# Assessing Fibropapillomatosis Trends in Green Sea Turtles (*Chelonia mydas*) On Hawai‘i Island

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**ABSTRACT:** Green sea turtles (*Chelonia mydas*) in Hawai‘i are a threatened species protected by both state and federal regulations. Hawaiian green sea turtles face a wide variety of issues, including Fibropapillomatosis (FP). FP is a disease characterized by tumors affecting both external and internal organs, with the herpesvirus Chelonid herpesvirus 5 (ChHV5) identified as the likely causative agent. This study investigated spatial and temporal trends in FP among Hawaiian green turtles, focused on seven locations across Hawai‘i Island with known coastal sea turtle populations including Onekahakaha Beach Park, Carlsmith Beach Park, Leleiwi Beach Park, Richardson Ocean Park, Punalu‘u Beach, and Puakō Bay. Snorkel surveys were conducted from September to March in 2023-2024 (n=24). During each survey, data were collected on the number of turtles observed, size of each turtle observed, and the presence and severity of FP tumors using standardized protocols from previous studies. Replicate water samples (n=3) were collected at each survey location and temperature, dissolved oxygen, and salinity were measured for each sample. Water samples were then filtered and analyzed for levels of nitrate and nitrite. Sea turtle survey data were compared with historical data from snorkel surveys using the same methodology from 2007-2022 (n=194). Turtle abundance was found to be greatest at Punalu‘u with 10.9 ± 7.0 turtles (mean ± SD) ($H = 68.327$, $p < 0.001$). Variations in tumor severity across locations were observed during this study ($H = 19.034$, $p = 0.004$). The highest average tumor Puakō score per survey was found at Leleiwi (2.17± 3.33) whereas Puakō exhibited the lowest average tumor score per survey (0.84 ± 0.74). This variation may be due to differences in environmental conditions and water quality parameters between locations. Results indicate a decrease in the presence of FP tumors over time from 2007 to 2024 ($H = 39.040$, $p < 0.001$) with 2007 having the highest number of FP incidences and 2019 having the lowest number of FP incidences. Throughout the years, a relatively steady decline was observed with the addition of relatively small spikes in 2010 and 2022. These results indicate that occurrences of FP throughout Hawai‘i Island have decreased significantly over the past decade. Turtles of small sizes were found to have lower tumor severity during this study ($H = 6.231$, $p = 0.044$). No relationships were observed between water temperature, salinity, or nutrients. Findings from this study provide valuable insight into the spatial and temporal patterns of FP in Hawaiian green sea turtles across Hawai‘i that will be valuable for conservation and management efforts aimed at protecting this species.

**Key Words**: Fibropapillomatosis ◆ Green turtle ◆ *Chelonia mydas*◆ Hawai‘i

## Introduction

*Chelonia mydas*, green turtles, are the most common species of sea turtles found in the Hawaiian Islands (Work et al. 2001). Green sea turtles are a threatened species that have been protected under Hawaiian Law since 1974 and under the Endangered Species Act since 1984 (Balazs & Chaloupka 2004). The Hawaiian green sea turtle genetic stock have been steadily increasing since their protection, but threats such as climate change, pollution, and disease remain causes for concern (Balazs & Chaloupka 2004). Green turtles have complex life histories that cause them to occupy a variety of habitats during their lifespans (Lutz et al., 1997; Mansfield, Wyneken, & Luo, 2021). During their early life history, green turtles will occupy pelagic environments, and in their later years they will spend their time in coastal areas feeding on macroalgae where they face a wide variety of threats including the development of Fibropapillomatosis  (Balazs 1980, Bolten 2003, Ene et al., 2005, Foley et al., 2005, Herbst, 1994, Patrício et al., 2016).

Fibropapillomatosis (FP) is a neoplastic disease associated with the herpes virus, Chelonid herpesvirus 5, that impacts marine turtles globally (Lackovich et al. 1999, Work et al. 2014, Shaver et al. 2019).  FP was first documented in 1938 at a Florida aquarium and in the 1980s emerged as a global epidemic being found in all ocean basins and in every species of marine turtle (Smith & Coates 1938, Herbst 1994, Aguirre and Lutz 2004, Jones et al. 2016). The development of this disease commonly occurs upon the recruitment of  turtles to neritic waters. (Ene et al 2005). FP is characterized by tumors often found externally on the skin, eyes, carapace, or oral cavity of a turtle (Jacobson et al. 1989, Brooks et al. 1994, Herbst 1994, Work et al. 2004, Flint et al. 2010). External tumors caused by FP may lead to negative impacts including impaired locomotion, obstruction of vision, and feeding hindrance (Herbst 1994; Work et al. 2004). Internal FP tumors can also develop on a variety of organs including the lungs, kidneys, heart, spleen, stomach, and liver (Aguirre et al. 1998; Herbst 1994; Work et al. 2004). Internal tumors have not been observed in turtles without eternal FP tumors, but this may be due to sampling bias in stranded turtles (Work et al. 2004). Both external and internal tumors have been linked to secondary complications such as decreased body condition, pneumonia, necrosis of the salt gland, bacterial infections, and inflammation with vascular flukes which can potentially be lethal to the turtle (Work et al. 2004, Santos et al. 2010). In Hawaiʻi for example, oral tumors, commonly found in the glottis, have been thoroughly studied and found to increase the likelihood of secondary complications such as pneumonia for green sea turtles in Hawaiʻi, whereas these oral tumors are less common in places such as Floridia (Bresette et al. 2003; Work et al. 2004). FP leads to a series of potentially lethal complications for sea turtles, and understanding the risk factors associated with the development of the disease is crucial for mitigation efforts.

Sea turtle size and age have both been identified as potential risk factors for FP. FP is most commonly found in turtles of intermediate size as the disease often increases with the size of the turtle, peaks, and then decreases (Work et al. 2001, Foley et al. 2005, Van Houtan et al. 2010, Patrício et al. 2016). Previous studies have found that the likelihood of having FP increased with straight carapace length until about 57 cm, then plateaued, and finally began decreasing with increasing carapace length (Patrício et al. 2016). This suggests that FP tumors develop in intermediately sized turtles and may regress in larger turtles (Patrício et al. 2016). FP is also most commonly found in juvenile turtles, but it is unclear if this is because the disease is fatal, killing juvenile and subadult turtles before adulthood, or if this is indicative of recovery (Work et al. 2004, Van Houtan et al. 2010, Patrício et al. 2016). Additionally, there are no reports of FP in pelagic post-hatchlings or new recruits that have recently moved to inshore foraging habitats (Herbst, 1994).

Environmental factors such as degraded water quality, land use and runoff, eutrophication, and algal blooms have all been identified as potential risk factors in the development of FP tumors (Herbst 1994; Adnyana et al. 1997; Aguirre & Lutz 2004. Foley et al. 2005; Chaloupka et al. 2009; Santos et al. 2010; Van Houtan et al. 2010, 2014; Dujon et al. 2021; Manes et al. 2022). Excess amounts of nitrogen may act as a particularly important risk factor as nitrogen is a fertilizer that can enhance algal growth, plays a part in eutrophication, and can help promote potentially toxic blooms (Van Houtan et al. 2010, 2014, Dujon et al. 2021). In multiple locations, elevated presence of FP has been identified in highly urbanized areas with habitat degradation and pollution. (Foley et al. 2005, Bastos et al. 2022). High prevalence of FP has been linked to exposure to harmful algal blooms encouraged by elevated water temperatures and eutrophication (Dujon et al. 2021). Green sea turtles are herbivores that recruit to foraging grounds where they spend much of their time feeding on macroalgae in shallow coastal areas and bays (Bolten 2003). Some macroalgae species such as *Hypnea musciformis* and *Ulva fasciata*, that grow in areas with excess nutrients have elevated arginine levels, which has been identified as immunosuppressants that may impact the development of FP (McDermid, Stuercke & Balazs, 2007, Van Houtan et al. 2010, 2014). Another factor that may influence tumor development and growth is water temperature. Temperature changes significantly impact viral infections in ectothermic vertebrates (Gilad et al. 2003). In some regions, warmer water seems to promote tumor growth, and sea surface temperature can be used as a predictor for FP (Herbst 1994, Manes et al. 2022). Over the last 50 years, the global rise in FP prevalence has coincided with an average ocean temperature increase of one-third of a degree (Lafferty et al. 2004). FP may also be influenced by seasonal changes in water temperature (Herbst 1994). In Florida, FP prevalence was highest during the fall and winter suggesting that tumor growth was the most rapid during the warmest time of the year, reaching debilitating sizes by the fall months (Herbst 1994). Variations in environmental conditions imapct the development of FP at various locations including in Hawai‘i.

The first case of FP in Hawai‘i was identified in Kāne‘ohe Bay on O‘ahu in 1958, since then, FP prevalence in Hawai‘i rapidly increased during the 1980s, appeared to peak during the 1990s, and has since been declining (Balazs 1991, Bennett et al. 1999, Work et al. 2001, Chaloupka et al. 2008, 2009). FP has occurred on all seven populated islands in Hawai‘i: O‘ahu, Maui, Hawai'i Island, Lāna‘i, Moloka‘i, and Kaua‘i with O‘ahu having the greatest tumor incidences (Chaloupka et al. 2008, Van Houtan et al. 2010). Variations in FP prevalence and severity in Hawai'i occurs both between islands, and across individual islands (Van Houtan et al. 2010). Historically, from 1982-2003 FP was identified as the main cause of stranding on the islands of O‘ahu, Maui, and Kaua‘i (Chaloupka et al. 2008). On Hawai‘i Island, FP occurrence and severity is more prevalent along the windward side (East side) than along the leeward side (West side) (Murakawa et al. 2000, Van Houtan et al. 2010). Much of the spatial variation in FP trends is due to changes in environmental conditions across areas including precipitation patterns (Jones et al. 2016). The windward side of the island receives plentiful and consistent rainfall that can transport more land-based pollutants to rivers, and the discharge from urbanized areas along these rivers may disrupt the immune system of green turtles, increasing their susceptibility to FP (Manes et al., 2022). In Hawai‘i, areas with excess nutrients may have increased incidences of FP as various species of the macroalgae have elevated levels of arginine which may act as an immunosuppressant impacting the development of FP (Van Houtan et al. 2010, 2014).

