

Fibropapillomatosis Affecting Green Turtles (*Chelonia mydas*) Research Report



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Abstract

Fibropapillomatosis is a disease that is affecting sea turtles all around the world. Turtles that are most at risk are those that live in near-shore waters and lagoons, especially areas next to large human populations with poor sewage treatment facilities. In this research project the main focus is fibropapillomatosis (FP) and green sea turtles captured by netting in Lac Bay, Bonaire. The research goal was to see how turtles living in Bonaire are affected by the disease. The two main research questions were: "What is the true rate of fibropapillomatosis affecting green sea turtles in Lac Bay?" and "What is the difference between healthy turtles and infected turtles that are caught by netting?" To determine the true rate of FP, the percentage of diseased turtles is calculated as the percentage of captured turtles with FP compared to the whole amount captured during netting conducted from 2006 until 2014. In 2006 rates of FP were 20 percent, the infection rates then decreased dramatically, even reaching zero percent in 2010. FP rates started increasing again after 2012, and in 2014 the rates of FP now stand at 34 percent (n=89). It is still uncertain what causes FP to increase. To determine the difference between healthy turtles and diseased turtles, the length, weight, and overall growth rates have been assessed. Recapture rates were also assessed, to determine if diseased turtles were captured more, because of their limitations. There was a significant difference ($p < 0.001$) found between recapture rates of healthy and diseased turtles indicating that healthy turtles are recaptured more often than diseased turtles. Assessed length and weight of diseased turtles are not significantly different than from healthy turtles ($p < 0.001$). The growth rate in this research was not significantly different between healthy and diseased turtles. Overall there was no significant difference found between healthy turtles and diseased turtles living in Lac Bay, not in length, weight or in growth rates. The implications of this research suggest that the overall survival rate of turtles with FP on Bonaire is relatively high in comparison to other areas of the Caribbean. This could be due to the tumors not restricting the turtles to such a degree that they are unable to forage or flee.

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1. Introduction

Three of the seven species of sea turtles are found around Bonaire's coast, green turtles (*Chelonia mydas*), hawksbill turtles (*Eretmochelys imbricata*), and loggerhead turtles (*Caretta caretta*). Green and hawksbill turtles can be seen the entire year around, whilst loggerheads mostly visit during nesting seasons. Very rarely leatherback turtles (*Dermochelys coriacea*) are also spotted around the coast of Bonaire. The organization Sea Turtle Conservation Bonaire (STCB) is constantly trying to monitor and protect these critically endangered creatures (IUCN Red List, 2014). Sea turtles have survived for over 150 million years, but they are now in danger of extinction. They are threatened by multiple factors including; pollution, coastal development, hunting, and fisheries (Sea Turtle Conservation Bonaire, 2010).

Fibropapillomatosis (FP) is one of the newer threats for the turtle population. It is a disease that has slowly spread and is now infecting sea turtles all around the world. The first sign that a turtle is infected are small white lesions, mostly around the neck and shoulders. Typically, in less than a year tumors can develop externally and internally. Fibropapilloma (tumors) that are visible mostly grow around the shoulders and on the eyes of the turtles. When the tumor grows (ranging from 0.1 cm to > 30 cm in diameter) it gradually slows down the sea turtle's movement and reduces their eyesight substantially. Internal tumors may also be present, which over time may disrupt the normal organ functions leading to death (Lawrence *et al.* 1995; Turtle Hospital 2013).

Buergelt *et al.* (1991) and Brown *et al.* (1999) determined that the virus that causes fibropapillomas appeared to be a herpes-like virus, for it has many similarities. Patricio *et al.* (2012) stated molecular studies suggest that FP has existed in sea turtle populations for millions of years. Recent studies found the herpes virus to be connected with a retrovirus (which have the ability to integrate their own genome into the germline, passing it on to the following generation), and an unknown primary etiological agent. Research of Brown *et al.* (1995) and Balazs *et al.* (2003) implied that fibropapillomatosis can be transferred from turtle to turtle by bodily fluids, water, or perhaps via parasites.

