Abstract—Loggerhead sea turtles (Caretta caretta) are migratory, long-lived, and slow maturing. They are difficult to study because they are seen rarely and their habitats range over vast stretches of the ocean. Movements of immature turtles between pelagic and coastal developmental habitats are particularly difficult to investigate because of inadequate tagging technologies and the difficulty in capturing significant numbers of turtles at sea. However, genetic markers found in mitochondrial DNA (mtDNA) provide a basis for predicting the origin of juvenile turtles in developmental habitats. Mixed stock analysis was used to determine which nesting populations were contributing individuals to a foraging aggregation of immature loggerhead turtles (mean 63.3 cm straight carapace length [SCL]) captured in coastal waters off Hutchinson Island, Florida. The results indicated that at least three different western Atlantic loggerhead sea turtle subpopulations contribute to this group: south Florida (69%), Mexico (20%), and northeast Florida-North Carolina (10%). The conservation and management of these immature sea turtles is complicated by their multinational genetic demographics.

Origin of immature loggerhead sea turtles (Caretta caretta) at Hutchinson Island, Florida: evidence from mtDNA markers

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North Atlantic loggerhead sea turtles have extended and complex developmental life histories (Musick and Limpus, 1997). After emerging from their nests, hatchling loggerhead sea turtles enter the surf and eventually move into the pelagic environment for several years before returning to inshore benthic coastal waters. The accepted hypothesis is that these hatchlings are passively transported to the eastern Atlantic by major current systems and these turtles would eventually return to coastal benthic habitats in the western Atlantic by the North Atlantic gyre when they attain 25–60 cm or an estimated 3–10 years old (Carr, 1986, 1987; Musick and Limpus, 1997).

The loggerhead sea turtle (Caretta caretta) is listed as threatened under the United States Endangered Species Act of 1973 and subsequent amendments. Although the loggerhead sea turtle nesting population in the southeastern United States is one of the largest in the world, other distinct nesting populations (as defined by genetic divergence) exist in the northwestern Atlantic Ocean. These known sub-populations are found in the Yucatan, northwest Florida, south Florida, and from north Florida to North Carolina. The east central coast of Florida supports the largest nesting subpopulation of loggerhead turtles and is a highly dynamic coastal area with multiple sea turtle species in various postpelagic developmental stages (Witzell, 1987). Sea turtles may hatch in one country, grow through adolescence in a second or more countries, feed and reproduce as adults in a third jurisdiction, and swim through a dozen more territorial waters enroute to and from these destinations (Bowen et al., 1995). Tagging studies, unfortunately, are only capable of providing glimpses of these complex changes in developmental habitats because of high tag loss rates and rare opportunities of recapturing tagged turtles thousands of kilometers away in the pelagic environment several years later (Chaloupka and Musick, 1997).

The likelihood that turtles from genetically distinct stocks share coastal and pelagic developmental habitats may raise doubts regarding the effectiveness of conservation strategies based on geographical or political boundaries (Carr and Stancyk, 1975; Bowen and Witzell, 1996). Consequently, sea turtle biolo-
gists and marine resource managers are presented with complex challenges that reinforce the need for complete life history information. In particular, the origin of immature loggerhead sea turtles foraging in coastal nearshore waters needs to be determined for the development of effective regional conservation and management strategies. Recent research has demonstrated that most sea turtle nesting colonies are genetically distinct as indicated by mitochondrial (mt) DNA haplotype frequency shifts. This finding allows the possibility of using mtDNA data to identify rookery cohorts on feeding grounds (Bass et al., 1998; Broderick et al., 1994). By using an existing database (Encalada et al., 1998) and molecular techniques, tissue samples from juvenile marine turtles can be analyzed to estimate the origin of animals inhabiting developmental habitats. These data are collected and analyzed faster than results from tagging studies and may provide information on cryptic migratory behavior (Bowen et al., 1995; Bolten et al., 1998). Both pelagic and coastal benthic zones are believed to be essential developmental habitats for sea turtles, and molecular markers have recently been used to document shifts in the demographic composition between these habitats (Laurent et al., 1998). This article examines the mtDNA composition of juvenile loggerhead sea turtles using the coastal waters off Hutchinson Island, Florida, to determine whether the turtles are primarily from the adjacent nesting subpopulation or whether this foraging population is composed of individuals from multiple rookeries.

