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Tutor: __J.Iyamu & G.Bell __

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Student signature:

C. Lawrence.....

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Abbreviations

(FP)	Fibropapillomatosis
(STSSN)	Sea Turtle Stranding and Salvage Network
(NOAA)	National Oceanic and Atmospheric Administration
(NESDIS)	National environmental satellite DATA, Information Service
(OISST)	Optimal Interpolation Sea Surface Temperature Database
FWS	Florida Fish and Wildlife Conservation Commission
DNA	Deoxyribonucleic Acid
(ChHV5)	Chelonid Alphaherpesvirus 5
CO ₂	Carbon Dioxide
(TSD)	Temperature-Dependent Sex Determination
(HHV8)	Human Herpesvirus 8
T-cells	Thymus Cells
TS	Turtle Strandings
ST	Sea Temperature
MAPK	Mitogen-Activated Protein Kinase

Abstract

In recent years, marine turtles (*Chelonioidae*) have become very popular, due in part to their roles in the film industry. In addition, a number of turtles have been observed on beaches and in locations where they have never been seen before, such as a Kemp's ridley turtle (*Lepidochelys kempii*) that washed up on a Welsh beach in 2021. This phenomenon is known as cold stunning, in which a turtle is exposed to abnormal temperatures that render it motionless (Bentivegna, *et al.*, 2002). Once a turtle has been cold-stunned, a reaction within its body causes its immune system to battle itself, resulting in fibropapillomatosis (FP), a neoplastic infectious disease (Herbst, 1994).

FP causes tumours to form in the turtle's softer tissues to the point that it is unable to utilise its limbs, leading to death (Arthur, *et al.*, 2006). With the disease having a correlation to cold stunning, knowing what sea surface temperature range activates this reaction within the body will help to plan for solutions and give teams the ability to react to potential mass incidents, but most importantly, it will help to understand what causes this disease, so that one day all the data will add up to a potential cure or solution saving the species before it's too late (Work, *et al.*, 2015). Data from the Sea turtle stranding and salvage network (STSSN), the National Oceanic and Atmospheric Administration (NOAA) (NOAA, n.d.), the National Centre for Atmospheric Research (NCAR) (UCAR, 2023), the National environmental satellite DATA, Information Service (NESDIS, 2016), the Optimal Interpolation Sea Surface Temperature database (OISST) (NOAA NCEI, n.d.), and the Climate Data store (Copernicus, n.d.) was first analysed, it was then cross-referenced with the data on species stranding to discover the causal factors and relationship between these incidences and temperature variations (Hayashi, 2021).

The second part of the analysis was to examine the data from the rescue centres in Florida to determine how many of these rescues presented with papilloma (FP) which along with records and statistics from journals such as (Ene, *et al.*, 2005), (Hirama & Ehrhart, 2007), (Foley, *et al.*, 2005) & (Aguirre & Lutz, 2004) was used to determine the severity of fibropapillomatosis on the marine turtle species. To improve awareness and emphasis on the potential severity of this mostly unknown disease, prediction models have been built that highlight the increasing percentage of turtle strandings, increased/decreased water temperatures, and an increasing number of turtles with

fibropapillomatosis. These prediction models can be used to aid the species and develop effective treatments. However, they can also be used for further research, as the data has been utilised in numerous studies which enhances its validity thus creating these prediction models, allowing researchers and professionals to see the big picture and investigate specifics such as temperature in specific regions, water contaminants, tumour location/formation, or even a cure for this disease.

The Emergence of Fibropapillomatosis in Marine Turtles (Chelonioidae) is Attributable to Rising Ocean Temperatures

Introduction

Climate Change

In literature, television, and film, the word climate change has always been associated with impending doom, which, when attained, will be irreversible and lead to extinction on a global scale (Bernstein, 2013). However, this will not occur in accordance with film and television models as the world is comprised of a complex system of life in which one species ultimately lives in a symbiotic relationship with another. These relationships coexist to give the world a homeostatic atmosphere in which all forms of life can flourish (McNutt, 2013).

This homeostatic atmosphere, as discussed previously, can be easily disrupted, with even the smallest environmental alterations leading to catastrophe (McNutt, 2013). Once a change has happened within the trophic levels that govern an organism's position it occupies within the food web and its advances have negatively shifted the mean homeostatic values, the little alteration becomes irreversible without intervention, affecting mother nature and the evolutionary wheel (Reid, *et al.*, 2009). An example of this can be seen in a current issue seen in the world's oceans where sea temperatures are rising causing species of all kinds to be affected leading them either adapt or fight for their very survival just like Darwin's iconic theory of survival of the fittest to determine which of these species will be able to adapt to the newer environment (Hawkes, *et al.*, 2009).

Risk of Extinction

However, if a species is unable to adapt to these changes, the consequences might be disastrous and could result in extinction (Curry, 2014). When an event like this happens a ripple effect develops throughout the trophic levels of any species that have ever interacted with it as prey, predator, or ally. These trophic levels are interdependent, relying on one another to provide the ideal habitat for species to survive (Cousins, 1987). They play a critical role in not just limiting the population through predation, but also managing predators through prey availability. When these

trophic levels function in unison, they regulate the surrounding surroundings (Ruess & Lussenhop, 2005). Depending on the extent of contact, species may undergo the same evolutionary process of adapting for survival or suffer the same fate, forming a continuous chain throughout fauna and flora until one species ends its adaptations and restores balance to a new food chain (Hamann, *et al.*, 2013).

Marine Turtles (*Chelonioidea*)

One group of species that are currently having to deal with this process is the marine turtle (*Chelonioidea*) population where climate change is forcing the oceans temperatures to fluctuate resulting in an increased amount of cold stunning events and also causing the ocean's currents to change their historic paths (Thompson, 2010), this, in turn, will affect most of the species within the ocean negatively, especially the oceans turtles who rely on the oceans warm currents to travel to nesting sites thousands of kilometres away from their natural feeding grounds which consequently will also be affected (Poloczanska, *et al.*, 2009) (see figure one, two & three).

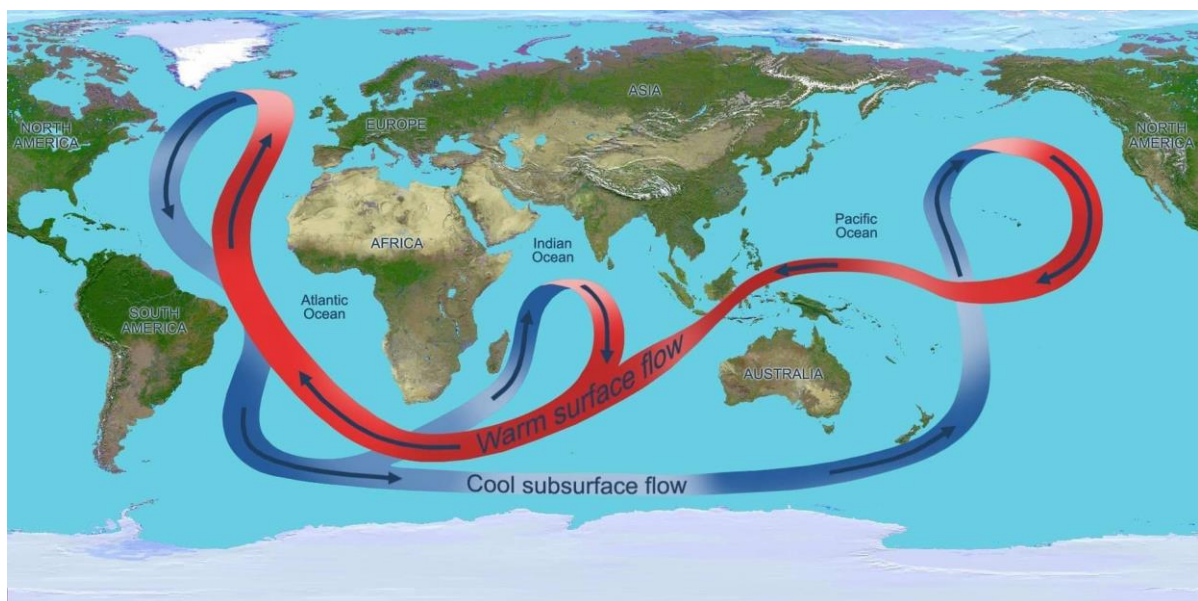


Figure One: This image represents an example of the worldwide conveyor belt of the sea and demonstrates its significance in heat transport between the various oceans (Graff, 2022).

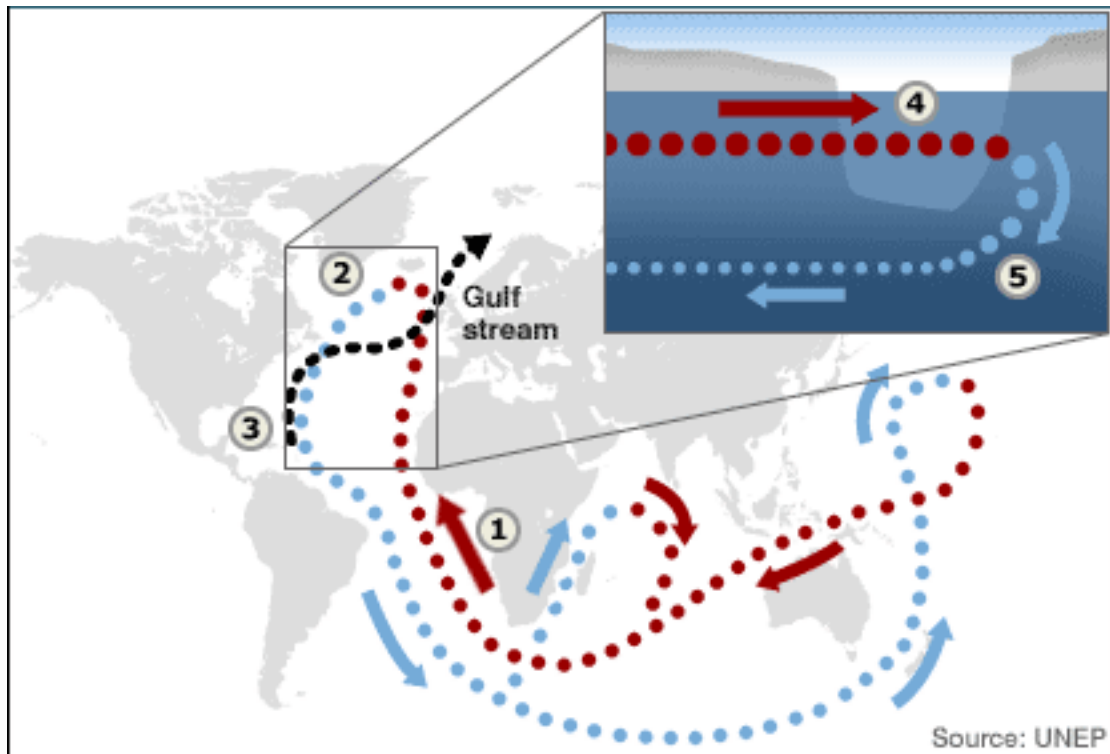


Figure Two: This picture illustrates how the global conveyor operates and how climate change has altered the Gulf Stream by slowing and altering the current that propels the global conveyor. 1 The warm, salty water of the tropics is transported by surface currents. 2 The water cools, accumulates density, and falls to the ocean depths. 3 The cold water returns to the equator, contributing to the Gulf Stream, which warms northern Europe. 4 As the ice melts, freshwater dilutes the warm, salty water from the tropics. 5 The less dense water lowers more slowly, so weakening the "conveyor" and perhaps altering the Gulf Stream (BBC News, 2009).



Figure Three: This picture demonstrates the directional flow and most popular marine turtle (*Chelonioides*) migration pathways inside the sargasso sea (The University of North Carolina at Chapel Hill, 2023).

Most of all marine turtles are ectothermic, meaning that their body temperature is controlled by the surrounding temperature, in the case of marine turtles the temperature of the ocean dictates their body temperature which when within range allows them to achieve homeostasis and a homeostatic life (Wallace & Jones, 2008). However, when the turtles are subjected to changes in temperatures that are out of their natural range, they do not bode well causing them to experience biological behaviour like that of the frozen green iguanas (*Iguana iguana*) in Florida. (Krysko, *et al.*, 2007). However, one species of turtle has broken the mould The leatherback sea turtle (*Dermochelys coriacea*), has evolved to become endothermic, allowing it to control its body temperature like most terrestrial mammals (Van Buskirk & Crowder, 1994). This ability is advantageous for this species as it is not affected by the same temperature restrictions and changes which can be caused by climate change as most other turtles. Even though most of the species are reliant on the sea's temperature and the leatherback can regulate its temperature there is still a threshold within their natural ranges that once crossed will have an adverse effect on each of the species (Friar, *et al.*, 1972).

