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Estimation of Green Turtle (*Chelonia mydas*) Growth Rates from Length-Frequency Analysis

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Length-frequency analysis (using the computer program MULTIFAN) was used to estimate growth rates and number of age classes in a population of immature green turtles (*Chelonia mydas*) at Great Inagua in the southern Bahamas. Results from MULTIFAN were compared with growth rates estimated from nonlinear regression analysis on capture-recapture data and with calculated growth rates for 5-cm carapace length increments for the same population of green turtles. MULTIFAN underestimated growth rates of the smaller green turtles but yielded a better estimate of asymptotic size than did the nonlinear regression analysis. Estimates generated by MULTIFAN and by nonlinear regression analysis for the number of years it takes a green turtle at Great Inagua to grow from 30 to 70 cm carapace length fell within the 95% confidence limits of the calculated growth data from capture-recapture data. When the number of length-frequency samples was reduced sequentially from 10 years to two years, MULTIFAN was successful in estimating the number of age classes in the population for each of the nine sample sets. Length-frequency analysis shows promise as a method to rapidly estimate the number of year classes in a population of immature sea turtles and to estimate von Bertalanffy growth parameters.

LENGTH-FREQUENCY analysis has been used for many years to estimate growth rates, age structure, and mortality in fish populations (Ricker, 1975). The development of computer software for the analysis of length-frequency data has resulted in a rapid increase in the use of this technique (Pauly and Morgan, 1987; Terceiro et al., 1992). Length-frequency analysis relies on the assumption that length frequencies have modes, each of which represents a single age class. The modes are usually most distinct at young ages. When the modes are identified, the mean length of each age class in a population can be determined, and growth models may be fit to the lengths-at-age data. Although a single sample of length frequencies may be analyzed in this manner, the evaluation of multiple, sequential samples from the same population allows a more powerful analysis by following modes through the samples in a time series.

Most studies of growth rates in wild sea turtles have been based on long-term, labor-intensive, capture-recapture studies (Balazs, 1982; Frazer and Ehrhart, 1985; Limpus, 1992). Two of us (KAB and ABB) are conducting one such long-term study with green turtles (*Chelonia mydas*) in the southern Bahamas (Bjorndal and Bolten, 1988, 1989; Bolten et al., 1992). In that study, immature green turtles are captured on their foraging grounds during an approximate two-week period each year.

In this paper, we analyze length frequencies of green turtles sampled annually from 1983 to 1992 at Great Inagua, Bahamas, with the computer program MULTIFAN (Fournier et al., 1990, 1991). We then compare the results of MULTIFAN length-frequency analysis with those obtained from our capture-recapture study of tagged green turtles in the same population over the same time period. This comparison allows us to determine whether length-frequency analysis is a reliable method for estimating sea turtle growth rates.

Methods

Green turtles were captured over their foraging pastures of the seagrass *Thalassia testudinum* on the north coast of Great Inagua, Bahamas, by chasing them briefly with a motor boat and then jumping onto them from the bow of the boat. Turtles were measured, marked in the trailing edge of their flippers with tags bearing identification numbers, and released at site of capture. The measurement used in this study is standard straight-line carapace length (SCL, from nuchal notch to posterior tip of posterior marginal) measured with anthropometer calipers (GPM model 101) to the nearest 0.1 cm. The study area has been described in previous publications (Bjorndal, 1980; Bjorndal and Bolten, 1988).

MULTIFAN (version 3.10; Otter Research

Ltd., Nanaimo, British Columbia, Canada) uses a maximum likelihood method to estimate the parameters of the von Bertalanffy growth model and the number of age classes (modes) in the sample population (Otter Research Ltd., 1992). The von Bertalanffy growth equation as modified by Fabens (1965) is:

$$L_r = L_\infty - (L_\infty - L_c)e^{-Kd} \quad (1)$$

where L_r is length at recapture, L_∞ is asymptotic length, L_c is length at capture, e is the base of the natural logarithms, K is an intrinsic growth rate variable, and d is the time interval between capture and recapture.

MULTIFAN requires that the following parameters be specified: expected number of age classes; expected initial K values; mean length of the mode representing the youngest age class; and standard deviation of a distinct mode. We let the number of age classes be 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 years, and let initial values of K be 0.01, 0.05, 0.1, and 0.5. The mean length of youngest age class was set to 27.5 cm (in 1988 sample), and standard deviation of mode width was set to 1.5 for all initial values of age classes and K. Because initial MULTIFAN runs indicated that there were significant length-dependent trends in standard deviation of length-at-age, this parameter was included in the model for all analyses reported here.

