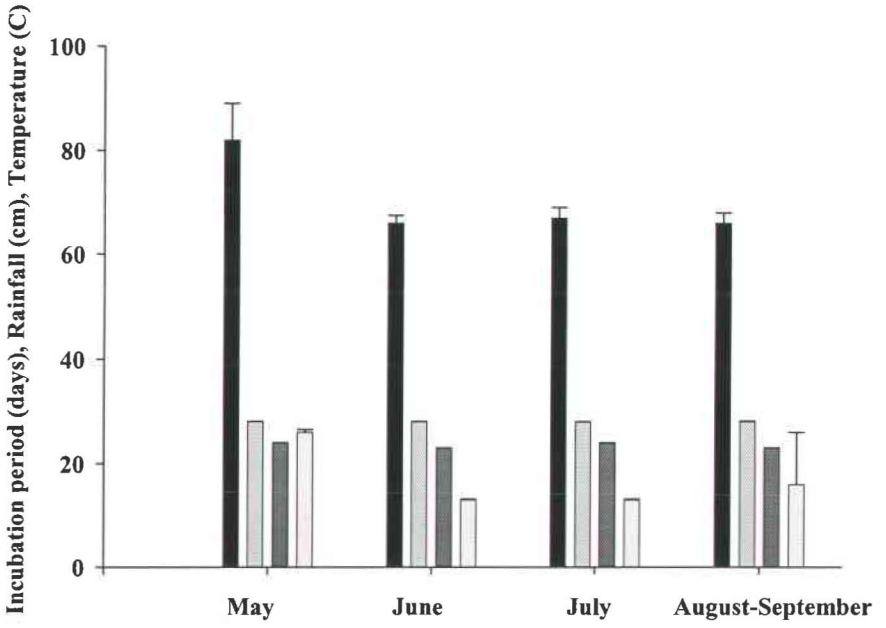


Figure 2.3. Average incubation period (black bars), maximum (gray bars) and minimum (dark gray bars) temperature (°C) per day and average rainfall (cm*100) light gray bars) for the nests during the end of May and June, July, August and September (± SE).

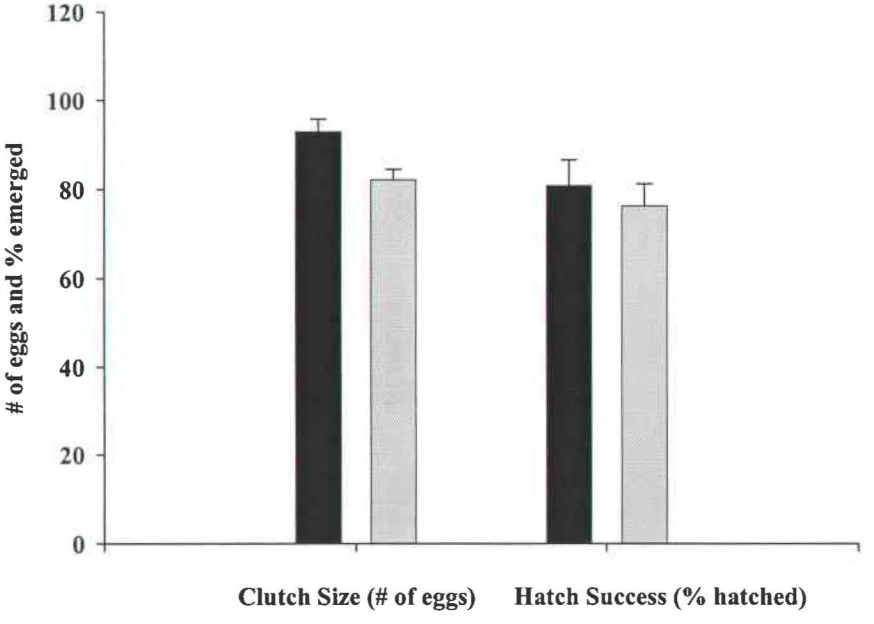


Figure 2.4. Overall average clutch size and nest success of turtles nesting at Tern Island (± SE). Black bars are non-FP and gray bars are FP.

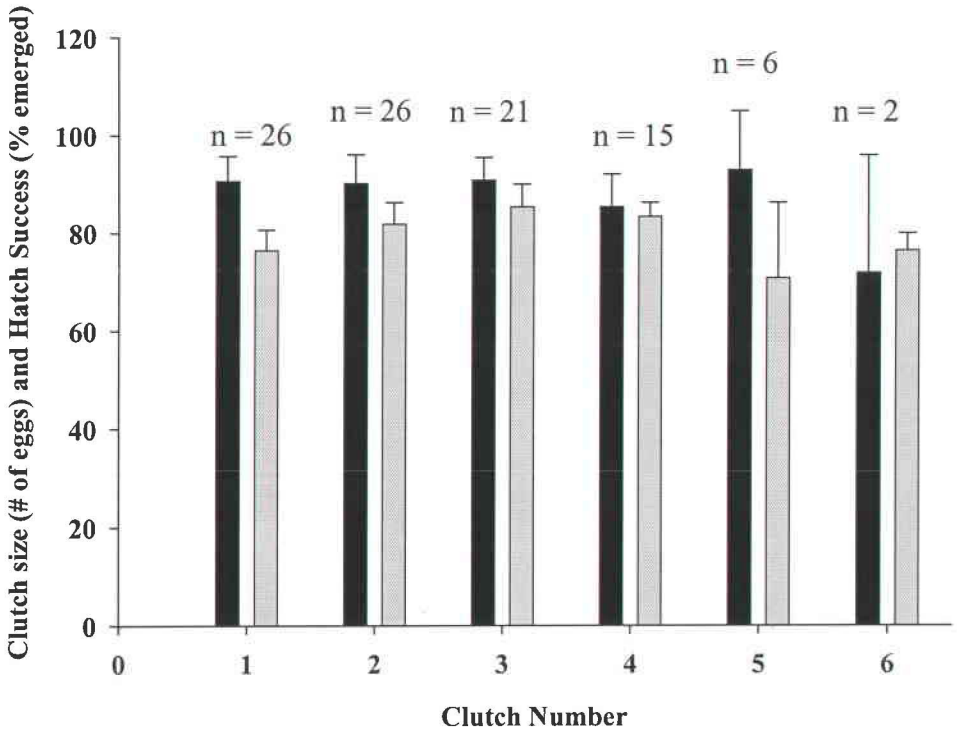


Figure 2.5. Average clutch sizes and nest successes of all nesting green turtles clutches 1 – 6 (± SE). Black bars are clutch size and gray bars are nest success.

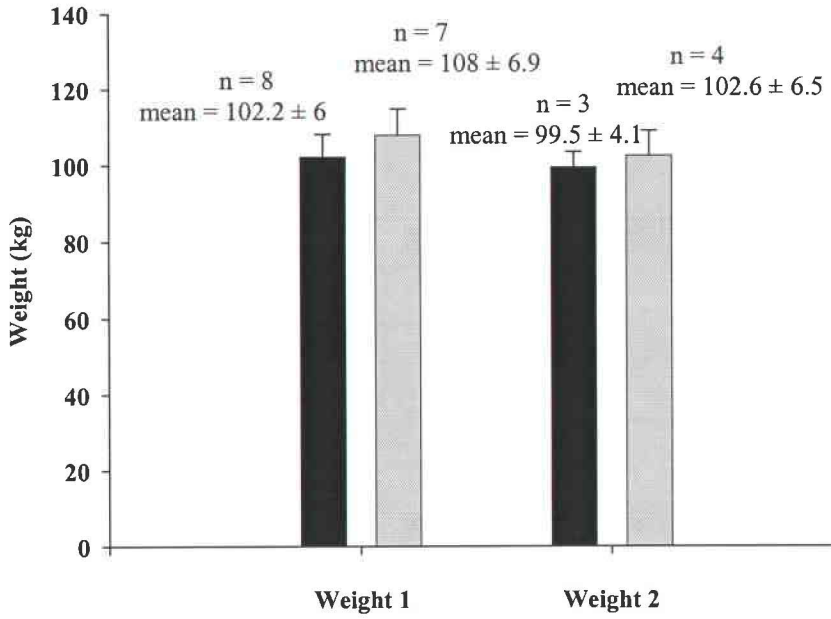


Figure 2.6. Initial weights (Weight 1) and weights after third nest laid (Weight 2) by green turtles. Black bars are non-FP and gray bars are FP.

# CHAPTER THREE
# IMPACT OF TURTLE FIBROPAPILLOMATOSIS ON EGG PRODUCTION AND COMPOSITION BY GREEN TURTLES (*CHELONIA MYDAS*) AT FRENCH FRIGATE SHOALS, HAWAIIAN ISLANDS NATIONAL WILDLIFE REFUGE

## ABSTRACT

Nesting activity of green turtles was monitored during the 1999 season on Tern Island, French Frigate Shoals Hawaiian Islands National Wildlife Refuge to determine the potential effects of fibropapillomatosis (FP) on reproductive success and egg composition. Of a total of 157 nesting turtles, 24 were observed to have FP. Throughout the season, 8 turtles without FP and 7 with FP were monitored; and all nests laid per individual were recorded, marked, and mapped. Clutch size (number of eggs) and hatch success (percent) were not significantly different between turtles with and without FP. Average egg weight (g) was greater ($p < 0.01$) but egg volume (ml) was the same for turtles without FP and with FP. Crude fat was significantly less in eggs from turtles without FP than with FP ($p < 0.05$). Percent moisture content, ash and protein were similar for turtles with and without FP, respectively. Percent carbohydrate (CHO), estimated by difference and gross energy (GE) calculated based on nutrient composition, were not significantly different for turtles with and without FP. We suspect FP turtles place more energy into individual eggs versus non-FP as compensation for decreased clutch size and hatch success

# INTRODUCTION

Hawaiian green turtles (*Chelonia mydas*) are a distinct and unique genetic haplotype (Bowen et al., 1992) and are present throughout the entire island chain from Hawai'i to Kure Atoll (Balazs, 1976). More than 90% of all Hawaiian green turtles nest on the remote atoll French Frigate Shoals (FFS) located at 23°45'N X 166°10'W and which is managed as part of the Hawaiian Islands National Wildlife Refuge by the U.S. Fish and Wildlife Service. Under the Endangered Species Act, green turtles are listed as threatened except for Florida and the Pacific coast of Mexico where they are endangered. Additionally, an epizootic disease called turtle fibropapilloma (FP) affects marine turtles worldwide (Herbst and Klein, 1995; Quackenbush et al., 1998; Balazs, 1991; Herbst, 1994).

Fibropapilloma (FP) is a neoplastic condition consisting of three main lesions; fibromas, cutaneous papillomas and fibropapillomas (Herbst, 1994; Balazs, 1991). On an individual turtle there may be a single or many lesions that range from a size of 0.1 cm to greater than 30 cm (Aguirre et al., 1998; Herbst and Klein, 1995; Herbst, 1994). The lesions occur on the flippers, base of the tail, neck, chin, eyes, inguinal, axillary regions and, in Hawai'i, the glottis (Herbst, 1994). By 1991, populations of turtles within the main Hawaiian Islands had records indicating up to 92% of green turtles exhibited FP, depending on time, method and area sampled (Balazs, 1991). Turtles with FP in Hawai'i and Florida also had herpesviral DNA in tumor tissues, while turtles without tumors had no viral DNA (Aguirre et al., 1998; Quackenbush et al., 1998). The viral etiology is still unproven; however, fibropapillomas are closely associated herpesvirus (Herbst and

Klein, 1995; Quackenbush et al., 1998) in green and loggerhead turtles (*Caretta caretta*) (Lackovich et al., 1999). Additionally, turtles with FP are immunosuppressed (Aguirre et al., 1995; Work et al., 2000; Work et al., 2001). Work et. al. (2001) showed that turtles with more advanced and numerous tumors had significantly impaired cell mediated immune status.

Maternal investment in reptiles can be divided into two categories, 1) as energy invested in embryogenesis (Congdon, 1989) and 2) producing excess yolk to ensure that there is enough material needed by the offspring to reach independence (Congdon and Gibbons, 1985). Additionally, there is no parental care by turtles; eggs represent the total reproductive investment (Congdon et al., 1983b), and turtles must do all parental investment prior to oviposition (Marlen and Fischer, 1999). Most reptiles maximize their lifetime reproductive output by laying many eggs and never returning to them (Hays and Speakman, 1991). Turtle eggs provide the energy and material for the development and maintenance of the embryo and for growth of the hatchling until independence (Congdon and Gibbons, 1990). The two major features of reptile eggs are a highly developed calcareous shell and a large proportion of the egg devoted to the yolk (Congdon and Gibbons, 1990). *C. mydas* eggs consist of 4.3% shell, 67.3% yolk and 28.4% albumen (Miller, 1985). High lipid reserves in the egg yolk supply the offspring with enough energy to ensure survival after they leave the egg (Marlen and Fischer, 1999).

If tumors impact the immune system, there may be impaired metabolism and reduced reproduction. The objectives of this study were to determine if 1) maternal energy resources decline from the beginning to the end of the nesting season and 2) the size, volume, and nutrient composition of the eggs and energy expenditures during reproduction differed in turtles with and without FP.

## METHODS

Eight non-FP and seven FP turtles at French Frigate Shoals were followed throughout the entire 1999 nesting season. Turtles were captured before the first nesting and again after the third nesting. At the time of first capture, the turtles received a thorough tumor check using tumor score classification (Work and Balazs, 1999). The turtles were weighed (kg); and the straight carapace length, curved carapace length, straight and curved carapace width on the $6^{th}$ scute, and head width and girth (cm) measured. Before the turtles were released, Passive Integrated Transponder (PIT) tags were inserted subcutaneously into the dorsal side of both hind flippers. At the time of egg-laying, three eggs from each clutch were collected and frozen. Additionally, each nest laid by turtles was marked by a piece of flagging tape with the nest number and turtle identification number written on it and dropped within the nest. A ball (nest number & identification number written on it) attached to a long piece of wire was placed within the egg chamber at the time of laying. A stake was placed one meter inland from nest marker ball and mapped in a field notebook. Nests were excavated three days after signs of hatching or on the $102^{nd}$ day after the nest laid (Niethammer et al., 1997).

