A Histological Comparison of Tumor Characteristics in Adult Nesting Female and Foraging Juvenile Hawaiian Green Sea Turtles (*Chelonia mydas*): Is Regression Related to Age?

With a Partial Historical Analysis of Tumor Trends on East Island and a Map of the Global Distribution of Fibropapillamatosis.

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Note of Importance

All 2005 field research took place in the Hawaiian Islands National Wildlife Refuge with the permission of the United States Fish and Wildlife Service (USFW), Department of the Interior. The support and permission of the United States Fish and Wildlife Service was essential to the completion of this project.

Division of Responsibilities

My master's project benefited a great deal from work that was not my own and from data collected by others previous to the start of my thesis.

Dr. Terry Spraker of Colorado State University analyzed all of the tumor samples. He was responsible for diagnosing all of the histological characteristics.

All of the juvenile tumor samples were collected by George Balazs and Alonso Aguirre in Kaneohe Bay in 2000. I collected the adult tumor samples on East Island in 2005.

The National Marine Fisheries Service and the United States Fish and Wildlife Service graciously allowed me to analyze over 30 years of past data. Shawn Murakawa and George Balazs of the National Marine Fisheries Service (NMFS) retrieved the data. Without this data I would not have been able to give an accurate picture of the historical trends of nesting and tumor rates on East Island.

For the past three years I collected field data on East Island with three other biological technicians: Erin Green, Chris Nappi, and Lisa Canty. Many other biologists, chief among them George Balazs, collected field data for the past 33 years on East Island. Much of the data in this thesis is a result of their effort.

O. Roger Anderson and Martin Stute helped in the statistic analysis of data.

John Michelson of the Center for International Earth Science Information Network (CIESIN) helped a great deal with the mapping portion of the project.

This thesis was a collaborative effort in every sense.

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Abstract

Fibropapillomatosis is a neoplastic disease with a worldwide distribution primarily affecting green sea turtles (*Chelonia mydas*). A global database and map of the disease was assembled from published reports to serve as a baseline measurement of the extent of FP. The etiologic agent of the disease has not been isolated, and more study is needed to understand the nature of the disease. Two field surveys were conducted in the Hawaiian Islands in order to collect tumor samples from two distinct age groups of green sea turtles. Tumor samples were collected from 11 adult turtles nesting on East Island and from 30 juvenile turtles foraging in Kaneohe Bay. In all, 17 tumor samples were collected from nesters and 52 tumor samples were collected from juveniles. Histologically, 15 of 17 adult tumors were characterized as regressing and 1 of 52 juvenile tumors was characterized as regressing. Statistical analysis revealed a significant difference between the rate of regression in the tumors of adult nesters (88%) and those of foraging juveniles (2%). This is the first comparative histological study of rates of regression in adult and juvenile sea turtles of the same species.

Introduction

Fibropapillomatosis (FP) is a neoplastic disease with a worldwide distribution that primarily affects green sea turtles (*Chelonia mydas*) (Herbst 1994; Williams et al. 1994). The disease is characterized by external and internal tumors, ranging from 1 mm to greater than 30 cm in size, which can negatively affect vision, respiration, cardiopulmonary function, digestion, feeding, and locomotion (Herbst 1994; Balazs and Pooley 1991; Aguirre et al. 2002). Severely afflicted turtles have demonstrated lower immune response, immunosupression, higher chronic stress, slower growth rates, and lower overall health than turtles free of tumors (Aguirre et al. 1995, Balazs et al. 2000, Aguirre and Balazs, 2000; Work et al. 2001). However, recent work suggests turtles with FP may not have slower somatic growth rates (Chaloupka and Balazs 2005). Ultimately, the tumors may lead to increasingly debilitating conditions and death.

FP was first identified in wild turtles around the southern tip of Florida in the late 1930's (Smith and Coates 1938, Lucke 1938) and in the Hawaiian Islands in 1958 (Balazs and Pooley 1991). Over the last 20 years, reported incidence of the disease has dramatically increased. The disease has been reported in at least 36 countries and territories, and in every ocean located within the tropics. There is an absence of published sightings for the Mediterranean and the west coast of South America. There are only two published sightings from the east coast of Africa to the west coast of India. Though the disease has been reported in every species of sea turtle, populations of *C.mydas* in Australia, Florida, Hawaii, Indonesia, and the Caribbean are affected most severely (Aguirre et al. 2000, Ehrhart 1991; Balazs and Pooley, 1991; Adnyana 1997; Herbst 1994).

In order to display the extent and prevalence of the disease, an interactive GIS database and map were constructed from published reports of FP. This database will serve as a baseline from which the rate and spread of the disease can be chronicled over space and time. Eventually, the map will be placed online through Wildlife Trust and

CIESIN to serve as a global repository for data regarding the disease. A screenshot of the interactive map is included in Figure 1 and an interactive version of the map and database are included at the end of this thesis (Appendix, Compact Disc 1).

The etiology of fibropapillomatosis is unknown. Three viruses have been associated with the tumors: a herpes virus, a papillomavirus, and a retrovirus (Aguirre and Spraker 1996; Lu et al. 1998, 2000; Casey et al. 1997). Observations suggest the disease may be associated with disturbed coastal habitats near areas of high human density and areas of shallow wave activity (Adnyana et al. 1997, Foley et al. 2005). A number of cofactors have been proposed to play a role in tumor formation. These include bacteria, pollutants, biotoxins, uv light, and temperature (Aguirre et al. 1994, Herbst 1994, Landsberg 1999, Hermanussen 2004).

Understanding the disease could yield results that are broadly beneficial. Coastal ecosystems are an important resource for wildlife habitat, fisheries, and recreation; and represent an important habitat for sea turtles from the later juvenile years through adulthood. Anthropogenic forces, such as pollution, climate change, and fishing, have an effect on all marine ecosystems, including coastal ecosystems. Since 1970, disease reports for sea turtles have increased and may reflect changes to their environment (Ward et al. 2004). Sea turtles can be a valuable sentinel species because they are long lived, live in water but breath air, have high fidelity to foraging sites, are a flagship species that attracts public attention, and, though physically robust, are susceptible to biological and chemical insults (Aguirre and Lutz 2004). Understanding the epidemiology of FP is important because sea turtles could serve as an effective tool to monitor ecosystem health in local warm-water coastal habitats, but correlations with physical and chemical characteristics of water and other factors need to be made (Aguirre and Lutz 2004).

All seven species of sea turtles are either threatened or endangered. Sea turtles are migratory animals that may travel thousands of miles over their lifetime, utilizing various oceanic and coastal habitats during different life stages. As a result, they cannot be saved in any one place or by controlling any one phase in their life cycle (Carr 1984). Sea turtles need a clean and productive marine environment, as well as peace on the beaches, to ensure their survival (Carr 1984).

Hawaiian green sea turtles are a genetically distinct metapopulation that primarily nests on French Frigate Shoals with numerous distinct foraging grounds within the Hawaiian Archipelago (Figure 2) (Balazs and Chaloupka 2004). Studies of green sea turtles have been undertaken in the Hawaiian Archipelago for more than 30 years, revealing that FP affects turtles differently depending on location and age.

East Island is an 11-acre island within French Frigate Shoals, a coral atoll located approximately 550 miles from Oahu (Figure 3). Over 90 % of Hawaiian green sea turtles nest on the islets of the atoll, and over 50 % of these nest on East Island (Balazs 1976). The estimated number of turtles nesting on East Island has risen in a cyclical fashion from 1973 to 2005 (Figure 4, Table 1) (Balazs and Chaloupka 2004, Balazs and Chaloupka 2005, Balazs unpublished data). The increase is most likely a consequence of cessation of habitat damage at the rookery level from the 1950's onwards and also protection since the mid-1970's of turtles from harvesting in coastal waters around the main Hawaiian Islands (Balazs and Chaloupka 2004). The quasi-periodicity in nester abundance suggests that female green sea turtles resident in the numerous Hawaiian Archipelago foraging grounds migrate to nest at the French Frigate Shoals rookery most

often every 3 or 4 years (Balazs and Chaloupka 2004). The substantial annual fluctuations in the rise is a characteristic of green turtle nesting populations due to a variable proportion of females preparing to breed each year in response to strong spatially correlated ocean climate variability (Balazs and Chaloupka 2004).

Between 1973 and 2005, the number of turtles identified with tumors on East Island has ranged between zero and 18 % (Balazs and Chaloupka 2004, Balazs, NOAA, personal communication). The first known occurrence of a turtle with tumors on East Island occurred in 1974 (Davidson 2001). The first known histological diagnosis of a turtle with FP at French Frigate Shoals occurred in 1979 (Harshberger 1991). The increase in nester abundance on East Island has occurred despite the relative increase in FP in some foraging grounds in the main Hawaiian Islands (Balazs and Chaloupka 2004).

The prevalence of FP in different foraging grounds in Hawaii has varied over space and time. In Kaneohe Bay (Figure 5), a semi-enclosed disturbed habitat on the island of Oahu, prevalence of the disease ranged from 46 to 69 % between 1989 and 1997 (Balazs et al. 2000). The first reported case of FP in Hawaii occurred here in 1958 (Balazs and Pooley 1991). Palaau, on the southern coastline on Molokai, showed an increase in the mid-to-late 1980's, with prevalence decreasing in recent years (Balazs et al. 1998, Balazs, NOAA, personal communication). On the Kona Coast of the Big Island, a relatively undisturbed near shore environment with good coastal mixing, the disease has gone virtually unreported (Aguirre et al. 2000). Necropsies of 255 turtles stranded from 1993 to 2003 revealed the majority (74%) were found on the most populated island of Oahu (Work et al. 2004).

No cases of FP have been documented in hatchling or pelagic juvenile turtles (Balazs and Pooley 1991). Observations suggest when pelagic juveniles (35-45cm) recruit to near shore habitat they are free of tumors, which develop after time foraging within their new habitat (Balazs and Pooley 1991; Aguirre et al. 1994). The majority of stranded turtles found in Hawaii since 1982 have been juveniles (35-65 cm) (Murakawa et al. 2000, Work et al. 2004).

Discovering the cause of fibropapillamatosis remains elusive, and so studying how the disease evolves over time may yield clues to its beginnings. Observational and capture studies have documented a regression of tumors on individual turtles over time in Hawaii (Balazs et al. 2000; Bennet et al. 2001) and Florida (Hirama and Ehrhart 2001; Erhart 1991). Furthermore, observational evidence from Honokwai, Maui suggests that regression of tumors occurs more often in adults and subadults than in juveniles (Bennett et al. 2000).

Histological analysis of tumor tissue has revealed characteristics indicative of regression. Tumors taken from adult olive ridley turtles (*Lepidochelys olivacea*) revealed characteristics suggestive of regression in 48 % of the samples (Aguirre et al. 1999). In samples taken from juvenile green sea turtles in Hawaii, the same characteristics were seen in 2 % of the samples (Aguirre et al. 1994, 1998).

A histological study comparing the rates of regression between juvenile and adults of the same species has not been performed. This study will compare the rate of characteristics indicative of regression in the tumors of adult female Hawaiian green sea turtles nesting on East Island to those in the tumors of juveniles captured from Kaneohe Bay, Oahu. The objective is to determine whether regression is related to age.

Methods

Two separate field surveys were conducted to obtain tumor samples of nesting females and juvenile turtles. An intense survey of nesting females was undertaken on East Island, French Frigate Shoals from May 31 to July 10, 2005. Juvenile turtles were captured by hand during snorkel surveys conducted by George Balazs and Alonso Aguirre in Kaneohe Bay, Oahu during November of 2000.

Turtles were identified, tagged, measured, and checked for tumors. A description of the size, pigmentation, surface, number, and location of tumors was recorded.

