Fibropapillomatosis Prevalence and Distribution in Immature Green Turtles (Chelonia mydas) in Martinique Island (Lesser Antilles)

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Fibropapillomatosis prevalence and distribution in immature green turtles (*Chelonia mydas*) in Martinique Island (Lesser Antilles)

Abstract

Fibropapillomatosis (FP) threatens the survival of green turtle (*Chelonia mydas*) populations at a global scale and human activities are regularly pointed as causes of high FP prevalence. However, the association of ecological factors with the disease's severity in complex coastal systems has not been well established and requires further studies. Based on a set of 405 individuals caught over ten years, this preliminary study provides the first insight of FP in Martinique Island, which is a critical development area for immature green turtles. Our main results are: i) 12.8% of the individuals were affected by FP, ii) FP has different prevalence and temporal evolution between very close sites, iii) green turtles are more frequently affected on the upper body part such as eyes (41.4%), fore flippers (21.9%) and the neck (9.4%), and iv) high densities of individuals are observed on restricted areas. We hypothesise that turtle's aggregation enhances horizontal transmission of the disease. FP could represent a risk for immature green turtles' survival in the French West Indies, a critical development area, which replenishes the entire Atlantic population. Continuing scientific monitoring is required to identify which factors are implicated in this panzootic disease and ensure the conservation of the green turtle at an international scale.

Keywords: marine turtles, infectious disease, epizootiology, environmental quality

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1. Introduction

Green turtle *Chelonia mydas* (Linnaeus, 1758) populations have to face a wide range of anthropogenic threats such as bycatch, boat strike, seagrass meadows destruction, dredging operations, marine pollution, poaching, and tourism development (Domiciano et al., 2017; Herbst & Klein, 1995; Jones et al., 2015; Rossi et al., 2019) leading to decreasing trend for the global population. This resulted in the classification of the species as "endangered" on the IUCN Red List (Seminoff, 2004). In addition to these threats, the epizootic disease fibropapillomatosis (FP) is an emerging global threat for green turtles (Bjorndal, 1999; Herbst & Klein, 1995).

FP is a neoplastic disease characterised by the growth of tumours mostly on soft tissues and the shell (Herbst, 1994). The tumours can have a diameter of up to 30 cm and alter vision, swimming, foraging, orientation abilities or even breathing (Jones et al., 2015; Williams et al., 1994). In the disease's end stage, internal tumours can develop on the lungs, kidneys, heart or digestive tract, which can lead to death (Jones et al., 2015). FP has been observed on the seven existing species of marine turtles but has only reached a panzootic level in the green turtle (Jones et al., 2015). This disease spread to numerous regions worldwide in the 1980's, especially in the Atlantic, the Caribbean (i.e. Cayman Islands, Puerto Rico, Virgin Islands, Barbados, Venezuela, Colombia, Nicaragua, Costa Rica, Panama, and Belize), and in the Indo-Pacific region with prevalence varying from 1.4% to 92% (Adnyana et al., 1997; Herbst, 1994). FP's precise aetiology is not yet fully known. However, the Chelonid HerpesVirus 5 (ChHV5) has been regularly associated with this disease, and it is now a consensus that this virus could be the most likely cause of FP (Chaves et al., 2017; Domiciano et al., 2017; Jones et al., 2015). Moreover, the hypothesis of infection through horizontal transmission (i.e. from one individual to another) during turtle's settlement into coastal habitats has been widely recognized. Indeed, there has been no observation of FP clinical signs on recently recruited turtles (Jones et al.,

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2020; Patrìcio et al., 2016; Shaver et al., 2019). Furthermore, it has been proved that horizontal transmission can be promoted by parasite marine leech, which act as mechanical vectors of ChHV5 (Rittenburg et al., 2021).

Environmental conditions have been proposed to play an important role in the emergence of FP (Herbst & Klein, 1995; Herbst et al., 2004) because of the multiple reports of high variations of prevalence between very close geographic regions (Herbst, 1994; Jones et al., 2015). Herbst & Klein (1995) suggested that higher sea temperature could induce faster tumour growth that in turn would result in more severe FP in green turtles. FP is also frequently associated with poor water quality (e.g. pollution, eutrophication) in coastal areas near human activities and/or with low hydrodynamics (Hargove et al., 2016; Torezani et al., 2010). Metal contaminants (da Silva et al., 2016), persistent organic pollutants (Foley et al., 2005), and eutrophication coupled with a change in diet quality (Van Houtan et al., 2014) have also been suspected to enhance green turtles' susceptibility to FP.

Located in the Lesser Antilles of the West Indies in the eastern Caribbean, Martinique Island hosts an important population of immature green turtles in which clinical signs of FP infection (i.e. tumours) have been observed (Bonola et al., 2019). Moreover, Martinique has many sheltered bays that support the settlement of multi-species seagrass meadows on large shallow areas, particularly favourable to green turtles (Siegwalt et al., 2020). Indeed, the island is an important developmental area for these immatures, who show high fidelity to their foraging grounds for several years (Siegwalt et al., 2020) before performing their developmental migration in the Caribbean and the entire Atlantic (Chambault et al., 2018). In areas where green turtles tend to congregate for feeding, we hypothesize that positive interactions between individuals would facilitate horizontal transmission of FP. Moreover, an important urban development and poor sanitation of wastewater of this island lead to discharge

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releases of polluted water in the marine environment (Hily et al., 2010) that could drive enhanced environmental pollution and/or trigger an eutrophication phenomenon.

This study is the first one to focus on FP prevalence in Martinique. From 2010 to 2019, capture-mark-recapture (CMR) data as well as the presence of tumours have been collected on immature green turtles along the western coast of Martinique. During 2018 and 2019, data on animal aggregation were gathered whereas, in 2021, seawater samples were collected in order to describe environmental quality. These datasets offer a unique opportunity to analyse the emergence of FP in green turtles (based on symptomatic individuals) in the critical developmental area of Martinique. The aims of this preliminary study were (i) to describe FP evolution through time between different high-fidelity grounds, (ii) look for possible environmental variables affecting the dynamics of this disease, (iii) describe turtles' distribution and compare it to FP prevalence and dynamics.

2. Materials and methods

2.1. Study area

This study was conducted along 60 km of the western coast of Martinique (14°30'9.64'N, 61°5'11.85'W, France). Green turtles were mainly present in six bays of the Caribbean coast of the island that are, from north to south, Anse Noire, Anse Dufour, Grande Anse, Anse du Bourg, Anse Chaudière and Petite Anse (Fig. 1). These sites were identified as critical for immature green turtle foraging (Siegwalt et al., 2020).

2.2. Capture-mark-recapture program (CMR)

The CMR program used for this study is based on the tagging of animals by the injection of passive integrated transponders (PIT). Since 2010, with the exception of 2014, immature green turtles have been captured by freedivers at depths up to 25 m (Fig. 1). Capture

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methodology and procedures are described in Nivière et al. (2018) and in Bonola et al. (2019). Date and geographical coordinates were recorded for each turtle capture. The presence of a PIT was checked using a universal reader (GR251, TROVAN). In case of absence of a transponder, a PIT (ID-100, TROVAN) was injected into the right triceps. The search for FP was conducted by carefully examining each turtle for the presence of external tumours looking like single or multiple raised masses. Tumours were located on the turtles' bodies according to the following body parts: eyes, head, nape, neck, shoulders, fore flippers, carapace, plastron, back flippers, and tail base.

The study met the French legal and ethical requirements. The protocol was approved by the Conseil National de la Protection de la Nature and the French Ministry for Ecology (permit numbers: 2013154-0037 and 201710-0005) and followed the recommendations of the Police Prefecture of Martinique.

2.3. Environmental conditions

Sea surface temperature (SST) data were obtained from the NOAA/OAR/ESRL PSL public database (Colorado, USA, https://psl.noaa.gov/ (accessed 5-10-2020)). Daily means of the 'Optimal Interpolation Sea Surface Temperature V2' were extracted for the closest coordinates to Martinique Island, which were 14.55° latitude and -61.25° longitude (Fig. S1). The NOAA 1/4° daily 'Optimum Interpolation Sea Surface Temperature' is an analysis constructed by combining observations from different platforms (satellites, ships, buoys, and Argo floats) on a regular global grid (0.25° latitude x 0.25° longitude grid). These values were averaged per trimester from 2010 to 2019 and applied to every site since we had only a single sample point close to Martinique Island (Fig. S1).

Net primary production (NPP) data were collected from the Ocean Productivity public database (https://sites.science.oregonstate.edu/ocean.productivity/index.php). Monthly means

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of NPP, based on the Vertically Generalized Production Model (Behrenfeld & Falkowski, 1997) from MODIS satellite measures, were extracted for six points alongside Martinique's West coast (Fig. S1). Each turtle's capture site was assigned the NPP means of the nearest sampling point.

