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Spatial distribution of fibropapillomatosis in green turtles along the Queensland coast and an investigation into the influence of water quality on prevalence

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Abstract

Fibropapillomatosis (FP) is a tumor-forming disease which affects all species of marine turtle, but predominantly the green turtle (*Chelonia mydas*). Expression of this disease is thought to be precipitated by poor environmental conditions and often linked to anthropogenically induced environmental changes. Although FP is a globally distributed disease, targeted studies on the spatial distribution of the disease in Australia are limited. Here, we present the first comprehensive report of FP prevalence in Queensland, Australia. A retrospective analysis of 25,645 capture records for 15 sites along the Queensland coast were used to determine FP prevalence and trends in foraging green turtles. Within this data set, 791 turtles (3.1%) with FP tumors were recorded. Our analysis showed that prevalence varies between sites and years, with juvenile turtles being the most frequently affected by the disease. We found that survey method has a significant influence on the apparent FP prevalence detected at each site. That is, surveys which were explicitly FP-targeted detected higher numbers of individual turtles with FP, and therefore generated higher prevalence rates than comprehensive population surveys. We also report the first attempt at developing water quality indices (WQIs) to compare with FP prevalence data in foraging green turtles. The WQIs were built from metrics published in a range of peer-reviewed papers, reports, and based on expert opinion. Despite utilizing an extensive data set, a relationship between FP prevalence and WQI rankings at each site could not be quantified. The analysis was confounded by a range of limitations, including data gaps, varying temporal scales and data capture methods in the FP prevalence, and water quality data sets. This study has significant implications for management as it highlights the benefits of designing and collecting centralized data that can be integrated and used across multiple projects or programs.

KEYWORDS

Chelonia mydas, Fibropapillomatosis, green turtle, water quality, water quality index, wildlife disease

1 | INTRODUCTION

Marine turtles are recognized as a flagship species for ecosystem health (Aguirre & Lutz, 2004), yet they can be afflicted by diseases that are not well understood. Fibropapillomatosis (FP), a neoplastic condition associated with chelonid alphaherpesvirus 5 (ChHV5) infection, is one such disease. This disease has been reported in all species of marine turtles, but it predominantly affects the endangered green turtle (*Chelonia mydas*) (Jones et al., 2016). This disease is characterized by the growth of tumors on the soft tissue, viscera, carapace, plastron, and/or cornea. The tumors may limit or obstruct the vision, feeding, and locomotive ability of affected turtles (Jones et al., 2016) and as a result, these turtles may be at increased risk of predation, starvation, and boat-strike. The consequences of tumors on infected individuals can vary, with both mortality (Chaloupka et al., 2008) and complete recovery reported (Limpus et al., 2016; Machado Guimarães et al., 2013) and modeled (Kelley et al., 2022). In depth understanding of the effects of this disease on marine turtle populations as a whole is still a missing piece in the evidence-based management of this endangered species.

Reports of FP in marine turtle populations have been documented globally, with prevalence of this disease varying both spatially and temporally (Jones et al., 2016). Some regions have established in-depth trends through retrospective studies of long-term FP data sets (Chaloupka et al., 2008; Chaloupka et al., 2009; Jones et al., 2021) and comprehensive health assessments with a strong FP focus (Page-Karjian et al., 2020). Researchers have considered several factors which may help explain the discrepancies involved in FP development. Currently, water temperature and seasonality (Herbst, 1994; Herbst, 1995; Jones et al., 2021), heavy metals (da Silva et al., 2016), metals and organic pollutants (Aguirre et al., 1994; Keller et al., 2014; Sánchez-Sarmiento et al., 2017), and dietary influences (Arthur et al., 2006; Arthur et al. 2008a; Landsberg et al., 1999; Van Houtan et al., 2010; Van Houtan et al., 2014) have all been investigated as possible co-factors in FP development. While some studies identified correlations with FP, results and conclusions vary. The most consistently reported correlation is that between FP prevalence and areas of reduced water quality (Adnyana et al., 1997; Chaloupka et al., 2009; dos Santos et al., 2010; Espinoza-Rodríguez & Barrios-Garrido, 2022; Foley et al., 2005; Hargrove et al., 2016; Herbst, 1995; Jones et al., 2016; Miguel et al., 2022; Van Houtan et al., 2014). Typically, these sites are associated with catchment outflow from areas with high anthropogenic influences like agriculture, urbanization, and/or industrialization. Anthropogenic effects on wildlife diseases are well documented (Hamede et al., 2020), but conclusive links are often difficult to establish. Hamede et al. (2020) highlight the need for increased disease surveillance efforts and data collection to address the increasing occurrence of wildlife disease linked to anthropogenic activities and sudden environmental changes. In the case of FP, surveillance and data collection have been hampered by the complexities of in-water surveys and access to water quality data.

Water quality is a complex field; any physical, chemical, or biological property that influences the suitability of water for natural ecological systems or use by humans can be considered a water quality variable (Boyd, 2015). These variables can be natural or anthropogenic, and may work alone or in synergy to influence water quality. While an extensive array of these variables exist, they can be subdivided into the broad categories of nutrients, suspended solids, pesticides, and metals (Boyd, 2015). Due to the intricacies associated with determining the concentrations of water quality parameters spatially and temporally, many studies rely on other measures as proxies. Water quality indices (WQIs) are designed to convert selected water quality parameters into a dimensionless number for a particular location and time. This number transforms an otherwise elaborate concept into a simple and easily understandable value, which can then be compared between locations and years to monitor changes (Sutadian et al., 2016) and the effectiveness of management arrangements to improve water quality.

Although understanding and managing this disease is a priority research area for marine turtle conservation (Hamann et al., 2010), published reports of FP prevalence in Australia are largely incidental data included in other studies (Ariel et al., 2017; Bell, 2003; Bell et al., 2019; Flint et al., 2010; Flint et al., 2015; Glazebrook & Campbell, 1990; Hamann et al., 2006; Jones et al., 2020; Limpus et al., 1993; Limpus et al., 2005; WWF-Australia, 2018) and other brief reports or conference proceedings (Limpus et al., 2016; Limpus & Miller, 1994). Experts recommend that continued monitoring of FP is essential to detecting changes in the distribution, occurrence, and severity of the disease (Hargrove et al., 2016). Additionally, although water quality along the Queensland coast has been studied extensively and reported through a range of long-term monitoring programs from several institutions (Brodie & Waterhouse, 2012), a relationship between FP prevalence and water quality has never been investigated in Australia.