Biopsies were collected from fibropapillomas of the neck, flippers, tail, and body with either a 6 mm dermal punch or by excisional biopsy with a scalpel. A topical antibiotic ointment was applied after each biopsy with wounds left uncovered for drainage in accordance with veterinary advice. All tumors and normal skin samples were placed in 10 % neutral phosphate-buffered formalin. In the lab, the tissues were embedded in paraffin, sectioned to 5-6-um thick, and stained with hematoxylin and eosin.

Histopathologic characteristics were recorded for each tumor based on criteria previously published (Jacobsen et al. 1989, Aguirre et al. 1994). There are nine characteristics of the dermis diagnostically related to the regression process. A tenth characteristic, regression, was diagnosed by a trained pathologist who took all nine of the previously mentioned characteristics into account and made a decision based on the state of the tumor. A chi-square test was performed using StatView 512+ software (SAS Institute, Inc., Cary, NC) to determine whether the difference between any of ten characteristics was significant. Yates' correction for continuity was applied to tables when any of the cells had frequencies less than five in order to obtain an arbitrary, conservative adjustment to chi-square. Significance was based on a 95 % confidence interval, in which a P Value $\leq .05$ was judged to be statistically significant.

Additional steps in gathering and analyzing data were taken for the field survey on East Island. A brief description of the full procedures for working up nesting females, developed by George Balazs of the National Marine Fisheries Service, follows.

On East Island, an individual number was harmlessly etched on the carapace of each identified turtle with a dremel mototool (Balazs 1995). The scratched surface was then painted to allow the turtle to be identified from a distance during subsequent nesting attempts. All four flippers were checked for metal tags. The hind flippers were checked for pit tags using a scanner. Those turtles lacking pit tags were tagged during appropriate nesting activity. The carapace of each turtle was measured for straight carapace length with calipers and curved carapace length with flexible tape. The turtle was checked for tumors by running the hands along the flippers and body, and by shining a partially covered flashlight over the animal. It should be noted that the occurrence of tumors on East Island turtles is likely underreported due to the constraints presented by working alone in a remote field location at night with a threatened species during a vital reproductive activity. The ventral surfaces of the turtles were not checked for tumors as it would have required turning the animals over. It should also be noted that 2005 was the first year in which identification and sampling of the tumors was part of an outside project, which may have led to an increased emphasis on identification. Tumors were assigned one of four approximate sizes in the field using a hand reference method (Figure 6). Those tumors sampled for histology were measured with a ruler. All sampled tumors

were photographed and are included in the photographic database included at the end of this thesis (Appendix, Compact Disc 2). All information taken in the field was recorded in field notebooks and then transferred to data sheets.

A brief historical analysis of tumored turtles was performed using data provided by the National Marine Fisheries Service. The size, location, and number of tumors were recorded in the field as described above and then entered into a comprehensive database developed by Shawn Murakawa and George Balazs of the National Marine Fisheries Service. Records from 2000 to 2005 were retrieved with the help of Shawn Murakawa. The size, location, and number of tumors were tabulated for each of the past six years. In addition, one of four tumor scores was assigned to each turtle based on a previously developed method (Work and Balazs, 1999). The scores reflected a spectrum of severity that ranged from non-afflicted (0), to lightly (1), to moderately (2), to heavily afflicted (3). The tumor scores were then tabulated for each year. It is likely that the number of turtles scored zero is over reported due to the field constraints mentioned in the previous paragraph and the fact that a portion of the turtles identified each year may not be checked for tumors due to time constraints.

Regression analysis was performed using Excel Software to determine whether any trends in tumor size or tumor score between 2000 and 2005 were statistically significant. Significance was based on a 95 % confidence interval, in which a P Value $\leq$ .05 was judged to be statistically significant.

Access to the National Marine Fisheries Service Database also allowed the history of previously tagged 2005 tumored turtles to be analyzed. Due to time and logistical constraints, only previously tagged tumored turtles had their tag histories retrieved. Previously tagged turtles seen in 2005 with no tumors identified did not have their histories retrieved. It is possible that turtles with FP in the nesting population are underreported because turtles identified without tumors in 2005 may have had the disease previously and their tumors have regressed.

Results

Field Observations

During the 2005 field survey on East Island there were 333 turtles identified, and all but eight of these were examined for tumors. Thirty-two (9.6%) of the 333 turtles identified had tumors.

The Ozobranchus leech, a parasite and possible mechanical vector of FP (Greenblatt et al. 2005), was identified on the eyes of an untumored nesting turtle.

During the 2000 field survey in Kaneohe Bay, Oahu, a total of 30 turtles were selectively captured with tumors.

Gross Pathology and Historical Data

On East Island, skin and tumor specimens were collected from 11 of the 32 tumored turtles; with 17 tumors being examined from these 11. The total number of observed tumors for all 32 turtles was 115. The average curved carapace length of turtles

with FP was 98.3 cm and the average straight carapace length was 91.4 cm (31 out of 32 were measured for straight carapace length).

Tumors sampled ranged from approximately 1 cm to greater than 6 cm in diameter. Tumors identified ranged from 1 cm to greater than 10 cm in diameter. Of the 115 tumors identified in 2005, approximately 62 % were categorized as size 1, 28 % as size 2, 10 % as size 3, and less than one % as size.

From 2000 to 2005, there were 500 tumors identified on 244 turtles (Figure 7, Table 2). Some tumored turtles came up and were counted more than once during this six year timespan. Of the 500 tumors recorded between 2000 and 2005, 253 (50.6%) were categorized as size 1, 172 (34.5%) were categorized as size 2, 60 (12%) were categorized as size 3, and 15 (3%) were categorized as size 4. In each individual year the percentage of tumors of each size decreased as the tumor size category increased. Statistical regression analysis, performed on the percentage of tumors of each size on an annual scale, revealed that the number of size 1 tumors increased over the six year span, while the number of size 2, 3, and 4 tumors decreased over the same span (Figure 8, Table 3). However, none of the P Values obtained from statistical regression analysis proved to be statistically significant.

Most of the turtles identified with FP in 2005 were lightly tumored, though some were severely afflicted, with one turtle having more than 20 tumors. Of the 32 tumored turtles identified in 2005, 66 % were lightly afflicted (score 1), 22 % were moderately afflicted (score 2), and 12 % were heavily afflicted (score 3). In 2005, lightly afflicted turtles had a longer average straight carapace length than moderately and heavily afflicted turtles, though the relationship between straight carapace length and tumor score varied with each year analyzed (Table 4).

For the 2160 turtles identified between 2000 and 2005,
1915 were scored 0 (88.66%), 174 (8.05%) were scored 1, 50 (2.4%) were scored 2, and 21 (.87%) were cored 3 (Figure 9, Table 5). For each individual year from 2000 to 2005, the percentage of turtles of each score decreased as the magnitude of the score increased. Statistical regression analysis revealed that the percentage of untumored turtles (score 0) increased over the six year span while the percentage of tumored turtles scored 1, 2, and 3 decreased over the same time period (Figure 10, Table 6). The increase in untumored turtles over the six year span proved to be statistically significant, with a P Value of .04. Statistical regression analysis of the percentage of tumored turtles over that same time span yielded a P value (.0525) that was borderline significant. However, these values were indicative of a downward trend in a longer wavelike pattern.

When statistical regression analysis was performed on a longer timescale, it was revealed that the number of tumored turtles has increased since 1990 in a manner that is borderline statistically significant (P Value = .05) (Figure 11, Table 7). However, when the *percentage* of tumored turtles was analyzed in the same manner over the same timescale, though there was an increase, the resulting P Value was not statistically significant (P Value = .44) (Figure 12, Table 8).

In 2005, tumors were identified on the jaw (1, 1%), eyes (23, 20%), front flippers (69, 60%), neck (13, 11%), hind flippers (5, 4%), cloaca (2, 2%), and other areas (2, 2%). The general location of tumors agreed with statistics kept for turtles from 2000-2005, with the majority of tumors found on the anterior portion of the body and no bias between tumors found on the right and left side (Figure 13, Table 9). Tumors identified in 2005

were white, pink, gray, yellowish, and consistent with the color of the skin. They were papillary, smooth, flaky, and pedunculated.

Of the 32 tumored turtles identified in 2005, 12 were tagged prior to 2005 (Figures 14 A and B, Table 10). Of these twelve, half were identified with tumors in previous nesting seasons. Of these six, two had less tumors identified prior to 2005, two had more tumors identified prior to 2005, and two had the same number of tumors identified prior to 2005. All of the previously tagged turtles had no tumors identified in the first year they came up to nest on East Island.

In Kaneohe Bay, skin and tumor samples were collected from 30 turtles, with 52 tumors being examined from these turtles. All 30 turtles were juveniles, and had curved carapace lengths between 45 and 65 centimeters. The tumors were found on various parts of the turtle and were papillary, smooth, flaky, and pedunculated.

Histopathology

In all, 17 tumors from adults and 52 tumors from juveniles were diagnosed as fibropapillomas. The characteristics associated with the process of regression are shown in Figure 15 and Table 11. (A listing and corresponding glossary for all of the diagnosed characteristics of the tumors can be found in Figure 16 and Table 12.)

Of the nine characteristics used to diagnose regression, four had statistically significant differences between juveniles and adults. The difference between adults and juveniles for the characteristic of cellularity was highly significant, with a (P Value = .0001). In adults, the dermis was characterized by very low cellularity in 12 out of 17 tumors. The dermis of juveniles was characterized by moderate cellularity in 48 out of 52 tumors. There were three patterns of fibroplastic proliferation seen within the dermis. The only anomalous pattern of cellular architecture that showed a significant difference between juvenile and adult tumors was the haphazard arrangement of fibroblasts (P Value = .0001). In adults, only 2 out of seventeen tumors displayed a haphazard arrangement of proliferating cells, while 49 out of the 52 juvenile samples displayed the same characteristic. Lymphocytic cuffing of vessels within the tumor was determined to be statistically significant (P Value = .0096). Most of the adult tumors, 14 out of 17, had vessels surrounded by lymphoid cells within the tumor. In juvenile tumors, less than half (22 out of 52) had lymphoid cells within the tumor. However, comparing the rates of vessels cuffed by lymphocytes under the tumor did not yield a statistically significant number. Neovascularization within the tumor was determined to be statistically significant (P Value = .0158). While most of the adult tumors, 14 out of 17, had proliferation of blood vessels in tissue not normally containing them, all 52 of the juvenile tumors displayed the same characteristic.

The rate at which small foci of necrosis were found within the tumor was relatively low for both populations surveyed, and the difference between the two populations was not statistically significant. Inflammation in the tumor was not analyzed because it was not examined in juvenile tumors.

The difference between adults and juveniles for the characteristic of regression was highly statistically significant with a P Value of .0001. Fifteen out of 17 adult tumors were diagnosed as regressing, and only one out of 52 juvenile tumors was

diagnosed as regressing. In other words, only 2 % of juvenile tumors were diagnosed as regressing while approximately 88 % of adults were diagnosed as regressing.

Discussion

The present study is the first histological comparison of tumor regression between adult and juvenile green sea turtles. Prior studies analyzing the histological features of FP concentrated on either juveniles or adults. In a survey of nesting adult female olive ridley sea turtles (Lepidochelys olivacea), 48% (20 of 42) had extensive areas of lymphocytic inflammation within the tumor tissue, and nine of the remaining tumors were marked by histologic changes of inflammation with mild degeneration within the tumor (Aguirre et al. 1998). In comparison, surveys of juveniles collected during field situations in the Hawaiian Islands resulted in only 1 of 52 tumors (2%) showing similar characteristics (Aguirre et al. 1994).

The current study is in agreement with prior results, but is more exact in that it details the rate of characteristics suggestive of regression in adults and juveniles of the same species living within the same population. Over 88% (15) of the adult nesting females had tumors characterized as regressing, while only 2% (1) of juveniles had tumors characterized as regressing. There were fewer tumors sampled from the adult population (17 tumors from 11 turtles) than from the juvenile population (52 tumors from 30 turtles). Still, such a low P Value related to the diagnosis of regression suggests the difference between the two populations should be taken seriously.