To test for the effect of decreasing the number of sampling years, MULTIFAN analyses were repeated as most recent sampling years were successively dropped from the time series. That is, analyses were conducted on time series with 10 samples (1983–1992) down to two samples (1983–1984). MULTIFAN requires at least two length-frequency distribution samples. Estimated initial values of number of age classes, K, and standard deviation of a distinct mode remained the same. Once the 1988 sample was omitted, the mean length of youngest age class was set to 29.5 cm (in 1984 sample).

To test the importance of including the month with the youngest animals in the analysis, MULTIFAN analyses were repeated on all sample years except 1988 (the sample with the youngest turtles) with identical estimated initial values except that mean length of youngest age class was set to 29.5 cm (in 1984 sample).

Growth curves were generated from the capture-recapture data ($n = 524$) with two methods. First, time intervals (mean and 95% confidence limits) between 5-cm SCL increments were calculated from mean growth rates for each 5-cm size class for our capture-recapture study. Throughout this paper, this procedure will be referred to as the MEASURED growth rates. Second, capture-recapture data were fit to a von Bertalanffy model (Equation 1) by nonlinear regression (SAS PROC NLIN; SAS Institute, 1982). This second procedure will be referred to as SASNLIN throughout this paper.

Von Bertalanffy growth curves generated from length-frequency data (MULTIFAN) and from capture-recapture data (SASNLIN) were plotted using the standard von Bertalanffy equation:

$$L_t = L_\infty(1 - be^{-Kt}) \quad (2)$$

where L_t is length at age t, b is a variable related to size at birth, and other variables are as in Equation 1. The values for b were calculated by solving Equation 2 using length at hatching (age 0) equal to 5.0 cm (Hirth, 1980).

The number of years that it takes a green turtle to grow from 30 to 70 cm was calculated by setting L_c equal to 30 and L_r equal to 70 and solving for d in Equation 1. The ability of MULTIFAN and SASNLIN to estimate growth rates of green turtles at Great Inagua was evaluated by determining whether the results of these analyses fell within 95% confidence limits of MEASURED growth data.

Results

The length-frequency distributions, sample sizes, and month of capture for 1983–1992 are shown in Figure 1. Numbers of turtles in each length-frequency sample varied from 45–152. The sample from April 1988 contained the youngest animals.

The estimates of number of age classes, L_∞, and K generated by MULTIFAN and selected by the maximum likelihood function to represent the best fit to the length-frequency data for all years are presented in Table 1. The year class modes assigned by MULTIFAN as the best fit for all years are superimposed on the length frequencies in Figure 1.

The growth curves from length-frequency analysis (MULTIFAN), nonlinear regression (SASNLIN) of capture-recapture data, and MEASURED mean growth rates for 5-cm SCL increments from the capture-recapture data over the size range of turtles measured in this study (30–70 cm) are shown in Figure 2. Values for the MEASURED 5-cm incremental growth rates are plotted as upper and lower 95% confidence limits. The SASNLIN growth curve falls within the 95% confidence limits of MEASURED data throughout the size range. Estimates of the number of years it takes Great Inagua green turtles to grow from 30 to 70 cm generated from the two von Bertalanffy curves