Fibropapillomatosis was first discovered in 1938 in captive green turtles in Florida. Later during 1980, fibropapillomas had substantially increased and has become an epidemic in numerous areas, including Hawaii and Florida. According to Spotila (2004), fibropapillomas now occur in every ocean. Turtles that are most at risk are turtles that live in near-shore waters and lagoons, especially the areas next to large human populations with poor sewage treatment facilities. Water pollution adds excessive nutrients and disease agents into the sea, which can lower the immune system of the turtle and make them more vulnerable to the disease (Herbst *et al.* 1995; Spotila 2004).

Fibropapillomatosis is most commonly seen in juvenile turtles and sub-adults (Turtle Hospital 2013). It was presumed that green sea turtles were the only species affected by the virus, although recently it has been detected in other turtle species. It was believed that the hawksbill was a species not yet affected by fibropapillomatosis, however the first cases of fibropapillomatosis in hawksbills were reported in 1996 in Brazil (Amato *et al.* 2000).

Since fibropapillomatosis is affecting green turtles on such a large scale, it is important to monitor fibropapillomatosis rates and note whether the disease is increasing over the years. According to Aguirre *et al.* (2004); the prevalence of the disease is associated with heavily polluted coastal areas, areas of high human density, agricultural runoff, and/or bio-toxin producing algae, suggesting that in the future the rates of FP could be used as an indicator of ecosystem health.

In this research project the main focus is fibropapillomatosis in green sea turtles captured by netting. The netting was carried out in Lac Bay, also known as Lac Cai or Lac Cay, and is a large lagoon with a rich ecosystem. Lac Bay is also a very important feeding ground for the green turtle and is where the highest amount of turtles live around the island (Sea Turtle Conservation Bonaire, 2012). For more information see section 2.1 in this document.

The following questions will be examined:

- What is the true rate of fibropapillomatosis affecting Bonaire's green sea turtles?
 - What is the percentage of diseased green turtles caught in the net at Lac Bay in the period of 2006 until 2014?

- What is the difference between healthy turtles and infected turtles that are caught by netting?
 - Are green turtles with fibropapillomatosis more likely to be caught in the net than healthy turtles?
 - Do green turtles with fibropapillomatosis have reduced weight and size in contrast to healthy turtles?
 - Do green turtles with fibropapillomatosis have a different growth rate in Lac Bay than healthy turtles?

2. Methods

2.1 Lac Bay

On 14th April 2014 the turtles were captured by the use of a net.

Capture surveys of turtles in Lac Bay using a net are usually conducted by STCB in April and November each year. The netting is conducted in Lac Bay (Figure 1). Lac Bay is located in the south-west of Bonaire, and is protected under the Ramsar Wetland of International Significance. Lac Bay also contains Bonaire's most important sea grass ecosystem and has significant mangrove forests (Stinapa, 2011). STCB has revealed that the turtles foraging in this lagoon will grow an average of eight centimeters per year, this is more than the average Caribbean turtle, which only grows approximately three centimeters per year (Sea Turtle Conservation Bonaire, 2012).



Figure 1. Lac Bay (red square), is located in the southeast of Bonaire. It is known for its sea grass ecosystem and mangroves (STINAPA, 2011).

2.2 Determine true rate of infection

To determine the true rate of fibropapillomatosis affecting Bonaire's sea turtles, the percentage of diseased turtles was calculated as the percentage of captured turtles with fibropapillomatosis compared to the total captured during netting. This was conducted with data collected between 2006 and 2014. STCB started recording turtle activity in Lac Bay in 2003, however it was not until 2006 that fibropapillomatosis was consistently documented.

Netting

The net was approximately 200 meters in length by 5 meters depth and was deployed from a boat in the bay. The coordinates were also noted. It was visually determined where to start netting, according to murkiness, turtle activity, and depth. After deployment the boat drove the turtles towards the net. The net was constantly monitored by STCB staff and volunteers by swimming alongside the net and removing the turtles from the net as soon as possible. This is important, for if the turtle is underwater in the net too long it will drown. When the turtles were captured and untangled, they were placed on the boat. The net is normally deployed for a maximum of one hour. When the net was removed the coordinates were documented. Once the net was removed from the water the turtles were taken to shore to be measured and documented. The turtles were also checked for tags to see if they were captured in the years before, by comparing the tags with

previous capture records. The turtle was then measured for its weight and length. To weigh the turtle, it was placed into a bag and weighed with a spring scale. Its features were also recorded, as almost every turtle has specific features that make them unique. The turtle was then photographed for a photographic identification database and checked for health including the presence of any fibropapillomas. The checking of tumors is done visually, (initially when the turtle comes on board, so it can be separated from healthy turtles, and again during the on-shore assessment) and if the turtle was infected, the size of the fibropapillomas was also measured.