While the results do show that adult nesting females on East Island have a higher rate of regressing tumors than juveniles from Kaneohe Bay, there are other factors to consider. It is important to consider that adult nesting females in Hawaii have to travel hundreds of miles to reach French Frigate Shoals, and so it is a possibility that there are more adults with regression because turtles severely afflicted with FP, or that have an advancing case of FP, are unable to make the journey. The high proportions of small tumors and of lightly tumored turtles seen between 2000 and 2005, as well as the fact that the majority of tumors sampled were regressing, could be used to support this hypothesis. Also, the frequency of fibropapillomatosis in nesting turtles may be an inappropriate indication of infection within a population because severely affected adults may not reach the necessary fat condition for egg production and nesting (Limpus and Miller 2004).

Why is the rate of regression higher among nesting females than juveniles? It is possible that adult turtles could be more resistant to the disease (Aguirre et al. 1999). It could also be that juveniles susceptible to the disease may have died off and only those with resistance to the tumors have survived to adulthood. Adult turtles may have survived the disease as juveniles and their tumors are now in regression (Aguirre et al. 1999).

The tag database provided by National Marine Fisheries Service allowed the history of the 2005 tumored turtles to be obtained, which may give some clues to the progression of the disease. In the first year each of the 2005 previously tagged tumored turtles were seen on East Island no tumors were identified. No turtle was identified as having tumors, coming up in a later year without tumors, and then returning subsequently with tumors identified. This data is supported by the fact that no published record was found of a turtle having all of its tumors regress, and then developing new tumors.

The historical data presented here suggests that the turtles may pick up FP as adults. At least two of the tumored turtles on East Island in 2005 were witnessed without tumors for a period spanning at least 19 years before they developed tumors. The history of these turtles has large gaps, and it is unknown whether these turtles may have had tumors as juveniles or in years between nesting attempts. Still, the data does provide evidence that adults may develop tumors, and show characteristics indicative of regression, in a relatively short time period.

It is important to note that turtles with no tumors identified in 2005 did not have their histories retrieved to determine if they had tumors in previous seasons. Turtles that have had FP in the nesting population are likely underreported because the history of previously tagged untumored turtles was not reviewed. Photographic evidence compiled at the Honokwai foraging site suggests that turtles with FP in the Hawaiian population are underreported because many turtles that had the disease have tumors that have undergone full regression (Bennet et al. 2002). Documented cases of regression in Honokwai, Maui show that full tumor regression can occur in a period of less than a year (Bennet et al. 2002).

That a significant percentage of adult tumors were found to be in a state of regression supports the hypothesis that a portion of the population may be able to cope with the tumors. Whether regression of tumors indicates the turtle is able to permanently deal with the disease is unknown.

Sampling surveys have revealed a lower percentage of turtles with FP in at least one foraging area in recent years (Balazs, NOAA, personal communication). The decrease in the percentage of nesting tumored turtles over the last six years, as revealed by regression analysis, may be a factor of this. However, there are relatively few long term surveys of foraging grounds due to the difficulty and expense of such surveys so caution must be taken when interpreting this figure. How the disease is affecting turtles in various foraging grounds around the Hawaiian Islands is not known. Increased studies in different foraging areas would help in understanding the effect of FP on the overall population. However, monetary and logistical constraints are limiting factors.

The increase in nester abundance on East Island has occurred despite the relative increase in FP in some foraging grounds in the main Hawaiian Islands and the increase in the number of strandings in the main Hawaiian Islands (Balazs and Chaloupka 2004, Murakawa et al. 2000). Though the annual number of turtles with FP has increased on East Island over at least the last fifteen years, the percent of turtles affected shows no significant increase. The annual number of turtles stranding in the main Hawaiian Islands has increased from 10-20 cases in 1982 to 200-300 cases a year in the late 1990's and early 2000's (Murakawa et al. 2000, Balazs unpublished). Fibropapillomatosis has been a major cause of these strandings as determined by hundreds of necropsies by veterinary researchers (Murakawa 2000). The percentage of stranded turtles with FP ranged from 47 to 69 % between 1982 and 1998 (Murakawa et al. 2000). Of the 3860 stranded turtles identified between 1982 and 2003, the annual number with FP has increased, though the percentage of stranded turtles with FP shows no specific trend (Balazs, NOAA, personal communication).

This is not to say that the increase in the nesting population indicates that the disease does not have an impact on the population. It is to say that the number of nesting females continues to increase as a result of protection of habitat and protection from

fishing take, despite the increase of the disease in some foraging grounds and the increase in the number of turtles stranded with FP. It is important to stress that the species continues on the way to recovery in spite of the presence of this debilitating disease. Continued and increased study is needed to address how the disease will affect the population as it continues to grow.

This study demonstrated that the rate of regression in nesting adult females is significantly higher than the rate of regression in foraging juveniles. Additional sampling and histological analysis of tumors taken from adults foraging in the same pastures as juveniles will help in understanding whether all adults have a higher rate of regression than juveniles.

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Alonso Aguirre was amazing in his willingness to meet with me on short notice and give quick and valuable feedback. His help narrowing down a topic and offering his personal resources helped make my job much easier. His expertise and advice were key to the success of this project. This project would not have been possible without the resources and encouragement he provided. I am especially grateful for his help and guidance.

Most of all I am thankful for the help and encouragement of my five classmates and family.

Glossary

Definitions and descriptions are general and are formed from a combination of sources.

Acanthosis – An increase in the thickness of the stratum spinosum of the epidermis

Acantholysis – Separation of the prickle cell layer of the stratum spinosum of the epidermis, resulting in atrophy of the prickle cell layer.

Bacteria – One of two major classes of prokaryotic organisms. Bacteria are small non-compartmentalized organisms with circular DNA and ribosomes.

Blister between SS, SB, and SC – A local swelling of the skin between or within any of the three layers of the epidermis

Cellularity – The degree, quality, or condition of cells that are present

Cleft – A split within the tissue

Collagen – the fibrous protein constituent of bone, cartilage, tendon, and other connective tissue that converts into gelatin by boiling

Cytoplasmic Vacuolar Degeneration – the formation of vacuoles in the cytoplasm of cells of a tissue

Deep Fibroblastic Reaction – Profound inflammation of the fibrous tissue

Dense or Loose Dermis - The state of cells in the dermis, either they are crowded closely together, or relatively loosely packed, or both in different places

Fibroblastic – Producing fibrous tissue

Fibrous tissue - Most connective tissue has threadlike elements, but the term usually refers to tissue laid down at a wound site. Usually it is well vascularized (with a lot of blood vessels) at first (granulation tissue), but later avascular (not supplied by or associated with blood vessels) and dominated by collagen rich extracellular matrix, forming a scar.

Fibroplastic Proliferation

- Haphazard – Dermal cells arranged in a scattered manner that has no set pattern
- Sheets – Dermal cells arranged in a broad horizontal or vertical pattern
- Interweaving Bundles – Dermal cells arranged in connected spiral or ring-like patterns

Foci of Lymphocytic Inflammation – Visible center or origin of white blood cell intrusion

Foci of Necrosis – The visible origin or center of cell death

Foci of Sarcoma – Center of an abnormal growth arising from supportive tissue such as bone, cartilage, fat, or muscle

Fungus – A group of eukaryotic protists, including mushrooms, yeasts, molds, etc., which are characterized by the absence of chlorophyll and by the presence of a rigid cell wall composed of chitin, mannans, and cellulose

Granuloma – chronic inflammatory lesion (traumatic discontinuity of tissue or loss of function of a part) characterized by a large number of cells of various types (lymphocytes, fibroblasts, giant cells, parasites), some degrading and some repairing the tissue

Individual Cell Necrosis – Sum of changes indicating individual cell death

Inflammation – A localized protective response illicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent and the injured disease. Histologically inflammation involves the dilation of arteries and veins, with increased blood flow, exuding of fluids containing bloodclotting proteins and white blood cells into the inflammatory focus

Intercellular Edema – the presence of abnormally large amounts of fluid between the cells

Intracellular Edema – the presence of abnormally large amounts of fluid within the cells

Keratin Pearls – a focus of central keratinization (development of a horny cell layer) within concentric layers of abnormal squamous cells

Lymphocytic of S.B. – Presence of a type of white blood cell within the Stratum Basale (bottom layer of the epidermis)

Mites – Ectoparasites of the phylum arthropoda and the class arachnida related to spiders. Common parasites of animals and humans

Margination of Chromatin – Visible division of chromatin (the stainable substance of a cell nucleus consisting of DNA, RNA, and various proteins)

Metapopulation – A group of spatially separated populations which interact at some level.

Mitotic Figures – the microscopic appearance of a cell undergoing mitosis, or, a cell of which the chromatin is visible by light microscope

Necrosis – The sum of the morphological changes indicative of cell death and caused by progressive degradation action of enzymes, it may affect groups of cells or part of a structure of an organ

Neoplastic – The pathological process that results in abnormal new growth of tissue that grows by cellular proliferation more rapidly than normal, continues to grow after stimuli ceases, shows lack of structural organization with normal tissue, and forms a distinct mass which may either be benign or malginant

Neovascularization – Proliferation of blood vessels in tissue not normally containing them

Nucleoli – plural of nucleolus – a small dense body within the nucleus of the eukaryotic cell, visible during interphase (the stage of the cell when it is not in mitosis). Contains RNA and protein and is the site of synthesis of ribosomal RNA. The nucleolus surrounds a site of one or more chromosomes in which are the repeated copies of DNA coding for ribosomal RNA.

Orthokeratotic Hyperkeratosis – A straight or upward growth of the keratin layer of skin

Papillary Projection Epidermis – A fingerlike projection into the epidermis

Pigment – Any one of the colored substances found in animal or plant tissue

Pseudoepitheliomatous Hyperplasia – A benign marked increase and downgrowth of epidermal cells

Regression – a return to a former or earlier state. A subsidence of symptoms or of a disease process

Squamous Cells – flat cells that look like fish scales that are normally found within the epidermis

Swelling of Nuclei – Abnormal increase in the size of the nucleus

Thrombosed Vessels – Blocked or clogged blood vessels

Trematodes – An extensive order of parasitic worms found on/inside animals and on the gills of fishes. They usually have a flattened body with a chitonous skin, and are furnished with two or more suckers for adhesion.

