Mixed stock analysis of juvenile loggerheads (*Caretta caretta*) in Indian River Lagoon, Florida: implications for conservation planning

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Introduction

Conservation of marine animals often is limited by the ability of researchers to identify biological trends and potential threats to organisms that make long distance migrations. For example, salmon production in California may be affected by logging hundreds of miles inland (Caffereta and Spittler 1998) and marine turtle bycatch during Mediterranean shrimping operations decreases breeding populations on nesting beaches in Florida and Mexico (Laurent et al. 1998). Attempts to solve this problem by censusing rookeries, while logistically feasible, fail to incorporate predictive models and primarily record the results of trends displayed by juveniles from distinct foraging areas (termed here as juvenile aggregations) in the prior decade. In the case of Florida marine turtles, the primary measure of population change is the number of nests deposited on a beach, which ignores the effects of juvenile mortality caused by disease (Work 2001), commercial fisheries (Crowder 1995), and pollution on the future breeding population. Understanding the factors dictating juvenile recruitment permits a more forward looking, predictive approach that incorporates the effects of pollution, disease, natural disasters, and commercial fisheries by-catch on juvenile populations to predict future trends. Our detailed mixed-stock analysis of a large juvenile aggregation with several contributing rookeries reveals a novel approach to understanding these factors and describes patterns that are broadly applicable to regional and possibly global loggerhead rookeries.

Loggerheads nest on sandy beaches throughout temperate latitudes. The species was federally listed as threatened in the North Atlantic in 1978 and is a CITES Appendix I listed species. The Marine Turtle Specialist Group and the IUCN Red List consider the loggerhead to be endangered throughout much of its range (Marine Turtle Specialist Group 1996). Atlantic loggerheads leave their nesting beaches and enter oceanic current systems such as the Gulf Stream which later becomes part of the North Atlantic Gyre. After circumnavigating the Atlantic for 3–10 years they recruit to a juvenile foraging area for the next 10–12 years (Carr 1986; Musick and Limpus 1997). Some of the juvenile (foraging) loggerheads in the North Atlantic recruit to juvenile foraging areas in the Azores and Madeira (Bolten et al. 1998), but many individuals enter foraging areas in Indian River Lagoon (IRL) on the east coast of central Florida.

Aggregations of juvenile marine turtles may include individuals from nesting beaches around the globe, but until very recently, biologists had not devised a method for modeling contributions of local rookeries to juvenile aggregations (Norrgard and Graves 1995; Lahanas et al. 1998; Bass

and Witzell 2000; Witzell et al. 2002; Luke et al. 2004). Bowen et al. (2004) suggested that on the scale of the North Atlantic, juvenile loggerhead recruitment to a foraging area was heavily influenced by distance from the natal rookery and natal rookery size. Juvenile loggerheads make two types of migrations, one a reproductive migration and one a migration to a juvenile foraging area proximal to their natal beach (Bowen et al. 2004). Loggerheads nesting in Florida comprise 86% of all nesting by this species on Atlantic beaches (Ehrhart et al. 2003). However, whether juvenile loggerheads from nesting beaches around the world use IRL as a foraging area remained unknown.

The large juvenile aggregations in IRL potentially describe how monitoring trends in juvenile recruitment (e.g., Bowen et al. 2004) can influence marine turtle conservation on the nesting beach. By understanding the mechanisms that influence rookery contributions to a mixed-source juvenile foraging area, and the threats posed to those juvenile aggregations, marine turtles can be managed with a pro-active strategy. Implementing the results of this study into management practices would incorporate predictive demographic data without the lag time required for trends to manifest in nesting adult populations.