Eggs were dried individually in a forced air oven at 55° C until the samples had reached a constant weight. Because of the high lipid content of the eggs after drying, they were frozen, individually homogenized with liquid nitrogen, and stored at -80° C. Eggs were analyzed for moisture, ash, crude protein, and crude fat (soxhlet) (AOAC, 1990). Crude protein analysis was done by Agricultural Diagnostic Services Center at the University of Hawaii, Honolulu, HI using automated combustion methods (Horneck and Miller, 1998). Carbohydrates (CHO) were estimated by difference where:

CHO(%) = 100% dry matter (DM) – (% crude protein + % crude fat + % ash) (Pond et al., 1995)

Gross energy (GE) of eggs was calculated using the following:

GE (kcal $gDM^{-1}$) = (% crude protein * 4.5) + (% crude fat * 9.5) + (% CHO * 3.75) values for eggs from Atwater system (Council, 1989).

Duplicate analyses were done on each subsample. Energy expenditure was calculated by:

1) Individual turtle's energy expenditure/nest = Clutch size * avg. wt. eggs * avg. calc. GE of those eggs.

2) Entire season energy expenditure = sum of all of the individual turtle's energy expenditure/nest.

3) Total kilocalories expended/kg of body weight = (Entire season energy expenditure/body weight)/1000.

General Linear Methods (Proc GLM) and least squares mean (SAS version 8, 1999) were used to compare overall differences between FP and non-FP turtles and differences between nests laid.

## RESULTS

The mean weight of turtles in this study was 102.9 ± 2.9 (± SE) kg with a curved carapace length of 97.0 ± 1.0 cm. There was no significant difference between the clutch size and hatch success between non-FP and FP turtles, although the FP turtles tended to lay fewer eggs and have a lower hatch success. Turtles without FP laid a mean of 98.3 ± 5.8 (SE) eggs with a mean hatch success of 85.4 ± 2.5% and turtles with FP laid a mean 88.6 ± 5.4 eggs with a mean hatch success of 82.3 ± 3.9%. The mean clutch size for an individual adult did not vary over all clutches laid in the season (Figure 3.1). Five of the seven FP turtles had a tumor score of 1 (Table 3.1).

Egg weight and volume were highly correlated in this study ($r = 0.916$, $r^2 = 0.839$) (Figure 3.2). Mean weight of eggs from FP turtles was significantly greater than that of non-FP eggs ($p<0.01$) (Table 3.2). The non-FP turtles had a lower overall mean egg weight for the first nest, 47.0g ± 0.6g, versus 54.4 ± 0.8g for FP eggs (Figure 3.3).

There was a curvilinear trend where the first and fifth nests had a lower percent of fat than the second, third and fourth nests (Figure 3.4). The mean percent crude fat in non-FP eggs was 27.8% and 28.5% in FP eggs (Table. 3.2, $p<0.05$). Eggs from FP turtles were consistently higher in fat than eggs from non-FP turtles however, showing a significant difference only in the third clutch laid (Figure 3.4). The results of the rest of the composition analysis followed the same curvilinear trend throughout all of the nests laid by both non-FP and FP turtles (Figures 3.5 through 3.8). Although most of the results are not significant, the trend for treatment means and the changes in composition with increasing clutch number are very similar. Additionally, the total energy expended

throughout the nesting season was not significantly different between non-FP and FP turtles with approximately 786 kcal/kg of body weight (Figure 3.11). There was no significant difference between non-FP and FP turtles in kcal/kg expended throughout all of the clutches combined (Figure 3.12).

## DISCUSSION

There is no parental care by turtles and thus, the eggs are representative of the total reproductive investment (Congdon et al., 1983b). Reproductive effort has been defined as the proportion of an animal's resource budget that will be allocated to reproduction, and parental investment as the relative amount of energy allocated to each offspring (Marlen and Fischer, 1999). Turtles do all parental investment prior to oviposition (Marlen and Fischer, 1999). Additionally, for animals that lay multiple clutches throughout the nesting season there may be an increased risk (*e.g.*, predation) to the adult female during the reproductive period (Frazer and Richardson, 1986). For example, tiger sharks are present in the FFS area due to the albatross fledging period and monk seal pupping. Furthermore, among individuals that produce more than one offspring per reproductive event, the clutch size and frequency of nesting are controlled by environmental factors such as available resources and utilization, age of the individual and changes in body size (Gibbons et al., 1982; Congdon, 1989). Each turtle nesting may produce the minimum number of hatchlings needed to emerge from the nest, but still be able to have an increased potential for greater nest success (Frazer and Richardson, 1986).

Egg diameter was not a factor in reproductive output in loggerhead turtles (Tiwari and Bjorndal, 2000), and for Hawaiian green turtles in this study, egg diameter also seems not to be a factor. Egg diameters seen here were similar to those of other studies (Carr and Hirth, 1962; Bjorndal, 1982). In this study there appeared to be a trend toward a lower clutch size for turtles with FP (non-FP 98 ± 5.8 eggs (n = 23), FP 88.9 ± 5.7 eggs (n = 19)) and a trend toward a slightly reduced hatch success (non-FP 85 ± 2.7%, FP 82 ± 4.0%). Conversely, the turtles with FP had significantly greater egg weights and crude fat content than the turtles without FP (Table 3.2). In Costa Rica, egg weights were lower in the first clutch and then increased in subsequent clutches until a decrease in the last nests (Bjorndal and Carr, 1989). The non-FP turtles followed the pattern described above, but the FP turtles followed a different pattern and had heavier eggs in the first clutch followed by a decrease in subsequent clutches. Taking into account the total nest mass (mean clutch size * mean weight of egg * mean hatch success) for non-FP and FP nests, the results are 439.6 ± 30.5kg and 398.1 ± 35.6kg, respectively. While there is not a significant difference, there appears to be a trend that turtles with FP possess a decreased reproductive output.

Does increased fat account for all of the increase in egg weights in FP clutches? There are poor correlations between egg weight versus clutch size ($r = 0.2$, $r^2 = 0.04$) (Figure 3.9) and egg weight versus percent crude fat ($r = 0.19$, $r^2 = 0.04$) (Figure 3.10). The amount of energy expended per nest in egg production is not significantly different between non-FP and FP turtles (Table 3.3). Both FP and non-FP turtles showed a decline in energy expended from the first nest to the last nest.

Previous studies of ovipositioned eggs of *C. mydas* have reported nutrient content as 16.5% protein, 11.6% fat, 0% fiber and 1.87% ash on a wet matter basis (Miller, 1985; Penyapol, 1958). The results of our study were similar when results were converted to a wet matter basis with approximately 12.9% protein, 7 % fat, 3% carbohydrates, and 3% ash. In this study, the egg shells were homogenized with the entire egg, which would account for this study's higher mineral content (*sic* ash). The mean energy content of green turtle eggs was 259.7 kJ/egg (Bjorndal, 1982). The calculated mean energy content of eggs was 332.8 kJ/egg (with egg shell) in this study.

Turtles with FP laid eggs with a significantly greater percent of fat than turtles without FP. The eggs of FP turtles showed the same trend, but were, on average, higher in fats than those of non-FP turtles, but only with a significant difference in the third clutch (Figure 3.4). These results are similar to other studies of reptiles where it has been noted that the concentration of lipids is less in the first clutch laid and then higher in the subsequent clutches (Ji and Braña, 1999). There are high lipid reserves in the egg yolk to supply the offspring with enough energy to ensure survival after they leave the egg (Marlen and Fischer, 1999; Nagle et al., 1998). There has to be enough lipids primarily, triacylgycerols, which are stored lipids to ensure the success of the hatchling (Rowe et al., 1995).

There appeared to be no significant difference between the total energy expended by non-FP and FP turtles using the initial weight of the turtle for the entire nesting season (Figure 3.11). However, there may be a slight trend when looking at the individual clutches laid (Figure 3.12). There appeared to be slightly lower energy expended per clutch by FP turtles ($p>0.5$). Overall, the non-FP and FP turtles are expending

approximately the same amount of energy and are losing approximately the same amount of weight throughout the entire nesting season.

Perhaps FP turtles are putting more energy into each individual egg and therefore reducing the number of eggs they lay in order to maximize their reproductive effort. Previously, it has been speculated that debilitated turtles are less likely to nest (Limpus and Miller, 1994). According to these data, turtles with mild cases of FP do not have a significantly different reproductive output than healthy turtles. However, the turtles with FP that nested on Tern Island were turtles that had a low overall tumor score. We observed several heavily tumored turtles on Tern Island, but we did not observe them nesting. Evidently, a healthy turtle lays, on average, 98 eggs/clutch while a lightly tumored turtle lays, on average, 88 eggs/clutch and a heavily tumored turtle, which may be immunosuppressed, does not have the energy with which to reproduce.

This study shows that turtles with FP, if the tumor score is low and have an overall lower reproductive effort than non-FP, are not energeticially impaired during the reproductive season. Throughout the years of research conducted on green turtles at FFS, very few turtles with an overall tumor score of three have been observed there. The majority of turtles observed nesting at FFS with FP have an overall tumor score of one. Balazs & Work (pers. com.) have hypothesized that since there have been very few turtles with an overall tumor score of three observed at FFS, afflicted turtles may not have the ability to undertake/or survive the migration to the breeding area to mate and nest. The main implication of this hypothesis is that while there is a trend for tumored turtles to exhibit lower reproduction, the etiology of FP still needs to be determined.

## TABLES

Table 3.1. Scores of individual tumors and overall tumor scores of individual turtles with turtle fibropapilloma nesting at French Frigate Shoals (n=7).

| | Tumor size | | | | |
|---|---|---|---|---|---|
| | < 1cm | 1-4 cm | > 4 cm – 10 cm | > 10 cm | Overall Score |
| Turtle 1 | 3 | 1 | 0 | 0 | 1 |
| Turtle 2 | 1 | 0 | 0 | 0 | 1 |
| Turtle 3 | 3 | 8 | 2 | 1 | 2 |
| Turtle 4 | 2 | 1 | 1 | 1 | 2 |
| Turtle 5 | 3 | 0 | 0 | 0 | 1 |
| Turtle 6 | 0 | 1 | 0 | 0 | 1 |
| Turtle 7 | 0 | 1 | 0 | 0 | 1 |

Table 3.2. Average egg weight, volume, moisture content, ash, crude fat, and crude protein on a dry matter basis of eggs laid by Hawaiian green turtles with and without turtle fibropapilloma[cd].

| Variables | Non-FP n = 68 | FP n = 66 |
|---|---|---|
| Egg Weight (g) | 51.4 ± 0.57[x] | 53.3 ± 0.41[y] |
| Egg Volume (ml) | 55.8 ± 0.52 | 56.7 ± 0.43 |
| Egg Diameter (cm) | 4.7 ± 0.02 | 4.7 ± 0.02 |
| Moisture Content (%) | 75.0 ± 0.19 | 74.8 ± 0.43 |
| Ash (%) | 11.9 ± 0.11 | 11.8 ± 0.12 |
| Crude Fat (%) | 27.8 ± 0.2[a] | 28.5 ± 0.22[b] |
| Crude Protein (%) | 51.4 ± 0.38 | 50.9 ± 0.27 |
| Carbohydrates (%) | 11.8 ± 0.44 | 10.6 ± 0.48 |
| Gross Energy (kcal/g) | 5.38 ± 0.03 | 5.40 ± 0.02 |

[c] Values are given as the mean ± SE.
[ab] p-value ($p < 0.05$).
[xy] p-value ($p < 0.01$).
[d] Carbohydrates and gross energy were calculated.

Table 3.3. Average kcal/kg (mean ± SE) used by green turtles with and without turtle fibropapilloma for individual clutches laid in egg production.

| Clutch | non-FP (n) | FP (n) |
|---|---|---|
| 1 | 27.8 ± 2.8 (8) | 26.8 ± 2.8 (7) |
| 2 | 28.8 ± 4.2 (7) | 26.8 ± 3.1 (7) |
| 3 | 28.0 ± 2.7 (5) | 24.0 ± 1.5 (5) |
| 4 & 5 | 22.1 ± 4.7 (3) | 23.3 ± 7.5 (3) |
| Overall Avg. | 786 ± 146 (8) | 787 ± 82 (7) |

## FIGURES

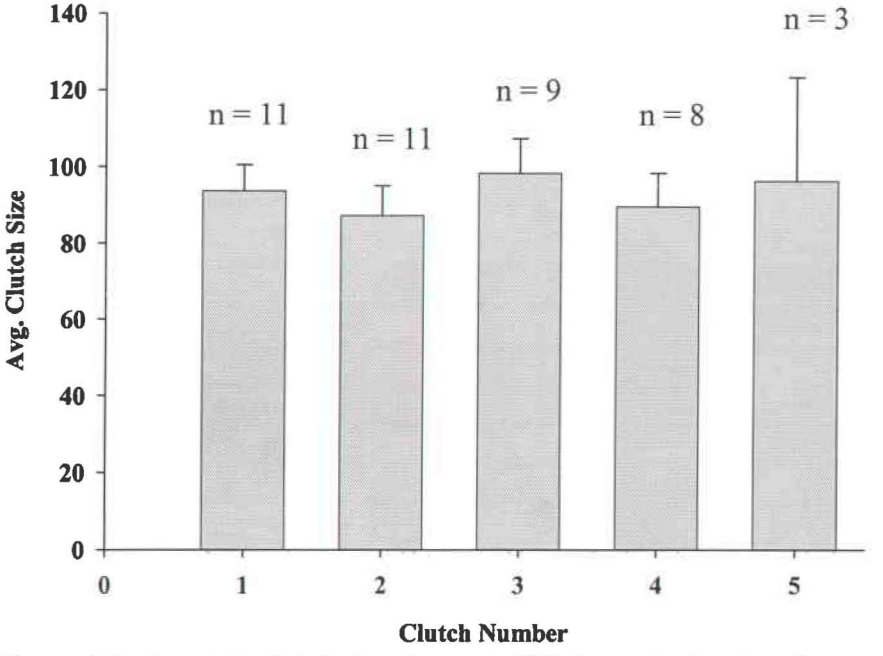


Figure 3.1. Average clutch size (mean ± SE) through clutches from green turtles nesting at French Frigate Shoals.