Fibropapillomatosis

In the late 19th century, reports of turtles with growths on their bodies led to the discovery of fibropapillomatosis (FP) near Florida. In 1938, over a century after its discovery, it was first found in green sea turtles (*Chelonia mydas*) and described in the scientific literature (NOAA, n.d.). This exposes that no records of FP for this disease existed prior to 1938; after its publication, reports of FP being recorded in the green sea turtle around the world began to emerge (Jones, *et al.*, 2016). In 1987, the first report of FP in olive ridley turtles (*Lepidochelys olivacea*) urged widespread inter calibrations amongst scientists to establish how this disease had transmitted from one species to another and how harmful and contagious it could be to the turtle and neighbouring animals (Herbst, 1994).

With this increased attention in FP, other discoveries have been made, including the fact that it has spread to the majority of sea turtle species, including the leatherback, the most resilient of all turtles (Huerta, *et al.*, 2002). Through DNA markers, Alfaro-Nunez's (2014) study also connected the sickness to the Chelonid Herpesvirus 5

(ChHV5), which is activated when a catalyst, for example; cold-stunning, affects the turtle's immune system. Once the virus has taken hold, tumours will begin to grow; these tumours are established within the epidermal layer of the skin, which means they can develop either within or outside the body (see figure four).



Figure Four: This photograph depicts a green sea turtle (*Chelonia mydas*) that has contracted fibropapillomatosis and grown tumours around the limbs, mouth, and eyes (Nuwer, 2014).

Through metastasis, malignant tumours can spread throughout the body in groups of tens to hundreds (Yetsko, *et al.*, 2021). However, once these tumours have formed, they might impede the turtle's movement, leading it to become immobile, which will eventually result in death. The problem that climate change has on the ocean however plagues all the marine turtle species causing an increase in cold stunning events (Doney, *et al.*, 2012). These cold stunning events cause turtles to be subdued to a state of homeostatic shock, this reaction causes the turtle's immune system to react at its full potential to try to find and stop whatever is causing the issue (Alfaro-Nunez, *et al.*, 2014). However, when their immune system is overreacting because of the slowed blood flow this causes the immune system to attack cells and dormant viruses that the turtles are known to carry, in particular, the Chelonid alphaherpesvirus 5 (ChHV-5) which once activated can then lead to the development of a benign tumorous disease known as Fibropapillomatosis (Page-Karijan, *et al.*, 2012).

Fibropapillomatosis is extremely discomfoting for the turtles that unfortunatly present with it because it causes benign tumours to form inside and outside of the body (Page-Karijan, *et al.*, 2015). There is however one saving grace in that if the turtle is found and rescued the tumours can be surgically removed giving the turtle a high chance of an extended life and then after some rehabilitation the turtle can be released to carry on with its natural life cycle, however, there is a strong possibility that these tumours will come back because the virus was activated initially, in most cases the turtles that have been operated on have gotten to a severe state before there rescue so they will be situated in a rehabilitation centre for longer where the potential growth rate of the disease can be monitored to determine a potential appointment for further surgery even if it has been released back into the wild (Spotila, 2004).

Implications of Fibropapillomatosis and Management Techniques

As previously stated, the severity of fibropapillomatosis in animals can result in death without intervention, a study by Page-Karijan, *et al.*, (2019) shows that even when operated on and rehabilitated turtles that were affected by fibropapillomatosis only 35% survival rate. The tumours grow to such a size that the turtle loses the ability to move its flippers, rendering it unable to swim. Additionally, the location of the tumours alters the turtle's buoyancy. This causes the turtle's daily life to become increasingly difficult until the turtle's demise, which is primarily the result of further cold-stunned since they cannot swim to warmer waters where they belong (Foley, *et al.*, 2005) (see table one).

Table One: This table outlines some of the negative repercussions of contracting fibropapillomatosis, as well as the harmful impacts on a turtle's daily existence (Jones, *et al.*, 2021).

Negative effects of fibropapillomatosis	Adverse effects
Tumours present on organs	Tumours are masses of abnormal tissue that arise spontaneously from pre-existing body cells, serve no function, and have an inherent propensity for

	<p>unregulated and uncontrolled growth. When these tumours grow, they can create their own blood supply from existing veins, which can divert blood away from the organ itself. A tumour can also use these newly created pathways to metastasise, transferring itself wherever in the body where blood flows.</p>
<p>Tumours present on skin and eyes</p>	<p>As stated previously, tumours are masses of unregulated growth, which means that as these tumours begin to grow, it is impossible to predict how large or where they will grow. In most cases, tumours are present around the softer tissue near the limbs, eyes, and mouth, which can cause lack of movement and swimming ability, lack of food because the turtle cannot get food into its mouth, and loss of eyesight if the tumours are too large.</p>
<p>Inability to forage, swim, and avoid predation</p>	<p>When a turtle is unable to execute its daily responsibilities, it has been handed a death sentence and will eventually cease to exist, as these essential innate behaviours are what helps turtles to survive.</p>
<p>Inability to mate</p>	<p>Once the tumours have formed, depending on their location surrounding or within the body, the turtle's ability to mate is diminished, leading to a fall in its bloodline as well as the depletion of the species.</p>

Decreased life span	Most turtles with fibropapillomatosis are juveniles, which indicates that most turtles do not reach adulthood since turtles with fibropapillomatosis do not survive treatment, resulting in the death of juvenile turtles.
Infectious	When a turtle has Fibropapillomatosis or comes into touch with one that does, the disease is extremely contagious and will spread even without contact through viral shedding, meaning it can readily and rapidly spread among a nearby population.
Aquatic flora	Some species of turtles carry an entire world of microflora on their carapaces, transporting them hundreds of miles along their voyages; along the route, pieces of these microflora fall off and create new areas of flora while also pollinating existing flora.

However, there is a severe secondary problem with this disease in that it is highly transmissible to other turtles. This disease can be transmitted through direct contact or by the turtle performing viral shedding around other turtles, and this can have a devastating effect on the turtles in the area, causing the disease to spread uncontrollably throughout the turtle population (Aguirre & Lutz, 2004). Over the last Nine years reports of cold stunning events have increased by an astonishing 134% across the species this is also tied in with an average mortality rate of 28% of those cold-stunned turtles found (SWOT, 2021) (see figure five).

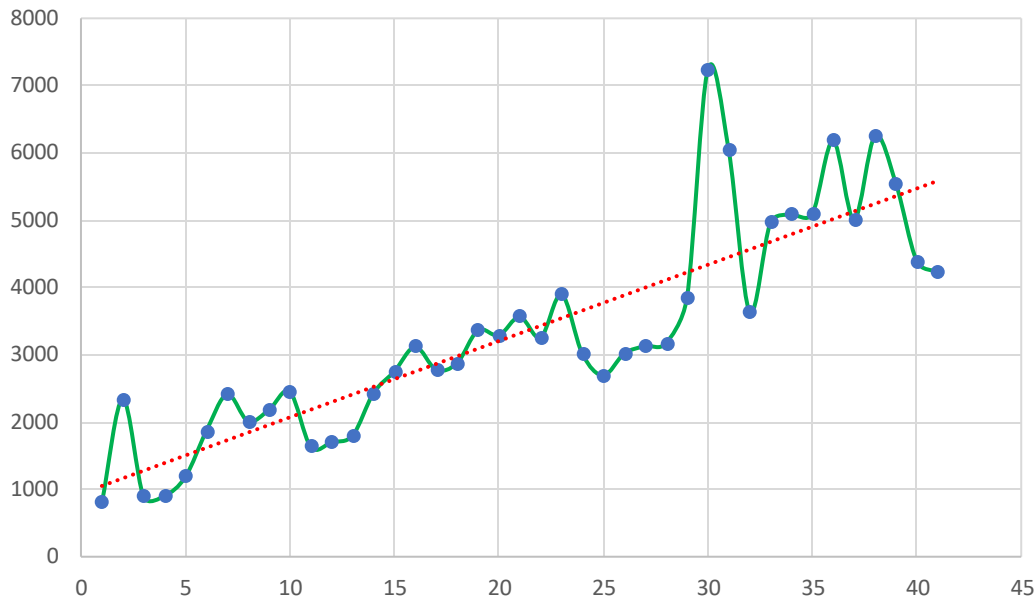


Figure Five: This graph depicts the severity of the number of cold-stunned turtles over the past 41 years, with the trend line indicating that this phenomenon is expected to continue.

One of the major issues is that cold stunning can cause a reaction that sparks the disease, but the tumorous disease goes undetected visually until it has already taken full effect (Foley, *et al.*, 2007). This means that the percentage of turtles that have or will present with fibropapillomatosis as a result of cold stunning is extremely hard to predict however models can predict that the number of turtles that were cold-stunned are more likely to present with this disease because of prolonged exposure to the decreased temperature drop where they have been stunned (Aguirre & Lutz, 2004). This piece of research is based on the fact that this can be measured, whereas viral shedding can only be measured under laboratory conditions; therefore, it is unrealistic to assume what percentage of animals would be affected in the wild, as each animal would have to be monitored for every interaction (Farrell, *et al.*, 2021). However, a model can be constructed to predict the average number of turtles that will present with fibropapillomatosis in areas where a temperature drops of a certain amount occurred. This would then direct teams to their location to potentially stop the cold stunning or provide immediate aid to turtles that have been stunned but would ultimately reduce the number of turtles presenting with this disease and assist the species in returning to its thriving permanency (Kelley, *et al.*, 2022).

Study Purpose

This study illustrates how little research has been conducted on other marine turtle species. In order to highlight the association between cold stunning and fibropapillomatosis, the purpose of this study will be to initially concentrate on all marine turtles and subsequently narrow the attention to a specific species.

Literature Review

This study is primarily inspired by an issue posed by the theoretical framework technique (Connelly, 2014). The terms fibropapillomatosis in marine turtles, Climate change and its effects on marine turtles, and how changing sea temperatures can affect marine turtles were entered into search engines such as google scholar and UCNL EBSCO (EBSCO Industries, Inc, 2023) were utilized to obtain the initial results. The results were then further analysed using the snowball sampling technique, which brought forward further important studies through the citations and reference. The identified journals and studies were then subjected to an inclusion or exclusion procedure in which their significance and relevance of key phrases were judged to facilitate a more directly critical research methodology (see table two).

Table Two: This table details the inclusion and exclusion criteria used to choose the most pertinent and precise data for this research.

Inclusion criteria	Exclusion criteria
Due to lack of studies Journals, books, or articles published on fibropapillomatosis / papilloma between 1950-2022 will be considered	Journals and articles will be excluded if published before 1950 on fibropapillomatosis and papilloma
Data set on strandings from 1980 onwards via the sea turtle stranding network STSSN (NOAA Fisheries , n.d.) and Florida Fish and Wildlife Conservation Commission (FWC, 2023)	Data will be excluded on strandings if they are from other sources than stated in the inclusion criteria
Journals, articles, DATA or studies published after 1980 on sea surface	Journal articles, DATA or studies will be excluded if published prior to the year 1980 on sea surface temperatures and

temperatures and 1995 on climate change and its implications	the year 1995 on climate change and its implications
For an accurate picture of current changes and trends, sea temperature data for the whole ocean will be used, although data from National Centre for Atmospheric Research (NCAR) (UCAR, 2023), (NOAA) the National environmental satellite DATA and Information Service (NESDIS, 2016), Optimal Interpolation Sea Surface Temperature database (OISST) (NOAA NCEI, n.d.), National Oceanic and Atmospheric Administration (NOAA) (NOAA, n.d.), and the Climate Datastore (Copernicus, n.d.)	Data from other sources on the oceans sea surface temperature will not be used unless there is evidence that crosses over with the data from the selected sources within the inclusion criteria

Climate Change and Cold Stunning

This was crucial information for future researchers, as scientists were aware that climate change had had a major impact, but the water was relatively calm, with climate change problems becoming nearly a constant in an ever-changing world. Griffin *et al.* (2019) identified a problem in the ocean and conducted a study that incorporated sea temperature as a new key factor within the Kemp ridleys turtles (*Lepidochelys olivacea*) that were increasingly being exposed to cold stunning events. These cold stunning events are increasing in the turtle species and can lead to death or serious consequences for any turtle that is exposed to these out-of-the-norm temperatures. In addition to causing the ocean to warm, this study demonstrates that climate change also leads to cooler temperatures in locations where they would normally be steady (Burke, *et al.*, 1991). This is further highlighted by Griffin *et al* (2019) within their study where they assessed the species of Kemp's Ridleys Turtles (*Lepidochelys kempii*) for their response to warming seas which also showed an increase in cold stunning events.

