

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

3 Running title: Fibropapillomatosis in Hawaiian Green Sea Turtles

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22 Keywords: Fibropapillomatosis, Site fidelity, Green Sea Turtles, Hawaii, *Chelonia mydas*

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24 **Abstract**

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

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47 **Introduction**

48           The Hawaiian green sea turtle (*Chelonia mydas*) is the most abundant species of  
49 turtle in the Hawaiian Archipelago. They are found throughout all islands and are  
50 commonly found in French Frigate Shoals in the Northwestern Hawaiian Islands, which  
51 is their main nesting location (Chaloupka et al. 2008). Green sea turtles have been  
52 known to forage in benthic, coastal environments on algae, and sea grasses around all  
53 Hawaiian Islands (Brill et al. 1995, Quakenbush et al. 1998, Work 2001). The eggs and  
54 meat of *C. mydas* were used for food, and the shell was used for special decoration in old  
55 Hawaii (Chaloupka et al. 2008a). Green sea turtles all over the world have experienced a  
56 decline in the past few decades caused by many factors including anthropogenic and  
57 natural causes including over-harvesting for their eggs, meat, leather, and shells, nesting  
58 habitat destruction, entrapment by fishing lines and nets, collisions with boats, and from  
59 dredging operations (Chaloupka et al. 2008, Herbst et al. 1995, Jones 2004, Broderick et  
60 al. 2007). *C. mydas* was considered threatened and listed on the Endangered Species List  
61 in 1978. Since then, there has been a population increase to an almost healthy levels  
62 (Chaloupka et al. 2008). Accompanying anthropogenic and natural causes of death,  
63 fibropapillomatosis negatively affects the survival of green sea turtles (Santos et al.  
64 2010).

65           Fibropapillomatosis is a disease that is commonly found in green sea turtles  
66 around the Hawaiian Islands that is caused by a herpes-like retrovirus. This retrovirus  
67 produces tumors (fibropapillomas) as one of the most recognizable symptoms. In a  
68 study done in Florida, four different types of the virus were found in different locations  
69 (Ene et al. 2005). About 50%-90% of juvenile green sea turtles in Hawaii die as a result

70 of fibropapillomatosis (Aguirre et al. 1998, Quackenbush et al. 1998, Work et al. 2001,  
71 Jones 2004). FP was first reported in the Caribbean in 1938, and has been increasingly  
72 documented after 1980 (Brill et al. 1995, Landsberg et al. 1999, Jones 2004). The disease  
73 appears to have peaked in Hawaii in the mid 1990's and has steadily declined since then  
74 (Chaloupka et al. 2009). In Hawaii, FP was first documented in 1958, followed by an  
75 outbreak in the 1980's (Work et al. 1999). Exact cause of fibropapillomatosis are  
76 unknown however it has been found that turtles with tumors tend to have a higher  
77 parasite load, are immunosuppressed, and bacteraemic (Santos et al. 2010). Juvenile  
78 turtles have been shown to contract the virus once associated with a neritic environment  
79 after being in the deep sea (Herbst et al. 1995, Santos et al. 2010). One possible  
80 mechanism for the spreading of the virus is by direct contact between individual turtles  
81 (Landsberg et al. 1999). Ingestion of the dinoflagellate *Prorocentrum lima* from the  
82 algae that the turtles eat potentially increases the chances of tumor growth in turtles that  
83 already harbor the virus (Landsberg et al. 1999). This is due to the okadaic acid produced  
84 by these dinoflagellates. Environmental changes such as a toxic algal bloom or increase  
85 in iron occurring in the near shore environments from local ground flow are also potential  
86 causes for production of this acid (Landsberg et al. 1999, Work et al. 2001, Chaloupka et  
87 al. 2008). Another factor in the possible spreading mechanism is the cleaner wrasse  
88 *Thalassoma duperry*. This particular wrasse is known to feed on barnacles that are  
89 burrowed into green sea turtles and leave behind a small wound. This leaves the turtle  
90 open to infectious agents. Cleaners could potentially be carriers of the virus, they move  
91 from individual to individual potentially passing the virus (Losey G et al. 1994). The  
92 tumors are benign; however, when enlarged, they can impair the turtles' mobility, vision,

93 foraging ability, and also the internal organs, such as the lungs, esophagus or intestines  
94 (Aguirre et al. 1998, Quackenbush et al. 1998, Landsberg et al. 1999, Jones 2004).  
95 Although tumors have been known to grow externally and internally, recent studies show  
96 tumors to have little effect on somatic growth, behavior, or diet (Chaloupka et al. 2009).  
97 However, studies in Florida show diseased turtles to be significantly smaller in size than  
98 non-diseased individuals (Hirama and Ehrhart, 2007). Tumor placement on turtles is  
99 known, however, geographic variation shows differences in severity of FP on the basis of  
100 size, location and quantity of tumors (Santos et al. 2010). Studies suggest the causative  
101 agent(s) are most likely found within the neritic foraging locations (Chaloupka et al.  
102 2009)

