Loggerhead turtles (*Caretta caretta*) in Florida Bay: An assessment of origin based on genetic markers

Prepared by
Anna L. Bass[1],
Meredith Clinton[2],
and
Brian W. Bowen[1]

2 January 1998

[1] Department of Fisheries and Aquatic Sciences
7922 NW 71st Street
Gainesville, Florida 32653

[2]Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

Introduction

Advances in the application of genetic techniques to the study of marine turtles have resulted in the acquisition of more information pertaining to aspects of their natural history (Bowen et al., 1992), systematics (Karl and Bowen, 1998; and behavior (FitzSimmons, 1996; Peare & Parker, 1996). In addition genetic markers provide a means of assessing the population structure and composition of foraging grounds. Previous investigations have examined the contributions of loggerhead nesting locations to foraging areas (Bowen et al., 1995; Bolten et al., 1997; Rankin-Baransky, 1997). This report includes results from an analysis of loggerhead turtles (Caretta caretta) from Florida Bay, to estimate contributions of regional rookeries to this major foraging area. This research is intended to provide critical demographic information for the management of loggerhead turtles, and to allow the development of hypotheses concerning developmental habitats and movement between such habitats during various life-history stages.

Materials and Methods

Blood (~1 ml) was taken from the cervical sinus region of captured animals (Owens & Ruiz 1980), placed in 9 ml of lysis buffer (100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; pH 8.0), and stored at room temperature (White and Densmore, 1992). DNA isolations were conducted using standard phenol/chloroform methodology (Hillis et al., 1996). A 391 bp fragment of the mitochondrial DNA control region was amplified with polymerase chain reaction methodology (PCR; Mullis and Faloona, 1987) using primers TCR-5 and TCR-6 of Norman et al. (1994) and the following temperature regime: 95(1 min) and 35 cycles of 93(30s)+55(15s)+72(30s) and a 3 min extension at 72. Standard precautions, including negative controls (template-free PCR reactions), were used to test for contamination in the PCR reaction.

Cycle sequencing was conducted with an ABI Prism kit and florescently labeled dideoxynucleotides at the University of Florida DNA Sequencing Core and the labeled extension products were analyzed with an automated DNA sequencer (Applied Biosystems model 373A) in the DNA Sequencing Core at the University of Florida. Sequences were aligned and corrected using Sequencher (v.3.0). Misalignments were corrected by visual inspection and removal of unnecessary gaps. Polymorphic sites were identified within the sample of 80 individuals and then compared to polymorphic sites identified at nesting locations by Encalada et al. (1998). Sequences which matched known haplotypes were collated for analysis whereas unique haplotypes were re-sequenced to assure the accuracy of haplotype designations.

To test for statistical differences among haplotype frequencies at rookeries and the foraging ground, Chi-square tests were performed with the program CHIRXC and probabilities were generated using Monte Carlo randomization (Zaykin and Pudovkin, 1993). Relative contributions of the surveyed Atlantic-Mediterranean loggerhead turtle rookeries in Northwest Florida, Southwest Florida, Southeast Florida, Northeast Florida, Georgia, South Carolina, North Carolina, Yucatan (Mexico), Bahia (Brazil) and Kiparrisia Bay (Greece) [see Encalada et al. (1998) for location information] were estimated using the maximum likelihood (ML) programs UCON and GIRLSEM (see Masuda et al., 1991). These programs use an iterative algorithm to estimate the most likely contributors to a mixed population based on the haplotype frequencies in the source populations (nesting colonies) and the mixed population (foraging ground). Standard deviations were generated using the infinitesimal jackknife procedure in UCON and GIRLSEM. The difference between these two programs is that UCON uses an iteratively re-weighted least squares approach to converge upon the maximum likelihood estimate while GIRLSEM uses an EM algorithm (Pella and Milner, 1987).

In the original survey of loggerhead turtle nesting locations by Encalada et al. (1998), the haplotype frequencies of nesting populations at several locations were not significantly different from each other as determined by a G-

test of independence. As a result, geographic locations were grouped into regional population units and these units were used as the potential source populations in the maximum likelihood analysis and chi-square tests. As a consequence there are six identified population (or management) units that could potentially contribute to the Florida Bay foraging area: Northwest Florida (NWFL), South Florida (SFL), North Carolina to Northeast Florida (NC-NEFL), Mexico, Brazil, and Greece.