The purpose of the present study was to assess spatial and temporal trends in FP in Hawaiian Green Turtles on the island of Hawai'i. Specifically, this study aims to (1) asses spatial trends in green sea turtle populations and FP prevalence over short-term (monthly) timescales (September 2023 to February 2024), (2) determine whether FP trends across short temporal scales are related to water quality measures: (dissolved oxygen, water temperature, salinity, nutrients), and (3) compare spatial trends in green sea turtle populations and FP prevalence overlong-term (annual).

## Methods

### *Data Collection*

Snorkel surveys were conducted in Hawaiʻi, USA at Onekahakaha Beach Park, Carlsmith Beach Park, Leleiwi Beach Park, Richardson Ocean Park, Punaluʻu Beach, and Puakō in order to assess the number of turtles observed, the size of the turtles, and the presence and severity of FP tumors (Fig 1). Survey locations were chosen due to accessibility for snorkel surveys and are areas where green sea turtles are historically found to aggregate.

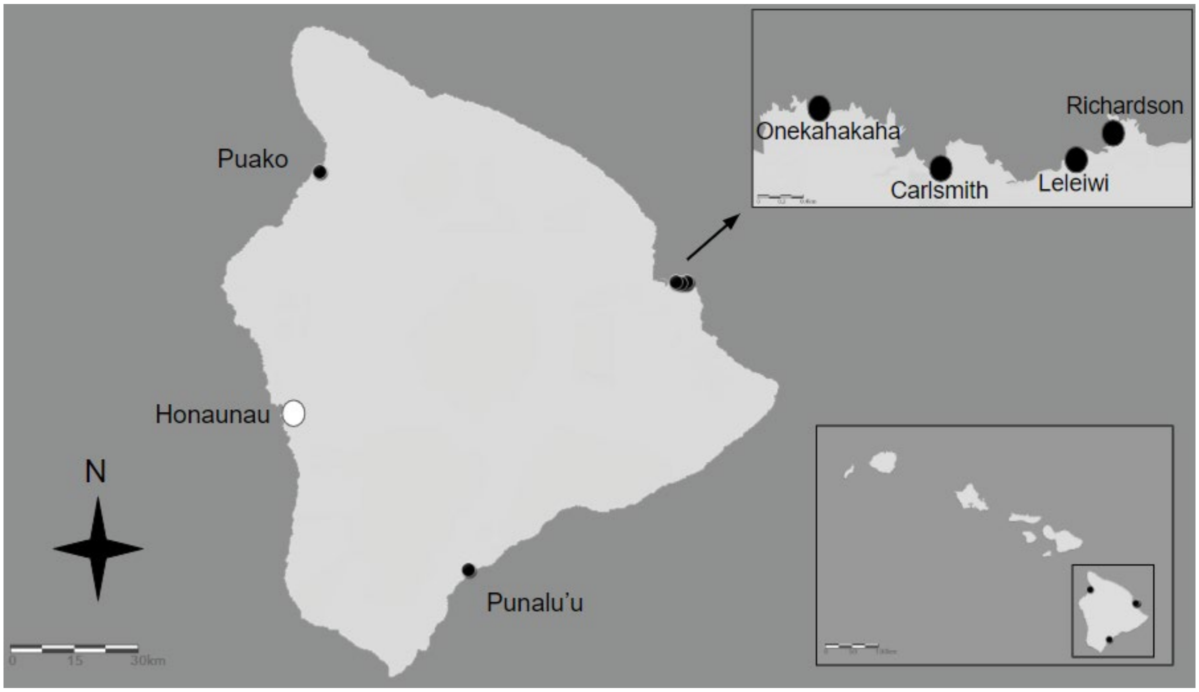



Figure 1. Survey locations across Hawai'i: Onekahakaha, Carlsmith, Leleiwi, Richardson, Punalu'u, Puakō, and Honaunau (indicated by the white circle, not surveyed during 2023-2024)

Surveys were conducted haphazardly for approximately 90 minutes at each location from September 2023 - February 2024. During each survey, turtles were counted and assessed for species, size, tumor score, sex (when possible), and any other relevant external characteristics were recorded. Size of each turtle was estimated as S, M, or L (S indicates turtles of small size, < 60.96 cm; M indicates turtles of medium size, 60.96-83.82 cm; L indicates turtles of large size > 83.82 cm) following the National Marine Fisheries Service (NMFS), Honolulu Lab, Marine Turtle Research Sea Turtle Sighting Form. FP tumor severity was also recorded during surveys, tumors were scored using the NOAA sighting form on a scale of 0-3 (0= no tumors present, 1= light tumor presence, 2= moderate tumor presence, 3= heavy tumor presence). FP tumor severity index was then calculated by taking the sum of all tumor scores for each survey in order to adjust for the number of turtles with each tumor score.

*Water Quality*

Replicate water samples (n=3) were collected per survey during 2023-2024 using 1L plastic bottles, which were immediately transported to shore for further analysis. Water was transferred to a 100 mL graduated cylinder and quality parameters (temperature, %DO, salinity) were measured using a YSI Pro 2030. Three sets of 12 mL of water from each location were filtered into 15 mL acid-washed test tubes using 60 mL acid-washed syringes and a GF/F filter (Whatman) and water samples were then immediately placed on ice and frozen at -5 °C until analysis. Samples were later thawed and processed for nitrate and nitrite using EPA method 353.2 by the University of Hawaiʻi at Hilo Analytical Laboratory. Historic water temperature and nutrient data were also obtained at survey locations and during survey years to use for additional analysis (PacIOOS' Water Temperature Buoy Observations: Hilo, Hawaiʻi Island, Abaya et al., 2018; Aguiar et al., 2023; Carlson & Wiegner, 2016; Wiegner et al., 2021; Nakoa III, 2022).

*Historical Data*

In addition to data collected during 2023 and 2024, historical data was also obtained to compare to recent surveys. Snorkel surveys were conducted by students in the University of Hawaiʻi at Hilo MARE 490- Sea Turtle Conservation and Ecology class from 2007-2022 at Onekahakaha Beach Park, Carlsmith Beach Park, Leleiwi Beach Park, Richardson Ocean Park, Punaluʻu Beach, Honaunau Bay, and Puakō using the same survey techniques listed above. Sea turtle stranding data collected on turtles stranded on Hawaiʻi Island by members of the Pacific Islands Fisheries Science Center under the US National Marine Fisheries Services, the University of Hawaiʻi at Hilo Sea Turtle Stranding Response Team, and the Hawaiʻi Preparatory

Academy Sea Turtle Response Program were also used to compare the presence of FP in stranded and *in situ* turtles.

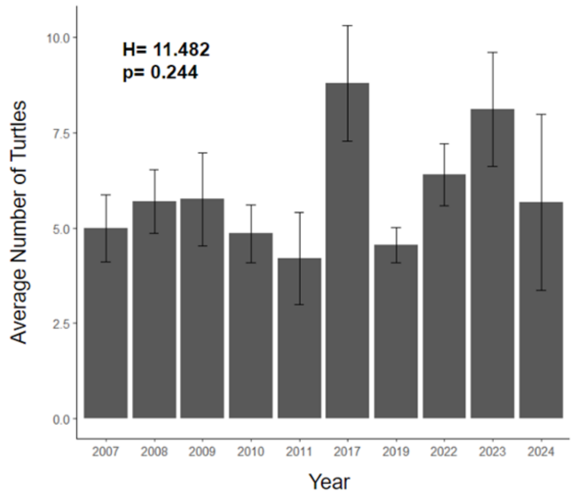
*Statistical Analysis*

Analysis of variance (ANOVA) tests, Kruskal Wallis tests, and subsequent post-hoc multiple comparisons tests (Tukey and Mann-Whitney) were used to determine whether sea turtle abundance differed by turtle size, tumor score, location, and by year. Pearson's correlations were used to test linear relationships between turtle abundance, turtle size, tumor score, and water quality variables (salinity, temperature, and nutrients). All analyses were performed using RStudio 2023.09+494 with an alpha of 0.05.

## Results

Among years, the average number of turtles observed during each survey did not differ significantly ($H = 11.482$, $p = 0.244$) (Fig 1A). The average number of turtles observed at each location during all of the years combined however did differ significantly ($H = 68.327$, $p < 0.001$) (Fig 1B). Onekahakaha had the lowest number of turtles observed, followed by Richardson, then Carlsmith and Leleiwi, and finally Punaluʻu and Puakō (Fig 1B).

A.




B.