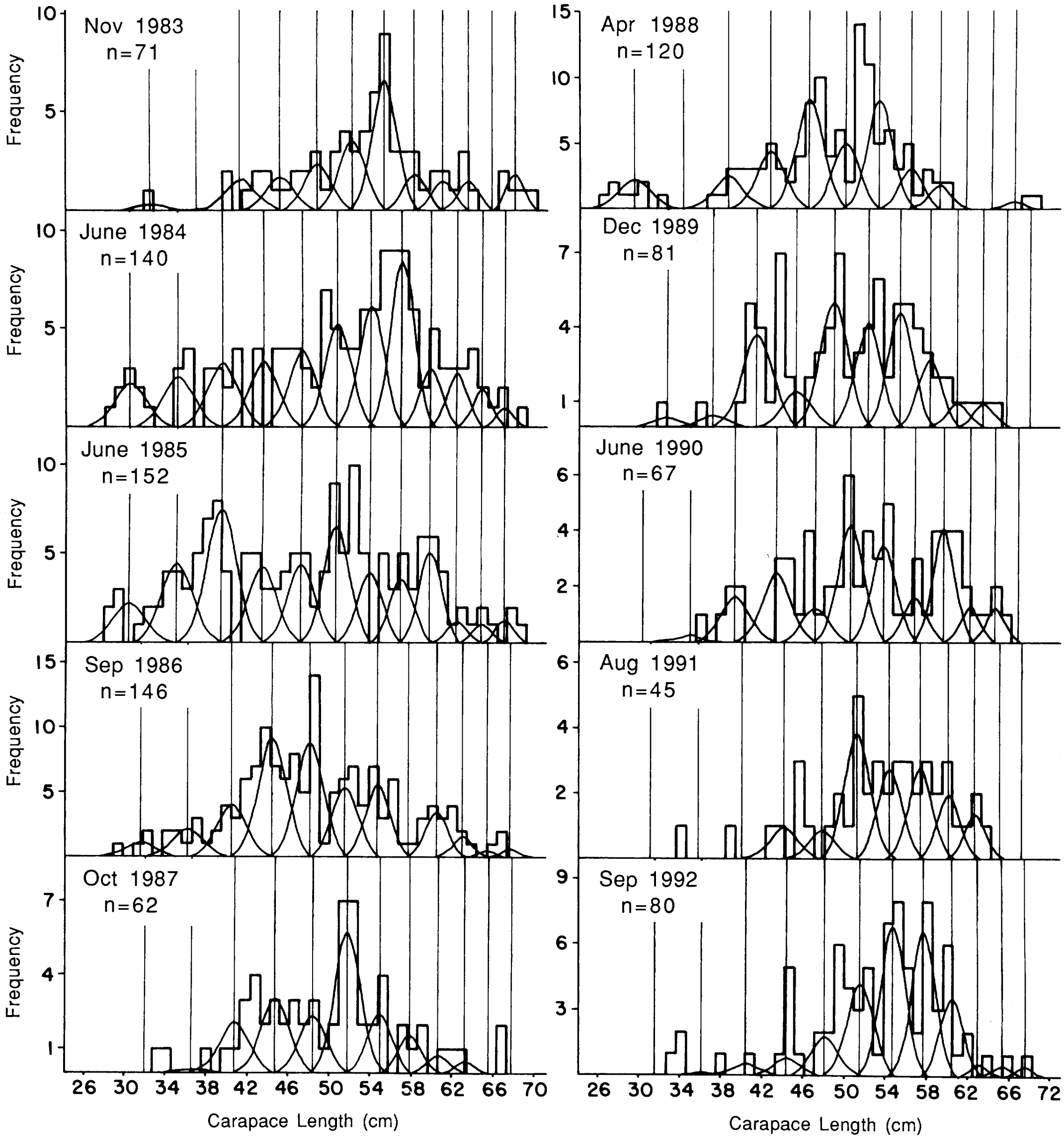

Fig. 1. Length-frequency distributions (straight carapace length) of green turtles at Great Inagua, Bahamas, for each of the 10 sample years. Curves represent modes assigned by length-frequency analysis (MULTIFAN); vertical lines indicate mean value of each mode.

(MULTIFAN and SASNLIN) fall within the 95% confidence limits of the MEASURED data (Fig. 2; Table 1).

The growth curve generated by MULTIFAN from data from all 10 years did not fall completely within the 95% confidence interval of the MEASURED growth data (Fig. 2). The growth in smaller size classes was underestimated with MULTIFAN. However, the estimate of asymptotic size (98.3 cm) was a more reasonable value than the estimate from SASNLIN analysis and fell above the 95% confidence interval for the SASNLIN estimate (72.33–76.17).

If the analysis requires data from a period of 10 years, as that presented here, the application of length-frequency analysis will be limited. Results of MULTIFAN analyses on data with successively fewer number of sample years are presented in Table 1. The growth curves from these MULTIFAN analyses are compared with the 95% confidence limits of the MEASURED data in Figure 3. Growth curves for all years and nine years were identical (Fig. 3) because the

TABLE 1. PARAMETER ESTIMATES FROM LENGTH-FREQUENCY ANALYSIS (MULTIFAN) FOR THE YEARS INDICATED, FROM NONLINEAR REGRESSION ANALYSIS (SASNLIN) OF CAPTURE-RECAPTURE DATA AND FROM CALCULATED GROWTH RATES FOR 5-cm SCL INCREMENTS (MEASURED). N is sample size; number of modes is only determined by MULTIFAN; years 30–70 cm is number of years to grow from 30 to 70 cm; 95% confidence interval is in parentheses for MEASURED.