Recaptures

If a turtle was recaptured, the previous capture records were cross-referenced with the new measurements to assess the growth rate. Turtle recaptures that are identified with the disease are counted and referenced with the numbers of recaptured healthy turtles. The hypothesis examined was that if there are more turtles captured with fibropapillomatosis, it could indicate that the disease makes them easier to capture because of the loss of eyesight and restriction of movement.

2.3 Comparison of healthy and infected turtles

To indicate what the difference is between healthy turtles and infected turtles the weight, size, and the turtle's growth rate was calculated. This will show if infected turtles are different from healthy turtles, which could affect their way of life.

Correlating weight and shell size

Weight and size of healthy green turtles is referenced with the weight and size of diseased green turtles, for the period 2006 until 2014. This would indicate if the green turtles with fibropapillomatosis have a reduced weight/size in contrast to healthy turtles.

Calculating Growth rate

To indicate differences between weight and the length gained over the years, the growth rates were calculated. Growth rates were calculated by taking the weight and length of recaptured turtles and calculating the average they had grown in the years since they had first been captured. By doing so it can be determined if healthy turtles have faster growth rates than diseased turtles. Turtles that were captured healthy and recaptured diseased were not included, as it could not be established when the exact onset of the disease had been.

3. Results

3.1 Percentage of diseased green turtles

From 2006 until 2014 the percentage of fibropapillomatosis was calculated. Only cases where it could be determined if they had FP have been used for this research, uncertain cases were not included in the results. Table 1 shows the rates of FP per year and total captured turtles. In 2006 rates of FP were 20 percent, between 2007 and 2010 the rates of FP decrease dramatically even reaching zero percent in 2010. FP rates started increasing again after 2012. In 2014 the rates of FP were 34 percent (n=89).

Table 1. Calculated fibropapillomatosis rates with netting in Lac Bay.

Captured total	FP	Year captured	% FP captured
91	18	2006	20%
81	4	2007	5%
89	3	2008	3%
108	1	2009	1%
130	0	2010	0%
84	2	2011	2%
105	22	2012	21%
109	36	2013	33%
89	30	2014	34%

3.2 Difference between healthy turtles and infected turtles

Recaptures

Figure 2 shows the calculated percentages of turtle recaptures per year. Recapture rates of diseased turtles appear to be lower than healthy recapture rates. Whilst the healthy recapture rates vary between four to eleven percent, the FP recapture rates range from only two to zero percent. For an overview of all recaptures and percentages, see Appendix 1. An independent T-test was used to calculate differences between healthy and diseased turtles (Appendix 2). There was a significant difference between groups ($t = 23.353$, $df = 71.186$, $p < 0.001$) indicating that healthy turtles are recaptured more often than diseased turtles.

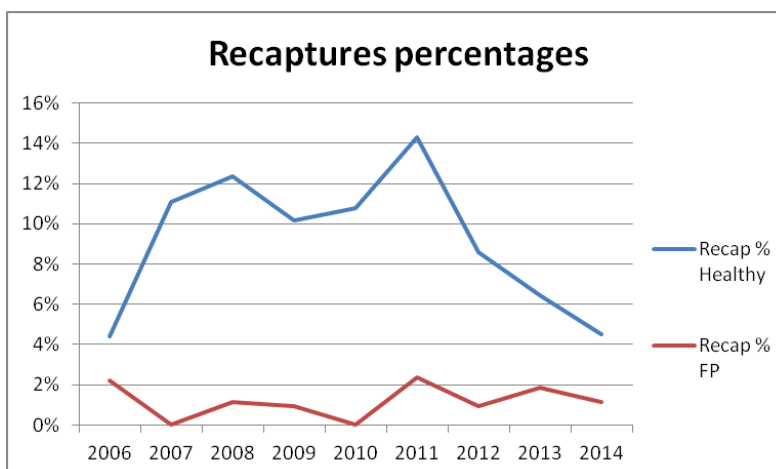


Figure 2. Calculated percentages recapture rates between healthy and FP turtles per year.