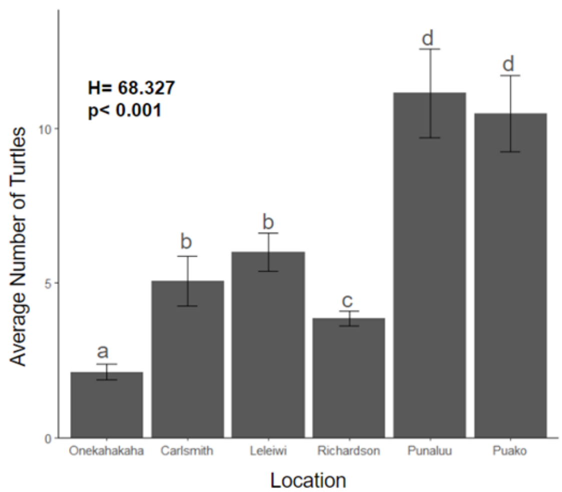
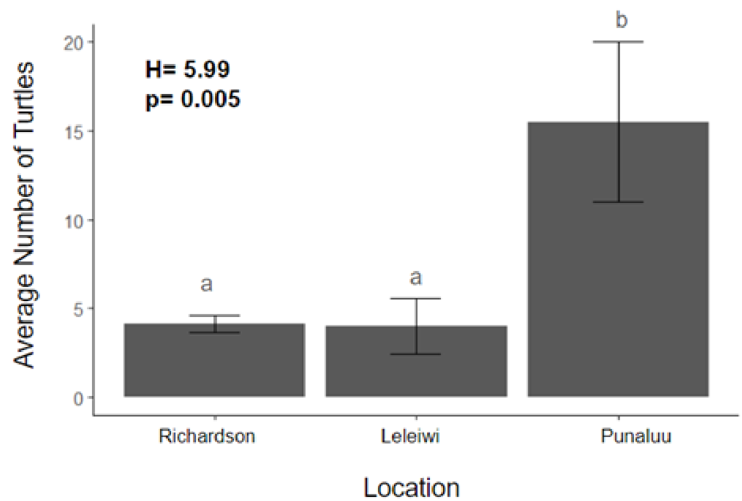


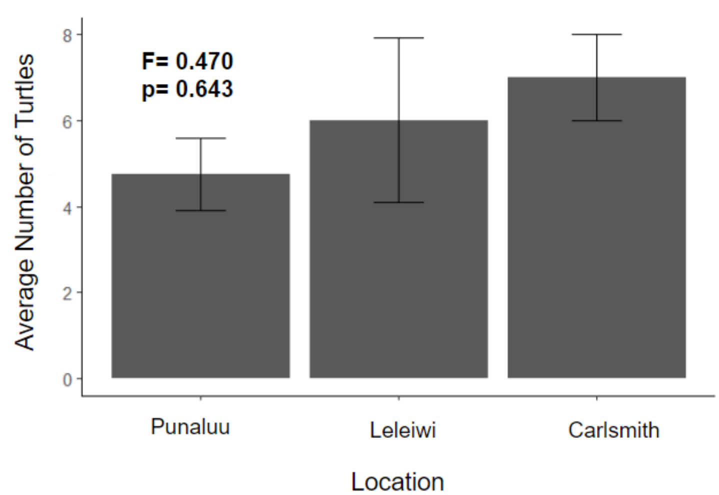

Fig 1. Mean number of turtles (±SE) compared among A) years and B) locations. Results from a Kruskal Wallis statistical test and Mann-Whitney pairwise comparisons. Statistical differences are represented by different letters above each column with α=0.05.

The average number of turtles observed varied significantly across the various locations in 2007 with Punaluʻu having the greatest number of turtles (H= 5.990, p = 0.005) (Fig 2A). In 2011, the average number of turtles observed varied significantly across the various locations with Puakō having the greatest number of turtles (H= 5.963, p = 0.051) (Fig 2E). In 2017, the average number of turtles observed varied significantly across the various locations with Punaluʻu having the highest numbers of turtles and Onekahakaha having the fewest number of turtles (H= 17.099, p < 0.001) (Fig 2F). During 2019, the average number of turtles observed varied significantly across the various locations with Puakō having the highest number of turtles (H= 18.522, p < 0.001) (Fig 2G). In 2022, the average number of turtles observed varied significantly across the various locations with Puakō also having the highest number of turtles observed (H= 23.697, p < 0.001) (Fig 2I).

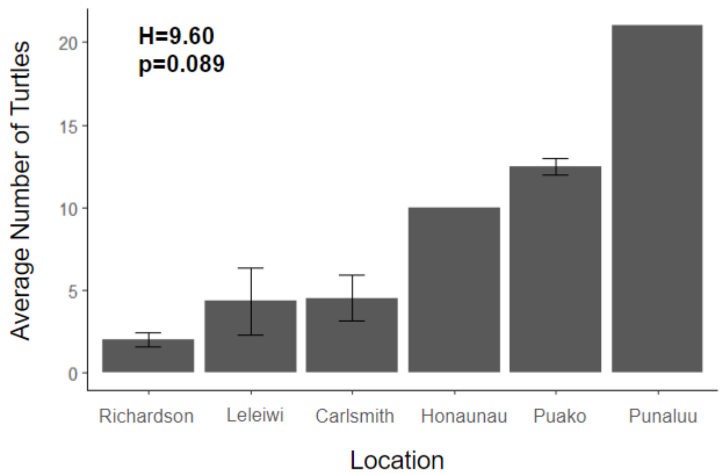
A. (2007)



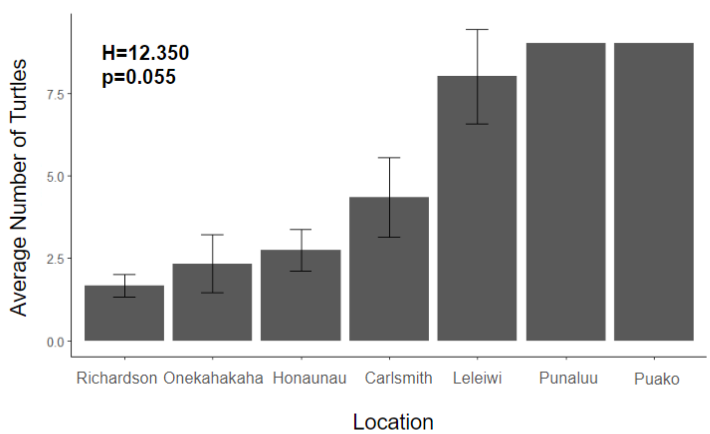

B. (2008)



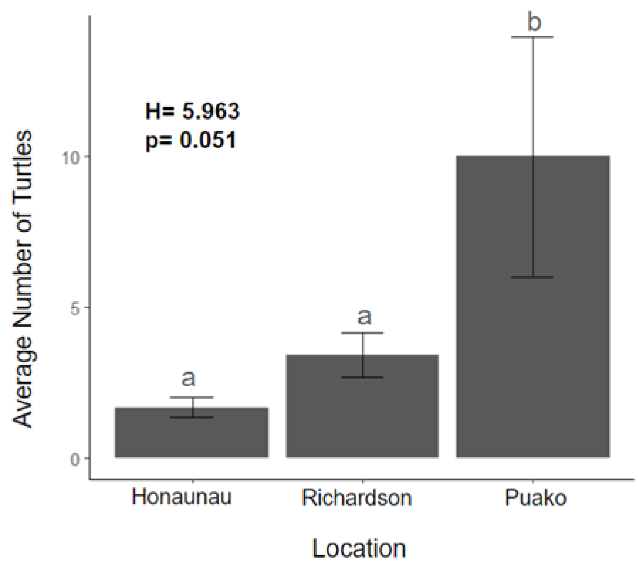
C. (2009)


H=9.60
p=0.089
Average Number of Turtles
0
5
10
15
20
Richardson
Leleiwi
Carlsmith
Honaunau
Puako
Punaluu
Location

D. (2010)

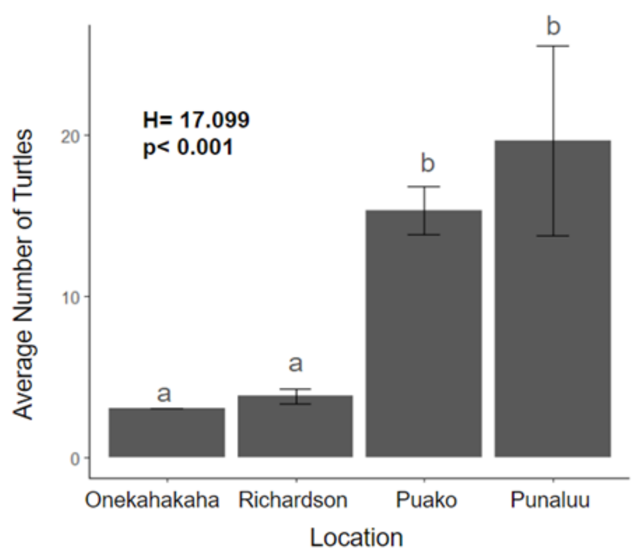

H=12.350
p=0.055
Average Number of Turtles
0.0
2.5
5.0
7.5
Richardson
Onekahakaha
Honaunau
Carlsmith
Leleiwi
Punaluu
Puako
Location

E. (2011)



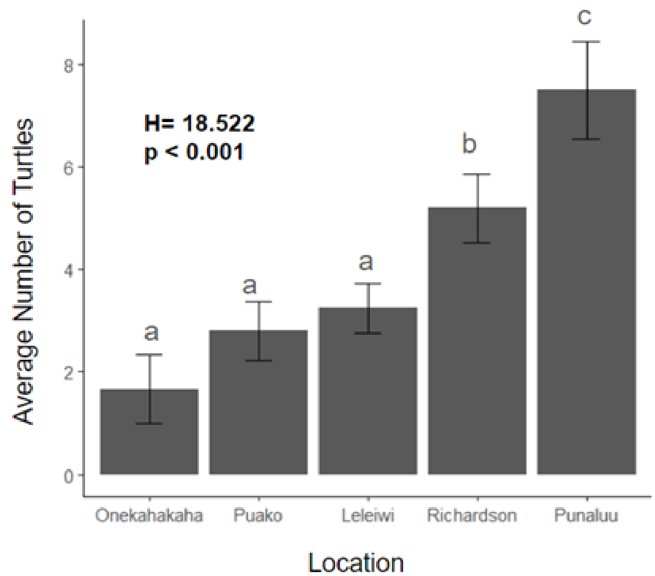
H= 5.963
p= 0.051
Average Number of Turtles
10
5
0
a
a
b
Honaunau
Richardson
Puako
Location


