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RESEARCH ARTICLE

Milani Chaloupka · George Balazs

Modelling the effect of fibropapilloma disease on the somatic growth dynamics of Hawaiian green sea turtles

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Abstract The effect of the tumour-forming disease, fibropapillomatosis, on the somatic growth dynamics of green turtles resident in the Pala'au foraging grounds (Moloka'i, Hawai'i) was evaluated using a Bayesian generalised additive mixed modelling approach. This regression model enabled us to account for fixed effects (fibropapilloma tumour severity), nonlinear covariate functional form (carapace size, sampling year) as well as random effects due to individual heterogeneity and correlation between repeated growth measurements on some turtles. Somatic growth rates were found to be nonlinear functions of carapace size and sampling year but were not a function of low-to-moderate tumour severity. On the other hand, growth rates were significantly lower for turtles with advanced fibropapillomatosis, which suggests a limited or threshold-specific disease effect. However, tumour severity was an increasing function of carapace size—larger turtles tended to have higher tumour severity scores, presumably due to longer exposure of larger (older) turtles to the factors that cause the disease. Hence turtles with advanced fibropapillomatosis tended to be the larger turtles, which confounds size and tumour severity in this study. But somatic growth rates for the Pala'au population have also declined since the mid-1980s (sampling year effect) while disease prevalence and severity increased from the mid-1980s before levelling off by the mid-1990s. It is unlikely that this decline was related to the increasing tumour severity because growth rates have also declined over the last 10–20 years for other green turtle populations resident in Hawaiian waters that have low or no disease prevalence. The declining somatic growth rate trends evident in the Hawaiian stock are more likely a density-dependent effect caused by a dramatic increase in abundance by this once-seriously-depleted stock since the mid-1980s. So despite increasing fibropapillomatosis risk over the last 20 years, only a limited effect on somatic growth dynamics was apparent and the Hawaiian green turtle stock continues to increase in abundance.

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M. Chaloupka (✉)
Ecological Modelling Services Pty Ltd,
University of Queensland, PO Box 6150, St Lucia,
Queensland 4067, Australia
E-mail: m.chaloupka@uq.edu.au
Fax: +61-7-33657299

G. Balazs
NOAA National Marine Fisheries Service,
Pacific Islands Fisheries Science Center,
2570 Dole Street, Honolulu,
HI 96822-2396, USA

Introduction

The green turtle (*Chelonia mydas*) is a threatened marine turtle species with a broad pantropical distribution and distinct regional metapopulation substructures (Bowen et al. 1992). The green turtle is the most abundant large long-lived marine herbivore (Bjorndal 1997) and has been subject to a long history of human exploitation for meat and eggs (Parsons 1962). An emerging worldwide threat to green turtles is fibropapillomatosis (Herbst 1994), which is a pandemic tumour-forming disease that is commonly associated with herpesviruses (Jacobson et al. 1991; Quackenbush et al. 1998; Herbst et al. 1999). Fibropapillomatosis is a debilitating disease that involves proliferation of multiple connective tissue tumours on or in the eyes, mouth, nasal passages, skin, carapace, plastron and visceral organs (Herbst 1994; Aguirre et al. 1998, 2002). The prevalence of this disease has increased significantly over the last 25 years, especially in green turtle populations in eastern Australia, Indonesia, Florida, the West Indies and Hawai'i (Balazs 1991; Herbst 1994; Limpus et al. 1994; Adnyana et al. 1997; Aguirre et al. 1998; Quackenbush et al. 2001).

The Hawaiian green turtle stock comprises a spatially disjunct metapopulation with numerous coral reef and

coastal foraging grounds within the Hawaiian Archipelago, which comprises 132 islands and reefs spanning 9° latitude and 2,400 km (Fig. 1). Fibropapilloma-free green turtles recruit to these foraging grounds from ca. 35 cm straight carapace length (SCL) after a developmental period of ca. 6 years in the northern Pacific Ocean (Zug et al. 2002). The disease is apparently contracted following recruitment from an oceanic habitat to various neritic habitats throughout the Archipelago (Balazs 1991). Fibropapillomatosis is most prevalent amongst immature green turtles resident in coastal habitats around the southeastern part of the island chain (Balazs 1991; Balazs et al. 1998, 2000; Murakawa et al. 2000).

It is widely held that the disease causes depressed somatic growth for green turtles (Balazs 1991; Balazs et al. 1998; Zug et al. 2002), and many turtles that wash ashore or strand in the southern Hawaiian islands are emaciated and have the disease (Murakawa et al. 2000; Work et al. 2004). Fibropapillomatosis is also often considered to be the primary cause of mortality for many green turtles that strand in the Hawaiian Archipelago (Aguirre et al. 1998). However, while clearly identified as a major hazard (Herbst 1994; Aguirre et al. 1998), the effect of fibropapillomatosis on sea turtle population dynamics is not well understood (Herbst 1994; Herbst and Klein 1995; Adnyana et al. 1997). In fact there are no reliable estimates of mortality or depressed reproductive rates attributable to the disease for any sea turtle population and there are only a few studies of the effects of the disease on sea turtle somatic growth dynamics (Balazs et al. 1998, 2000).