**Materials and methods**

Sea turtles are routinely captured in the canal that supplies cooling water at the St. Lucie Power Plant on Hutchinson Island, Florida (Fig. 1). The power plant intake operates year round—collecting sea turtles from 365 m offshore and providing an excellent opportunity to sample sea turtles in the nearshore developmental habitat (Bass and Witzell, 2000). Power plant biologists collect these turtles daily in an ongoing research and conservation program. Turtles are measured, flipper-tagged, and released in good condition promptly into the adjacent coastal waters.

A total of 109 juvenile loggerhead sea turtles were sampled at the St. Lucie power plant from January through May 1999 for our study. The minimum straight carapace length (SCL) was measured with calipers. Tissue samples were collected by using a 6-mm biopsy punch and placed in 15 mL of saturated salt preservation buffer developed...
Table 1

Haplotype distribution among loggerhead sea turtle nesting groups compiled by Encalada et al. (1998). Haplotypes K and M were identified previously in the Madeira and Azores foraging assemblages (Bolten et al., 1998). Haplotype N was identified previously in a sample of stranded individuals from the Atlantic U.S. coast (Rankin-Baransky et al., 2001).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>NWFL</th>
<th>SFL</th>
<th>NEFL-NC</th>
<th>Mexico</th>
<th>Greece</th>
<th>Brazil</th>
<th>St. Lucie, Florida</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>34</td>
<td>22</td>
<td>104</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>40</td>
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<tr>
<td>B</td>
<td>4</td>
<td>24</td>
<td>1</td>
<td>11</td>
<td>19</td>
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<td>C</td>
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<td>G</td>
<td>2</td>
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<td>5</td>
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<td>2</td>
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<tr>
<td>J</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
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<tr>
<td>K</td>
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<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

by Amos and Hoelzel (1991). Samples were transferred to the University of Florida for analysis. Standard phenol and chloroform DNA isolation protocols were conducted on the tissue samples (Hillis et al., 1996). A 380-bp fragment of the mitochondrial DNA control region was amplified using primers

TCR5 (5'-TTGTACATCTACTTAATTACCAC-3'), and HDCM2 (5'-GCAAGTAAAACCTACCGTATGCCAGGTTA-3') designed for sea turtles (Encalada et al., 1996; Norman et al., 1994). Cycling parameters were as follows: 94°C 1 min, 25 cycles of (94°C/45s + 52°C/30s + 72°C/45s) and 3-min extension at 72°C.

Individuals were compared to known loggerhead sea turtle haplotypes and assigned a letter designation based on Encalada et al. (1998) and Bolten et al. (1998). To test for statistical differences among haplotype frequencies at rookeries and the foraging location, chi-square analyses were performed with the program CHIRXC (Zaykin and Pudovkin, 1993) and probabilities were generated with a Monte Carlo randomization procedure (Roff and Bentzen, 1989).

Maximum likelihood (ML) analysis for mixed stock identification (Grant et al., 1980) was used to estimate the contributions of nesting populations to the foraging habitat adjacent to the St. Lucie Power Plant on Hutchinson Island. This method estimates the most likely contributions of source populations based on the haplotype frequencies in the source populations and in the mixed population. The source populations and frequencies of associated haplotypes used in the analysis were those of Encalada et al. (1998). It should be noted that the addition of new nesting population data could change the results presented in our study. The addition of new nesting data will always be a problem when conducting these types of analyses but should not preclude the use of this technique to determine potential contributors to a foraging population. The maximum likelihood program GIRLSEM was used (Masuda et al., 1991). As a starting point in ML iterations with GIRLSEM, it was assumed that all source populations had an equal probability of contributing (i.e. population size, distance from the foraging location, etc. did not have an impact on the percentage of animals recruiting to a particular area). Standard errors and 95% confidence intervals of the point estimates were generated from 100 bootstraps of the stock and mixture data sets with GIRLSEM (Pella et al., 1998).