103         Site fidelity for green sea turtles is best described as a constant association or  
104 attachment to a specific site for their daily activities such as foraging, travelling or  
105 sleeping, and can be comparable to a site preference for an individual within a  
106 population. Site fidelity of the Hawaiian green turtle is understudied, but is useful for  
107 implementation of conservation efforts (Broderick et al. 2007). Site fidelity in *C. mydas*  
108 is most commonly determined by photographic evidence or tagging efforts (Bennet et al.  
109 1999, Pelletier et al. 2003, Broderick et al. 2007). Bennet et al. (1999) identified 247  
110 turtles over a decade with photographic evidence, 37% of who were “resights” (turtles  
111 that have been seen more than once at a particular location). The importance of this  
112 finding is that 73% of the resights were identified to have the fibropapillomatosis virus by  
113 the existence and abundance of tumors. It is unknown whether these resight turtles had  
114 tumors at the beginning of the study. Fidelity or association to particular locations could

115 be the possible link to the spread of the disease which could be shown by turtles that are  
116 resights that obtain tumors after disappearing for a couple of years.

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

## 129 **Materials and Methods**

### 130 *Sites*

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

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138 *Experimental Design*

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

161 **Results**

162 Total individual turtles photo-captured were 129 (this number excludes the multiples of  
163 turtles photo-recaptured). Site Fidelity was established in 13 individual turtles (Table 2)  
164 at two of the three East Hawaii locations; two individuals at Richardson's and 11 at 4-  
165 Mile. Turtle 4 was photo-recaptured the most, four times and also has the highest tumor  
166 score, three. Turtle 7 and 9 were recaptured three times, tumor score of 0 for turtle 7 and  
167 1 for turtle 9. In recaptured turtles at Richardson's, 50% had tumors (tumor score of 1)  
168 and 18% at 4-Mile (tumor score of 1 and 3). East Hawaii, sites 14% of the total  
169 individuals have tumors, all other sites showed no external signs of fibropapillomatosis.  
170 Mean values of individual turtles were highest at Punaluu and Puako followed by 4-Mile  
171 and Leleiwi with the least amount at Richardson's (Figure 3). Mean abundance of  
172 tumored turtles is highest at Leleiwi followed by 4-Mile. Richardson's had one  
173 individual with a tumor score of one and Puako, Punaluu and Honaunau had no  
174 frequency of tumored turtles. Data were transformed using the square root function and a  
175 one-way ANOVA was used to test these data. Data indicates a significant difference  
176 between tumor score and location with a p-value of 0.006 (Figure 4). Mean abundance  
177 individuals in the large size category had the highest value followed by medium then  
178 small. Another one-way ANOVA showed there to be no significant difference between  
179 tumor score and turtle size with a p-value of 0.226 (Figure 5). Turtles that fell into the  
180 size category of small were 27.1%, medium were 52.7% and large with 20.1%. Of these  
181 sizes, 2.8% of small have tumors, 1.4% of medium have tumors and the large size had  
182 15.3% tumors. The highest tumor score was turtle 7, which was photo-recaptured the  
183 most with a tumor score of three.