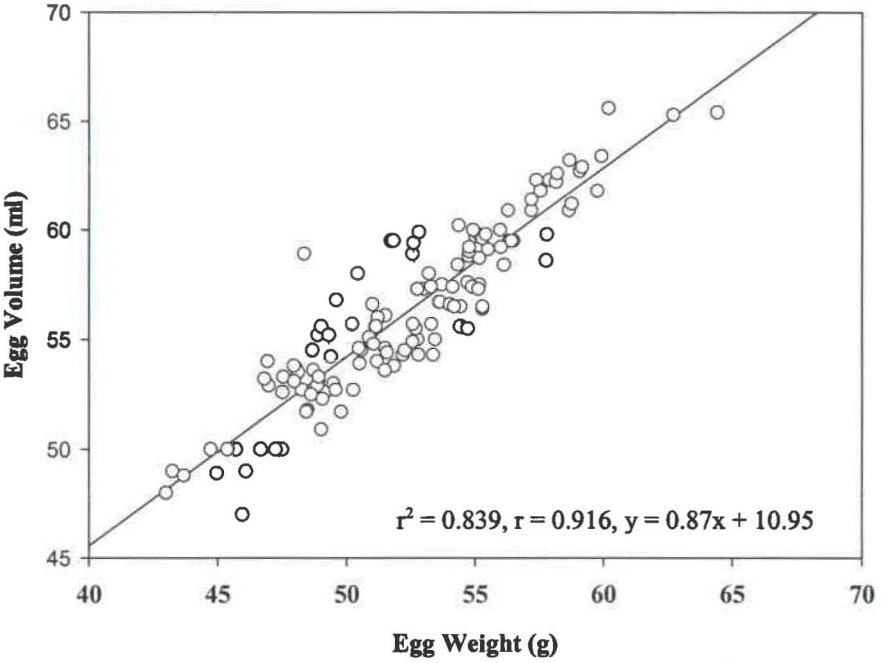


Fig. 3.2. Correlation of egg weights versus egg volumes from green turtles with and without turtle fibropapilloma nesting at French Frigate Shoals.

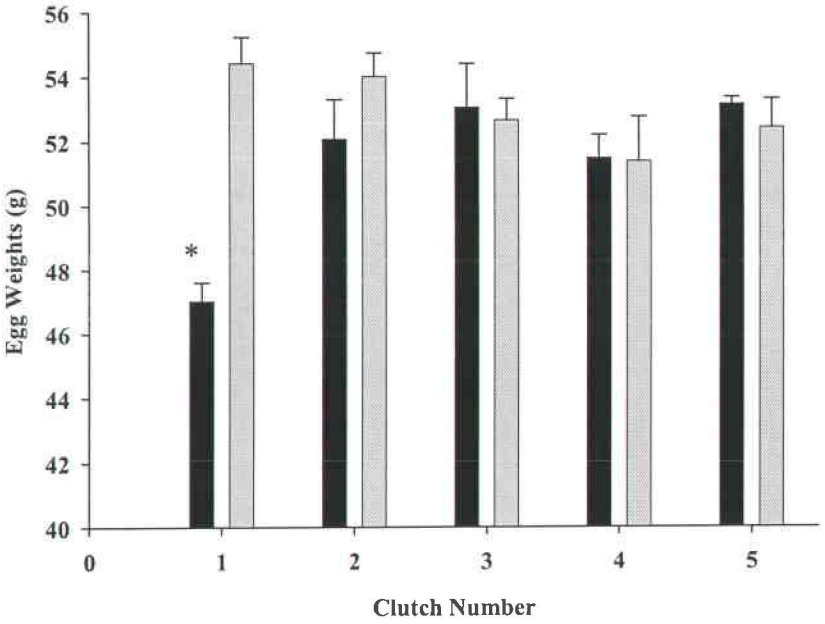


Figure 3.3. Egg weights (g) (mean ± SE) of eggs from nesting green turtles with and without FP. Black bars are non-FP and gray bars are FP (Fig. 3-3 through Fig. 3-8 have the same sample sizes; Clutch 1 non-FP n = 15, FP n = 18, Clutch 2 non-FP n = 20, FP n = 18, Clutch 3 non-FP n = 15, FP n = 18, Clutch 4 non-FP n = 12, FP n = 9, Clutch 5 n = 6, FP n = 3) (* $p = 0.06$).

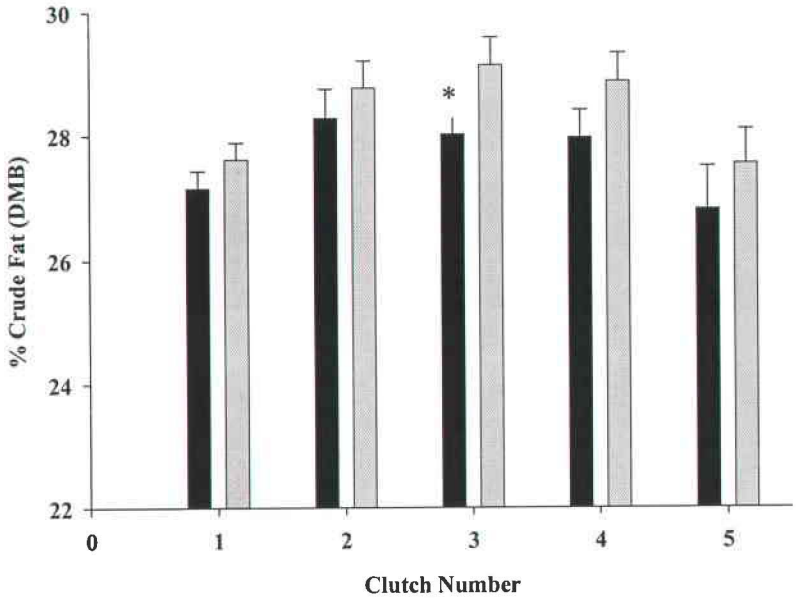


Figure 3.4. Percent Crude fat (mean ± SE) of eggs from nesting green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP (* $p < 0.01$).

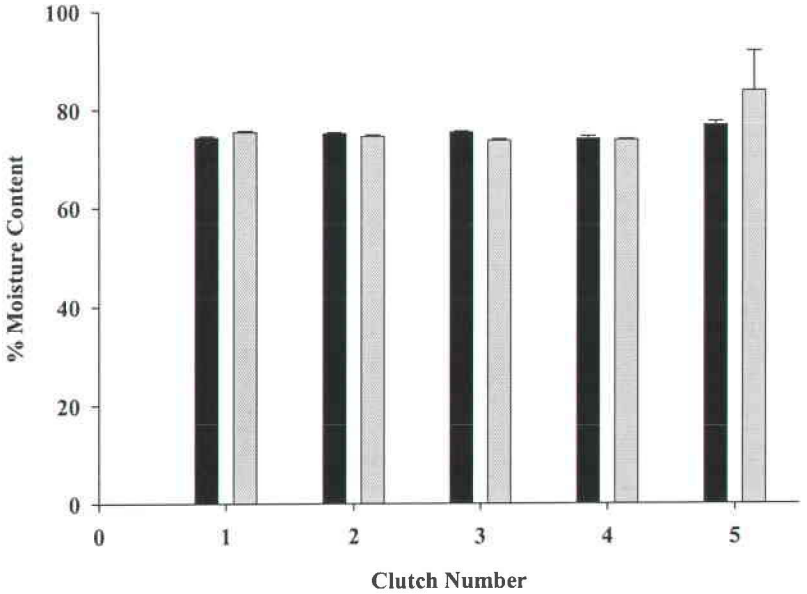


Figure 3.5. Percent moisture content (mean ± SE) of eggs from nesting green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP.

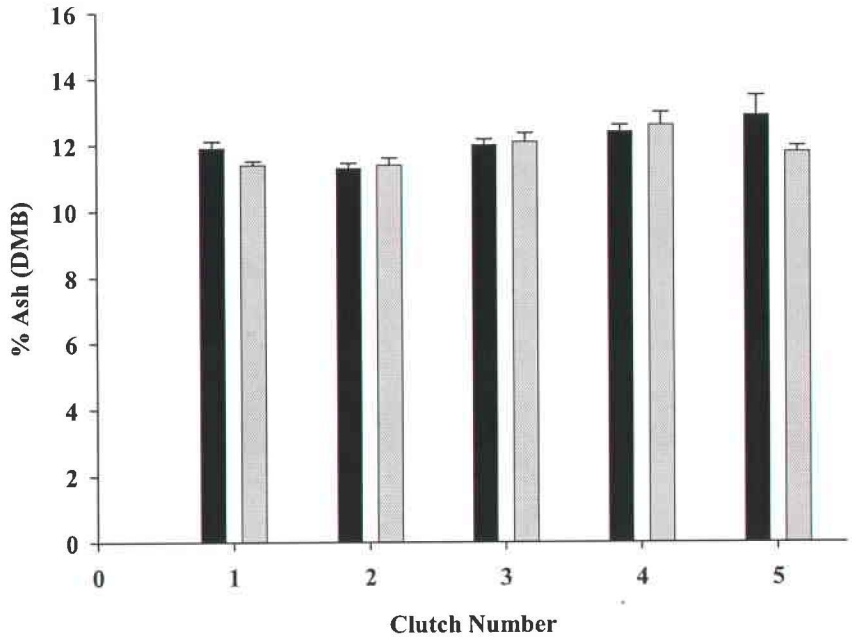


Figure 3.6. Percent ash (mean ± SE) of eggs from nesting green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP.

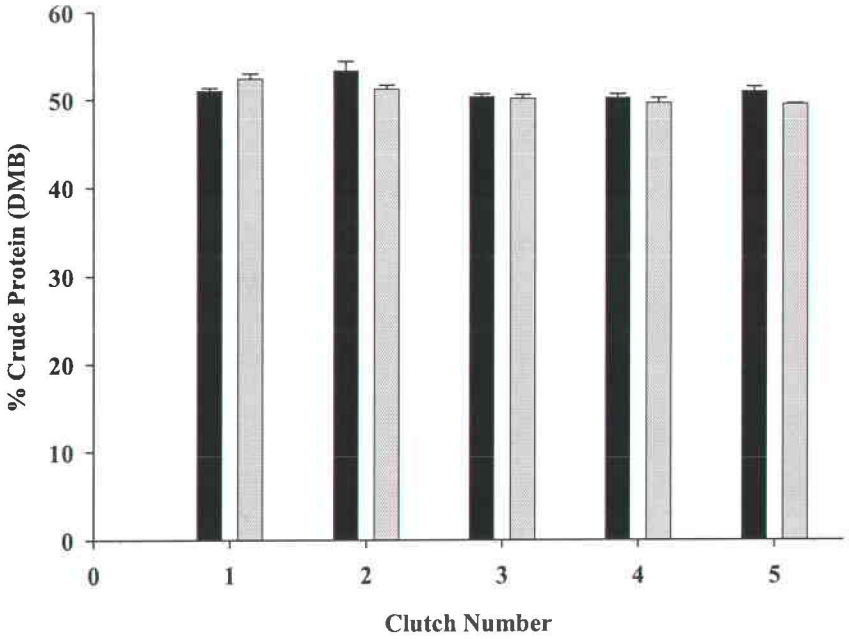


Figure 3.7. Percent crude protein (mean ± SE) of eggs from nesting green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP.

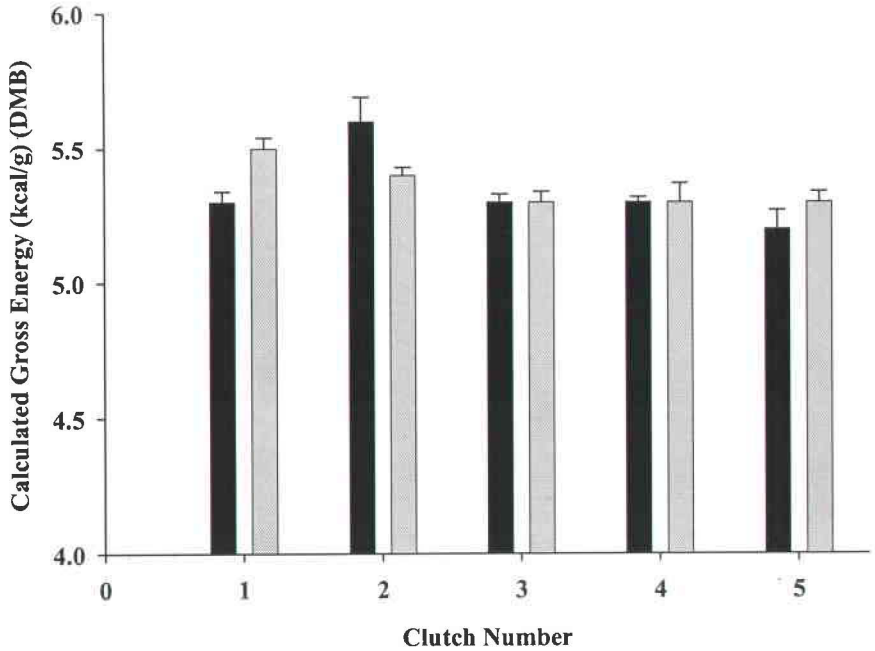


Figure 3.8. Percent calculated gross energy (mean ± SE) of eggs from nesting green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP.

