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► To cite this version:

Thibaut Roost, Jo-Ann Schies, Marc Girondot, Jean-Patrice Robin, Pierre Lelong, et al.. Fibropapillomatosis Prevalence and Distribution in Immature Green Turtles (*Chelonia mydas*) in Martinique Island (Lesser Antilles). *EcoHealth*, 2022, 19 (12), pp.190-202. 10.1007/s10393-022-01601-y . hal-03692770

HAL Id: hal-03692770

<https://hal.science/hal-03692770v1>

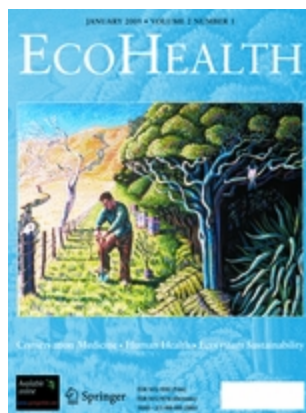
Submitted on 17 Jun 2022

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Fibropapillomatosis prevalence and distribution in immature green turtles in Martinique

Journal:	<i>EcoHealth</i>
Manuscript ID	ECH-21-0147.R1
Manuscript Type:	Original Contribution
Keywords:	marine turtles, infectious disease, epizootiology, environmental quality

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Manuscripts

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4 1 **Fibropapillomatosis prevalence and distribution in immature green turtles**
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6 2 **(*Chelonia mydas*) in Martinique Island (Lesser Antilles)**
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9 4 **Abstract**

10 5 Fibropapillomatosis (FP) threatens the survival of green turtle (*Chelonia mydas*) populations
11 6 at a global scale and human activities are regularly pointed as causes of high FP prevalence.
12 7 However, the association of ecological factors with the disease's severity in complex coastal
13 8 systems has not been well established and requires further studies. Based on a set of 405
14 9 individuals caught over ten years, this preliminary study provides the first insight of FP in
15 10 Martinique Island, which is a critical development area for immature green turtles. Our main
16 11 results are: i) 12.8% of the individuals were affected by FP, ii) FP has different prevalence and
17 12 temporal evolution between very close sites, iii) green turtles are more frequently affected on
18 13 the upper body part such as eyes (41.4%), fore flippers (21.9%) and the neck (9.4%), and iv)
19 14 high densities of individuals are observed on restricted areas. We hypothesise that turtle's
20 15 aggregation enhances horizontal transmission of the disease. FP could represent a risk for
21 16 immature green turtles' survival in the French West Indies, a critical development area, which
22 17 replenishes the entire Atlantic population. Continuing scientific monitoring is required to
23 18 identify which factors are implicated in this panzootic disease and ensure the conservation of
24 19 the green turtle at an international scale.
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48 21 **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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3 49 2020; Patricio et al., 2016; Shaver et al., 2019). Furthermore, it has been proved that horizontal
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5 50 transmission can be promoted by parasite marine leech, which act as mechanical vectors of
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8 51 ChHV5 (Rittenburg et al., 2021).
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10 52 Environmental conditions have been proposed to play an important role in the
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12 53 emergence of FP (Herbst & Klein, 1995; Herbst et al., 2004) because of the multiple reports of
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14 54 high variations of prevalence between very close geographic regions (Herbst, 1994; Jones et
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16 55 al., 2015). Herbst & Klein (1995) suggested that higher sea temperature could induce faster
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18 56 tumour growth that in turn would result in more severe FP in green turtles. FP is also frequently
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20 57 associated with poor water quality (e.g. pollution, eutrophication) in coastal areas near human
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22 58 activities and/or with low hydrodynamics (Hargrove et al., 2016; Torezani et al., 2010). Metal
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24 59 contaminants (da Silva et al., 2016), persistent organic pollutants (Foley et al., 2005), and
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26 60 eutrophication coupled with a change in diet quality (Van Houtan et al., 2014) have also been
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28 61 suspected to enhance green turtles' susceptibility to FP.
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33 62 Located in the Lesser Antilles of the West Indies in the eastern Caribbean, Martinique
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35 63 Island hosts an important population of immature green turtles in which clinical signs of FP
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37 64 infection (i.e. tumours) have been observed (Bonola et al., 2019). Moreover, Martinique has
38
39 65 many sheltered bays that support the settlement of multi-species seagrass meadows on large
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41 66 shallow areas, particularly favourable to green turtles (Siegwalt et al., 2020). Indeed, the island
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43 67 is an important developmental area for these immatures, who show high fidelity to their
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45 68 foraging grounds for several years (Siegwalt et al., 2020) before performing their
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47 69 developmental migration in the Caribbean and the entire Atlantic (Chambault et al., 2018). In
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49 70 areas where green turtles tend to congregate for feeding, we hypothesize that positive
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51 71 interactions between individuals would facilitate horizontal transmission of FP. Moreover, an
52
53 72 important urban development and poor sanitation of wastewater of this island lead to discharge
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73 releases of polluted water in the marine environment (Hily et al., 2010) that could drive
74 enhanced environmental pollution and/or trigger an eutrophication phenomenon.

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

85

86 **2. Materials and methods**

87 **2.1. Study area**

88 This study was conducted along 60 km of the western coast of Martinique
89 ($14^{\circ}30'9.64''\text{N}$, $61^{\circ}5'11.85''\text{W}$, France). Green turtles were mainly present in six bays of the
90 Caribbean coast of the island that are, from north to south, Anse Noire, Anse Dufour, Grande
91 Anse, Anse du Bourg, Anse Chaudière and Petite Anse (Fig. 1). These sites were identified as
92 critical for immature green turtle foraging (Siegwalt et al., 2020).

93

94 **2.2. Capture-mark-recapture program (CMR)**

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

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3 98 methodology and procedures are described in Nivière et al. (2018) and in Bonola et al. (2019).
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5 99 Date and geographical coordinates were recorded for each turtle capture. The presence of a PIT
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8 100 was checked using a universal reader (GR251, TROVAN). In case of absence of a transponder,
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10 101 a PIT (ID-100, TROVAN) was injected into the right triceps. The search for FP was conducted
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12 102 by carefully examining each turtle for the presence of external tumours looking like single or
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14 103 multiple raised masses. Tumours were located on the turtles' bodies according to the following
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16
17 104 body parts: eyes, head, nape, neck, shoulders, fore flippers, carapace, plastron, back flippers,
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19 105 and tail base.

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21 106 The study met the French legal and ethical requirements. The protocol was approved
22
23 107 by the Conseil National de la Protection de la Nature and the French Ministry for Ecology
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25 108 (permit numbers: 2013154-0037 and 201710-0005) and followed the recommendations of the
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27 109 Police Prefecture of Martinique.
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32 33 111 **2.3. Environmental conditions**

34
35 112 Sea surface temperature (SST) data were obtained from the NOAA/OAR/ESRL PSL
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37 113 public database (Colorado, USA, <https://psl.noaa.gov/> (accessed 5-10-2020)). Daily means of
38
39 114 the 'Optimal Interpolation Sea Surface Temperature V2' were extracted for the closest
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41 115 coordinates to Martinique Island, which were 14.55° latitude and -61.25° longitude (Fig. S1).
42
43 116 The NOAA 1/4° daily 'Optimum Interpolation Sea Surface Temperature' is an analysis
44
45 117 constructed by combining observations from different platforms (satellites, ships, buoys, and
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47 118 Argo floats) on a regular global grid (0.25° latitude x 0.25° longitude grid). These values were
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49 119 averaged per trimester from 2010 to 2019 and applied to every site since we had only a single
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51 120 sample point close to Martinique Island (Fig. S1).
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56 121 Net primary production (NPP) data were collected from the Ocean Productivity public
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58 122 database (<https://sites.science.oregonstate.edu/ocean.productivity/index.php>). Monthly means
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3 123 of NPP, based on the Vertically Generalized Production Model (Behrenfeld & Falkowski,
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5 124 1997) from MODIS satellite measures, were extracted for six points alongside Martinique's
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8 125 West coast (Fig. S1). Each turtle's capture site was assigned the NPP means of the nearest
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10 126 sampling point.

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12 127 For seawater quality, two (for Anse Noire, Anse Dufour, Anse Chaudière) to three (for
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14 128 Grande Anse, Anse du Bourg, Petite Anse) sampling points per foraging site were chosen prior
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16
17 129 to field work (Siegwalt et al., 2020). On each sampling location, seawater was sampled multiple
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19 130 times with a plunger sampler ($V = 1$ L) at 5 m depth in order to fill a 2 L vial for total
20
21 131 chlorophyll *a* analysis, a 250 mL vial for bacterial analysis (*E. coli* and enterococci) and four
22
23 132 150 mL vials for chemical analysis (ammonium, nitrites, nitrates and phosphates). Chlorophyll
24
25 133 *a* vials were protected from any light by wrapping them in aluminium paper. Vials were stored
26
27 134 in coolers away from sunlight. All samples were collected on the same day in 2021 and sent to
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29
30 135 the Laboratoire Territorial d'Analyses de Martinique. Chemical and chlorophyll *a* analyses
31
32 136 were done according to Aminot & Chaussepied (1983) while *Escherichia coli* (*E. coli*) and
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34 137 enterococci were measured following European standards NF EN ISO 9308-3 and NF EN ISO
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36 138 7899-1, respectively. We looked at chemical and biological parameters in order to seek for
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38 139 potential eutrophication and at European standards bacterial parameters to describe seawater
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41 140 sanitary safety.