Tumor Pattern Papillary – Cellular growth and generation in fingerlike projections

Tumor Pattern Smooth – Cellular growth and generation occurring in a straight pattern

Vessels Surrounded by Lymphocytes – Tumor – The presence of white blood cells around blood vessels within the area of the tumor

Vessels Surrounded by Lymphocytes – Under Tumor - The presence of white blood cells under the affected area

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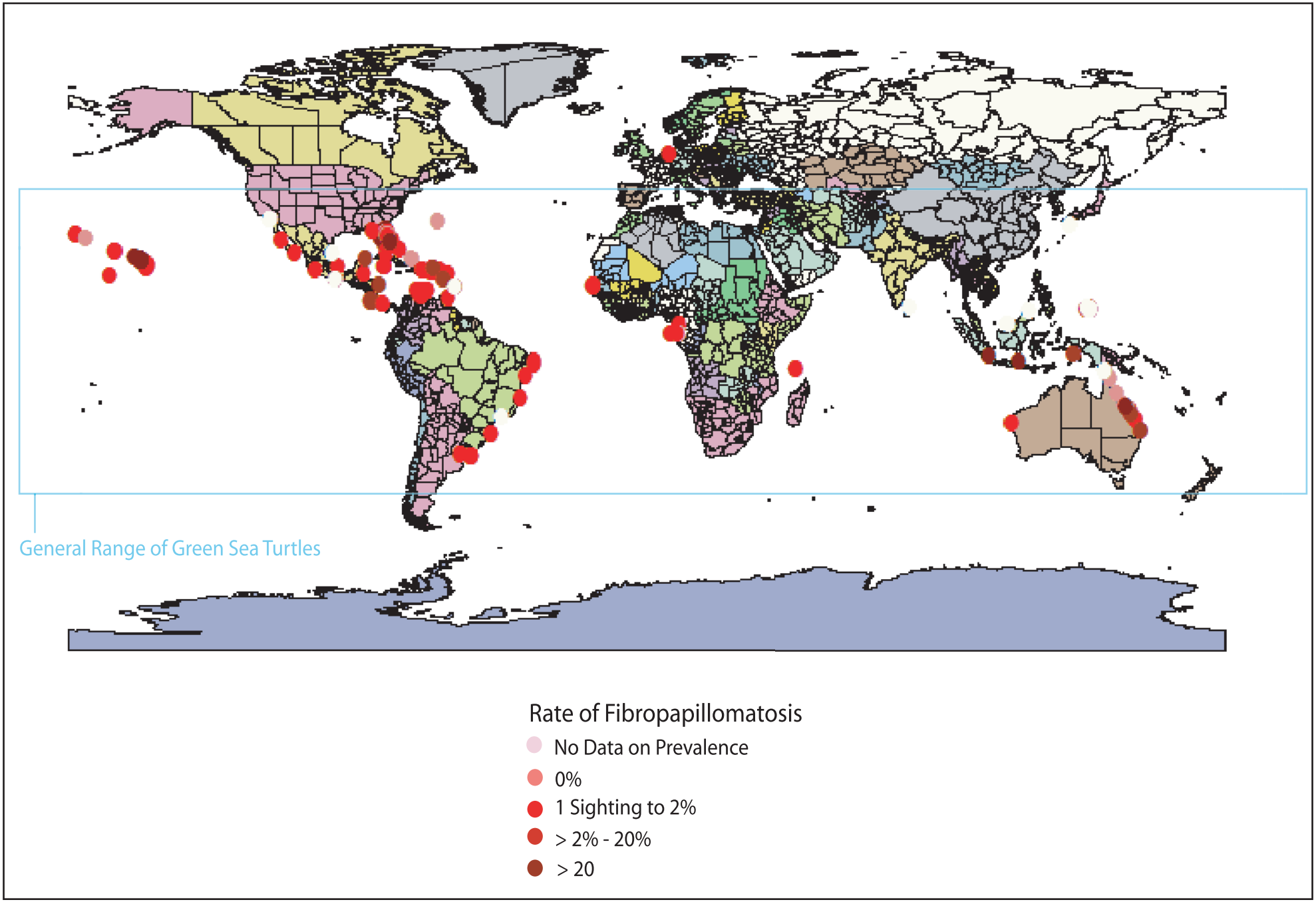