For seawater quality, two (for Anse Noire, Anse Dufour, Anse Chaudière) to three (for Grande Anse, Anse du Bourg, Petite Anse) sampling points per foraging site were chosen prior to field work (Siegwalt et al., 2020). On each sampling location, seawater was sampled multiple times with a plunger sampler (V = 1 L) at 5 m depth in order to fill a 2 L vial for total chlorophyll *a* analysis, a 250 mL vial for bacterial analysis (*E. coli* and enterococci) and four 150 mL vials for chemical analysis (ammonium, nitrites, nitrates and phosphates). Chlorophyll *a* vials were protected from any light by wrapping them in aluminium paper. Vials were stored in coolers away from sunlight. All samples were collected on the same day in 2021 and sent to the Laboratoire Territorial d'Analyses de Martinique. Chemical and chlorophyll *a* analyses were done according to Aminot & Chaussepied (1983) while *Escherichia coli* (*E. coli*) and enterococci were measured following European standards NF EN ISO 9308-3 and NF EN ISO 7899-1, respectively. We looked at chemical and biological parameters in order to seek for potential eutrophication and at European standards bacterial parameters to describe seawater sanitary safety.

2.4. Density surveys and mapping

Population density surveys of green turtles were carried out in 2018 and 2019 on several bays: Anse Noire, Anse Dufour, Grande Anse, Anse du Bourg, Anse Chaudière and Petite Anse. In order to count a maximum number of turtles under the water surface, one diver (observer) was connected by a 10 m rope to a boat. Transects specific to each bay, parallel to the beach and arranged from coast to sea, were previously created and integrated into a GPS

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(GarmineTrex) in order to follow a predefined course. The chosen distance between transects (10 to 30 m depending on the visibility) was intended to cover the greatest area and sample the largest number of individuals and to minimize the risk of missing an individual. Standardised hand signals have been established so that the observer could communicate with the operators aboard the boat to report the number of observed turtles. Each observation has been associated with its GPS coordinates.

Density maps were created to visualise the spatial distribution and density of green turtles using the software QGIS 2.18 (2016) and the legal CRS RGAF09 (EPSG:5490) in use at that time for French West Indies. Turtle sighting points and predefined transects were placed on Mapbox Satellite v9 satellite image in order to set up grids of hexagonal 1 ha-cells using the QMarxan extension, whose extents correspond to the surveyed areas of each bay. Then, turtle densities were determined in each cell.

2.5. Data analysis

FP data were obtained through CMR surveys and visual detection of FP-indicating tumours. Given the movements of individuals between Anse Noire and Anse Dufour (< 500 m apart; Siegwalt et al., 2020), the two sites were considered as a single entity called Anse Noire/Dufour. With the same considerations, Anse du Bourg and Anse Chaudière became Anse du Bourg/Chaudière.

Relationships between FP prevalence and environmental conditions were assessed with generalised linear models (GLM). Thus, in order to describe geographic disparities between close bays, interannual evolution, look for SST's influence on the disease's severity, and NPP's influence according its relationship with immature green turtle's body mass (Bonola et al., 2019), fixed factors included *capture site*, *year*, *mean SST,* and *mean NPP,* respectively. *Year* was considered as a discrete factor instead of a numeric variable. The distribution of the

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explained variable was binomial (presence or absence of FP) and a logit link was therefore used. A model selection was performed with the use of the Akaike information criterion (AIC; Akaike, 1973) and relative Akaike weight. Models with a difference lower than 2 in their respective AIC were considered similar (Burnham & Anderson, 2002).

To highlight which individual body parts were the most affected by FP, we calculated infection proportions per body part by dividing the number of turtles with the presence of tumour(s) on the specific body part by the total number of turtles affected by FP. Individuals captured several times were counted as one turtle having FP.

Generalised Linear Mixed Models (GLMMs) were used to explore potential differences in the seven seawater variables measured (i.e. ammonium, nitrites, nitrates, phosphates, total chlorophyll *a*, *E. coli*, and enterococci) between field sites. As for FP analysis, Anse Noire/Dufour and Anse du Bourg/Chaudière were considered unique sites. Sampling replication was included as a random effect for every GLMM to account for non-independence of data. Models were fitted using the *lme4* package (Bates et al., 2012). Likelihood Ratio Tests (LRTs) were performed using the *lmtest* package (Hothorn et al., 2015) to select the best-fit model between the nul GLMM and the one with capture site fixed effect. Post-hoc estimations and comparisons were performed when the *capture site* effect was kept using Estimated Marginal Means (EMMs) with the *emmeans* package (Lenth et al., 2021). Degrees of freedom were calculated using the Kenward-Roger method and p-values were adjusted for multiple comparisons with Tukey adjustment. Significance thresholds were fixed at 0.05. All data analysis was performed using R version 4.0.2 (R Core Team, 2020).

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3. Results

3.1. Fibropapillomatosis prevalence and body distribution

From 2010 to 2019, 539 immature green turtle catches were performed on the Caribbean coast of Martinique, corresponding to 405 distinct individuals. The vast majority of the individuals were captured at Grande Anse (n = 302), Anse du Bourg/Chaudière (n = 181) and Anse Noire/Dufour (n = 56). The change in occurrence of FP could not be properly evaluated at Le Prêcheur, Saint-Pierre, Le Carbet, Cap enragé, and Petite Anse (Fig. S2). First, immature green turtles' abundance seemed to be weaker at Le Prêcheur, Saint-Pierre, Le Carbet, and Cap enragé. Second, at Petite Anse, captures were less successful since animals escaped more often, perhaps because of important human activities in this bay (pers. obs.).

A significant increase of immature green turtles' FP prevalence from 0.000 (IC95% [0.000; 0.278]) in 2011 to 0.168 (IC95% [0.108; 0.253]) in 2019 was observed when considering the three major sites together (Odd-ratio : 1.72, p-value < 0.001; Fig. 2). Post-hoc Tukey between capture sites highlighted significant differences of prevalence between Grande Anse and Anse du Bourg/Chaudière ($p < 0.001$). Indeed, FP prevalence remained close to zero from 2011 to 2019 in Grande Anse and also from 2015 to 2019 in Anse Noire/Dufour. In fact, the major increase in FP prevalence on the Martinique coast seemed to be restricted to Anse du Bourg/Chaudière, where no individual with FP being observed in 2011 and 2012 and with FP prevalence increasing from 2013 (0.11) to 2019 (0.50).

A total of 128 observations on 52 individuals were used to calculate the percentage distribution of tumours on individual body parts (Fig. 3) as tumour's body location was not reported during the first years of the CMR program. For the majority of turtles, lesions were observed on several parts of the body. Eyes were the most frequently affected body parts (21% of individuals for the left and 20% for the right). The front fins and neck's ventral part were

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also frequently affected, with 9% of the individuals being affected on the left fin, 13% on the right fin, and 9% on the neck.

3.2. Fibropapillomatosis and environmental cofactors

AICs, ΔAICs and relative Akaike weights for the 11 tested GLMs are presented in Table 1. The null model is the model without effect and FP prevalence is therefore supposed constant. The lowest AIC (79.74) has been observed for the model with *capture site* and *year* effects. This model had a relative Akaike weight of 0.45. When adding *NPP* to the two previous fixed effects (Fig. S3), the model had a ΔAIC < 2 (1.70) and a relative Akaike weight of 0.19. *Temperature* effect (Fig. S4) associated with *capture site* and *year* resulted with a ΔAIC of exactly 2 compared to the model with the lowest AIC. All other models had a ΔAIC > 2 and lower relative Akaike weight values.

To validate the pertinence of the two selected models (*capture site* + *year* & *capture site* + *year* + *NPP*) we used them to predict FP prevalence. These predictions were compared with the observed prevalence (Fig. S5ab). Indeed, despite large CI, the vast majority of predicted values for both models were very close to those observed. However, some points diverged, such as an observed value at 1.00 but predicted at 0.55 or those observed at 0 and predicted at 0.25. Then, predicted values of both models were compared graphically (Fig. S5c). The relationship between predicted FP prevalence of the model *capture site* + *year* and those from *capture site* + *year* + *NPP* seemed to be nearly linear with a slope close to 1. Focusing on the *capture site* + *year* + *NPP* model, the regression between NPP and FP prevalence (Fig. S5d) was positive and greater values of NPP were associated with higher FP prevalence. However, regression's CI happened to be important.

Ammonium and nitrites levels were significantly different between sites (Fig. 4; Table 2). Ammonium levels were significantly lower at Grande Anse compared to Petite Anse with

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respective means of 0.17 µmol.L^{-1} and 1.49 µmol.L^{-1} (Table 3). For nitrites, significant higher levels were observed at both Grande Anse and Anse du Bourg/Chaudière (0.07 µmol.L-1 and 0.11 µmol.L-1, respectively) than at Anse Noire/Dufour and Petite Anse (Table 3). Nitrates levels were quite similar and there has been no significant differences between capture sites, with values ranging from 0.50 µmol.L-1 for Grande Anse and Anse du Bourg/Chaudière to 0.29 µmol.L-1 for Petite Anse (Fig. 4). No significant differences between sites were measured for phosphate levels even if it was significantly predicted by the capture sites (Table 3). Chlorophyll *a* means were not significantly different and close to 0.30 µg.L^{-1} for Anse Noire/Dufour, Anse du Bourg/Chaudière, and Petite Anse. Grande Anse had a slightly higher level (0.43 µg.L^{-1}; Fig. 4). There was no significant difference between locations for bacterial parameters (Table 2). However, a strong value for *E. coli* (mean = 1548 MPN.100mL^{-1}) was observed at Grande Anse. Anse du Bourg/Chaudière had an important presence of *E. coli* too (mean = 1836 MPN.100mL^{-1}) with three values out of five exceeding 1000 MPN.100mL^{-1} and it was the only site where the presence of enterococci was measured (mean = 5 MPN.100mL^{-1}).