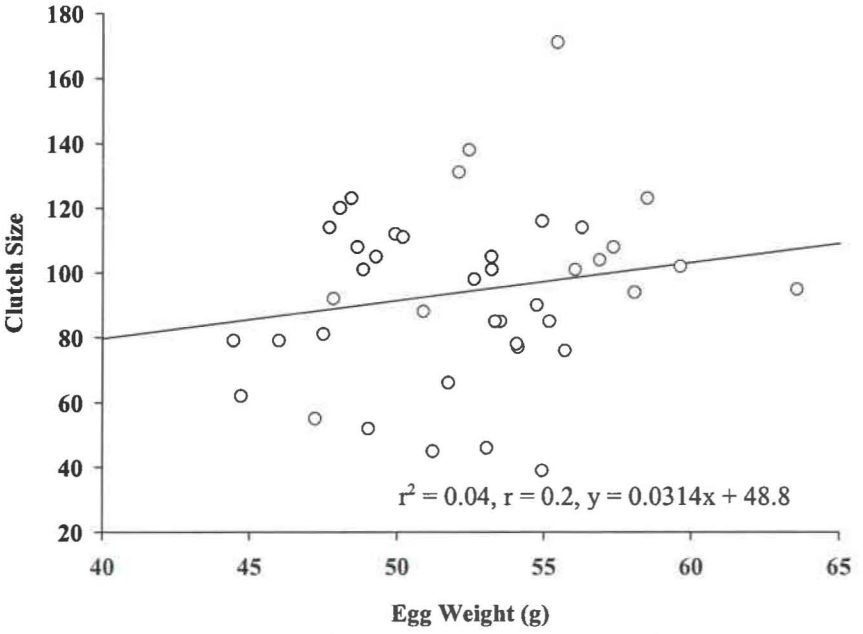


Figure 3.9. Correlation of egg weights versus clutch size from Hawaiian green turtles with and without turtle fibropapilloma.

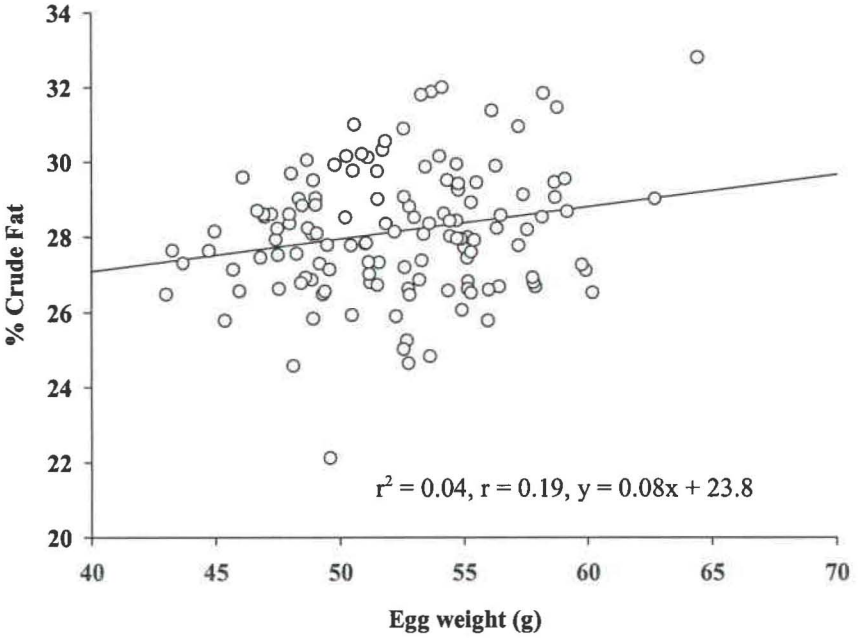


Figure 3.10. Correlation of egg weights versus percent crude fat from Hawai'ian green turtles with and without turtle fibropapilloma..

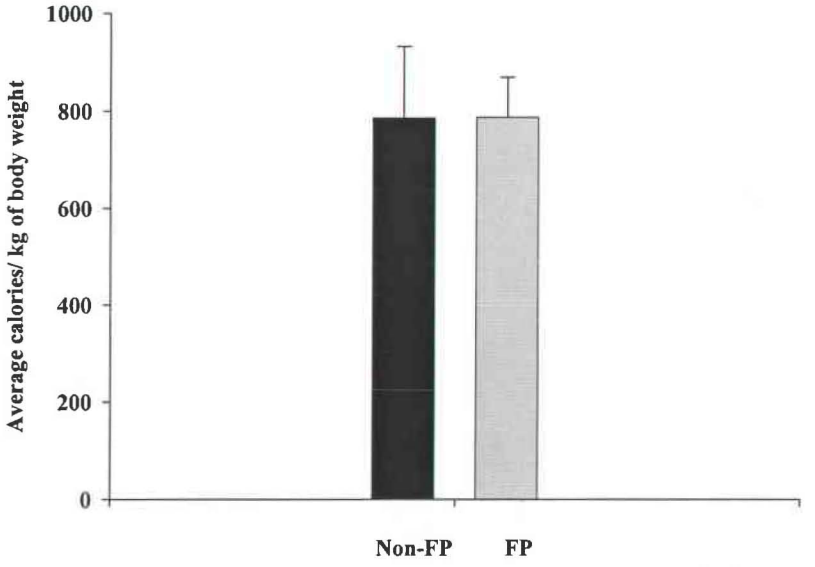


Figure 3.11. Average calories/ kg of maternal body weight (mean ± SE) used during entire nesting season in egg production in Hawaiian green turtles with and without turtle fibropapilloma. Black bars are non-FP and gray bars are FP.

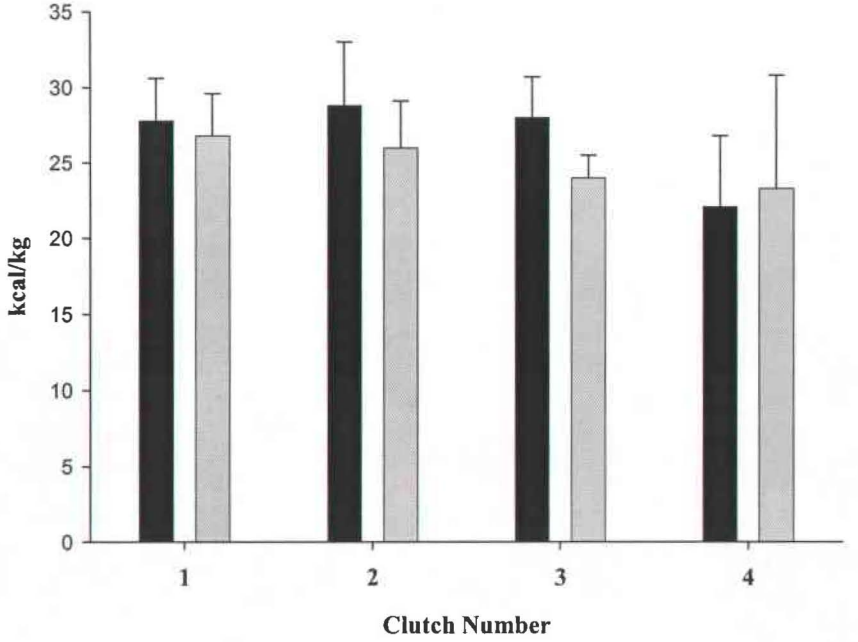


Figure 3.12. Average kcal/kg of maternal body weight (mean ± SE) used by green turtles with and without turtle fibropapilloma for individual clutches laid in egg production. Black bars are non-FP and gray bars are FP.

# CHAPTER FOUR

## SUMMARY

Although this was a single year survey, the results are comparable with previous studies conducted at French Frigate Shoals (Balazs, 1980; Niethammer et al., 1997). Additionally, since the etiology of FP has not been determined yet, it is important to know whether the reproductive success of the population is impaired. The information from this study can be used further the Recovery Plan for the Pacific green turtle (NMFS and USFWS, 1998).

A total of forty three turtles were observed throughout the nesting season of 1999. The morphometric data were not significantly different between turtles with and without FP. According to the results of this study, hatching success is mildly impaired in turtles with FP which is illustrated by a decrease in clutch size and a slight decrease in hatch success. To explain, when looking at all forty three turtles, there was a significant difference in clutch size between FP and non-FP, however, when looking at a sub-sample for egg composition analysis, there was not a significant difference. Additionally, the hatch successes were not significantly different between turtles with and without FP in either the total or subsample group, but there was a trend towards a reduced hatch success in turtles with FP. It is difficult to analyze the data from a single year when there is a large variation in clutch sizes among individual turtles, and the sample sizes are relatively small. Several years of surveys or a greater sample size would be needed to better determine the extent of how the reproductive success is impaired by FP. For this reason, the $\alpha$, or the level of significance, was increased to 0.10.

Since eggs, and subsequent hatchlings, are the representation of the total reproductive investment of the turtle; it is very important to look at the egg composition and energy content of those eggs. Theories of the optimal clutch size have been discussed extensively and in this literature review. Briefly, smaller eggs may have a lower chance of survival, and an increase in egg size may give only a marginal increase in survival because there is a marginal decrease in the total number of eggs produced (Hays et al., 1993). However, there are an optimal number of eggs that most reptiles produce where the maximum reproductive success is achieved without impairing the survival of the adult. Turtles with FP lay fewer eggs, but invest approximately the same amount of energy into egg production as do the turtles without FP. However, FP turtles lay eggs that weigh significantly more and have significantly more fat. This could be a physiological adaptation to deal with the effects of FP and ensure the success of the hatchlings.

The curvilinear trends of the crude fat in successive clutches may indicate that the turtles invest more in the middle clutches than in the first and last clutches. This is consistent with previous results in the literature.

In conclusion, overall clutch size is impacted by FP, but the hatch success is not. This may be due to increased egg weight and increased fat, which gives the hatchlings extra energy to endure the FP virus. This study suggests that 1) there is a physiological adaptation by the turtles affected by FP to cope with the disease by changing their nesting strategy and 2) there may be a tumor threshold beyond which nesting does not occur.

*Suggested Future Studies*

Additional studies would be helpful to better understand reproductive investment in green turtles. A study that 1) covered multiple years, in order to account for annual variability, and increase the sample size and 2) studied each year to see if there is a difference in overall reproductive output between turtles with and without FP. It would also be very interesting to learn whether or not a turtle's migration interval is longer if it is infected with FP. A comparison of the initial fat composition of a turtle as she reaches the nesting area and the subsequent loss of fat over the nesting season utilizing a total body water study would be beneficial. The etiology of FP is still unknown, but it would be important to determine whether the herpesvirus is passed from the mother via the egg to the hatchling.