The overall capture rates were also determined. Figure 3 depicts that despite the fact that healthy turtles are recaptured more than diseased turtles, the rates of captured diseased turtles is increasing. From 2011 there is a drop by 20 percent in healthy turtles and an increase of 32 percent in captured diseased turtles.

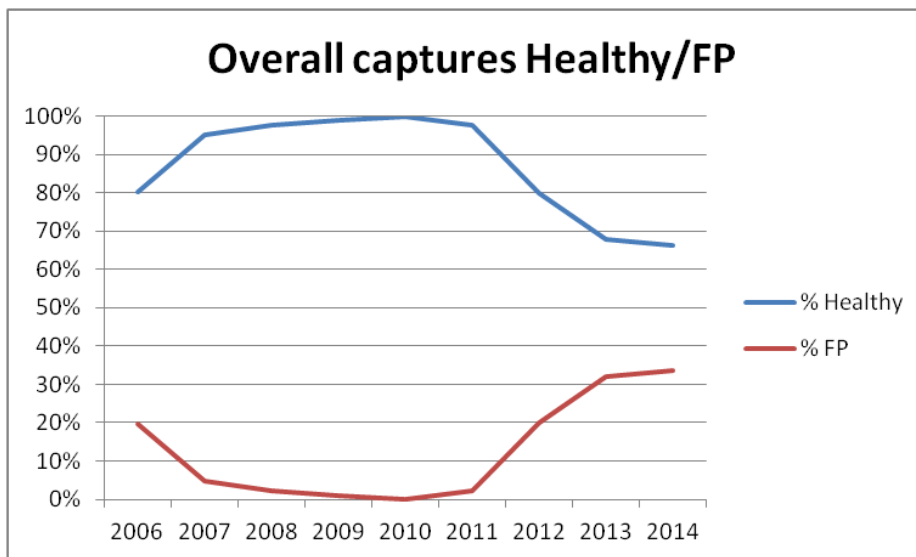


Figure 3. Overall capture rates in percentages. An increase in captured diseased turtles is seen in 2011, and has increased ever since.

Cumulative recapture percentages were also calculated. Healthy recapture rates were fairly consistent, around ten to eleven percent ($M = 8.89, SD = 3.65$). The recapture rate for FP were not consistent, ranging from 0 to 100 percent ($M = 1, SD = 0.70$), which could be due to the fact that in 2009 and 2011 only one turtle was recaptured, which was also the same amount of captured FP turtles in that year, resulting in a cumulative percentage of 100 percent (Figure 4).

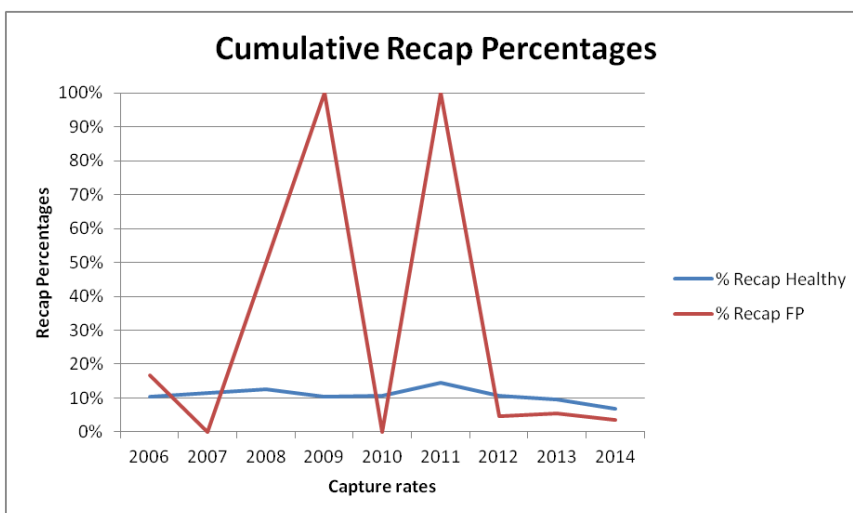


Figure 4. Cumulative recapture rates. Healthy turtle recapture rates are around ten to eleven percent, ranging between 3 to 14 recaptures each year. Diseased turtle recapture rates vary highly per year, ranging between 1 to 2 recaptures each year.