184 **Discussion**

185 *Tumor Score and Turtle Size*

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

203 *Site Fidelity and Tumor Score and Location*

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

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

230 **Conclusion**

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

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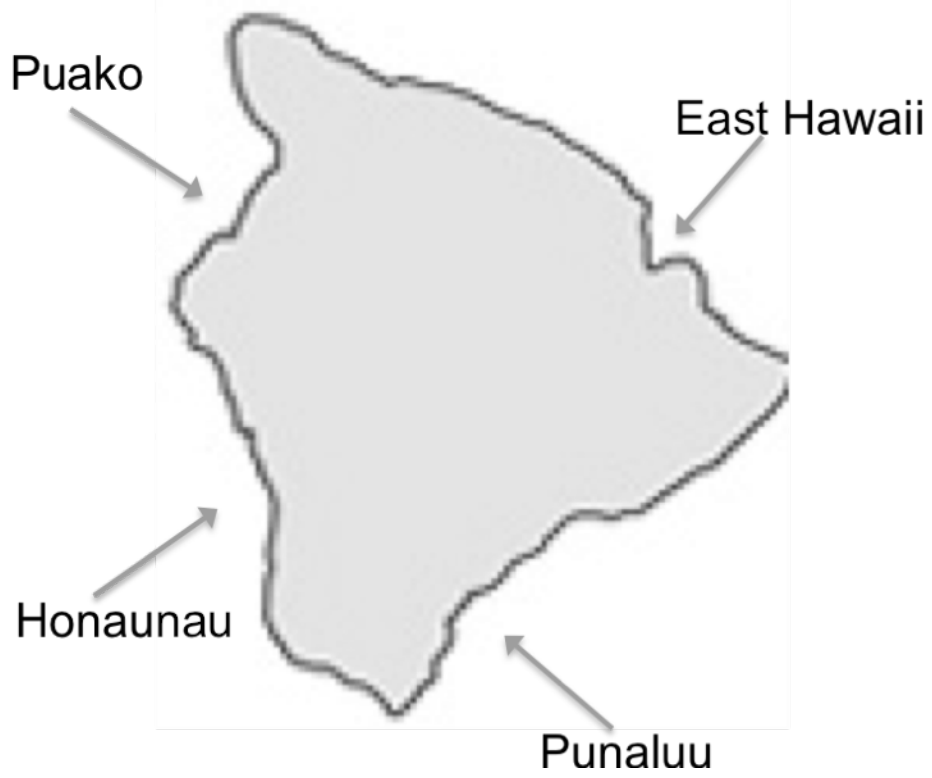
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312 **Figure 1 Map of Hawaii Island with different sample locations**

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**Sea Turtle Sightings**

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

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

<sup>B</sup> 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

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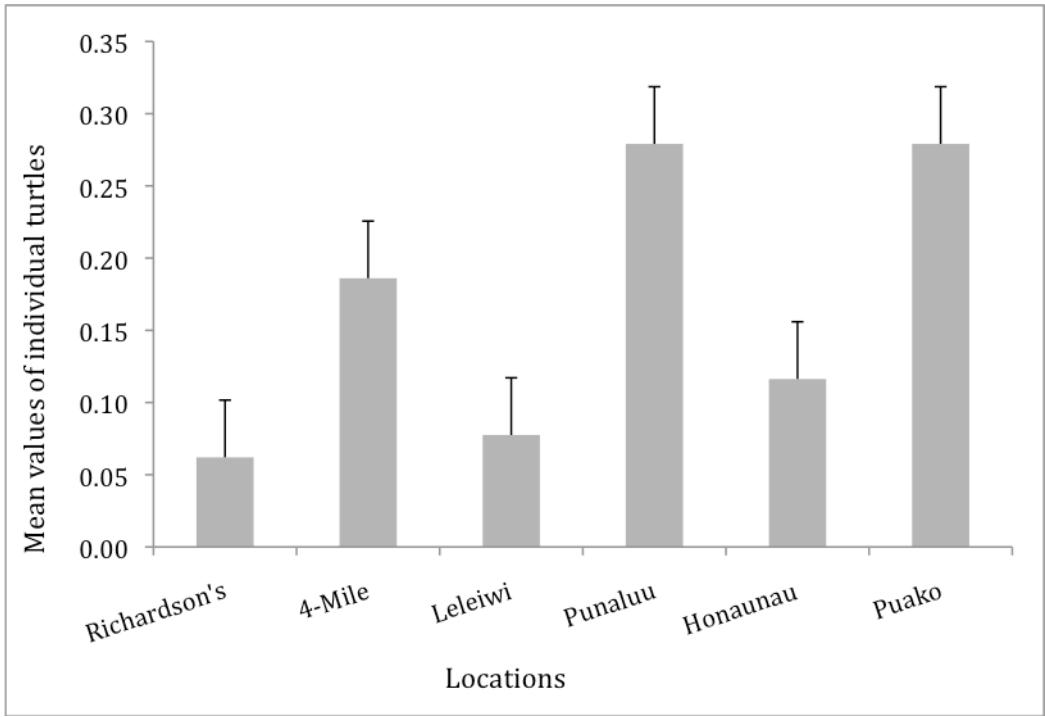
Figure 2 Summary data sheet used in the field to record data such as time, location, weather, frequency of tumors, turtle size and frequency of individual turtles

322 Table 1 Used to assign values to tumors on individual turtles. It is read top to bottom (Work and  
 323 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