Indian River Lagoon comprises more than 1/3rd of Florida's eastern coastline and extends 250 km, from Ponce de Leon Inlet to Jupiter Inlet. The lagoon system spans 5900 km^2 and is the nation's most diverse estuarine system (Dybas 2002). Its waters are shallow (3–4 m deep) and the prevailing current is caused by wind rather than tides (Trocine and Trefry 1993). Canals that drain heavily irrigated farm and livestock lands further inland contribute massive quantities of freshwater to the brackish water system, artificially decreasing its salinity by 20–80% in some areas (Trocine and Trefry 1993). This freshwater influx brings pesticides, heavy metals, dissolved nitrogen, and fertilizer residues into IRL (Trocine and Trefry 1993; MacDonald et al. 1996), with certain areas having heavy metal concentrations up to ten times greater than natural levels (Trocine and Trefry 1993). Pollutants contributed to eutrophication of IRL from a clear, oyster bed-supporting system in the early 1900s to its current low visibility, red/green algae-dominated community (Trocine and Trefry 1993). Polluted conditions are thought to have contributed to a high prevalence (up to 70%) of fibropapillomatosis in resident juvenile green turtles (Ehrhart 1991).

Here, we use mitochondrial DNA haplotypes and a Bayesian Markov-Chain Monte Carlo (MCMC) mixed stock analysis to investigate whether the juvenile loggerhead (*Caretta caretta*) aggregation in IRL, Florida represents an investment of several nesting beach rookeries outside the United States, or strictly Florida and Mexico nesting beaches. Similar MCMC analyses have been used to estimate stock composition and rookery contribution in other marine turtles and fishes (Fernandez et al. 2002; Fillatre et al. 2003; Herwerden et al. 2003; Luke et al. 2004; Ruzzante et al. 2004). We also test the hypothesis that large rookeries in close geographic proximity to juvenile aggregations contributed more individuals to those populations than would be expected, given their relative size and geographic proximity. We tested both hypotheses with the most extensive quantitative and temporal sampling of juvenile marine turtles to date. Our study directly addresses how management units are linked through juvenile foraging areas in the North Atlantic.

Methods

Sampling and DNA analyses

We conducted mitochondrial DNA d-loop sequence analysis on 9 years of samples comprising 168 juvenile loggerheads from IRL, approximately 2 km south of Sebastian Inlet, Indian River County, Florida. We used large-mesh tangle nets to capture juvenile turtles monthly from 1993 to 2004. We stored blood samples obtained from the dorsal cervical sinus (Owens and Ruiz 1980) in lysis buffer at room temperature and extracted DNA using standard phenol/chloroform extraction techniques (Hillis et al. 1996). Because Florida nesting populations were previously defined by Pearce (2001). Based on a 400 bp fragment of the mitochondrial d-loop (Table 1), we analyzed this fragment using primers TCR5 and TCR6 (Norman et al. 1994). We subjected purified DNA to polymerase chain reaction (PCR) in 25 μl reactions by denaturing at 93 °C for 3 min, followed by 39 cycles of (1) DNA denaturing at 93 °C for 30 s, (2) primer annealing at 52 °C for 30 s, and (3)

Table 1. Frequency of loggerhead mtDNA control region haplotypes described by Bolten et al. (1998) and Pearce (2001). Fourteen haplotypes describe these rookery regions and the Indian River Lagoon juvenile aggregation.

	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10	CCA13	CCA14	CCA20	Novel haplotypee	Total
Northeast Florida	44	13	1				2		1						61
Southeast Florida	32	28		1	1		1		1				1		65
Southwest Florida	24	67	4		2	2	2		1						102
Northwest Florida	36	7	1		2										46
Mexico		11	2				1	1	5						20
Brazil				11											11
Mediterranean		78	13				1								92
Rookery totals	136	204	21	12	5	2	5	2	2	7			1		397
Indian River Lagoon	81	75	3				2			3	1	1	1	1	168

primer extension at 72 °C for 30 s, with a final primer extension cycle at 72 °C for 10 min. We visualized PCR products on an agarose gel by comparison to a 1 kb ladder and removed and purified the 400 bp fragment using MinElute Gel Extraction Kits (Qiagen). We quantified purified PCR products postpurification using an agrose gel with a low mass quantitative standard (Promega). We sequenced the purified products on a Beckman CEQ 8000 automated sequencer following the manufacturer's protocols. We manually edited sequence data in Sequencher and aligned the sequence data in the program GeneDoc (http://www.cris.com/~Ketchup/genedoc.shtml). We calculated haplotype frequencies and a 95% plausible parsimony network in TCS v1.13 (Clement et al. 2000).