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 1 | 88.2 | 1 | 87 | 0.862 | 1 | 73.63636 | 0.83488 | 88.7 | 16.2 | 12 | 43.1 |
| 40 | 1 | . | 2 | 84 | 0.464 | 2 | . | . | . | . | . | . |
| 45 | 0 | 99 | . | . | . | 2 | 93.18182 | 0.94123 | . | . | . | . |
| 48 | 0 | 98.5 | 1 | 102 | 0.853 | 2 | 104.0909 | 1.05676 | . | . | . | . |
| 48 | 0 | . | 2 | 17 | 0.471 | . | . | . | . | . | . | . |
| 48 | 0 | . | 3 | 74 | 0.73 | . | . | . | . | . | . | . |
| 48 | 0 | . | 4 | 89 | 0.618 | . | . | . | . | . | . | . |
| 50 | 0 | 96.2 | 1 | 102 | 0.775 | 1 | 83.18182 | 0.86468 | 93 | 17.5 | 13.5 | 45.5 |
| 50 | 0 | . | . | . | . | 2 | 93.18182 | 0.96863 | . | . | . | . |
| 51 | 0 | 99 | 1 | 117 | 0.35 | 1 | 124.0909 | 1.25344 | . | . | . | . |
| 51 | 0 | . | 3 | 117 | 0.632 | 2 | 60.90909 | 0.61524 | . | . | . | . |
| 51 | 0 | . | 4 | 118 | 0.949 | * | * | * | . | . | . | . |
| 52 | 0 | 98 | 1 | 120 | 0.843 | 1 | 99.54545 | 1.01577 | . | . | . | . |
| 52 | 0 | . | 1 | . | . | 2 | 91.36364 | 0.93228 | . | . | . | . |
| 52 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 52 | 0 | . | 2 | 81 | 0.816 | . | . | . | . | . | . | . |
| 52 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 52 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 52 | 0 | . | 3 | 79 | 0.861 | . | . | . | . | . | . | . |
| 52 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 52 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | 93 | 1 | . | . | 1 | 99.09091 | 1.06549 | . | . | . | . |
| 54 | 1 | . | 1 | . | . | 2 | 88.63636 | 0.95308 | . | . | . | . |
| 54 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | . | 2 | 77 | 0.444 | . | . | . | . | . | . | . |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 54 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 57 | 1 | 99.8 | . | . | . | 1 | 125.9091 | 1.26161 | 99.5 | 19 | 12.7 | 44 |
| 58 | 0 | 103.3 | 1 | 105 | 0.77 | 1 | 114.0909 | 1.10446 | 102.1 | 19.1 | 13.3 | 45.7 |
| 58 | 0 | . | 1 | . | . | 2 | 117.7273 | 1.13966 | . | . | . | . |
| 58 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 2 | 45 | 0.889 | . | . | . | . | . | . | . |
| 58 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 3 | 94 | 0.989 | . | . | . | . | . | . | . |
| 58 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 4 | 85 | 0.95 | . | . | . | . | . | . | . |
| 58 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 5 | 46 | 0.512 | . | . | . | . | . | . | . |
| 58 | 0 | . | 5 | . | . | . | . | . | . | . | . | . |
| 58 | 0 | . | 5 | . | . | . | . | . | . | . | . | . |
| 59 | 0 | 97.2 | 2 | 112 | 0.822 | 1 | 115.9091 | 1.19248 | 87.3 | 16.7 | 12.7 | 45.1 |
| 59 | 0 | . | 2 | . | . | 2 | 104.5455 | 1.07557 | . | . | . | . |
| 59 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 59 | 0 | . | 3 | 123 | 0.932 | . | . | . | . | . | . | . |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 59 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 59 | 0 | . | 4 | 114 | 0.851 | . | . | . | . | . | . | . |
| 59 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 59 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 60 | 0 | 92.9 | . | . | . | 1 | 101.3636 | 1.05368 | 91.5 | 18.8 | 12.7 | 43 |
| 61 | 0 | 96.4 | 1 | 108 | 0.709 | 1 | 79.54545 | 0.82516 | 88 | 14.4 | 12.5 | 44.7 |
| 61 | 0 | . | 1 | . | . | 2 | 85.90909 | 0.89117 | . | * | . | . |
| 61 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 61 | 0 | . | 2 | 90 | 0.933 | . | . | . | . | . | . | . |
| 61 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 61 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 63 | 0 | 88 | 1 | 62 | 0.935 | 1 | 104.0909 | 1.18285 | 88.4 | 17.2 | 13 | 45.6 |
| 63 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 63 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 64 | 0 | 96.3 | . | . | . | 1 | 107.2727 | 1.11394 | 96.4 | 17.1 | 12.7 | 43.7 |
| 65 | 0 | 99.6 | 2 | 123 | 0.881 | 1 | 122.7273 | 1.2322 | 94 | 18.4 | 12.5 | 43.4 |
| 65 | 0 | . | 2 | . | . | 2 | 102.7273 | 1.0314 | . | . | . | . |
| 65 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 65 | 0 | . | 3 | 171 | 0.735 | . | . | . | . | . | . | . |
| 65 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 65 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 65 | 0 | . | 4 | 131 | 0.746 | . | . | . | . | . | . | . |
| 65 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 65 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 65 | 0 | . | 5 | 105 | 0.886 | . | . | . | . | . | . | . |
| 65 | 0 | . | 5 | . | . | . | . | . | . | . | . | . |
| 65 | 0 | . | 5 | . | . | . | . | . | . | . | . | . |
| 66 | 0 | 98.1 | 2 | 95 | 0.944 | 1 | 74.77273 | 0.76221 | 89.4 | 13.6 | 12.4 | 45.4 |
| 66 | 0 | . | 2 | . | . | 2 | 98.63636 | 1.00547 | . | . | . | . |
| 66 | 0 | . | 3 | 115 | 0.982 | . | . | . | . | . | . | . |
| 66 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 66 | 0 | . | 3 | . | . | . | . | . | . | . | . | . |
| 66 | 0 | . | 4 | 98 | 0.98 | . | . | . | . | . | . | . |
| 66 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 66 | 0 | . | 4 | . | . | . | . | . | . | . | . | . |
| 68 | 1 | 99.4 | 1 | 102 | 0.866 | 1 | 77.27273 | 0.77739 | 91.5 | 20.7 | 13.1 | 43.6 |
| 68 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 68 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 68 | 1 | . | 3 | 76 | 0.775 | . | . | . | . | . | . | . |
| 68 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 68 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 69 | 0 | 95.6 | 1 | 79 | 0.635 | 1 | 106.8182 | 1.11734 | 90 | 14.6 | 12.9 | 43.3 |
| 69 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 69 | 0 | . | 1 | . | . | . | . | . | . | . | . | . |
| 69 | 0 | . | 2 | 92 | 0.966 | . | . | . | . | . | . | . |
| 69 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 69 | 0 | . | 2 | . | . | . | . | . | . | . | . | . |
| 71 | 0 | 95.5 | 1 | . | . | 1 | 69.54545 | 0.72822 | 88.8 | 13.7 | 12.8 | 44.5 |
| 72 | 0 | 95.5 | 1 | 108 | 0.981 | 1 | 107.2727 | 1.12327 | 87 | 15.2 | 13.1 | 43.2 |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 72 | 0 | . | 2 | 100 | 0.97 | . | . | . | . | . | . | . |
| 72 | 0 | . | 3 | 96 | 1 | . | . | . | . | . | . | . |
| 72 | 0 | . | 5 | 88 | 0.943 | . | . | . | . | . | . | . |
| 72 | 0 | . | * | 48 | 0.8 | . | . | . | . | . | . | . |
| 73 | 0 | 101.2 | 2 | 100 | 0.95 | 1 | 116.3636 | 1.14984 | 92.3 | 20.3 | 12.7 | 45.6 |
| 73 | 0 | . | 3 | 102 | 0.971 | . | . | . | . | . | . | . |
| 73 | 0 | . | 4 | 97 | 0.732 | . | . | . | . | . | . | . |
| 73 | 0 | . | 5 | 95 | 0.989 | . | . | . | . | . | . | . |
| 73 | 0 | . | 6 | 96 | 0.729 | . | . | . | . | . | . | . |
| 81 | 0 | 104.8 | . | . | . | 1 | 136.3636 | 1.30118 | 94.4 | 19.1 | 14 | 48 |
| 82 | 0 | 89 | 1 | 72 | 0.917 | 1 | 84.54545 | 0.94995 | 82 | . | 12.9 | 43 |
| 82 | 0 | . | 2 | 70 | 0.957 | 2 | 76.36364 | 0.85802 | . | . | . | . |
| 82 | 0 | . | 3 | 57 | 0.86 | . | . | . | . | . | . | . |
| 87 | 0 | 101.8 | 1 | 96 | 0.896 | 1 | 111.3636 | 1.09395 | 93.6 | 17.5 | 12.8 | 44.8 |
| 87 | 0 | . | 2 | 99 | 0.949 | . | . | . | . | . | . | . |
| 89 | 1 | 95.5 | 2 | 109 | 0.971 | 1 | 115 | 1.20419 | 91.5 | 17.1 | 13.2 | 46 |
| 89 | 1 | . | 2 | . | . | 2 | 103.1818 | 1.08044 | . | . | . | . |
| 89 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 3 | 114 | 0.917 | . | . | . | . | . | . | . |
| 89 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 4 | 85 | 0.824 | . | . | . | . | . | . | . |
| 89 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 5 | 138 | 0.902 | . | . | . | . | . | . | . |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | 1 | . | 5 | . | . | . | . | . | . | . | . | . |
| 89 | 1 | . | 5 | . | . | . | . | . | . | . | . | . |
| 91 | 0 | 100.8 | 1 | 110 | 0.827 | 1 | 113.1818 | 1.12284 | 93.5 | 20.1 | 13.4 | 45.1 |
| 91 | 0 | . | 2 | 109 | 0.835 | . | . | . | . | . | . | . |
| 91 | 0 | . | 3 | 57 | 0.0351 | . | . | . | . | . | . | . |
| 92 | 1 | 98.6 | 1 | 78 | 0.904 | 1 | 108.6364 | 1.10179 | 93.6 | 19.4 | 13.4 | 45.6 |
| 92 | 1 | . | 1 | . | . | 2 | 108.1818 | 1.09718 | . | . | . | . |
| 92 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 2 | 104 | 0.929 | . | . | . | . | . | . | . |
| 92 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 3 | 85 | 0.9 | . | . | . | . | . | . | . |
| 92 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 4 | 55 | 0.82 | . | . | . | . | . | . | . |
| 92 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 92 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | 104.5 | 1 | 116 | 0.937 | 1 | 138.6364 | 1.32666 | 90.5 | 14.5 | 14.5 | 48.8 |
| 99 | 1 | . | 1 | . | . | 2 | 120 | 1.14833 | . | . | . | . |
| 99 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | . | 2 | 39 | 1 | . | . | . | . | . | . | . |
| 99 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | . | 3 | 112 | 0.929 | . | . | . | . | . | . | . |
| 99 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |

| Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
| 99 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | . | 4 | 66 | 0.984 | . | . | . | . | . | . | . |
| 99 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 99 | 1 | . | 4 | . | . | . | . | . | . | . | . | . |
| 106 | 0 | 95.3 | 2 | 88 | 0.773 | 1 | . | . | 91 | 18.3 | 13.5 | 44.4 |
| 106 | 0 | . | 3 | 85 | 0.918 | 2 | 102.2727 | 1.07317 | . | . | . | . |
| 106 | 0 | . | 4 | 85 | 0.753 | . | . | . | . | . | . | . |
| 110 | 0 | 97.7 | 1 | 122 | 0.926 | 1 | 113.1818 | 1.15846 | 94.2 | 18.4 | 12.6 | 44.2 |
| 110 | 0 | . | 2 | 112 | 0.893 | 2 | 104.5455 | 1.07007 | . | . | . | . |
| 110 | 0 | . | 3 | 114 | 0.895 | . | . | . | . | . | . | . |
| 113 | 0 | 104.2 | 1 | 120 | 0.833 | 1 | 129.5455 | 1.24324 | 95 | 17.4 | 14 | 48 |
| 113 | 0 | . | 2 | 112 | 0.893 | 2 | 120.9091 | 1.16036 | . | . | . | . |
| 113 | 0 | . | 3 | 94 | 0.968 | . | . | . | . | . | . | . |
| 113 | 0 | . | 4 | 87 | 0.989 | . | . | . | . | . | . | . |
| 113 | 0 | . | 5 | 86 | 0.0233 | . | . | . | . | . | . | . |
| 118 | 1 | 98.2 | 1 | 52 | 0.553 | 1 | 108.1818 | 1.10165 | 86.7 | 16.3 | 12.6 | 44.4 |
| 118 | 1 | . | 1 | . | . | 2 | 98.63636 | 1.00444 | . | . | . | . |
| 118 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 118 | 1 | . | 2 | 101 | 0.969 | . | . | . | . | . | . | . |
| 118 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 118 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 118 | 1 | . | 3 | 88 | 0.795 | . | . | . | . | . | . | . |
| 118 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 118 | 1 | . | 3 | . | . | . | . | . | . | . | . | . |
| 118 | 1 | . | 4 | 83 | 0.747 | . | . | . | . | . | . | . |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 119 | 0 | 99.6 | 1 | 110 | 0.873 | 1 | 113.6364 | 1.14093 | 92.3 | 19.6 | 13.9 | 45.6 |
| 119 | 0 | . | 2 | 104 | 0.962 | 2 | 102.2727 | 1.02683 | . | . | . | . |
| 119 | 0 | . | 3 | 87 | 0.989 | . | . | . | . | . | . | . |
| 119 | 0 | . | 4 | 54 | 0.796 | . | . | . | . | . | . | . |
| 126 | 0 | 94.8 | 1 | 99 | 0.354 | 1 | 84.09091 | 0.88703 | 92.5 | 17.3 | 12 | 41.5 |
| 126 | 0 | . | 2 | 189 | 0.82 | 2 | 104.5455 | 1.1028 | . | . | . | . |
| 126 | 0 | . | 3 | 48 | 0.938 | . | . | . | . | . | . | . |
| 128 | 0 | 96.4 | 1 | 85 | 0.871 | 1 | 109.5455 | 1.13636 | 90.2 | 17.8 | 12.8 | 47 |
| 128 | 0 | . | 2 | 115 | 0.904 | 2 | 120.9091 | 1.25424 | . | . | . | . |
| 128 | 0 | . | 3 | 170 | 0.118 | . | . | . | . | . | . | . |
| 139 | 1 | 103.7 | 1 | 140 | 0 | 1 | . | . | . | . | 13.2 | . |
| 139 | 1 | . | 2 | 123 | 0 | 2 | 108.1818 | 1.04322 | . | . | . | . |
| 141 | 1 | 102 | 1 | 101 | 0.417 | 1 | 112.7273 | 1.10517 | 87 | 18.7 | 13.4 | 45.3 |
| 141 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 141 | 1 | . | 1 | . | . | . | . | . | . | . | . | . |
| 141 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 141 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 141 | 1 | . | 2 | . | . | . | . | . | . | . | . | . |
| 147 | 1 | 98.2 | 1 | 43 | 0.791 | 1 | 102.2727 | 1.04147 | 90.2 | 17.5 | 12.7 | 43.5 |
| 150 | 0 | 92 | . | . | . | 1 | 85.90909 | 0.93379 | 81.5 | 18.2 | 12.7 | 42.8 |
| 154 | 0 | 91.2 | 1 | 85 | 0.894 | 1 | 81.81818 | 0.89713 | 81.8 | 16.1 | 12.1 | 42.5 |
| 154 | 0 | . | 2 | 143 | 0.671 | 2 | 75.90909 | 0.83234 | . | . | . | . |
| 154 | 0 | . | 3 | 91 | 0.945 | . | . | . | . | . | . | . |
| 154 | 0 | . | 4 | 35 | 0.771 | . | . | . | . | . | . | . |
| 155 | 1 | 101 | 1 | 24 | 0.208 | 1 | 108.6364 | 1.07561 | 88.8 | 12.9 |  | 42.6 |