F. (2017)

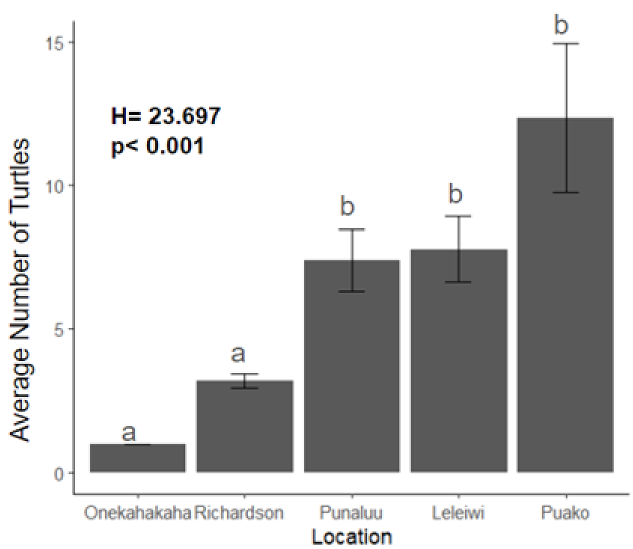


H= 17.099
p< 0.001
Average Number of Turtles
20
10
0
a
a
b
b
Onekahakaha
Richardson
Puako
Punaluu
Location

G. (2019)


H= 18.522
p < 0.001
Average Number of Turtles
0
2
4
6
8
a
a
a
b
c
Onekahakaha
Puako
Leleiwi
Richardson
Punaluu
Location

H. (2022)


H= 23.697
p< 0.001
Average Number of Turtles
0
5
10
15
a
a
b
b
b
Onekahakaha
Richardson
Punaluu
Leleiwi
Puako
Location

I. (2023)

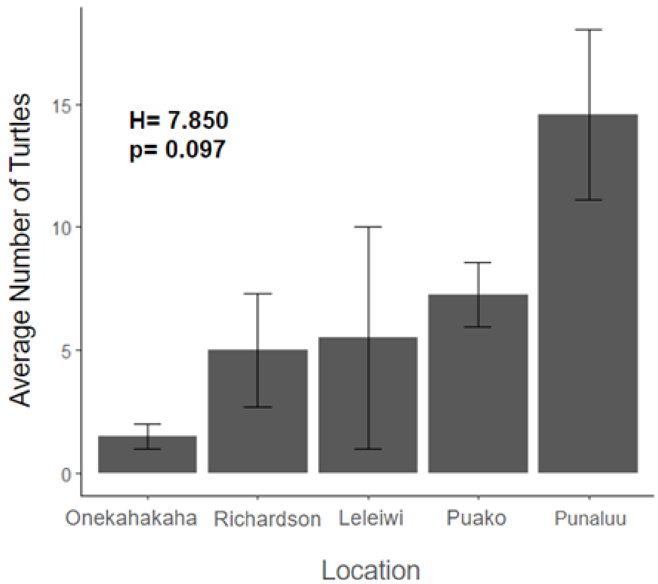



Fig 2. Mean number of turtles (±SE) per survey compared among locations during A) 2007, B) 2008, C) 2009, D) 2010, E) 2011, F) 2017, G) 2019, H) 2022, I) 2023. Results from a Kruskal Wallis statistical test and Mann-Whitney pairwise comparisons. Statistical differences are represented by different letters above each column with α=0.05

A total of 1277 turtles were surveyed from 2007-2024. In June-July 2007, 61 turtles (49%) of the 125 surveyed turtles had external tumors present whereas in 2023 a total of 118 turtles were observed, only 10 (8%) of which had external FP tumors, and in 2024 a total of 35 turtles were surveyed and 6 of these turtles (17%) had FP tumors (Fig 3).

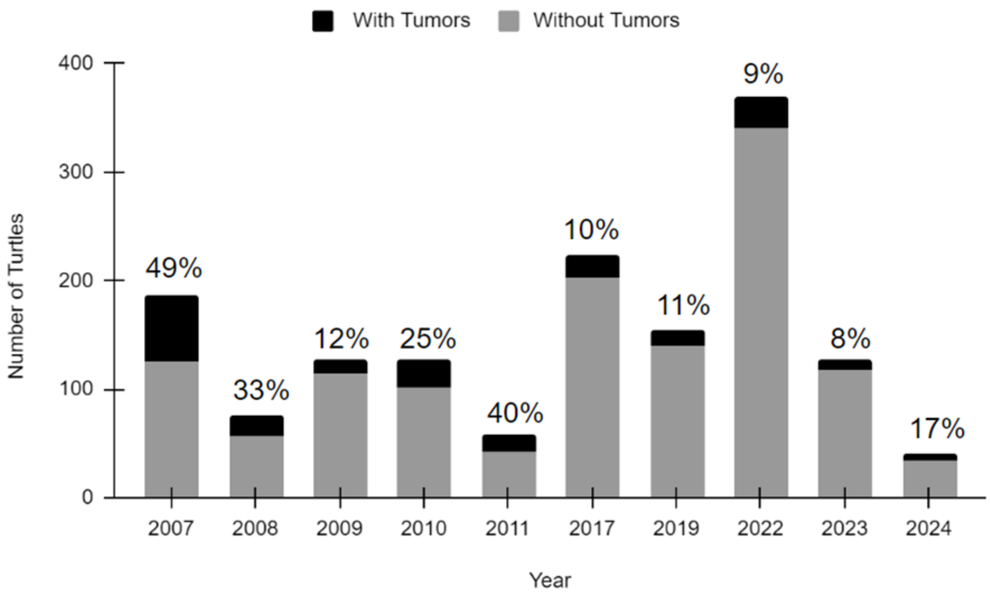
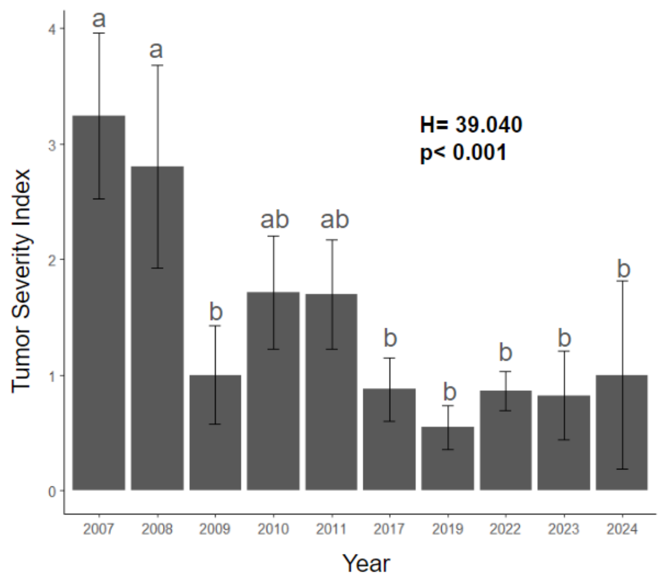



Fig. 3 Total number of turtles surveyed each year among all locations (Onekahakaha, Carlsmith, Leleiwi, Richardson,Puakō, Punalu'u, and Honaunau).

Differences in tumor severity indexes have occurred from 2007 to 2023 with an overall general decline in the severity of FP tumors occurring as the highest mean FP tumor severity index was observed in 2007 and the lowest severity index was observed in 2019 ($H= 39.040$, $p<0.001$) (Fig 4A). The tumor severity indexes in 2009, 2017, 2019, 2022, 2023, and 2024 were significantly lower than the severity in 2007 and 2008. When tumor severity indexes were assessed with all of the years combined, differences among locations were also recorded ($H= 19.034$, $p= 0.004$) (Fig 4B). The lowest tumor severity indexes were observed at Puakō and Onekahakaha while Leleiwi had the highest. Tumor severity was significantly higher at Richardson and at Leleiwi when compared to the other locations with some overlap among Carlsmith and these two locations.

A.




B.

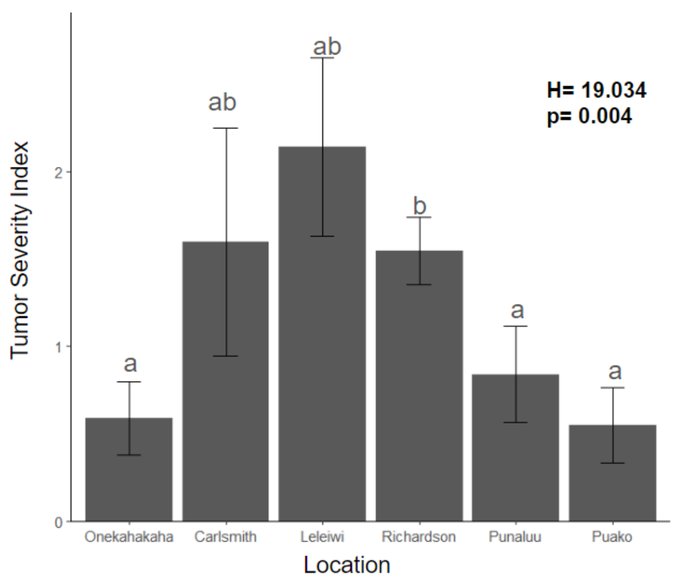
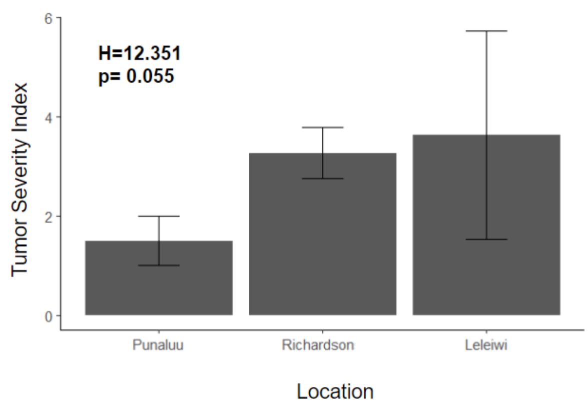