We compared haplotypes observed in IRL to those found on 12 nesting beaches that encompass all nesting regions of Florida, plus Quintana Roo, Mexico. Currently, 11 loggerhead mitochondrial haplotypes define the nesting beaches of Florida and the Yucatan Peninsula of Mexico and were used by Pearce (2001) as a possible basis for delineating management units. Despite the fact that these haplotypes are widespread and common throughout the North Atlantic, they do occur in significantly different frequencies when grouped by management units recognized in the United States Fish and Wildlife Service recovery plan for the loggerhead (Figure 1). We also included Brazilian and Mediterranean nesting beach haplotypes (Bolten et al. 1998; Laurent et al. 1998) in the mixed stock analyses to determine their contribution of juveniles to IRL.

Data analysis

To estimate the contribution of various nesting beaches to the IRL juvenile aggregation, we performed a MCMC mixed stock analysis using the program BAYES (Pella and Masuda 2001). We used even prior expected distributions for all analyses to avoid biasing the results by incorporating rookery size or distance into the model. This method allows rookeries to contribute rare haplotypes even when those haplotypes were not found in the juvenile aggregation sample (Pella and Masuda 2001). Bayesian MCMC methods also yield more accurate probabilistic estimates of contribution than maximum-likelihood point estimates (see Bolker et al. 2003; Luke et al. 2004). We calculated estimated rookery contributions to IRL juveniles based on 23,598 resamplings (as determined by BAYES) of one stock mixture expected to recruit juveniles from five nesting beach aggregations. Estimated contributions excluded the Brazil rookery because its sole haplotype was not found in IRL. We also excluded Mediterranean rookeries for two reasons: (1) no endemic control region haplotypes are available that indicate their contributions, and (2) while juvenile loggerheads from Atlantic rookeries spend extended periods in the Mediterranean Sea, the
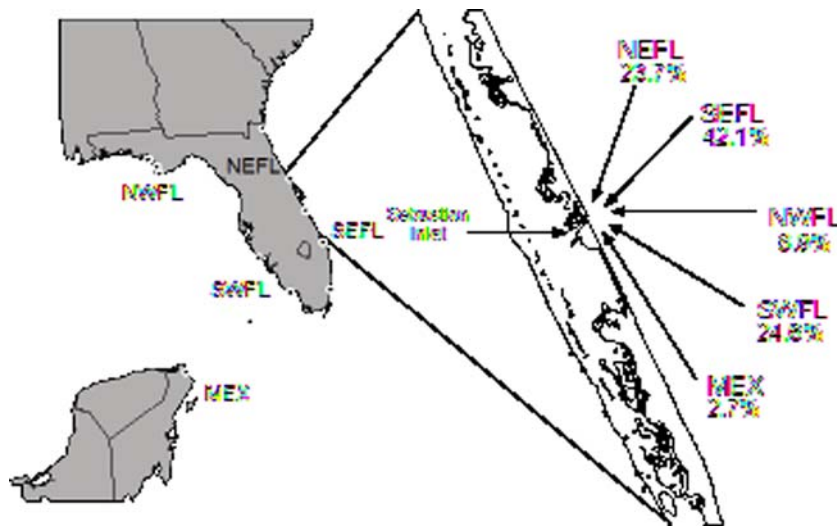
Figure 1. Southeastern United States, Yucatan Peninsula and Indian River Lagoon (enlarged), with estimated contributions from nesting beach regions. NEFL = Northeast Florida, SEFL = Southeast Florida, SWFL = Southwest Florida, NWFL = Northwest Florida, MEX = Mexico.

opposite is not suggested in the literature; Mediterranean-born loggerheads may spend their entire juvenile period in that basin (Laurent et al. 1998; see Discussion).