Figure 1 - Map of Global Distribution of Fibropapillomatosis

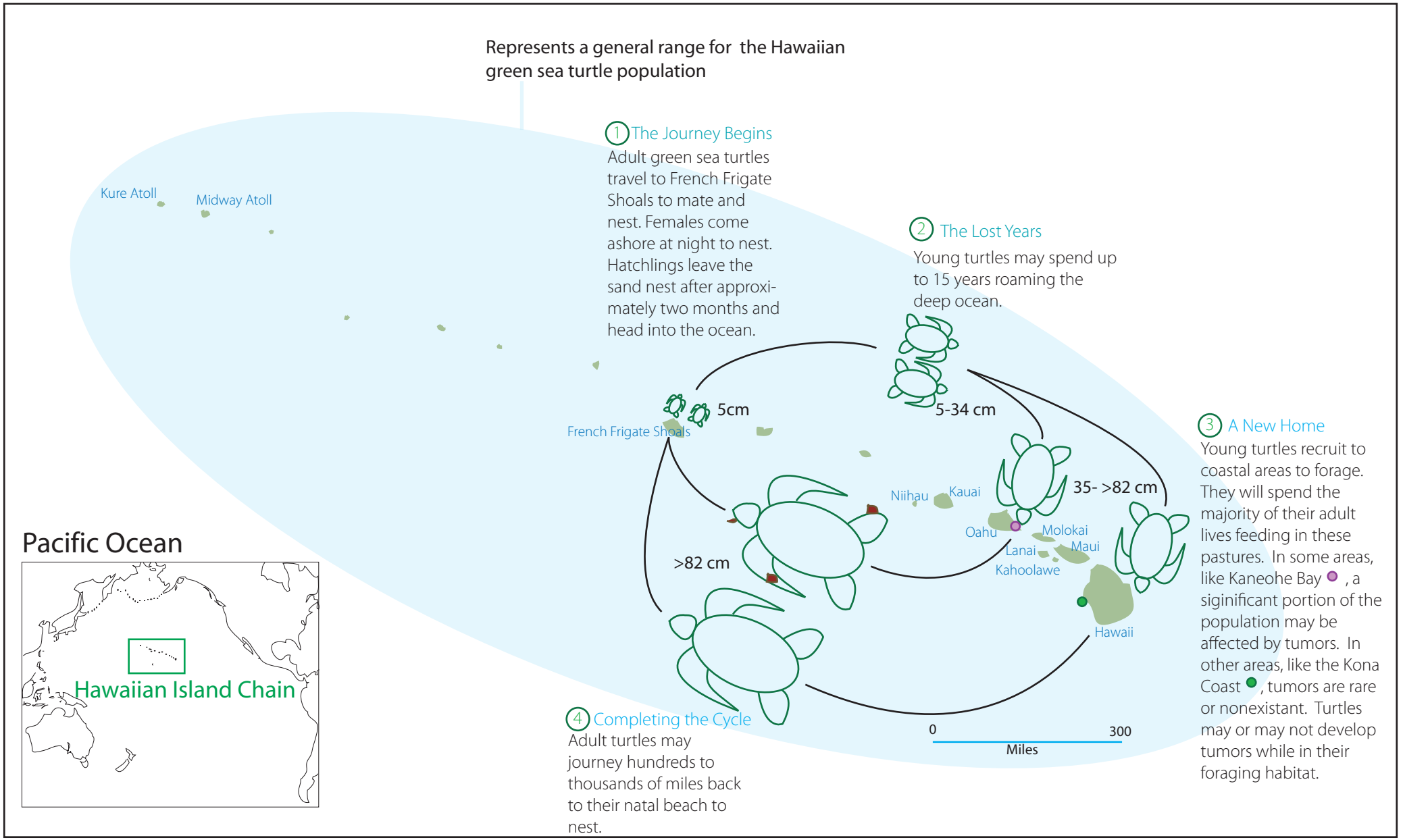

Figure 2 - The Life Cycle of a Hawaiian Green Sea Turtle

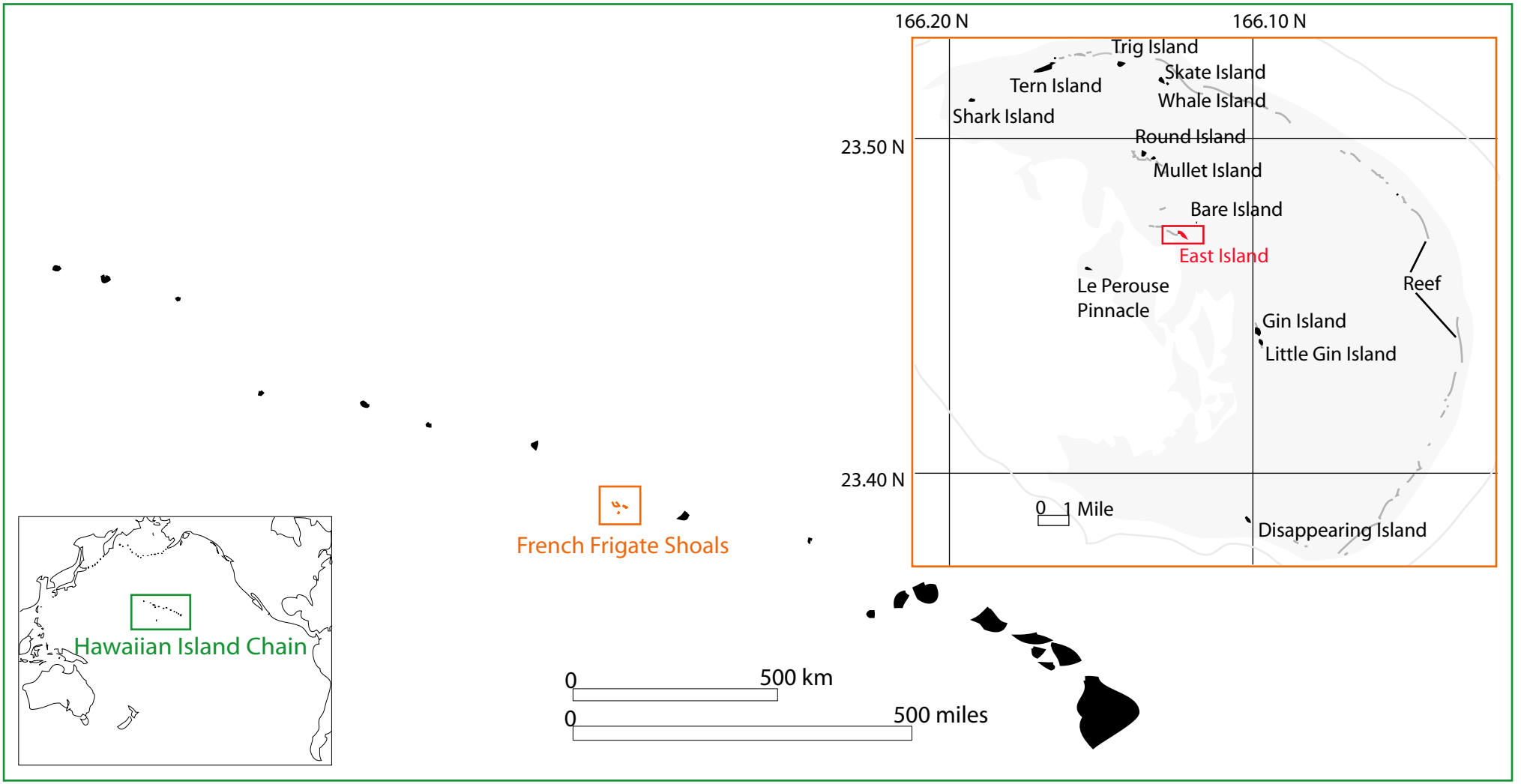

Figure 3 - Map of East Island

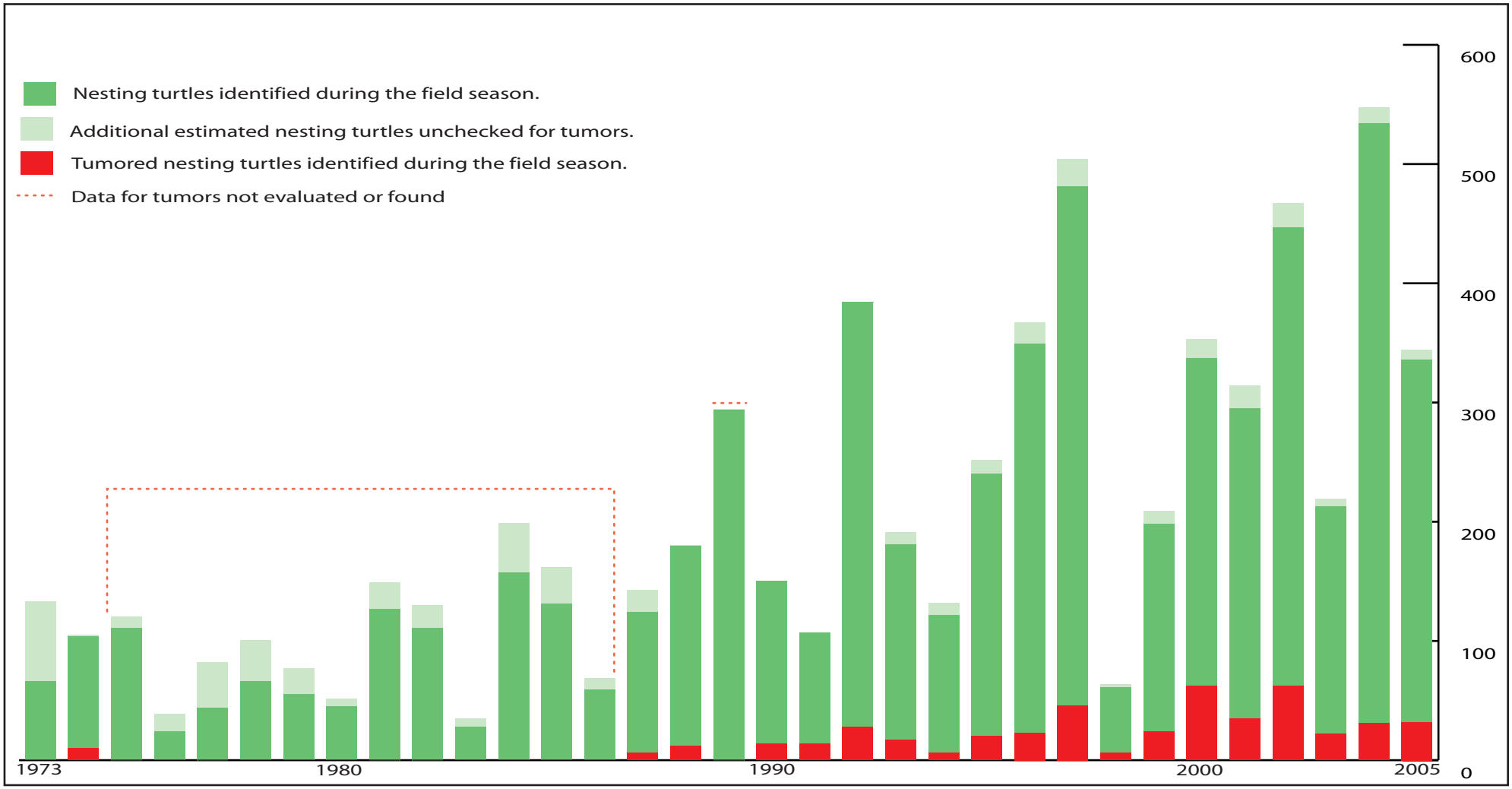

Figure 4 - A 33 Year History of Nesting Green Sea Turtles on East Island

Surveys of nesting females have been conducted for 33 years on East Island (Balazs and Chaloupka 2004). The length of the field season is variable, and so a formula was developed to estimate the approximate number of nesting turtles each year based on the annual number of nesters witnessed during the field season (Wetherall 1998, Balazs and Chaloupka 2004, Balazs and Chaloupka 2005, Balazs, NOAA, personal communication). This graph shows the number of turtles identified during each field season, the number of additional turtles estimated for the entire nesting season, and the number of turtles identified with tumors. Data for some years was not evaluated due to time and logistical constraints.

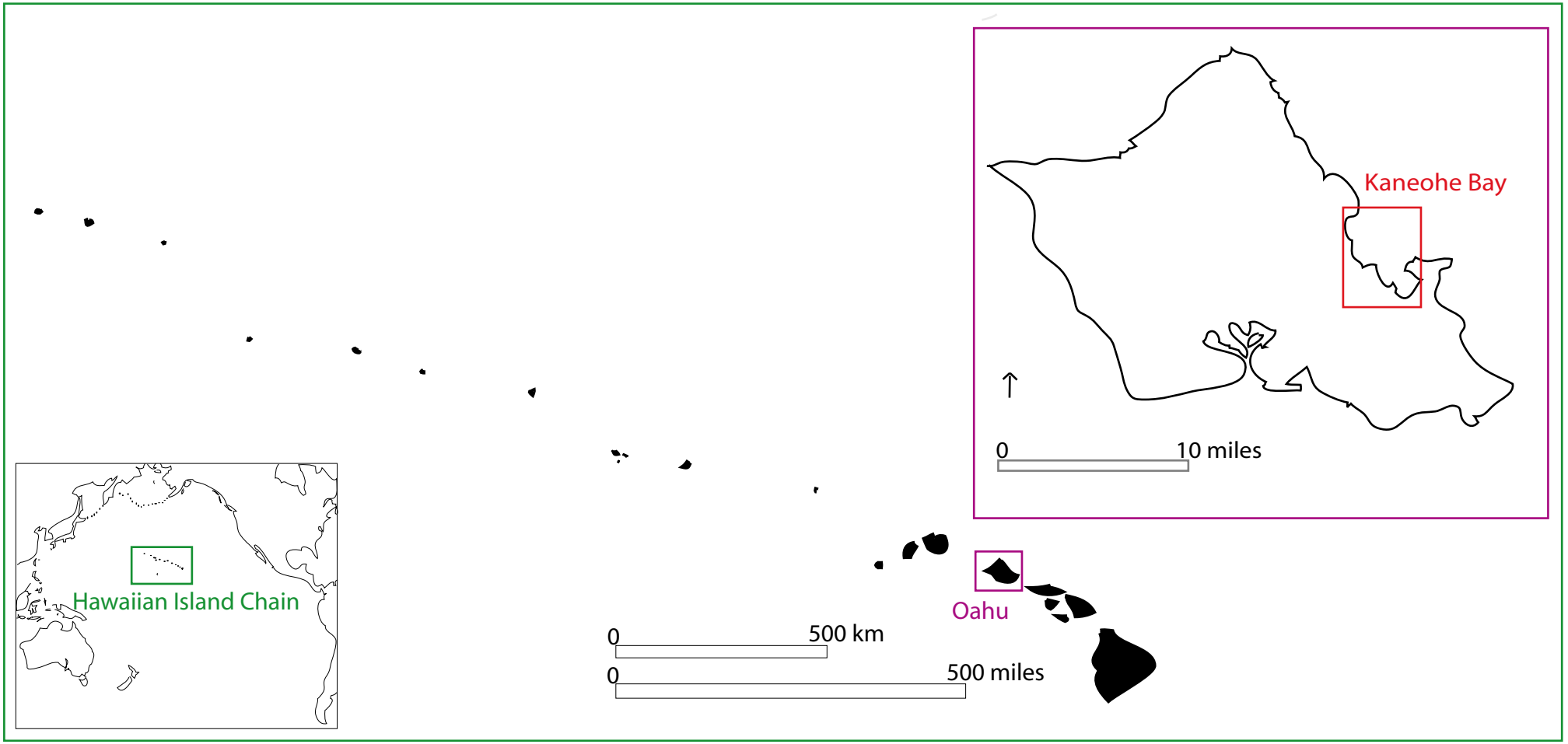

Figure 5 - Map of Kaneohe Bay

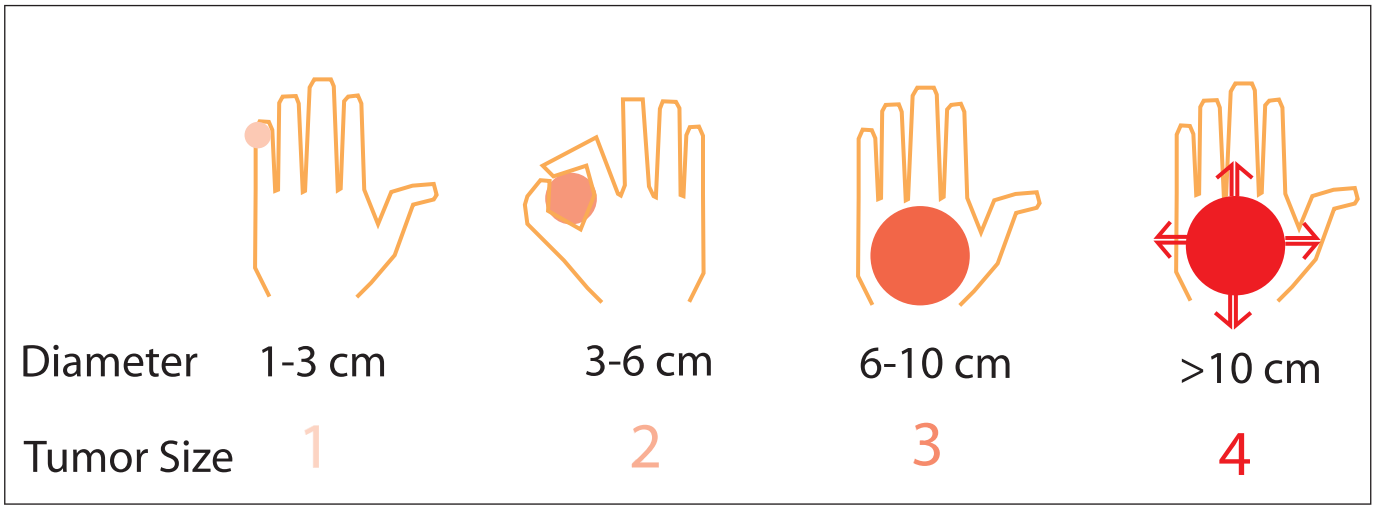

Figure 6 - Field Guide for Sizing Tumors.
All tumors identified on East Island between 2000 and 2005 were ranked on a scale (size 1-4) developed by George Balazs of the National Marine Fisheries Service. Due to the time constraints presented by working alone in the field with live animals, unsampled tumors were measured on an approximate scale using various parts of the hand as a reference.