We present a robust statistical analysis of the somatic growth dynamics of green turtles resident in one of the major Hawaiian foraging ground populations that is seriously afflicted with fibropapillomatosis. The sampled population was resident in the Pala'au foraging grounds that are located on the south side of the island of Moloka'i, Hawai'i (Fig. 1), where there has been a high prevalence of the disease since the late 1980s (Balazs et al. 1998). Our analysis provides a basis for developing an understanding of the effect of fibropapillomatosis on green turtle somatic growth dynamics, which are yet to be considered in stochastic simulation modelling of green turtle demography (Chaloupka and Musick 1997; Chaloupka 2002, 2004).

Materials and methods

Data set and sampling design

The data set comprised 198 growth records for 172 green turtles from the Hawaiian genetic stock that were tagged in a long-term capture-mark-recapture programme between 1983 and 1998 (Balazs and Chaloupka 2004b). This specific green turtle foraging ground sample was from Pala'au, which is a coastal, algal and seagrass habitat on the south side of Moloka'i (Fig. 1). The Pala'au green turtle population has a much higher prevalence of fibropapillomatosis than most other well-studied foraging ground populations that comprise the Hawaiian green turtle genetic stock (Balazs et al. 1998; Balazs and Chaloupka 2004b). Capture and recapture of

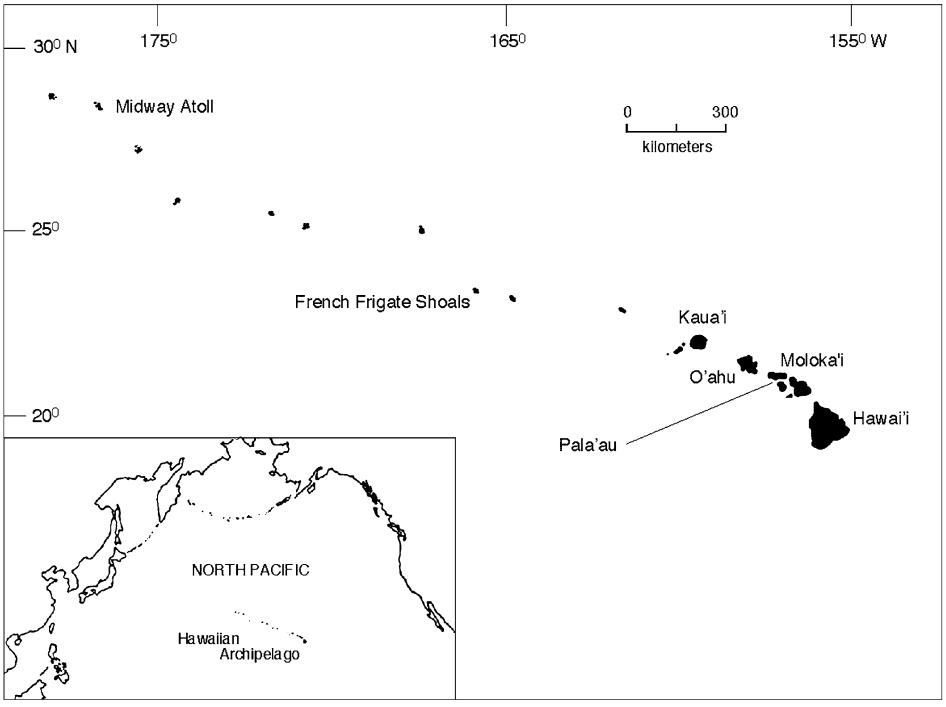

Fig. 1 *Chelonia mydas*. Location of the Pala'au foraging ground sampling site in the Hawaiian Archipelago. The major rookery of the Hawaiian green turtle stock is at French Frigate Shoals located in the middle of the Archipelago. See Balazs and Chaloupka (2004b) for more details of other foraging ground populations comprising the Hawaiian green turtle stock

green turtles from the Pala'au population were undertaken using a specialised non-selective bullpen net (Balazs et al. 1998). Prior to 1996, all turtles were double-marked with uniquely coded Inconel flipper tags but since then the turtles were double-marked with passive integrated transponder (PIT) tags. Chaloupka and Musick (1997) provide a review of sea turtle tagging issues and the potential sources of tag loss. There is no evidence of any significant tag loss from the Pala'au capture-mark-recapture programme. Details of the capture, handling, measurement and tagging methods for this study can be found in Balazs and Chaloupka (2004b). The data included the growth records spanning the post-oceanic phase from 39 cm to ca. 86 cm SCL (ca. 6–70 kg, see Balazs and Chaloupka 2004b) with 12% of turtles recaptured on two or more of the 16 annual sampling occasions. Hence, the implicit sampling design comprised mixed longitudinal sampling with repeated growth measurements for some turtles, which confounds year and cohort effects since age was unknown for all individuals (Chaloupka and Musick 1997).

Capture–recapture profiles recorded for each turtle included the following—carapace size recorded to the nearest millimetre as straight carapace length (SCL, cm) at first capture and recaptures using a metal caliper marked in 0.1 cm intervals, year of first capture and years-at-large since first capture or previous recapture. Only turtles with recapture intervals >12 months were included to minimize the effect of measurement error on growth rate estimation. Recapture intervals ranged from 1 year to 13 years with the median recapture interval ca. 3 years. Absolute growth rates were derived from the capture–recapture profiles with negative or zero growth rates included since there is no reason to do otherwise (Limpus and Chaloupka 1997).