We grouped haplotypes that characterize Florida and Mexico rookeries into northeast, southeast, northwest, and southwest Florida regions and Mexico (Table 1) according to the findings in Pearce (2001), which suggested that control region haplotype frequency differences were sufficient to consider each of these regions as separate management units. We estimated distance from each rookery to Sebastian Inlet using GIS software. We estimated rookery size as the total number of nests recorded by the Florida Index Nesting Beach Program from 1988–2002. We used linear regression to test whether Bayesian MCMC estimated contributions depended on rookery size or distance, and log-transformed independent variables to meet analysis assumptions. We used Chi-squared tests to compare IRL haplotype frequencies predicted by our MCMC model with those values expected under equal contributions by each rookery.

Results

We observed 8 loggerhead haplotypes in 168 individuals (Table 1) in IRL: CCA1 (48.2%),

CCA2 (44.6%), CCA3 (1.8%), CCA7 (1.2%), CCA10 (1.8%), CCA13 (0.6%), CCA14 (0.6%), and CCA20 (0.6%). In a single individual, we observed a novel haplotype (GenBank Accession Number DQ060038) that was one bp distant from CCA2 at site 157 of 380 where there has been a T→C mutation. These haplotypes can be compared to 35 published Atlantic loggerhead haplotypes at http://accstr.ufl.edu/ccmtdna.html. The haplotype network is given in Figure 2 and is
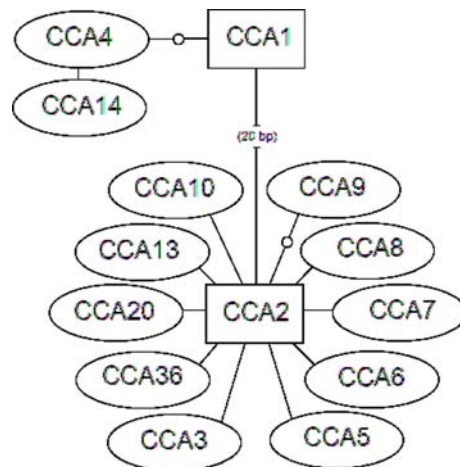

Figure 2. Relationships among haplotypes observed in Atlantic rookeries and the Indian River Lagoon juvenile aggregation. This 95% plausible parsimony network includes a novel haplotype, here named CCA36.

characterized by two central haplotypes separated by 20 point mutations (including one 6 bp insertion as a single mutation).

Results of Bayesian MCMC analyses using even prior expected distributions indicated that the IRL juvenile aggregation is a genetically diverse assemblage with contributions of juveniles from nesting beaches in Florida and Mexico (Table 2) and possibly the Mediterranean (Table 1; haplotypes CCA2 and CCA3). However, due to the lack of endemic control region haplotypes and the absence of evidence in the literature to support Mediterranean contributions in juvenile loggerhead aggregations, we tested our second hypothesis using only northern Atlantic rookeries. There was no support for contributions from southern Atlantic rookeries in Brazil due to the absence of the sole haplotype which described that rookery in Bolten et al. (1998). Haplotypes CCA13 and CCA14 were each observed in a single individual. These haplotypes have never been documented on a nesting beach, however they have been observed in juvenile aggregations at the St. Lucie Power Plant (southeast Florida; Witzell et al. 2002) and the Azores and Madeira (Bolton et al. 1998).

Primary contributions were from southeast Florida, where most loggerhead nesting in the Atlantic occurs. Chi-squared tests between expected haplotype frequencies in IRL (based on even distributions) and those observed in each rookery were all significantly different at $\alpha = 0.05$; indicating that no single rookery was likely to have contributed the vast majority of individuals to the IRL aggregation. The proportion of estimated contributions regressed significantly on both $\log_{10}$ distance ($F_{1,3} = 20.7$, $P < 0.020$, $r^2 = 0.86$) and $\log_{10}$ rookery size ($F_{1,3} = 21.6$, $P < 0.019$, $r^2 = 0.88$). Distance and rookery size were negatively correlated albeit not significantly ($r^2 = 0.74$, $P > 0.063$).

These results confirm the importance of rookery distance from foraging areas and rookery size in predicting contributions to juvenile aggregations.