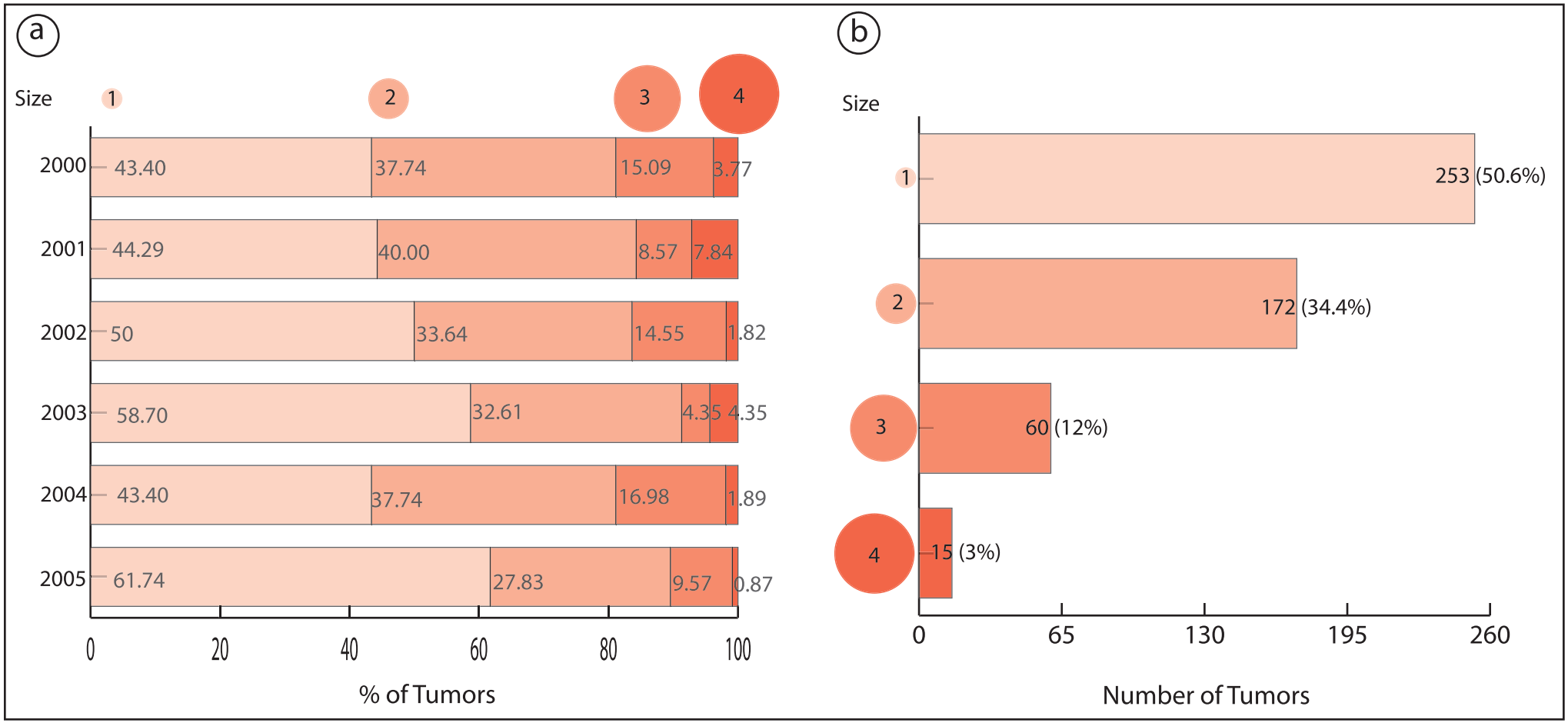

Figure 7 - Tumor Size.
a) Graph showing the annual percentage of tumors of each size from 2000 to 2005. b) Graph showing the percentage distribution by size of all 500 tumors seen between 2000 and 2005. Some turtles had tumors sized and counted in multiple years.

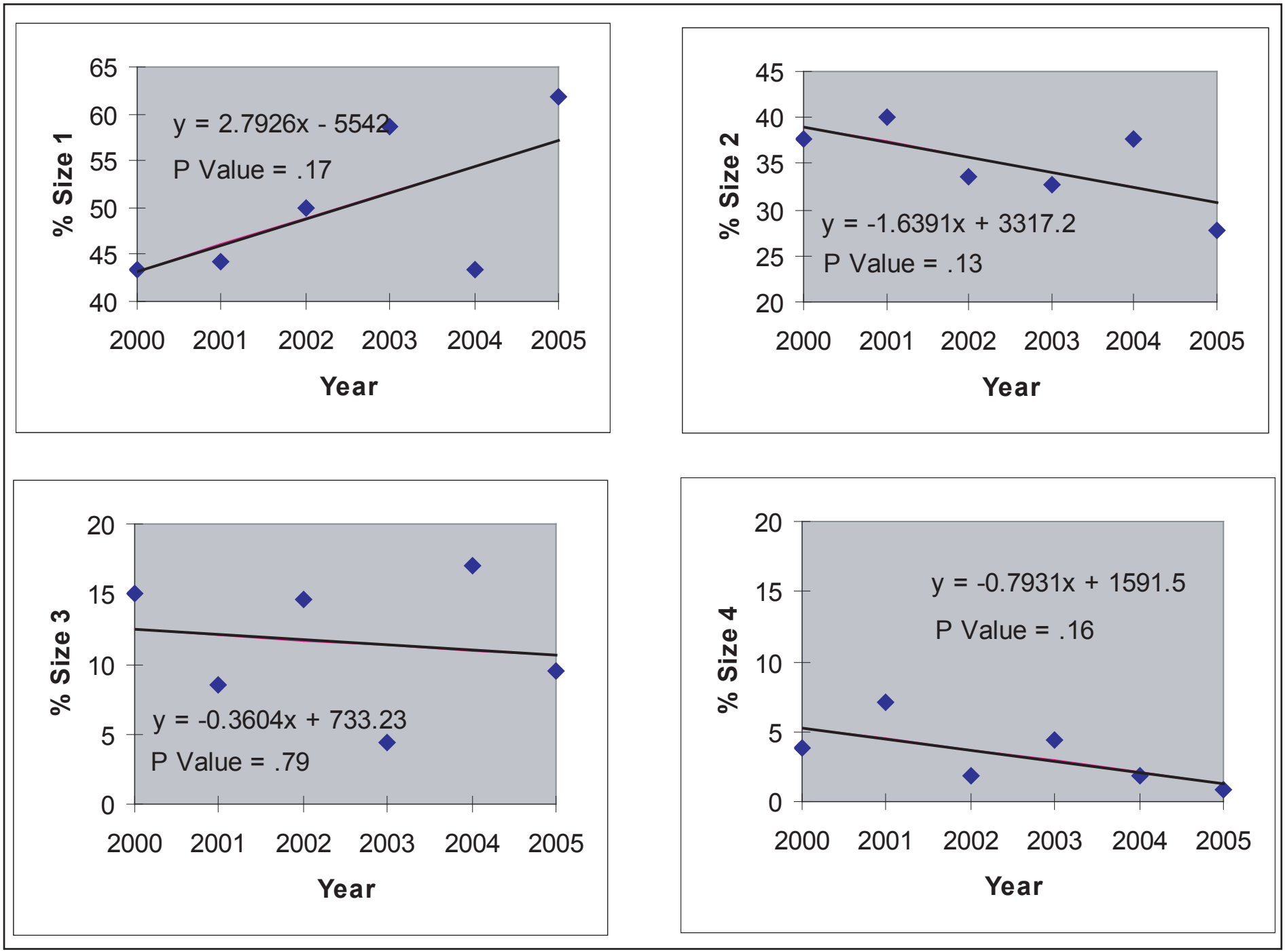

Figure 8 - Regression Graphs of Tumor Size Percentage by Nesting Season.

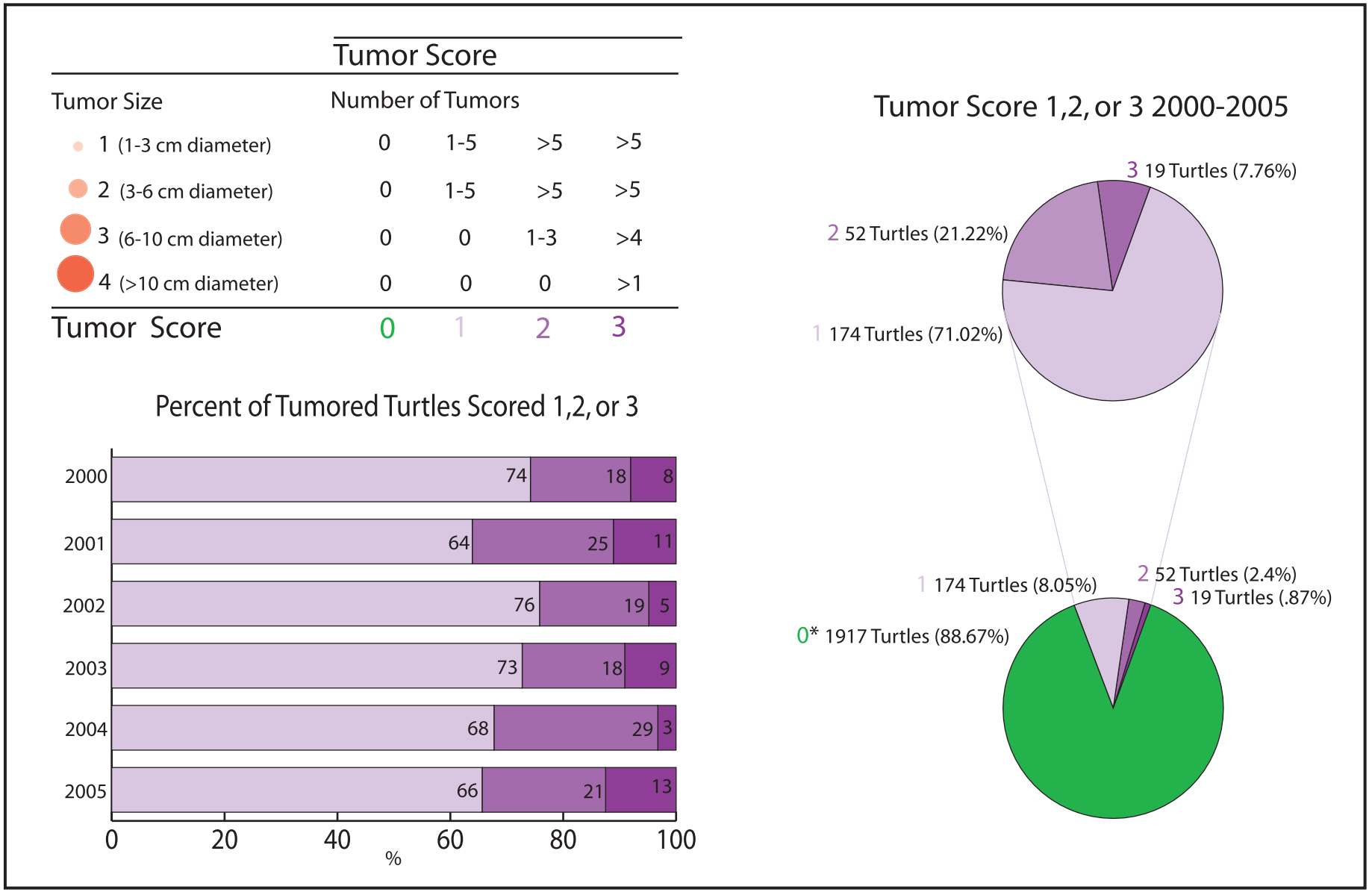

Figure 9 - Tumor Score
Turtles were given a score from 0 - 3 based on the number and size(s) of their tumor(s). The scale is based on the tumor score scale developed by Work and Balazs (1999), though tumor size ranks 1-4 are slightly different than those used by Work and Balazs. * A portion of turtles listed as 0 went unchecked for tumors. In 2005 it was 2.4%. In 2004 it was 7.7%. The totals from 2003-2000 have not been tabulated.