Rising Sea Temperatures

Throughout the literature, on sea turtles and fibropapillomatosis, a key theme has arisen showing that the temperature fluctuations within the ocean which have been caused by climate change have had a significant effect on the marine turtle species causing increasing cold stunning events that lead to the development of FP. Reid *et al* (2009) conducted a study on the impacts of the ocean on climate change highlighting the purpose of the world's oceans in climate change, they highlighted that the ocean is the world's largest heat and carbon store, comprising 97% of the earth's water and covering 71% of the earth's surface, this causes the oceans to heat up and in the last few decades the oceans have seen the biggest rise, and when tied with the increased amount of carbon dioxide, which the ocean stores up to about 40% of anthropogenic-sourced CO₂ from the atmosphere the ocean cannot perform its duty of cleansing the air and maintaining its homeostatic nature (Helm, *et al.*, 2011).

The world has reported on how serious climate change has become a serious worry because it has started to affect human's daily life from the melted ice caps, and worryingly hot summers to increasingly cold winters, but these changes have affected species of animals far before they have affected the humans leading to some animals becoming extinct in the process (McMichael, *et al.*, 2006). However, has the ignorance of man caused this climate change or has this been inevitable, within their study Reid *et al* (2009) make a statement that because man does not know enough about the processes of the ocean regarding climate change any kind of understanding apart from the basic is conspicuously inadequate.

One thing that is known is these changes cause currents to shift and salinity to change within the ocean (Hays, 2017). when currents change animals that follow those currents for things such as feeding, or migration routes then must adapt to new conditions possibly out of the realms of normality. furthermore, Reid *et al* (2009) also emphasise that the sea surface temperature has had a warming trend over the last 50 years which has resulted in increased areas of warming in the Arctic and along the equator but a slower rate of warming in summer months around the Southern Ocean in the northwest Atlantic. This is also backed up by griffin *et al* (2019) who they too noticed a raise in sea temperature in the Gulf of Mexico of 2.2 degrees the study also highlights some of the key points when considering how the ocean reacts to climate

change and how the ocean tries to process the problem to restore normality within the earth's atmosphere.

They also highlight that the sex ratio is not affected in their model by this increased nesting temperature whereas this may be true in the leatherback in other marine turtle species these increases in temperatures can cause a severe shift (Fuller, *et al.*, 2013). Blechschmidt *et al* (2020) show that turtle species with temperature-dependent sex determination (TSD) are highly affected by a temperature rise of just 1 degree, take the green sea turtle for example their hatchling's sex is determined in the incubation period when they are buried in the beach the temperature of where the eggs are laid determines if the hatchling will be male or female by one single degree, one degree higher will result in an abundance of female hatchlings.

This means that if temperatures continue to increase around nesting sites for marine turtles the sex ratio of turtles will take a negative shift towards more females being hatched than males and in turn, if these changes are not acted on it could lead to the same fate as the northern white rhino where there are just two females left of that species and no males meaning the only way the species can continue is by the intervention of man or cross breeding which has its risks and flaws (Hillman-Smith, *et al.*, 1986).

Consideration of the Impact of the Herpes Virus

Studies have been conducted throughout the years since the initial discovery of fibropapillomatosis (FP) relating to how, what, when, and why; however, the problem with these studies is that they have been specific to one species of turtle take this study conducted by Jones *et al* (2016) a review of fibropapillomatosis in Green turtles (*Chelonia mydas*); in this study they examine the Epidemiology that fibropapillomatosis has on the green turtles, and how This work demonstrates the association between FP and ChHV5, which strengthens our understanding of the causal agent. However, the authors also note that marine chelonids carry herpesviruses one, five, and six, but only herpesvirus 5 causes FP.

Although this study is relatively one-sided in that the green turtle has developed FP and that it is detrimental to the species, it does not assess many of the issues

surrounding FP, with the exception of one crucial point when discussing the infectious nature of FP: a study by Herbst & Klein (1995) transferred FP between different species by using cell-free lesion extracts to inoculate turtle species that were not infected with FP. This study revealed that three-quarters of the experimental groups contracted FP, indicating that this disease is more contagious than previously believed. However, this led Herbst & Klein (1995) to conclude that FP is classified as an infectious disease and that additional research is required to elucidate its role and the environmental cofactors that influence it. Their hypothesis consisted of attempting to understand what factors led to the development of FP where reports of the disease were sporadic in nature until a common theme was relevant in that in the green turtle there were more cases of FP reported in the shallower waters nearer to humans where contaminants were thought to be a possible causative agent; however, blood toxicology showed no difference between the turtles with and without FP.

This indicated that no one fully understood what caused the immune systems of turtles to react in this manner, resulting in FP; something must have prompted this response. This was also supported by a study by Duffy & Martindale (2019) in which they took a different approach using perspectives on the expansion of human precision oncology and genomic approaches to sea turtle fibropapillomatosis and also found that a causative agent was not identified. However, they did note within this study that there were ongoing studies that looked at pathogens being the causative agent, but no conclusion has been reached to date. Prior to this discovery, researchers knew that turtles were presenting with FP in several different species, including species that were considered endangered or vulnerable, with no common causative agents other than the fact that these turtles all had the herpesvirus 5 in their bloodstreams. At the same time that this disease was spreading, the ocean was undergoing rigorous testing and observations due to the bleaching of corals, which was causing sections of the ocean to become barren of tetrapod's (*Tetrapoda*) (Lesser, 2007).

This resulted in the publication of research that highlighted the effects of climate change, with new evidence of rising ocean temperatures taking centre stage. Brown *et al.* (2019) examined the long-term effects of rising sea temperatures and sea levels on shallow water coral communities over a 40-year period, where the sea temperature was a key factor; they even highlighted the peak temperatures that led to the mass

bleaching of marine corals in 2010 for seven weeks, which reached 31 degrees Celsius.

Molecular Basis of Fibropapillomatosis

The creation of these tumours has been connected to immunosuppression, where it is hypothesised that lymphocyte T-cells begin or worsen a mutation that causes a proto-oncogene to change into an oncogene at the proto-oncogene level (Pappou & Ahuja, 2010). Proto-oncogenes are responsible for encoding the proteins that regulate the different stages of the cell cycle. Once this mutation, which is typically caused by point mutations during the G2 division phase, occurs, the gene becomes inactive and loses its function (Johnson & Walker, 1999). A point mutation is a single nucleotide alteration, insertion, or deletion within the DNA sequence. Point mutations are often referred to as missense mutations and nonsense mutations. These mutations are dominant, which means that only one gene copy must be mutated for a proto-oncogene to become an oncogene (Furth, *et al.*, 1994).

Once this response occurs within the MAPK-associated genes, it transforms into this malignant condition characterised by papillary cell hyperplasia and excessive fibrous connective tissue in both the epidermal and dermal skin layers, or more specifically, dermal fibroblast proliferation and epidermal keratocyte growth (Duffy, *et al.*, 2018). Yetsko *et al.* (2021) investigated this further in their research Molecular characterisation of a marine turtle tumour epizootic, profiling exterior, internal, and postsurgical regrowth tumours, highlighting each of these aspects by their transcriptional profile.

One aspect of their results that stood out was Herpesvirus infection related with Kaposi sarcoma was one of the viral profiles identified. Kaposi sarcoma is a type of cancer that develops on the skin, lymph nodes, and organs of immunocompromised people and is associated with human herpesvirus 8. (HHV8) They discovered that the viral and immune response signalling cycles revealed by transcriptomics are consistent with the theory that ChHV5 drives FP tumour growth in sea turtles in the same way that HHV8 reacts to an external environmental or immune responsive trigger in humans (Yetsko, *et al.*, 2021).

Research Methodologies

Climate Change and Cold Stunning

Griffin *et al.* (2019) utilised a novel framework in their study in which they utilised various methods such as Random Forest and Bayesian count model and validation for analysing their collected DATA. Using this framework, they were able to develop prediction models that can be used in future research. The results provided a good level of analysis because it used data and results from multiple sources with vast amounts of data, such as the sea turtle stranding network and sea surface temperatures (NOAA Fisheries, n.d.) & (NOAA). The advantage of this framework is that the accuracy and results can be followed easily for verification and also used in future research, the fact that they also created future models on the prevalence of predicted value increases is a great example of what research should strive for (Shuttleworth, 2023). This model will be a perfect asset to this study. However, the amount listed within their prediction model has already been exceeded in as little as five years, and it is more likely that there will be more than 10,000 incidents by their predicted 2031 (Herbst, 1994).

Rising Sea Temperatures

Reid *et al.* (2009) used a similar method and prediction model idea as Griffin *et al.* (2019) to evaluate the potential severity and future suggestions to prevent or reverse the effects of climate change on the ocean before it is too late. The benefit of their results is that they can be easily evaluated and validated, which demonstrates their validity for anyone utilising the same data set or similar approach. Another advantage is that there are no actual disadvantages or constraints to adopting this framework based on the outputs of these data sets and prediction models; the data speaks for itself once the data sets have been analysed.

Consideration of the Impact of the Herpes Virus

Saba *et al.* (2012) emphasises and comprehend future climate change forecasts for sea turtle species using the prediction model approach. The prediction models used in this study predict the overall effects that climate change has on the leatherback sea turtle and show that the leatherback may be fine as only a slight decrease in population is predicted as a result of some of these climate change occurrences. One of these

predictions is that on their model, an increase of 2.5 degrees warming of their nesting sites showed the greatest contributor to the decline of the population by reducing hatchling success.

As a result of the correlation between Saba *et al* (2012) & Jones *et al* (2016) studies were they both highlight the lack of literature on the subject of fibropapillomatosis correlating with the rising sea temperature, a gap has been created, necessitating the conduct of this study to not only highlight the issue's importance and urgency, but also to potentially allow for solutions so that the species can save itself before the marine turtle population reaches a point of chaos. There are several gaps in the literature regarding fibropapillomatosis in turtles and its severity within the marine turtle population, which prompted this study to emphasise in its Hypothesis that there will be a significant association between marine turtles exposed to cold stunning and later diagnosed with fibropapillomatosis and those diagnosed with fibropapillomatosis who were not exposed to cold stunning (Allman, 1995).

However, when compared to a similar study by Patricio *et al* (2021), where they adopted a more methodical approach by analysing existing written peer-reviewed literature they utilized 202 peer-reviewed papers which accounted for 75% of the literature available on the research question to draw their results and conclusion, this approach gives their study a more compelling and strong validity because each study they have used has gone through its process of verification limiting the possibility of doubt in the data, although this process does make this data a strong and reliable data set there is still limitations *and* questions regarding the human error and specifics of recording. whereas griffin *et al* (2019) adopted a more statistical methodology using physically collected data statistics to establish and verify their results. This approach gives the study an eyewitness account for the data collection which holds no stronger validity which for a research study is by far the best for results (Faculty Development and Instructional Design Centre, n.d.), however, there are still some limitations albeit decreased but still relevant one of these limitations would be seeing a smaller picture more focused on the actual questions whereas the previous could form more future research questions as a by-product (NHS sabp, 2020). Even though both studies have limitations which hinder their validity for further outreach, however, both studies although different in the methodology concisely utilise the data to draw a valid conclusion that can be verified and utilised for future research. Both papers are

methodologically sound particularly in their data analysis which is why both methodologies will be utilized within this study to combine both angles of data analysis and produce the most concise and precise data (Laughlin, 2019). One thing that stands out with this study is that the section on current knowledge, research, advances, and knowledge gaps goes into depth on the specific subject matter highlighting the key points of the research question. In this section at reference point 4.7, the chelonid herpesvirus 5 is mentioned and similarly to Griffin *et al* (2019), they examine the disease in some detail within the literature where they highlight that the disease is driven by either climatic or anthropogenic factors and that stressors can increase and exacerbate its potential uptake. Climate change is also mentioned in this section as a factor that can alter host-pathogen interactions by either impairing the host's immune system or altering the virulence of pathogens. However, one thing that stands out is that they refer back to a study that they are part of Patricio *et al* (2016) Novel insights into the dynamics of green turtle fibropapillomatosis which they have used as verification to their statement although widespread, FP currently does not seem to pose a major threat to marine turtles, which contradicts all most all of the current literature including Griffin *et al*'s (2019) study where they took special care in highlighting the devastating effects this disease is having and future impacts on the species. This is a bold move by the researchers in that they have justified their statement with their previous study which shows no grounds for legitimacy especially when that study highlights the severity of the prevalence of the disease in as little as three years the population of green sea turtles in the Indian river hit 75% with an average of 28-66 % across 1989-1997.