Discussion

Previous studies of juvenile loggerhead aggregations suggest multiple contributions primarily from local rookeries (Norrgard and Graves 1995; Witzell et al. 2002). However, recruitment of juvenile loggerheads from Florida to the Azores and Madeira suggest that individuals are capable of migrating to a juvenile foraging area upwards of 6400 km from their natal nesting beach (Bolten et al. 1998). Despite these long-distance migrations, a study of multiple rookeries in the North Atlantic indicated that juvenile loggerheads primarily recruit to foraging areas near their natal beaches (Bowen et al. 2004). Our data support the findings of Bowen et al. (2004) and are consistent with a pattern by which IRL juveniles originate from Florida and Mexico rookeries based on rookery size and distance from IRL. However, the resolution offered by mitochondrial d-loop haplotypes is insufficient to rule out minimal contributions from the Mediterranean. Some juveniles hatched in Florida and Mexico recruit to juvenile aggregations in the Mediterranean (Laurent et al. 1993, 1998). It is possible that the opposite also occurs, however we feel it is somewhat unlikely given the biological reasons outlined in Laurent et al. (1998) and the historical and genetic relationships outlined in Reece et al. 2005. A more detailed study of hypervariable nuclear loci (e.g., microsatellites) may further resolve the contributions of Mediterranean rookeries to juvenile aggregations in IRL.

Under the assumption that Mediterranean rookeries do not contribute significantly to IRL

Table 2. Estimated contributions of five rookeries to the juvenile loggerhead aggregation in Indian River Lagoon, Florida, based on Bayesian Markov-chain Monte Carlo (MCMC) mixed stock analyses. Rookery sizes are based on average number of nests deposited from 1988 to 2002. Only haplotypes from IRL that previously were identified on a nesting beach were incorporated in this analysis.

Nesting stock	Rookery size	Distance to IRL	Mean	SD
Northwest Florida	711	1333	6.9%	0.112
Southwest Florida	5479	704	24.6%	0.175
Northeast Florida	2425	272	23.7%	0.196
Southeast Florida	59485	123	42.1%	0.250
Mexico	1230	1354	2.7%	0.044

aggregation, our results demonstrate that IRL receives individuals from nesting beaches throughout Florida and Mexico with contributions proportional to rookery size and distance from the foraging area. This result is surprising, particularly given that the southeastern Florida aggregation, which comprises 86% of all Florida and Mexico loggerhead nesting and is significantly closer to IRL than all other rookeries, only contributed an estimated 42.1% of individuals in IRL. Thus, our results generally confirm the patterns suggested by Bowen et al. (2004). Similar studies of green turtles suggested that distance either did not factor into recruitment (Luke et al. 2004) or was not as important as rookery size (Lahanas et al. 1998). Bass and Witzell (2000) did find a correlation between rookery size/distance and contribution of juveniles, with distance having the greatest effect. Our study focused on a species with distinctively different life history patterns than the green turtle studies listed above and the lack of consensus among similar studies of green turtles is likely due to small sample sizes and short sampling duration. We incorporated more than twice as many samples as the studies listed above. The larger sample size provides greater resolution of rare alleles, which may significantly affect estimated contributions. Our study also incorporated 108 continuous months of sampling, whereas the previous studies cited (Lahanas et al. 1998; Bass and Witzell 2000; Luke et al. 2004) were 1–27 months duration. We suggest that longer periods of genetic sampling and greater sample size increase the accuracy of population assessment and support strong effects of rookery size and distance from the juvenile foraging area.