3.3. Turtles density distribution

The following number of turtles were observed in each bay in 2018 and 2019, respectively : 10 and 24 in Anse Noire, 8 and 3 in Anse Dufour, 93 and 100 in Grande Anse, 95 and 76 in Anse du Bourg, 14 and 7 in Anse Chaudière, 95 and 114 in Petite Anse. Densities of turtles per 1-ha cell were high in some restricted areas of the bays, with some variations between 2018 and 2019 (Fig. 5). Higher density cells were found in Anse du Bourg in 2018 (10-12 individuals.ha^{-1}) and Petite Anse in 2019 (13-15 individuals.ha^{-1}). More precisely, medium to high-density patches were located in the northern and central parts of Anse du Bourg, in the south of Petite Anse, and in the central and northern parts of Grande Anse, where

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individuals seemed more dispersed in 2019 than in 2018. Anse Noire, Anse Dufour and Anse Chaudière had lower turtle concentrations for both years compared to the other sites (0-6 individuals.ha^{-1}). Anse du Bourg (2018) and Petite Anse (2019) seemed to be the site with the highest heterogeneity considering the distribution of immature green turtles, with 1-ha cells reaching 10-15 individuals.ha^{-1} right next to cells with no individuals observed.

4. Discussion

This study provides the first long-term study on FP prevalence over time in immature green turtles in the Lesser Antilles. Looking at the spatio-temporal distribution of FP, we noted an increase of global FP prevalence between 2011 and 2019 for Anse du Bourg/Chaudière, Anse Noire/Dufour and Grande Anse. Our data suggested differences between FP evolution patterns through time of these geographically close sites. While NPP was slightly positively associated with FP prevalence, *mean SST* had no effect on FP prevalence. In parallel, we demonstrated clear differences in seawater quality between the different bays. The heterogeneous distribution of tumours on the body of individuals was highlighted. Indeed, the majority of tumours was observed on the eyes, fore fins and the neck. Finally, the analysis of immature green turtles density distributions underscored their tendency to cluster in relatively small areas in each bay. Indeed, individuals were strongly concentrated in a restricted zone in Anse du Bourg. This could be one of the reasons explaining the higher FP prevalence in Anse du Bourg/Chaudière than in other capture sites, because of the proximity between individuals which could induce an increase of FP horizontal transmission. However, this assumption needs to be verified by further studies. Our study demonstrated a significant increase of the global FP prevalence on three sites with high densities of turtle : Anse Noire/Dufour, Grande Anse, and Anse du Bourg/Chaudière. The temporal patterns of FP prevalence seemed to be different between these three sites, which are very close geographically (i.e. from 0.36 to 7.2 km, for

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distance's details in Siegwalt et al., 2020, Table S1). It is possible that FP prevalences were underestimated as we based our diagnosis on external lesions while it has been demonstrated that asymptomatic turtles can present high loads of ChHV5 (Chaves et al., 2017; Page-Karjian et al., 2015). In comparison, FP clinical signs were observed on half of the green turtles located in the Indian River lagoon , but none seemed to be affected at Sabellariid Worm Reef located one kilometer away in Florida (Herbst, 1994). A significant increase of FP prevalence over time was also highlighted for the same species in Texas with a prevalence under 5% before 2015 and rising to 35.2% in only three years (Shaver et al., 2019). At Pala'au, Molokai Island, the prevalence increase was quite similar to the one we observed at Anse du Bourg/Chaudière, rising from 1% to 61% in eight years (Jones et al., 2015). Thus, the literature suggests an important influence of local conditions on the disease's development and global trends toward an increase of FP prevalence.

Higher NPP values, but not temperature, were associated with higher FP prevalence. However, the positive link between NPP and FP prevalence was slight and associated with high uncertainty. Herbst (1994) proposed that tumour growth was more important in spring and summer because of higher water temperature. Murakawa et al. (2000) and Chaloupka et al. (2008), though, found no intra-annual variation of tumour size in stranded green turtles in Hawaii. Furthermore, Torezani et al. (2010) did not find an effect of temperature on FP prevalence with a range of temperatures from 27.5°C to 33.5°C. We had only a single SST sample point for the entire Martinique Island and mean SST values had a maximum variation of 2.7°C. This range was probably not sufficient to have a notable effect on FP-associated tumour growth. Thus, the influence of temperature on FP dynamics has not been evidenced at this time. However, Anse Noire/Dufour, Grande Anse, Anse du Bourg/Chaudière and Petite Anse are shallow bays where water temperature could greatly vary at a local scale compared

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to our SST sample point located further at sea. Finer scale temperature data are necessary to verify whether SST influences FP prevalence in Martinique.

Herbst (1994) suggested that human activities such as agriculture, industry and urban development should impact the development of tumours through different pathways and mechanisms. Santos et al. (2010) found that the high FP prevalence in a green turtle developmental area was associated with its poor water quality (EEI = 2, ecological evaluation index based on benthic macrophyte) in Espírito Santo Bay off Brazil. Concomitantly, on Oahu, Maui and Hawaii islands, high prevalence areas corresponded to those with a greater nitrogen footprint (Van Houtan et al., 2010). We therefore analysed seawater chemical, biological and bacterial parameters in the different bays where we captured turtles in order to characterise their environmental quality. The high level of NO_2 in Anse du Bourg/Chaudière might be associated with the high prevalence observed at this site. Despite the lack of significant differences between capture sites regarding bacterial parameters, we could also notice that Anse du Bourg/Chaudière was the only site where the presence of enterococci was observed, which reflect faecal contamination and poor water quality. This capture site also had several samples exceeding the European sanitary threshold of 1000 MPN.100mL^{-1} for *E. coli*.

The present results highlighted a significant difference in seawater quality between the capture sites where immature green turtles are most present. The presence of a damaged outfall releasing wastewater from a sewage treatment plant in Anse du Bourg (Impact Mer, 2016), as well as an important pressure of pleasure boats could be responsible for the releasing of faecal matter and nitrogen-enriched content (pers. obs.). Therefore, further environmental quality studies are necessary to seek a possible link with the FP outbreak in Martinique. Indeed, higher levels of arginine, an amino-acid known to enhance the emergence of tumours in some cases, have been found in marine algae in watersheds with a higher nitrogen footprint due to human land use (Hargove et al., 2016). Considering the above-mentioned environmental context and

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the high fidelity of immature green turtles to their feeding zone in the south-western bays of Martinique (Siegwalt et al., 2020), Anse du Bourg/Chaudière could provide an optimal environment for the contraction, persistence and transmission of this disease in green turtles.

By studying the density of individuals within each capture site, we have highlighted the presence of areas with high densities of individuals while others were left vacant. Differences in FP prevalence between locations could therefore be explained by the fact that some sites have higher turtle densities than others and that individuals are not uniformly dispersed within the bays. This could be especially the case in Anse du Bourg/Chaudière, where immature green turtles were highly concentrated in the North part of this small shallow bay while CMR data showed the highest FP prevalence for this location. Head rubbing or higher concentration of the ChHV5 in seawater in Anse du Bourg/Chaudière due to highly clustered individuals are possible explanations for the FP situation in this bay. On the other hand, at Anse Noire/Dufour where turtle's densities were the lowest, no FP outbreak has been recorded by the CMR monitoring.

The previous hypothesis of horizontal transmission is reinforced by our results on the relative distribution of tumours over the different body parts. Similar to Rossi et al. (2019), our results show that the neck, fore fins, and eyes, were the most frequently affected body parts. This result highlighted the possibility that turtle aggregations in restricted areas influence the prevalence of FP (Patrìcio et al., 2016) through positive interactions between individuals. Indeed, videos from cameras fixed on green turtles' shells have shown that green turtles sometimes rub their heads against each other and their upper body parts get consequently in contact during interactions occuring on feeding areas (pers. obs.). Moreover, the DNA of ChHV5 has also been detected in saliva and ocular secretion of green turtles affected by FP, thus representing another possibility of viral excretion and FP transmission, even if direct

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transmission from one turtle to another is not fully understood for the moment (Domiciano et al., 2017; Patrìcio et al., 2016; Rossi et al., 2019).

5. Conclusion and perspectives

Fibropapillomatosis is a potentially debilitating condition, depending on the severity of the lesions, that affects green turtle populations worldwide. We observed an evolution of the FP prevalence in Martinique with an increase of 50% in seven years in one particular bay: Anse du Bourg/Chaudière. We supposed that the high density of immature green turtles in restricted areas enhances positive interactions between individuals and can therefore promote the transmission of FP from one turtle to another. We hypothesised that the addition of factors promoting FP such as lower water quality (i.e. possible eutrophication due to high nutrient loads, high bacterial parameters) or the presence of a wastewater discharge is responsible for the disease's outbreak at Anse du Bourg/Chaudière. Further studies regarding seawater quality (e.g. eutrophication, pollutant presence), the presence and quantity of ChHV5 in the environment, and fine scale currentology, SST, and NPP in the different capture sites are necessary to find clear evidence on how these parameters influence FP dynamics.