It is clear that our limited understanding of the factors influencing the prevalence of FP in Australia restricts the ability to make informed management decisions for minimizing the negative impacts of FP. This study aims to fill this knowledge gap and better inform management of marine turtles by 1) characterizing FP

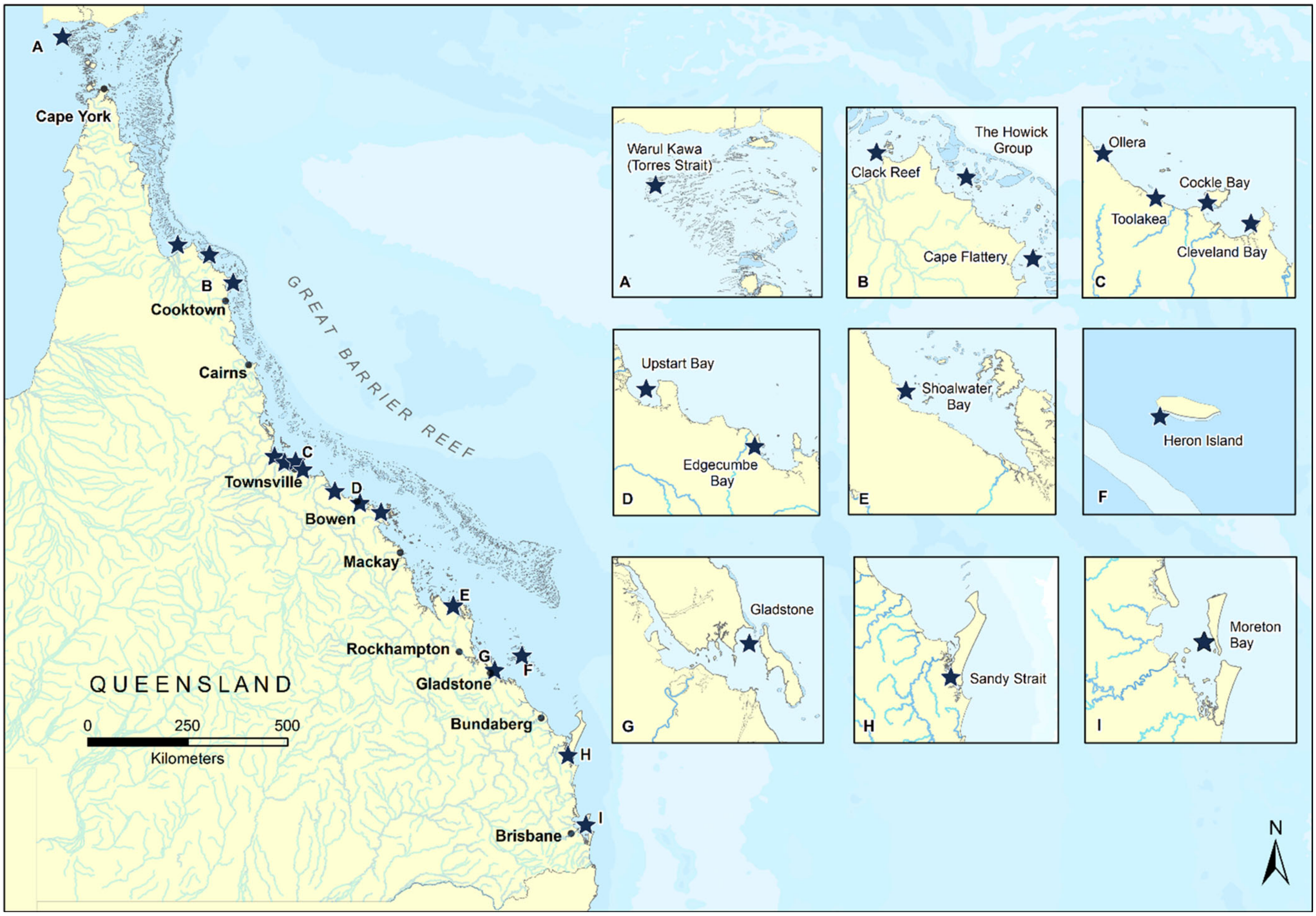

FIGURE 1 Marine turtle capture sites along the Queensland coastline, with more detailed site maps in inset. Sites include waters adjacent to: Warul Kawa (a); clack reef, the Howick group, cape flattery (b); Ollera, Toolakea, Cockle Bay, and Cleveland Bay (c); Upstart Bay and Edgecumbe Bay (d); Shoalwater Bay (e); Heron Island (f); Gladstone (g); Sandy Strait (h); and Moreton Bay (i)

prevalence, determined by tumor presence, at a range of sites spanning the Queensland coastline, 2) investigating any link between water quality and FP along the Queensland coast.

2 | MATERIALS AND METHODS

2.1 | FP prevalence

2.1.1 | Study sites

Marine turtle capture data were obtained from 15 sites spread along the Queensland coast, with the most distant sites being separated by more than 2300 km (Figure 1). Sites were selected based on data availability. This huge expanse of coast encompasses study sites both close to and distant from the coast, in addition to catchments which support remote, rural, urban, and industrialized communities.

2.1.2 | Data collection

Retrospective turtle capture data from a range of sites was extracted from both the Queensland Turtle Conservation database (maintained by Queensland Government's Department of Environment and Science; DES) and the James Cook University (JCU) Turtle Health Research database. Records from two additional marine turtle population surveys in waters adjacent to Warul Kawa in the Torres Strait were obtained from the Torres Strait Regional Authority (TSRA).

The Queensland Turtle Research project commenced in 1968 while the JCU Turtle Health Research team has been studying marine turtle health in Queensland since 2011, often in collaboration with the Department of Environment and Science. The primary objective of the foraging turtle surveys conducted by JCU are more targeted to detecting FP, rather than the comprehensive population surveys conducted by DES and TSRA, which aimed to better understand the demographics of the overall population. Most comprehensive population surveys were conducted over an intensive, two to four-week period with hundreds of turtles of all age-classes captured and processed across multiple habitat types within each study site. However, other comprehensive population surveys were conducted with varying frequency (4 to 25 times per year) of varying duration and attendance by veterinarians. The FP-targeted surveys are typically conducted more sporadically as 1 day fieldtrips several times a year at localized habitats, with smaller numbers of predominantly juvenile turtles captured. With the health-focus of the FP-targeted surveys, each turtle captured is meticulously examined for tumor presence, with any case of FP documented thoroughly (photographic records along with size, location and severity of each tumor). Considering the varying degree of survey intent and extent, the data collected were broken down into two categories: Data generated from 1) Comprehensive population surveys and 2) FP-targeted surveys.

The final data set contained data from turtles captured during foraging population surveys either by turtle rodeo technique from vessels (Limpus & Reed, 1985) or walking captures in the shallows (beach jump) with in-season-recaptured turtles removed from the data set to ensure each turtle was only counted once on an annual basis; the status of the turtle was considered to be "FP" if it was reported to have FP tumors on any in-season capture; for example, if the turtle was recaptured five times within a year but had FP tumors during only one of these capture events then it was considered to be an FP turtle during that year. Upon capture, the curved carapace length (CCL) was measured with flexible tape (±2 mm) and used to determine age-class; juvenile (CCL <65.0 cm), sub-adult (CCL 65.0–90.0 cm), and adult-sized (>90.0 cm) (Limpus et al., 1994; Limpus & Chaloupka, 1997). A total of 23,423 green turtle capture records were included in the final data set. These capture records were used to determine the prevalence of FP at 15 sites along the Queensland Coast (hereby referred to as "Grouped Data"). In addition, capture records from 412 hawksbill (*Eretmochelys imbricata*) and 1810 loggerhead (*Caretta caretta*) turtles were also included, resulting in a total of 25,645 records. For turtles in the Grouped Data, prevalence was calculated for each study site as the total number of individual turtles with FP tumors relative to the total number of turtles captured throughout all the years that site was sampled. Prevalence was calculated for all study species. However, data for hawksbill and loggerhead turtles could only be obtained from a subset of the study sites, and this limited sample size prevented further analysis and conclusions for hawksbill and loggerhead turtles. Therefore, the rest of this study focused on the green turtle data set.