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46 47 142 **2.4. Density surveys and mapping**

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49 143 Population density surveys of green turtles were carried out in 2018 and 2019 on several
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51 144 bays: Anse Noire, Anse Dufour, Grande Anse, Anse du Bourg, Anse Chaudière and Petite
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53 145 Anse. In order to count a maximum number of turtles under the water surface, one diver
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55 146 (observer) was connected by a 10 m rope to a boat. Transects specific to each bay, parallel to
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57 147 the beach and arranged from coast to sea, were previously created and integrated into a GPS
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3 148 (GarminTrex) in order to follow a predefined course. The chosen distance between transects
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5 149 (10 to 30 m depending on the visibility) was intended to cover the greatest area and sample the
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8 150 largest number of individuals and to minimize the risk of missing an individual. Standardised
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10 151 hand signals have been established so that the observer could communicate with the operators
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12 152 aboard the boat to report the number of observed turtles. Each observation has been associated
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14
15 153 with its GPS coordinates.

16
17 154 Density maps were created to visualise the spatial distribution and density of green
18
19 155 turtles using the software QGIS 2.18 (2016) and the legal CRS RGAF09 (EPSG:5490) in use
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21 156 at that time for French West Indies. Turtle sighting points and predefined transects were placed
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24 157 on Mapbox Satellite v9 satellite image in order to set up grids of hexagonal 1 ha-cells using the
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26 158 QMarxan extension, whose extents correspond to the surveyed areas of each bay. Then, turtle
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28 159 densities were determined in each cell.

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32 33 161 **2.5. Data analysis**

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35 162 FP data were obtained through CMR surveys and visual detection of FP-indicating
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38 163 tumours. Given the movements of individuals between Anse Noire and Anse Dufour (< 500 m
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40 164 apart; Siegwalt et al., 2020), the two sites were considered as a single entity called Anse
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42 165 Noire/Dufour. With the same considerations, Anse du Bourg and Anse Chaudière became Anse
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44 166 du Bourg/Chaudière.

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47 167 Relationships between FP prevalence and environmental conditions were assessed with
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49 168 generalised linear models (GLM). Thus, in order to describe geographic disparities between
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52 169 close bays, interannual evolution, look for SST's influence on the disease's severity, and NPP's
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54 170 influence according its relationship with immature green turtle's body mass (Bonola et al.,
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56 171 2019), fixed factors included *capture site*, *year*, *mean SST*, and *mean NPP*, respectively. *Year*
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58 172 was considered as a discrete factor instead of a numeric variable. The distribution of the
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3 173 explained variable was binomial (presence or absence of FP) and a logit link was therefore
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5 174 used. A model selection was performed with the use of the Akaike information criterion (AIC;
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7 Akaike, 1973) and relative Akaike weight. Models with a difference lower than 2 in their
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9 175
10 176 respective AIC were considered similar (Burnham & Anderson, 2002).

11
12 177 To highlight which individual body parts were the most affected by FP, we calculated
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14 178 infection proportions per body part by dividing the number of turtles with the presence of
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16 179 tumour(s) on the specific body part by the total number of turtles affected by FP. Individuals
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18 180 captured several times were counted as one turtle having FP.

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20
21 181 Generalised Linear Mixed Models (GLMMs) were used to explore potential differences
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23 182 in the seven seawater variables measured (i.e. ammonium, nitrites, nitrates, phosphates, total
24
25 183 chlorophyll *a*, *E. coli*, and enterococci) between field sites. As for FP analysis, Anse
26
27 184 Noire/Dufour and Anse du Bourg/Chaudière were considered unique sites. Sampling
28
29 185 replication was included as a random effect for every GLMM to account for non-independence
30
31 186 of data. Models were fitted using the *lme4* package (Bates et al., 2012). Likelihood Ratio Tests
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33 187 (LRTs) were performed using the *lmtest* package (Hothorn et al., 2015) to select the best-fit
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35 188 model between the nul GLMM and the one with capture site fixed effect. Post-hoc estimations
36
37 189 and comparisons were performed when the *capture site* effect was kept using Estimated
38
39 190 Marginal Means (EMMs) with the *emmeans* package (Lenth et al., 2021). Degrees of freedom
40
41 191 were calculated using the Kenward-Roger method and p-values were adjusted for multiple
42
43 192 comparisons with Tukey adjustment. Significance thresholds were fixed at 0.05. All data
44
45 193 analysis was performed using R version 4.0.2 (R Core Team, 2020).

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198 3. Results

199 3.1. *Fibropapillomatosis prevalence and body distribution*

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

208 A significant increase of immature green turtles' FP prevalence from 0.000 (IC95%
209 [0.000; 0.278]) in 2011 to 0.168 (IC95% [0.108; 0.253]) in 2019 was observed when
210 considering the three major sites together (Odd-ratio : 1.72, p-value < 0.001; Fig. 2). Post-hoc
211 Tukey between capture sites highlighted significant differences of prevalence between Grande
212 Anse and Anse du Bourg/Chaudière (p < 0.001). Indeed, FP prevalence remained close to zero
213 from 2011 to 2019 in Grande Anse and also from 2015 to 2019 in Anse Noire/Dufour. In fact,
214 the major increase in FP prevalence on the Martinique coast seemed to be restricted to Anse du
215 Bourg/Chaudière, where no individual with FP being observed in 2011 and 2012 and with FP
216 prevalence increasing from 2013 (0.11) to 2019 (0.50).

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

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

225 **3.2. *Fibropapillomatosis and environmental cofactors***

226 AICs, Δ AICs and relative Akaike weights for the 11 tested GLMs are presented in Table

227 1. The null model is the model without effect and FP prevalence is therefore supposed constant.

228 The lowest AIC (79.74) has been observed for the model with *capture site* and *year* effects.

229 This model had a relative Akaike weight of 0.45. When adding *NPP* to the two previous fixed

230 effects (Fig. S3), the model had a Δ AIC < 2 (1.70) and a relative Akaike weight of 0.19.

231 *Temperature* effect (Fig. S4) associated with *capture site* and *year* resulted with a Δ AIC of

232 exactly 2 compared to the model with the lowest AIC. All other models had a Δ AIC > 2 and

233 lower relative Akaike weight values.

234 To validate the pertinence of the two selected models (*capture site + year* & *capture*

235 *site + year + NPP*) we used them to predict FP prevalence. These predictions were compared

236 with the observed prevalence (Fig. S5ab). Indeed, despite large CI, the vast majority of

237 predicted values for both models were very close to those observed. However, some points

238 diverged, such as an observed value at 1.00 but predicted at 0.55 or those observed at 0 and

239 predicted at 0.25. Then, predicted values of both models were compared graphically (Fig. S5c).

240 The relationship between predicted FP prevalence of the model *capture site + year* and those

241 from *capture site + year + NPP* seemed to be nearly linear with a slope close to 1. Focusing on

242 the *capture site + year + NPP* model, the regression between NPP and FP prevalence (Fig.

243 S5d) was positive and greater values of NPP were associated with higher FP prevalence.

244 However, regression's CI happened to be important.

245 Ammonium and nitrites levels were significantly different between sites (Fig. 4; Table

246 2). Ammonium levels were significantly lower at Grande Anse compared to Petite Anse with

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3 247 respective means of $0.17 \mu\text{mol.L}^{-1}$ and $1.49 \mu\text{mol.L}^{-1}$ (Table 3). For nitrites, significant higher
4
5 248 levels were observed at both Grande Anse and Anse du Bourg/Chaudière ($0.07 \mu\text{mol.L}^{-1}$ and
6
7 249 $0.11 \mu\text{mol.L}^{-1}$, respectively) than at Anse Noire/Dufour and Petite Anse (Table 3). Nitrates
8
9 250 levels were quite similar and there has been no significant differences between capture sites,
10
11 251 with values ranging from $0.50 \mu\text{mol.L}^{-1}$ for Grande Anse and Anse du Bourg/Chaudière to
12
13 252 $0.29 \mu\text{mol.L}^{-1}$ for Petite Anse (Fig. 4). No significant differences between sites were measured
14
15 253 for phosphate levels even if it was significantly predicted by the capture sites (Table 3).
16
17 254 Chlorophyll *a* means were not significantly different and close to $0.30 \mu\text{g.L}^{-1}$ for Anse
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19 255 Noire/Dufour, Anse du Bourg/Chaudière, and Petite Anse. Grande Anse had a slightly higher
20
21 256 level ($0.43 \mu\text{g.L}^{-1}$; Fig. 4). There was no significant difference between locations for bacterial
22
23 257 parameters (Table 2). However, a strong value for *E. coli* (mean = $1548 \text{MPN.100mL}^{-1}$) was
24
25 258 observed at Grande Anse. Anse du Bourg/Chaudière had an important presence of *E. coli* too
26
27 259 (mean = $1836 \text{MPN.100mL}^{-1}$) with three values out of five exceeding $1000 \text{MPN.100mL}^{-1}$ and
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29 260 it was the only site where the presence of enterococci was measured (mean = 5MPN.100mL^{-1}).
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263 3.3. *Turtles density distribution*

