Site fidelity and fibropapillomatosis tumor incidence in green sea turtles (*Chelonia mydas*) on Hawaii Island.

Running title: Fibropapillomatosis in Hawaiian Green Sea Turtles

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Abstract

Fibropapillomatosis (FP), a viral disease causing tumors, was first discovered in Green Sea turtles in the Caribbean in 1938, in Hawaii in the 1950's, and has since been found in all oceans. Although the spreading mechanism is currently unknown, the most commonly accepted hypothesis is that turtles are spreading the disease through direct contact. I investigated tumors on Green Sea Turtles at locations in East Hawaii (Richardson's, Leleiwi and 4-Mile), West Hawaii (Puako and Honaunau), and South East Hawaii (Punaluu). At each site, individual turtles were scored for tumor severity to determine the distribution of tumored turtles at these locations. Site Fidelity was studied to determine the possibility of a point source location for FP. Turtle size was analyzed in conjunction with tumor score to evaluate patterns of infection across the study period at these locations. Data were collected by snorkel surveys and photo-capture techniques from October 2009-February 2010. Results indicate that there was no significant difference in tumor score among sizes of the turtles ($F=1.51$, $p=0.226$); however, there was a significant difference between tumor score and turtle of sea turtle habitat location ($F=3.49, p=0.006$). Tumor scores vary by location, however size is not indicated as a factor. Locations that have a high prevalence of tumors could indicate a point source for the virus compared to locations that lack the virus altogether. These data support the hypothesis that either significant mortality may occur in infected juveniles prior to adulthood or that adults have the ability to recover from the disease.

Introduction

The Hawaiian green sea turtle (*Chelonia mydas*) is the most abundant species of turtle in the Hawaiian Archipelago. They are found throughout all islands and are commonly found in French Frigate Shoals in the Northwestern Hawaiian Islands, which is their main nesting location (Chaloupka et al. 2008). Green sea turtles have been known to forage in benthic, coastal environments on algae, and sea grasses around all Hawaiian Islands (Brill et al. 1995, Quakenbush et al. 1998, Work 2001). The eggs and meat of *C. mydas* were used for food, and the shell was used for special decoration in old Hawaii (Chaloupka et al. 2008a). Green sea turtles all over the world have experienced a decline in the past few decades caused by many factors including anthropogenic and natural causes including over-harvesting for their eggs, meat, leather, and shells, nesting habitat destruction, entrapment by fishing lines and nets, collisions with boats, and from dredging operations (Chaloupka et al. 2008, Herbst et al. 1995, Jones 2004, Broderick et al. 2007). *C. mydas* was considered threatened and listed on the Endangered Species List in 1978. Since then, there has been a population increase to an almost healthy levels (Chaloupka et al. 2008). Accompanying anthropogenic and natural causes of death, fibropapillomatosis negatively affects the survival of green sea turtles (Santos et al. 2010).