Results

Mitochondrial DNA analysis

Of the 109 tissue samples collected, 106 produced readable sequences (Table 1). Eighty five of the samples consisted of the most common haplotypes A and B. Haplotype C, another haplotype found in multiple rookeries at low frequency, was found in six of the individuals sampled. The remaining seven individuals possessed haplotypes (K, M, and N) observed previously, but only from surveys of foraging or stranded individuals.

The haplotype frequencies of the foraging juveniles were significantly different from all nesting loggerhead sea turtle populations except for the SFL nesting population ($\chi^2=9.51, P=0.40$). Although the haplotype frequencies were similar to that of the SFL nesting population, the presence of haplotypes not associated with the SFL popu-
Table 2

Maximum likelihood estimates of contribution by source populations to immature loggerhead sea turtles from the St. Lucie Power Plant (n=99). Estimates were generated by using UCON software (Masuda et al., 1991) Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM. NWFL = northwest Florida; SFL = south Florida; and NEFL-NC = northeast Florida to North Carolina.

<table>
<thead>
<tr>
<th>Source population</th>
<th>Contribution</th>
<th>Standard error</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWFL</td>
<td>0.0912</td>
<td>0.1896</td>
<td>0.0479–0.1345</td>
</tr>
<tr>
<td>SFL</td>
<td>0.6681</td>
<td>0.3072</td>
<td>0.5979–0.7383</td>
</tr>
<tr>
<td>NEFL-NC</td>
<td>0.0366</td>
<td>0.1363</td>
<td>0.0055–0.0677</td>
</tr>
<tr>
<td>Yucatan</td>
<td>0.2040</td>
<td>0.1125</td>
<td>0.1783–0.2297</td>
</tr>
<tr>
<td>Brazil</td>
<td>0.0000</td>
<td>0.0000</td>
<td>—</td>
</tr>
<tr>
<td>Greece</td>
<td>0.0000</td>
<td>0.1555</td>
<td>0.0000–0.0355</td>
</tr>
</tbody>
</table>

Table 3

Maximum likelihood estimates of contribution by source populations to immature loggerhead sea turtles from the St. Lucie power plant (n=99). Estimates were generated by using UCON software (Masuda et al., 1991). Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture by using GIRLSEM. The source populations NWFL, Brazil and Greece, were removed from the analysis. SFL = south Florida; and NEFL-NC = northeast Florida to North Carolina.

<table>
<thead>
<tr>
<th>Source population</th>
<th>Contribution</th>
<th>Standard error</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFL</td>
<td>0.6965</td>
<td>0.2211</td>
<td>0.6527–0.7403</td>
</tr>
<tr>
<td>NEFL-NC</td>
<td>0.0984</td>
<td>0.1151</td>
<td>0.0756–0.1212</td>
</tr>
<tr>
<td>Yucatan</td>
<td>0.2050</td>
<td>0.1296</td>
<td>0.1793–0.2307</td>
</tr>
</tbody>
</table>

lation indicates that this nesting population is not the sole contributor to this foraging population.

Maximum likelihood analysis

The initial maximum likelihood analysis yielded estimates of contribution that were associated with high standard errors (Table 2). The estimate for NWFL had an extremely high standard error. This high standard error is an indication of sampling error associated with the source population (Epifanio et al., 1995). Because of the large standard errors, an attempt was made to reduce the complexity of solutions that were involved in the maximum likelihood algorithm’s search. Removing a potential source population decreases the potential number of “answers,” consequently reducing the standard errors about the mean estimates.

The maximum likelihood analysis was repeated, removing the smallest and most distant source populations (NWFL, Brazil, and Greece) as potential contributors (Table 3). In addition to the statistical assumption that the large variation associated with several estimates is partially due to sampling error, we also made some biological assumptions. By removing the source populations we assumed that they were not contributing to this foraging area at levels sufficient to detect in this ML analysis. The nesting effort in NWFL may include 100–200 turtles annually (Meylan et al., 1995), as compared to tens of thousands of turtles that nest in southern Florida. Hence, the Florida panhandle is an important nesting area, but is probably too small to detect with precision in ML analyses. The removal of Greece as a potential source population was based on the results of the first analysis that indicated that this source population did not contribute at detectable levels (Table 1). Brazil was also removed from the analysis because there were no indications (either from the observed haplotypes or from the initial ML analysis) that this population contributed individuals to the foraging population at Hutchinson Island.