Further analysis of the proportion of FP among age-classes of green turtles (hereby referred to as "Age-class Data Subset") was conducted with available data. An annual breakdown of juvenile, subadult, and adult-sized turtles captured at three sites (western Shoalwater Bay, Heron Island and Moreton Bay) between 1987 and 2014 was generated using the same formula as for the Grouped Data. Within each age-class, the number of turtles with FP was compared to the total number captured for a particular year, with the results expressed as a percentage.

	Water quality indices (WQIs)				
Study site	**DIN**	**TSS**	**Pesticides**	**Metals**	**Overall**
Warul Kawa	1	1	1	1	1
Clack Reef	1	2	1	1	1
Howick Group	1	1	1	1	1
Cape Flattery	1	2	1	1	1
Ollera	2	3	3	2	3
Toolakea	2	3	3	2	3
Cockle Bay	3	4	4	4	4
Southern Cleveland Bay	3	4	4	4	4
Upstart Bay	3	5	5	2	4
Edgecumbe Bay	2	2	3	2	2
Western Shoalwater Bay	1	2	2	1	2
Heron Island	1	1	1	1	1
Gladstone	5	4	3	4	4
Sandy Strait	3	3	4	3	3

TABLE 1 Sub-index scores for DIN, TSS, pesticide, and metal exposure at the 14 sites examined in this study. An aggregated score which reflects the overall water quality at each site (by considering the four parameters) is also shown. Sites are listed in approximately north to south order. The lowest score (1) indicates low levels of exposure to a particular parameter whereas the highest score (5) denotes high levels of exposure

2.2 | Development of water quality indices

WQIs were developed to explore the potential relationship between FP prevalence and water quality along the Queensland coast. These indices were then compared with FP prevalence in green turtles at the same sites. This method is highly reliant on available data for study sites of interest. All but one of the 15 sites in this study comprise a proposed management province of the Great Barrier Reef (GBR) (Brodie & Pearson, 2016). This proposed province, which includes the overall catchment of this region, encompasses large areas of contiguous ecosystems which are ecologically connected (Brodie & Pearson, 2016; Johnson et al., 2018). It is significant to the spatial ecology of green turtles, which extends beyond the current boundary of the Great Barrier Reef World Heritage Area into the contiguous ecosystems within the proposed province (Brodie & Pearson, 2016; Johnson et al., 2018). Due to this connectivity of ecosystems, these sites are typically studied and managed together. As a result, available water quality data for these regions are considered to be comparable. In order to align with available water quality data, further analysis in the present study was restricted to only those sites located within this management province. WQIs were therefore developed for all sites except Moreton Bay

TABLE 2 Mean prevalence rates of fibropapillomatosis in marine turtles at foraging grounds along the Queensland coastline. Values greater than zero are highlighted in bold. The distribution of capture records used for analysis, including study site, survey type, species and number of turtles captured, capture type, and time period that the records span are also provided. Sites are listed in approximately north to south order, and are divided into green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), and loggerhead (*Caretta caretta*) turtle records

Study site	Survey type	Species	Mean prevalence of FP (%)	95% confidence interval	Turtles with FP tumors	Total captured	Capture method	Time period spanned
Warul Kawa	Comprehensive	Green	**3.4**	1.7–6.0	**11**	325	Walking	2016–2017
	Comprehensive	Hawksbill	0.0	0.0–52.2	0	5	Walking	2016–2017
Clack Reef	Comprehensive	Green	**0.1**	0.0–0.5	**1**	1126	Rodeo	1987–1997
Howick Group	Comprehensive	Green	0.0	0.0–0.1	0	3850	Rodeo	1996–2016
Cape Flattery	Comprehensive	Green	0.0	0.0–7.9	0	45	Rodeo	2000
Ollera	FP-targeted	Green	0.0	0.0–6.2	0	58	Walking	2011–2018
Toolakea	FP-targeted	Green	0.0	0.0–3.1	0	117	Walking	2011–2017
Cockle Bay	Comprehensive	Green	**0.7**	0.1–2.0	**3**	444	Rodeo	2002–2016
	FP-targeted	Green	**11.6**	6.8–18.1	**16**	138	Rodeo	2011–2018
	FP-targeted	Hawksbill	0.0	0.0–18.1	0	9	Rodeo	2011–2018
Southern Cleveland Bay	Comprehensive	Green	**1.9**	0.2–6.5	**2**	108	Rodeo	2014–2016
Upstart Bay	Comprehensive	Green	**1.6**	0.7–3.3	**7**	430	Rodeo	2012–2016
	FP-targeted	Green	0.0	0.0–5.7	0	63	Rodeo	2012–2014
Edgecumbe Bay	Comprehensive	Green	**0.7**	0.3–1.3	**10**	1386	Rodeo	2000–2016
	FP-targeted	Green	**7.9**	5.8–10.6	**43**	541	Rodeo	2011–2017
	FP-targeted	Loggerhead	**50.0**	1.3–98.7	**1**	2	Rodeo	2011–2017
Western Shoalwater Bay	Comprehensive	Green	**1.6**	1.3–2.0	**99**	6124	Rodeo	1987–2012
Heron Island	Comprehensive	Green	**0.3**	0.1–0.6	**10**	3204	Rodeo	1989–1999
	Comprehensive	Loggerhead	**1.3**	0.6–2.5	**9**	675	Rodeo	1989–1999
	Comprehensive	Hawksbill	0.0	0.0–1.0	0	360	Rodeo	1989–1999
Gladstone	Comprehensive	Green	**3.0**	1.4–5.4	**10**	338	Rodeo	2011–2014
Sandy Strait	Comprehensive	Green	**3.6**	0.8–10.2	**3**	83	Rodeo	1996–2011
Moreton Bay	Comprehensive	Green	**10.5**	9.6–11.3	**527**	5043	Rodeo	1990–2014
	Comprehensive	Loggerhead	**3.4**	2.4–4.6	**38**	1132	Rodeo	1990–2014
	Comprehensive	Hawksbill	**2.6**	0.1–13.8	**1**	38	Rodeo	1990–2014

($n = 14$), which is located outside of this management province (Brodie & Pearson, 2016; Johnson et al., 2018).

Although there is no standardized method of developing water quality indices (WQI) (Sutadian et al., 2016; Uddin et al., 2021), the general steps outlined by Abbasi and Abbasi (2012) were followed to develop the WQIs in the present study. Briefly, WQI development involved selection of parameters, obtaining sub-index values, and aggregation of sub-indices to produce a final index.