Fig 4. Mean FP tumor severity index (±SE) compared among A) years and B) locations. Results from a Kruskal Wallis statistical test and Mann-Whitney pairwise comparisons. Statistical differences are represented by different letters above each column with α=0.05

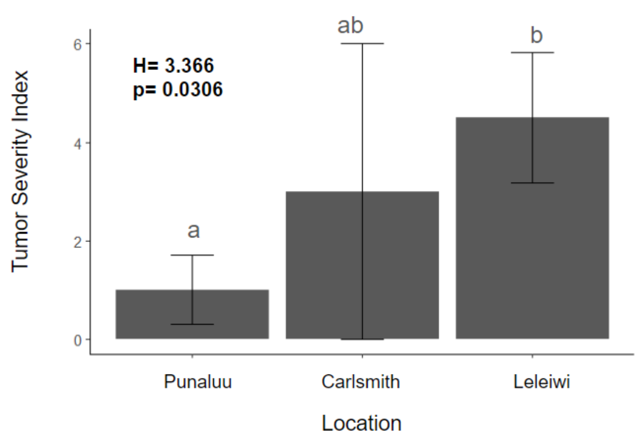
Mean FP tumor severity indexes did not differ by location in 2007, 2009, 2010, 2011, 2017, 2022, or 2023, but a significant difference was observed during 2008 (H= 3.366, p =

0.055)  (Fig 5B). During 2008, the tumor severity index was higher at Leleiwi than at Punalu'u. Across years, Leleiwi had the highest tumor severity index in four of the nine surveyed years. During 2019, FP tumors were only recorded at Richardson.

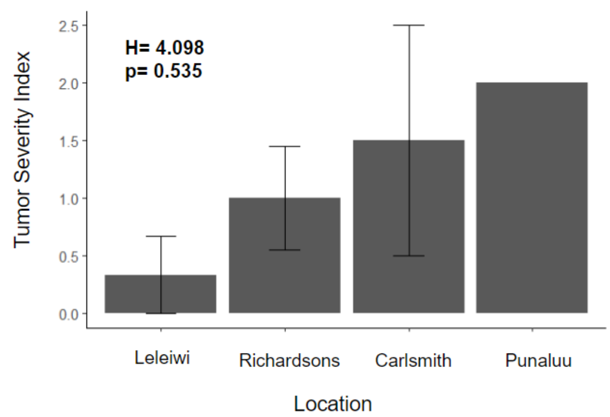
A. (2007)



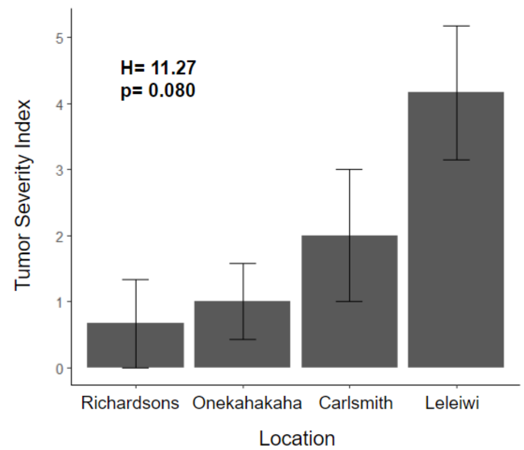

B. (2008)



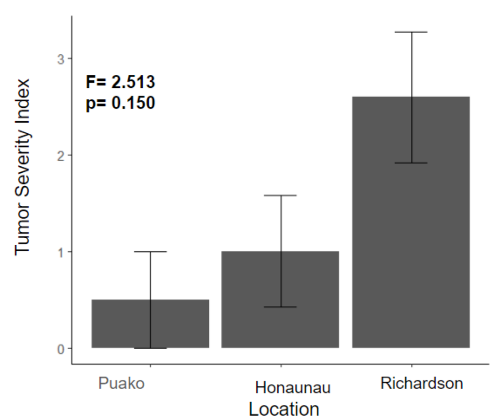
C. (2009)



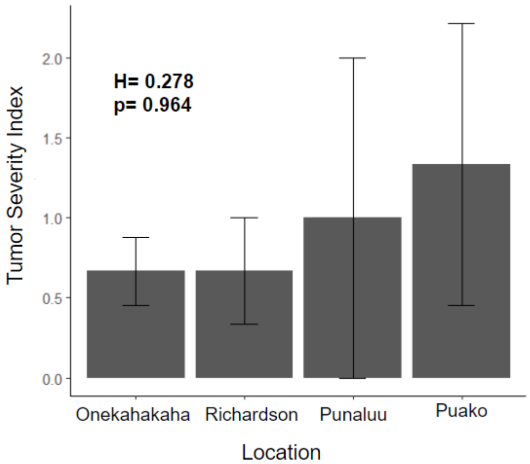

D. (2010)



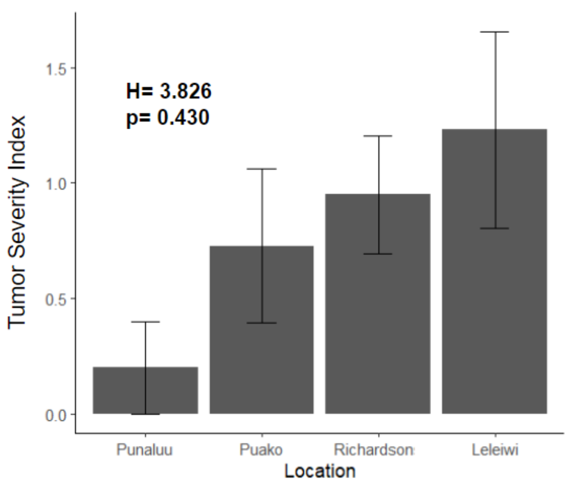

E. (2011)



F. (2017)


H= 0.278
p= 0.964
Tumor Severity Index
2.0
1.5
1.0
0.5
0.0
Onekahakaha
Richardson
Punaluu
Puako
Location

G. (2022)


H= 3.826
p= 0.430
Tumor Severity Index
1.5
1.0
0.5
0.0
Punaluu
Puako
Richardson
Leleiwi
Location

H. (2023)

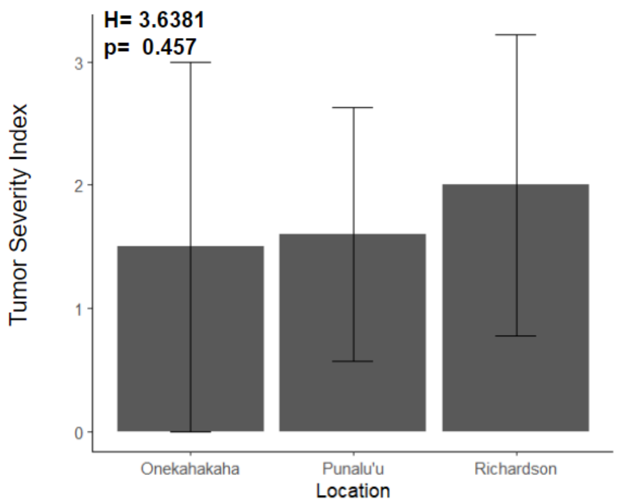



Fig. 5 Mean FP tumor severity index (±SE) compared among locations during A) 2007, B) 2008, C) 2009, D) 2010, E) 2011, F) 2017, G) 2022, H) 2023. Results from a Kruskal Wallis statistical test and Mann-Whitney pairwise comparisons. Statistical differences are represented by different letters above each column with α=0.05

Across size classes, tumor severity differed significantly (H= 6.231, p= 0.044). Tumor severity was lowest in small turtles of sizes less than 24 inches (Fig 6).

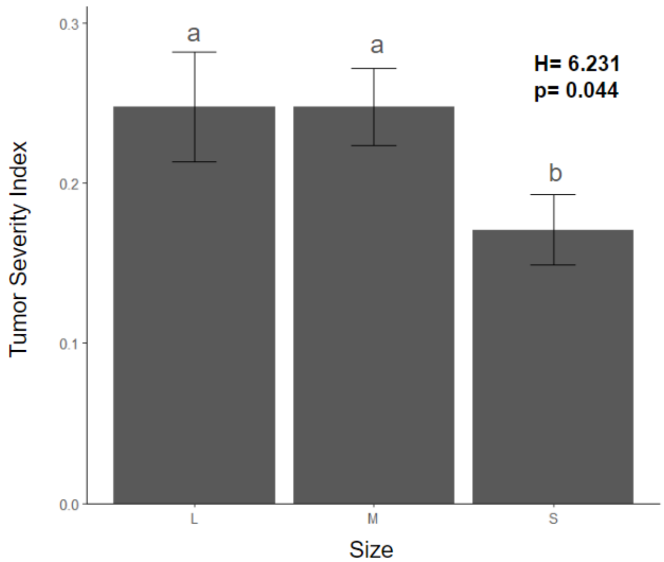
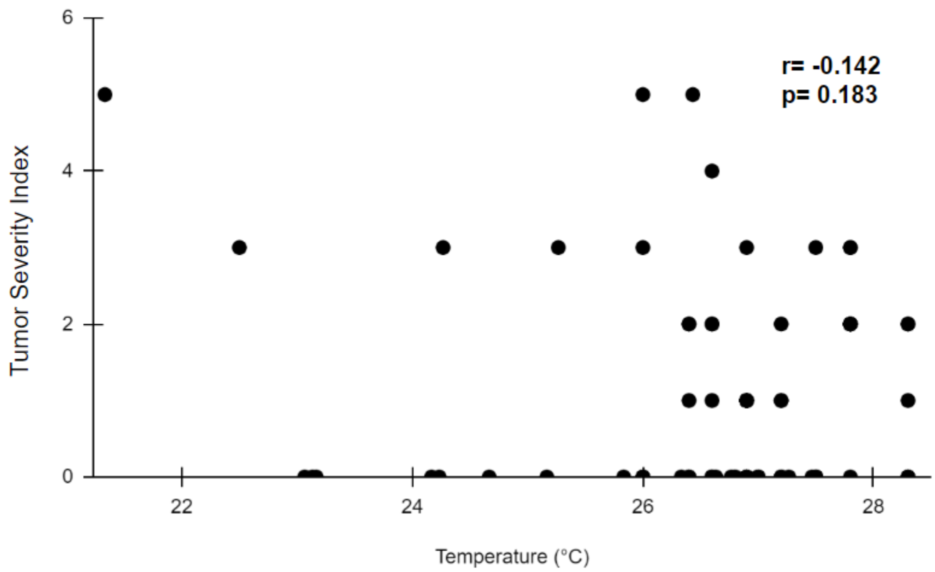