The mixed stock composition of juvenile loggerheads in IRL suggests that management units described by the United States Fish and Wildlife Service Recovery Plan for the Loggerhead Turtle are linked though this important foraging area. Overall, our results demonstrate the potential importance of IRL as a primary resource for juvenile loggerheads from the entire region. The large size of IRL, its abundant and high quality food sources (Holloway-Adkins 2001), and lack of most pelagic predators make it an ideal foraging area for both loggerhead and green turtles. Moreover, strong correlation of estimated contributions with distance and rookery size indicates that juvenile marine turtle aggregations will recruit to contributing rookeries in a predictable manner. Commercial fishing impacts, pollution, and diseases affecting these juvenile aggregations will have measurable outcomes for the rookeries that depend on them. We suggest that these parameters be incorporated into predictive population-dynamic models based on monitoring of juvenile aggregations. Currently, neither the 1991 Recovery Plan, nor the current draft includes provisions for protecting foraging areas local to important nesting beaches. While it is clear that nesting beaches are critical to marine turtle recovery, our findings have confirmed a pattern by which nesting beaches are likely to benefit from the protection of local foraging areas. This pattern is clearly demonstrated by Bowen et al. (2004) and supported through our detailed examination of the IRL and surrounding rookeries.

The fact that multiple rookeries contribute to a limited number of juvenile aggregations makes marine turtles as a group, and specifically the loggerhead, particularly vulnerable to the effects of pollutants in degraded juvenile foraging habitats such as IRL. Currently, we have little data on the effects degraded ecosystems, such as IRL, have on the long term development and fitness of juvenile turtles. Fibropapillomatosis is not as prevalent in juvenile loggerheads as it is in green turtles, however it occurred in 4.5% of individuals captured from 1982 to 2004 (unpublished data). Juvenile loggerheads potentially recruit from numerous nesting beaches (possibly from all over the world) to a select few foraging areas, so threats to localized foraging areas are relevant to global population dynamics. We suggest that future management plans focused on nesting beach populations include known juvenile foraging areas proximal to those beaches as critical habitat. Our results and those of Bowen et al. (2004) confirm a pattern by which inclusion of proximal foraging areas would likely protect juveniles originating from protected nesting beaches and thus ensure the continued recruitment of mature nesting females.

References

Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast: Evidence from mtDNA markers. *Herpetologica*, **56**, 357–367.

Bolker B, Okuyama T, Bjorndal K, Bolten A (2003) Sea turtle stock estimation using genetic markers: Accounting for sampling error of rare genotypes. *Ecol. Appl.*, **13**, 763–775.

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada SE, Bowen BW (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecol. Appl.*, **8**, 1–7.

Bowen BW, Bass AL, Chow SM, Bostrom M, Bjorndal KA, Bolten AB, Okuyama T, Bolker BM, Epperly S, Lacasella E, Shaver D, Dodd M, Hopkins-Murphy SR, Musick JA, Swingle M, Rankin-Baransky K, Teas W, Witzell WN, Dutton PH (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Mol. Ecol.*, **13**: 3797–3808.

Caffereta PH, Spittler TE (1998) *Logging impacts of the 1970s vs. the 1990s in the Caspar Creek Watershed.* California Department of Forestry and Fire Protection.

Carr AF (1986) Rips, FADS, and little loggerheads *Bioscience*, **38**, 92–100.

Clement M, Posada D, Crandall KA (2000) TCS: A computer program to estimate genealogies. *Mol. Ecol.*, **9**, 1657–1659.

Crowder LB (1995) Effects of turtle excluder devices (TEDs) on loggerhead sea turtle strandings with implications for conservation *Copeia*, **4**, 773–779.

Dybas CL (2002) Florida's Indian River Lagoon – An Estuary in Transition *BioScience*, **52**, 555–559.

Ehrhart LM (1991) Research Plan for Marine Turtle Fibropapilloma *NOAA-TM-NMFS-SWFSC*, **156**, 59–61.

Ehrhart LM, Bagley DA, Redfoot WE (2003) Loggerhead turtles in the Atlantic Ocean: Geographic distribution, abundance, and population status. In: Loggerhead Sea Turtles (eds. Bolten Ab, Witherington BE), pp. 157–174. Smithsonian Institution, Washington, DC.

Fernandez C, Ley E, Steel MJ (2002) Bayesian modeling of catch in a north-west Atlantic fishery. *Appl. Statist.*, **51**, 257–280.