The presence of this infectious disease on Martinique Island is of great concern since it is a key developmental area for green turtles. The knowledge of the population's health status is critical to establish conservation programs for this species. FP emergence is recent compared to the CMR program. Thus, the prosecution of the capture program for several years and with more captures in every location will allow us to describe whether Anse du Bourg/Chaudière is the only site affected by FP in Martinique. Moreover, an important number of recaptures will permit comparison of survival rates between healthy and sick turtles to determine whether FP has an impact on immature green turtle population dynamics in Martinique. However, the presence of ChHV5 DNA in green turtles without external tumours (Chaves et al., 2017; Page-

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Karjian et al., 2015) suggests that FP prevalence based on external tumours is underestimated compared to real prevalence. Monitoring the health status of immature green turtles in Martinique using serological methods (Work et al., 2020; Sposato et al., 2021) and quantitative PCR (Page-Karjian et al., 2015) will be a more efficient way to take into account asymptomatic individuals in FP prevalence measurement and would allow a better understanding of FP disease.

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Figures and tables

Figure 1: Martinique map showing capture sites of immature green turtles *Chelonia mydas* (black dots).

Figure 2: Annual fibropapillomatosis prevalence from 2010 to 2019 (except 2014) of immature green turtles on the Caribbean coast of Martinique: Anse Noire/Dufour (n = 56), Grande Anse (n = 302), and Anse du Bourg/Chaudière (n = 181). Vertical bars represent 95% confidence interval.

Figure 3: Anatomical distribution of fibropapillomatosis tumours on the dorsal (left) and ventral (right) sides based on CMR data from 2010 to 2019. The percentages refer to the relative number of individuals showing tumours on the different body areas (n = 128 affected body parts on 52 distinct green turtles).

Figure 4: Water quality parameters in 2021 for several capture sites: Anse Noire/Dufour (n = 4), Grande Anse (n = 3), Anse du Bourg/Chaudière (n = 5), and Petite Anse (n = 3). Dots represent individual measures, columns the means, and vertical bars 95% confidence intervals. Ammonium, nitrites, nitrates, total nitrogen and phosphates are expressed in $\mu mol.L^{-1}$, total chlorophyll *a* in $\mu g.L^{-1}$, *Escherichia coli* and enterococci in $MPN.100mL^{-1}$.

Figure 5: Green turtle density maps of Anse Noire, Anse Dufour, Grand Anse, Anse du Bourg, Anse Chaudière and Petite Anse in 2018 (left) and 2019 (right). Each hexagon represents one hectare and darker hexagonal colour shades indicate a higher turtle concentration (expressed as the number of individuals per hectare).

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Table 1: Synthetic results of generalised linear models applied to observed fibropapillomatosis prevalence. Models without results had too many parameters compared to the sample size. Best fit models are in bold. ΔAIC represents the difference between the AIC of a model and the one from the model with the highest relative Akaike weight.

Model	**AIC**	**ΔAIC**	**Relative Akaike weight**
Null	167.94	88.19	< 0.0001
Capture site	101.51	21.77	< 0.0001
Year	144.13	64.39	< 0.0001
Temperature	145.65	65.91	< 0.0001
NPP	167.37	87.62	< 0.0001
Capture site + year	**79.74**	**0.00**	**0.45**
Capture site + temperature	88.79	9.05	0.0049
Capture site + NPP	102.84	23.10	< 0.0001
Capture site + year + temperature	81.74	2.00	0.16
Capture site + year + NPP	**81.44**	**1.70**	**0.19**
Capture site + year + temperature + NPP	82.42	2.68	0.12

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Table 2: Results of likelihood ratio tests performed on generalised linear mixed models of seven different seawater parameters. Sampling replication (Replicate) was included as random effect.

Model	**df**	**LogLik**	**χ^2**	**p-value**
Ammonium ~ (1|Replicate)	3	-15.35		
Ammonium ~ Capture site + (1|Replicate)	6	-9.12	12.47	0.006
Nitrites ~ (1|Replicate)	3	22.91		
Nitrites ~ Capture site + (1|Replicate)	6	37.01	28.20	< 0.001
Nitrates ~ (1|Replicate)	3	7.12		
Nitrates ~ Capture site + (1|Replicate)	6	9.77	5.31	0.150
Phosphates ~ (1|Replicate)	3	26.95		
Phosphates ~ Capture site + (1|Replicate)	6	31.84	9.78	0.021
Chlorophyll *a* ~ (1|Replicate)	3	12.35		
Chlorophyll *a* ~ Capture site + (1|Replicate)	6	14.85	5.00	0.172
E. coli ~ (1|Replicate)	3	-130.18		
E. coli ~ Capture site + (1|Replicate)	6	-126.89	6.57	0.087
Enterococci ~ (1|Replicate)	3	-41.08		
Enterococci ~ Capture site + (1|Replicate)	6	-39.92	2.31	0.510

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Table 3: Post-hoc comparisons between capture sites according estimated marginal means for ammonium, nitrites and phosphates. AB/C is Anse du Bourg/Chaudière, AN/D Anse Noire/Dufour, GA Grande Anse, and PA Petite Anse. Degrees of freedom were calculated according Kenward-Roger method and p-values adjusted using Tukey's method for multiple comparison. Significant differences are in bold.

Comparison	Estimate	SE	df	t-ratio	p-value
Ammonium					
AB/C - AN/D	-0.733	0.356	9.64	-2.06	0.233
AB/C - GA	0.293	0.382	9.35	0.77	0.867
AB/C - PA	-1.033	0.382	9.35	-2.70	0.091
AN/D - GA	1.026	0.415	10.12	2.47	0.125
AN/D - PA	-0.301	0.415	10.12	-0.73	0.884
GA - PA	**-1.327**	**0.424**	**9.17**	**-3.13**	**0.049**
Nitrites					
AB/C - AN/D	**0.107**	**0.016**	**9.64**	**6.54**	**< 0.001**
AB/C - GA	0.038	0.018	9.35	2.16	0.205
AB/C - PA	**0.107**	**0.018**	**9.35**	**6.09**	**< 0.001**
AN/D - GA	**-0.069**	**0.019**	**10.12**	**-3.62**	**0.020**
AN/D - PA	0.000	0.019	10.12	0.00	1.000
GA - PA	**0.069**	**0.020**	**9.17**	**3.54**	**0.026**
Phosphates					
AB/C - AN/D	0.027	0.023	9.64	1.14	0.674
AB/C - GA	0.074	0.025	9.35	2.97	0.061
AB/C - PA	0.004	0.025	9.35	0.16	0.998
AN/D - GA	0.048	0.027	10.12	1.76	0.346
AN/D - PA	-0.023	0.027	10.12	-0.83	0.838
GA - PA	-0.070	0.028	9.17	-2.53	0.119

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Fibropapillomatosis prevalence and distribution in immature green turtles (*Chelonia mydas*) in Martinique Island (Lesser Antilles)

Figures

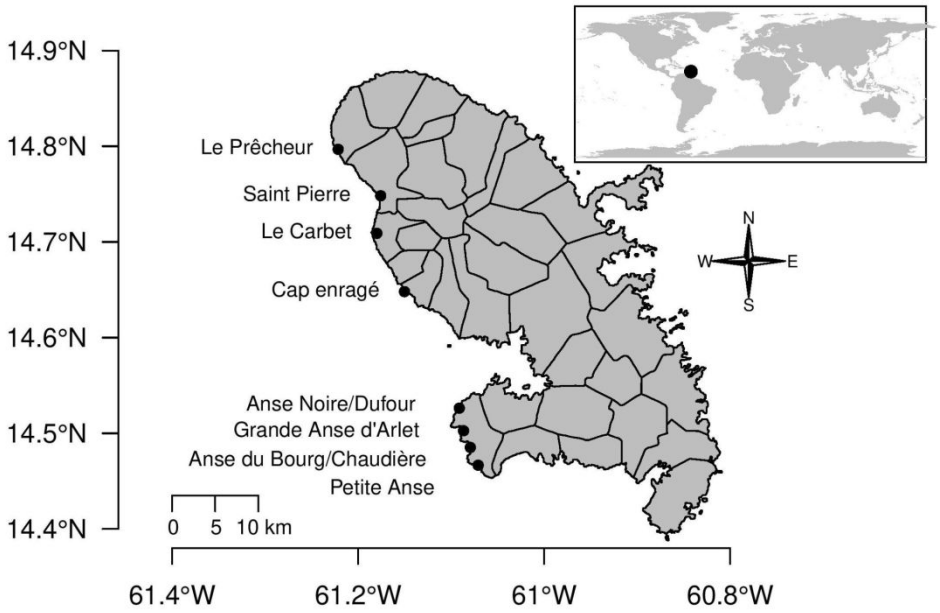

Figure 1: Martinique map showing capture sites of immature green turtles *Chelonia mydas* (black dots).