Fibropapillomatosis is a disease that is commonly found in green sea turtles around the Hawaiian Islands that is caused by a herpes-like retrovirus. This retrovirus produces tumors (fibropappillomas) as one of the most recognizable symptoms. In a study done in Florida, four different types of the virus were found in different locations (Ene et al. 2005). About 50%-90% of juvenile green sea turtles in Hawaii die as a result of fibropapillomatosis (Aguirre et al. 1998, Quackenbush et al. 1998, Work et al. 2001, Jones 2004). FP was first reported in the Caribbean in 1938, and has been increasingly documented after 1980 (Brill et al. 1995, Landsberg et al. 1999, Jones 2004). The disease appears to have peaked in Hawaii in the mid 1990's and has steadily declined since then (Chaloupka et al. 2009). In Hawaii, FP was first documented in 1958, followed by an outbreak in the 1980's (Work et al. 1999). Exact cause of fibropapillomatosis are unknown however it has been found that turtles with tumors tend to have a higher parasite load, are immunosuppressed, and bacteraemic (Santos et al. 2010). Juvenile turtles have been shown to contract the virus once associated with a neritic environment after being in the deep sea (Herbst et al. 1995, Santos et al. 2010). One possible mechanism for the spreading of the virus is by direct contact between individual turtles (Landsberg et al. 1999). Ingestion of the dinoflagellate *Prorocentrum lima* from the algae that the turtles eat potentially increases the chances of tumor growth in turtles that already harbor the virus (Landsberg et al. 1999). This is due to the okadaic acid produced by these dinoflagellates. Environmental changes such as a toxic algal bloom or increase in iron occurring in the near shore environments from local ground flow are also potential causes for production of this acid (Landsberg et al. 1999, Work et al. 2001, Chaloupka et al. 2008). Another factor in the possible spreading mechanism is the cleaner wrasse *Thalassoma duperry.* This particular wrasse is known to feed on barnacles that are burrowed into green sea turtles and leave behind a small wound. This leaves the turtle open to infectious agents. Cleaners could potentially be carriers of the virus, they move from individual to individual potentially passing the virus (Losey G et al. 1994). The tumors are benign; however, when enlarged, they can impair the turtles' mobility, vision, foraging ability, and also the internal organs, such as the lungs, esophagus or intestines (Aguirre et al. 1998, Quackenbush et al. 1998, Landsberg et al. 1999, Jones 2004). Although tumors have been known to grow externally and internally, recent studies show tumors to have little effect on somatic growth, behavior, or diet (Chaloupka et al. 2009). However, studies in Florida show diseased turtles to be significantly smaller in size than non-diseased individuals (Hirama and Ehrhart, 2007). Tumor placement on turtles is known, however, geographic variation shows differences in severity of FP on the basis of size, location and quantity of tumors (Santos et al. 2010). Studies suggest the causative agent(s) are most likely found within the neritic foraging locations (Chaloupka et al. 2009)

Site fidelity for green sea turtles is best described as a constant association or attachment to a specific site for their daily activities such as foraging, travelling or sleeping, and can be comparable to a site preference for an individual within a population. Site fidelity of the Hawaiian green turtle is understudied, but is useful for implementation of conservation efforts (Broderick et al. 2007). Site fidelity in *C. mydas* is most commonly determined by photographic evidence or tagging efforts (Bennet et al. 1999, Pelletier et al. 2003, Broderick et al. 2007). Bennet et al. (1999) identified 247 turtles over a decade with photographic evidence, 37% of who were “resights” (turtles that have been seen more than once at a particular location). The importance of this finding is that 73% of the resights were identified to have the fibropapillomatosis virus by the existence and abundance of tumors. It is unknown whether these resight turtles had tumors at the beginning of the study. Fidelity or association to particular locations could be the possible link to the spread of the disease which could be shown by turtles that are resights that obtain tumors after disappearing for a couple of years.

Studies have shown site fidelity at breeding locations due to the dependencies at those sites (Aguirre et al. 1998, Hays 2004, Broderick et al. 2007, Chaloupka et al. 2008). The majority of green sea turtles travel to the Northwest Hawaiian Islands which range from 90 to 345 km between foraging and nesting (Parker et al. 2009). It is possible for the turtles to become infected with the virus in transit as they forage on the way, however, there have not been many studies that examined whether or not the green turtle has a specific attachment to their foraging ground (Aguirre et al. 1998, Hays 2004, Broderick et al. 2007, Chaloupka et al. 2008). In this study, site fidelity at foraging will be determined from tumor scores (number and severity) and individual markings on Hawaii Island. By establishing site fidelity, this could support the theory turtles are obtaining the fibropapillomas by direct contact with other individuals, which could also indicate a point source for a specific location around Hawaii Island.

Materials and Methods

Sites

Data were collected at six sites around the island of Hawaii (Fig. 1). East Hawaii locations are Richardson's Beach Park (~65 m transect), Leleiwi (~42 m transect) and 4-Mile (~170 m transect). West Hawaii locations are Honaunau (~183 m transect) and Puako (~93 m transect). Southeast location is Punaluu (Black Sand Beach, ~120 m transect).