Water quality variables deemed likely to have a direct, or indirect, effect on turtles were selected as parameters. Metals and pesticides have the potential to have a direct toxic effect on turtles, while dissolved inorganic nitrogen (DIN) and total suspended solids (TSS) may detrimentally affect seagrass growth, one of several primary food sources for green turtles (Read & Limpus, 2002), and indirectly affect the health and presence of green turtles in their foraging grounds. Please refer to Appendix 2 for additional detail on these parameters.

A detailed explanation of WQI development is outlined in the Supplementary Material. In short, each sub-index was developed using a combination of available data in the published literature and expert opinion was elicited where needed, consistent with similar studies (Uddin et al., 2021). The expert consulted had over 40 years of experience studying water quality, and was an acknowledged world authority on water quality along the Queensland coast; this expert had published over 100 peer-reviewed articles and over 300 technical reports, books and book chapters during their career. Consistent with other WQI development studies in Australia (Jahan & Strezov, 2017; Ladson et al., 1999), all data obtained were then scored on a 5-point scale. In the present study the lowest score (1) indicated low levels of exposure to a particular parameter while the highest score (5) indicated high levels of exposure. Proximity to sources of parameters of interest were considered, including proximity to river mouths and land-based activities conducted within the catchment associated with particular river systems (e.g., cropping and grazing). However, distinct river plumes formed during high river discharge events typically move northward along the Queensland coast (Waterhouse et al., 2017) and as such, rivers to the south of a study site are often more influential than the ones to the north. This was also considered when developing the sub-indices. None of the parameters were weighted as data availability limited such depth of

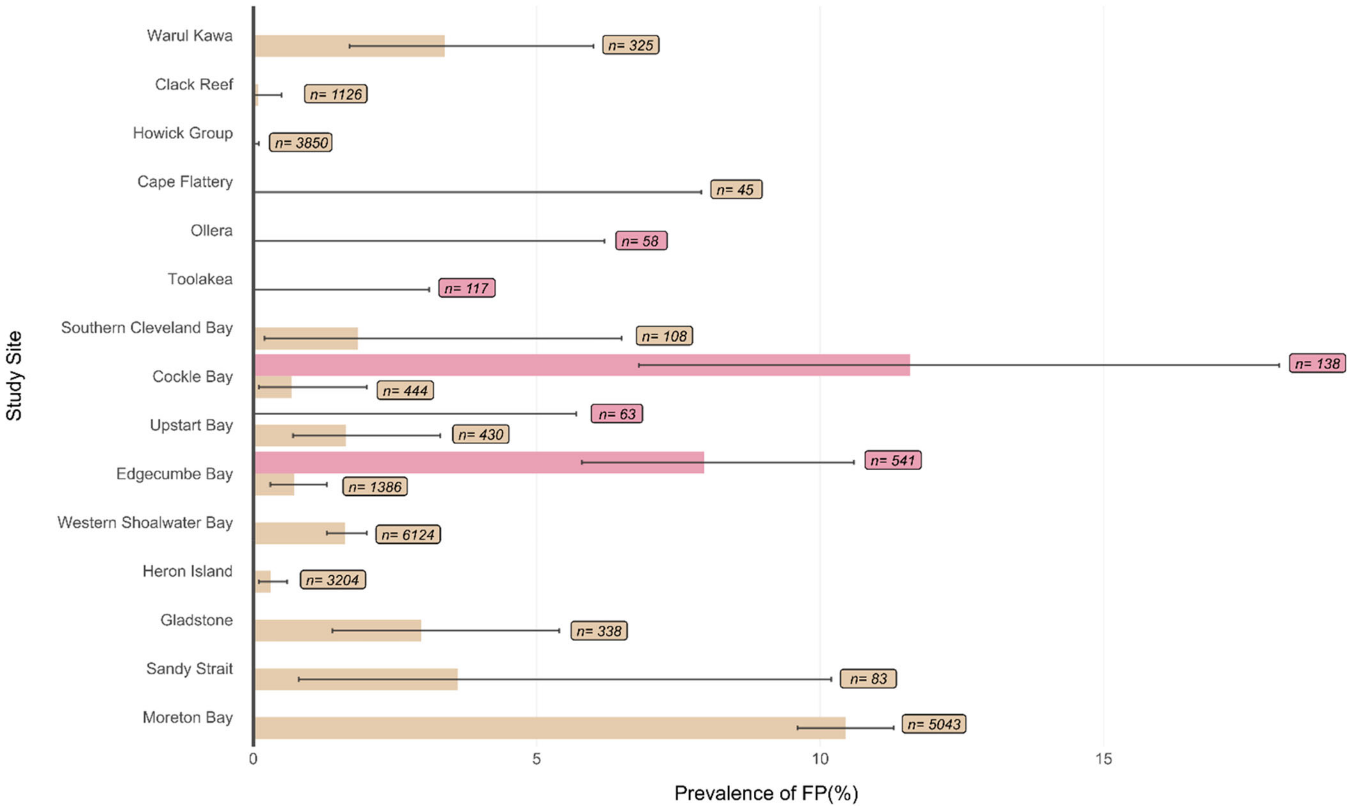

FIGURE 2 Retrospective prevalence rates of fibropapillomatosis in green turtles at foraging grounds along the Queensland coastline. The difference between FP prevalence recorded by comprehensive population surveys (beige) and FP-targeted surveys (pink) is also indicated. Error bars denote the 95% confidence interval

analysis. The final data set, including the sub-index and aggregated scores for each study site, is outlined in Table 1.

2.3 | Statistical analysis

Generalized linear models were used to investigate factors in the data set which influenced FP prevalence. As the response variable (FP prevalence) is a proportion derived from the turtle counts, a logistic regression model was used to investigate factors which may influence FP prevalence. Significant overdispersion was accounted for using the quasibinomial family to model data dispersion. As the number of independent data points (study sites) was small relative to the number of potential explanatory variables, and several explanatory variables included some missing values, it was not possible to evaluate a model including all explanatory variables. We therefore examined the association of each explanatory variable with FP prevalence separately. Those variables which appeared to show an association were then examined in a combined model. All analyses used R (R Core Team, 2018), via the glm() function in the stats package to fit the models, and the Anova() function in the car package to execute analyses of deviance.

The FP prevalence data sets were analyzed alongside the WQI developed for each site in order to determine if there was a relationship between water quality and FP prevalence at these locations. The association between DIN, TSS, pesticides, metals and the overall WQI was examined, both separately and together. Initial analysis of the grouped data demonstrated that turtle age-class and survey method both influenced the recorded FP prevalence, so these explanatory variables were also included where available.