Appendix 1. Life history information of Hawaiian green turtles nesting on Tern Island, French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Time Weighed | Weight (kg) | BMI (kg/cm) | CCW 6th | HL | HW | HG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 155 | 1 | . | 2 | 27 | 0.04 | . | . | . | . | . | . | . |
| 158 | 1 | 100.5 | . | . | . | 1 | 127.7273 | 0.61963 | 96.6 | 18.9 | 13.6 | 45.1 |
| 161 | 1 | 95.7 | 1 | 57 | 0.877 | 1 | 95 | 0.99269 | 90.5 | 18.8 | 12.9 | 40.7 |
| 167 | 1 | 97 | . | . | . | 1 | 96.81818 | 0.99813 | 88.5 | 18.3 | 13.2 | 44.5 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Time Weighed | Wt. (kg) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Egg no | Egg Wt (g) | Egg Vol (ml) | 90 degree 1 (mm) | 90 degree 2 (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 52 | 0 | 1 | 99.55 | 98 | 1 | 120 | 0.843 | 1 | 47.506 | 52.6 | 4.8 | 4.6 |
| 52 | 0 | 1 | 99.55 | 98 | 1 | 120 | 0.843 | 2 | 47.419 | 50 | 4.8 | 4.75 |
| 52 | 0 | 1 | 99.55 | 98 | 1 | 120 | 0.843 | 3 | 49.175 | 52.6 | 4.6 | 4.75 |
| 52 | 0 | 1 | 99.55 | 98 | 2 | 81 | 0.816 | 1 | 46.967 | 52.9 | 4.6 | 4.65 |
| 52 | 0 | 1 | 99.55 | 98 | 2 | 81 | 0.816 | 2 | 46.809 | 53.2 | 4.6 | 4.6 |
| 52 | 0 | 1 | 99.55 | 98 | 2 | 81 | 0.816 | 3 | 48.666 | 54.5 | 4.7 | 4.7 |
| 52 | 0 | 2 | 91.36 | 98 | 3 | 79 | 0.919 | 1 | 43.248 | 49 | 4.4 | 4.6 |
| 52 | 0 | 2 | 91.36 | 98 | 3 | 79 | 0.919 | 2 | 47.483 | 50 | 4.7 | 4.4 |
| 52 | 0 | 2 | 91.36 | 98 | 3 | 79 | 0.919 | 3 | 47.240 | 50 | 4.65 | 4.7 |
| 54 | 1 | 1 | 99.09 | 93 | 1 | . | . | 1 | 55.005 | 59.7 | 4.8 | 4.8 |
| 54 | 1 | 1 | 99.09 | 93 | 1 | . | . | 2 | 52.563 | 58.9 | 4.75 | 4.8 |
| 54 | 1 | 1 | 99.09 | 93 | 1 | . | . | 3 | 51.005 | 56.6 | 4.7 | 4.6 |
| 54 | 1 | 1 | 99.09 | 93 | 2 | 77 | 0.444 | 1 | 52.810 | 59.9 | 4.7 | 4.7 |
| 54 | 1 | 1 | 99.09 | 93 | 2 | 77 | 0.444 | 2 | 55.143 | 59.8 | 4.75 | 4.75 |
| 54 | 1 | 1 | 99.09 | 93 | 2 | 77 | 0.444 | 3 | 54.364 | 60.2 | 4.8 | 4.8 |
| 54 | 1 | 2 | 88.64 | 93 | 3 | . | . | 1 | 48.250 | 52.7 | 4.8 | 4.5 |
| 54 | 1 | 2 | 88.64 | 93 | 3 | . | . | 2 | 51.116 | 55.6 | 4.7 | 4.6 |
| 54 | 1 | 2 | 88.64 | 93 | 3 | . | . | 3 | 49.485 | 53 | 4.6 | 4.7 |
| 58 | 0 | 1 | 114.1 | 103.3 | 1 | 105 | 0.777 | 1 | 48.879 | 55.2 | 4.65 | 4.7 |
| 58 | 0 | 1 | 114.1 | 103.3 | 1 | 105 | 0.777 | 2 | 48.704 | 53.6 | 4.7 | 4.6 |
| 58 | 0 | 1 | 114.1 | 103.3 | 1 | 105 | 0.777 | 3 | 50.206 | 55.7 | 4.8 | 4.7 |
| 58 | 0 | 1 | 114.1 | 103.3 | 2 | 45 | 0.889 | 1 | 50.432 | 58 | 4.75 | 4.65 |
| 58 | 0 | 1 | 114.1 | 103.3 | 2 | 45 | 0.889 | 2 | 51.724 | 59.5 | 4.75 | 4.65 |
| 58 | 0 | 1 | 114.1 | 103.3 | 2 | 45 | 0.889 | 3 | 51.512 | 56.1 | 4.7 | 4.8 |
| 58 | 0 | 2 | 117.7 | 103.3 | 3 | 94 | 0.989 | 1 | 56.484 | 59.5 | 4.75 | 5 |
| 58 | 0 | 2 | 117.7 | 103.3 | 3 | 94 | 0.989 | 2 | 58.652 | 60.9 | 5 | 4.8 |
| 58 | 0 | 2 | 117.7 | 103.3 | 3 | 94 | 0.989 | 3 | 59.072 | 62.7 | 4.95 | 4.9 |
| 58 | 0 | 2 | 117.7 | 103.3 | 4 | 85 | 0.950 | 1 | 52.995 | 57.3 | 4.7 | 4.9 |
| 58 | 0 | 2 | 117.7 | 103.3 | 4 | 85 | 0.950 | 2 | 54.743 | 58.8 | 4.8 | 4.7 |
| 58 | 0 | 2 | 117.7 | 103.3 | 4 | 85 | 0.950 | 3 | 52.797 | 54.3 | 4.8 | 4.8 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Time Weighed | Wt. (kg) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Egg no | Egg Wt (g) | Egg Vol (ml) | 90 degree 1 (mm) | 90 degree 2 (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 58 | 0 | 2 | 117.7 | 103.3 | 5 | 46 | 0.512 | 1 | 52.190 | 54.3 | 4.6 | 4.7 |
| 58 | 0 | 2 | 117.7 | 103.3 | 5 | 46 | 0.512 | 2 | 53.370 | 54.3 | 4.8 | 4.7 |
| 58 | 0 | 2 | 117.7 | 103.3 | 5 | 46 | 0.512 | 3 | 53.606 | 56.7 | 4.8 | 4.7 |
| 59 | 0 | 1 | 115.9 | 97.2 | 2 | 112 | 0.822 | 1 | 51.819 | 59.5 | 4.7 | 4.75 |
| 59 | 0 | 1 | 115.9 | 97.2 | 2 | 112 | 0.822 | 2 | 48.345 | 58.9 | 4.6 | 4.6 |
| 59 | 0 | 1 | 115.9 | 97.2 | 2 | 112 | 0.822 | 3 | 49.589 | 56.8 | 4.6 | 4.65 |
| 59 | 0 | 2 | 104.5 | 97.2 | 3 | 123 | 0.932 | 1 | 49.009 | 55.6 | 4.6 | 4.6 |
| 59 | 0 | 2 | 104.5 | 97.2 | 3 | 123 | 0.932 | 2 | 46.932 | 54 | 4.5 | 4.6 |
| 59 | 0 | 2 | 104.5 | 97.2 | 3 | 123 | 0.932 | 3 | 49.301 | 55.2 | 4.65 | 4.55 |
| 59 | 0 | 2 | 104.5 | 97.2 | 4 | 114 | 0.851 | 1 | 48.922 | 53.1 | 4.6 | 4.6 |
| 59 | 0 | 2 | 104.5 | 97.2 | 4 | 114 | 0.851 | 2 | 48.040 | 53.7 | 4.6 | 4.7 |
| 59 | 0 | 2 | 104.5 | 97.2 | 4 | 114 | 0.851 | 3 | 46.092 | 49 | 4.55 | 4.55 |
| 61 | 0 | 1 | 79.55 | 96.4 | 1 | 108 | 0.709 | 1 | 48.112 | 53.5 | 4.7 | 4.7 |
| 61 | 0 | 1 | 79.55 | 96.4 | 1 | 108 | 0.709 | 2 | 48.858 | 52.9 | 4.7 | 4.6 |
| 61 | 0 | 1 | 79.55 | 96.4 | 1 | 108 | 0.709 | 3 | 48.917 | 53.3 | 4.7 | 4.7 |
| 61 | 0 | 2 | 85.91 | 96.4 | 2 | 90 | 0.933 | 1 | 54.440 | 56.5 | 4.9 | 4.9 |
| 61 | 0 | 2 | 85.91 | 96.4 | 2 | 90 | 0.933 | 2 | 52.604 | 59.4 | 4.8 | 4.7 |
| 61 | 0 | 2 | 85.91 | 96.4 | 2 | 90 | 0.933 | 3 | 57.197 | 60.9 | 4.7 | 4.8 |
| 63 | 0 | 1 | 104.1 | 88 | 1 | 62 | 0.935 | 1 | 45.709 | 50 | 4.6 | 4.5 |
| 63 | 0 | 1 | 104.1 | 88 | 1 | 62 | 0.935 | 2 | 43.685 | 48.8 | 4.5 | 4.5 |
| 63 | 0 | 1 | 104.1 | 88 | 1 | 62 | 0.935 | 3 | 44.732 | 50 | 4.55 | 4.5 |
| 65 | 0 | 1 | 122.7 | 99.6 | 2 | 123 | 0.881 | 1 | 60.196 | 65.6 | 4.8 | 5.1 |
| 65 | 0 | 1 | 122.7 | 99.6 | 2 | 123 | 0.881 | 2 | 57.384 | 62.3 | 4.9 | 4.9 |
| 65 | 0 | 1 | 122.7 | 99.6 | 2 | 123 | 0.881 | 3 | 57.885 | 62.3 | 4.9 | 4.9 |
| 65 | 0 | 2 | 102.7 | 99.6 | 3 | 171 | 0.735 | 1 | 55.967 | 60 | 4.85 | 4.85 |
| 65 | 0 | 2 | 102.7 | 99.6 | 3 | 171 | 0.735 | 2 | 55.164 | 58.7 | 4.9 | 4.8 |
| 65 | 0 | 2 | 102.7 | 99.6 | 3 | 171 | 0.735 | 3 | 55.157 | 57.5 | 4.85 | 4.8 |
| 65 | 0 | 2 | 102.7 | 99.6 | 4 | 131 | 0.746 | 1 | 52.754 | 57.3 | 4.8 | 4.7 |
| 65 | 0 | 2 | 102.7 | 99.6 | 4 | 131 | 0.746 | 2 | 52.263 | 54.5 | 4.6 | 4.75 |
| 65 | 0 | 2 | 102.7 | 99.6 | 4 | 131 | 0.746 | 3 | 51.209 | 56 | 4.7 | 4.6 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Time Weighed | Wt. (kg) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Egg no | Egg Wt (g) | Egg Vol (ml) | 90 degree 1 (mm) | 90 degree 2 (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 65 | 0 | 2 | 102.7 | 99.6 | 5 | 105 | 0.886 | 1 | 53.201 | 58 | 4.7 | 4.7 |
| 65 | 0 | 2 | 102.7 | 99.6 | 5 | 105 | 0.886 | 2 | 53.631 | 56.7 | 4.7 | 4.7 |
| 65 | 0 | 2 | 102.7 | 99.6 | 5 | 105 | 0.886 | 3 | 52.763 | 55 | 4.8 | 4.7 |
| 66 | 0 | 1 | 74.77 | 98.1 | 2 | 95 | 0.944 | 1 | 64.410 | 65.4 | 5.1 | 5.1 |
| 66 | 0 | 1 | 74.77 | 98.1 | 2 | 95 | 0.944 | 2 | 62.699 | 65.3 | 5.1 | 5.05 |
| 66 | 0 | 1 | 74.77 | 98.1 | 3 | 115 | 0.982 | 1 | 56.328 | 59.5 | 4.9 | 4.8 |
| 66 | 0 | 1 | 74.77 | 98.1 | 3 | 115 | 0.982 | 2 | 57.546 | 61.8 | 4.9 | 4.9 |
| 66 | 0 | 1 | 74.77 | 98.1 | 3 | 115 | 0.982 | 3 | 58.154 | 62.2 | 5 | 4.9 |
| 66 | 0 | 2 | 98.64 | 98.1 | 4 | 98 | 0.980 | 1 | 52.691 | 55.5 | 4.7 | 4.75 |
| 66 | 0 | 2 | 98.64 | 98.1 | 4 | 98 | 0.980 | 2 | 51.847 | 53.8 | 4.8 | 4.75 |
| 66 | 0 | 2 | 98.64 | 98.1 | 4 | 98 | 0.980 | 3 | 53.296 | 55.7 | 4.8 | 4.8 |
| 68 | 1 | 1 | 77.27 | 99.4 | 1 | 102 | 0.824 | 1 | 59.916 | 63.4 | 5 | 4.9 |
| 68 | 1 | 1 | 77.27 | 99.4 | 1 | 102 | 0.824 | 2 | 59.758 | 61.8 | 5 | 5 |
| 68 | 1 | 1 | 77.27 | 99.4 | 1 | 102 | 0.824 | 3 | 59.162 | 62.9 | 5 | 4.95 |
| 68 | 1 | 1 | 77.27 | 99.4 | 3 | 76 | 0.724 | 1 | 56.418 | 59.5 | 4.8 | 5 |
| 68 | 1 | 1 | 77.27 | 99.4 | 3 | 76 | 0.724 | 2 | 54.700 | 57.6 | 4.8 | 4.9 |
| 68 | 1 | 1 | 77.27 | 99.4 | 3 | 76 | 0.724 | 3 | 55.999 | 59.2 | 4.95 | 4.95 |
| 69 | 0 | 1 | 106.8 | 95.6 | 1 | 79 | 0.635 | 1 | 42.995 | 48 | 4.4 | 4.5 |
| 69 | 0 | 1 | 106.8 | 95.6 | 1 | 79 | 0.635 | 2 | 45.380 | 50 | 4.5 | 4.5 |
| 69 | 0 | 1 | 106.8 | 95.6 | 1 | 79 | 0.635 | 3 | 44.965 | 48.9 | 4.5 | 4.55 |
| 69 | 0 | 1 | 106.8 | 95.6 | 2 | 92 | 0.966 | 1 | 47.547 | 53.3 | 4.5 | 4.6 |
| 69 | 0 | 1 | 106.8 | 95.6 | 2 | 92 | 0.966 | 2 | 47.965 | 53.1 | 4.75 | 4.8 |
| 69 | 0 | 1 | 106.8 | 95.6 | 2 | 92 | 0.966 | 3 | 47.953 | 53.8 | 4.7 | 4.6 |
| 89 | 1 | 1 | 115 | 95.5 | 2 | 101 | 0.971 | 1 | 54.022 | 56.6 | 4.8 | 4.8 |
| 89 | 1 | 1 | 115 | 95.5 | 2 | 101 | 0.971 | 2 | 50.877 | 55.1 | 4.6 | 4.7 |
| 89 | 1 | 1 | 115 | 95.5 | 2 | 101 | 0.971 | 3 | 54.702 | 57.6 | 4.8 | 4.85 |
| 89 | 1 | 2 | 103.2 | 95.5 | 3 | 114 | 0.917 | 1 | 56.124 | 58.4 | 4.8 | 5 |
| 89 | 1 | 2 | 103.2 | 95.5 | 3 | 114 | 0.917 | 2 | 55.502 | 59.1 | 5 | 4.5 |
| 89 | 1 | 2 | 103.2 | 95.5 | 3 | 114 | 0.917 | 3 | 57.191 | 61.4 | 4.85 | 5 |
| 89 | 1 | 2 | 103.2 | 95.5 | 4 | 85 | 0.824 | 1 | 58.678 | 63.2 | 4.9 | 4.85 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Time Weighed | Wt. (kg) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Egg no | Egg Wt (g) | Egg Vol (ml) | 90 degree 1 (mm) | 90 degree 2 (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | 1 | 2 | 103.2 | 95.5 | 4 | 85 | 0.824 | 2 | 56.277 | 60.9 | 4.8 | 4.7 |
| 89 | 1 | 2 | 103.2 | 95.5 | 4 | 85 | 0.824 | 3 | 50.568 | 54.5 | 4.55 | 4.6 |
| 89 | 1 | 2 | 103.2 | 95.5 | 5 | 138 | 0.902 | 1 | 51.507 | 54.6 | 4.65 | 4.7 |
| 89 | 1 | 2 | 103.2 | 95.5 | 5 | 138 | 0.902 | 2 | 54.190 | 56.5 | 4.7 | 4.8 |
| 89 | 1 | 2 | 103.2 | 95.5 | 5 | 138 | 0.902 | 3 | 51.570 | 54.4 | 4.65 | 4.7 |
| 92 | 1 | 1 | 108.6 | 98.6 | 1 | 78 | 0.904 | 1 | 54.427 | 55.6 | 4.5 | 4.9 |
| 92 | 1 | 1 | 108.6 | 98.6 | 1 | 78 | 0.904 | 2 | 54.320 | 58.4 | 4.7 | 4.9 |
| 92 | 1 | 1 | 108.6 | 98.6 | 1 | 78 | 0.904 | 3 | 53.446 | 55 | 4.85 | 4.6 |
| 92 | 1 | 1 | 108.6 | 98.6 | 2 | 104 | 0.929 | 1 | 58.757 | 61.2 | 5 | 4.8 |
| 92 | 1 | 1 | 108.6 | 98.6 | 2 | 104 | 0.929 | 2 | 53.698 | 57.5 | 4.8 | 4.8 |
| 92 | 1 | 1 | 108.6 | 98.6 | 2 | 104 | 0.929 | 3 | 58.198 | 62.6 | 4.9 | 4.85 |
| 92 | 1 | 2 | 108.2 | 98.6 | 3 | 85 | 0.900 | 1 | 52.564 | 54.9 | 4.7 | 4.7 |
| 92 | 1 | 2 | 108.2 | 98.6 | 3 | 85 | 0.900 | 2 | 54.122 | 57.4 | 4.7 | 4.8 |
| 92 | 1 | 2 | 108.2 | 98.6 | 3 | 85 | 0.900 | 3 | 53.286 | 57.4 | 4.8 | 4.7 |
| 92 | 1 | 2 | 108.2 | 98.6 | 4 | 55 | 0.82 | 1 | 49.016 | 50.9 | 4.6 | 4.6 |
| 92 | 1 | 2 | 108.2 | 98.6 | 4 | 55 | 0.82 | 2 | 45.959 | 47 | 4.45 | 4.55 |
| 92 | 1 | 2 | 108.2 | 98.6 | 4 | 55 | 0.82 | 3 | 46.669 | 50 | 4.55 | 4.5 |
| 99 | 1 | 1 | 138.6 | 104.5 | 1 | 116 | 0.937 | 1 | 54.885 | 57.4 | 4.7 | 4.8 |
| 99 | 1 | 1 | 138.6 | 104.5 | 1 | 116 | 0.937 | 2 | 54.721 | 55.5 | 4.8 | 4.8 |
| 99 | 1 | 1 | 138.6 | 104.5 | 1 | 116 | 0.937 | 3 | 55.131 | 57.3 | 4.8 | 4.7 |
| 99 | 1 | 1 | 138.6 | 104.5 | 2 | 39 | 1.000 | 1 | 55.287 | 59.6 | 4.8 | 4.8 |
| 99 | 1 | 1 | 138.6 | 104.5 | 2 | 39 | 1.000 | 2 | 54.770 | 59 | 4.8 | 4.8 |
| 99 | 1 | 1 | 138.6 | 104.5 | 2 | 39 | 1.000 | 3 | 54.767 | 59.2 | 4.9 | 4.85 |