Method	n	No. of modes	von Bertalanffy			Years 30–70 cm
			L_∞	K	b	
MULTIFAN						
All years	964	12	98.3	.074	.949	11.96
9 years	884	12	98.3	.074	.949	11.96
8 years	839	12	99.4	.072	.950	12.03
7 years	772	13	92.6	.082	.946	12.29
6 years	691	15	168.0	.025	.970	13.51
5 years	571	12	82.2	.122	.939	11.91
4 years	509	11	158.6	.035	.968	10.79
3 years	363	11	162.8	.033	.969	11.12
2 years	211	12	84.4	.114	.941	11.55
Recoded	964	12	98.7	.073	.949	11.95
SASNLIN						
All years	524	—	74.25	.180	.933	13.05
MEASURED						
All years	524	—	—	—	—	13.04 (10.76–18.38)

parameter estimates were the same (Table 1). The seven- and eight-year growth curves were very similar to the curve for all years (Fig. 3). Curves for the six-, four-, and three-year samples were characterized by higher asymptotes. Curves for two- and five-year analyses fell close to or within the 95% confidence limits across the size range (Fig. 3). Estimates of the number of years it takes green turtles to grow from 30 to 70 cm for each of the MULTIFAN analyses fall within the 95% confidence limits of the MEASURED data (Fig. 3; Table 1). However, estimation of asymptotic size was less accurate with fewer length-frequency samples (Table 1). For analyses with six or fewer years, estimates of asymptotic size were either unacceptably high (six years, four years, three years) or low (five years and two years).

For MULTIFAN analysis, the number of length-frequency samples may be more important than the number of years over which the samples are collected. MULTIFAN assumes that there is one period of recruitment to the population each year and analyzes modal shifts over an annual cycle. This was demonstrated by reanalyzing our 10 samples as if they were collected during a single year (assignment of month remains the same), so that the data include one sample from April, three from June, one from Aug., two from Sept., one from Oct., one from Nov., and one from Dec. collected during one year. The results generated by MULTIFAN are in Table 1 for the "Recoded" sample. The similarity of these results to those of the all-years sample, and the fact that the estimation of the number of years between 30 cm and 70 cm SCL falls within the 95% confidence interval of the MEASURED growth data, suggest that a series of length-frequency samples collected over a one-year period may be sufficient to estimate the number of year classes in a sea turtle population. However, this conclusion must be tested.

MULTIFAN analysis on all years except 1988 (the sample with the youngest turtles) yielded parameter estimates (12 modes, L_∞ = 98.1, K = 0.074) very similar to those generated for all years (Table 1). Therefore, it is not necessary to have a length-frequency distribution from the month in which the youngest animals are recruited into the population. It is only necessary that, of those months included in the sample, the month with the youngest animals be correctly identified.

DISCUSSION

Von Bertalanffy models have been used in several studies to describe sea turtle growth and have a better fit than other growth equations

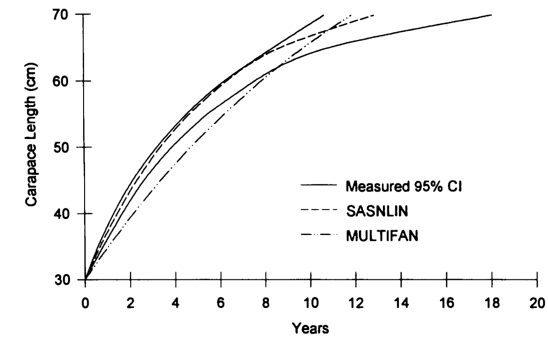

Fig. 2. Growth curves for green turtles from 30 to 70 cm straight carapace length (SCL) at Great Inagua, Bahamas, generated from length-frequency analysis (MULTIFAN), nonlinear regression analysis (SASNLIN), and mean growth rates for 5-cm SCL increments (MEASURED). MEASURED curves are plotted as 95% confidence interval (95% CI).