3 | RESULTS

3.1 | FP prevalence along the Queensland coast

3.1.1 | Grouped data

A total of 23,423 green turtle capture records were used to determine FP prevalence and trends at 15 sites along the Queensland coast. Within this data set, 791 individual turtles with FP tumors were recorded. Mean prevalence of FP for all age-classes of green turtles at study sites ranged from 0% to 11.6% (Table 2). High frequencies of FP were found in Cockle Bay, Edgecumbe Bay, and Moreton Bay (Table 2). Cockle Bay had the highest prevalence recorded overall (11.6%), determined from FP-targeted surveys over 8 years, while the second highest report (10.5%) was from Moreton Bay, determined from comprehensive population surveys conducted over 25 years

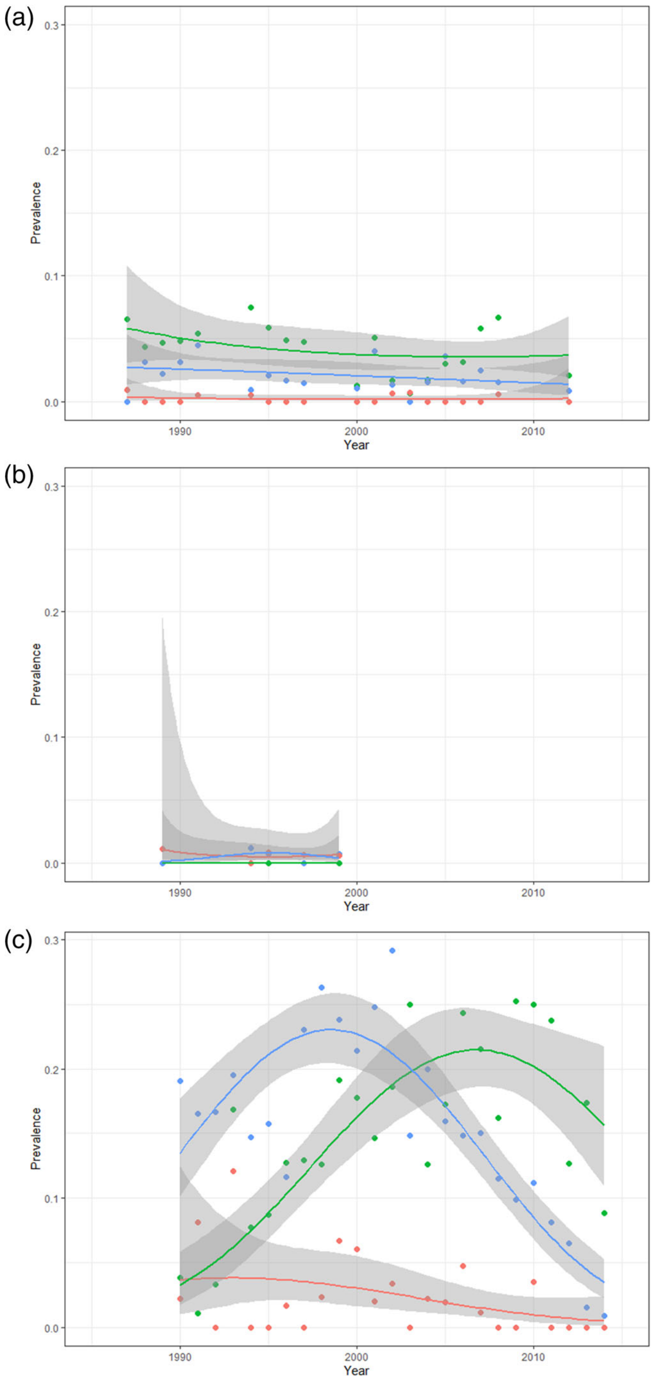

FIGURE 3 Annual age-class distribution of fibropapillomatosis (FP) between turtles at western Shoalwater Bay (a), Heron Island (b), and Moreton Bay (c). Prevalence as a proportion of the turtles collected with FP in age-class is shown. Data collected during comprehensive population surveys between 1987 and 2014, with juvenile (green), sub-adult (blue), adult-sized (red) are represented

(Table 2). FP prevalence was unevenly distributed among study sites and no evidence of a latitudinal north–south cline in prevalence was observed (Figure 2).

The explanatory variables examined individually were study site, survey method (comprehensive population survey and FP-targeted survey), the average age-class of the turtles at each study site (average age-class), and the median year of the study undertaken at each study site (median year) (see Appendix 1: Table S1). Of these, only survey method (Likelihood Ratio [LR] $\chi2 = 10.778$, $df = 1$, $p = .001$) and median year (LR $\chi2 = 5.5173$, $df = 1$, $p = .019$) were significantly associated with FP prevalence. Comprehensive population surveys that targeted broad areas with diverse habitats gave much lower estimates of FP prevalence than the FP-targeted surveys which were eight times more likely to detect FP (odds ratio 0.13). FP prevalence tended to increase with the subsequent year of the survey; however, this effect disappeared when both variables were included in the model, indicating that the apparent temporal trend was probably due to the FP-targeted surveys only being initiated in 2012. Data availability precluded further analysis of age-class or temporal trends in this data set.

Despite the database containing over 25,000 capture records across 15 study sites, further statistical analysis regarding factors influencing FP prevalence was restricted due to insufficient data and varying temporal scales.

3.1.2 | Age-class data subset

Using the available data, an annual age-class breakdown of FP detection was generated for three sites from the comprehensive population surveys (western