264 The following number of turtles were observed in each bay in 2018 and 2019,
265 respectively : 10 and 24 in Anse Noire, 8 and 3 in Anse Dufour, 93 and 100 in Grande Anse,
266 95 and 76 in Anse du Bourg, 14 and 7 in Anse Chaudière, 95 and 114 in Petite Anse. Densities
267 of turtles per 1-ha cell were high in some restricted areas of the bays, with some variations
268 between 2018 and 2019 (Fig. 5). Higher density cells were found in Anse du Bourg in 2018
269 ($10\text{-}12 \text{individuals.ha}^{-1}$) and Petite Anse in 2019 ($13\text{-}15 \text{individuals.ha}^{-1}$). More precisely,
270 medium to high-density patches were located in the northern and central parts of Anse du
271 Bourg, in the south of Petite Anse, and in the central and northern parts of Grande Anse, where

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3 272 individuals seemed more dispersed in 2019 than in 2018. Anse Noire, Anse Dufour and Anse
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5 273 Chaudière had lower turtle concentrations for both years compared to the other sites (0-6
6
7 274 individuals.ha⁻¹). Anse du Bourg (2018) and Petite Anse (2019) seemed to be the site with the
8
9 275 highest heterogeneity considering the distribution of immature green turtles, with 1-ha cells
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11 276 reaching 10-15 individuals.ha⁻¹ right next to cells with no individuals observed.
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17 278 **4. Discussion**

19 279 This study provides the first long-term study on FP prevalence over time in immature
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21 280 green turtles in the Lesser Antilles. Looking at the spatio-temporal distribution of FP, we noted
22
23 281 an increase of global FP prevalence between 2011 and 2019 for Anse du Bourg/Chaudière,
24
25 282 Anse Noire/Dufour and Grande Anse. Our data suggested differences between FP evolution
26
27 283 patterns through time of these geographically close sites. While NPP was slightly positively
28
29 284 associated with FP prevalence, *mean SST* had no effect on FP prevalence. In parallel, we
30
31 285 demonstrated clear differences in seawater quality between the different bays. The
32
33 286 heterogeneous distribution of tumours on the body of individuals was highlighted. Indeed, the
34
35 287 majority of tumours was observed on the eyes, fore fins and the neck. Finally, the analysis of
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37 288 immature green turtles density distributions underscored their tendency to cluster in relatively
38
39 289 small areas in each bay. Indeed, individuals were strongly concentrated in a restricted zone in
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41 290 Anse du Bourg. This could be one of the reasons explaining the higher FP prevalence in Anse
42
43 291 du Bourg/Chaudière than in other capture sites, because of the proximity between individuals
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45 292 which could induce an increase of FP horizontal transmission. However, this assumption needs
46
47 293 to be verified by further studies. Our study demonstrated a significant increase of the global FP
48
49 294 prevalence on three sites with high densities of turtle : Anse Noire/Dufour, Grande Anse, and
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51 295 Anse du Bourg/Chaudière. The temporal patterns of FP prevalence seemed to be different
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53 296 between these three sites, which are very close geographically (i.e. from 0.36 to 7.2 km, for
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3 297 distance's details in Siegwalt et al., 2020, Table S1). It is possible that FP prevalences were
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5 298 underestimated as we based our diagnosis on external lesions while it has been demonstrated
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7 299 that asymptomatic turtles can present high loads of ChHV5 (Chaves et al., 2017; Page-Karjian
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9 300 et al., 2015). In comparison, FP clinical signs were observed on half of the green turtles located
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11 301 in the Indian River lagoon , but none seemed to be affected at Sabellariid Worm Reef located
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13 302 one kilometer away in Florida (Herbst, 1994). A significant increase of FP prevalence over
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15 303 time was also highlighted for the same species in Texas with a prevalence under 5% before
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17 304 2015 and rising to 35.2% in only three years (Shaver et al., 2019). At Pala'au, Molokai Island,
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19 305 the prevalence increase was quite similar to the one we observed at Anse du Bourg/Chaudière,
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21 306 rising from 1% to 61% in eight years (Jones et al., 2015). Thus, the literature suggests an
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23 307 important influence of local conditions on the disease's development and global trends toward
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25 308 an increase of FP prevalence.

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30 309 Higher NPP values, but not temperature, were associated with higher FP prevalence.
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32 310 However, the positive link between NPP and FP prevalence was slight and associated with high
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34 311 uncertainty. Herbst (1994) proposed that tumour growth was more important in spring and
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36 312 summer because of higher water temperature. Murakawa et al. (2000) and Chaloupka et al.
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38 313 (2008), though, found no intra-annual variation of tumour size in stranded green turtles in
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40 314 Hawaii. Furthermore, Torezani et al. (2010) did not find an effect of temperature on FP
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42 315 prevalence with a range of temperatures from 27.5°C to 33.5°C. We had only a single SST
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44 316 sample point for the entire Martinique Island and mean SST values had a maximum variation
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46 317 of 2.7°C. This range was probably not sufficient to have a notable effect on FP-associated
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48 318 tumour growth. Thus, the influence of temperature on FP dynamics has not been evidenced at
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50 319 this time. However, Anse Noire/Dufour, Grande Anse, Anse du Bourg/Chaudière and Petite
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52 320 Anse are shallow bays where water temperature could greatly vary at a local scale compared
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3 321 to our SST sample point located further at sea. Finer scale temperature data are necessary to
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5 322 verify whether SST influences FP prevalence in Martinique.

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7 323 Herbst (1994) suggested that human activities such as agriculture, industry and urban
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9 324 development should impact the development of tumours through different pathways and
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11 325 mechanisms. Santos et al. (2010) found that the high FP prevalence in a green turtle
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13 326 developmental area was associated with its poor water quality (EEI = 2, ecological evaluation
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15 327 index based on benthic macrophyte) in Espirito Santo Bay off Brazil. Concomitantly, on Oahu,
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17 328 Maui and Hawaii islands, high prevalence areas corresponded to those with a greater nitrogen
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19 329 footprint (Van Houtan et al., 2010). We therefore analysed seawater chemical, biological and
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21 330 bacterial parameters in the different bays where we captured turtles in order to characterise
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23 331 their environmental quality. The high level of NO₂ in Anse du Bourg/Chaudière might be
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25 332 associated with the high prevalence observed at this site. Despite the lack of significant
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27 333 differences between capture sites regarding bacterial parameters, we could also notice that
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29 334 Anse du Bourg/Chaudière was the only site where the presence of enterococci was observed,
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31 335 which reflect faecal contamination and poor water quality. This capture site also had several
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33 336 samples exceeding the European sanitary threshold of 1000 MPN.100mL⁻¹ for *E. coli*.

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35 337 The present results highlighted a significant difference in seawater quality between the
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37 338 capture sites where immature green turtles are most present. The presence of a damaged outfall
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39 339 releasing wastewater from a sewage treatment plant in Anse du Bourg (Impact Mer, 2016), as
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41 340 well as an important pressure of pleasure boats could be responsible for the releasing of faecal
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43 341 matter and nitrogen-enriched content (pers. obs.). Therefore, further environmental quality
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45 342 studies are necessary to seek a possible link with the FP outbreak in Martinique. Indeed, higher
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47 343 levels of arginine, an amino-acid known to enhance the emergence of tumours in some cases,
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49 344 have been found in marine algae in watersheds with a higher nitrogen footprint due to human
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51 345 land use (Hargove et al., 2016). Considering the above-mentioned environmental context and
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3 346 the high fidelity of immature green turtles to their feeding zone in the south-western bays of
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5 347 Martinique (Siegwalt et al., 2020), Anse du Bourg/Chaudière could provide an optimal
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7 348 environment for the contraction, persistence and transmission of this disease in green turtles.
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10 349 By studying the density of individuals within each capture site, we have highlighted the
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12 350 presence of areas with high densities of individuals while others were left vacant. Differences
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14 351 in FP prevalence between locations could therefore be explained by the fact that some sites
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16 352 have higher turtle densities than others and that individuals are not uniformly dispersed within
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18 353 the bays. This could be especially the case in Anse du Bourg/Chaudière, where immature green
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20 354 turtles were highly concentrated in the North part of this small shallow bay while CMR data
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22 355 showed the highest FP prevalence for this location. Head rubbing or higher concentration of
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24 356 the ChHV5 in seawater in Anse du Bourg/Chaudière due to highly clustered individuals are
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26 357 possible explanations for the FP situation in this bay. On the other hand, at Anse Noire/Dufour
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28 358 where turtle's densities were the lowest, no FP outbreak has been recorded by the CMR
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30 359 monitoring.
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35 360 The previous hypothesis of horizontal transmission is reinforced by our results on the
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37 361 relative distribution of tumours over the different body parts. Similar to Rossi et al. (2019), our
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39 362 results show that the neck, fore fins, and eyes, were the most frequently affected body parts.
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41 363 This result highlighted the possibility that turtle aggregations in restricted areas influence the
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43 364 prevalence of FP (Patricio et al., 2016) through positive interactions between individuals.
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45 365 Indeed, videos from cameras fixed on green turtles' shells have shown that green turtles
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47 366 sometimes rub their heads against each other and their upper body parts get consequently in
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49 367 contact during interactions occurring on feeding areas (pers. obs.). Moreover, the DNA of
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51 368 ChHV5 has also been detected in saliva and ocular secretion of green turtles affected by FP,
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53 369 thus representing another possibility of viral excretion and FP transmission, even if direct
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3 370 transmission from one turtle to another is not fully understood for the moment (Domiciano et
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5 371 al., 2017; Patricio et al., 2016; Rossi et al., 2019).