Fillatre EK, Etherton P, Heath DD (2003) Bimodal run distribution in a northern population of sockeye salmon (*Oncorhynchus nerka*): Life history and genetic analysis on a temporal scale. *Mol. Ecol.*, **12**, 1793–1805.

Herwerden LV, Benzie J, Davies C (2003) Microsatellite variation and population genetic structure of the red throat emperor on the Great Barrier Reef. *J. Fish. Biol.*, **62**, 987–999.

Hillis DM, Moritz C, Mable BK (1996) *Molecular Systematics*, 2nd edn. Sinaur Associates, Sunderland, MA.

Holloway-Adkins KG (2001) A Comparative Study of the Feeding Ecology of *Chelonia mydas* (Green Turtle) and the Incidental Ingestion of *Prorocentrum* spp. MS thesis, University of Central Florida, Orlando, Florida, USA.

Lahanas PN, Bjorndal KA, Bolten AB, Encalada SE, Miyamoto MM, Valverde RA, Bowen BW (1998) Genetic composition of a green turtle feeding ground population: Evidence for multiple origins. *Mar. Biol.*, **130**, 345–352.

Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D, Abd El-Mawla EM, Hadoud DA, Gomati HE, Domingo M, Hadjichristophorou M, Kornaraky L, Demirayak F, Gautier CH (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: A case study in the Mediterranean. *Mol. Ecol.*, **7**, 1529–1542.

Laurent L, Lescure J, Excoffier L, Bowen B, Domingo M, Hadjichristophorou M, Kornaraki L, Trabuchet G (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. Paris*, **316**, 1233–1239.

Luke K, Horrocks JA, LeRoux RA, Dutton PH (2004) Origins of green turtle (*Chelonia mydas*) feeding aggregations around Barbados, West Indies. *Mar. Biol.*, **144**, 799–805.

MacDonald DD, Carr RS, Calder FD, Long ER, Ingersoll CG (1996) Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology*, **5**, 253–278.

Marine Turtle Specialist Group (1996) *Caretta caretta*. In: IUCN 2004. *2004 IUCN Red List of Threatened Species* < redlist.org > .

Musick JA, Limpus CJ (1997) Habitat utilization and migration in juvenile sea turtles. In: The Biology of Sea Turtles (eds. Lutz P.L., Musick J.A.), pp. 137–163. CRC Press, Boca Raton, FL.

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: Genetic markers for ecological studies of marine turtles. *Mol. Ecol.*, **3**, 363–373.

Norrgard JW, Graves JE (1995) Determination of the natal origin of a juvenile loggerhead turtle (*Caretta caretta*) population in Chesapeake Bay using mitochondrial DNA analysis. *Proceedings of International Sea Turtle Genetics Symposium*, 129–136.

Owens DW, Ruiz GJ (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica*, **36**, 17–20.

Pearce AF (2001) Contrasting Population Structure of the Loggerhead Turtle (*Caretta caretta*) Using Mitochondrial and Nuclear DNA Markers. MS thesis, University of Florida, Gainesville, FL.

Pella M, Masuda J (2001) Bayesian methods for analysis of stock mixtures from genetic characters. *NOAA Fish. Bull.*, **99**, 151–167.

Reece JS, Castoe TC, Parkinson CL (2005) Historical Perspectives on population genetics and conservation of three marine turtle species. *Con Gen.*, **6**, 235–251.

Ruzzante DE, Hansen MM, Meldrup D, Ebert KM (2004) Stocking impact and migration pattern in an anadromous brown trout (*Salmo trutta*) complex: Where have all the stocked spawning sea trout gone?. *Mol. Ecol.*, **13**, 1433–1445.

Trocine RP, Trefry JH (1993) Final Report to the St. Johns River Water Management District and the Indian River Lagoon National Estuary Program.

Witzell WN, Bass AL, Bresette MJ, Singewald DA, Gorham JC (2002) Origin of immature loggerhead sea turtles (*Caretta caretta*) at Hutchinson Island, Florida: Evidence from mtDNA markers. *Fish. Bull.*, **100**, 624–631.

Work TM (2001) Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii *J. Wild. Dis.*, **37**, 574–581.