Difference in weight and length

Data from 2006 to 2014 of all recorded captures in Lac Bay using the net was used to make a scatter plot graph (Figure 5). The range of healthy turtles was the largest, ranging from 2.2 kg - 25.3 cm up to 88 kg - 81.5 cm. Diseased turtles range between 5.8 kg - 44 cm up to 74 kg - 78 cm. It appeared that most rates of FP are seen in juveniles between 11 kg - 45.3 cm and 50.0 kg - 72.5 cm. There are only five documented turtles below the 11 kg mark and eight that exceed further than the 50.0 kg mark.

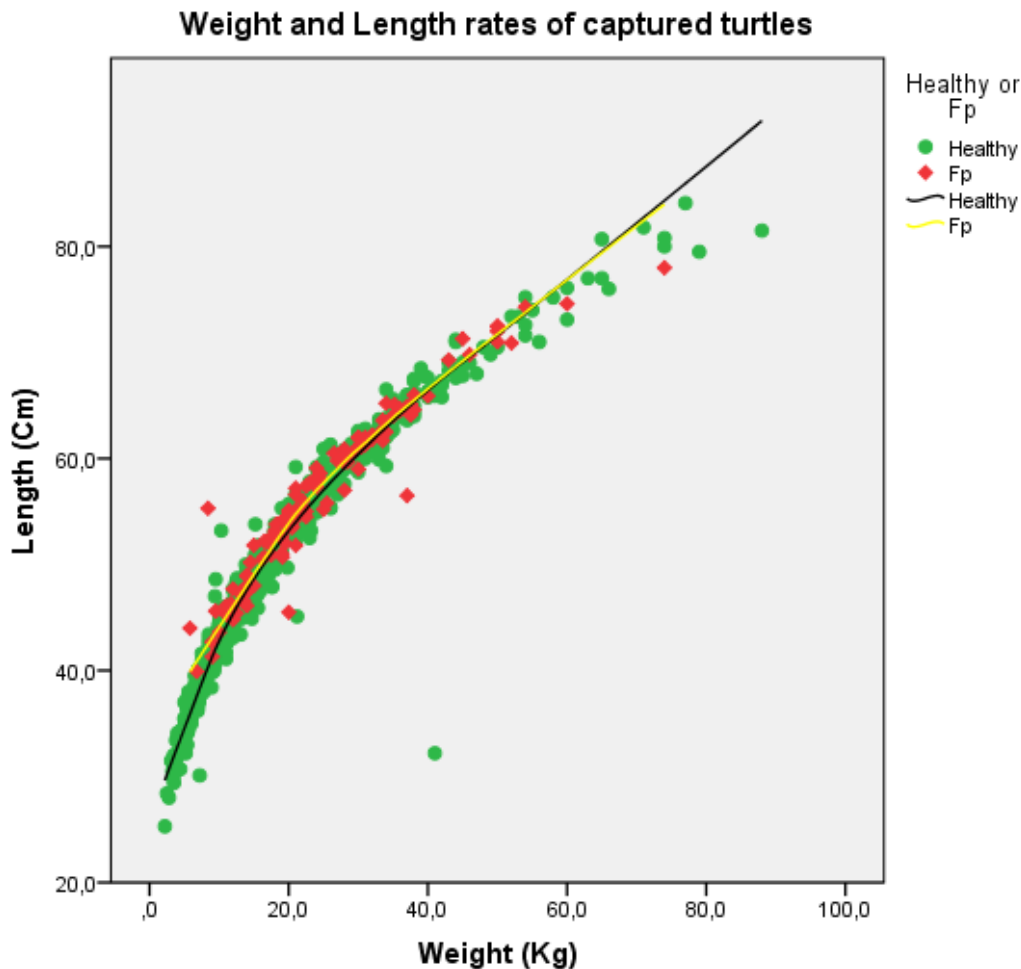


Figure 5. Documented length and weight from all captured turtles in Lac Bay whilst netting during 2006 -2014. The black line is an interpolation line showing the average dispersion of length and weight of all healthy captures. The yellow line is the interpolation line for the diseased turtles. Most rates of FP are seen in juveniles between 11 kg - 45.3 cm and 50 kg - 72.5 cm.