Experimental Design

Data were collected between September 2009-February 2010 by snorkel transect at each site. East Hawaii sites were sampled two to three times per month, Honaunau and Puako three times between September 2009-February 2010, and Punaluu twice during the study period. Transect distances vary between site, and were determined by natural barriers, i.e. lava rock, and shelves in the water. Locations were chosen based on the lack of research done on Hawaii Island and the accessibility of the site for the researchers and the knowledge of turtles foraging at those locations. All transects followed the natural contours of the shoreline. Data were collected on free-ranging turtles at high tide whenever possible. Water depth varied by location and tidal cycle. Once a turtle was found, characteristics were recorded on a standardized tumor score sheet (Figure 2) and assigned tumor severity (Table 1) using an underwater slate including; size (S-<0.61 m, M-0.61-0.83 m, L >0.83 m), tumor score (0-no tumors, 1-lightly afflicted, 2-moderately afflicted, 3-heavily afflicted, sex (only if the turtle falls in the Large size range is sex able to be determined) and any distinguishable markings (e.g. bite out of fins, epizootic coralline algal growth, pit tags, etc.). Photos were taken with an Olympus camera in an underwater housing. Photos were taken from a safe distance from the turtle unless approached by the animal (15 feet away). Photos were of the entire turtle as well as any distinguishing characteristics that would allow researchers to identify them back in the lab such as tumors or notches in the shell or in the flippers. Each individual was named and kept on file on the computer to determine site fidelity of the species. Site fidelity was determined by comparing the photos of all sizes and the characteristics of each individual turtles.

Results

Total individual turtles photo-captured were 129 (this number excludes the multiples of turtles photo-recaptured). Site Fidelity was established in 13 individual turtles (Table 2) at two of the three East Hawaii locations; two individuals at Richardson's and 11 at 4-Mile. Turtle 4 was photo-recaptured the most, four times and also has the highest tumor score, three. Turtle 7 and 9 were recaptured three times, tumor score of 0 for turtle 7 and 1 for turtle 9. In recaptured turtles at Richardson's, 50% had tumors (tumor score of 1) and 18% at 4-Mile (tumor score of 1 and 3). East Hawaii, sites 14% of the total individuals have tumors, all other sites showed no external signs of fibropapillomatosis. Mean values of individual turtles were highest at Punaluu and Puako followed by 4-Mile and Leleiwi with the least amount at Richardson's (Figure 3). Mean abundance of tumored turtles is highest at Leleiwi followed by 4-Mile. Richardson's had one individual with a tumor score of one and Puako, Punaluu and Honaunau had no frequency of tumored turtles. Data were transformed using the square root function and a one-way ANOVA was used to test these data. Data indicates a significant difference between tumor score and location with a p-value of 0.006 (Figure 4). Mean abundance individuals in the large size category had the highest value followed by medium then small. Another one-way ANOVA showed there to be no significant difference between tumor score and turtle size with a p-value of 0.226 (Figure 5). Turtles that fell into the size category of small were 27.1%, medium were 52.7% and large with 20.1%. Of these sizes, 2.8% of small have tumors, 1.4% of medium have tumors and the large size had 15.3% tumors. The highest tumor score was turtle 7, which was photo-recaptured the most with a tumor score of three.

Discussion

Tumor Score and Turtle Size

Data on turtle size and tumor prevalence of FP in this study are concurrent with previous studies done by Santos et al. (2010). A paper discussing low tumor frequencies in juvenile individuals and higher frequencies in adults could be explained by the following hypotheses: 1) Causative agents are found in the pelagic zone or neritic zones and have a long dormant period before first signs of exposure are noticeable, and 2) juveniles are not exposed to the causative agents until they have reached the neritic zone (Herbst 1994). More data is needed to determine which of these, if any, hypotheses are correct. An alternate hypothesis may simply be that turtles in the juvenile size class (small) may be seen with worse tumors and have higher mortality rates and individuals in the adult size class (large) may have the ability to recover from this disease which is documented in Florida as well as Hawaii (Santos et al. 2010). Data from the present study supports all three hypotheses therefore it will be difficult to determine until the etiologic agent is determined (Santos et al. 2010). Alternatively, studies done in Florida in the Indian River Lagoon show differences in data compared to studies done in Hawaii. The presence of FP seems to decrease with increasing size of the turtle and the intermediate size turtles were the most heavily afflicted size. Turtles also possessed a much lower frequency of oropharyngeal tumors in Florida than turtles in Hawaii (Hirama and Ehrnart 2007).