| 99 | 1 | 2 | 120 | 104.5 | 3 | 111 | 0.929 | 1 | 49.779 | 51.7 | 4.65 | 4.8 |
| 99 | 1 | 2 | 120 | 104.5 | 3 | 111 | 0.929 | 2 | 50.507 | 53.9 | 4.6 | 4.6 |
| 99 | 1 | 2 | 120 | 104.5 | 3 | 111 | 0.929 | 3 | 50.255 | 52.7 | 4.7 | 4.7 |
| 99 | 1 | 2 | 120 | 104.5 | 4 | 66 | 0.984 | 1 | 51.180 | 54 | 4.7 | 4.7 |
| 99 | 1 | 2 | 120 | 104.5 | 4 | 66 | 0.984 | 2 | 52.575 | 55.7 | 4.7 | 4.7 |
| 99 | 1 | 2 | 120 | 104.5 | 4 | 66 | 0.984 | 3 | 51.493 | 53.6 | 4.65 | 4.65 |
| 118 | 1 | 1 | 108.2 | 98.2 | 1 | 52 | 0.533 | 1 | 49.387 | 54.2 | 4.6 | 4.7 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Time Weighed | Wt. (kg) | CCL (cm) | Clutch | Size of Clutch | Nest Success | Egg no | Egg Wt (g) | Egg Vol (ml) | 90 degree 1 (mm) | 90 degree 2 (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 118 | 1 | 1 | 108.2 | 98.2 | 1 | 52 | 0.533 | 2 | 49.061 | 52.3 | 4.55 | 4.55 |
| 118 | 1 | 1 | 108.2 | 98.2 | 1 | 52 | 0.533 | 3 | 48.613 | 52.5 | 4.55 | 4.6 |
| 118 | 1 | 1 | 108.2 | 98.2 | 2 | 101 | 0.969 | 1 | 48.477 | 51.8 | 4.6 | 4.6 |
| 118 | 1 | 1 | 108.2 | 98.2 | 2 | 101 | 0.969 | 2 | 49.573 | 52.7 | 4.7 | 4.6 |
| 118 | 1 | 1 | 108.2 | 98.2 | 2 | 101 | 0.969 | 3 | 48.428 | 51.7 | 4.6 | 4.6 |
| 118 | 1 | 2 | 98.64 | 98.2 | 3 | 88 | 0.795 | 1 | 51.145 | 55.6 | 4.6 | 4.6 |
| 118 | 1 | 2 | 98.64 | 98.2 | 3 | 88 | 0.795 | 2 | 50.474 | 54.6 | 4.6 | 4.7 |
| 118 | 1 | 2 | 98.64 | 98.2 | 3 | 88 | 0.795 | 3 | 51.049 | 54.8 | 4.65 | 4.7 |
| 118 | 1 | 2 | 98.64 | 98.2 | 4 | 83 | 0.747 | . | . | . | . | . |
| 141 | 1 | 1 | 112.7 | 102 | 1 | 101 | 0.416 | 1 | 54.917 | 60 | 4.7 | 4.8 |
| 141 | 1 | 1 | 112.7 | 102 | 1 | 101 | 0.416 | 2 | 57.805 | 59.8 | 4.8 | 4.8 |
| 141 | 1 | 1 | 112.7 | 102 | 1 | 101 | 0.416 | 3 | 55.410 | 59.8 | 5 | 4.8 |
| 141 | 1 | 1 | 112.7 | 102 | 2 | . | . | 1 | 55.287 | 56.4 | 4.9 | 4.7 |
| 141 | 1 | 1 | 112.7 | 102 | 2 | . | . | 2 | 57.767 | 58.6 | 4.9 | 5 |
| 141 | 1 | 1 | 112.7 | 102 | 2 | . | . | 3 | 55.293 | 56.5 | 4.75 | 4.9 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Egg no | Dry Wt % | Ash % DMB | EE % DMB | Protein % DMB | % Carb | Projected GE (cal/g) |
|---|---|---|---|---|---|---|---|---|
| 52 | 0 | 1 | 26.37 | 11.44 | 27.52 | 52.95 | 15.03 | 5607.84 |
| 52 | 0 | 2 | 26.02 | 10.81 | 27.92 | 51.81 | 14.22 | 5567.72 |
| 52 | 0 | 3 | 26.20 | 12.40 | 27.29 | 51.21 | 13.57 | 5210.58 |
| 52 | 0 | 1 | 24.70 | 11.51 | 28.53 | 64.36 | 24.32 | 6518.68 |
| 52 | 0 | 2 | 25.13 | 11.57 | 27.45 | 69.22 | 31.06 | 6806.07 |
| 52 | 0 | 3 | 25.73 | 11.89 | 30.05 | 53.70 | 11.76 | 5711.90 |
| 52 | 0 | 1 | 24.00 | 11.64 | 27.64 | 49.50 | 10.22 | 5236.43 |
| 52 | 0 | 2 | 25.32 | 11.33 | 28.22 | 50.53 | 10.73 | 5379.65 |
| 52 | 0 | 3 | 23.13 | 11.80 | 28.60 | 51.04 | 10.64 | 5412.66 |
| 54 | 1 | 1 | 22.91 | 10.71 | 27.72 | 52.35 | 13.92 | 5511.65 |
| 54 | 1 | 2 | 24.00 | 11.17 | 25.02 | 59.29 | 23.26 | 5901.50 |
| 54 | 1 | 3 | 24.54 | 10.84 | 27.82 | 52.92 | 15.80 | 5470.39 |
| 54 | 1 | 1 | 23.81 | 12.73 | 26.46 | 51.01 | 11.40 | 5276.63 |
| 54 | 1 | 2 | 22.69 | 12.15 | 27.99 | 51.06 | 10.92 | 5366.39 |
| 54 | 1 | 3 | 22.99 | 11.95 | 26.57 | 51.00 | 12.48 | 5286.95 |
| 54 | 1 | 1 | 24.77 | 12.44 | 27.55 | 51.69 | 11.69 | 5381.94 |
| 54 | 1 | 2 | 26.03 | 11.78 | 30.12 | 48.69 | 8.40 | 5213.65 |
| 54 | 1 | 3 | 24.56 | 11.72 | 27.78 | 49.83 | 10.33 | 5269.05 |
| 58 | 0 | 1 | 25.13 | 11.31 | 28.08 | 51.08 | 12.55 | 5355.72 |
| 58 | 0 | 2 | 25.18 | 11.13 | 28.23 | 49.44 | 10.08 | 5284.30 |
| 58 | 0 | 3 | 25.18 | 11.35 | 28.52 | 50.10 | 7.65 | 5270.52 |
| 58 | 0 | 1 | 26.54 | 12.07 | 27.77 | 50.29 | 10.45 | 5292.85 |
| 58 | 0 | 2 | 24.90 | 9.09 | 30.32 | 52.95 | 13.54 | 5770.95 |
| 58 | 0 | 3 | 24.05 | 10.67 | 29.01 | 51.54 | 11.86 | 5520.39 |
| 58 | 0 | 1 | 22.92 | 12.37 | 28.58 | 49.01 | 8.05 | 5222.44 |
| 58 | 0 | 2 | 23.65 | 11.46 | 29.46 | 49.63 | 8.71 | 5359.12 |
| 58 | 0 | 3 | 23.85 | 12.24 | 29.55 | 48.99 | 7.20 | 5281.89 |
| 58 | 0 | 1 | 23.62 | 12.35 | 28.52 | 50.37 | 10.51 | 5273.24 |
| 58 | 0 | 2 | 23.03 | 11.92 | 29.30 | 50.09 | 8.88 | 5370.35 |
| 58 | 0 | 3 | 23.69 | 11.96 | 28.81 | 49.95 | 9.92 | 5374.52 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Egg no | Dry Wt % | Ash % DMB | EE % DMB | Protein % DMB | % Carb | Projected GE (cal/g) |
|---|---|---|---|---|---|---|---|---|
| 58 | 0 | 1 | 22.60 | 12.95 | 28.13 | 49.71 | 8.63 | 5233.01 |
| 58 | 0 | 2 | 23.40 | 13.42 | 28.07 | 49.96 | 8.47 | 5232.42 |
| 58 | 0 | 3 | 19.50 | 10.06 | 28.36 | 51.74 | 13.79 | 5494.78 |
| 59 | 0 | 1 | 25.01 | 11.00 | 30.56 | 51.76 | 10.21 | 5614.85 |
| 59 | 0 | 2 | 26.56 | 10.79 | 29.02 | 51.22 | 11.41 | 5490.06 |
| 59 | 0 | 3 | 25.97 | 11.70 | 22.12 | 51.01 | 17.20 | 5041.59 |
| 59 | 0 | 1 | 25.68 | 11.07 | 29.03 | 52.29 | 12.19 | 5568.06 |
| 59 | 0 | 2 | 26.47 | 11.52 | 28.60 | 48.75 | 8.63 | 5233.95 |
| 59 | 0 | 3 | 25.81 | 11.16 | 26.47 | 51.05 | 13.42 | 5314.70 |
| 59 | 0 | 1 | 27.62 | 12.00 | 29.51 | 48.26 | 7.41 | 5190.85 |
| 59 | 0 | 2 | 29.10 | 12.03 | 29.69 | 48.22 | 6.50 | 5233.81 |
| 59 | 0 | 3 | 28.87 | 12.15 | 29.59 | 48.78 | 8.58 | 5182.32 |
| 61 | 0 | 1 | 25.97 | 12.42 | 24.57 | 48.54 | 11.30 | 4966.13 |
| 61 | 0 | 2 | 25.84 | 11.61 | 26.86 | 50.43 | 11.96 | 5269.52 |
| 61 | 0 | 3 | 25.38 | 11.93 | 25.83 | 51.29 | 13.11 | 5293.87 |
| 61 | 0 | 1 | 23.57 | 11.35 | 28.02 | 50.41 | 11.04 | 5343.93 |
| 61 | 0 | 2 | 24.62 | 11.87 | 27.19 | 49.81 | 10.75 | 5227.29 |
| 61 | 0 | 3 | 24.04 | 11.70 | 27.77 | 52.89 | 13.43 | 5521.92 |
| 63 | 0 | 1 | 23.70 | 12.59 | 27.13 | 50.53 | 10.81 | 5256.55 |
| 63 | 0 | 2 | 25.59 | 13.69 | 27.30 | 49.99 | 9.00 | 5181.10 |
| 63 | 0 | 3 | 23.41 | 10.77 | 27.63 | 50.50 | 12.10 | 5351.54 |
| 65 | 0 | 1 | 23.66 | 11.32 | 26.52 | 52.48 | 14.42 | 5442.76 |
| 65 | 0 | 2 | 24.39 | 10.88 | 29.13 | 51.52 | 12.49 | 5575.26 |
| 65 | 0 | 3 | 24.69 | 10.73 | 26.68 | 55.20 | 17.69 | 5691.29 |
| 65 | 0 | 1 | 25.01 | 11.53 | 25.78 | 49.71 | 12.40 | 5150.53 |
| 65 | 0 | 2 | 25.39 | 12.89 | 26.82 | 49.98 | 10.27 | 5181.77 |
| 65 | 0 | 3 | 24.65 | 13.25 | 26.62 | 51.32 | 12.60 | 5202.13 |
| 65 | 0 | 1 | 25.92 | 11.72 | 26.62 | 50.44 | 11.91 | 5263.16 |
| 65 | 0 | 2 | 26.02 | 13.04 | 25.89 | 51.24 | 11.64 | 5265.47 |
| 65 | 0 | 3 | 26.20 | 12.08 | 26.79 | 52.71 | 13.84 | 5435.73 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Egg no | Dry Wt % | Ash % DMB | EE % DMB | Protein % DMB | % Carb | Projected GE (cal/g) |
|---|---|---|---|---|---|---|---|---|
| 65 | 0 | 1 | 24.26 | 13.13 | 26.86 | 51.80 | 11.81 | 5325.29 |
| 65 | 0 | 2 | 25.32 | 14.07 | 24.83 | 49.24 | 10.34 | 4962.28 |
| 65 | 0 | 3 | 23.91 | 13.99 | 24.64 | 52.66 | 14.04 | 5236.66 |
| 66 | 0 | 1 | 22.29 | 12.36 | 32.80 | 50.91 | 10.91 | 5326.32 |
| 66 | 0 | 2 | 22.63 | 12.17 | 29.02 | 49.65 | 9.62 | 5368.32 |
| 66 | 0 | 1 | 24.30 | 12.69 | 28.23 | 50.78 | 9.86 | 5336.68 |
| 66 | 0 | 2 | 24.39 | 11.99 | 28.19 | 52.70 | 12.51 | 5519.11 |
| 66 | 0 | 3 | 24.25 | 12.80 | 28.53 | 48.61 | 7.28 | 5170.70 |
| 66 | 0 | 1 | 24.21 | 14.47 | 25.25 | 52.75 | 12.37 | 5298.70 |
| 66 | 0 | 2 | 27.59 | 12.53 | 28.35 | 50.08 | 9.19 | 5291.69 |
| 66 | 0 | 3 | 25.80 | 12.28 | 27.37 | 49.74 | 10.08 | 5216.51 |
| 68 | 1 | 1 | 22.73 | 11.91 | 27.11 | 51.44 | 12.42 | 5356.16 |
| 68 | 1 | 2 | 22.88 | 12.01 | 27.26 | 51.46 | 12.20 | 5362.44 |
| 68 | 1 | 3 | 22.63 | 12.14 | 28.68 | 48.93 | 8.95 | 5183.33 |
| 68 | 1 | 1 | 24.17 | 11.97 | 26.68 | 51.25 | 14.52 | 5203.17 |
| 68 | 1 | 2 | 24.11 | 11.90 | 28.43 | 52.12 | 11.79 | 5488.30 |
| 68 | 1 | 3 | 24.18 | 11.98 | 26.59 | 51.33 | 12.77 | 5315.00 |
| 69 | 0 | 1 | 27.86 | 12.44 | 26.47 | 52.50 | 13.59 | 5386.76 |
| 69 | 0 | 2 | 26.72 | 12.77 | 25.78 | 52.54 | 13.80 | 5349.32 |
| 69 | 0 | 3 | 26.20 | 11.48 | 28.14 | 51.84 | 12.22 | 5463.60 |
| 69 | 0 | 1 | 26.09 | 10.92 | 26.62 | 52.79 | 15.25 | 5476.60 |
| 69 | 0 | 2 | 26.36 | 11.94 | 28.36 | 50.76 | 10.46 | 5371.10 |
| 69 | 0 | 3 | 26.80 | 11.41 | 28.60 | 53.19 | 13.18 | 5605.03 |
| 89 | 1 | 1 | 25.56 | 9.44 | 30.15 | 51.17 | 11.58 | 5601.31 |
| 89 | 1 | 2 | 26.91 | 10.12 | 30.21 | 51.67 | 11.33 | 5620.00 |
| 89 | 1 | 3 | 24.80 | 10.48 | 29.94 | 50.91 | 10.49 | 5529.05 |
| 89 | 1 | 1 | 26.30 | 10.14 | 31.38 | 52.61 | 11.10 | 5764.77 |
| 89 | 1 | 2 | 26.01 | 10.64 | 29.45 | 50.21 | 10.12 | 5436.59 |
| 89 | 1 | 3 | 27.36 | 10.72 | 30.95 | 51.30 | 8.77 | 5658.91 |
| 89 | 1 | 1 | 25.18 | 12.02 | 29.06 | 49.95 | 9.81 | 5287.70 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Egg no | Dry Wt % | Ash % DMB | EE % DMB | Protein % DMB | % Carb | Projected GE (cal/g) |
|---|---|---|---|---|---|---|---|---|
| 89 | 1 | 2 | 25.81 | 11.78 | 29.90 | 49.04 | 7.36 | 5323.08 |
| 89 | 1 | 3 | 26.91 | 10.40 | 31.00 | 50.37 | 8.97 | 5548.18 |
| 89 | 1 | 1 | 23.99 | 11.60 | 26.72 | 49.42 | 11.10 | 5178.12 |
| 89 | 1 | 2 | 24.57 | 12.15 | 28.62 | 49.61 | 8.84 | 5282.52 |
| 89 | 1 | 3 | 0.00 | 11.64 | 27.32 | 49.57 | 9.01 | 5316.45 |
| 92 | 1 | 1 | 25.87 | 11.74 | 28.42 | 49.69 | 10.67 | 5227.56 |
| 92 | 1 | 2 | 24.34 | 11.63 | 29.51 | 49.64 | 8.50 | 5355.69 |
| 92 | 1 | 3 | 25.44 | 12.29 | 29.87 | 49.95 | 7.79 | 5377.81 |
| 92 | 1 | 1 | 26.28 | 11.30 | 31.46 | 50.29 | 8.70 | 5466.99 |
| 92 | 1 | 2 | 27.20 | 12.06 | 31.88 | 48.32 | 3.02 | 5315.86 |
| 92 | 1 | 3 | 26.25 | 10.93 | 31.84 | 47.37 | 4.61 | 5329.03 |
| 92 | 1 | 1 | 27.76 | 12.69 | 30.89 | 47.44 | 3.86 | 5213.64 |
| 92 | 1 | 2 | 27.13 | 15.04 | 32.00 | 46.76 | -0.42 | 5141.57 |
| 92 | 1 | 3 | 26.97 | 12.72 | 31.80 | 47.04 | 2.52 | 5232.74 |
| 92 | 1 | 1 | 27.14 | 13.66 | 28.85 | 47.76 | 5.25 | 5087.51 |
| 92 | 1 | 2 | 25.97 | 14.28 | 26.56 | 47.39 | 4.55 | 5015.65 |
| 92 | 1 | 3 | 26.32 | 12.98 | 28.70 | 47.87 | 6.92 | 5070.97 |
| 99 | 1 | 1 | 28.12 | 10.98 | 27.94 | 52.43 | 13.93 | 5495.91 |
| 99 | 1 | 2 | 25.95 | 10.71 | 27.95 | 52.56 | 13.89 | 5541.31 |
| 99 | 1 | 3 | 25.94 | 10.91 | 27.44 | 55.08 | 16.72 | 5712.80 |
| 99 | 1 | 1 | 26.79 | 11.02 | 28.92 | 49.93 | 10.89 | 5317.29 |
| 99 | 1 | 2 | 26.06 | 10.78 | 29.25 | 51.68 | 11.66 | 5541.39 |
| 99 | 1 | 3 | 27.18 | 10.69 | 29.42 | 49.59 | 9.48 | 5381.79 |
| 99 | 1 | 1 | 27.83 | 11.59 | 29.92 | 49.07 | 7.56 | 5334.30 |
| 99 | 1 | 2 | 27.25 | 11.72 | 29.77 | 47.98 | 7.48 | 5173.38 |
| 99 | 1 | 3 | 27.75 | 11.38 | 30.16 | 50.60 | 9.10 | 5479.83 |
| 99 | 1 | 1 | 26.64 | 11.65 | 27.01 | 52.86 | 11.36 | 5641.04 |
| 99 | 1 | 2 | 25.60 | 13.14 | 29.07 | 50.18 | 7.97 | 5319.41 |
| 99 | 1 | 3 | 26.20 | 13.16 | 29.75 | 50.86 | 7.26 | 5452.15 |
| 118 | 1 | 1 | 25.93 | 11.45 | 26.55 | 53.71 | 15.71 | 5528.46 |