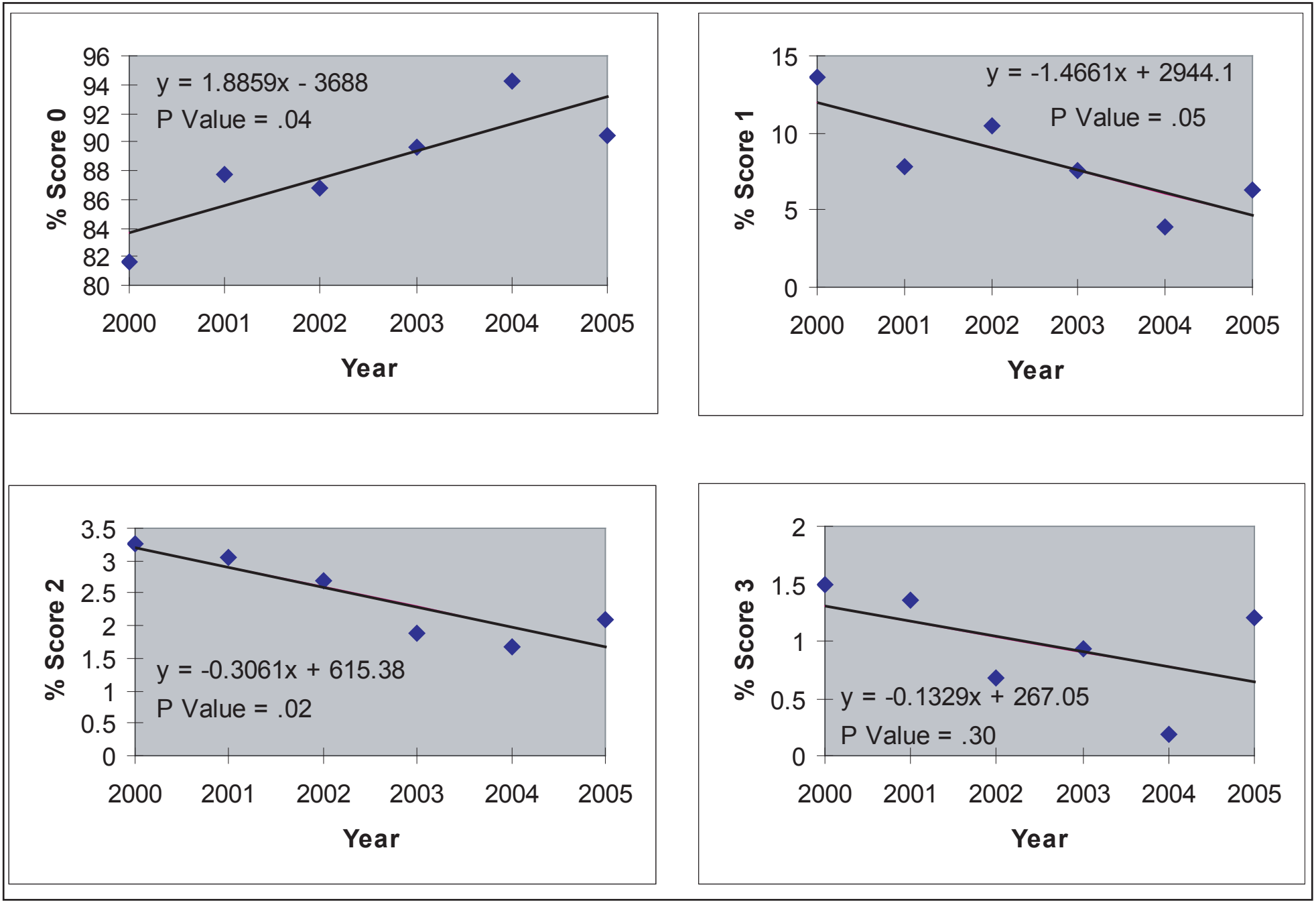

Figure 10 - Regression Graph of Tumor Score by Nesting Season

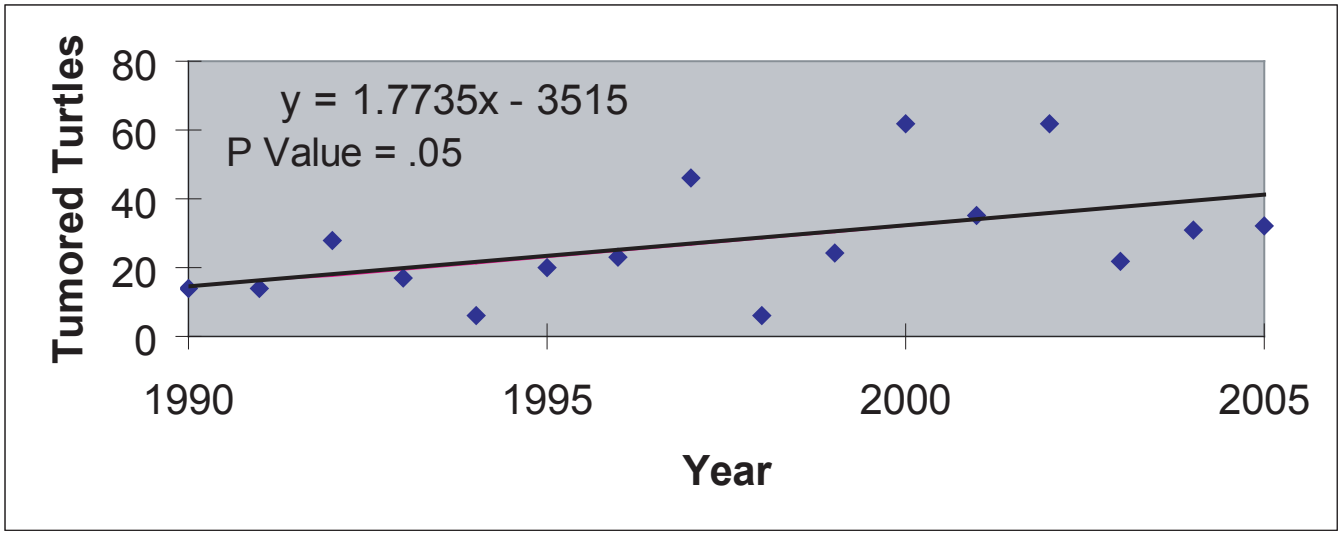

Figure 11 - Regression Graph of Number of Tumored Turtles Per Nesting Season

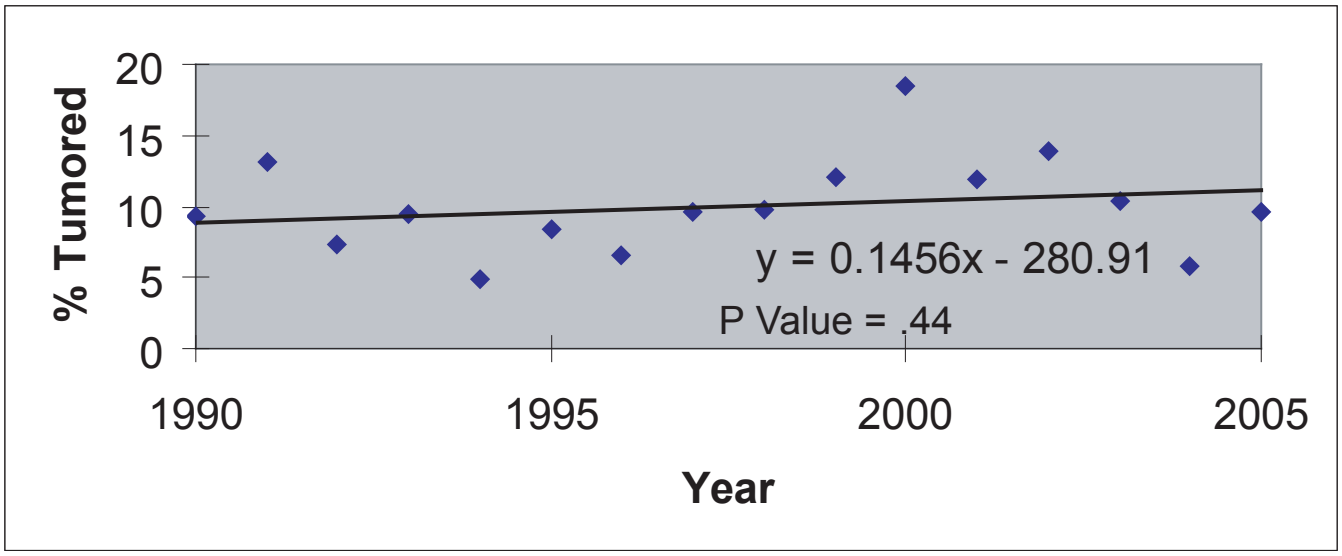

Figure 12 - Regression Graph of Percentage of Tumored Turtles Per Nesting Season

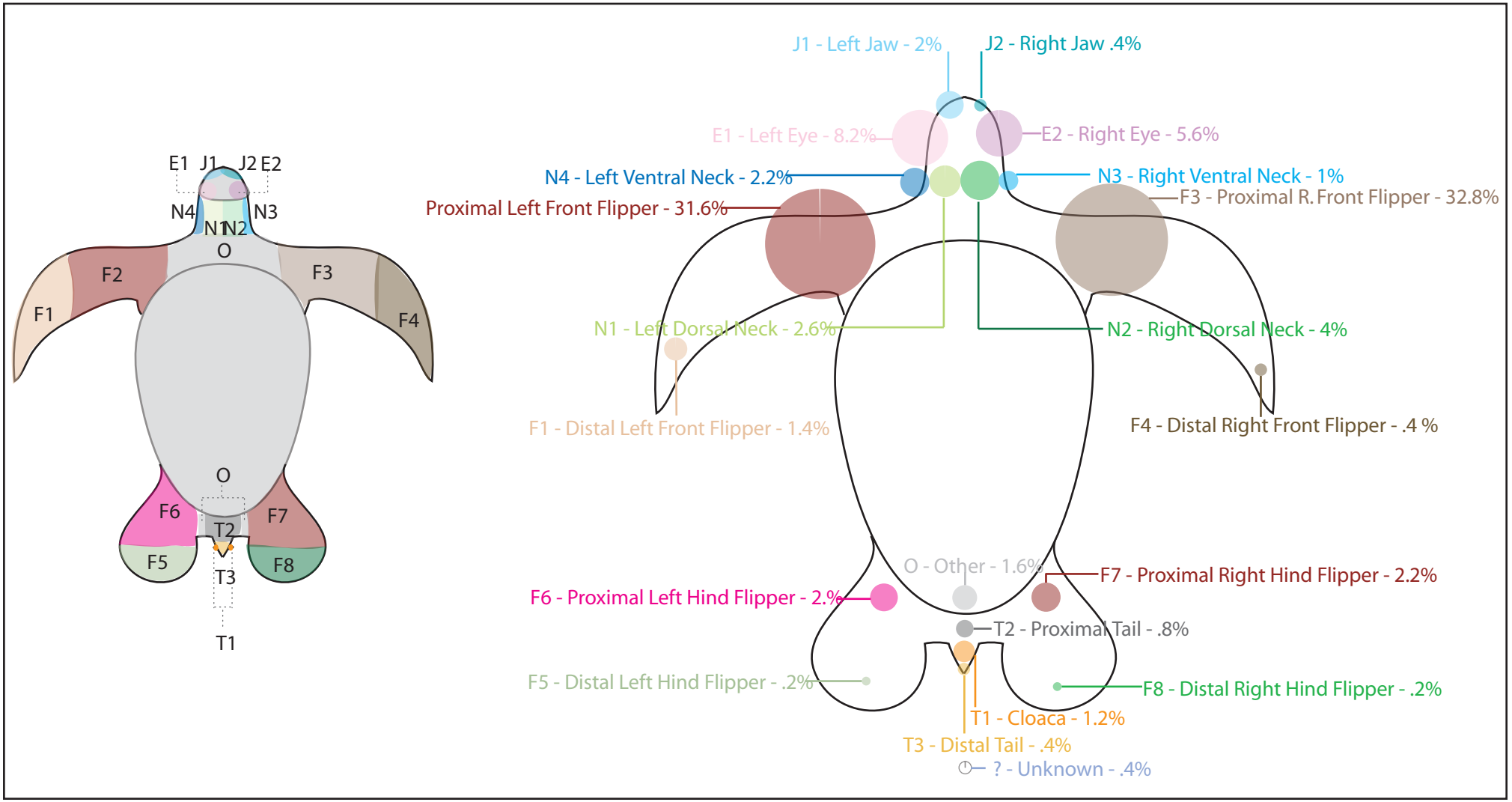

Figure 13 - Tumor Location

A system for identifying and logging tumors by location was developed by George Balazs and Shawn Murakawa of the National Marine Fisheries Service. Each tumor was given one of twenty individual location codes based on subjective identification in the field. The above graphic shows the location codes and the percent distribution of the 500 tumors identified between 2000 and 2005 by location.