Site Fidelity and Tumor Score and Location

Site Fidelity is most commonly studied in nesting and resting turtles but is not in foraging turtles, particularly on Hawaii Island. In a study done at Kapoho, Hawaii, turtles were captured and PIT tags were used to re-identify turtles and showed 84% site fidelity (KT

Valdez, unpublished data). A study done on Loggerhead (*Caretta caretta*) and green turtles in Cyprus showed site fidelity for foraging, migrating, and wintering sites in both species of turtles using satellite data. The study took place over two migrations and nesting females passed suitable foraging grounds en route and potentially stopping (Broderick et al. 2007). This photo-recapture study showed there to be a significant difference between location and tumor scores and an occurrence of site fidelity in two of the three locations tested. This data is concurrent with previous studies including one in Florida. Tumor prevalence of 50% was found in a lagoon and adjacent, ocean side of this location, has no tumor prevalence (Herbst et al. 1995, Hirama and Ehrnart 2007). These data suggest the possible importance of environmental cofactors in the spreading of this disease, which could also be affected by agricultural activities, urban, and industrial development with catchment areas (Herbst et al. 1995). Environmental contaminants are difficult to relate to FP due to the toxicity level in the green turtles is unknown and also because the only data collected tends to be on chemicals that bioaccumulate. This poses a problem because organisms come into contact with chemicals that are in sparse quantities that do not always bioaccumulate. Another reason environmental contaminants are difficult to relate is because exact toxic effects are difficult to model in the lab because there are other factors in the wild that are potentially unknown. Lastly, biological effects are not strictly related to one chemical and may be due to a combination or another singular compound (Herbst et al. 1995). To better understand FP, it is necessary to understand where the turtles are becoming affected. Each location has different characteristics such as varying salinity, temperature, sedimentation rates, and sewage inputs (Herbst et al. 1995).

Conclusion

The present study supports the hypothesis of site fidelity at specific locations for the Hawaiian green sea turtle on the East side of Hawaii island but not at Southeast or West Hawaii. Location appears to be of importance for the transmission of the virus and could be due to the differing characteristics at each site. Further research is needed to determine the specific cause to the higher prevalence of tumors at one location and not at others. Tumor score was not shown to be significantly different among the sizes. These data show tumor size increase with increasing size but with lower severity. A few hypotheses were discussed for the possible explanation as to the different sizes acquiring the disease, however, it could simply be juveniles have a higher mortality rate and adults have the ability to recover. A long-term study should be considered to show a potential regression of tumored turtles on Hawaii Island compared to other locations.

References

Aguirre AA, Spraker TR, Balazs GH, Zimmerman B (1998) Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. J Wildlife Dis 34:91-98

Bennet P, Bennet UK, Balazs GH (1999) Photographic evidence for the regression of fibropapillomatosis afflicting green turtles at Honokowai, Maui, in the Hawaiian Islands. NMFS-SEFSC 443:37-39

Brill RW, Balazs GH, Holland KN, Chang RC, Sullivan S, George S (1995) Daily movements, habitat use, and submergence intervals of normal and tumor-bearing juvenile green turtles (*Chelonia mydas* L.) within a foraging area in the Hawaiian Islands. J Exp Mar Biol Ecol 185: 203-218

Broderick AC, Coyne MS, Fuller WJ, Glen F, Godley BJ (2007) Fidelity and over-wintering of sea turtles. Proc R Soc B 274: 1533-1539

Chaloupka M, Balazs GH, Work TM (2009) Rise and fall over 26 years of a marine epizootic in Hawaiian green sea turtles. J Wildlife Dis 45:1138-1142

Chaloupka M, Bjorndal KA, Balazs GH, Bolten AB, Ehrhart LM, Limpus CJ, Suganuma H, Troeng S, Yamaguchi M (a 2008) Encouraging outlook for recovery of a once severely exploited marine megaherbivore. Global Ecol Biogeogr 17:297-304