Statistical modelling approach

The growth rate predictors (covariates) used here were (1) mean sampling year and (2) mean carapace size or length (SCL, cm) recorded from capture and subsequent next recapture and (3) an ordinal 4 level fibropapilloma tumour severity score (FPS 0–3), where 0 = no tumours and 3 = severe affliction (Balazs 1991; Work and Balazs 1999; Coberley et al. 2001). The FPS is a readily measured qualitative indicator of disease severity that correlates with a broad range of pathologic, haematologic and physiologic parameters that reflect deteriorating immunocompetence or health status of Hawaiian green turtles with an increasing tumour affliction (Aguirre et al. 1995; Work and Balazs 1999; Aguirre and Balazs 2000; Work et al. 2001, 2003, 2004).

The size effect (carapace length) was included here, as it is known that growth rates for the Pala'au population are a significant nonlinear function of size (Balazs and Chaloupka 2004b). Body mass (kg) could have also been used as a metric of somatic growth in addition to carapace length but there are insufficient weight measurements available for green sea turtles in the Hawaiian Archipelago (Balazs and Chaloupka 2004b). The sampling year covariate reflects the calendar year of each growth rate estimate and was included here to account for the implicit time-dependent sampling design. Year effect is also a source of growth variability due to environmental factors but is confounded with cohort effects because of the mixed longitudinal sampling design with unknown age inherent in this study (Chaloupka and Musick 1997). Moreover, year effect is imprecise because not all growth records were for 1 and only 1-year duration. Recall that the median recapture interval was ca. 3 years. Nonetheless, the year covariate as defined here is a useful proxy of the year effect and should be included as it is a sampling design constraint inherent in capture-mark-recapture programmes (Chaloupka and Musick 1997). The mean size covariate is the arithmetic mean of the carapace size at first capture and subsequent recapture and is the appropriate metric for indexing size-specific growth (Chaloupka and Limpus 1997).

We modelled the functional relationship between the 198 growth rates (response variable) recorded from 172 individual turtles and three growth rate predictors (FPS, carapace size, sampling year) using a Bayesian generalised additive mixed model (GAMM) regression modelling approach (Fahrmeir and Lang 2001). This approach enables robust analysis of regression models with parametric or fixed effects (FPS), nonparametric or nonlinear covariates (carapace size, sampling year) and nonnormal error terms. Turtle-specific random effects were included to account explicitly for individual heterogeneity and potential correlation inherent in the mixed longitudinal data set. This model is specifically a Bayesian semiparametric GAMM regression (Fahrmeir and Lang 2001).

We also modelled the functional relationship between FPS recorded for each turtle at capture and all recaptures and two predictors of fibropapillomatosis risk (carapace size, sampling year) using a Bayesian GAMM regression. This model comprised nonlinear and turtle-specific random effects but no fixed effects and so is a Bayesian nonparametric GAMM regression. This model is similar to the generalised additive models or GAM regressions (Hastie and Tibshirani 1990), which have been used in other sea turtle growth studies that did not explicitly account for random effects inherent in mixed longitudinal sampling designs (Chaloupka and Limpus 1997; Limpus and Chaloupka 1997; Bjorndal et al. 2000; Seminoff et al. 2002).

The GAMM regressions used here were fitted in a Bayesian inference framework with program BayesX using Markov chain Monte Carlo (MCMC) simulation techniques (Brezger et al. 2003). Bayesian regression modelling via MCMC simulation methods is the only practical approach for simultaneous estimation of fixed, nonparametric (nonlinear) and random effects (Lang and Sunder 2003). The Bayesian GAMM regression models estimated here comprised (1) an identity link, (2) a Gaussian error function and (3) Bayesian cubic

P-splines with random walk smoothness priors (Fahrmeir and Lang 2001) to model the functional form between response variables (growth rates, FPS) and nonlinear predictors or covariates (carapace size, sampling year). A thorough account of penalised B-splines (P-splines) with application to GAM regressions can be found in Marx and Eilers (1998). The important extension to Bayesian P-splines within the GAMM regression modelling framework was given by Lang and Brezger (2004).

All unknown model parameters are assumed to be stochastic random variates in Bayesian modelling and so prior (initial assumed) distributions must be specified. We used diffuse or noninformative priors for the fixed parameters and second-order random walk smoothness priors for the nonlinear functions, which are common priors used in nonparametric Bayesian regression modelling (Fahrmeir and Lang 2001; Lang and Sunder 2003). We assumed that the turtle-specific parameters were independent Gaussian with a common variance (Lang and Sunder 2003). All estimated parameter effects were evaluated by fitting a series of models of increasing complexity to determine if inclusion of that parameter improved the model fit. Model selection was then based on the deviance information criterion (DIC) approach proposed for Bayesian statistical models in which the best-fit model was indicated by the lowest DIC (Speilgelhalter et al. 2002). The posterior distribution of the parameter effects for all models was sampled from 50,000 MCMC iterations following an initial burn-in period of 2000 iterations (Brezger et al. 2003). Inference concerning the parameter effects was then based on the posterior distribution of each parameter derived from the best-fit model, which was then summarised using the posterior mean, standard deviation, median (50% quantile), and 95% credible intervals based on the posterior 2.5% and 97.5% quantiles.