Results and Discussion

All of the 80 samples sequenced matched previously identified loggerhead haplotypes. Individual haplotypes, cross-referenced to the field tag numbers, are given in Table 1. Four Atlantic-Mediterranean loggerhead haplotypes were identified in the 80 individuals sampled (Table 2).

Table 2. Florida Bay loggerhead turtle haplotypes and management units where these haplotypes are found (Encalada et al., 1998).

Haplotype	# of individuals	Management Unit(s)
A	36	NWFL, SFL, NC-NEFL
B	39	NWFL, SFL, NC-NEFL, MEXICO, GREECE
C	3	NWFL, SFL, MEXICO
J	2	MEXICO

The Florida Bay loggerhead foraging population was significantly different from five of the six management units. Chi-square results indicate that the haplotype frequencies of the foraging ground population is not significantly different from the haplotype frequencies of the South Florida management unit although probability of homogeneity was low (p=0.535).

Both UCON and GIRLSEM were used to estimate the contributions from management units under the null hypothesis that all management units, as defined by Encalada et al., (1998) contribute equally to the Florida Bay loggerhead foraging population (see Chapman, 1996). The two types of ML analyses did not produce significantly different results and indicate that the major contributor to this foraging area is the South Florida (SFL) management unit (84% ± 13%). Only two other management units contributed at detectable levels using GIRLSEM; North Carolina to Northeast Florida at 8% (±10%) and Mexico at 8% (±6%).

Table 3. ML estimates of the contributions of management units to the Florida Bay loggerhead foraging population.

Source Management Unit	GIRLSEM (+ S.D.)	UCON (+ S.D.)
NWFL	0.000000 (0.000000)	0.000004 (0.000002)
SFL	0.835397 (0.132036)	0.841334 (0.123941)
NC-NEFL	0.083678 (0.104478)	0.082129 (0.101584)
MEXICO	0.080919 (0.056079)	0.076307 (0.051003)
BRAZIL	0.000000 (0.000000)	0.000000 (0.000000)
GREECE	0.000005 (0.000018)	0.000226 (0.000153)

Due to the extremely low contribution from the Greece population and evidence indicating that loggerheads from the Mediterranean do not traverse the

Atlantic to forage in US waters (Laurent et al, 1993) , the ML analyses were repeated excluding Greece as a possible source. Removal of the Greece population did not significantly change the estimates. The SFL management unit again contributed the largest percentage to the foraging area and the estimates from NC-NEFL and Mexico remained approximately the same.

Table 4. ML estimates of the contributions of management units to the Florida Bay loggerhead foraging population (excluding Greece).

Source Management Unit	GIRLSEM (+ S.D.)	UCON (+ S.D.)
NWFL	0.000001 (0.000000)	0.000024 (0.000011)
SFL	0.835387 (0.132162)	0.844405 (0.125179)
NC-NEFL	0.083684 (0.104622)	0.079540 (0.102792)
MEXICO	0.080928 (0.056056)	0.076031 (0.050931)
BRAZIL	0.000000 (0.000000)	0.000000 (0.000000)

In conclusion, it appears that the Florida Bay loggerhead foraging population is composed primarily of individuals from the South Florida management unit. Smaller contributions are detected from the Mexican and northern Atlantic (NC-NEFL) nesting populations, but the large standard deviations of these estimates do not allow this conclusion to be stated with finality. We conclude that three sampled nesting populations are represented by individuals found in Florida Bay. The potential for other populations to contribute to this foraging area is a necessary caveat with the occurrence of small (unsampled) nesting locations in Cuba, the Bahamas, and elsewhere. It is possible that a finer scale of resolution may be reached with the use of other molecular techniques and genetic markers. Microsatellites or other rapidly evolving regions of the nuclear genome may provide additional molecular markers to resolve the dispersal of the loggerhead in North American waters.