Chaloupka M, Work TM, Balazs GH, Murakawa S, Morris R (b 2008) Cause specific temporal and spatial trends in green sea turtle strandings in the Hawaiian Archipelago (1982-2003). Mar Biol 10: 5-27

Ene A, Su M, Lemaire S, Rose C, Schaff S, Moretti R, Lenz J, Herbst LH (2005) Distribution of Chelonid fibropapillomatosis-associated herpesvirus variants in Florida:

Molecular genetic evidence for infection of turtles following recruitment to neritic developmental habiats. J Wild Dis 41 (3): 489-497

Hays GC (2004) Good news for sea turtles. Trends Ecol Evo 19:349-351

Hays GC (2008) Sea turtles: a review of some key recent discoveries and remaining questions. J Exp Mar Biol Ecol 356:1-7

Herbst LH, Klein PA (1995) Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors.

Hirama S, Ehrhart LM (2007) Description, prevalence and severity of green turtle fibropapillomatosis in three developmental habitats on the east coast of Florida. Florida Scient 70(4):435-448

Jones AG (2004) Sea turtles: old viruses and new tricks. Cur Biol 14:842-843

Landsberg JH, Balazs GH, Steidinger KA, Baden DG, Work TM, Russell DJ (1999) The potential role of natural tumor promoters in marine turtle fibropapillomatosis. J Aquat Health 11:199-210

Losey GS, Balazs GH, Privitera LA (1994) Cleaning symbiosis between the wrasse, *Thalassoma duperry*, and the green turtle, *Chelonia mydas*. Amer Soc Icthy and Herpet 3:684-690

Parker DM, Balazs GH, King CS, Ktahira L, Gilmartin W (2009) Short-range movement of hawksbill turtles (*Eretmochelys imbricate*) from nesting to foraging areas within the Hawaiian Islands. Pac Sci 63:371-382

Pelletier D, Roos D, Ciccione S (2003) Oceanic survival and movement of wild and captive-reared immature green turtles (*Chelonia mydas*) in the Indian Ocean. Aquat Living Resource 16:35-41

Santos RG, Martins AS, Torezani E, Baptistotte C, Farias JN, Horta PA, Work TM, Balazs GH (2010) Relationship between firbropapillomatosis and environmental quality: a case study with *Chelonia mydas* off Brazil. Dis Aquat Org 89:87-95

Quackenbush SL, Work TM, Balazs GH, Casey RN, Rovnak J, Chaves A, duToit L, Baines JD, Parrish CR, Bowser PR, Casey JW (1998) Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. Virology 246:392-399

Work TM, Balazs GH (1999) Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. J Wildlife Dis 35: 804-807

Work TM, Rameyer RA, Balazs GH, Cray C, Chang SP (2001) Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. J Wildlife Dis 37: 74-81