This is a complex area because of this one statement another study now needs to join the mix to further enhance and validate the basis of this study as questions could be drawn on the validity of their study. Koch *et al* (2013) use a slightly different methodology from both Griffin *et al* (2019) and Patricio *et al* (2021) but still statistical in nature as they performed actual data collecting performed on the beaches of their areas of study themselves and then enhanced the stranding data by conducting estimations of stranding probabilities to give them more of a representation of the magnitude of the turtle strandings in that area. All three of these studies have the reoccurring themes that the sea surface temperature plays a crucial role in the life of marine turtles and has also proven to be a consistent factor in the development of FP

within the species. In Koch *et al* (2013) study they have highlighted in contradiction to Patricio *et al* study that only 1.8% of the turtles recorded for cold stunning mortality were due to bycatch or boat strikes and that 93% were mortalities caused by cold stunning incidents, which again backs up the evidence that these cold stunning events are more detrimental to the species and as the main instigator in FP prevalence proves to be very damning for the species as a whole.

Molecular Basis of Fibropapillomatosis

The study by Yetsko *et al* (2021) uses a good solid rounded framework to gain the desired outcome, their use of research to boost and add to their study has been concluded in a way that leaves little room for error including the main question of their hypothesis because of the techniques used within the laboratory conditions again no error can really be sought although this study does highlight a finding that they were also surprised to see which was the apparent discovery of how the human herpesvirus 8 (HHV8) and the Chelonid Herpesvirus 5 react and signal in the same way. As their main part of the study had taken place in a laboratory the highest attention to detail would be expected so this discovery highlights the level of justification, they have within their study showing that its framework has worked in such an efficient way that they were able to perform their initial study and highlight a finding that was not expected.

Further Opportunities

Throughout this literature review the journals used proved to have delivered on a well-rounded outcome brought forward by utilising the best framework that was seen fit for their study. However there were some stand out limitations when it came to some of the studies that needed to be highlighted as important factors to consider for future research, one of these was Griffin *et al* (2019) the research and data were all based around Cape Cod on the Gulf of Maine which when considering the turtle species can travel far and wide for food and migration periods this holds back data that can be related to the further field and also this cannot be used as a full representation of how the species is effected because it focuses in on a small part of their habitat (Hays, *et*

al., 2002). Another stand out factor is that for all the journals that have used the STSSN DATA set, being as there is no other DATA base the recorded cold stunning events data is reported by the sea turtle stranding network which is either accomplished by trained professionals performing data recording surveys or by people calling in stranded turtles, this highlights not only are quite a few turtles that could have been cold-stunned that will not be accounted for as a result of non-observation (Paradis, *et al.*, 2016), but there is also the fact that the descriptions of these cold stunned turtles do not give an accurate field of view to what actually happened to the turtle and its condition at the same time there is also the need to account for human error which a more detailed description would account for (Theofanidis & Fountouki, 2018). Another limitation found was that the sea surface temperatures for some areas were recorded as mean Data rather than an accurate timeline, the data provided by the Optimal Interpolation Sea Surface Temperature database (OISST) (NOAA NCEI, n.d.) Provides accurate and mean data for a period of time but as climate change continues the sea surface temperatures can change rapidly within a day but then be recorded as the mean which would not account for the Sharpe drops and climb that could be the indicator for incidents that studies are looking for, these Sharpe changes would also prove to be very relevant to cold-stunning as a move in temperature either way could prove crucial for understanding what has happened, also temperatures of a slightly deeper nature more like where the turtle would swim and spend most of its time are not considered and rarely recorded however they are important just as seen in coral bleaching the temperatures of the deeper water can also change causing other variables to change adding to more possibilities that the trophic levels are of concern (Hoegh-Guldberg, 1999). The final limitation that has arisen from this literature review is that the data on cases of fibropapillomatosis is not officially recorded resulting in most studies giving average guesstimates to the prevalence and transmissibility of the disease. in most cases of this nature having no Data set would mean that a physical recording would need to take place or the Data would be dismissible however as this is crucial Data that is needed to not only test the hypothesis but to highlight this potential pandemic in the marine turtle species multiple sources could be used to conduct an average percentage of cases from small and large areas to use as a representation for the species (Gal & Rubinfeld, 2019).

Aims/Hypothesis

This study aims to discover if there is a correlation between climate change-induced rising sea temperatures and the occurrence of fibropapillomatosis in marine turtles as a result of their exposure to cold stunning events caused by rising sea temperatures (Zell, 2004) & (Gale, *et al.*, 2009).

The second objective of this study is to facilitate the construction of prediction models for sea surface temperatures, cold stunning events, and the prevalence of fibropapillomatosis that can be used to predict where a turtle is likely to be exposed to cold stunning and to forecast how many turtles may present with fibropapillomatosis in the coming years (Pearson & Dawson, 2003) & (Mousikos, *et al.*, 2021). Objectives of this study and their predicted outcomes can be found within table three.

Null Hypothesis

There will be no significant association between marine turtles that were involved in cold stunning incidents due to rising sea temperatures and thus presented with the disease fibropapillomatosis and those who present with fibropapillomatosis that were not involved in cold stunning incidents.

Alternate Hypothesis

There will be a significant association between marine turtles that were involved in cold stunning incidents due to rising sea temperatures and thus presented with the disease fibropapillomatosis and those who present with fibropapillomatosis that were not involved in cold stunning incidents.

Table Three: This table summarises the study's objectives and their related anticipated outcomes.

<u>Objectives of study</u>	<u>Predicted Result of objectives of the study</u>
Sea surface temperatures will be analysed around the habitats and migration routes of marine turtles to assess for critical changes in temperature or location (critical	The data analysis will reveal that temperatures have fluctuated throughout the habitat and migration routes of the typical marine turtle.

temperature changes will be 1°C above or below the average mean) (Huey & Hertz, 1984).	
Changes in migration routes or habitat preference as a result of sea surface temperature changes will be assessed for potential impacts on the species (Pepper , 2019).	The data will demonstrate that marine turtles have left their traditional habitats and migration patterns as a result of fluctuating temperatures, driving them to enter uncharted waters where they are susceptible to cold stunning.
Data will be assessed to see if there is any correlation between the incidents of cold stunning and the location where it may occur between different species (Roberts, <i>et al.</i> , 2014).	Through the data on cold stunning, the locations of every turtle reported for cold stunning will provide a correlation between the various species and specific locations where cold stunning is more prevalent.
Data for turtles with the disease fibropapillomatosis will be assessed to establish a baseline mean (Dujon, <i>et al.</i> , 2021).	The data will reveal the mean average of turtles that have been documented to have tumours or been diagnosed with fibropapillomatosis, hence revealing the baseline mean for a representation of sea turtles.
The mean data for turtles with fibropapillomatosis will be cross-referenced with the data for turtles that have been subjected to cold stunning to see if there is a correlation between the two (Herbst & Klein, 1995).	The data will demonstrate that the number of turtles that present or are diagnosed with fibropapillomatosis correlates with the number of turtles that were treated to cold stunning either that year or the previous year and will also provide an indication of how rapidly the condition develops.
All of the data will be cross-referenced to form a prediction model for the future that, highlights increase and decreases in sea surface temperature, forecasts for	Once the data have been cross-referenced, a prediction model will be created indicating that fibropapillomatosis cases in turtles will

<p>the occurrence of fibropapillomatosis in marine turtles (Beissinger & Westphal, 1998)</p>	<p>continue to rise and that temperature changes in certain locations indicate an increase in cold stunning, allowing rescue teams to predict where turtles will need assistance.</p>
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Methodology

This study will be evaluated using a method similar used by griffin *et al.* (2019) and Patricio *et al.* (2021) where DATA will be gathered from previous studies and literature containing information and data about marine turtles and cold stunning, marine turtles presenting with fibropapillomatosis, turtles found with tumours after cold stunning events, rising sea temperatures, and marine turtles impacted by climate change.

The additional part of this study will be a combination of the previous study and literature data combined with the new DATA sets provided by the Sea Turtle Stranding and Salvage Network (STSSN) (NOAA Fisheries , n.d.), the United States Environmental Protection Agency, and (NOAA) the National environmental satellite DATA and Information service (NESDIS, 2016), the National Centre for Atmospheric Research (NCAR) (NOAA NCEI, n.d.), and the Climate Data store (Copernicus, n.d.) to form a collective, up-to-date assessment of analysed DATA that can then be utilised via Microsoft excel and IBM SPSS Modelling software.

The Data used within this research has come from outside sources which all have protocols and stringent procedures to verify their Data for their own peer review studies and literature so that it can be used in scientific research (see appendix a & b).

Data on Fibropapillomatosis Found in Marine Turtles.

As there is no official data set with the primary aim of recording data on turtles found or rescued with fibropapillomatosis or tumours, this information will be gathered from peer-reviewed articles and reputable sources such as The Florida fish and wildlife conservation commission (FWC, 2023), Ene, *et al.*, (2005), Hirama & Ehrhart (2007), Foley, *et al.*, (2005), and Aguirre & Lutz, (2004). Due to the unreliability of some

sources, data that cannot be cross-referenced or has no legitimacy through peer reviewed literature will be removed.

Cold Stunned Marine Turtle Data

The sea turtle stranding and salvage network (STSSN) (NOAA Fisheries, n.d.) provided the data on cold-stunned turtles. This network educated and organised employees to monitor and record cold-stunned turtle data across the Atlantic Ocean, which connects the United States, Mexico, Canada, and Brazil. The data will cover the entire term of their existence, from 1980 to 2023. These years were chosen in order to develop a full model of the timeframe and changes related with cold-stunned turtles and rising water temperatures.

Sea Surface Temperature and Environmental Data

Sea surface temperatures were provided by the National Centre for Atmospheric Research (NCAR) (UCAR, 2023), (NOAA) the National environmental satellite DATA and Information Service (NESDIS, 2016), Optimal Interpolation Sea Surface Temperature database (OISST) (NOAA NCEI, n.d.), and the Climate Datastore (Copernicus, n.d.). Once the data has gone through the analysis stage where monthly, yearly and deviations have been isolated the process of zone analysis will be taken as turtles may inhabit the majority of the planet in a variety of circumstances; but, for the sake of this study, the area along the northern and southern American coastlines is where the greatest number of cold-stunned species, have been recorded which spans an area of around 15,000 square miles, this will be analysed down further to determine the most active zone within the data field, which in turn will give the best representation for the species. These data sets will then be taken and analysed to test the hypothesis and create prediction models.

Statistical Analysis

Once all of the data has been collected and excluded data has been removed, the data will be analysed to create monthly and yearly means of sea surface temperatures highlighting spikes and deviations. The stranding data will also be analysed for monthly and yearly totals using analysing software such as Microsoft excel (©

Microsoft, 2023) and IBM SPSS (IBM, n.d.), where data sets will be analysed through Kruskal-Wallis's test or ANOVA, bivariate regression tests, and regression analysis to establish correlative relationships. Once the hypothesis has been determined, prediction models similar to those developed by Patricio *et al.* (2016) will be developed to demonstrate the extent of what is likely to occur in the future of each model. These models will be able to predict 13-year look into the future according to the data on sea surface temperature, cold stunning incidents and the prevalence of FP. If the prediction models become obsolete or outperform those of Patricio *et al.* (2016), the bases of this work and the analysed Data can be used to develop a new prediction model with a solid, dependable foundation, as the data spans 41 years.