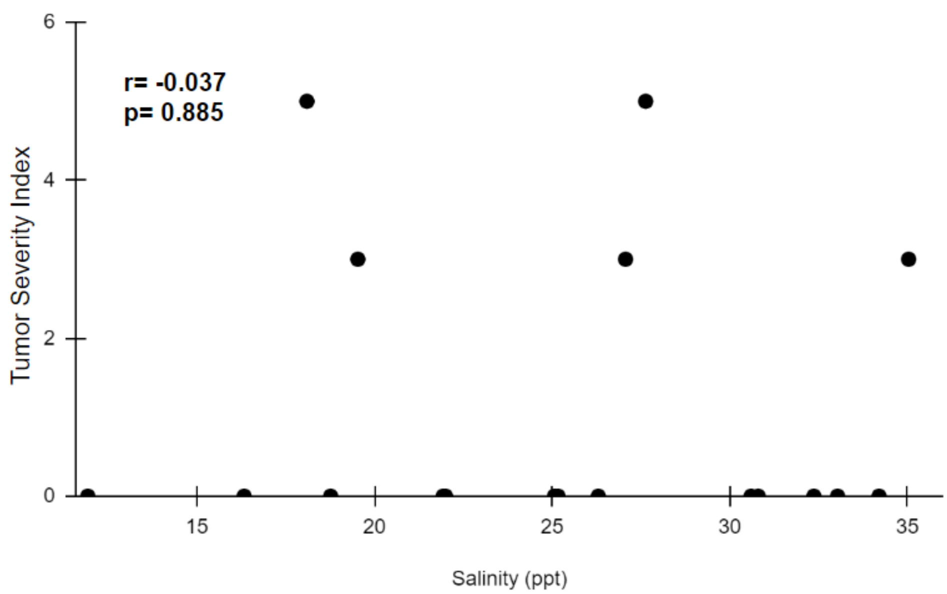

Fig. 6 Mean FP tumor severity (±SE) compared among size classes. Results from a Kruskal Wallis statistical test and Mann-Whitney pairwise comparisons. Statistical differences are represented by different letters above each column with $\alpha$=0.05

No significant relationships were found between water temperature (r= -0.142, p= 0.183), salinity (r= -0.037, p= 0.885) or $NO^{3-}$ + $NO^{2-}$ (r= -0.151, p= 0.135) concentrations and FP tumor severity (Fig 7). Among all three water parameters, points of relatively high tumor severity were spread across relatively low and relatively high salinity, temperature, and dissolved oxygen.

A.


r= -0.142
p= 0.183
Tumor Severity Index
Temperature (°C)
0
2
4
6
22
24
26
28

B.


r= -0.037
p= 0.885
Tumor Severity Index
Salinity (ppt)
0
2
4
6
15
20
25
30
35

C.

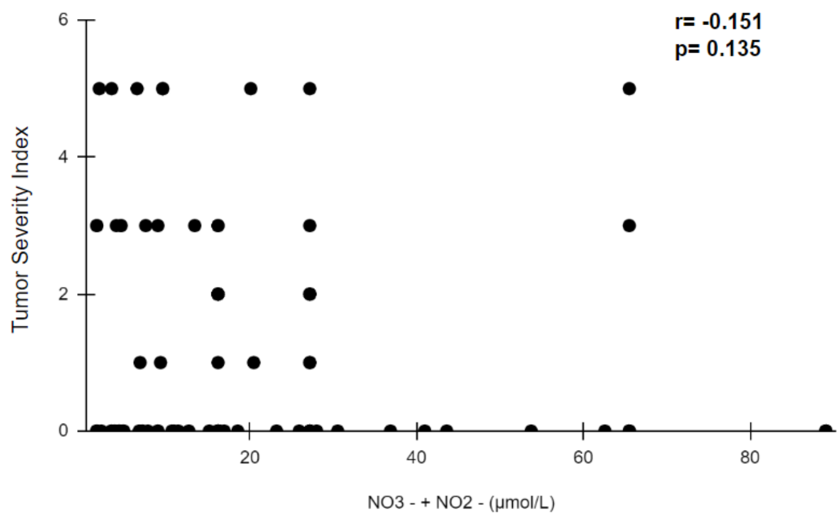
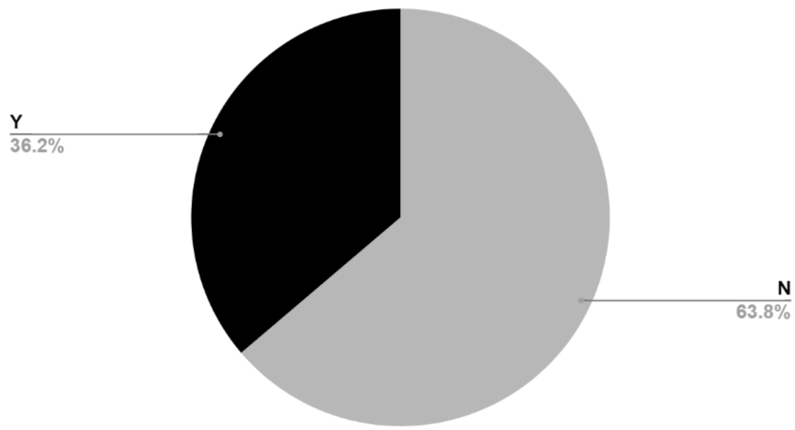



Fig. 7. Scatterplots showing comparison of A) temperature, B) salinity, C) $NO^{3-} + NO^{2-}$ and tumor severity indexes. Results are from a Pearson's correlation test (α=0.05). Additional temperature data obtained from:Water temperature buoy observations: Hilo, Hawai‘i Island: Pacioos, Nakoa III, 2022. Additional nutrient data obtained from: Abaya et al., 2018; Aguiar et al., 2023; Carlson & Wiegner, 2016; Wiegner et al., 2021; Nakoa III, 2022.

The percent of turtles with external FP tumors was greater in turtles that were stranded than in turtles that were surveyed *in situ* (Fig 8). Tumored turtles made up 36.2% of the stranded turtles whereas they only made up 17.2 % of the surveyed *in situ* turtles.

A. (Stranded)




B. (*In Situ*)

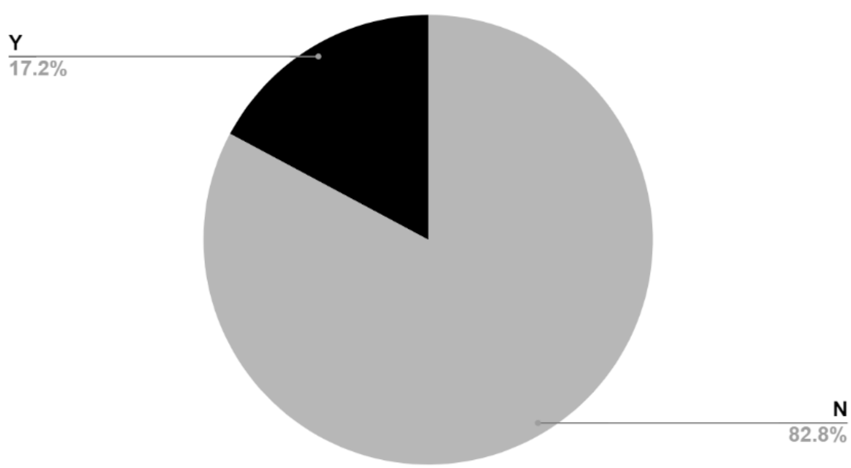
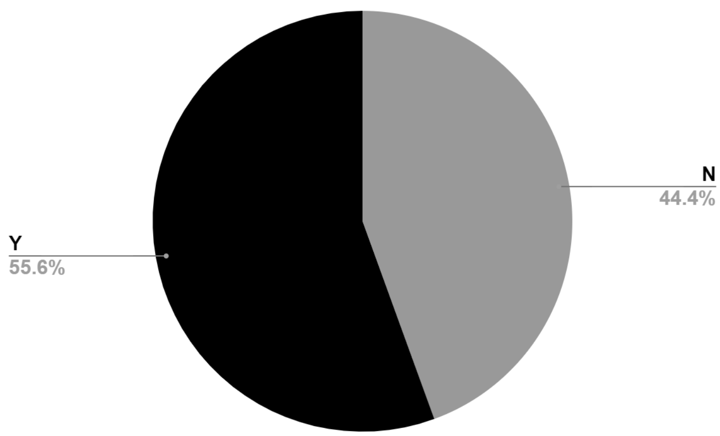


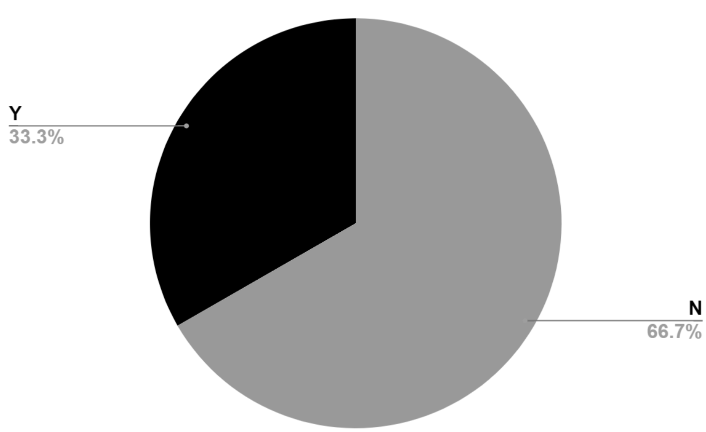

Fig. 8 Percent of A) stranded and B) *in situ* turtles with FP tumors in 2007, 2008, 2009,2010, 2011, 2017, 2019, and 2022 among all locations (Onekahakaha, Carlsmith, Leleiwi, Richardson, Puakō, Punalu'u). Stranded data was obtained from members of the Pacific Islands Fisheries Science Center under the US National Marine Fisheries Services, the University of Hawaiʻi at Hilo Sea Turtle Stranding Response Team, and the Hawaiʻi Preparatory Academy Sea Turtle Response Program.