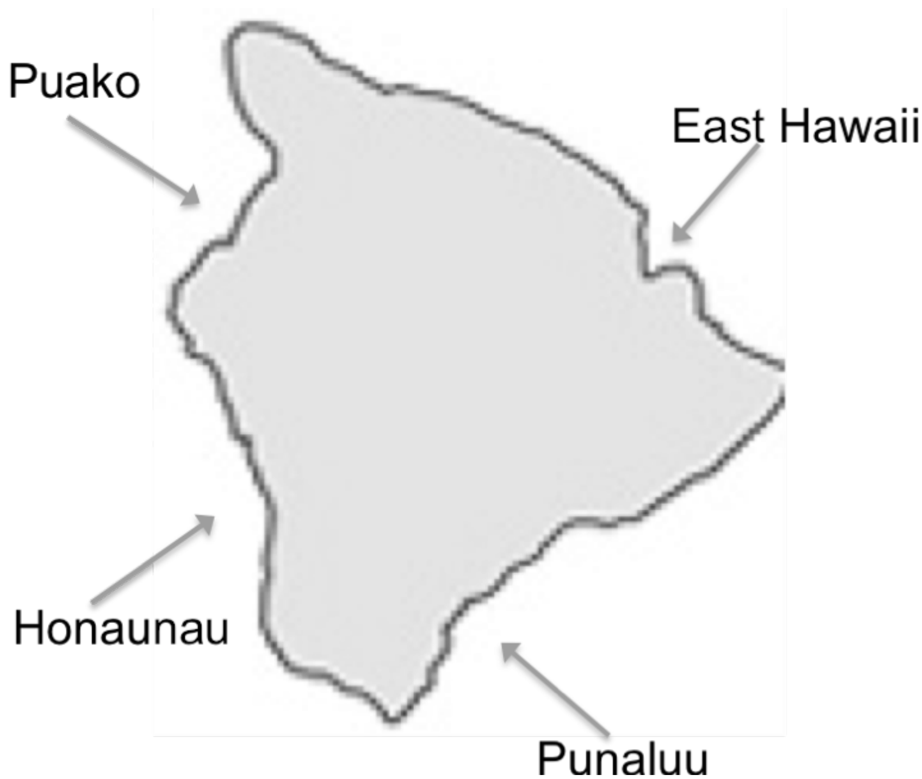


Figure 1 Map of Hawaii Island with different sample locations

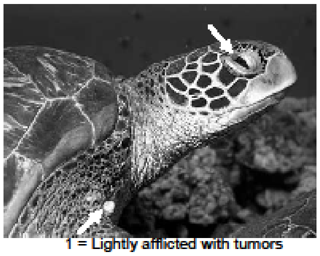
Sea Turtle Sightings

Date	Time of Sightings Scuba Snorkel Shore		Distance and Route covered	Sky, Wind, and Surf Conditions	Underwater Visibility	Total number of turtles seen	Size of turtles[A] (No. turtles)	Severity of tumors[B] (No. turtles)	Behaviors seen (No. turtles)
	START	STOP							
							S =	1 =	Swimming =
									Resting =
LOCATION and DEPTH:							M =	2 =	Feeding =
									Posing for cleaning =
							L =	3 =	Other (explain below) =

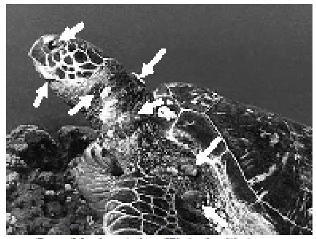
Other Comments and Descriptions: (Injuries, Hooks/Fishing line, Tags, etc.)

Sightings by (Name, address, phone number):

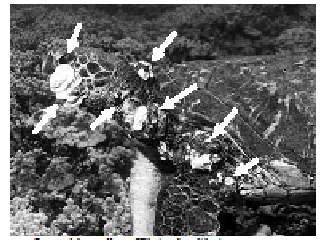
Use reverse side if more space needed



1 = Lightly afflicted with tumors



2 = Moderately afflicted with tumors



3 = Heavily afflicted with tumors

[A] Shell size; **S** = small, < 24inches (2 ft.); **M** = medium, 24 – 33 inches (2 – approx. 3 ft); L = Large, > 33 inches (3 ft or greater)

[B] **1** = Lightly afflicted, **2** = moderately afflicted, **3** = heavily afflicted

Note: **HARASSMENT AND DISTURBANCE ARE STRICTLY PROHIBITED.** All sea turtles in Hawaii are protected by State and Federal regulations. The distribution of this sighting form by the National Marine Fisheries Service is NOT a solicitation to conduct surveys or research. The objective is to record and obtain information from individuals who are normally encountering turtles during the course of their regular activities (recreational or visitor diving, hiking the shoreline, etc.)