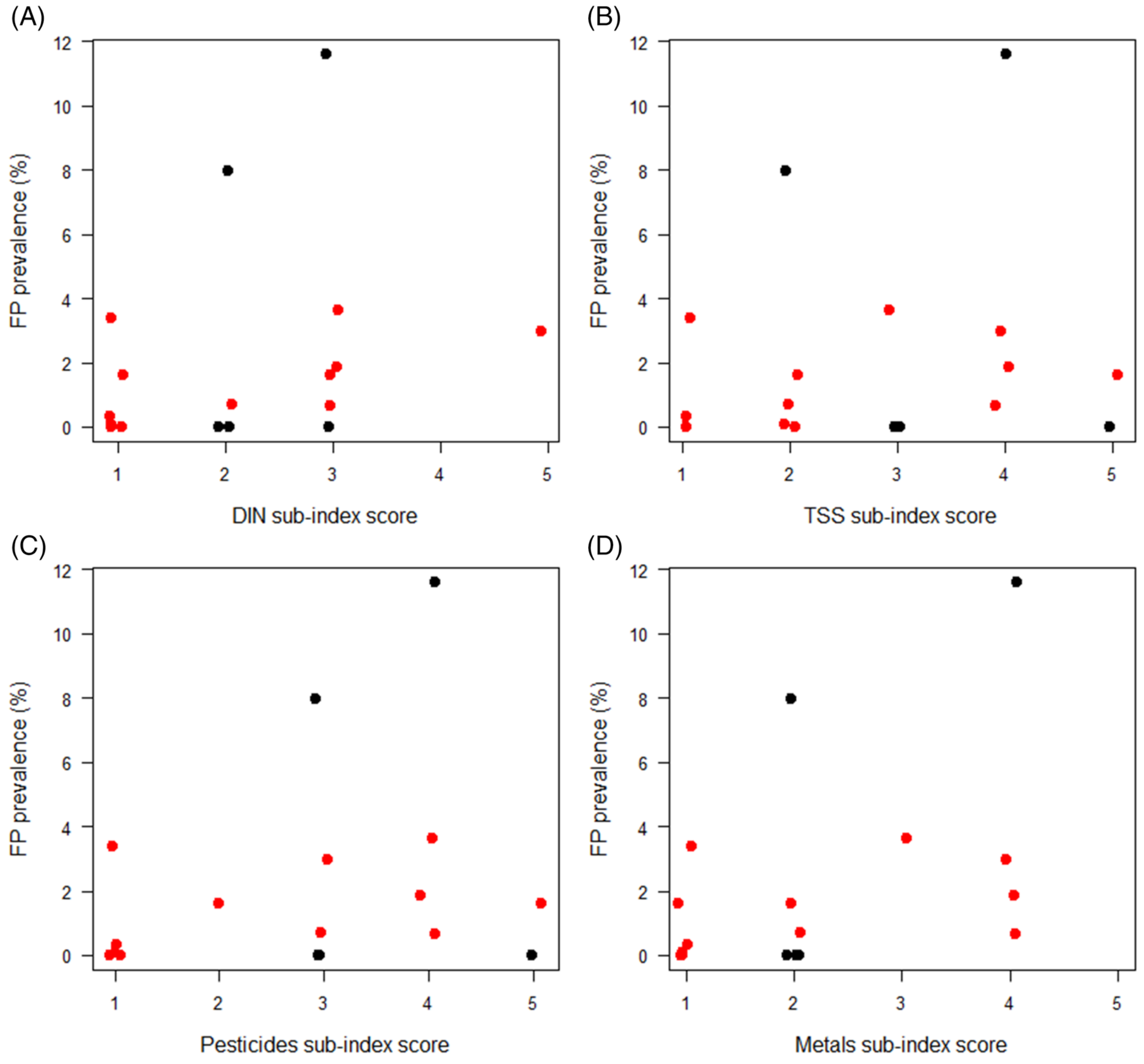

FIGURE 4 A jittered plot of dissolved inorganic nitrogen (DIN) (a), total suspended solids (TSS) (b), pesticides (c), and metals (d) sub-index scores against fibropapillomatosis (FP) prevalence. In each plot, FP-targeted survey results are shown in black and comprehensive population surveys in red

Shoalwater Bay, Heron Island and Moreton Bay). This data subset was collected over longer temporal scales, with higher numbers of individual turtles, which allowed for a better separation of trends. No comparable data set could be obtained from the FP-targeted survey methods.

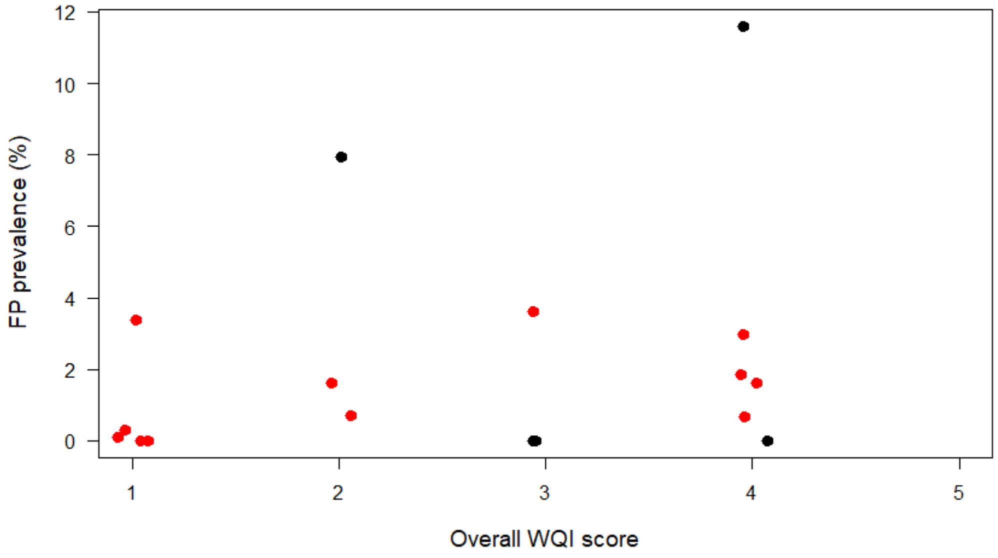

FIGURE 5 A jittered plot of the overall water quality index (WQI) scores against fibropapillomatosis (FP) prevalence. FP-targeted survey results are shown in black and comprehensive population surveys in red