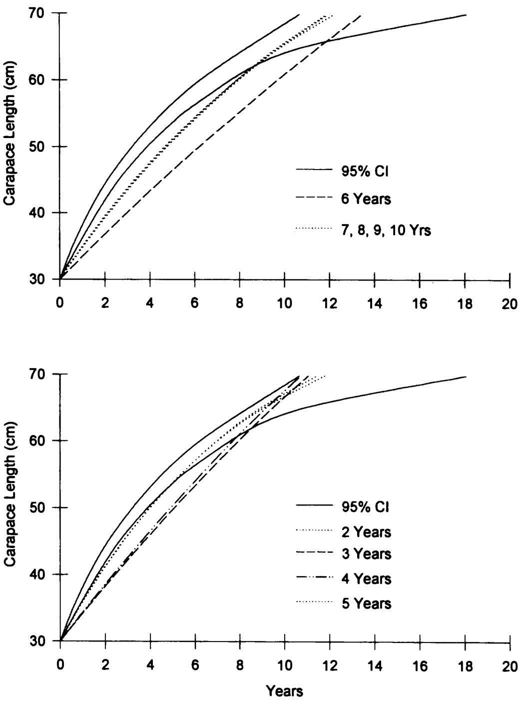

Fig. 3. Growth curves for green turtles from 30 to 70 cm straight carapace length at Great Inagua, Bahamas, generated from length-frequency analysis (MULTIFAN), for six-, seven-, eight-, nine-, and 10-year samples and for two-, three-, four-, and five-year samples. Because the curves for seven-, eight-, nine-, and 10-year samples and the curves for two- and five-year samples are indistinguishable in their respective figures, they are plotted with the same line style. For comparison, 95% confidence interval (95% CI) for MEASURED data are plotted.

(e.g., logistic, Gompertz) to data from populations of wild, immature sea turtles (Frazer and Ehrhart, 1985; Frazer and Ladner, 1986; Bjorndal and Bolten, 1988). In this study, SASNLIN successfully modeled growth of green turtles at Great Inagua between 30 cm and 70 cm straight carapace length (SCL).

However, the estimate of L_∞, or asymptotic size, of 74.25 cm from SASNLIN was clearly an underestimate. Hirth (1980) gave a range of mean values of SCL of adult Caribbean green turtles as 100–109 cm. Asymptotic values should be greater than the mean adult size. The underestimate of the asymptote in our study is not surprising. Witzell (1980) and Frazer et al. (1990) demonstrated that L_∞ will often be underestimated if large turtles are not included in the sample population. Large subadult green turtles move out of our study area, and, therefore, turtles larger than 70–75 cm are not included in our data.

We have demonstrated that length-frequency analysis is a valuable tool for modeling sea turtle growth rates and estimating the number of age classes present in a population. Our results indicate that data from many years are not required for length-frequency analysis to successfully estimate von Bertalanffy growth parameters. Thus, size frequency data for length-frequency analysis can be collected much more rapidly than capture-recapture data.

As has been discussed for nonlinear regression analysis of turtle growth data (Bjorndal and Bolten, 1988; Boulon and Frazer, 1990; Frazer et al., 1990), extrapolating beyond the size range included in the study may yield unreliable results in length-frequency analysis. Estimates of asymptotic size (which is beyond the size range of the population analyzed in this study) are less consistent than are estimates of number of year classes included in the size range measured for the population.

We want to encourage caution in the application of this analysis to sea turtle populations. First, despite the widespread use of length-frequency analysis, its use is controversial (Hilborn and Walters, 1992).

Second, MULTIFAN requires that initial values of several parameters be set. The success of the MULTIFAN analyses in this study is, to at least a certain extent, a result of our knowledge of this population after working with it for nearly 20 years. For example, correct designation of the month with the youngest (not necessarily the smallest) animals is critical to MULTIFAN analysis. We could be confident that the April 1988 sample contained the youngest turtles, not only because it was the month with the smallest turtles, but also because of plasma color. We often collect blood samples from the turtles we capture and centrifuge the blood to separate the cell fraction from the plasma. In green turtles that are feeding on an herbivorous diet, the plasma is bright yellow from the plant pigments (Nakamura, 1980). The smallest green turtles in the April 1988 sample had clear plasma, indicating that they had not yet shifted to a plant diet and had probably just recruited from pelagic to benthic foraging grounds (Bolten and Bjorndal, 1992).

Third, length-frequency analysis may be less successful with populations that include very large turtles than it was in this study of a population in which the largest turtles had carapace lengths of 70 cm. Because rate of growth in carapace length decreases with increasing length in green turtles (Frazer and Ehrhart, 1985; Bjorndal and Bolten, 1988), age class modes will lie closer together for large size classes and be more difficult to distinguish in populations that include large sea turtles.

Despite these caveats, length-frequency analysis is potentially a valuable method for the study of growth in sea turtles. Length-frequency analysis would be particularly useful in sea turtle tagging studies with low recapture rates (e.g., in pelagic areas) and for studies that involve terminal sampling, such as carcasses stranded on beaches and sea turtles harvested from foraging populations.

More studies are needed to evaluate the application of length-frequency analysis to sea turtle populations. Studies in which results of length-frequency analysis can be compared to measured growth rates or age structures will be of greatest value. Also, populations that include large subadult or adult turtles should be evaluated.

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