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9 10 373 **5. Conclusion and perspectives**

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12 374 Fibropapillomatosis is a potentially debilitating condition, depending on the severity of
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14 375 the lesions, that affects green turtle populations worldwide. We observed an evolution of the
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16 376 FP prevalence in Martinique with an increase of 50% in seven years in one particular bay: Anse
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18 377 du Bourg/Chaudière. We supposed that the high density of immature green turtles in restricted
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20 378 areas enhances positive interactions between individuals and can therefore promote the
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22 379 transmission of FP from one turtle to another. We hypothesised that the addition of factors
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24 380 promoting FP such as lower water quality (i.e. possible eutrophication due to high nutrient
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26 381 loads, high bacterial parameters) or the presence of a wastewater discharge is responsible for
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28 382 the disease's outbreak at Anse du Bourg/Chaudière. Further studies regarding seawater quality
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30 383 (e.g. eutrophication, pollutant presence), the presence and quantity of ChHV5 in the
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32 384 environment, and fine scale currentology, SST, and NPP in the different capture sites are
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34 385 necessary to find clear evidence on how these parameters influence FP dynamics.

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36 386 The presence of this infectious disease on Martinique Island is of great concern since it
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38 387 is a key developmental area for green turtles. The knowledge of the population's health status
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40 388 is critical to establish conservation programs for this species. FP emergence is recent compared
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42 389 to the CMR program. Thus, the prosecution of the capture program for several years and with
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44 390 more captures in every location will allow us to describe whether Anse du Bourg/Chaudière is
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46 391 the only site affected by FP in Martinique. Moreover, an important number of recaptures will
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48 392 permit comparison of survival rates between healthy and sick turtles to determine whether FP
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50 393 has an impact on immature green turtle population dynamics in Martinique. However, the
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52 394 presence of ChHV5 DNA in green turtles without external tumours (Chaves et al., 2017; Page-

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3 395 Karjian et al., 2015) suggests that FP prevalence based on external tumours is underestimated
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5 396 compared to real prevalence. Monitoring the health status of immature green turtles in
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7 397 Martinique using serological methods (Work et al., 2020; Sposato et al., 2021) and quantitative
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9 398 PCR (Page-Karjian et al., 2015) will be a more efficient way to take into account
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11 399 asymptomatic individuals in FP prevalence measurement and would allow a better
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13 400 understanding of FP disease.
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563 **Figures and tables**

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

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

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

574 Figure 4: Water quality parameters in 2021 for several capture sites: Anse Noire/Dufour (n =
575 4), Grande Anse (n = 3), Anse du Bourg/Chaudière (n = 5), and Petite Anse (n = 3). Dots
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578 chlorophyll *a* in $\mu\text{g.L}^{-1}$, *Escherichia coli* and enterococci in MPN.100mL^{-1} .

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

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

Model	AIC	Δ AIC	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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599 Table 2: Results of likelihood ratio tests performed on generalised linear mixed models of seven
 600 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 <i>a</i> ~ (1 Replicate)	3	12.35		
Chlorophyll <i>a</i> ~ Capture site + (1 Replicate)	6	14.85	5.00	0.172
<i>E. coli</i> ~ (1 Replicate)	3	-130.18		
<i>E. coli</i> ~ 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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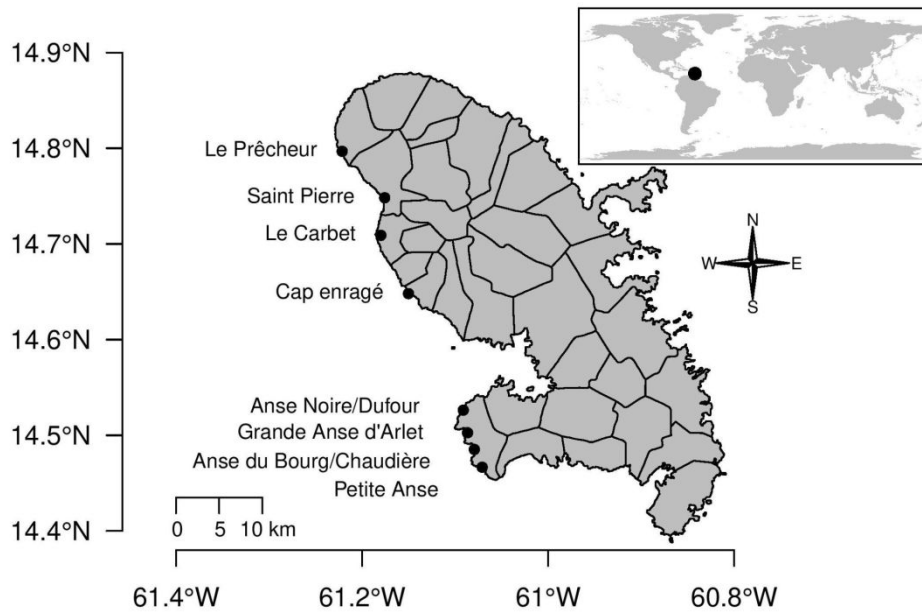
609 Table 3: Post-hoc comparisons between capture sites according estimated marginal means for
 610 ammonium, nitrites and phosphates. AB/C is Anse du Bourg/Chaudière, AN/D Anse
 611 Noire/Dufour, GA Grande Anse, and PA Petite Anse. Degrees of freedom were calculated
 612 according Kenward-Roger method and p-values adjusted using Tukey's method for multiple
 613 comparison. Significant differences are in bold.

Comparison	Estimate	SE	df	t-ratio	p-value
<i>Ammonium</i>					
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
<i>Nitrites</i>					
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
<i>Phosphates</i>					
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