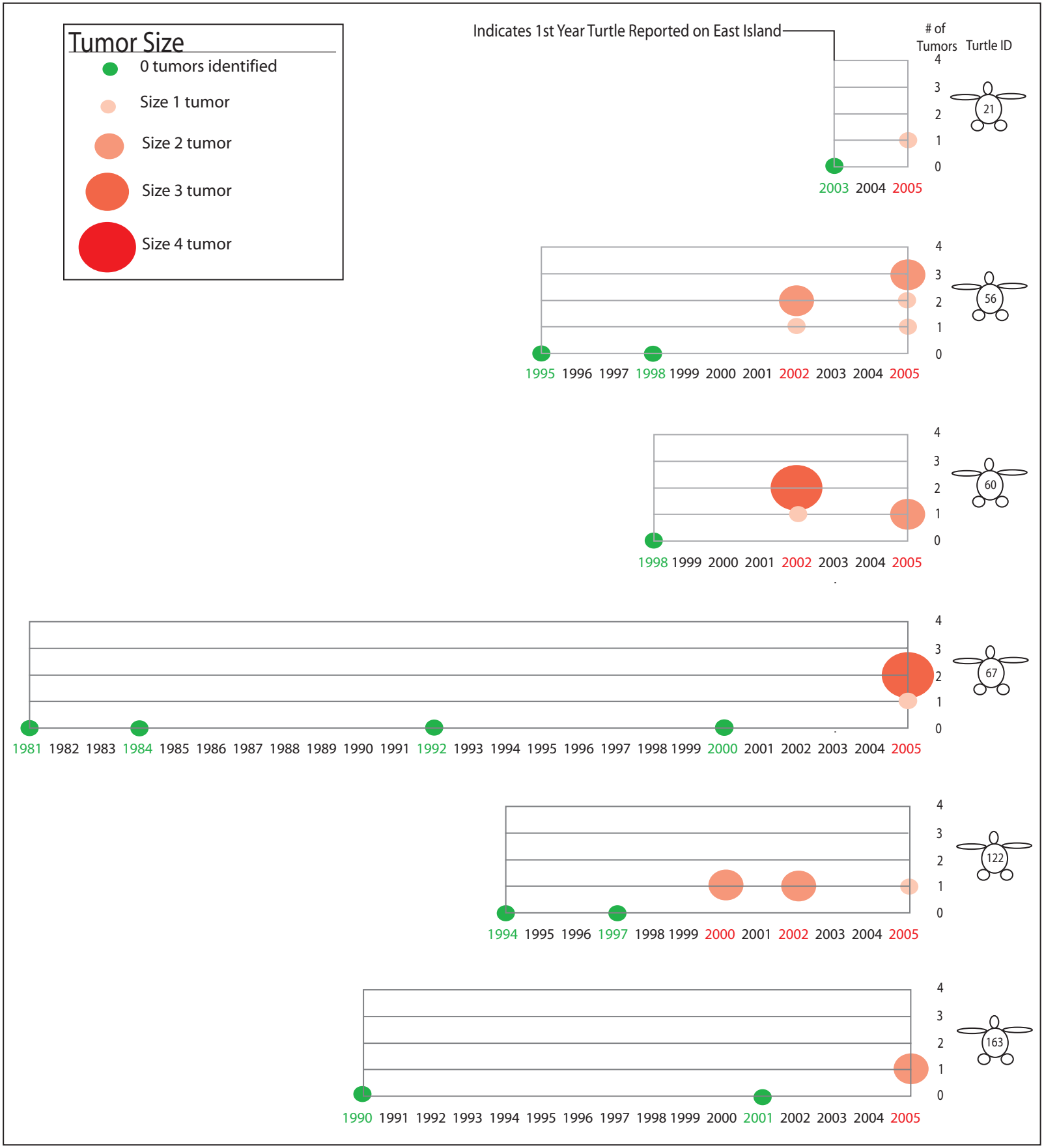

Figure 14 A - 2005 Previously Tagged Tumored Turtles

Twelve of the 32 tumored turtles identified on East Island in 2005 had been previously tagged and had their histories retrieved from the National Marine Fisheries Service database developed by Shawn Murakawa and George Balazs. Each turtle is listed with the size and number of tumors identified. A green or red circle above the year indicates the turtle was seen that year. It is possible turtles may nest and not be seen during the field season. Turtles are labeled based on the number assigned to them during the 2005 nesting season.

Figure 14 B - 2005 Previously Tagged Tumored Turtles

Twelve of the 32 tumored turtles identified on East Island in 2005 had been previously tagged and had their histories retrieved from the National Marine Fisheries Service database developed by Shawn Murakawa and George Balazs. Each turtle is listed with the size and number of tumors identified. A green or red circle above the year indicates the turtle was seen that year. It is possible turtles may nest and not be seen during the field season. Turtles are labeled based on the number assigned to them during the 2005 nesting season.

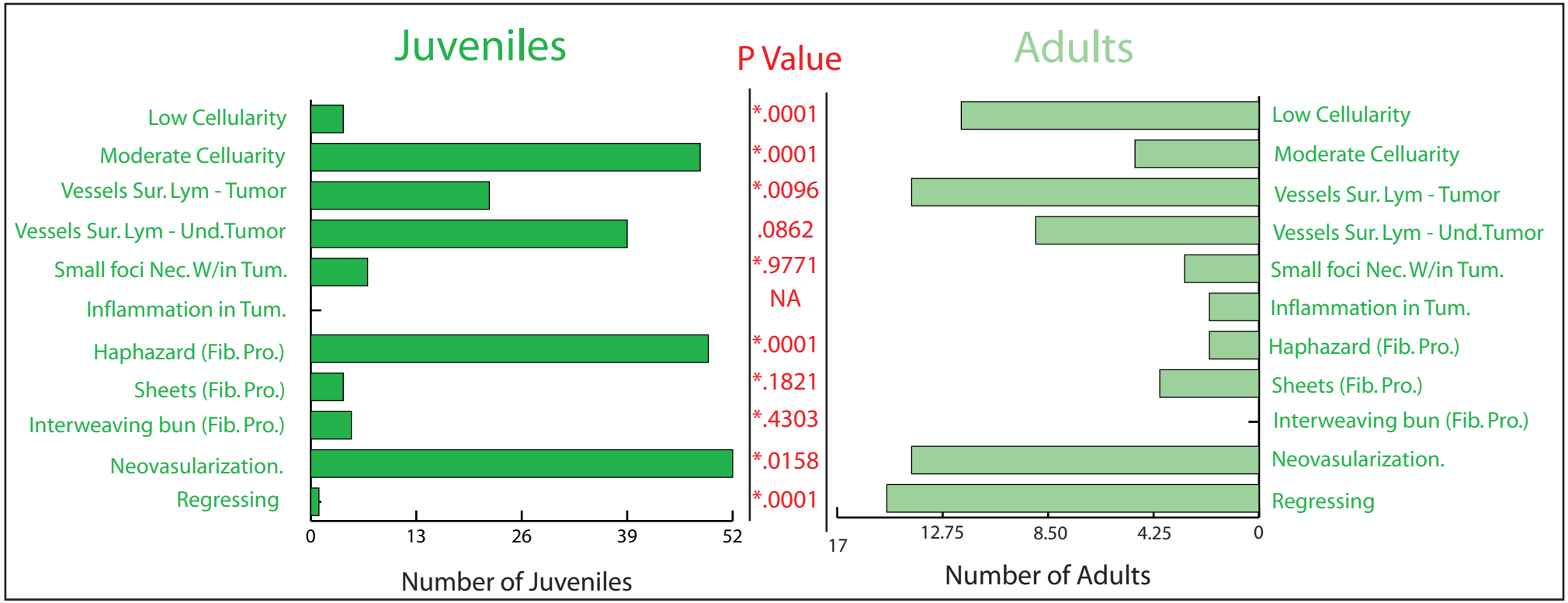

Figure 15 - HIstological Characteristics of Regression

Tumor samples were examined individually by Dr. Terry Spraker of Colorado State University. The number of adult and juvenile turtles with characteristics indicative of regression were compared statistically using Chi-Square in order to determine whether the differences were significant.

*Indicates continuity correction was used in estimating the P Value

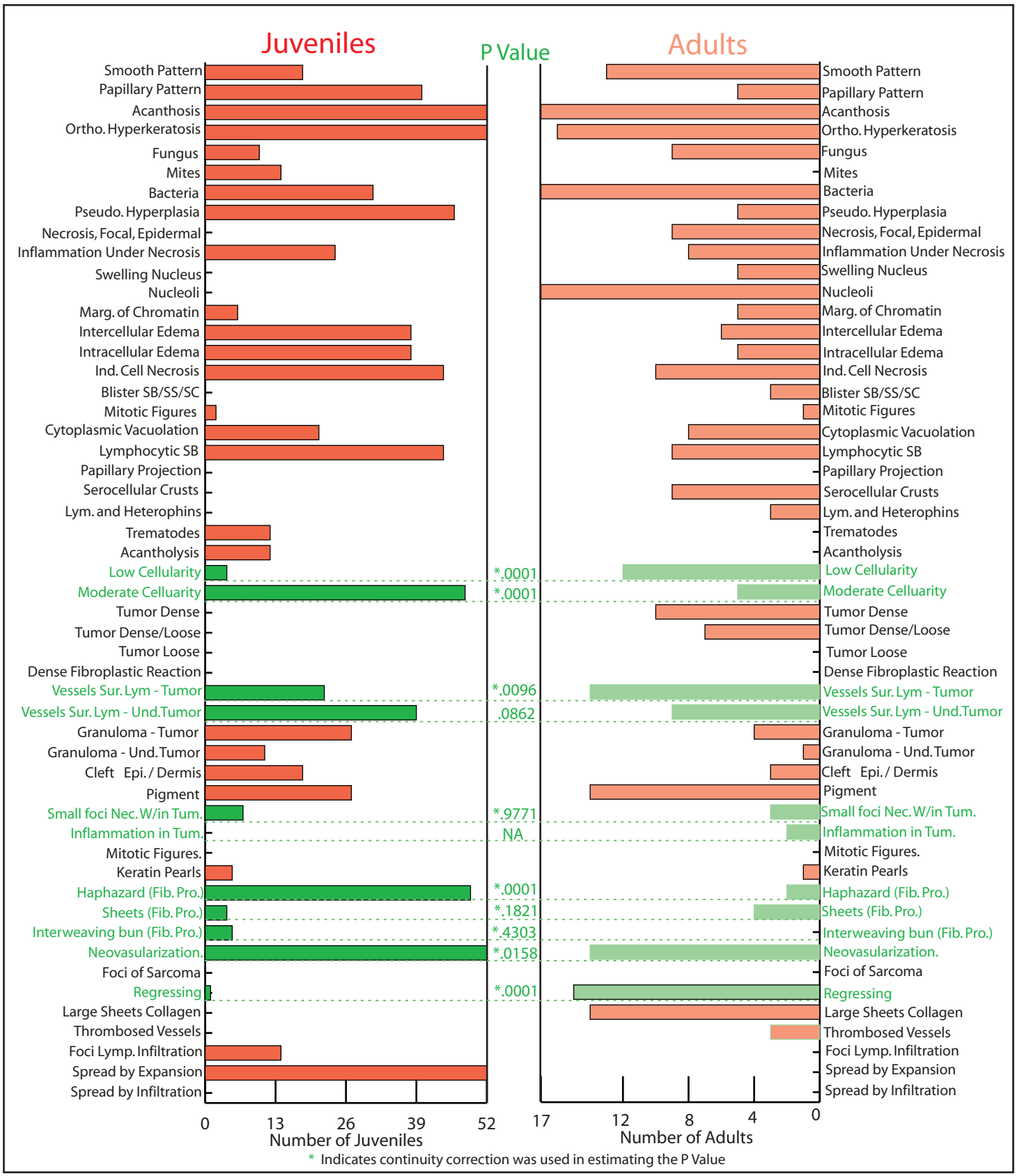

Figure 16 - HIstological Characteristics of Adult and Juvenile Tumors

Tumor samples were examined individually by Dr. Terry Spraker of Colorado State University and the characteristics of each sample were recorded. The number of juvenile and adult turtles with each characteristic are graphed below. Green bars represent characteristics that are diagnostically related to the regression process. The number of turtles with these characteristics were compared statistically using Chi-Square in order to determine whether the differences between adults and juveniles were significant.