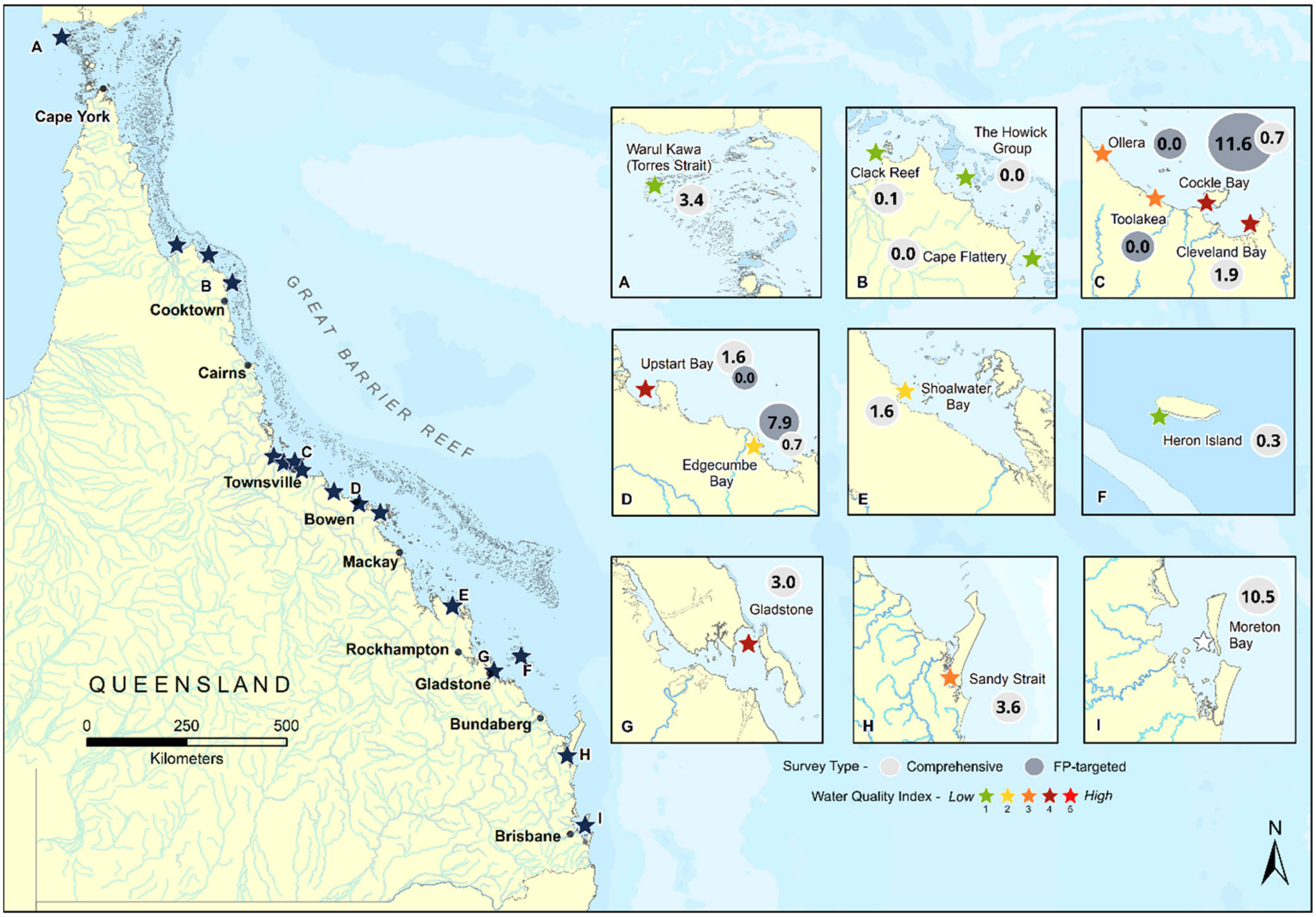

FIGURE 6 FP prevalence and aggregated water quality index (WQI) scores for each study site along the Queensland coast. Two survey methods were employed to generate the FP prevalence values: Comprehensive population (light gray) and FP-targeted (dark gray) surveys. Aggregated WQI scores are denoted by color, with the lowest scores being represented in green and the highest in red

At western Shoalwater Bay, the average prevalence of FP for all green turtles was 1.9% ± 1.0%, while among juvenile, sub-adult, and adult-sized turtles the prevalence was 4.2% ± 2.0%, 1.9% ± 1.3%, and 0.2% ± 0.3%, respectively. At Moreton Bay, where 12.5% ± 4.4% of all turtles were affected by FP, the breakdown among age-classes was 15.2% ± 7.0% of juvenile, 15.7% ± 7.1% of sub-adult, and 2.3% ± 3.1% of adult-sized turtles. No juvenile turtles with FP were recorded at Heron Island (Appendix 1: Table S2). Statistical analysis of the effect of age-class and sites showed that both were strongly associated with FP prevalence ($p < .001$ for both variables). Although prevalence varied annually at all sites, juvenile and sub-adult turtles were the age-classes with the highest proportion of FP (Figure 3). A significant interaction between age-class and study site ($p = .017$) was also detected, suggesting that the effect of age-class is not consistent between the sites. However, further exploration of this was precluded by limited data. A striking shift in FP prevalence over time was observed at Moreton Bay; subadults had a higher prevalence between 1990 and 2002, while juveniles had higher prevalence between 2003 and 2014 (Figure 3(c)). This was followed by a downward trend of FP prevalence in Moreton Bay after 2010 (Figure 3(c)). The curvature in the relationship between prevalence and time at Moreton Bay (modeled using a quadratic term in the equation) was statistically significant in both juveniles and sub-adults ($p < .001$).

3.1.3 | Investigation of the influence of water quality on FP prevalence

A subset of 18,380 individual capture records of green turtles, including 264 records of FP across 14 sites along the Queensland coast were analyzed in conjunction with WQIs to investigate a potential link between FP prevalence and water quality. Despite the analysis of this extensive data set, there was no clear trend between FP prevalence and WQI rankings at any of the sites. Both sub-index and overall WQI scores varied in relation to FP prevalence. The variable results for the sub-index and overall WQI scores are plotted in Figure 4 and 5, respectively. The lack of clear trend between FP prevalence and WQI score at each study site can be visualized in Figure 6. No statistically significant relationships between FP prevalence and the WQIs developed for this study were identified (Appendix 4: Table S9).

4 | DISCUSSION

This study provides a detailed description of FP prevalence at a range of marine turtle study sites in Queensland, Australia. We report that FP prevalence in Queensland varies between sites and years, with juvenile turtles the most frequently affected by FP. This study highlighted significant differences in survey methods with respect to FP detection, and this must be considered when interpreting results. The spatial distribution of FP in Queensland described in this study can be used to improve existing management plans through more site-specific approaches to abating this threat. We also investigated statistically significant relationships between water quality and FP prevalence in green turtles foraging along the Queensland coast. However, despite an extensive data set, no clear trends or statistical relationships between FP prevalence and the WQIs developed in this study were determined. This may have been due to a range of limitations in the data sets and/or in the development of the WQIs. During the development of the WQI, it became clear that there was limited agreement in the methodology used by different agencies monitoring water quality along the Queensland coast. These findings highlight significant deficiencies in the current monitoring of water quality in this region which need to be addressed to improve the management of all organisms inhabiting the Queensland coast, including the endangered green turtle.

Our results show that FP was considerably more common in juveniles than in adult-sized turtles. Reports of FP in sub-adults were most frequent in Moreton Bay between 1990 and 2002. Notably, the two sites examined in this study that were situated well offshore (Heron Island and Howick Group) recorded very low incidences of FP. Further, at Heron Island, despite a significant number of juvenile turtles ($n = 1047$) sampled, FP was never recorded in a juvenile turtle at this site. However, the overall trends observed in this study regarding FP distribution among age-classes are consistent with other reports of FP within these regions (Limpus et al., 2005; Limpus et al., 2016; Limpus & Miller, 1994) and around the world (Adnyana et al., 1997; Ene et al., 2005; Herbst, 1994; Herbst & Klein, 1995; Page-Karjian et al., 2014; Patrício et al., 2012; Work et al., 2004).

The consistent reports of FP predominantly affecting juvenile green turtles raises questions regarding what factors of this stage in their lifecycle may increase their susceptibility to FP. There is a high probability that the small juveniles are first exposed to FP factors as they recruit from the pelagic foraging life history phase in oceanic water into the benthic foraging life history phase in shallow nearshore habitats (Limpus et al., 2005) and by the time they reach adulthood, they have either died from the disease or recovered. Alternatively, upon recruitment to inshore areas from their pelagic existence, green turtles in Australia undergo an ontogenetic shift in diet (Arthur, Boyle, & Limpus, 2008). It may be possible that stress from the dietary shift from an omnivorous to herbivorous diet in new recruits is associated with the increase in susceptibility to FP (Bolten et al., 2003; Jones & Seminoff, 2013; Kelley et al., 2022). Moreover, as green turtles consume macroalgae, mangrove fruit, and seagrass (Arthur et al., 2009; Brand-Gardner et al., 1999; Prior et al., 2015; Read & Limpus, 2002), either food source or the combination of both could be contributing to this susceptibility.