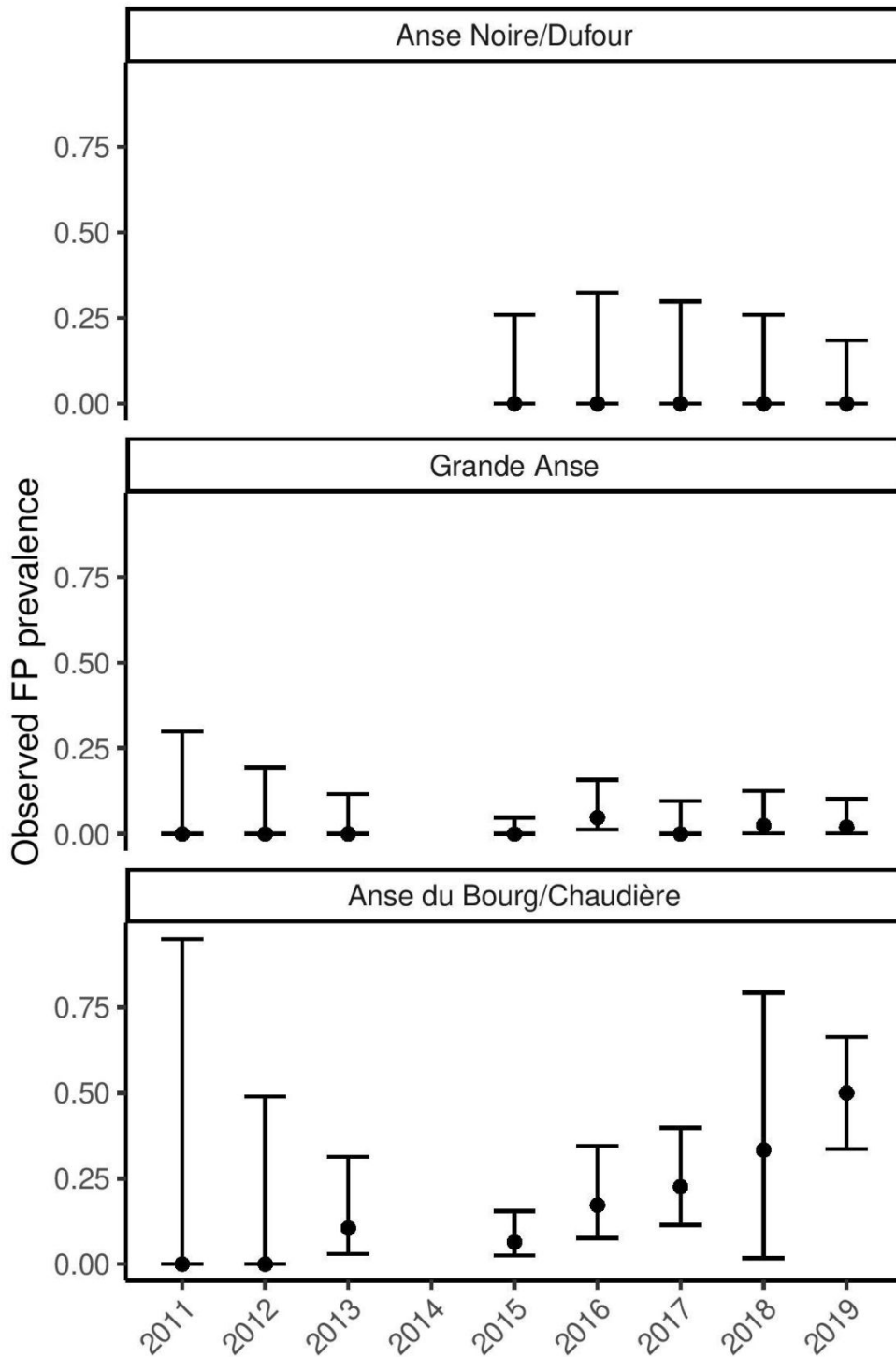
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1 **Fibropapillomatosis prevalence and distribution in immature green turtles**
2 **(*Chelonia mydas*) in Martinique Island (Lesser Antilles)**

4 **Figures**

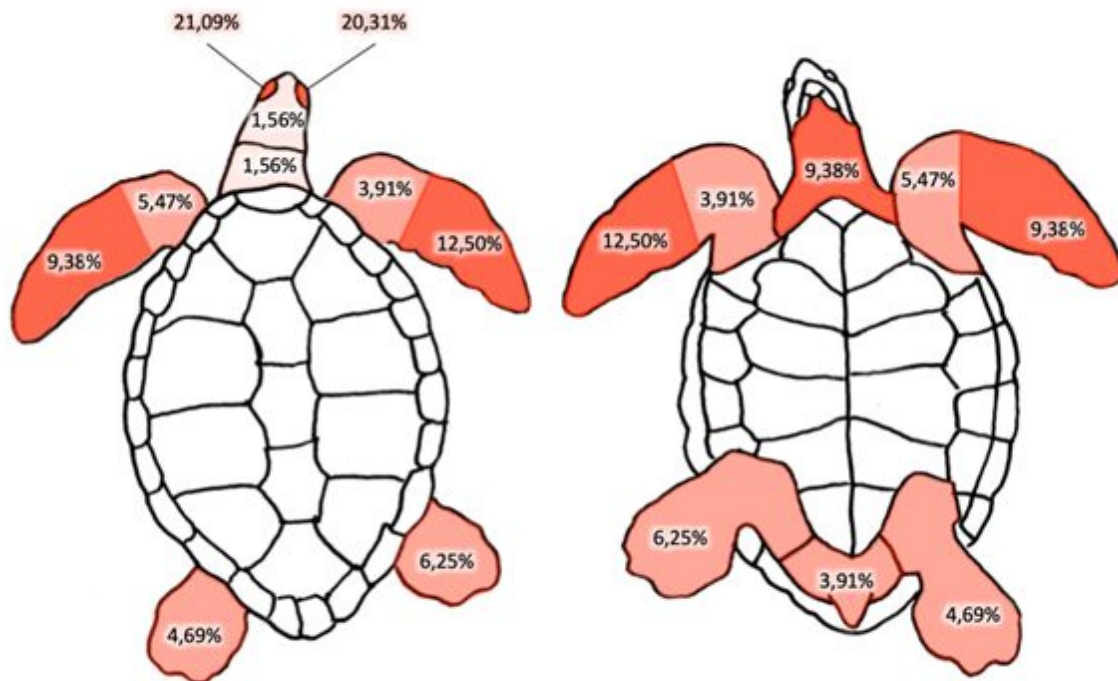


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6 Figure 1: Martinique map showing capture sites of immature green turtles *Chelonia mydas*
7 (black dots).



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



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

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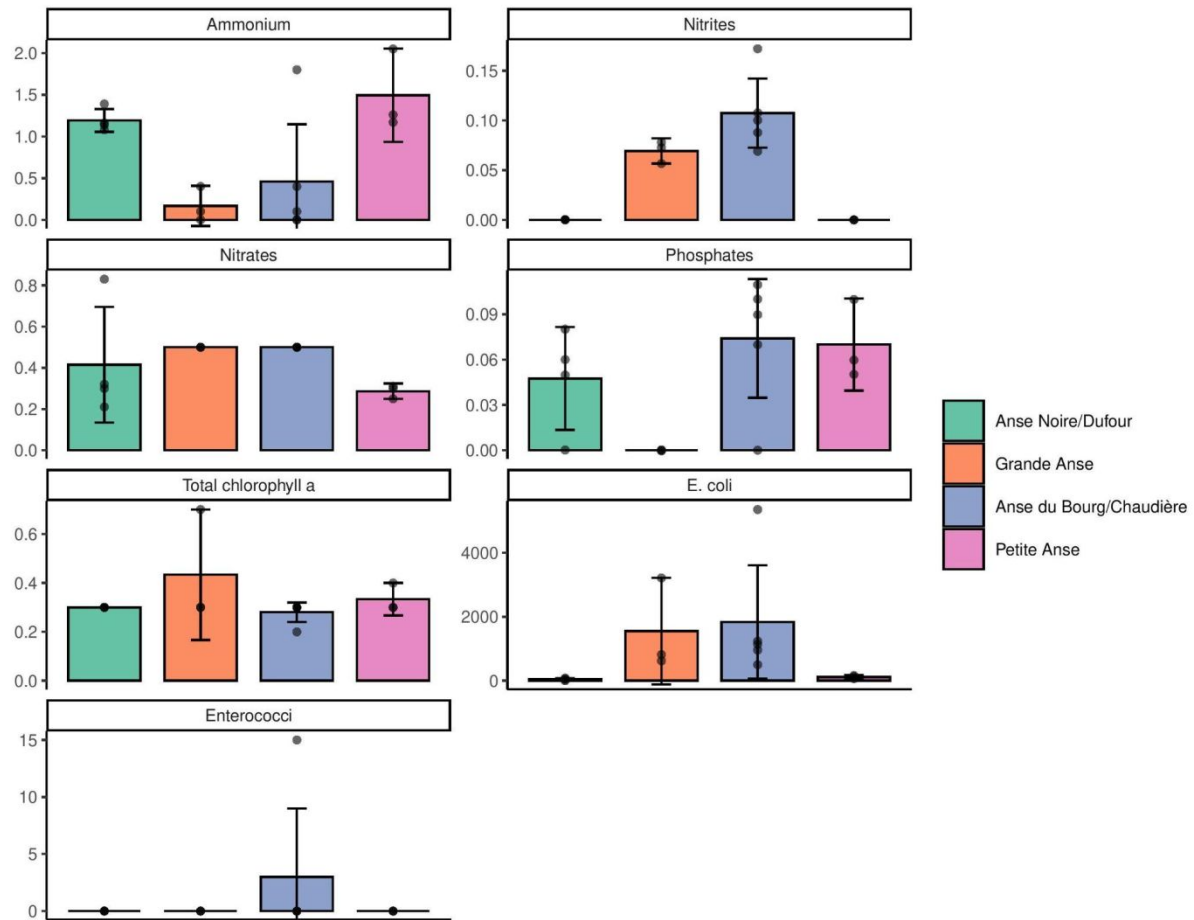
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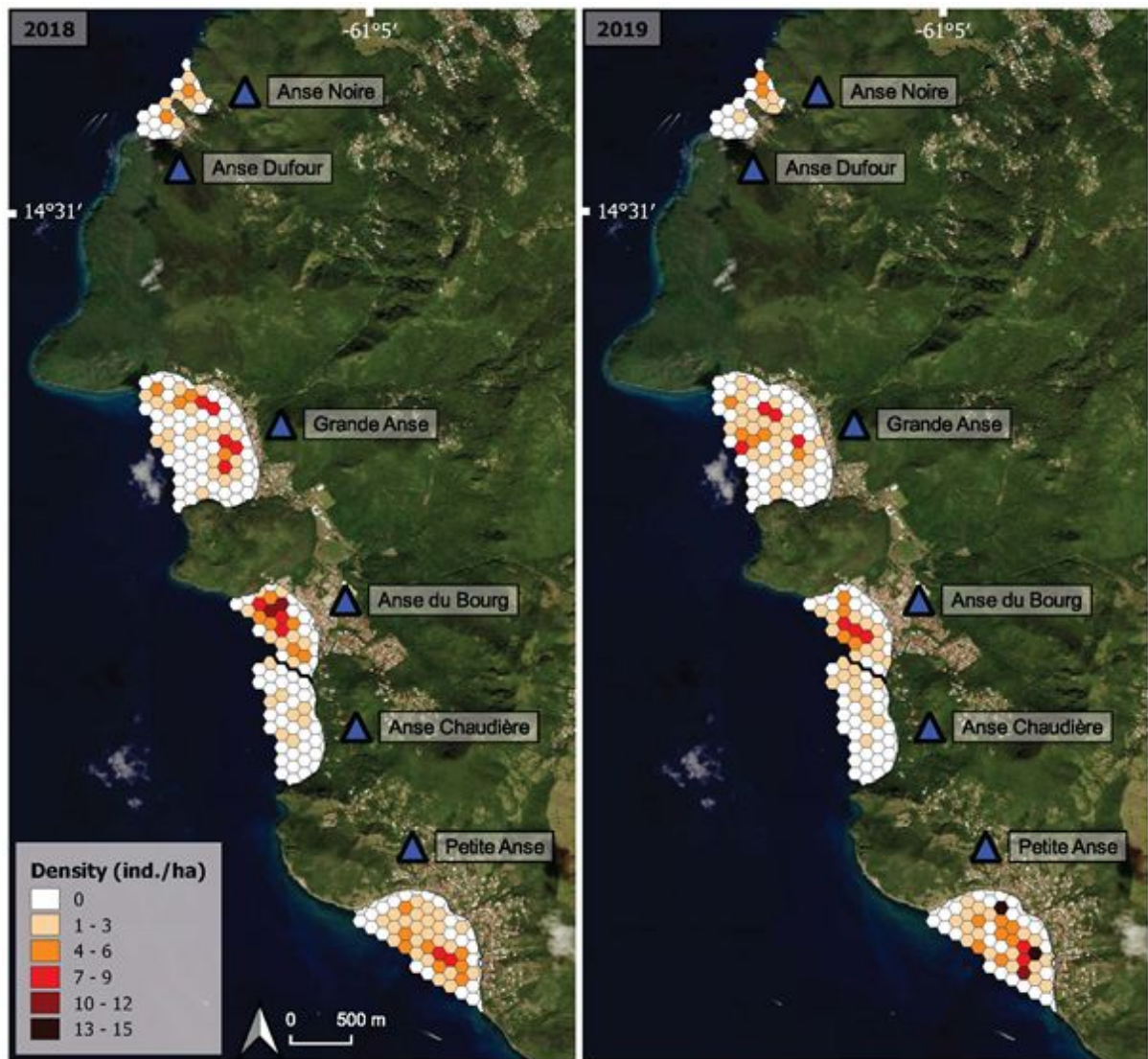
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34 Anse Chaudière and Petite Anse in 2018 (left) and 2019 (right). Each hexagon represents one
35 hectare and darker hexagonal colour shades indicate a higher turtle concentration (expressed
36 as the number of individuals per hectare).