SEND COPY TO:
NMFS, Honolulu Lab
Marine Turtle Research
2570 Dole Street
Honolulu, Hawaii 96822
Phone: 808-983-5733
Fax: 808-983-2902

Form design: Denise Parker 1/00
Photos: Ursula Keuper-Bennett, www.turtles.org

Figure 2 Summary data sheet used in the field to record data such as time, location, weather, frequency of tumors, turtle size and frequency of individual turtles

Table 1 Used to assign values to tumors on individual turtles. It is read top to bottom (Work and Balazs, 1999)

	Tumor Score			
	0	1	2	3
Tumor Size				
(A) <1 cm	0	1-5	>5	>5
(B) 1-4 cm	0	1-5	>5	>5
(C) >4-10 cm	0	0	1-3	>4
(D) >10 cm	0	0	0	>1

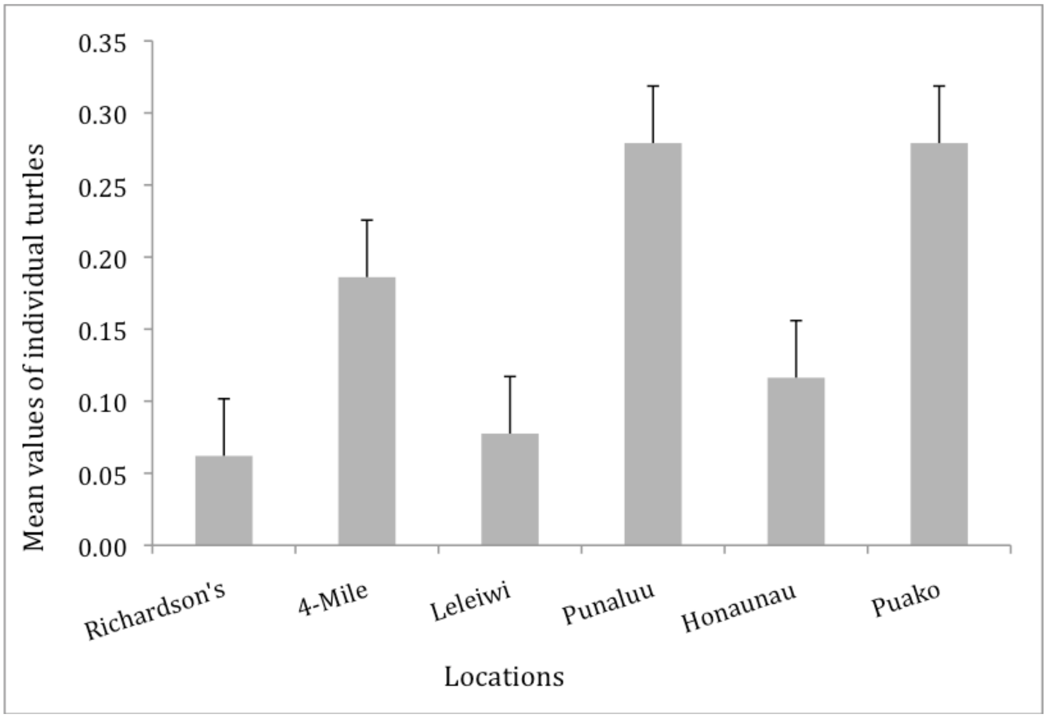


Figure 3 Mean number of turtles observed among sites

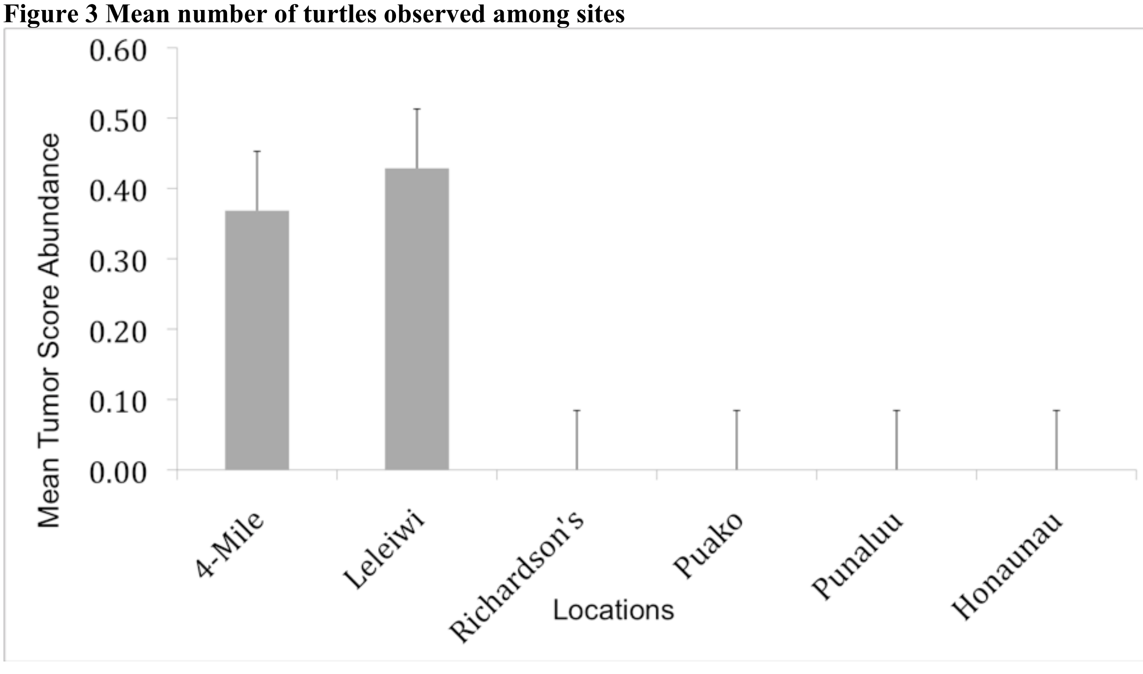


Figure 3 Graph of mean tumored turtles observed at each location (P-value 0.006).

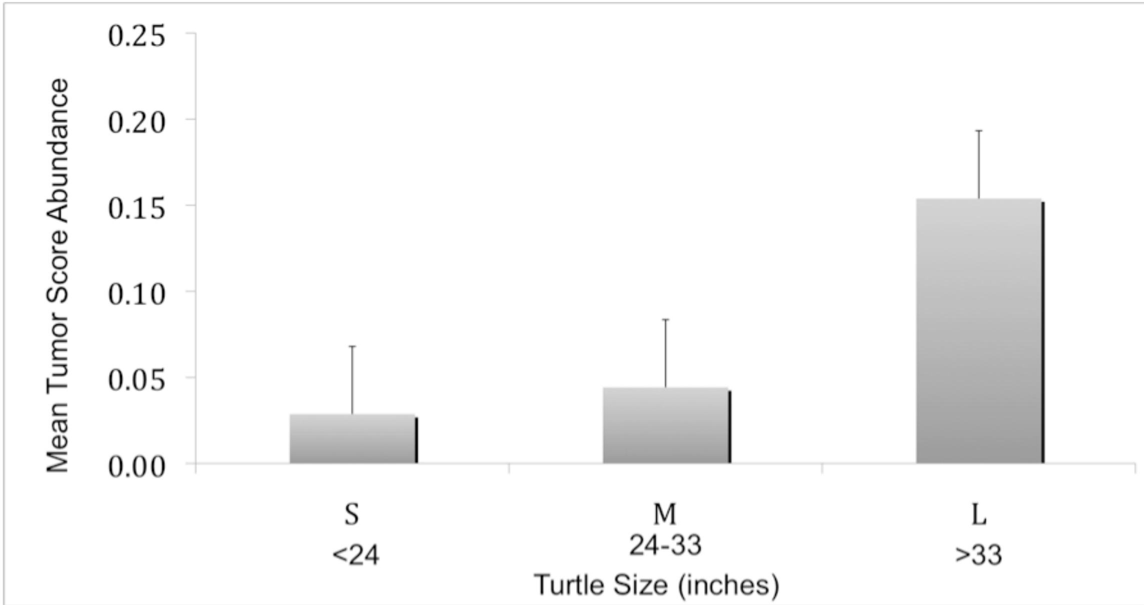


Figure 4 Graph of mean tumored turtles and the size of the turtle (P-value 0.226).

Table 2 Frequency of turtles photo-recaptured and individual tumor scores.

Turtle ID	Amount of resights	Tumor Score
1	2	0
2	2	1
3	2	0
4	4	3
5	2	0
6	2	0
7	3	0
8	2	0
9	3	1
10	2	0
11	2	0
12	2	0
13	2	0