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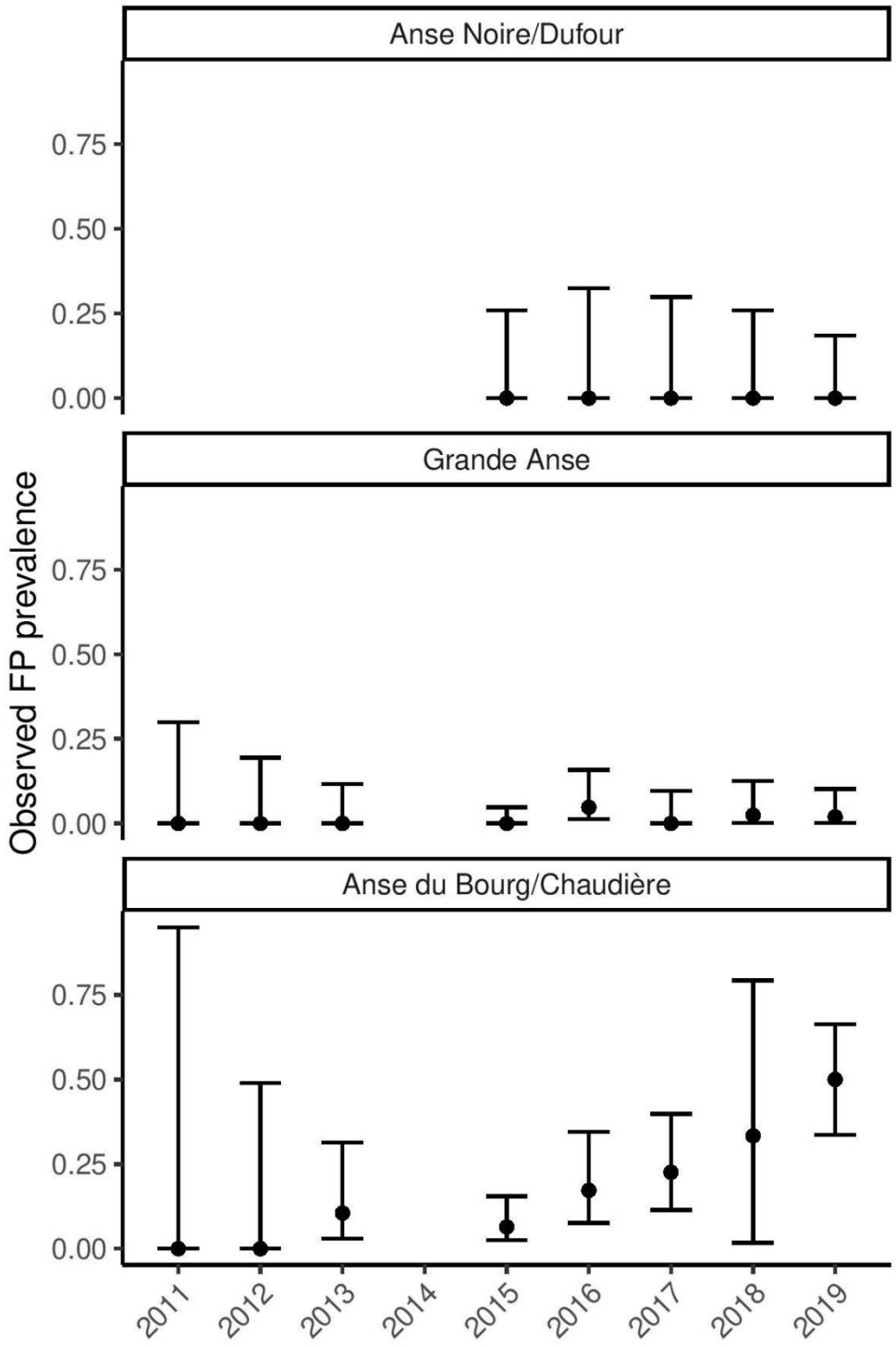

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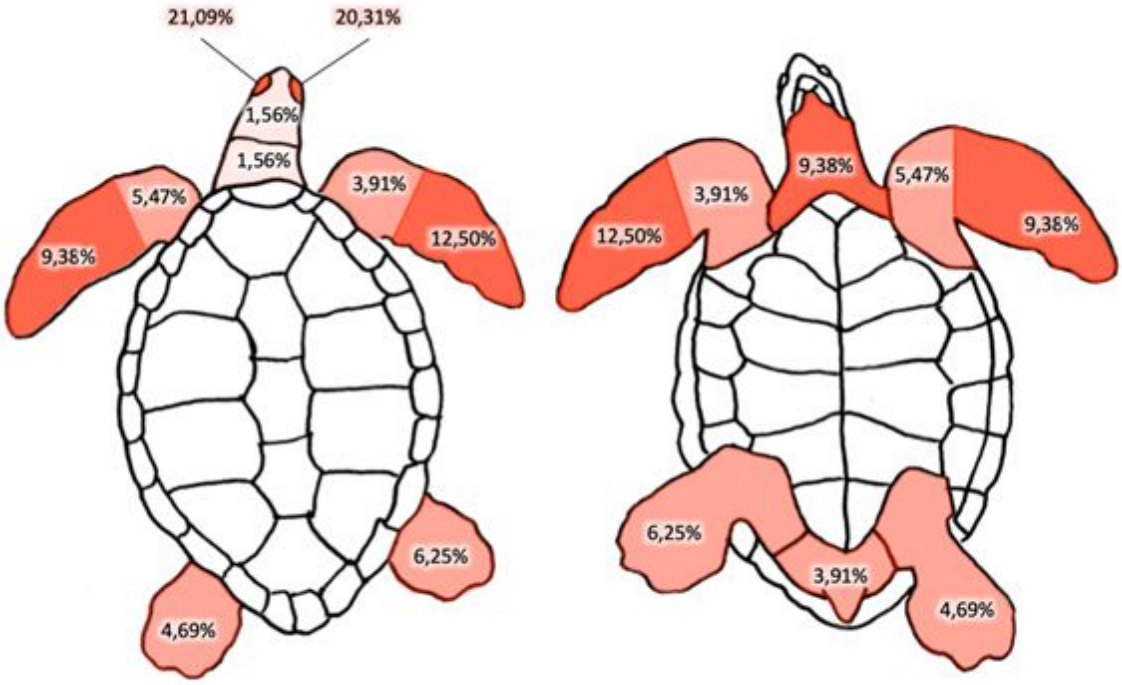

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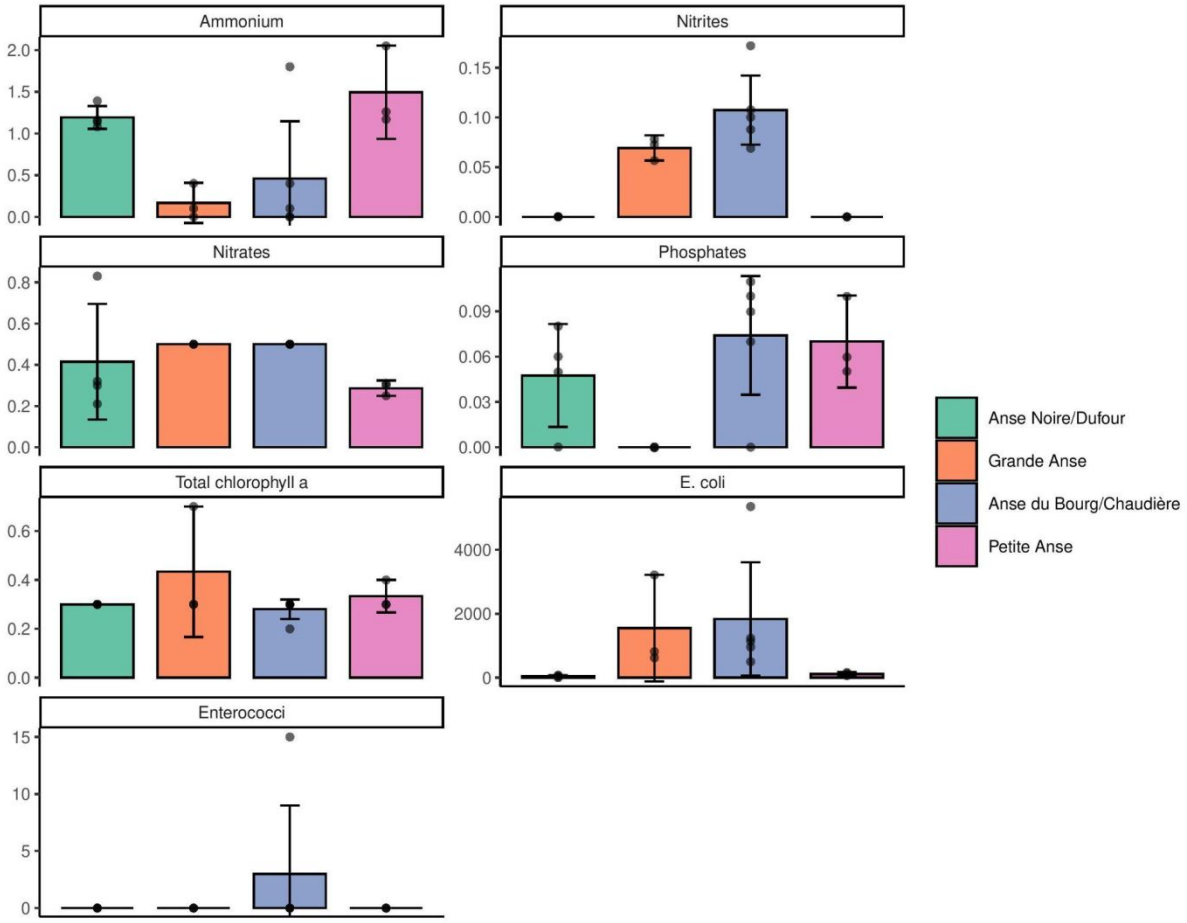