Growth rate

Growth rates were calculated by taking the weight and length of recaptured turtles and calculating the average gain between the years they had been captured. As can be seen in Table 2, the mean growth rate of healthy turtles over the period of 2006 to 2014 is 6.54 kg (SD = 6.23kg, n=70) and 6.05 cm in a year. For diseased turtles the mean growth rate was 5.98 kg (SD = 5.97kg, n=7) and 6.05 cm per year. An independent T-Test was used to calculate if there was a significant difference between mean weight and mean length between healthy and diseased turtles (Appendix 3). No significant difference was found between the weights of healthy and diseased turtles, $t = 0.236$, $df = 75$, $p = 0.814$. Neither was there a significant difference between lengths, $t = 0.001$, $df = 75$, $p = 0.999$. This suggests that there is no difference between the growth rates of healthy turtles, and turtles with FP.

Table 2. Calculated weight/length gain of green turtles in Lac Bay per year.

Healthy	Mean growth weight per year	SD weight	Mean length increase per year	SD length	(n)
2006 - 2014	6.54	6.23	6.05	3.96	70
FP	Mean growth weight per year	SD weight	Mean length increase per year	SD length	(n)
2006 - 2014	5.98	5.97	6.05	2.44	7

4. Discussion

In this study the main research questions were: “What are the true rates of fibropapillomatosis affecting Bonaire’s green sea turtles?” and “What is the difference between healthy green turtles and infected green turtles that are caught by netting in Lac Bay?”

The rates of FP were calculated based on the netting capture data of 2006 until 2014. Rates of FP in Lac Bay were calculated to be thirty-four percent in 2014. However this percentage was calculated with data from a half year, so it is possible this percentage may change with the inclusion of more turtle captures. Over the years FP has been increasing substantially on Bonaire (from twenty-one percent to thirty-four percent over a period of two years), but researchers are still unsure what causes it. Due to recent studies it has been revealed that a herpes virus, a retrovirus, and a primary etiological agent activate the disease, but it is still unknown what agent it is (Patricio *et al.* 2012) and what the specific cause is in Lac Bay. Until that agent is discovered and a treatment established, captured turtles with high cases of FP can be treated by tying or surgically removing their tumors. There is evidence to suggest that by doing so the auto immune system of the turtle can be activated and retreat may occur (George 1997; Guimaraes *et al.* 2013).

The difference between healthy green turtles and diseased turtles was tested considering the difference of recapture, difference in weight and length, and growth rate. It was hypothesized that turtles affected by FP may be more likely to be caught in the net, because of their reduced eyesight and/or restricted movement (Lawrence *et al.* 1995). Recapture rates were calculated to be fairly low, for both healthy and for diseased turtles. All newly captured turtles under fifty centimeters in carapace length are tagged only with pit tags. However, pit tags could be lost, therefore leading to marking some of the captured turtles as new, when they could be actually recaptures, (Limpus 1992). This could also affect the healthy recapture rates since recapture rates are around ten to eleven percent, which is also relatively low. Since recapture rates are low it can affect the validity and reliability of the analysis. STCB is already researching if the pit tags are indeed lost, by tagging new captured turtles with one pit tag and a metal tag. This should indicate if pit tags are being lost.

It is interesting that length and weight are not significantly different between healthy and diseased turtles. One possibility is that the overall survival rate of diseased turtles on Bonaire is relatively high compared to other areas that have been studied, such as Hawaii. The increased survival rate could be due to the high amount of food in Lac Bay, therefore limited food competition, and a low predator count. Another possibility is that the tumors do not restrict the turtle to such a degree that it is unable to forage. According to Herbst (1994) and Aguirre *et al.* (2002), certain tumors are also location specific in where they grow on the body of the turtle. For example, in Florida liver tumors seem to be more frequent in occurrence, whereas in Hawaii mouth tumors are more frequent. Both tumor types have been recorded to cause mortality, however it was stated that the liver tumors were not as deadly as mouth tumors (Herbst 1994; Aguirre *et al.* 2002). The mouth tumors can block food intake completely, thus causing a higher mortality rate.

Cases of tumor retreat have also been documented, so it could be possible that certain turtles do suffer from FP but due to tumor retreat do not suffer large side effects from the tumors. For example, Hirama *et al.* (2004) and Bennet *et al.* (1999) state that they documented turtles in Florida, as well as in Hawaii, had tumors either dramatically shrink in size (5 - 10 cm in size) or disappeared completely (< 1 - 5 cm in size) in a period of three years. Tumors above 10 centimeter did not appear to retreat in size. Tumor retreat could be the reason why diseased turtles above 50.0 kg - 72.5 cm occur infrequently on Bonaire (see Figure 5).