Significant differences in rates of FP prevalence were observed between survey methods, with FP-targeted surveys detecting FP at much higher rates than comprehensive population surveys. The comprehensive population surveys target all age-classes of turtles at a location to better understand and describe the demographics of the population. Conversely, the FP-targeted surveys in this study target juvenile turtles as they are most likely to be afflicted with this disease. These surveys are conducted on a smaller spatiotemporal scales than the comprehensive population surveys and each turtle captured is specifically checked for tumors. As FP predominantly affects juvenile turtles, the FP-targeted survey data are biased toward detecting FP while the detection rate in the comprehensive population surveys may be lower. This inherent bias, coupled with the variation in methods and temporal scales, make it difficult to draw accurate conclusions from this data set. While the comprehensive population surveys are arguably more reliable for drawing conclusions on populations as a whole, the FP-targeted survey data highlight that FP is present in higher numbers at certain specific sites and can be detected if a targeted approach is used (Figure 2). For example, at Edgecumbe Bay the comprehensive population surveys reported a prevalence of only 0.7%, while FP-targeted surveys reported a prevalence of 7.9%. However, the comprehensive population survey and FP-targeted survey at this site differed in sample size ($n = 1386$ and $n = 541$, respectively), temporal scale (17 years and 8 years, respectively), and survey method (whole population and targeted subset, respectively). Thus, the contrast between comprehensive population surveys and FP-targeted surveys makes it difficult to compare prevalence values. Yet, the number of individual turtles captured with FP reported by the FP-targeted survey was more than four times greater than that of the comprehensive population survey ($n = 43$ and $n = 10$, respectively). It is likely that the true prevalence lies somewhere between those reported from each survey type. This disparity could be addressed by employing a systematic guide to data collection using the minimum standards proposed by Hargrove et al. (2016). Some of these standards include, individual turtle identification (flipper tags, photo ID, PIT tags, etc.), standard measurements (length and weight), presence/absence of tumors, tumor severity, body condition, oral examination, method of capture, and effort (Hargrove et al., 2016).

High prevalence of FP in green turtles were found at Cockle Bay, Edgecumbe Bay, and Moreton Bay (Table 2), and within all three of these sites small areas with an increased likelihood of encountering the disease in captured turtles were noted. In Cockle Bay, turtles with FP were more likely to be found in a narrow section of the northern end of the bay while at Edgecumbe Bay, higher numbers of turtles with FP were captured in Brisk Bay, a small bay within Edgecumbe Bay. Moreton Bay has been reported as an average across a very diverse study site. While FP turtles were more frequently captured in the southern region of this bay, local knowledge suggests that FP occurrence at the Blue Pool-Henderson's gutter drainage within the Moreton Banks is consistently elevated well above the mean prevalence reported here. Unfortunately, some of this detail is minimized as a result of pooling across sub-habitats with differences in age-class composition. Temporal spikes in FP are also more difficult to detect in the grouped data set. For example, the age-class data subset which allowed for temporal analysis showed that up to 29% of sub-adults sampled at Moreton Bay in 2002 had FP tumors. However, the overall mean FP prevalence for green turtles at Moreton Bay between 1990 and 2014 was less than half of the 2002 prevalence (10.5%). Nonetheless, distinct localized incidences of FP are reported in this study. It is unclear whether these locations are the site of FP infection, or a refuge for infected turtles. The presence of such trends within a larger region highlights that although areas appear similar in regards to both environmental conditions and in proximity to human habitation, there may be some unique local character(s) that make them stand apart from the rest of that region. It also highlights the importance of a high geographic resolution in the capture data to differentiate the site of capture on a local scale. Recently, Jones et al. (2021) were able to separate FP prevalence on both fine and broad scales, highlighting the power of a high quality data set. Though challenging, future studies in Australia would benefit from addressing this gap.

Although our attempts to quantify a relationship between FP prevalence and the WQIs developed for this study were inconclusive, these results have implications for marine turtles and the Queensland coast as a whole. The inability to quantify a relationship between these factors may have been due to a range of limitations, including data gaps, varying temporal scales, and methods in both the FP prevalence and water quality data sets. The design of the WQIs, which were not able to account for several factors which have been suggested to influence FP development, may have also hampered our efforts to investigate this relationship. Variables that could not be included in the WQI scores include: seagrass coverage (Bell & Ariel, 2011; Meager & Limpus, 2012), xenobiotics (Heffernan et al., 2017), co-factor interaction (Jones et al., 2016) and temporal variation in water quality; possible seasonal influences in FP prevalence (Herbst, 1994), seasonal influence on water quality from discharge events into catchments of interest (Waterhouse et al., 2017), population density, and water circulation. It is also possible that any relationship between FP prevalence and water quality is caused by a single variable whose effect has been lost by grouping it into the broader category. Moreover, several of the water quality variables which were included in the WQIs were also highly confounded, which restricted our ability to narrow down the cause of any correlations detected.

The lack of integration between some of the research and monitoring programs conducted along the Queensland coast is a significant management challenge. These programs have largely been designed to address and report on specific issues, locations, or management initiatives, which often precludes the ability to source and use data from different programs. Access to a single long-term water quality monitoring data set for the expanse of the Queensland coast may have alleviated some of the limitations in this study's WQI development, but no such data set exists. There is also significant variation in reporting and outputs are challenging to interpret as there are no set guidelines or minimum standards which can be used to assess the status of water quality along the Queensland coast (Brodie & Waterhouse, 2012). The Reef 2050 Integrated Monitoring and Reporting Program (RIMReP), a key component of the Reef 2050 Long-Term Sustainability Plan (Great Barrier Reef Marine Park Authority & Queensland Government, 2015), aims to drive the coordination, alignment, and integration of existing monitoring, modeling, and reporting programs conducted on the GBR and its catchments. Such integration would capitalize on existing program investment, provide value for money, improve efficiency, and avoid duplication of effort. The inconclusive results from the present study underpin the value of RIMReP and highlight the need for integration and cross-disciplinary studies. Though expert opinion was employed in the present study to overcome the data availability challenges, it is hoped that improved coordination and internationally agreed guidelines for such programs will decrease the reliance of expert opinion in future work.

5 | FUTURE DIRECTIONS

Future programs would benefit from the adoption of targeted and consistent survey methods for sampling marine turtles and determining FP prevalence data. Long-term studies aimed to better understand temporal trends in FP prevalence along the Queensland coast will require this standardized monitoring to produce rich data sets. This will improve the reliability of the reported FP prevalence and assist managers to make more informed decisions regarding conservation efforts and the implementation of management measures. In order to establish links between specific environmental contaminants and FP prevalence, future studies should consider collecting long-term water quality reference samples alongside marine turtle surveys to better understand (or determine) whether a causal relationship exists between water quality and the prevalence of FP. This would allow direct comparison of FP prevalence and water quality using consistent methods and temporal scales. Consistent and centralized water quality monitoring along the expanse of the Queensland coast is also an essential component of effective management and needs to be addressed.

AUTHOR CONTRIBUTIONS

Karina Jones and Ellen Ariel conceived the study and Karina Jones, Ellen Ariel, Limpus Colin J., Shum Edith; and Ian P. Bell collected the data. Karina Jones, Brodie Jon, and Jones Rhondda advised on data analysis; and Karina Jones, Brodie Jon, Jones Rhondda, Limpus Colin J., and Read Mark advised on the interpretation of results. Karina Jones, Ellen Ariel, and Read Mark contributed to writing the original draft of the manuscript. All authors reviewed, edited, and accepted the final version of the manuscript.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data associated with this study are published herein.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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