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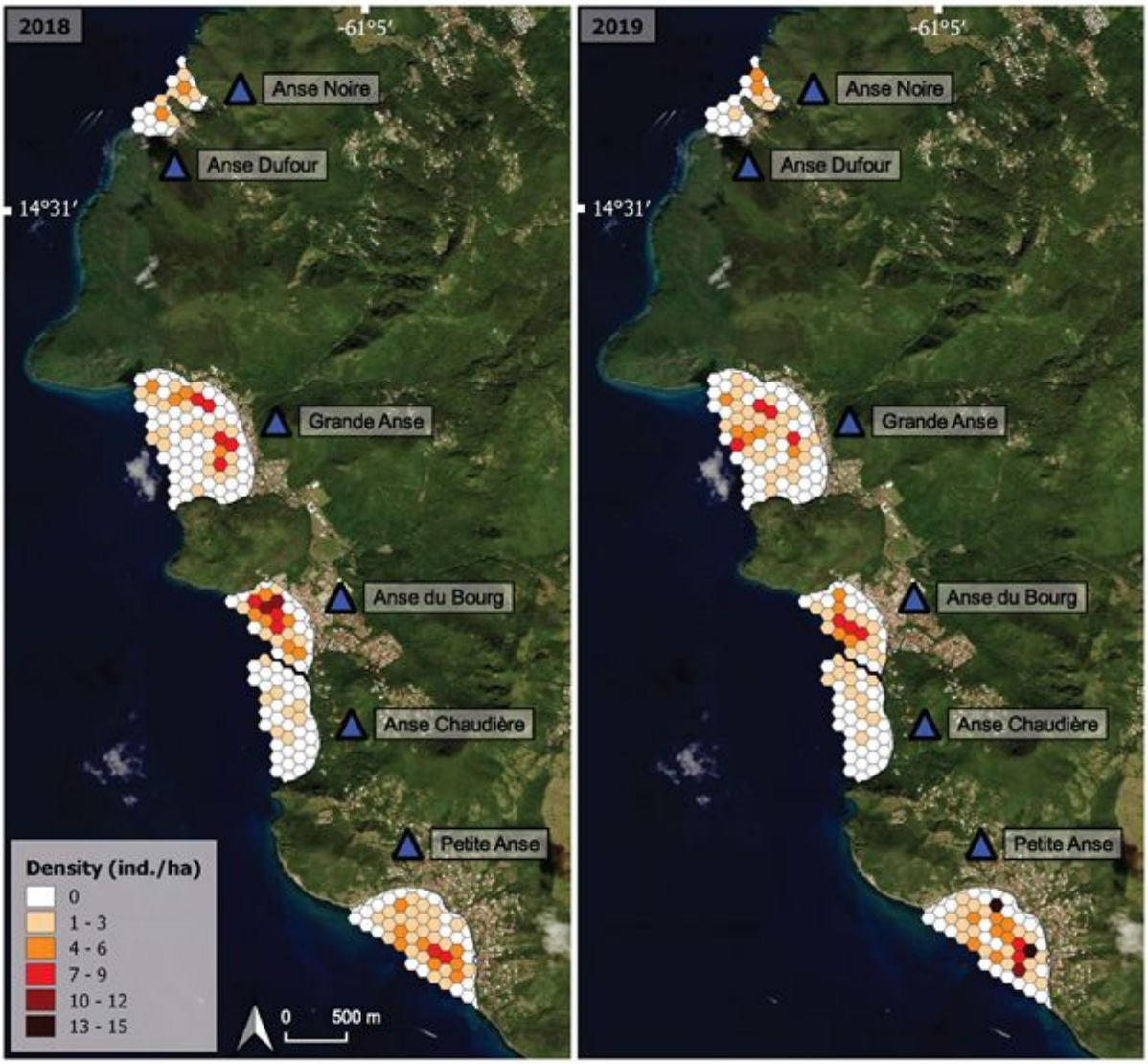

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Supplementary Material

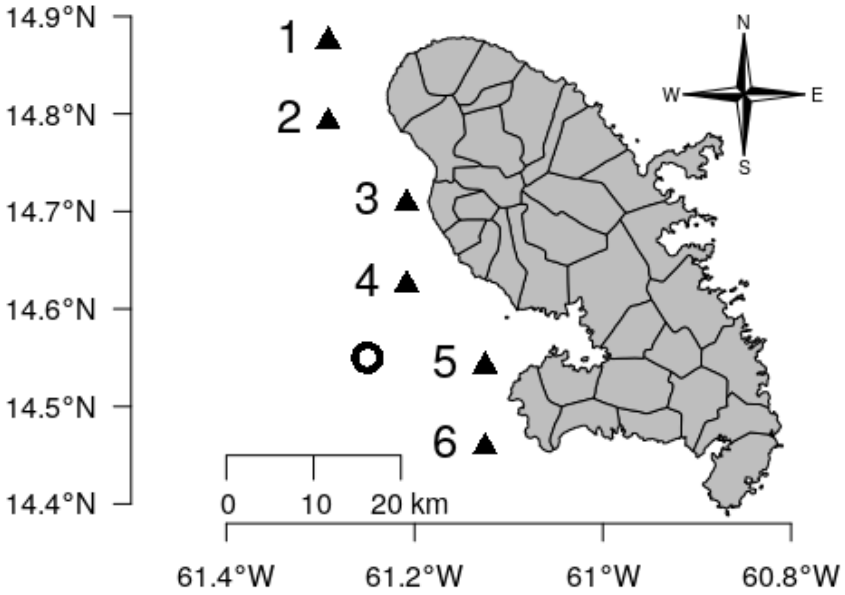

Figure 1: Sampling points of sea surface temperature (black circle) and net primary production (numbered triangles) on the west coast of Martinique Island.

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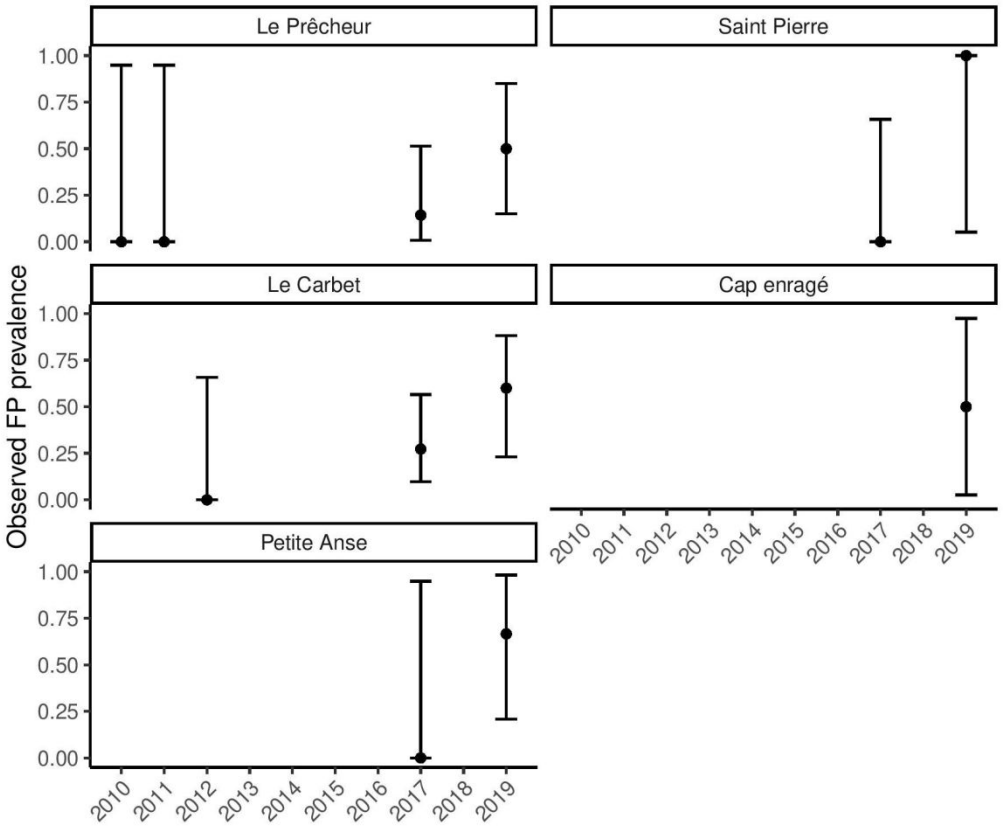

Figure 2: Annual fibropapillomatosis prevalence from 2010 to 2019 (except 2014) of immature green turtles on the Caribbean coast of Martinique where captures were weak: Prêcheur (n = 17), Saint-Pierre (n = 3), Carbet (n = 5), Cap enragé (n = 2), and Petite Anse (n = 4). Vertical bars represent 95% confidence interval.