The results from the final ML analysis provided the most realistic estimate of the genetic composition of this area (Table 3). This analysis indicated that the SFL subpopulations is a major contributor to the Hutchinson Island foraging population. In addition, the Yucatan nesting population also appears to contribute a significant percentage of individuals to this area. Although the small northeast Florida to North Carolina (NEFL-NC) rookery appears to contribute some individuals, the standard error is still large; therefore, the estimate is not precise. Individuals from the NEFL-NC nesting population are present, but an accurate estimate (finer than 7–12%) is not possible at this time.

The results of the analysis of the St. Lucie foraging population are provisional and there are several limitations to the maximum likelihood analysis. A major assumption of this type of analysis is that all potential source populations
have been identified (Pella and Milner, 1987). Although the source population data set represents most of the major nesting colonies in the Atlantic, other nesting colonies exist that may contribute individuals to this foraging ground assemblage. For example, there are small nesting loggerhead populations on the Cape Verde Islands, Cuba, and on the coast of Colombia for which data are not accessible and we have been unable to estimate their possible contributions. If these nesting populations were included in the analysis there is a chance that the relative contributions of the source populations included in our study could be altered. A second major assumption is that the source populations were sampled sufficiently to uncover all haplotypes (Pella and Milner, 1987). In the case of the south Florida nesting assemblage, this assumption may not hold. As previously mentioned, south Florida supports the largest nesting assemblage in the Atlantic Ocean and it is possible that all haplotypes of the assemblage have not been determined. As with the inclusion of more potential source populations, there is the chance that changes in the frequency and distribution of haplotypes could alter the relative contributions reported in our study. We see these results as a sound starting point for the eventual analysis of foraging loggerhead sea turtles along the coast of the eastern United States.

The mean length of the 109 sampled turtles was 63.3 cm (SCL) with a standard deviation of 6.0 cm (Fig. 2). The mean lengths for all haplotype groups were very similar: for haplotypes A, B, C, and G (south Florida), mean length was 62.9 cm (±6.2); for haplotypes I and J (Yucatan), it was 63.8 cm (±8.0); and for haplotypes K, M, and N (other), it was 63.7 cm (±3.8).

Discussion

Although the estimates of percent contributions of the source populations generated here are provisional (i.e. more sampling of loggerhead nesting subpopulations may eventually uncover previously unrecorded haplotypes), they indicate that sea turtles foraging in the nearshore waters of Hutchinson Island originate from at least three rookeries in the Northwest Atlantic: SFL (69%), Yucatan (20%), and NEFL-NC (10%). Although the majority of animals appear to originate from Florida nesting beaches, the maximum likelihood analysis indicates that a substantial proportion of the foraging juveniles are coming from the southern nesting population in the Yucatan. In addition, the northern population, comprising animals nesting on beaches from northeast Florida to North Carolina, also contributes a smaller percentage of individuals.

Sears et al. (1995) reported on the demographic composition of juvenile loggerhead sea turtles off Charleston, South Carolina, and Norrgard and Graves (1995) analyzed juvenile loggerhead sea turtles from Chesapeake Bay, Virginia. Restriction fragment length polymorphism (RFLP) analysis of mtDNA was used to identify the genotype of individuals in both studies. The data sets were used in conjunction with a geographic survey limited to nesting locations in the North Atlantic Ocean and Mediterranean Sea by Bowen et al. (1993) to estimate the composition of these two areas. The estimates of contribution by nesting populations to these foraging areas (Table 4) appear to differ from the results presented in the present study, particularly the large contributions from the NEFL-NC haplotype. This may either reflect real differences in haplotype composition,
also included several other nesting subpopulations in the resolution and small sample sizes of the RFLP studies. undoubtedly an artifact and are more likely due to the poor Atlantic Ocean, in particular nesting subpopulations in northwest Florida and the Yucatan of Mexico.