Results

Bayesian model summaries

The best-fit Bayesian GAMM regression model for evaluating the somatic growth rates as a function of various covariates was Model 4, which comprised (1) significant fixed effect (FPS), (2) significant nonlinear or nonparametric effects (carapace size, sampling year) and (3) a significant turtle-specific or random effect (Table 1). Overall, this semiparametric model was an adequate fit but there was still significant unexplained variability, which was indicated by a posterior mean deviance = 473.1 that was larger than the sample size = 198 (Speilgelhalter et al. 2002). Some of the unexplained variability is probably due to sex-specific effects that cannot be included here since the gender of each turtle could not be determined. As shown elsewhere, accounting for sex-specific differences in growth rates usually results in a substantial improvement in the fit of nonparametric models for sea turtle growth studies (Chaloupka and Limpus 1997; Limpus and Chaloupka 1997; Bjorndal et al. 2000; Chaloupka et al. 2004). The estimated fixed parameters for the FPS effect on somatic growth rates as well as the variance estimates for the nonlinear (size, year) and turtle-specific random effects from Model 4 are summarized in Table 2. The functional form of the nonlinear size-specific growth effect is shown in Fig. 2a with the functional form of the nonlinear year-specific growth effect shown in Fig. 2b. The fixed fibropapillomatosis-dependent growth effect is shown in Fig. 2c. The Bayesian GAMM fit for evaluating FPS as a function of carapace length, sampling year and turtle-specific random effects is shown in Fig. 3. Overall, this nonparametric model was an adequate fit for this data set but there was still significant unexplained variability (posterior mean deviance = 549.2, sample size = 198). Again inclusion of potentially informative covariates such as gender might improve model fit.

Table 1 *Chelonia mydas*. Summary of Bayesian semiparametric regression model fits to the growth rates data ($N = 198$) for the 172 individual turtles resident in the Pala'au foraging ground located on the southern coast of Moloka'i (Hawai'i)

Model	Nonlinear effects		Fixed effect	Random effect	Deviance	enp	DIC
	Size	Year	FPS	Turtle			
1	Yes	Yes	No	No	497.9	8.4	506.3
2	Yes	Yes	No	Yes	491.4	12.2	503.6
3	Yes	Yes	Yes	No	484.9	11.3	496.2
4	Yes	Yes	Yes	Yes	473.9	17.6	491.5

Each of the listed models shows the four possible effects that were included in the respective model. Comparative model fit assessed using the deviance information criterion or DIC = posterior mean deviance + enp, where enp = effective number of parameters. Best-fit model indicated by the lowest DIC (underlined). FPS = fibropapilloma tumour severity score and size = SCL in cm

Table 2 *Chelonia mydas*. Summary of constant parameter estimates for Bayesian semiparametric regression model 4 fitted to the Pala'au growth data (see Table 1)

Parameter	Mean	SD	*t*-value	Posterior quantiles		
				2.5%	Median	97.5%
Intercept	1.764	0.105	16.72	1.561	1.761	1.970
FPS (1 vs. 0)	0.072	0.159	0.45	−0.239	0.069	0.399
FPS (2 vs. 0)	−0.126	0.173	−0.73	−0.464	−0.124	0.232
FPS (3 vs. 0)	−0.737	0.208	−3.52	−1.140	−0.735	−0.331
Year variance	0.004	0.006	0.79	0.001	0.003	0.020
Size variance	0.006	0.008	0.77	0.001	0.004	0.029
Turtle variance	0.027	0.064	0.41	0.001	0.005	0.255

Mean = posterior mean, SD = posterior standard deviation, *t*-value = mean/SD. FPS (1 vs. 0) = regression model term for fibropapilloma tumour severity score 1 referenced to the no tumour score (FPS = 0) where a *t*-value > 2 based on the posterior mean and SD indicates a significant difference assuming that the parameter samples were drawn from a Gaussian probability density function. Not all parameters are well summarised using a Gaussian probability density function (e.g., variance parameters), so inference based on the posterior quantiles and 95% credible intervals is more appropriate