In 2007, 2010, and 2017 the percentage of tumored turtles was higher in stranded turtles than *in situ* turtles. In stranded turtles, from 2007 to 2009, percentages of tumored turtles decreased, then increased in 2010 and in 2017 (Fig 9). The percentage of tumored turtles *in situ* increased from 2007 to 2009, then decreased in 2010 and increased again in 2017 (Fig 10).

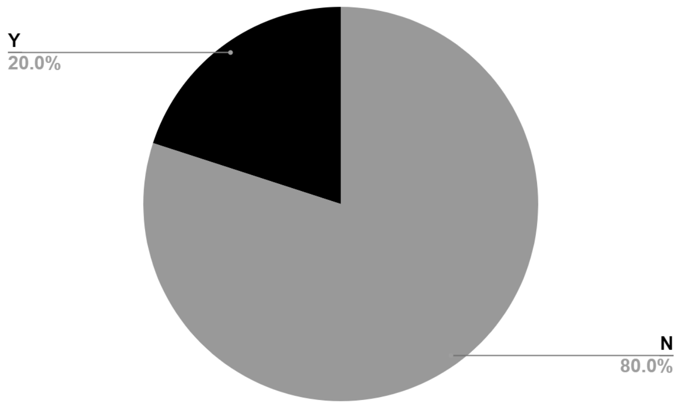
A. (2007)



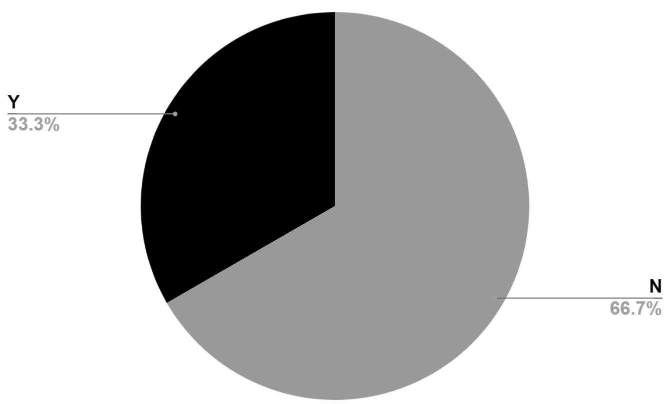

B. (2008)



C. (2009)




D. (2010)




E. (2017)

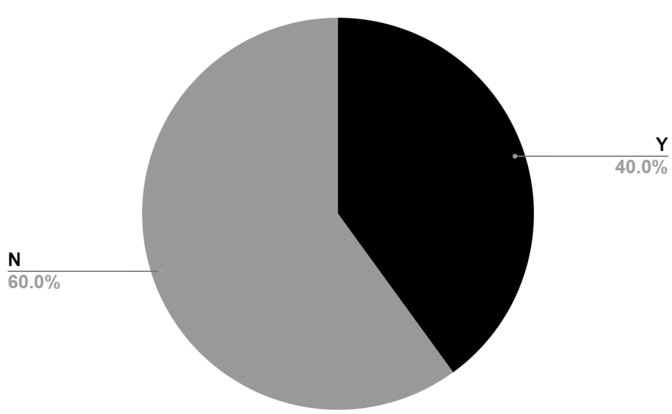
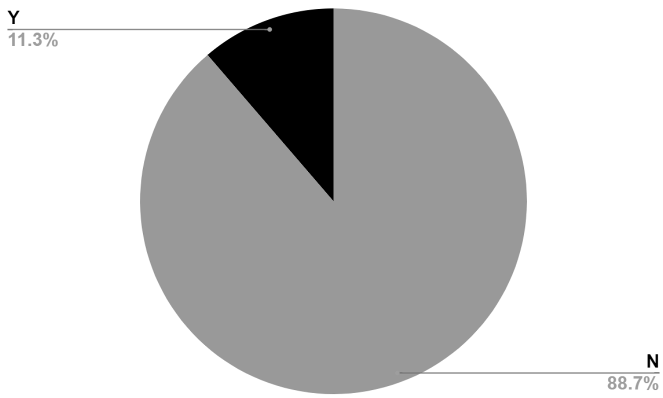
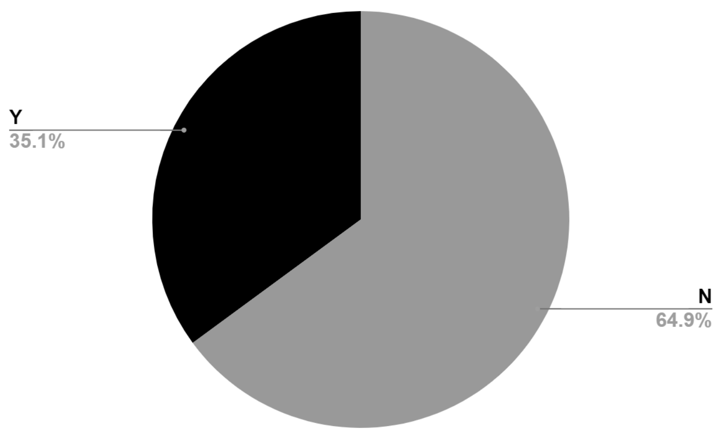



Fig. 9 Percent of stranded turtles with FP tumors in A) 2007, B) 2008, C) 2009, D) 2010, E) 2017 among all locations (Onekahakaha, Carlsmith, Leleiwi, Richardson,Puakō, Punalu'u).

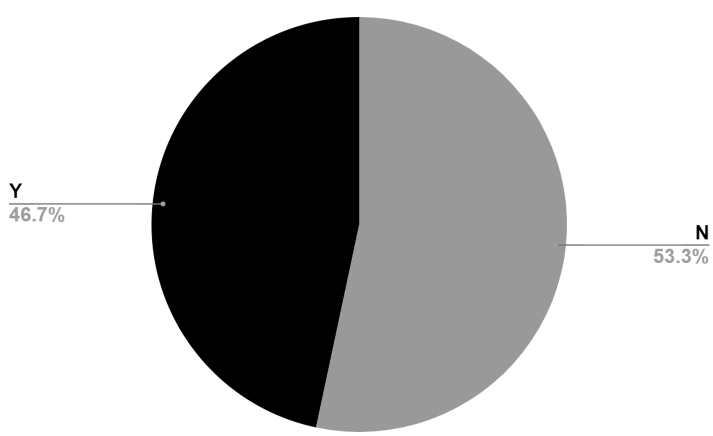
A. (2007)



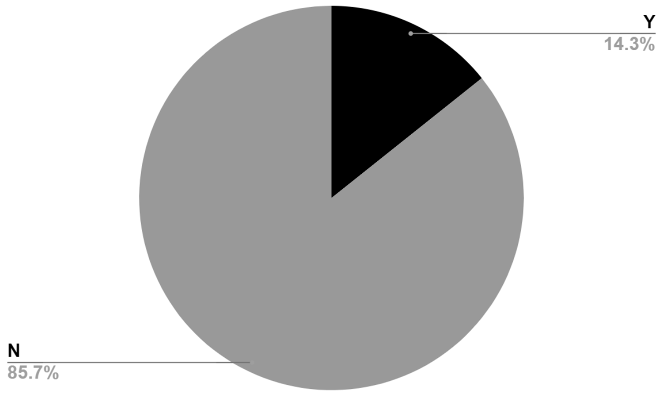

B. (2008)




C. (2009)



D. (2010)




E. (2017)

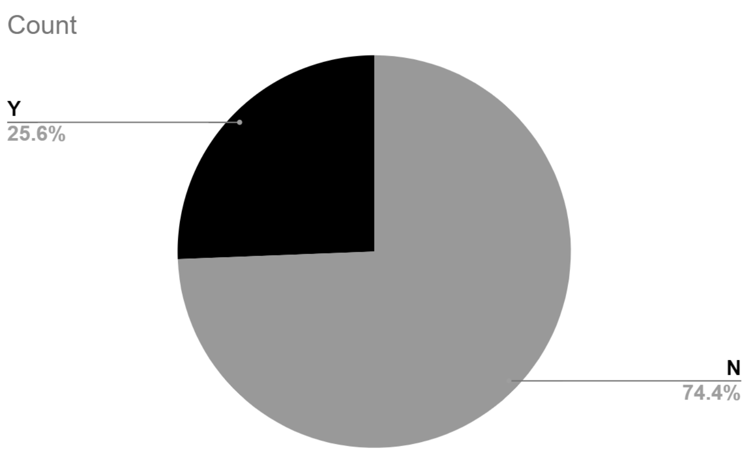



Fig. 10 Percent of *in situ* turtles with FP tumors in A) 2007, B) 2008, C) 2009, D) 2010, E) 2017 among all locations (Onekahakaha, Carlsmith, Leleiwi, Richardson, Puakō, Punalu'u). Stranded data was obtained from members of the Pacific Islands Fisheries Science Center under the US National Marine Fisheries Services, the University of Hawaiʻi at Hilo Sea Turtle Stranding Response Team, and the Hawaiʻi Preparatory Academy Sea Turtle Response Program