Results and Analysis

With the wide range in temperature in SSTs both seasonally and in years the first analysis was to establish the means for months and years as this would make the initial comparison more diverse, within this comparison spikes both higher and lower was sought to see if there were any anomalies that indicated more focus was needed (see figure six).

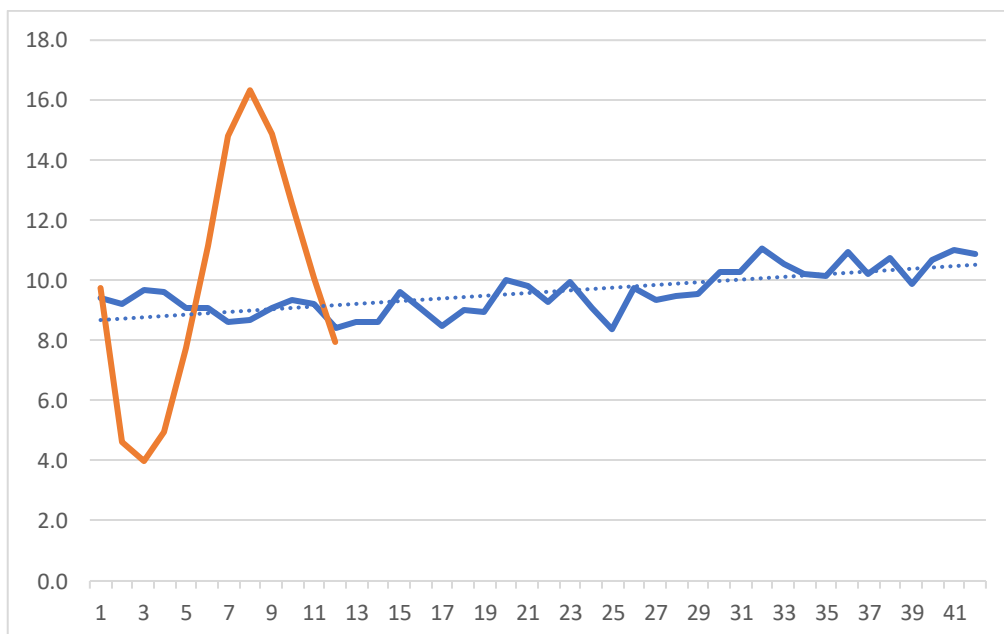


Figure Six: This graph depicts the average annual sea surface temperature and the average monthly high and low temperatures. The blue line reflects the annual average, whereas the red trend line

indicates a progressive rise in temperature. The orange line indicates the average monthly temperature spikes, illustrating the magnitude of the deviation from the typical baseline.

The stranding Data was first analysed to establish the total amount of turtles that had been recorded $n = 135,067.4$ (see figure seven & eight), further to this an analysis for each species was conducted to identify which turtles were most effected loggerhead turtle $n = 57,401$, green sea turtle $n = 45,915$, olive ridleys turtle $n = 11,587$, kemps ridleys $n = 2,062$, hawksbill turtle $n = 3,657$, leatherback $n = 3,434$, unidentified $n = 3,219$, these total numbers of stranding were then cross referenced with the area of most incidents the gulf of Maine which $n=55,582$, this was then further enhanced by detailing the monthly figures for strandings to give a more detailed analysis (see figure nine).

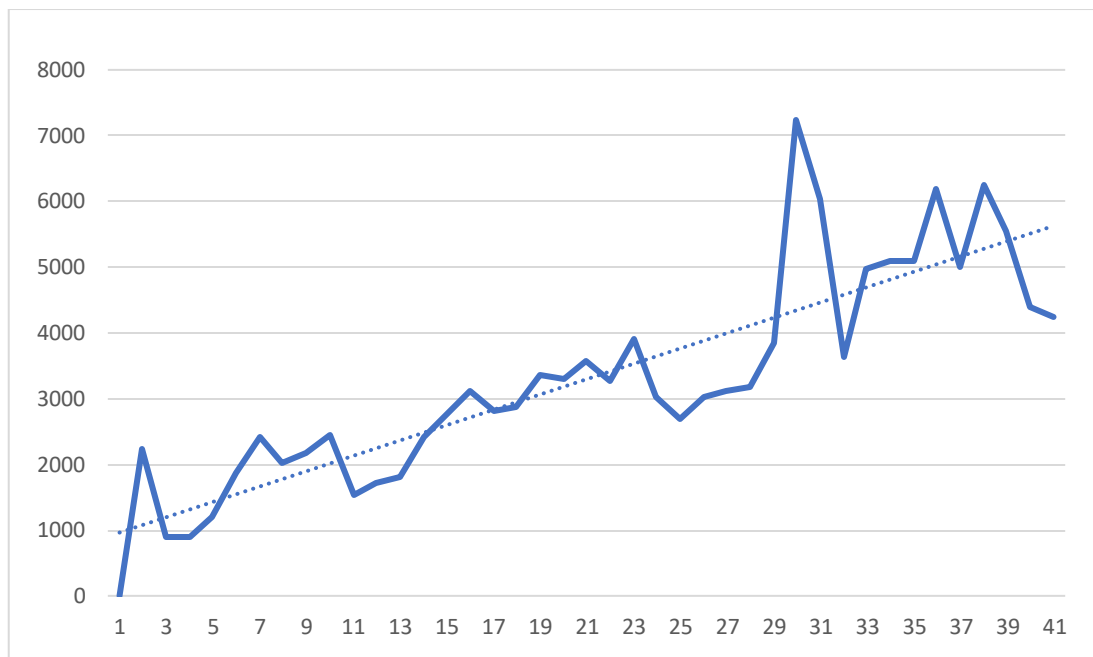


Figure Seven: This graph depicts the total number of strandings during a 41-year period, from year one = 1981 to year forty-one = 2022. The most remarkable part of this graph is that the highest number of cold stunnings in a single year happened in 2010 despite a progressive increase over the prior 41 years.

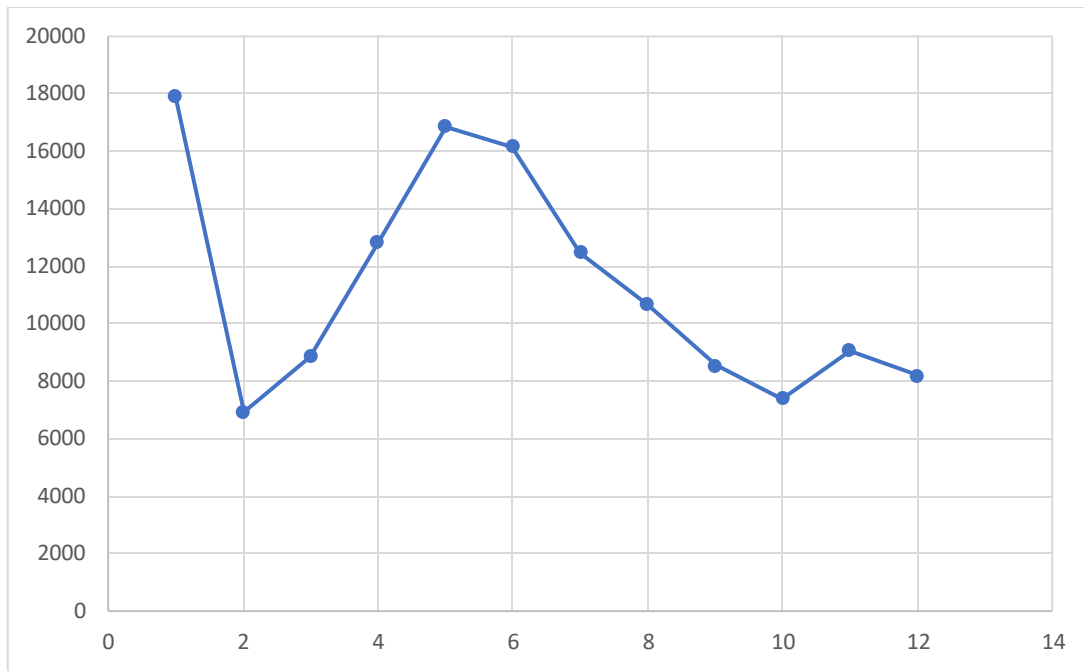


Figure Eight: This graph depicts the number of turtles cold stunned during a 41-year period, with each month represented by its corresponding number: 1 = January, 2 = February, 3 = March, 4 = April, 5 = May, 6 = June, 7 = July, 8 = August, 9 = September, 10 = October, 11 = November, 12 = December. This graph's peaks are separated by four months, indicating that something occurred at the end of the year and the beginning of the year to generate such a dramatic changes.

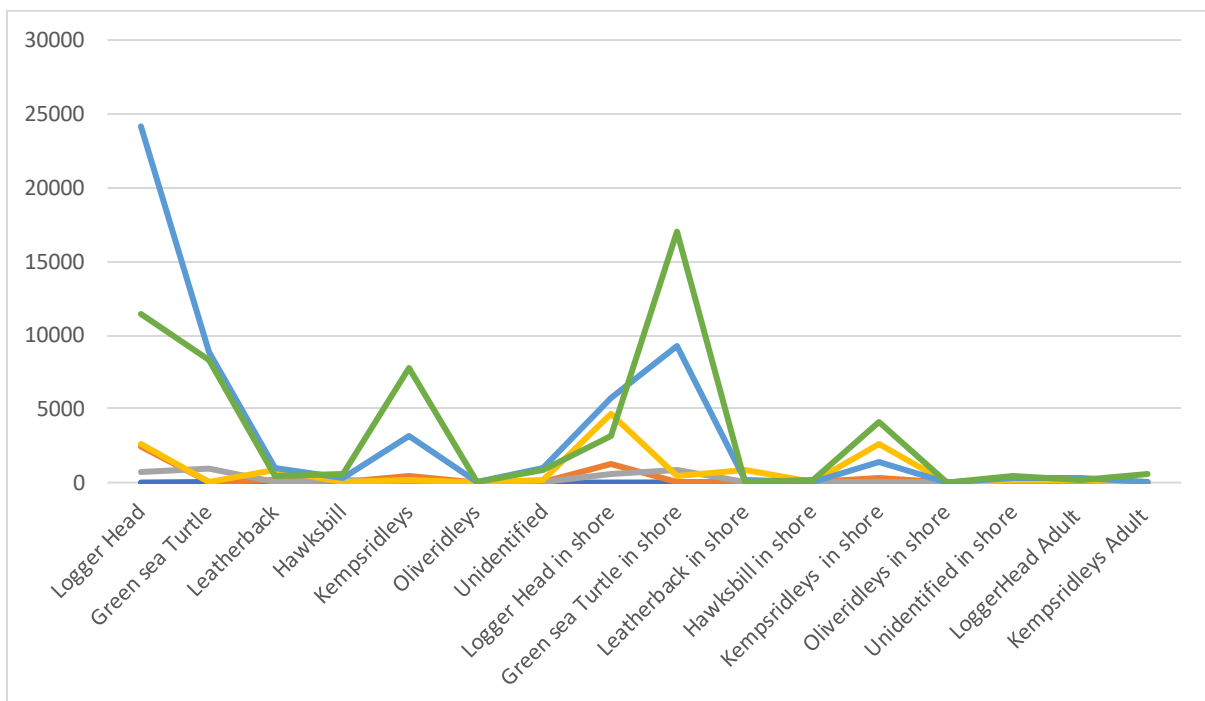


Figure Nine: This graph highlights the total strandings recorded by species and zone: dark blue for the US Caribbean, red for the South-eastern US Atlantic, grey for the Gulf of Mexico and the South-eastern US Atlantic, orange for the Northeast Atlantic, light blue for the South-eastern Atlantic (Gulf of Maine),

and green for the Gulf of Mexico. It is interesting to see that green sea turtles were thought to be the most affected by FP, but this graph demonstrates that Loggerhead turtles have really suffered the most from cold stunning.

The monthly data was cross-referenced with the average monthly sea surface temperature in the Gulf of Maine (see figure ten) to determine if any relationships existed between the results (see appendix c). Surprisingly, only three significant correlations were found among 37 non-significant correlations. This data was then analysed to determine if there were any correlations between the stranding data and the high and low temperatures in the Gulf of Maine; however, these results were also non-significant. This may be because the cases of recording stranded could be related to the preceding month's temperature, thus it was decided that the next step would be an examination of the total number of strandings over 40 years and the total average sea temperature over 40 years, which yielded similar results.