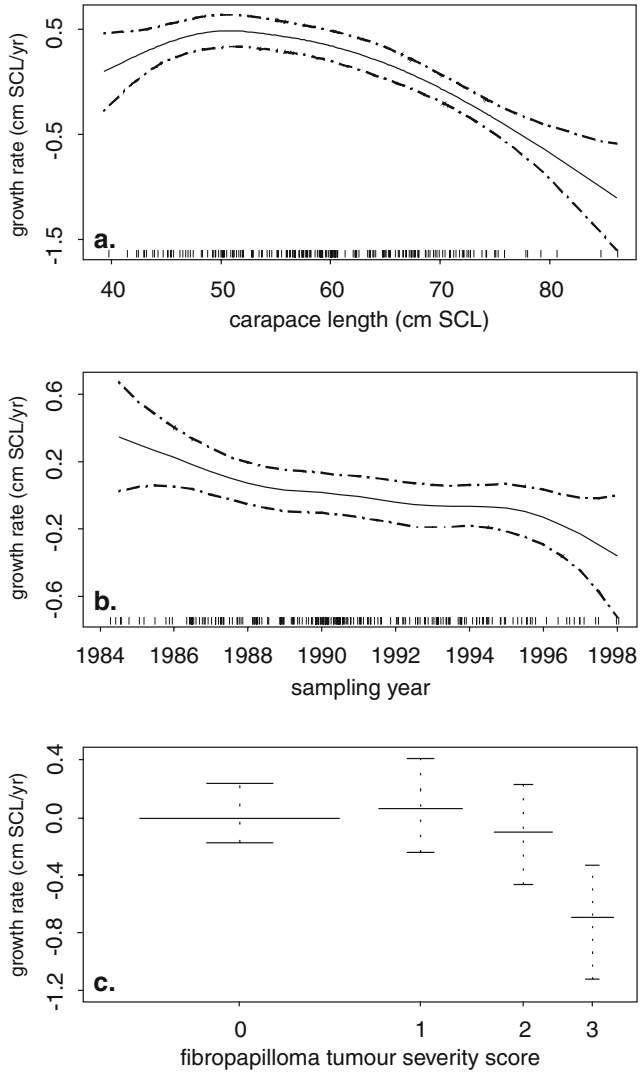

Fig. 2 *Chelonia mydas*. Graphical summary of Bayesian semiparametric GAMM fit for the Pala'au population growth rates conditioned on three predictors (carapace length, sampling year, fibropapilloma tumour severity score or FPS) and turtle-specific random effects (Model 4 in Table 1). The response variable (growth rate in cm SCL/year) is shown on the *y*-axis in each panel as a centred scale to ensure valid pointwise 95% credible intervals and comparison between the covariates across the three panels). The expected size-specific growth function (**a**) is shown by the *solid curve* and a 95% Bayesian credible interval by the *dashed curves*. The expected year-dependent growth function is shown in **b**. The *vertical bars* on the topside of the lower *x*-axis of **a**, **b** are known as a rug, which shows the data distribution within each panel. The width of the FPS categories in **c** are proportional to sample size with the 95% credible interval for each category shown by the *cross bars* based on the 2.5% and 97.5% quantiles summarised in Table 2

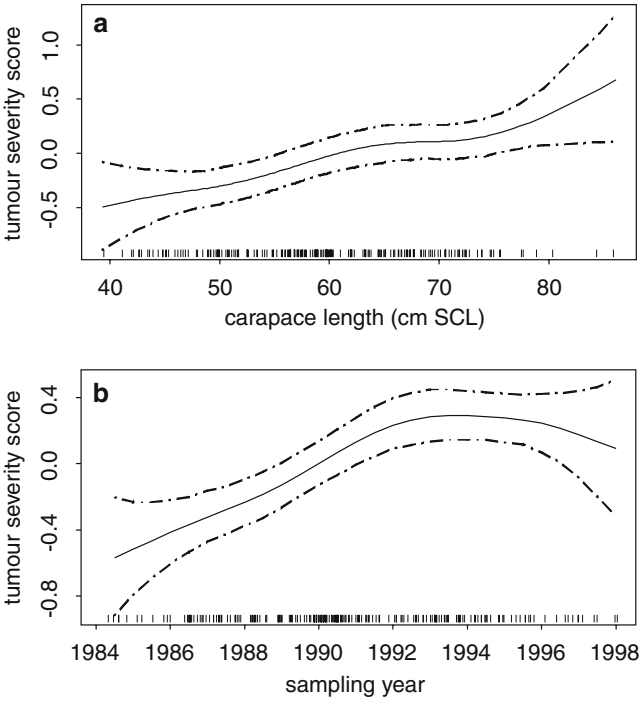

Fig. 3 *Chelonia mydas*. Graphical summary of a Bayesian nonparametric GAMM fit for the Pala'au population tumour scores conditioned on two predictors (carapace length, sampling year) and turtle-specific random effects. The response variable (fibropapilloma tumour severity score: FPS 0–3) is shown on the *y*-axis in each panel as a centred scale to ensure valid pointwise 95% credible intervals and comparison between the covariates across the two panels. The expected size-specific tumour severity function (**a**) is shown by the *solid curve* and a 95% Bayesian credible interval by the *dashed curves*. The expected year-dependent FPS function is shown in **b**. The data distribution within each panel is shown by the *rug or vertical bars* on the topside of the lower *x*-axis