## Discussion

The percentage of turtles with FP in recent years has been relatively low, < 20%, in 2019, 2022, 2023, and 2024. The percentage of turtles with FP has been relatively high > 25% with the exception of 2009. This is consistent with the relatively low percentages of FP that have been identified previously in Hawaiian green turtles, for example, out of a sample of 66 turtles observed during a study conducted by Bennett et al (1999), only 21, or 33.3% initially had tumors and these turtles were then subsequently observed without any tumors (Bennett et al. 1999). The sparse presence of FP on Hawaiʻi Island is also consistent with the one case of FP reported in 2022 for stranded green turtles on Maui (Cutt et al. 2023). In stranded Hawaiian green turtles, from 1975-2014 a total of 39.7% of stranded turtles were tumored turtles, 41.7% were non-tumored turtles, and 18.6% had unknown tumor presence. Tumored turtles comprised less than 40% of those seen from 1982 to 1987. From 1987 to 2004 the number of tumored turtles peaked at 52%. Then in 2005, the number of tumored turtles declined to 32% (Hargrove et al. 2016).

No significant changes in the number of sea turtles observed over the years was found in this study, but a previous study found an increase in the number of turtles since they were first protected (Balazs & Chaloupka 2004). Variation in the number of turtles surveyed at each location was found during this study. The average number of turtles observed per survey both per year, and at each location is crucial in better understanding FP tumor severity as during this study, higher numbers of turtles did not necessarily indicate higher tumor severity. For example, the highest number of turtles were observed in 2017 but tumor severity in 2017 was the second lowest. A similar trend was observed at Puakō which had the second highest turtle observations and lowest tumor severity.

The highest tumor severity in this study occurred in 2007 and 2008 and the lowest tumor severity occurred in 2019 and 2023. This is consistent with previous studies conducted on other Hawaiian Islands revealing decreases in tumor presence since the 1990s (Bennett et al. 1999, Work et al. 2001, Chaloupka et al. 2008, 2009, Cutt et al. 2023). In a study conducted on stranded turtles on Hawaiʻi Island found spikes in the number of stranded turtles with FP in 2007, 2010, 2012, and 2017 (Dentlinger et al. 2024). Temporal declines in the presence of FP may be due to the death of severely affected juveniles, the regression of tumors, or a combination of both (Foley et al. 2005, Van Houtan et al. 2010). Decreases in tumor presence were observed across years even during years with higher numbers of turtle observations and higher survey effort which suggests that the decrease in FP severity may be due to long-term tumor regression (Patrício et al., 2016). FP is not lethal to all green turtles, and tumor recovery may be possible in Hawaiian waters (Chaloupka et al. 2009). In Hawaiʻi, some photographic evidence of FP regression has been documented with 32% of turtles photographed having regressed tumors (Bennett et al. 1999). In Brazil, 32.8% of recaptured turtles showed tumor regression, and 24.6% of turtles showed both tumor progression and regression (Machado Guimarães et al. 2013). Data from this study also suggests that FP severity progresses and gets worse before it begins to regress and get better (Machado Guimarães et al. 2013). Other studies have suggested that FP tumor regression occurs naturally, and full recovery may be possible in some turtle populations (Ehrhart, 1991, Hargrove et al., 2016, Patrício et al., 2016). One of the strongest cases for full recovery was reported in Florida by Kelley et al (2022) in the Indian River Lagoon (IRL), where nearly every turtle in the IRL was affected by the disease and then recovered from the disease before emigrating from the IRL as they mature (Kelley et al. 2022). Strong temporal variations in FP have been observed in a wide variety of locations, and additional variation is present between locations both globally and locally.

The highest tumor severity was found at Carlsmith and Leleiwi located on the eastern side of the Island whereas the lowest severity was found at Onekahakaha located on the eastern side of the Island and Puakō located on the western side of the island. Previous studies conducted on Hawai'i Island also revealed variations in FP tumor presence across locations with FP severity documented as more prevalent along the windward side than along the leeward side (Balazs 1991, Murakawa et al. 2000, Van Houtan et al. 2010). FP severity was lowest at Onekahakaha, this may be due to low sampling effort or the low number of turtles observed during surveys. FP was only observed at Puakō during two of the sampling years, 2010 and 2017. Puakō was the only survey location located on the leeward side of the island making it difficult to determine if the lack of FP found at this location is impacted by the location of this survey site.

FP is most commonly found in turtles of intermediate size as the disease often increases with the size of the turtle, peaks, and then decreases (Work et al. 2001, Foley et al. 2005, Van Houtan et al. 2010, Patrício et al. 2016). During the present study, the lowest tumor severity was found in small sized turtles. Previously in Hawai'i, larger turtles were found to have higher tumor severity scores, but lower tumor growth rates (Balazs & Chaloupka 2004). These higher tumor scores were most likely due to extended exposure to disease causing factors (Balazs & Chaloupka 2004). This study found turtles less than 24 inches (60.96 cm) to have the lowest tumor severity. The data collected during this study is inconsistent with some other studies that have found the highest tumor severity in turtles between about 35cm- 60 cm (IRL), but is consistent with other studies that found the highest tumor severity in turtles between about 65 cm- 80cm. (Van Houtan et al. 2014).

No relationship was found between temperature and tumor severity. Previous studies have identified that warmer water may promote tumor growth, with higher water temperatures correlating with greater FP prevalence (Herbst 1994, Manes et al. 2022). Warmer water temperatures, especially during summer, may promote lesion growth, leading to larger lesions by autumn (Herbst, 1994; Herbst et al.1995). This seasonal pattern has been noticed in Florida, where a higher incidence of FP is seen in turtles stranded during winter (Herbst, 1994). In Hawaiʻi however, this relationship has not been observed most likely due to relatively stable water temperatures throughout the year (Work et al. 2001,Murakawa et al. 2000, Foley et al. 2005).

No relationship among salinity and tumor severity was observed. Salinity has been identified as a stressor for sea turtles, and plays an important role in the suitability of a habitat for an organism (Landsberg et al. 1999). In both Florida and Texas, salinity predicted the prevalence of FP, but this may be a side effect of river water discharge as a link between FP and salinity has not been observed elsewhere (Manes et al. 2022).

A relationship between nutrients and tumor severity was not identified during this study. Previous studies have shown that FP prevalence was often highest in areas with high nutrient concentrations especially when concentrations led to harmful algal blooms (Dujon et al., 2021). In Hawaiʻi, previous studies have suggested that excess nutrients can lead to elevated levels of arginine in invasive algae species (Van Houtan et al. 2010; Van Houtan & Schwaab, 2013). These elevated levels of arginine, when consumed by sea turtles, may promote the formation of FP tumors (Van Houtan et al 2010, 2014, Van Houtan & Schwaab, 2013). Among green turtle populations, FP has been identified as most prevalent in eutrophied and impaired waterways (Herbst, 1994; Van Houtan et al. 2010, 2014). Spatial variation in FP severity on Hawaiʻi Island may be attributed to differences in environmental conditions across locations (Jones et al. 2016). Excess amounts of nutrients and increased amounts of pollutants from river runoff at locations on east Hawaiʻi Island may increase the likelihood of FP development (Van Houtan et al. 2010, 2014, Dujon et al. 2021, Manes et al. 2022). A previous study conducted on Hawaiʻi Island found that locations on the leeward side of the island had fewer nutrient inputs and fewer invasive algae species that contain arginine (Van Houtan et al. 2010). The lack of relationship between nutrients and FP tumors found during this study may be due to the relatively few FP tumors observed during 2023-2024 when water samples were collected.

The percentage of turtles with tumors was higher in turtles that had stranded than in those observed during field surveys. This may be due to the inherent bias present when surveying stranded animals because they are usually either sick or injured. Turtles may strand due to impacts from the disease such as locomotion hindrance. Other studies conducted in Florida found that turtles with tumors are more likely to become entangled in fishing line than turtles without FP tumors (Foley et al. 2005). However, no correlation between FP and fishing gear strandings were found in the other main Hawaiian Islands (Chaloupka et al. 2008). From 2007-2009, the percentage of stranded tumored turtles decreased while the percentage of *in situ* tumored turtles increased. This may be indicative of tumor recovery. Turtles that have been debilitated by conditions such as advanced FP may be at higher risk to be found stranded (Foley et al. 2005, Chaloupka et al. 2008, Page-Karjian et al. 2014). Fewer stranded turtles with FP may have been observed over these years as fewer turtles had debilitating cases of FP and more *in situ* turtles had less severe cases of FP.

Overall, this study provides valuable insights into the spatial and temporal trends of FP in Hawaiian green sea turtles. A decrease in FP severity across years consistent with previous studies was found during this study with the decline in FP severity possibly being indicative of tumor regression and recovery. Spatial variations in FP severity were also observed among the various locations on Hawai‘i Island during this study. No relationships were found between tumor severity and temperature or nutrient levels. This was most likely due to the consistent water temperatures observed across the island and possibly due to the relatively low tumor severity observed in recent years. Higher percentages of turtles with FP were found in stranded turtles when compared to turtles surveyed *in situ*. Despite ongoing threats, the observed declines in tumor severity suggest that these turtles may have the ability to recover from FP. However, continued monitoring and conservation efforts are essential to ensure the long-term survival of this threatened species.

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