Literature Cited

Bolten, A.B., K.A. Bjorndal, H.R. Martins, T. Dellinger, M.J. Biscoito, S.E. Encalada, and B.W. Bowen. 1998. Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecol. Appl., *In Press*.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evol. 46:865-881.

Bowen, B.W., F.A. Abreu-Grobois, G.H. Balazs, N. Kamezaki, C.J. Limpus, and R.J. Ferl. 1995. Trans-Pacific migrations of the loggerhead turtle (Caretta caretta) demonstrated with mitochondrial DNA markers. Proc. of the Nat. Acad.of Sci. USA 92:3731-3734.

Chapman, R.W. 1996. A mixed stock analysis of the green sea turtle: the need for null hypotheses. Pp. 139-148 In Bowen, B.W. and W.N. Witzell (eds.) Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical Memorandum. NMFS-SEFSC-396. 173 pp.

Encalada, S.E., K.A. Bjorndal, A.B. Bolten, J.C. Zurita, B. Schroeder, E. Possardt, C.J. Sears, and B.W. Bowen. 1998. Population structure of loggerhead turtle (Caretta caretta) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Mar. Biol., *In Press*.

FitzSimmons, N.N. 1996. Use of microsatellite loci to investigate multiple paternity in marine turtles. Pgs. 69-78 In Bowen, B.W. and W.N. Witzell (eds.) Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical Memorandum. NMFS-SEFSC-396. 173 pp.

Karl, S.A. and B.W. Bowen. 1998. Evolutinary significant units versus geopolitical taxonomy: Molecular systematics of an endangered sea turtle (genus *Chelonia*). Submitted.

Hillis, D.M., B.K. Mable, A. Larson, S.K. Davis, and E.A. Zimmer. 1996. Nucleic Acids IV: Sequencing and Cloning. Pp 321-384 In Hillis, D.M., B.K. Mable, and C. Moritz (eds.) Molecular Systematics, second edition. Sinauer Associates, Sunderland, Massachusetts.

Laurent, L., J Lescure, L. Excoffier, B.W. Bowen, M. Domingo, M. Hadjichristophorou, L. Kornaraki, and G. Trabuchet. 1993. Etude genetique des relations entre les populations Mediterraneenne et Atlantique d'une tortue marine (*Caretta caretta*) a l'aide d'un marqueur mitochondrial. C.R. 'Acad. Sci. Ser. III Sci. Vie 316:1233-1239.

Masuda, M., S. Nelson, and J. Pella. 1991. User's Manual for GIRLSEM and GIRLSYM. USA-DOC-NOAA-NMFS Programs and user manual available from US- Canadian Salmon Program, 11305 Glacier Highway, Juneau, Alaska 99801, USA.

Mullis, K.B., and F. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Meth. Enzymol. 155:335-350.

Norman, J.A., C. Moritz, and C.J. Limpus. 1994. Mitochondrial DNA control region polymorphisms: Genetic markers for ecological studies of marine turtles. Mol. Ecol. 3(4):363-373.

Peare, T. and P.G. Parker. 1996. The use of multilocus minisatellite DNA fingerprinting to examine local genetic structure within green turtle rookeries. Pp 87-94 In Bowen, B.W. and W.N. Witzell (eds.) Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical Memorandum. NMFS-SEFSC-396. 173 pp.

Pella, J.J. and G.B. Milner. 1987. Use of genetic marks in stock composition analysis. Pp. 247-276 In N. Ryman and F. Utter (eds.). Population Genetics and Fishery Management. University of Washington Press, Seattle, WA. 420 pp.

Rankin-Baransky, K. 1997. Origin of loggerhead turtles (*Caretta caretta*) in the western north Atlantic ocean as determined by mtDNA analysis. MSc. Thesis, Drexel University. Philadelphia, Pennsylvania, USA.

White, P.S. and L.D. Densmore. 1992. Mitochondrial DNA isolation. Pp. 29-58 In A.R. Hoelzel (ed.) Molecular Genetic Analysis of Populations: A Practical Approach. IRL Press, Oxford University Press, N.Y. 315pp.

Zaykin, D.V. and A.I. Pudovkin. 1993. Two programs to estimate significance of X2 values using pseudoprobability tests. J. Hered. 84:152.