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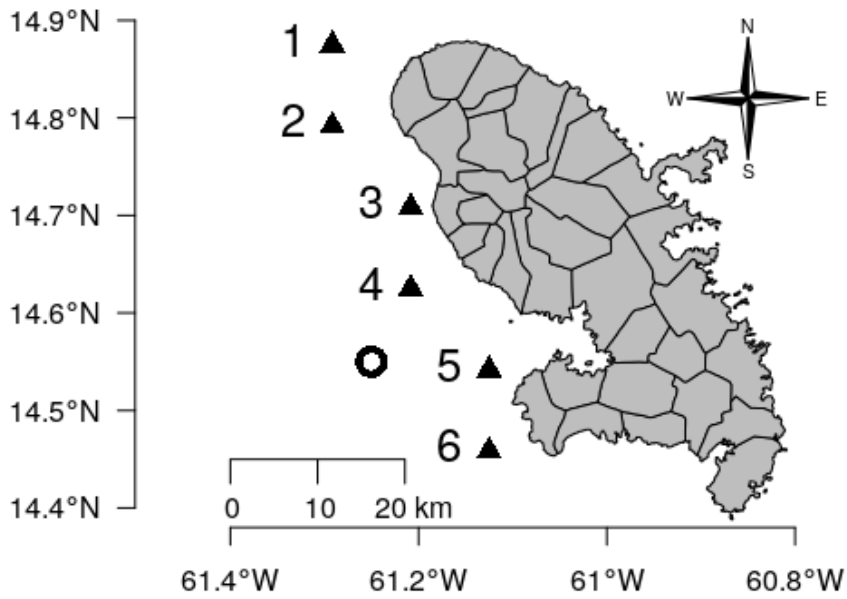
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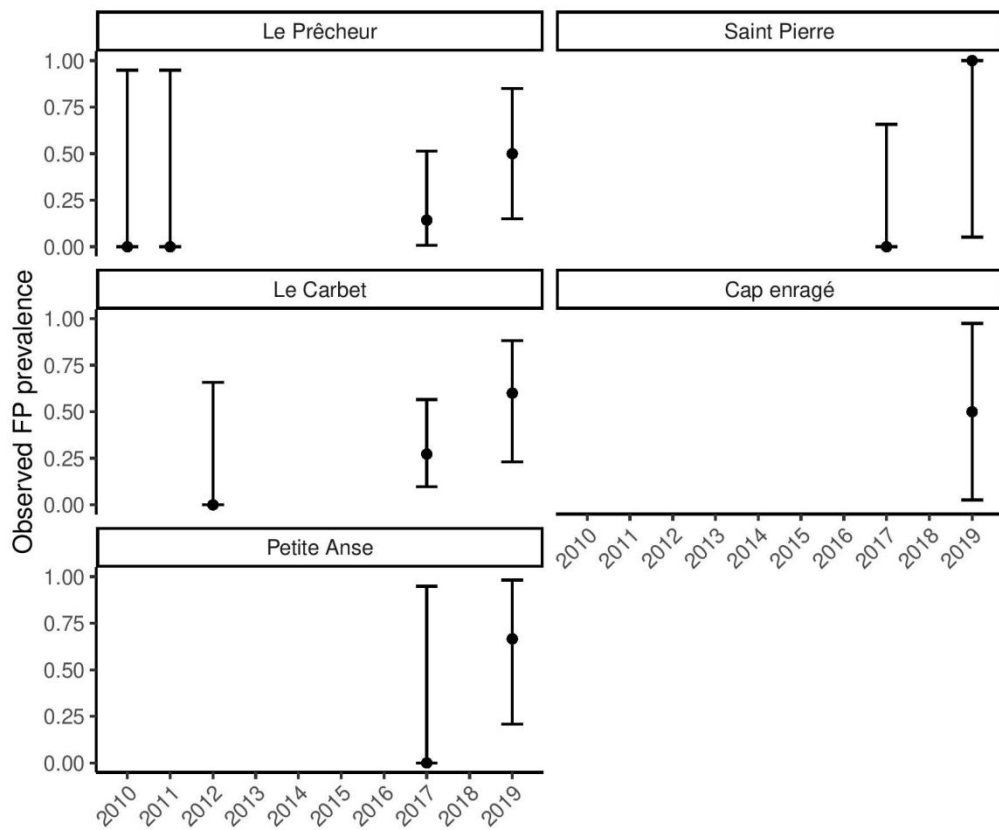
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43 **Supplementary Material**