Growth rate comparisons could indicate if diseased turtles take longer to grow than healthy turtles, recaptures of healthy turtles were higher ($n = 70$), than diseased recaptures ($n = 7$). The growth rate in this research was not significantly different between healthy and diseased turtles, which suggests that FP does not affect growth rates in Lac Bay.

Overall there was no significant difference found between healthy turtles and diseased turtles living in Lac Bay, not in length and weight and not in growth rates. This implies that turtles affected by FP in Lac Bay do not have a disadvantage in survival than the healthy turtles, since the growth rate and sizes are not significantly different in both cases.

5. Conclusions

According to this research, rates of fibropapillomatosis on Bonaire are increasing over the years. True rate of FP has increased by thirty-two percent 2011 to 2014. In 2014 rates of FP were thirty-four percent.

Recapture rates were assessed to determine if diseased turtles were captured more, because of their limitations. There was a significant difference ($p < 0.001$) found between recapture rates of healthy and diseased turtles indicating that healthy turtles are recaptured more often than diseased turtles. Turtles affected by the disease, according to capture rates, do not appear to have any difference in length and weight than healthy turtles.

Growth rate could indicate if the diseased turtles take longer to grow to a certain size than the healthy turtles. From diseased turtles seven turtles were recaptured to calculate the actual growth rate per year, but their growth rate did not significantly differ from their healthy variants that were recaptured. This implies that diseased turtles in Lac Bay show no significant difference in comparison to their healthy variants.

6. Recommendations

According to this research, length and weight between diseased and healthy turtles do not differ from one another. It is unsure however if there is a difference between the amount of time they need to grow to a certain size. More data is needed for establishing the growth rate. It could be possible to conduct a few additional east coast water surveys in the east of Bonaire, specifically capturing FP turtles with tags to increase this database.

A further suggestion is to keep treating captured diseased turtles. If the tumor is tied off or surgically removed there is a possibility that the auto immune system of the turtle is triggered, therefore inducing tumor retreat. If it is possible, retreat rates of treated cases could lead to valuable information if tumor-retreat is indeed induced.

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8. Appendices

Appendix 1

Rates of captured and recaptured turtles in the period of 2006 until 2014.

	New Healthy	Recap Healthy	New FP	Recap FP	Captured Total
2006	69	4	16	2	91
2007	68	9	4	0	81
2008	76	10	1	2	89
2009	96	11	0	1	108
2010	117	14	0	0	130
2011	70	12	0	2	84
2012	74	9	21	1	105
2013	66	7	34	2	109
2014	55	4	29	1	89

	Recap % Healthy	Recap % FP
2006	4%	2%
2007	11%	0%
2008	12%	1%
2009	10%	1%
2010	11%	0%
2011	14%	2%
2012	9%	1%
2013	6%	2%
2014	4%	1%

Appendix 2

Statistical test used to determine significance between recapture rates.

Group Statistics

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Percentages	New Healthy	81	,1010	,02888	,00321
	Recap Healthy	9	,0156	,00527	,00176

Independent Samples Test

	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Percentages	Equal variances assumed	7,252	,008	8,816	88	,000	,08543	,00969	,06617	,10469
	Equal variances not assumed			23,353	71,186	,000	,08543	,00366	,07814	,09273

Appendix 3

Statistical test used to determine if growth rates of healthy and diseased turtles are similar to one another.

Group Statistics

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Average_Weight	Healthy	70	6,5417	6,23029	,74466
	FP	7	5,9750	3,62736	1,37101
Average_Lenght	Healthy	70	6,0486	3,96536	,47395
	FP	7	6,0464	2,44380	,92367

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Average_Weight	Equal variances assumed	,096	,757	,236	75	,814	,56667	2,40357	-4,22150	5,35483
	Equal variances not assumed			,363	9,987	,724	,56667	1,56019	-2,91028	4,04361
Average_Lenght	Equal variances assumed	,283	,597	,001	75	,999	,00218	1,53243	-3,05057	3,05493
	Equal variances not assumed			,002	9,518	,998	,00218	1,03817	-2,32699	2,33134