Size-, year- and turtle-specific growth effects

Carapace size, sampling year and repeated growth measurements on some Pala'au green turtles (turtle-specific random effect) were all sampling design constraints included in the Bayesian models used to evaluate any disease effect on somatic growth dynamics. Green turtles from the Pala'au population display a distinct dome-shaped or nonmonotonic size-specific growth rate function that reflects an immature growth spurt ca. 50 cm SCL (Fig. 2a), which is characteristic of the foraging populations comprising the Hawaiian green turtle stock (Balazs and Chaloupka 2004b). A 50 cm SCL green turtle from the Hawaiian stock is ca 18 kg (Balazs and Chaloupka 2004b). Green turtles from all the Hawaiian stock hatch at ca. 5 cm SCL and then spend ca. 6 years growing up in an oceanic habitat prior to recruitment to a coastal or neritic habitat from ca. 35 cm SCL (Zug et al. 2002). Growth rates then increase rapidly after a number of years in the neritic habitat, peaking at ca. 50 cm SCL, then followed by declining growth rates approaching the onset of sexual and physical maturity at a carapace size >80 cm SCL (Fig. 2a). The expected somatic growth rate function for the Pala'au population was not only size-specific (Fig. 2a) but also was a significant nonlinear declining function of sampling year since the mid-1980s (Fig. 2b). A year-effect indicates significant inter-annual growth variability inferring an environmental effect on immature growth since turtles display negligible growth fol-

lowing the onset of adult size from 80 cm SCL onwards (Fig. 2a). The posterior mean variances of the size- and year-specific nonlinear growth effects are similar, irrespective of whether the posterior mean or median was used, and are smaller than the variance for the turtle-specific effect that reflects individual growth heterogeneity (Table 1). Including a turtle-specific or individual random effect in Model 4 was shown to improve model fit (Table 1). However, the magnitude of this parameter was smaller when evaluated using the median and 95% credible interval rather than the posterior mean (Table 2). This was because the probability density function of the 50,000 iterations for the turtle-specific variance was not Gaussian but was heavily right skewed. All other parameters in the best-fit model were adequately summarised by a Gaussian probability density function (Table 2).

Fibropapillomatosis-dependent growth effect

There was a significant difference in expected growth rates between fibropapilloma tumour severity scores FPS = 3 and FPS = 0 (the reference category) but not between any of the other tumour severity scores (Fig. 2c). In other words, only turtles with advanced fibropapillomatosis or severe tumour affliction (FPS = 3) exhibited slower growth rates compared to turtles with no tumour affliction (FPS = 0). The 95% credible interval for the fibropapilloma-dependent growth effect shown in Fig. 2c was based on the posterior 2.5% and 97.5% quantiles, as the turtle-specific random effect was shown to be nonnormal and better summarised by the posterior quantiles rather than the posterior mean and standard deviation (Table 2). However, the same conclusion holds when using standard *t*-value inference based on the ratio of the posterior mean and the standard deviation sampled from the 50,000 MCMC iterations (Table 2).

Size- and year-specific tumour severity

While somatic growth rates were a function of advanced fibropapillomatosis (FPS = 3, Fig. 2c), the tumour severity score (FPS) was itself a significant size-specific function. Larger turtles (> 65 cm SCL) were more likely to have advanced fibropapillomatosis than smaller turtles (Fig. 3a). Larger turtles may have more severe tumours because they are presumably older and so have had a longer exposure to the factors that cause the disease. Hence, the Pala'au turtles with advanced fibropapillomatosis or severe tumour affliction tended to be the larger turtles, which confounds the effects of size and FPS in our study. Much larger samples from the Pala'au population that comprise a greater proportion of larger turtles are needed to resolve this important issue. Recall that there were very few large turtles in the sample (Fig. 2a). Meanwhile, disease severity increased during the mid-1980s and early 1990s before levelling off by the mid-1990s (Fig. 3b), which is consistent with a slowing of the green turtle strandings rate recorded in the Hawaiian Archipelago since the early 1990s (Fig. 4). Recall that many green turtle strandings in the Archipelago are widely assumed to be directly attributable to fibropapillomatosis (Aguirre et al. 1998).

Discussion

The major findings derived from this long-term capture-mark-recapture study of green turtle growth dynamics for the Pala'au, Molokai population were (1) significant nonlinear size- and year-specific growth rate functions, (2) limited or threshold-specific fibropapilloma tumour severity effect on size-specific somatic growth that might be confounded with sampling effects and (3) a significant nonmonotonic long-term trend in fibropapilloma tumour severity since the mid-1980s.

Size and year effects

Carapace size and sampling year covariates were significant nonlinear predictors of somatic growth rates for the Pala'au population (Fig. 2a, b) and in fact were part of the sampling constraints imposed on the data set. Hence, inclusion of both effects in the Bayesian GAMM was necessary prior to evaluating whether there was an effect of FPS on somatic growth rates. This is important because the FPS used here (Balazs 1991; Work and Balazs 1999) was itself found to be a function of carapace size (Fig. 3a) and sampling year (Fig. 3b). Aguirre and Balazs (2000) and Work et al. (2001, 2003) also noted that FPS was a function of carapace size for Hawaiian green turtles from a Kane'ohe Bay, O'ahu population (Fig. 1). Moreover, some haematologic parameters of healthy sea turtles are also size-dependent (Frair and Shah 1982) so that clinical pathology and somatic growth studies need to account for size differences when evaluating any functional relationship between such parameters and FPS.