To test for correlation, the dependent variable sea surface temperature total was regressed on the predictive variable turtle strandings total. The results were $ST(1,39) = 34.900, p.001$, indicating that sea surface temperature can play an important role in shaping TS ($b=.000, p.001$). These results clearly indicate that water temperature has a positive effect on the total number of turtles strandings. Moreover, $R^2 = 0.472$ indicates that the model explains 47.2% of the total variance in TS.

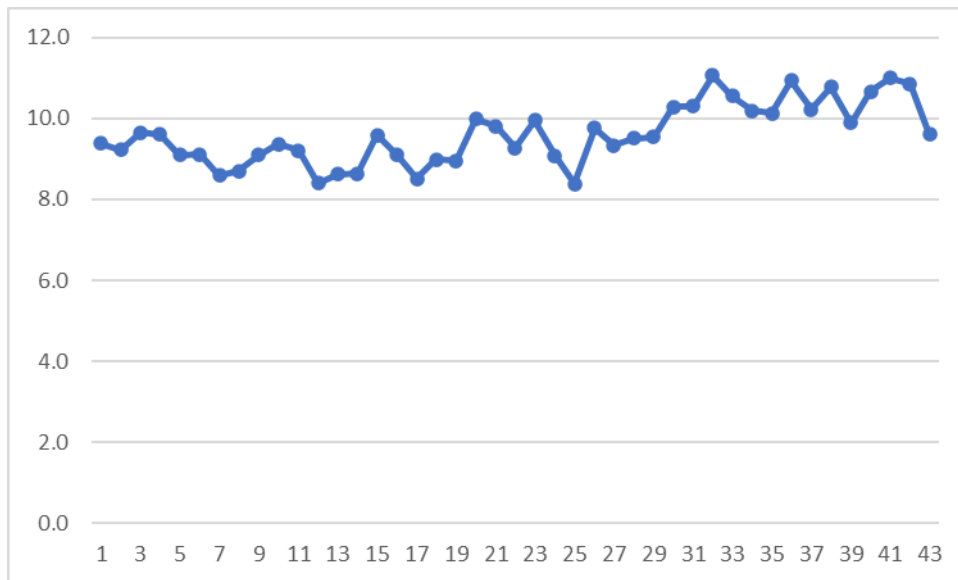


Figure Ten: This graph displays the mean average daily sea surface temperatures for the Gulf of Maine during a 41-year period.

Table Four: This table displays the results of an ANOVA and regression test comparing monthly high temperature to monthly strandings and monthly low temperature to monthly strandings. T41 corresponds to hot temperatures and T42 to low temperatures.

Tests	Regression Weight	Beta Coefficient	R ²	F	p-value	Hypothesis supported
T41	ST→TS	-5.95	.002	.025	p> 0.878	No
T42	ST→TS	.000	.024		p> 0.634	No

This result highlights that there is a significant correlation between sea surface temperature and the stranding of turtles, so next was to try and establish what is the prevalence of fibropapillomatosis within the turtle population. Because there is no official data set on turtle with fibropapillomatosis data was taken from previous studies and rescue facilities to produce an average, this is an out of the box way of thinking however because the data is published and correct it can be used to perform an average. Analysis was firstly taken upon the data provided by the Florida Fish and Wildlife Conservation Commission (FWC, 2023) this coincided with the area of analysis, so no data was excluded, within this data they recorded turtles with fibropapillomatosis as papilloma turtles n = 2,021.

However, this was a relatively small amount compared to the total turtles received by the facilities n = 129,197 however the results were still analysed to form a percentage of turtles that presented with fibropapillomatosis each year compared to the number of turtles received (see table five) the total amount of turtles was then analysed to give the average percent over the 14-year period n = 1.54% (see table four). This data was then further enhanced with data provided from journals that were conducted with the basis of identifying fibropapillomatosis for example, Teneria, *et al.*,(2022) recorded a prevalence of n=6.8% of 487, Patricio *et al* (2016) recorded a prevalence over the 18 year period of n = 20.65% of 218, Perrault *et al* (2021) gave a prevalence across two sites of n = 59.01% of 61 and sellera *et al* (2019) which was used for the reoccurrence rate n=95%. This brought in a prevalence rate once analysed of n= 22.00% prevalence of FP for the representation of the species which will form the basis of the start of the prediction model see figure.

Table Five: This table displays the analysed data extracted from the Florida Fish and Wildlife Conservation Commission's data (FWC, 2023). Highlighting the total number of turtles with papilloma, the total number of turtles received by the facilities, and the percentage calculated for turtles with FP and the corresponding year.

Florida turtle Rescue Centres Data	Turtles with Papilloma	Turtles Brought in to Rescue Centres	Percentage	Year
	90	4466	2.01%	06-07
	82	10359	0.79%	08-09
	67	13809	0.48%	07-08
	109	8870	1.02%	09-10
	111	10638	1.04%	11-12
	302	16480	1.22%	14-15
	424	27078	1.56%	15-16
	376	15078	2.49%	17-18
	206	6634	3.10%	18-19
	81	8736	0.92%	18-19
	173	7049	2.45%	19-20
Totals	2021	129197	1.54%	

Discussion and Synthesis

The aims of this research were to determine whether or not climate change, specifically the rising sea surface temperatures, has affected marine turtles by increasing and decreasing the water temperatures around them, thereby causing them to suffer from cold stunning incidents, that also proves to be one of the leading reactions to the development of fibropapillomatosis. Nonetheless, the second aim was to determine and create a predictive model for each of the data sets to utilise for future research.

Similarly, to the findings of Shaver *et al.* (2017), which also demonstrated a link between sea temperature and hypothermic stunning, the results demonstrate a significant correlation between changing sea surface temperatures and an increase in cold stunning occurrences. There has also been a significant increase in cold stunning

across all species of marine turtles, with juveniles being the majority. It is believed through studies such as Patricio *et al* (2016) that green sea turtles can have a slightly elevated internal temperature of 1.5 degrees when taken from waters of normalised or slightly higher temperatures, but most importantly, turtles have also experienced a decrease in body temperature (Heath & McGinnis, 1980),

In August, however, when summer temperatures reach new record highs, it is likely that the turtles will experience a similar effect to that of cold stunning, albeit in a slightly different manner, more akin to exhaustion, but still exhibiting the same symptoms and appearing to have been stunned to most observers (Achrai & Wagner, 2017). Since this is on the opposite end of the spectrum and as previously stated, turtles can be up to 1.5 degrees Celsius above their warmer water temperature, this would also contribute to the rising numbers, but ultimately, these fluctuating temperatures will continue to cause an increase in the population (Limpus *et al.*, 2003).

Sea Surface Temperature

Due to the fact that marine turtles spend their whole lives in the ocean, the temperature of the water is essential to their survival. As a result, marine turtles have a symbiotic relationship with the ocean (Heithaus, *et al.*, 2002). The results of this study reveal that sea surface temperatures have changed at higher and lower rates over the past eight years compared to the prior thirty years in fact the average yearly temperature has increased by an average one degree overall.

This study found that from April to September, sea surface temperatures increased on a monthly basis by an average of four degrees, with an overall increase of 14 to 15 degrees, this temperature proves to be a significant finding showing that the turtles are subjected to a severe spike in temperature. This shows that the turtles being an ectothermic species must deal with a fairly rapid change of almost double its lowest optimal temperature, which is a rare occurrence for any species, although marine turtles are able to adapt to this stress on an increasing temperature range (Goessling, *et al.*, 2019). However, unfortunately the temperature decreases from October to November, are a different story a gradual but unforgiving constant reduction by an average of 3 degrees every month until March, when the sea temperature is at its lowest. This consistent decline has become more prominent over the past 40 years, prior to this a more average temperature was attained with no sudden spikes, and a

smaller gradual reduction resulting in the turtles' flourishing (Hochscheid, *et al.*, 2002) & (Schofield, *et al.*, 2009). However, the results indicate that the turtles now have to deal with a world where they must endure both ends of the scale of extreme temperatures experiencing a temperature range from around three degrees Celsius up to 19 degrees and then back to around three degrees, these are temperature that the marine turtles will be susceptible to within a year which holds validity to the process of cold stunning becoming more prominent.

Nevertheless, if these turtles are caught off guard by unexpected fluctuations in sea temperature, they could undergo hypothermic shock on a daily basis, indicating that a turtle could endure cold stunning effects on a daily basis, this would increase the likelihood that the immune system reacts in a way that assaults the body, resulting in the turtles suffering the ramifications and FP becoming more prevalent (Poloczanska, *et al.*, 2009)

Marine turtles are ectothermic and rely on the surrounding temperature to thermoregulate. As with most ectothermic creatures, marine turtles have a controlled temperature range between 15 to 30, which is best for maintaining homeostasis (Rodgers, *et al.*, 2021). However, if a turtle spends an extended period of time outside of its natural range, it may suffer from an incident known as cold stunning, a condition in which its body and organs slow down restricting its movements and causing it to aimlessly float around until the point where it reaches warmer waters and begins to warm up enough to restore blood flow and move again, this is very similar to what is happening currently to Florida's green iguanas (Vanderstraeten, 2020).

These cold stunning events are problematic and as the results show these events are increasing at an alarming rate, in fact, the results show that the last 12 years have recorded the almost the same number of turtles being cold-stunned $n = 63,281$ as the preceding 23 years $n = 63,592$. While this is a worrying development the main concern comes that comes from cold stunning is even more concerning, the disease FP this benign tumorous disease which as seen in previous literature and highlighted within this study can generally occur around 45 days after the turtle has been subjected to a cold stunning incident (Dujon, *et al.*, 2021).

Location / Zone

The study highlights that over 110,000 occurrences of cold stunning were documented in the Gulf of Maine and the Gulf of Mexico over the past 41 years, respectively, this is primarily because this is the prime habitat and corridor for the turtles' life and migration route. This was taken into account when determining the best location for a representation of the species based on the results from the first process of analysis. These two locations account for more than 80 per cent of the total number of cold stunning cases recorded. This means the analysis of one of these two areas would yield the most representative results for the species, in turn allowing for the creation of a future prediction model to raise awareness for the species.

The Gulf of Maine was selected as the primary study area because it yielded more than 55,500 reports of cold stunning and also because the data for FP cases in rescue centres, the full sea surface temperature breakdown data, and the journal with fibropapillomatosis transmission cases are all located there. The data sets utilised have also been used in prior studies, including Patricio *et al.* (2016), foley *et al.* (2005), IR (2008-2010), Escobedo-Bonilla *et al.* (2022), and Thompson *et al.* (1988) showing a stronger validity for critique.

Within the data, it was noted that 54.3 per cent of the turtles afflicted by cold stunning were loggerhead turtles, closely followed by green sea turtles., Interestingly, according to the literature, the green sea turtle was the sea turtle with the most cases of fibropapillomatosis (Hirama & Ehrhart, 2007), thus, if cold stunning is the primary component in triggering the disease, the loggerhead may well become the species most seriously impacted based on these new statistics and forecasts over the next few years (Manes, *et al.*, 2022).

Within the data provided by the rescue facilities, it is also noted that the green sea turtle was the species most frequently diagnosed with fibropapillomatosis. This may be due to the fact that green sea turtles spend more time along the shore, whereas loggerheads prefer open waters, and that the majority of turtles were only held in the rescue facilities for one to ten days before they were released (FWC, 2023). This suggests that the turtle would not have appeared with FP for at least 45 days after being cold-stunned and taken to the institution for treatment unless it already had the disease. Theoretically, the turtles could have been put back into the ocean, where the

sickness then manifested, to be passed on and then perish without trace. This illustrates that the disease can spread uncontrollably in an area prior to detection, even if the animals are in the care of professionals (Ellwanger, *et al.*, 2019).

Fibropapillomatosis

According to Suárez-Dominguez, *et al.*, (2020), the first record of this disease was found in green sea turtles, and it has since spread throughout their population showing a higher prevalence of FP than any other marine turtle species; this study has found that there is a link between the sea surface temperature and the incidents of cold stunning which strengthens results highlighted by previous research, and also highlights the possibility that a higher prevalence of FP coincides with these findings.