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



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

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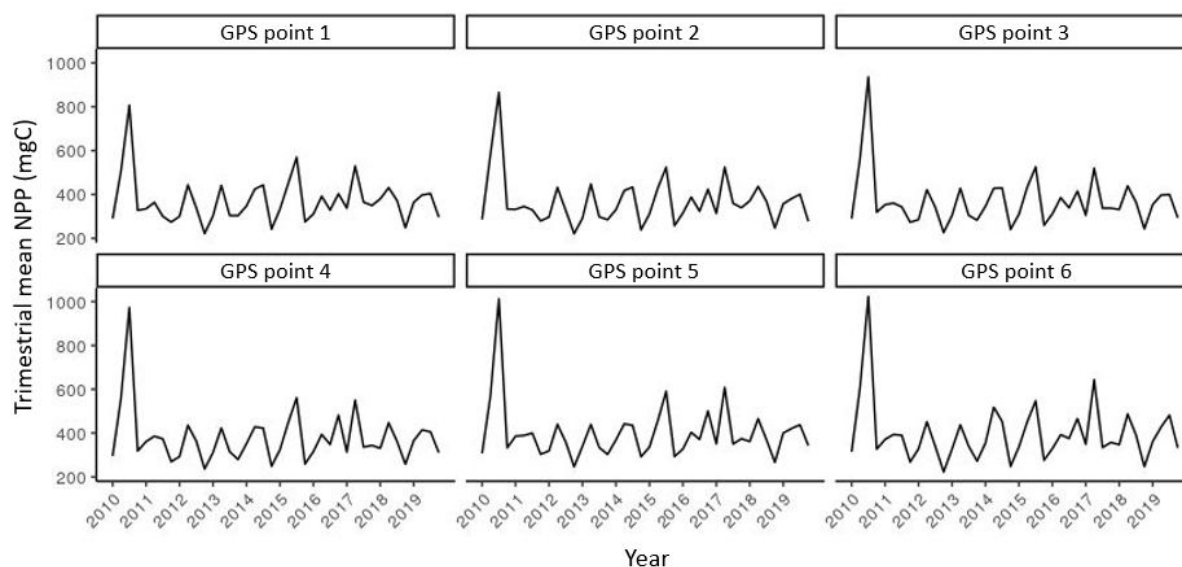
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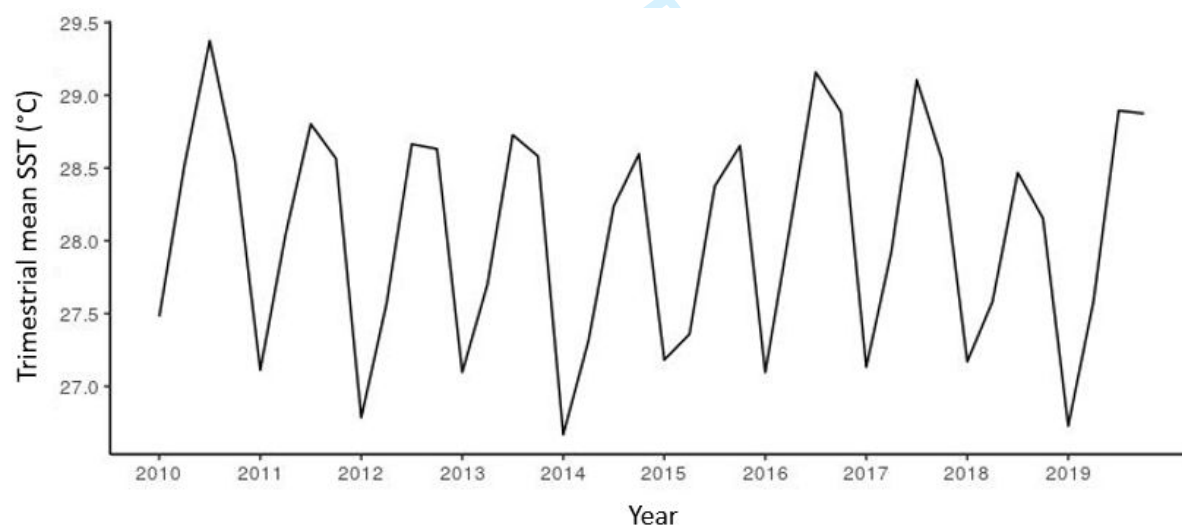
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62 Figure 3: Trimestrial mean net primary production (NPP) on the West coast of Martinique
 63 Island for six sampling points from 2010 to 2019 (2160 x 4320 grid).

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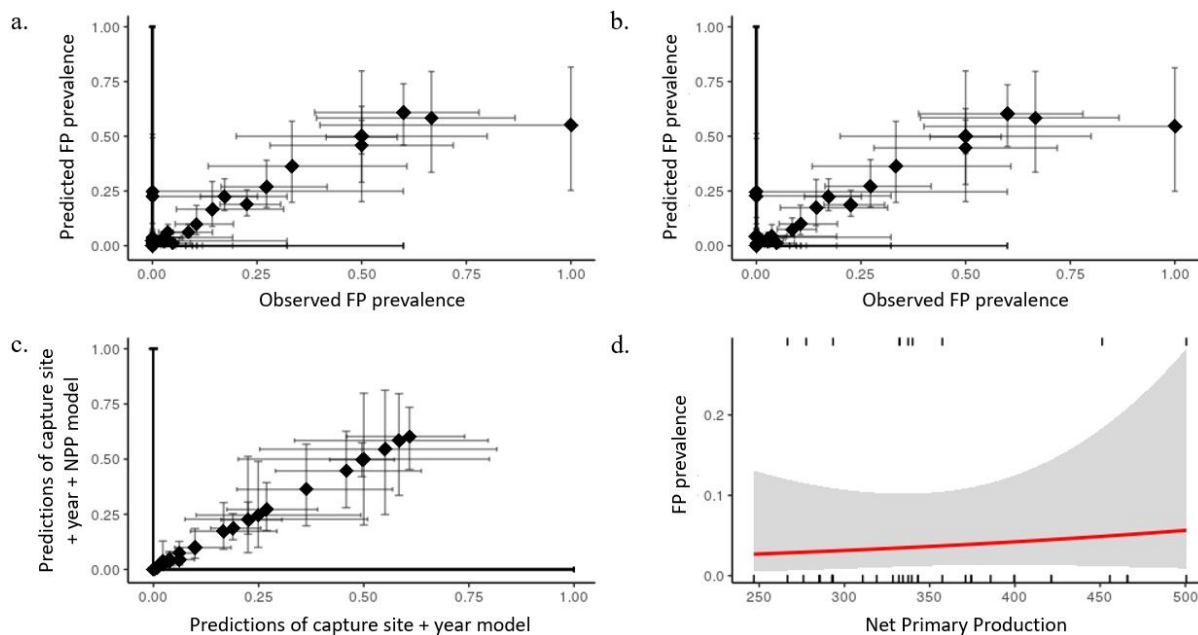
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66 Figure 4: Trimestrial mean sea surface temperature (SST) on the West coast of Martinique
 67 Island from 2010 to 2019 on the 14.55° latitude and -61.25° longitude point (0.25° x 0.25°
 68 grid).