Interestingly, the nonlinear size- and year-effect functions derived here using a Bayesian GAMM approach (Fig. 2a, b) are very similar to the nonlinear functions derived by Balazs and Chaloupka (2004b) for the same sample that did not explicitly account for multiple growth measurements on some turtles. In other words, accounting explicitly for turtle-specific random effects, which did improve model fit (Table 1), apparently had no significant effect on the estimation of the size- and year-specific somatic growth rate functions for the Pala'au population. This finding supports the use of the simpler generalised additive model (GAM) approach for modelling sea turtle growth that was proposed by Chaloupka and Limpus (1997).

Our primary reason for using a more complex Bayesian GAMM approach that requires MCMC simulation was to estimate a mixed nonparametric model that accounted explicitly for turtle-specific random effects. However, if inclusion of random effects has little effect on estimation, for example, of the nonlinear size-specific growth rate function, then the simpler GAM approach may well suffice for mixed longitudinal sampling designs commonly used in sea turtle growth studies (Chaloupka and Musick 1997).

Fibropapilloma disease effect

The etiologic agent or causal process that underlies fibropapillomatosis in green turtles has yet to be established but it is most likely a herpes virus (Jacobson et al. 1991; Quackenbush et al. 1998; Lackovich et al. 1999; Herbst et al. 1999). Elsewhere, green turtles contract other infectious herpes virus diseases such as lung-eye-trachea disease but there is no apparent relationship with fibropapillomatosis, which suggests different causal processes (Coberley et al. 2001). Green turtle fibropapillomatosis was first reported in Hawai'i in 1958 but the disease incidence has increased significantly since the early 1980s (Balazs 1991; Murakawa et al. 2000). The disease has reached epidemic proportions in some Hawaiian foraging grounds such as Kane'ohe Bay on O'ahu and Pala'au on Moloka'i (Brill et al. 1995; Balazs et al. 1998; Murakawa et al. 2000). Balazs et al. (1998) in a previous study of the Pala'au green turtle population found that somatic growth was a linear declining function of FPS while Balazs et al. (2000) found no disease effect for the Kane'ohe Bay population. However, neither study accounted for confounding factors and sampling design constraints when evaluating differences in expected or mean growth rates between tumoured and nontumoured turtles.

Furthermore, there is no association between the geographic or spatial pattern of disease prevalence and somatic growth rates throughout the Hawaiian Archipelago (Balazs and Chaloupka 2004b). For instance, low disease prevalence has been recorded for the Midway foraging ground (Balazs and Chaloupka 2004b) in the northwestern end of the Archipelago (Fig. 1). Yet, the Midway green turtle population is characterised by slower somatic growth at any carapace size compared with all other green turtle foraging ground populations that have been sampled in the Archipelago (Balazs and Chaloupka 2004b). Moreover, there is little or no disease prevalence in the southeastern end of the Archipelago along the western shore of the island of Hawai'i (Fig. 1), where size-specific somatic growth is similar to the populations with high disease prevalence such as Kane'ohe Bay and Pala'au (Balazs and Chaloupka 2004b).

The fibropapilloma tumour severity score (FPS 0–3) used in our study has been shown to reflect a range of haematologic parameters that are apparently indicative of disease chronicity and severity (Work and Balazs 1999; Aguirre and Balazs 2000). Increasing FPS is also apparently associated with increasing bacterial and vascular trematode parasite loads and depressed immune response that could further compromise the health status of Hawaiian green turtles (Aguirre et al. 1998; Work et al. 2001, 2003). Yet, we found only a limited disease-dependent or a threshold-specific FPS effect on somatic growth for the Pala'au turtle population (Fig. 2c) when using a robust regression modelling approach to control for size, sampling year and individual turtle heterogeneity (Table 2). A threshold-specific effect was apparent because only turtles with advanced fibropapillomatosis or severe tumour load (FPS = 3) displayed depressed somatic growth rates compared to turtles with no tumours (FPS = 0) or only a low-to-moderate affliction (Fig. 2c).

The conclusion of a threshold-specific disease effect must be regarded with some caution for the following reasons. Firstly, the number of green turtles with advanced fibropapillomatosis in this study was very small ($N = 22$) so that larger samples or similar findings of a threshold-specific effect for other populations are needed to support a strong inference about disease effects on green turtle somatic growth rates. Secondly, the tumour severity score (FPS 0–3) was itself a significant size-specific function where larger turtles (> 65 cm SCL) were more likely to have advanced fibropapillomatosis than smaller turtles (Fig. 3a). Tumour severity scores are probably higher in larger turtles because these individuals are presumably older and so have had longer exposure to the factors that cause the disease. Hence, the Pala'au turtles with advanced fibropapillomatosis or severe tumour affliction tended to be the larger turtles, which confounds size and FPS in our study.

Nevertheless, Hawaiian green turtles with advanced fibropapillomatosis are blood protein and iron deficient with low cholesterol and triglyceride levels (Aguirre and Balazs 2000; Work et al. 2001), which are indicators of chronic stress, starvation and immunosuppression given exposure to an infectious disease (Aguirre et al. 1995; Aguirre and Balazs 2000; Work et al. 2001, 2003). Hence the threshold-specific FPS effect on somatic growth for the Pala'au population (Fig. 2c) is consistent with haematologic and physiologic expectations. However, there are no significant differences in behavioural factors such as local movement and habitat use between tumoured and nontumoured Hawaiian green turtles in the Kane'ohe Bay population (Brill et al. 1995). Nor, apparently, is there any disease effect on breeding behaviour of Hawaiian green turtles as this once-depleted stock is now increasing at a much faster rate than previously expected for a green turtle metapopulation (Balazs and Chaloupka 2004a).