The disease is believed to be kickstarted by the immune system as a response to abnormalities within the body triggering a dormant virus Chelonid alpha herpesvirus 5 that a predicted 70% of turtles carry (James, *et al.*, 2021). The turtle's body is subjected to a level of stress that can only be compared to a near-death experience thus the turtle's immune system triggering the virus which creates reactions within the turtle's body that causes cells to overreact multiplying again and again causing benign tumours to form in the turtle's epithelium tissue (Reséndiz, *et al.*, 2021). The reality of things is that the real cause of this disease has never been documented, although this study provides solid evidence that cold stunning is one of the primary causing elements and hence aims to emphasise the significance of cold stunning as its primary contributor.

In Florida rescue centres, 1.54 per cent of the turtles received for rescue presented with tumours, which may seem low in the grand scheme of things, but when you consider that this was only turtles with tumours and the turtles that had been subjected to cold stunning, that were injured or sick were not tested for FP, there is a strong likelihood that after the incubation period of around 45 days, these too could have developed FP (Jones, *et al.*, 2021).

This means that if marine turtles are left to develop this disease to its full potential it could prove to be catastrophic to all turtles within that area and beyond, a study by Patricio *et al* (2016) showed the rate of transmission within an area of the Indian river where in as little as 3 years the infection rate had increased to 75% of the population.

The results of this study highlight and enhance the data analysed and demonstrate in these investigations that cold stunning and the spread of this tumorous disease pose a threat that could lead to the loss of 66.7% of the entire marine turtle population within five - thirteen years (Teneria, *et al.*, 2022).

Within the given literature such as Perrault *et al* (2021), there are many contradictions that state fibropapillomatosis is of little to no concern and that these animals will adapt to the changing temperatures just as they have for thousands of years; however, if this species is left to fend for itself, survival of the fittest always had an abundance of species on its side so the number of casualties was of no concern, whereas today species are declining, meaning that a species can become extinct before it has chance to evolve.

Since fibropapillomatosis does not have its own recorded data set, in part due to the incubation period of the disease and also due to the fact that not every turtle can be captured, tested, and held for up to two months, it is difficult to determine the severity and potency of this disease, especially considering that it was not well-known at the time that some of the contradictory studies were conducted. With a recurrence rate of 95%, even if a turtle receives treatment, the disease will nearly always return and kill it (Sellera, *et al.*, 2019).

Prediction Models

The by-product or secondary outcome of this study was to produce a prediction model of sea surface temperatures, a prediction model for cases of cold stunning on all species, and a prediction model of the that would highlight the potential cases of fibropapillomatosis for the future. Previous literature downplayed the severity of fibropapillomatosis, and previous prediction models for cold stunning have already been exceeded by eight or more years before their predicted dates. Therefore, it is imperative to raise awareness of the potential outcomes if there is no intervention or potential plan of action to save the marine turtle species from this fate (Griffin, *et al.*, 2019).

The best way to understand the importance of a prediction model is to look at what has happened to humans over the last three years through coronavirus (covid-19) a

virus that started in one area from as little as a few people and then became the world's biggest nightmare a global pandemic, At the beginning of covid-19, it demonstrates what can happen when a population has a transmissible virus that can go undetected with no current vaccine or medicine in place. The way this virus spread around the world demonstrates how easily a virus can spread, which is why a prediction model from this study could prove to be so important (Suryasa, *et al.*, 2021).

The results and prediction models highlight that sea surface temperatures are going to increase around 0.3 degrees over 10 years (see figure eleven) this may seem small however when you consider that one degree can change the sex of a turtle embryo or the fact that one degree has killed off thousands of corals within the great barrier reef, an increase of 0.3 on top of the already increased sea temperatures could prove to be catastrophic (Bernstein, 2013).

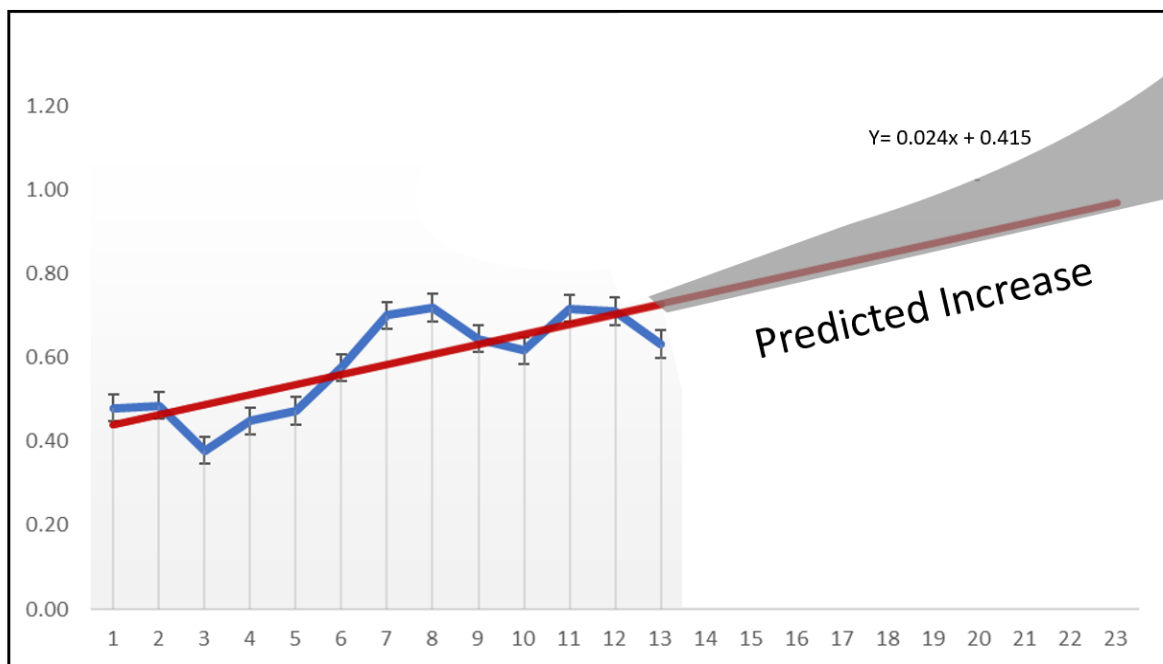


Figure Eleven: This is the model for predicting the rising sea surface temperature. The temperature anomalies of the past 13 years were evaluated, and the average was used as the prediction path. The trendline depicts the growth for the next 10 years, and a 0.3-degree Celsius increase is projected. The equation employed is located above the trend line.

As the sea temperature fluctuates in an area where turtles are known to reside, the turtles will be subjected to even more cold stunning events; therefore, if FP is to be avoided, the turtles must be intercepted or rescued before their immune system begins

to react, resulting in the development of FP. Models of predicted outcomes indicate that both cold stunning episodes and the prevalence of FP will continue to increase considerably over the next thirteen years (see figures twelve & thirteen). However, prediction models can be used to indicate that a team may need to be dispatched to intercept in areas where temperature spikes occur, thereby preventing the disease from taking hold or preventing its transmissibility between the species, thereby preventing the disease from spreading (Work, *et al.*, 2015).

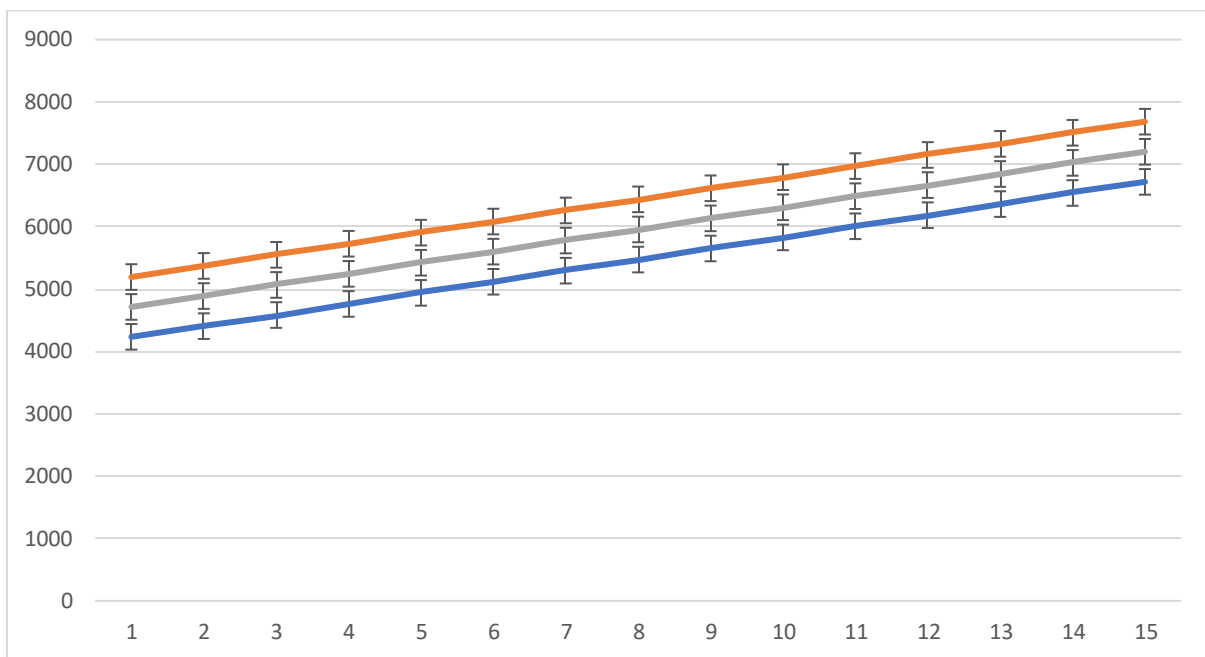


Figure Twelve: This is a model for predicting future cold stunning incidents. The orange line is increasing from the 13-year average, the blue line is increasing from the most recent number reported, and the grey line is the average of the two, which is used to predict the future occurrences of cold stunning. Using the average of cold stunning and the average rise over the past thirteen years, 3.42 percent $n = 177.94$ were utilised to calculate the prevalence for the prediction model. This number was then used to predict the increase over the next fifteen years, which revealed a consistent but gradual increase.

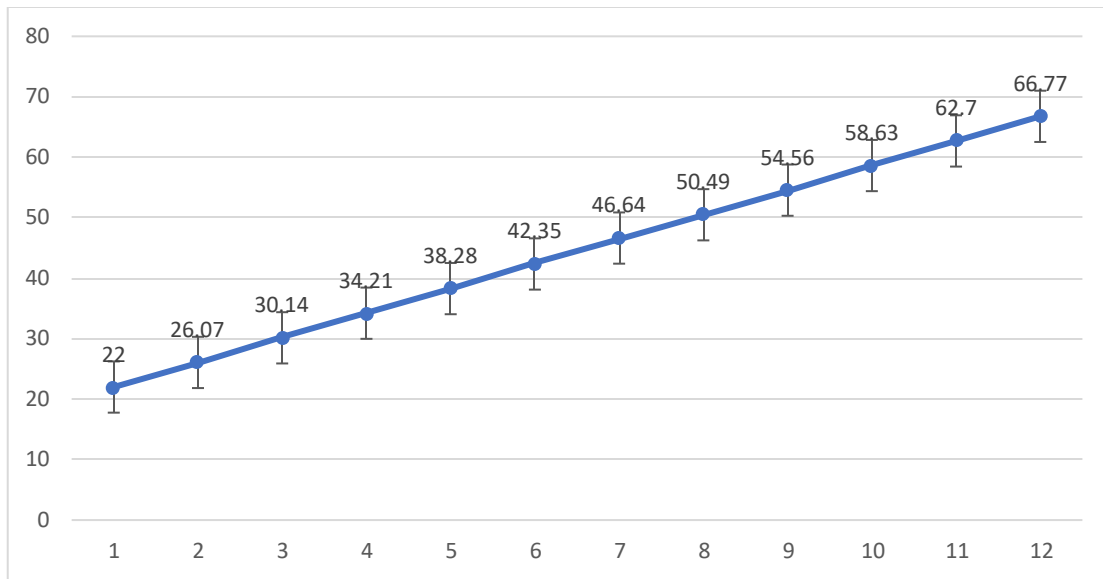


Figure Thirteen: This is a model for predicting the future prevalence of FP over the entire species of marine turtles. A forecast model based on the last 18 years of reported FP prevalence was developed by analysing the average high and low FP rates during the last 18 years, reducing the average rate of 6.07 by two to account for fatalities and anomalies, resulting in an increase of 4.07 percent per year.