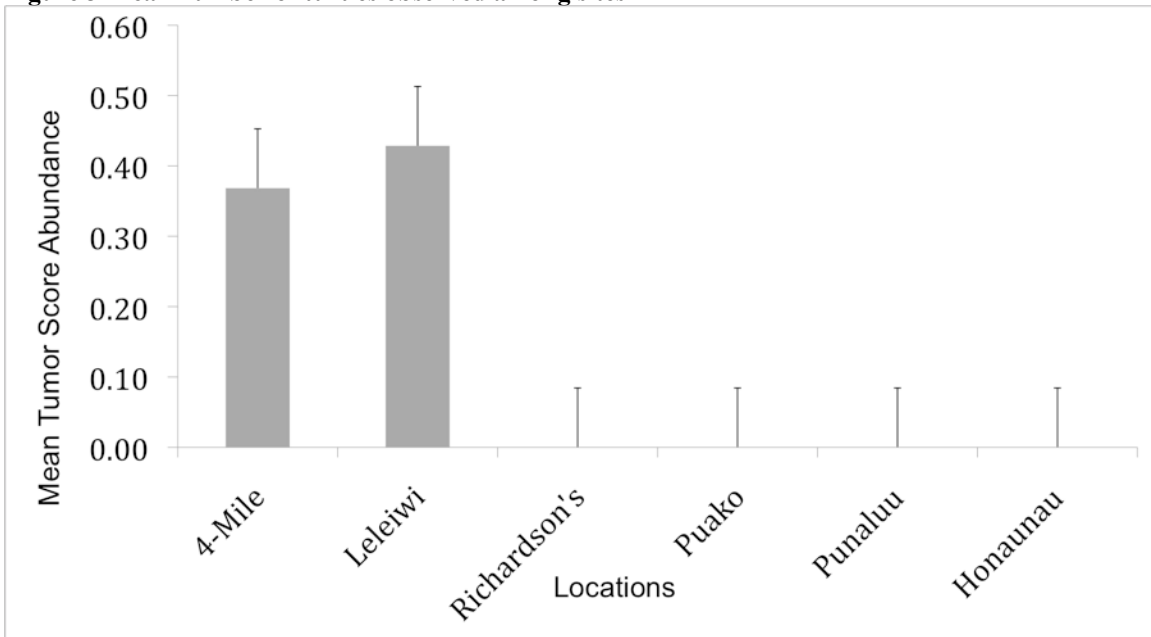
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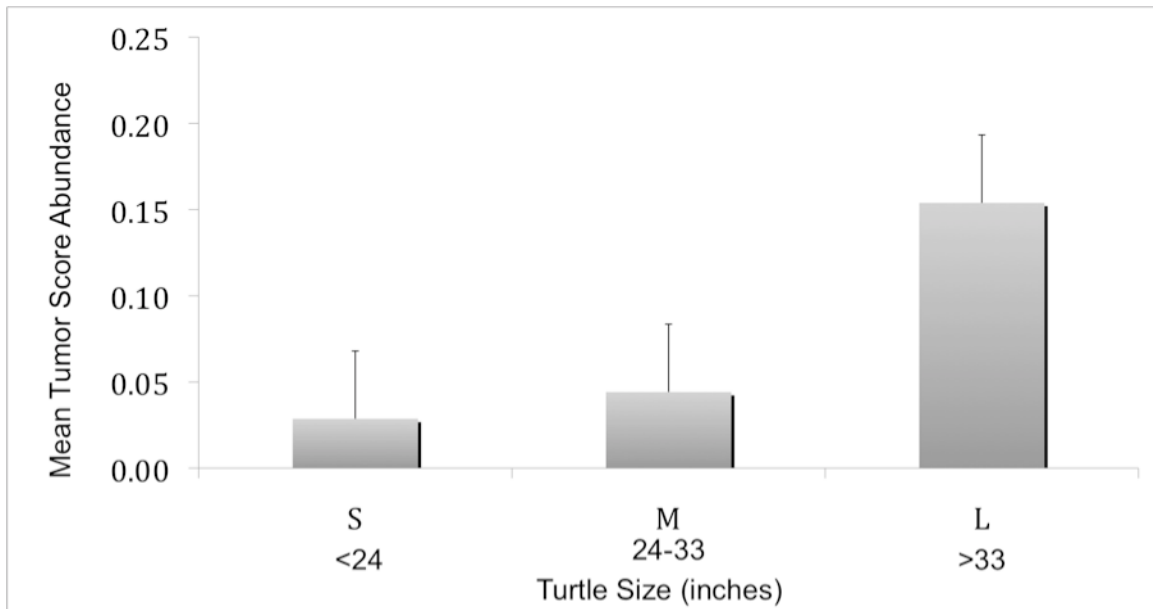
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326 **Figure 3 Mean number of turtles observed among sites**



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



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

331

332 **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

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