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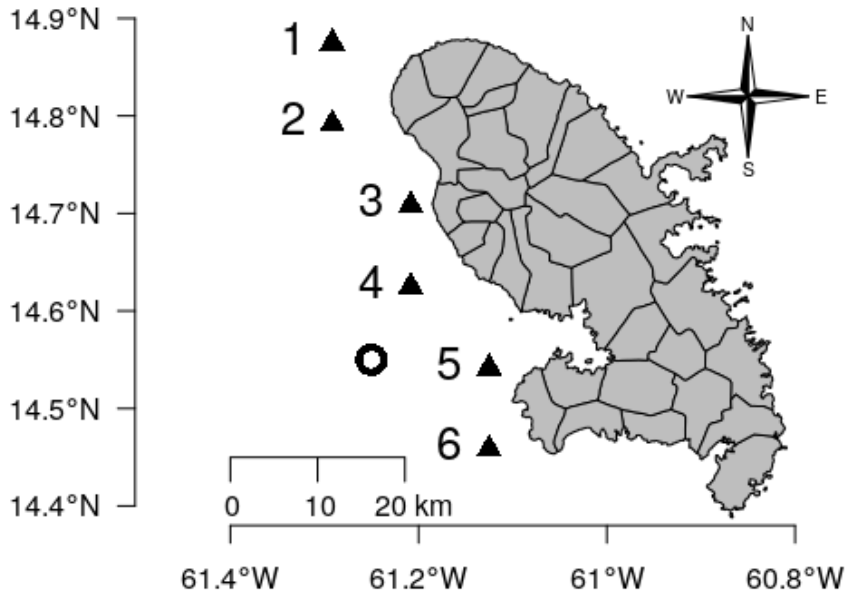
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73 Figure 5: Predicted versus observed fibropapillomatosis prevalence using the *capture site +*
 74 *year* model (a) and the *capture site + year + NPP* model (b), comparison between predicted
 75 values of these two models (c) and regression between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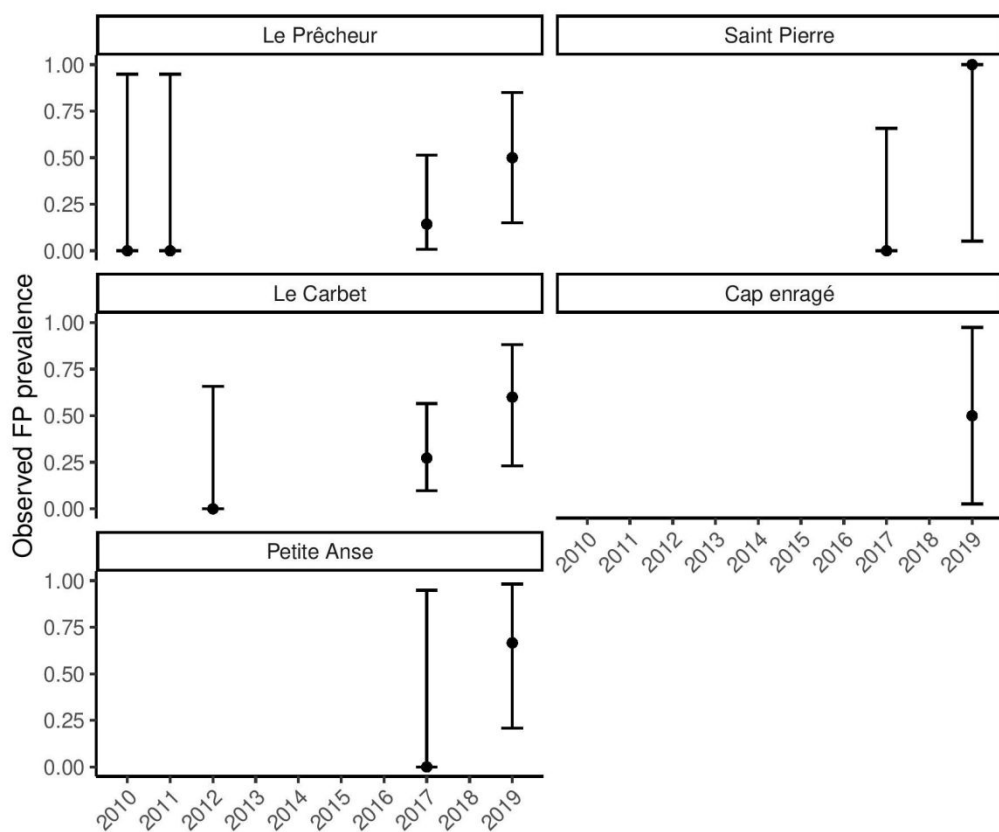
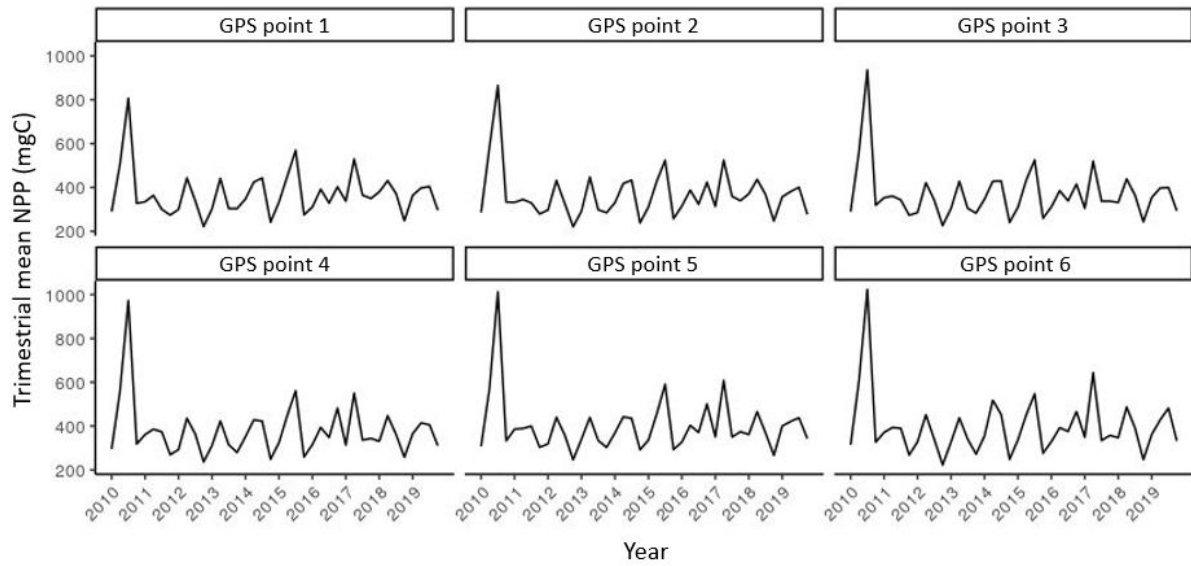


Figure 2: Annual fibropapillomatosis prevalence from 2010 to 2019 (except 2014) of immature green turtles on the Caribbean coast of Martinique where captures were weak: Prêcheur ($n = 17$), Saint-Pierre ($n = 3$), Carbet ($n = 5$), Cap enragé ($n = 2$), and Petite Anse ($n = 4$). Vertical bars represent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

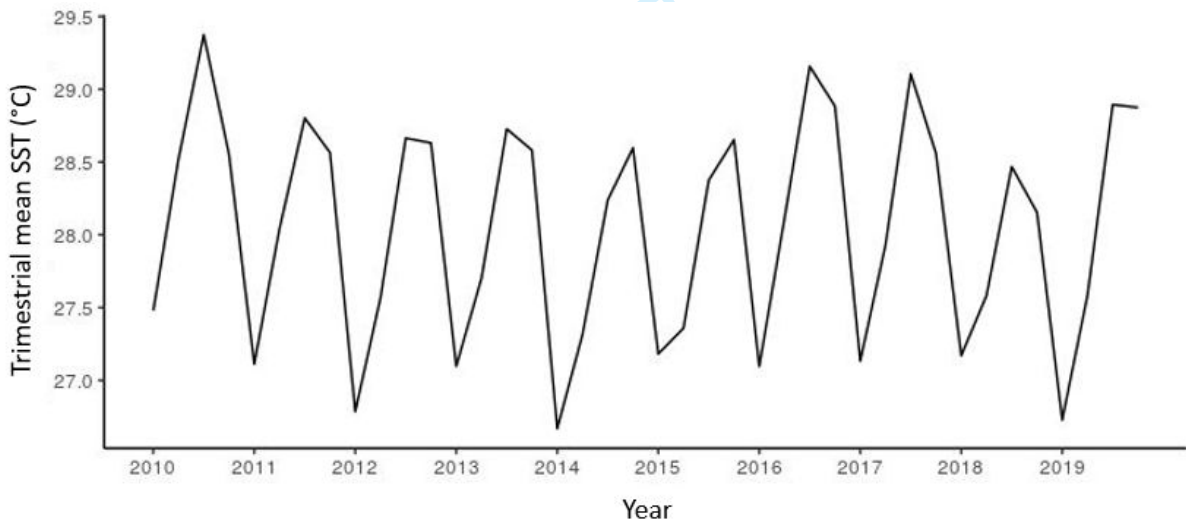
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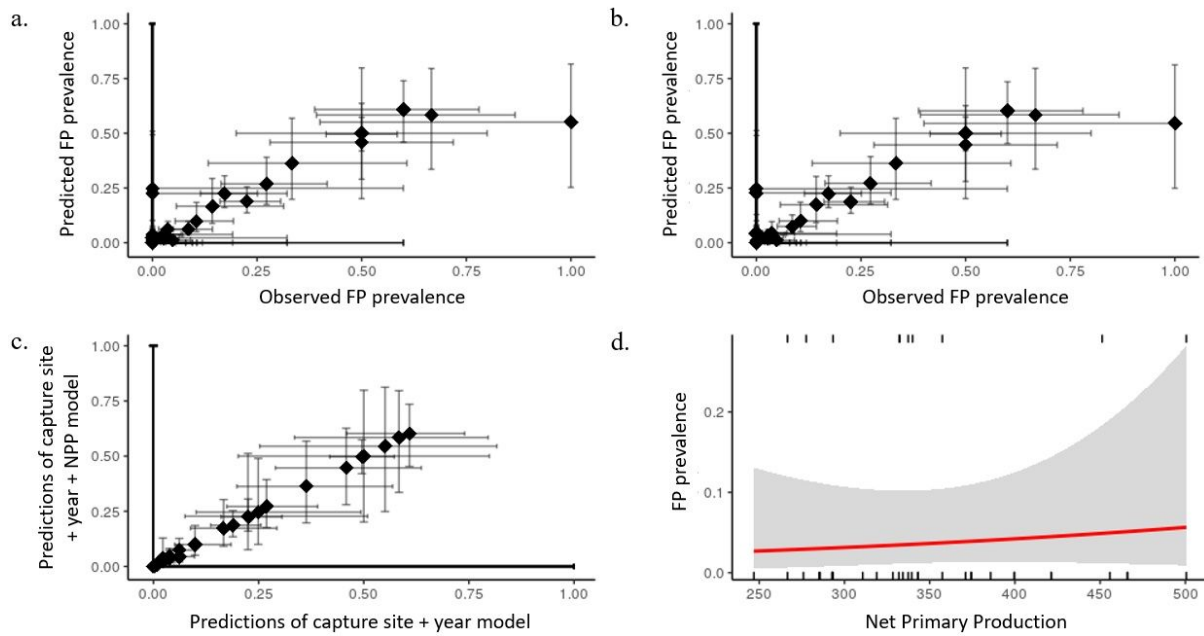
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