These prediction models also highlight a more realistic and representative image derived from the analysed data sets in an effort to garner the necessary attention and awareness for the development of future solutions (Steyerberg & Steyerberg, 2009).

Limitations Future Research

Throughout this study, there have been several limitations that have made it difficult to analyse the data for the purpose of testing the hypothesis; however, these limitations have also helped to think creatively to analyse and compute the data to provide an accurate representation of the numerous species of marine turtles involved in the hypothesis question.

The first limitation that caused an issue was selecting the number of years that would adequately depict changes and anomalies in sea surface temperature and whether or not there were sufficient recorded data sets. Once literature and journals such as Dujon, *et al.*, (2021), Griffin, *et al.*, (2019), Coles & Musick, (2000) were identified, the snowball method was utilised to identify further literature, journals, and sources such as Sea turtle stranding and salvage network (STSSN), the National Oceanic and Atmospheric Administration (NOAA) (NOAA, n.d.), the National Centre for

Atmospheric Research (NCAR) (UCAR, 2023), the National environmental satellite DATA, Information Service (NESDIS, 2016), the Optimal Interpolation Sea Surface Temperature database (OISST) (NOAA NCEI, n.d.), and the Climate Data store (Copernicus, n.d.). These data sets were structured and accurate enough to enable the discovery of a zone analysis, allowing for a greater level of specialised zone analysis. All of these data sets are well-known and widely utilised in previously published papers, which essentially guarantees the highest quality of results, since they have been scrutinised on several different occasions.

Another limitation involved cold-stunned turtle data and the number of years it would take for its representation, as well as its representation of the species of marine turtles that have been subjected to cold stunning, as there are not many variations of data available. Nevertheless, the national oceanic and atmospheric administration (NOAA) and the sea turtle stranding and salvage network (STSSN) (NOAA Fisheries, n.d.) provided Data for all recorded strandings from 1981 to 2023. It is important to note that the sea turtle stranding and salvaging network is the sole source of cold-stunned and stranding data, however it has been used in numerous published publications, which strengthens its validity.

There was no real data base for recording cases of fibropapillomatosis, the data provided from the STSSN and NOAA provided a very small description with some of the cold stunned data where they described some turtles presented with tumours, this referred to cases of fibropapillomatosis, but as their main concern is recording data for cold stunned turtles, this was not considered a significant limitation (NOAA Fisheries, n.d.), The Florida fish and wildlife conservation commission came forward with another data set for rescue centres in the Florida area where they had turtles with fibropapillomatosis listed as papilloma turtle (FWC, 2023), with this data and journal data such as Ene, *et al.*, (2005), Hirama & Ehrhart, (2007), Foley, *et al.*, (2005), & Aguirre & Lutz, (2004) this combination provided the most accurate representation for the prediction model for the awareness and need for solutions necessary to avoid the spread of the pandemic and the imminent threat of all marine turtle species.

Throughout these results, there have been challenges, but most significantly, there has been an accurate depiction of how sea surface temperatures impact marine turtles, causing them to experience cold stunning. Clearly, cold stunning is either the

cause of or a contributor to the present epidemic of FP in turtles. This research should enable scientists and researchers to comprehend, respond to, and combat the possibility of cold stunning as well as the spread of fibropapillomatosis before it becomes a global pandemic and causes the extinction of one or more marine turtle species.

Looking towards the future, further studies that could follow on from this one to enhance the knowledge around cold stunning and the development of FP, could consist of forming a physical data set of turtles with FP, this would show researchers and the world the extent of how much the disease has taken hold.

Another study for further research could look into how climate change has changed the currents within the ocean, affecting the heat transfer between oceans and that the fact that marine turtles use these currents to travel to and from the migration grounds, could this be a key to cold stunning?

The final possibility would be enhancing this study with a more biological look into the effects of cold stunning and the development of fibropapillomatosis to assess if there are any intercept points, where the reaction between the turtle's immune system and attacking itself can be stopped. There is also the possibility that the disease could be stopped at an early stage, and also if there is a cure rather than a temporary fix that prolongs their life rather than saves it.

For each of these future research studies or anyone interested in learning more about this topic, this study will provide a solid foundation for the key points that need to be investigated further, as well as a thorough literature review to increase awareness and attention before the marine turtle species reaches a point of no return and succumbs to the epidemic.

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Appendices

Appendix A: This is a portion of the 86,174 lines of turtle stranding data provided by the STSSN. (NOAA Fisheries , n.d.)

Sea Turtle Stranding by Zone Report																			
Report Type: Stranding and Cold Stun																			
Year 1982										Zone 21									
Region: GULF OF MEXICO (TX-FG)																			
State: TEXAS																			
Red Week Number: Not all States Reported																			
Period	OFF SHORE							IN SHORE							ALL ADULT SIZED				
	CC	CM	DC	EI	LK	LO	UN	TOT	CC	CM	DC	EI	LK	LO	UN	TOT	CC	LK	
1	01/01-01/09/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	01/10-01/16/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	01/17-01/23/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	01/24-01/30/1982																		

5	01/31-02/06/1982	1	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0
6	02/07-02/13/1982																		
7	02/14-02/20/1982																		
8	02/21-02/27/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	02/28-03/06/1982																		
10	03/07-03/13/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	03/14-03/20/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	03/21-03/27/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	03/28-04/03/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	04/04-04/10/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	04/11-04/17/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	04/18-04/24/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	04/25-05/01/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	05/02-05/08/1982	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
19	05/09-05/15/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

2005/16-05/22/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2105/23-05/29/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2205/30-06/05/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2306/06-06/12/1982	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2406/13-06/19/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2506/20-06/26/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2606/27-07/03/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2707/04-07/10/1982	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2807/11-07/17/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2907/18-07/24/1982	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
3007/25-07/31/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3108/01-08/07/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3208/08-08/14/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3308/15-08/21/1982																		
3408/22-08/28/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

35	08/29-09/04/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
36	09/05-09/11/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
37	09/12-09/18/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
38	09/19-09/25/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
39	09/26-10/02/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
40	10/03-10/09/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
41	10/10-10/16/1982																	
42	10/17-10/23/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
43	10/24-10/30/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
44	10/31-11/06/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
45	11/07-11/13/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
46	11/14-11/20/1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
47	11/21-11/27/1982	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	
48	11/28-12/04/1982	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	
49	12/05-12/11/1982																	

50	12/12-12/18/1982																		
51	12/19-12/25/1982																		
52	12/26-12/31/1982																		
TOTAL:		7	0	0	0	5	0	0	12	0	0	0	0	0	0	0	0	0	

Appendix B: This is a screenshot of one of the 45 pages of data that the Florida Fish and Wildlife Conservation Commission provides annually (FWC, 2023).

TABLE 3. MARINE TURTLE CAPTIVE FACILITY DATA (July 1, 2006 through June 30, 2007)

FACILITY CODE	SPECIES	#	ENTRY DATE	DATE ACQUIRED	DATE RELEASED	DATE DIED	DATE EUTHANIZED	DATE TRANSFERRED	N_T*	STATUS	COMMENTS
CMC											
	CM	1	J	06/05/06	06/07/21				N	rehab	stranding - monofilament around LFF
	CM	1	J	06/07/01				07/02/21	N	rehab	stranding - papilloma turtle, floating
	CC	1	A	82/12/27		06/08/15			N	pre-act	acquired from Pier 60/Clearwater Beach. Necropsy showed abscess on L neck, R lung partially collapsed, L lung = possible hemorrhaging, L kidney = no lobulations, R kidney = 1/3 size of L, 3 lobulations present, E. coli & Strept present in cultures.
	CC	1	S	06/11/22	07/06/14				N	rehab	stranding - possible red tide turtle, lethargic, epibiota
	CM	1	J	06/11/17			06/11/20		N	rehab	stranding - papilloma turtle, emaciated, epibiota.
	CM	1	S	07/06/22					TI	rehab	transferred back from MML - papilloma turtle, healed damage to L marginals.
	CM	1	J	06/10/29	07/06/19				N	rehab	stranding - buoyancy issues, boat damage (healed)
	EI	1	J	06/10/11	06/11/20				N	rehab	stranding - lethargic, no external anomalies. Not flipper tagged at time of release b/c too small for tags.
	CM	1	J	06/02/25				07/02/21	N	rehab	stranding - papilloma turtle, floating
	CC	1	A	06/05/19					N	rehab	acquired from St. Lucie PP - boat damage to posterior carapace (healing), 40% both FF missing (healed)
	CC	1	S	06/08/12					N	rehab	caught during trawling (Egmont Channel) - eagle ray barb in LRF (probably from coming in bag with bycatch)
	CC	1	J	06/05/06	06/07/21				N	rehab	stranding - LFF entangled in crab trap line, healed LRF damage
	CC	1	S	06/04/26	06/07/21				N	rehab	stranding - floating, no external anomalies, epibiota
	CM	1	J	06/12/23	06/12/23				TI	rehab	transferred from MML - papilloma turtle. Originally GA DNR stranding - transferred to MML for temporary treatment
	LK	1	A	06/04/17	06/07/21				N	rehab	stranding - monofilament around LRF (severe flipper damage)

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Appendix C: This table displays the results of the regression analysis tests for yearly comparisons of sea surface temperatures and marine turtle strandings throughout the selected 40-year time period.

Tests	Regression Weights	Beta Coefficient	R ²	F	P-Value	Hypothesis supported
T1	ST→TS	0.019	0.510	10.31	P < 0.009	Yes
T2	ST→TS	0.029	0.281	3.89	P > 0.077	no
T3	ST→TS	0.037	0.156	1.85	P > 0.203	no
T4	ST→TS	0.039	0.206	2.59	P > 0.138	No
T5	ST→TS	0.013	0.132	1.52	P > 0.246	no
T6	ST→TS	0.009	0.124	1.41	P > 0.262	no
T7	ST→TS	0.029	0.307	4.42	P > 0.062	no
T8	ST→TS	0.035	0.262	3.54	P > 0.089	No
T9	ST→TS	0.009	0.026	.270	P > 0.615	No
T10	ST→TS	0.011	0.024	.242	P > 0.634	no
T11	ST→TS	0.024	0.163	1.94	P > 0.194	no
T12	ST→TS	0.014	0.130	1.49	P > 0.249	no
T13	ST→TS	0.015	0.200	2.50	P > 0.145	no
T14	ST→TS	0.022	0.239	3.14	P > 0.107	no
T15	ST→TS	0.009	0.063	.673	P > 0.431	no
T16	ST→TS	0.012	0.135	1.56	P > 0.240	no
T17	ST→TS	0.008	0.085	.933	P > 0.357	no

T18	ST→TS	0.015	0.174	2.11	P > 0.177	no
T19	ST→TS	0.004	0.012	0.126	P > 0.730	No
T20	ST→TS	- 0.002	0.004	0.041	P > 0.844	No
T21	ST→TS	0.005	0.022	0.227	P > 0.644	No
T22	ST→TS	0.006	0.049	0.513	P > 0.490	No
T23	ST→TS	0.004	0.016	0.161	P > 0.697	No
T24	ST→TS	0.025	0.413	7.03	P < 0.024	Yes
T25	ST→TS	0.022	0.182	2.23	P > 0.166	No
T26	ST→TS	0.013	0.056	0.597	P > 0.458	No
T27	ST→TS	0.002	0.003	0.026	P > 0.876	No
T28	ST→TS	- 0.007	0.032	0.332	P > 0.577	No
T29	ST→TS	- 0.002	0.085	0.935	P > 0.356	No
T30	ST→TS	- 0.008	0.409	6.90	P < 0.025	Yes
T31	ST→TS	- 0.006	0.019	0.189	P > 0.673	No
T32	ST→TS	- 0.009	0.112	1.26	P > 0.287	No
T33	ST→TS	- 0.011	0.154	1.81	P > 0.207	No
T34	ST→TS	- 0.013	0.169	2.03	P > 0.184	No
T35	ST→TS	- 0.003	0.124	1.14	P > 0.262	No
T36	ST→TS	- 0.010	0.193	2.38	P > 0.153	No
T37	ST→TS	- 0.001	0.063	0.676	P > 0.430	No
T38	ST→TS	- 0.003	0.013	0.133	P > 0.723	No

T39	ST→TS	- 0.021	0.370	5.87	P < 0.036	Yes
T40	ST→TS	- 0.010	0.217	2.77	P > 0.127	No