Fibropapilloma severity trend

Fibropapillomatosis has been considered a major cause of mortality for immature green turtles that stranded

around the main islands in the southeastern end of the Hawaiian Archipelago (Aguirre et al. 1998, Work et al. 2004). The disease incidence has increased since the early 1980s in the Pala'au population (Balazs et al. 1998; Murakawa et al. 2000). The disease severity also increased for this population from the early 1980s before levelling off by the early 1990s (Fig. 3b) while the disease prevalence has declined in recent years (Balazs et al. 1998; Balazs unpublished). The rate of increase of the immature green turtle strandings in the southern Archipelago has also slowed since the late 1980s (Fig. 4), which might reflect a declining rate of exposure to the factors that cause the disease or a declining rate of infection as disease resistance accumulates in the population. Hence it is unlikely that the strandings rate is a reflection of the tumour severity trend shown in Fig. 3b that levels off much later than the strandings trend shown in Fig. 4. Moreover, as mentioned, the once-depleted Hawaiian green turtle stock is well on the way to recovery (Balazs and Chaloupka 2004a); thus, green turtle strandings around the main islands would be expected to increase initially as the stock recovers and then level off as the stock approaches foraging habitat carrying capacity in the southern Archipelago. The trend in number of immature green turtle strandings in the southeastern part of the Archipelago (Fig. 4), which implies a slowing of the rate of increase in the stranding rate, is consistent with the strandings expectation for a recovering stock.

The reason as to why the fibropapilloma tumour severity increased initially and then levelled off after about 10 years is unclear but possibly reflects a cohort-specific rather than a year-specific effect whereby a similar age (size) cohort of immature green turtles was exposed to a single disease outbreak in the early 1980s, with tumour severity increasing over time for afflicted turtles as they aged. Previous and subsequent cohorts of immature turtles were not exposed to the factors causing the disease and so the tumour severity levelled off or even decreased (Fig. 3b) as the infected cohort either declined due to mortality or recovered from the disease. In fact, some cases of tumour regression are well known for Hawaiian green turtles (Balazs et al. 2000; Bennett et al. 2000) and for other sea turtle species (Herbst 1994; Aguirre et al. 1999). Recall that the implicit sampling design in this study comprised mixed longitudinal sampling with repeated growth measurements for some turtles, which confounds year and cohort effects since age was unknown for all individuals (Chaloupka and Musick 1997). If age was known, then it might be possible to unravel the temporal trends underlying fibropapillomatosis progression in a green turtle population using nonparametric age-year-cohort regression models (Heuer 1997). Chaloupka and Limpus (1998) used such models to evaluate the temporal trends in green turtle survival probabilities where age was defined as years since first capture. It might be worthwhile exploring the use of a Bayesian age-year-cohort GAMM to help develop a better understanding of fibropapillomatosis progression within the Hawaiian green turtle stock.

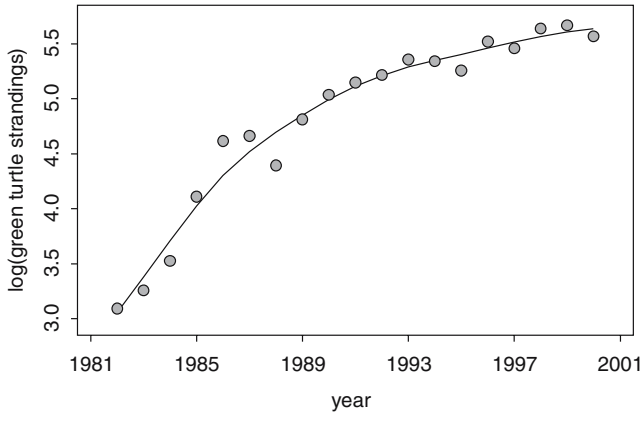

Fig. 4 *Chelonia mydas*. The annual number of green turtle strandings recorded since 1982 (*solid dots*) around the main islands in the southeastern part of the Hawaiian Archipelago—note that the log scale reflects the change in the rate of strandings. A smoothing spline (*solid curve*) has been superimposed to highlight the underlying trend. The rate of increase of the strandings has slowed since the mid- to late-1980s with the annual number of recorded strandings apparently starting to level out somewhere ca. 300–350

In conclusion, the Hawaiian green turtle stock has increased substantially since the mid-1980s (Balazs and Chaloupka 2004a) and continues to do so despite long-term exposure of some local populations to the fibropapilloma disease. Although turtles with advanced fibropapillomatosis might be stressed and immunocompromised, there are no apparent behavioural effects of the disease on Hawaiian green turtles (Brill et al. 1995). So it seems unlikely that fibropapillomatosis will slow the current recovery of this once-depleted stock. Nevertheless, ongoing monitoring of the health or disease status of the Hawaiian green turtle stock is warranted as it is possible that the disease effects have yet to be manifest given the long age to maturity estimated for this stock (Zug et al. 2002; Balazs and Chaloupka 2004b).

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