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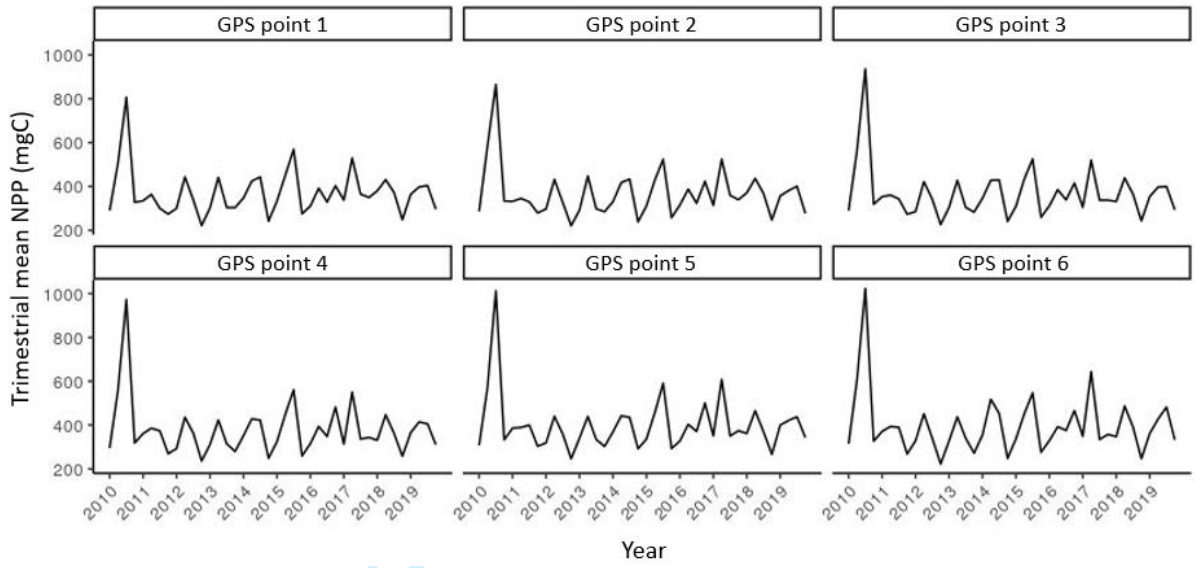

Figure 3: Trimestrial mean net primary production (NPP) on the West coast of Martinique Island for six sampling points from 2010 to 2019 (2160 x 4320 grid).

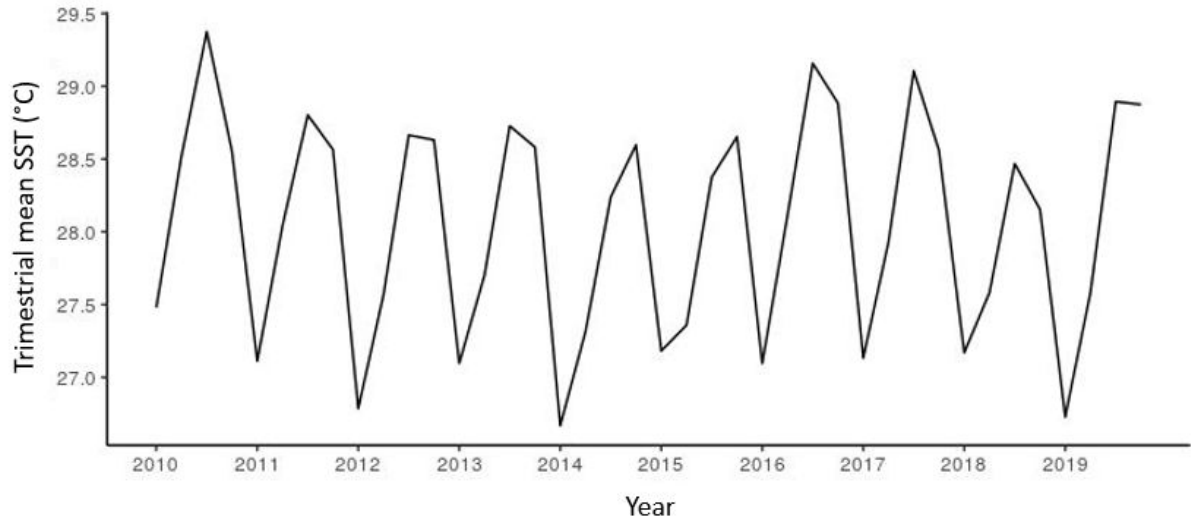

Figure 4: Trimestrial mean sea surface temperature (SST) on the West coast of Martinique Island from 2010 to 2019 on the 14.55° latitude and -61.25° longitude point (0.25° x 0.25° grid).

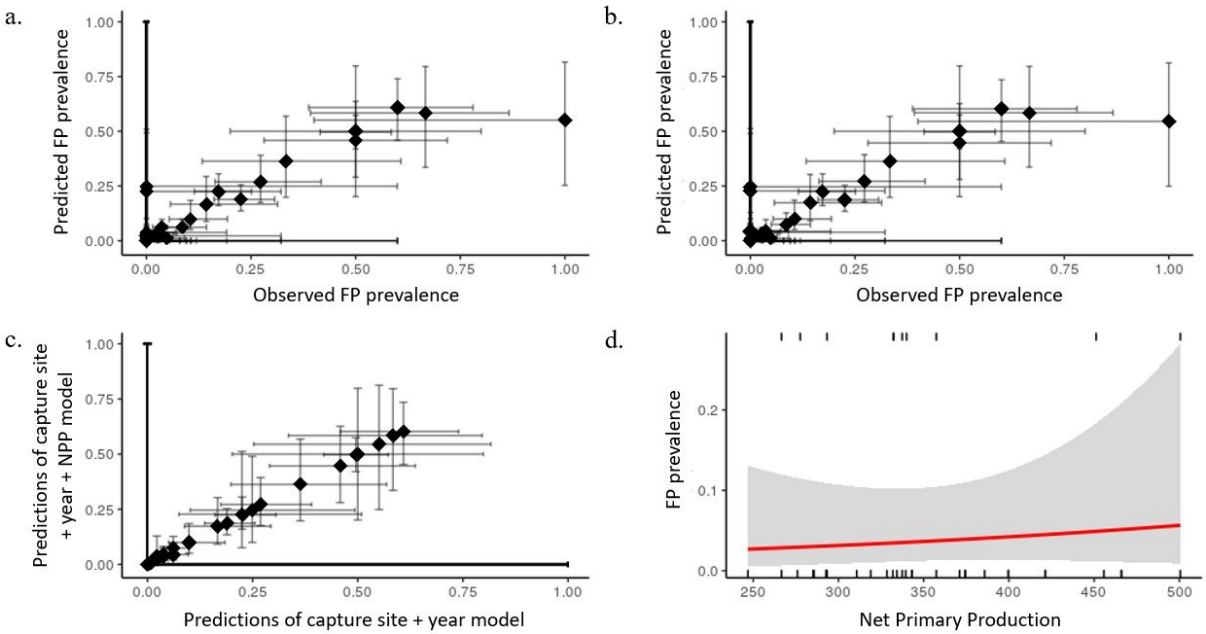

Figure 5: Predicted versus observed fibropapillomatosis prevalence using the *capture site + year* model (a) and the *capture site + year + NPP* model (b), comparison between predicted values of these two models (c) and regression between mean net primary production and the observed prevalence of the *capture site + year + NPP* model (d). Vertical and horizontal bars represent 66% confidence intervals. The regression's shaded area represents the 95% confidence interval.

Peer Review

Fibropapillomatosis prevalence and distribution in immature green turtles (*Chelonia mydas*) in Martinique Island (Lesser Antilles)

Supplementary Material

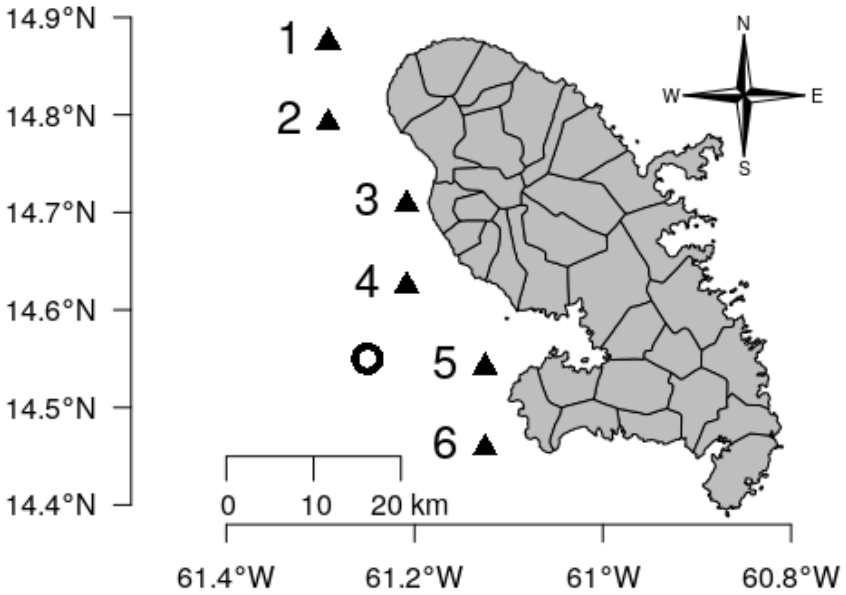

Figure 1: Sampling points of sea surface temperature (black circle) and net primary production (numbered triangles) on the west coast of Martinique Island.