Appendix 2. Reproductive information and egg nutrient composition of green turtles nesting at French Frigate Shoals.

| Turtle ID # | FP/Non-FP (1/0) | Egg no | Dry Wt % | Ash % DMB | EE % DMB | Protein % DMB | % Carb | Projected GE (cal/g) |
|---|---|---|---|---|---|---|---|---|
| 118 | 1 | 2 | 24.79 | 11.49 | 28.09 | 52.74 | 13.17 | 5535.98 |
| 118 | 1 | 3 | 25.30 | 11.07 | 26.92 | 53.70 | 15.71 | 5563.17 |
| 118 | 1 | 1 | 26.75 | 11.47 | 28.84 | 52.19 | 11.88 | 5533.72 |
| 118 | 1 | 2 | 26.51 | 11.35 | 27.13 | 51.70 | 13.22 | 5399.15 |
| 118 | 1 | 3 | 26.48 | 12.58 | 26.77 | 55.36 | 17.28 | 5562.23 |
| 118 | 1 | 1 | 25.97 | 13.10 | 27.33 | 50.75 | 10.33 | 5267.30 |
| 118 | 1 | 2 | 27.99 | 13.06 | 25.92 | 52.41 | 10.82 | 5474.56 |
| 118 | 1 | 3 | 26.75 | 12.92 | 27.84 | 50.03 | 9.17 | 5249.47 |
| 118 | 1 | . | . | . | . | . | . | . |
| 141 | 1 | 1 | 24.34 | 11.58 | 26.06 | 52.44 | 14.80 | 5389.81 |
| 141 | 1 | 2 | 23.55 | 11.66 | 26.79 | 52.99 | 12.86 | 5411.80 |
| 141 | 1 | 3 | 24.09 | 11.09 | 27.91 | 51.67 | 12.66 | 5451.64 |
| 141 | 1 | 1 | 24.01 | 12.41 | 27.60 | 51.50 | 11.48 | 5370.50 |
| 141 | 1 | 2 | 24.11 | 11.13 | 26.92 | 53.28 | 15.23 | 5525.98 |
| 141 | 1 | 3 | 24.66 | 12.33 | 26.51 | 53.89 | 15.05 | 5508.32 |

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