Juvenile loggerhead sea turtles from the Yucatan and NWFL are transported into the Atlantic by the Florida Current. *Lepidochelys kempii* scenarios as proposed for the Kemp's ridley sea turtle (*Lepidochelys kempii*) by Collard and Ogren (1990). These loggerhead turtles either stay in the Gulf of Mexico or are transported into the Atlantic by the Florida Current. Once in the Atlantic, they either stay on the Continental shelf area or are entrained in major current systems and are transported to the eastern Atlantic Ocean for an undetermined amount of time before presumably returning to western Atlantic coastal benthic habitats. The SFL and NEFL-NC rookeries are all situated near the western boundary of the Gulf Stream where the hatchlings are quickly transported away from beaches. Juvenile loggerhead sea turtles leave their pelagic habitat over a range of sizes, and presumably ages, depending on feeding success. This estimate size range (SCL) was from 25 to 60 cm (Carr, 1986, 1987; Martin et al., 1989; Musick and Limpus, 1997). Hays and Marsh (1997) deduced from drift studies that the pelagic phase ended “at least for some loggerheads in the north Atlantic” when they were around 50 cm. These postpelagic turtles are thought to become coastal benthic crustacean foragers (Musick and Limpus, 1997) and to move up and down the U.S. East Coast seasonally.

Movements of juvenile loggerhead sea turtles tagged at the St. Lucie Power Plant indicate that a substantial portion of these turtles appear to reside in the immediate vicinity of Hutchinson Island (M. Bresette, unpubl. data). However, a few turtles have been documented from as far north as North Carolina and as far south as Florida Bay in southwest Florida. Most recaptured juvenile loggerhead sea turtles were from the north and may indicate that Hutchinson Island is near the southern boundary of the seasonal migration (Ernest et al., 1989). Relatively few southern movements were recorded to Florida Bay. Over 47% of 3142 turtles under 85 cm captured at the St. Lucie Power Plant were captured from January through April 1976–2000 (Fig. 3). Captures then tapered off until December, consistent with seasonal north and south movements as suggested for immature turtles at nearby Cape Canaveral (Henwood, 1987). There has previously been no tagging evidence to indicate movements of these immature turtles outside the continental United States, yet these mtDNA data indicate a large contribution from Mexico.

The Hutchinson Island juvenile loggerhead sea turtles settle into this important coastal habitat at the same size, and presumably age, whatever their genetic origins are. This finding indicates that this particular coastal habitat may be critical for several western Atlantic populations of juvenile sea turtles, both loggerhead and green.

### Management implications

The NEFL-NC population is of particular interest to some U.S. conservation biologists because of an apparent decline in nesting females prior to 1990 (Turtle Expert Working Group, 2000). The goal of protecting these turtles while in various developmental habitats is extremely complex, particularly when they intermingle with large numbers of sea turtles from a healthy (large) breeding population (SFL), as well as with small (NWFL) and foreign populations (Yucatan). Management measures affecting immature loggerhead sea turtles in the east-central Florida foraging habitats need to take into account the contributions from other source populations that may not be as robust as that of the south Florida population. Specific measures will depend largely on the demographic composition of stranded turtles with known sources of mortality. The genetic compositions of juvenile loggerhead sea turtles that are impacted by disease, boat collisions, anthropogenic debris, channel dredging, and recreational and commercial fisheries need to be determined before resource managers can take appropriate action. Unfortunately it is difficult to determine specific causes of mortality from stranded individuals, and researchers are unable to accurately determine the activities that affect specific nesting aggregations. The conservative approach would be to regulate all known sources of mortality to immature coastal loggerhead sea turtles until more data become available through aggressive genetic sampling and necropsies of stranded animals.
The coastal foraging habitat near Hutchinson Island is also an important developmental habitat for endangered immature green sea turtles, *Chelonia mydas* (Bass and Witzell, 2000). This is a highly cosmopolitan species with contributions to the Florida foraging grounds from Costa Rica (53%), the United States and Mexico (42%), and Venezuela and Surinam (4%). It may be advisable for managers to designate specific areas, such as Hutchinson Island, as critical developmental habitats for immature sea turtles and to restrict public access.

The need for regional and internationally coordinated sea turtle research and management becomes obvious as genetic analysis reveals the complicated mixed stock of foraging turtle aggregations. International organizations, such as the International Union for the Conservation of Nature (IUCN) and the Intergovernmental Oceanographic Commission (IOCARIPE) need to become actively involved with the appropriate regional agencies of government to ensure the protection of sea turtle stocks in regional as well as international waters.

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