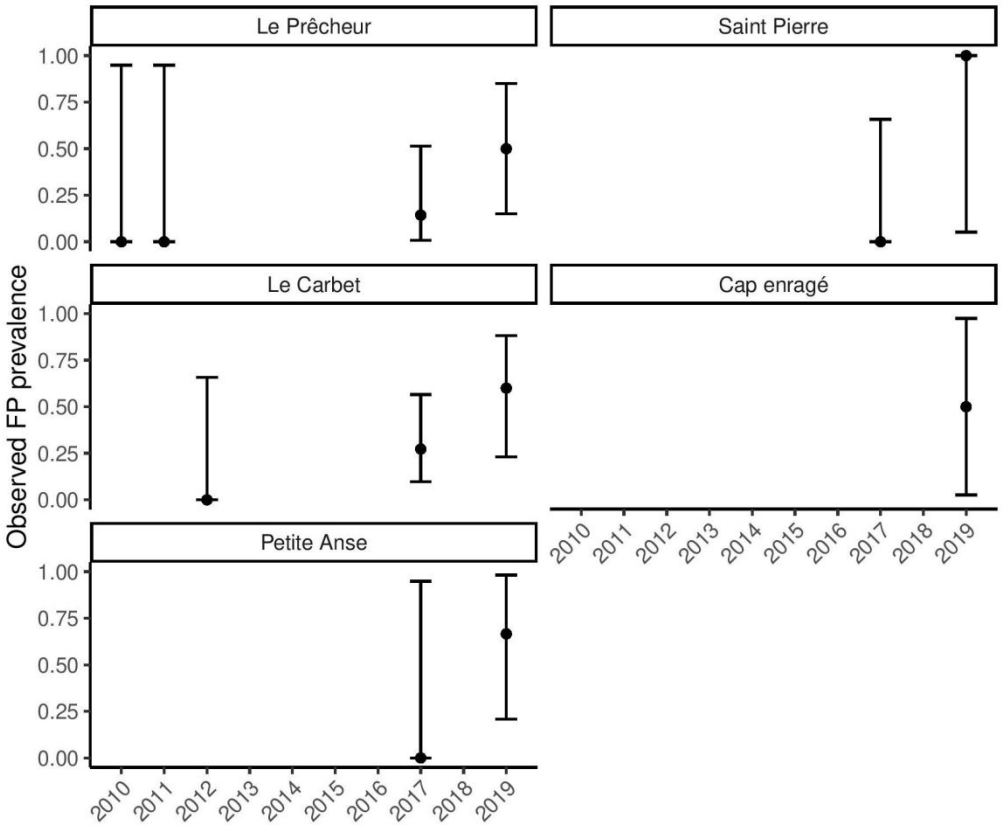

Figure 2: Annual fibropapillomatosis prevalence from 2010 to 2019 (except 2014) of immature green turtles on the Caribbean coast of Martinique where captures were weak: Prêcheur (n = 17), Saint-Pierre (n = 3), Carbet (n = 5), Cap enragé (n = 2), and Petite Anse (n = 4). Vertical bars represent 95% confidence interval.

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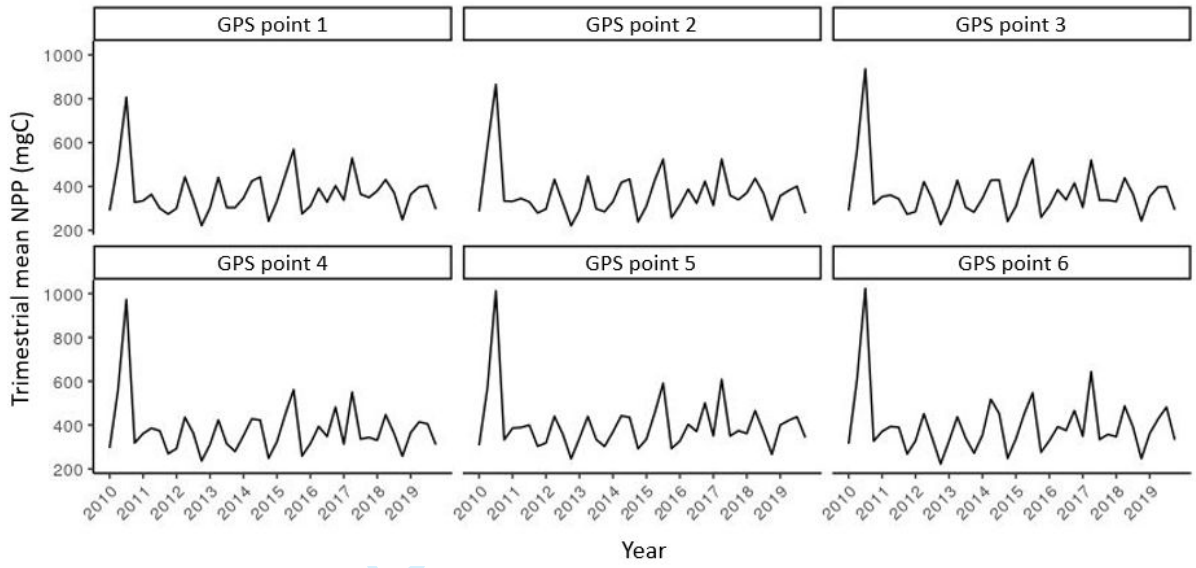

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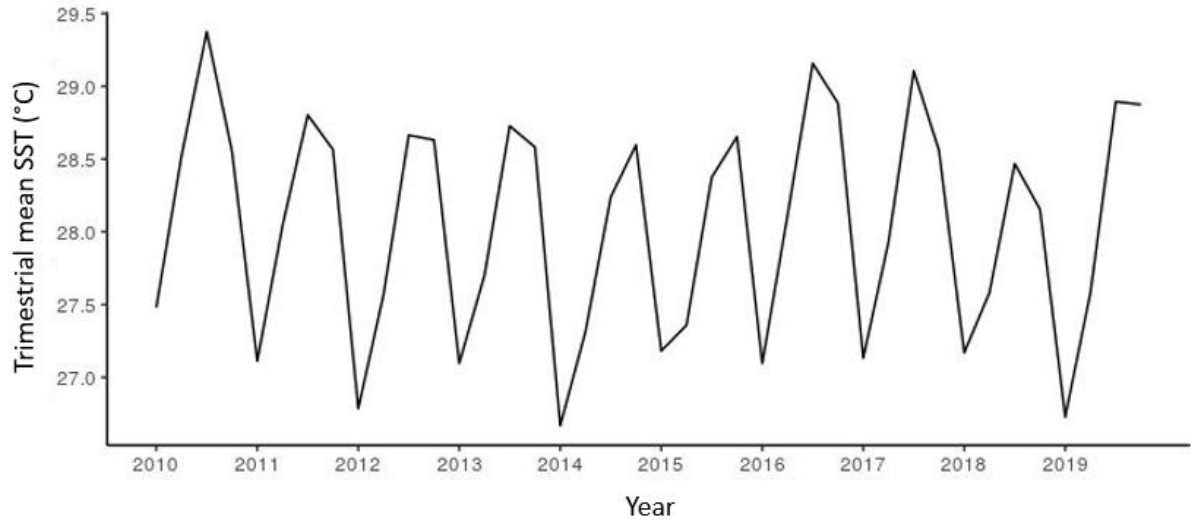

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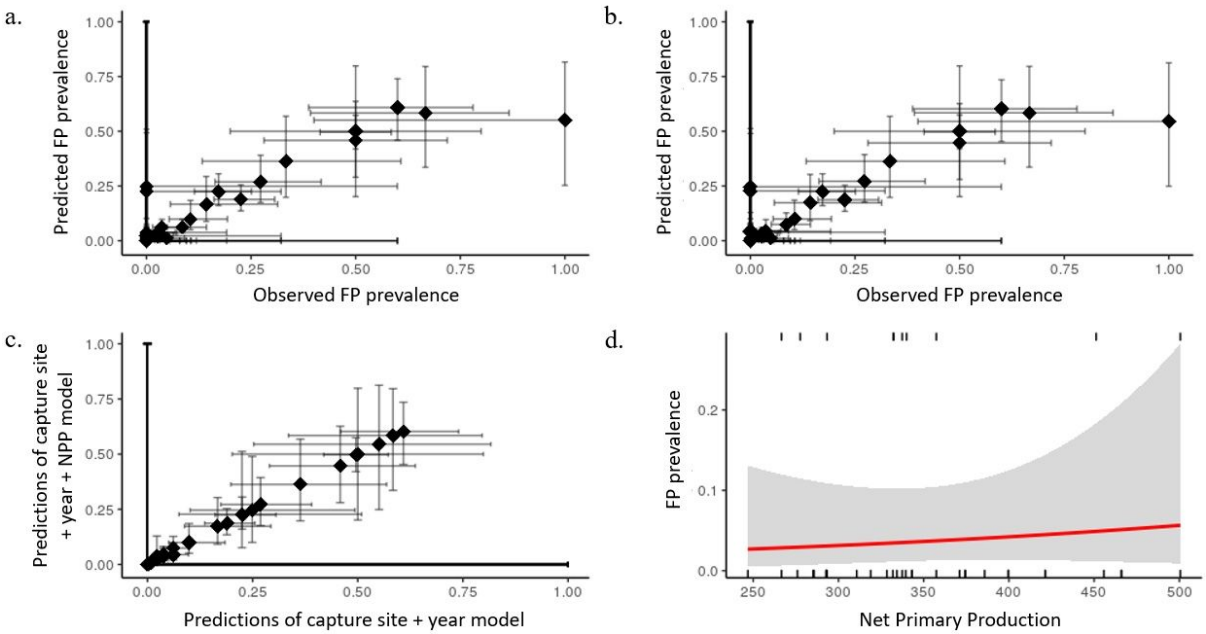

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