NOAA TECHNICAL MEMORANDUM NMFS-SEFSC-401

DESIGN FOR A MULTIPLE-METHOD APPROACH TO DETERMINE STOCK STRUCTURE OF BOTTLENOSE DOLPHINS IN THE MID-ATLANTIC

Report of a Workshop, 11-12 February 1997

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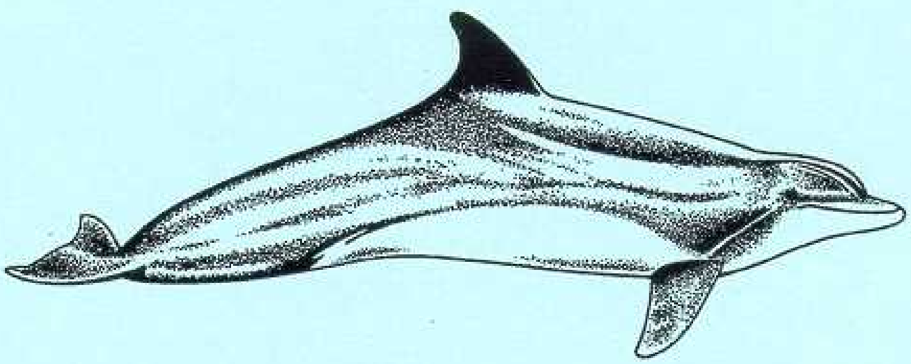

May 1997

U. S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Science Center
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NOTICE

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ABSTRACT

Relatively new information has provided the basis for designing a series of field studies that will allow determination of whether the coastal migratory stock of bottlenose dolphins, as currently defined, is actually more than one stock. To date, the methods that have contributed to our understanding include photo-identification, analysis of isotope ratios, morphometric characteristics, feeding habits, genetics, and freeze brands applied during live captures. One critical aspect contributing to success will be the analysis of matched samples (various tissues from an individual analyzed across the various methods) and the subsequent merging of the disassociated results. Existing information is sufficient to suggest an efficient geographic approach for collecting and analyzing samples: beginning at the north and south ends of the distribution of the coastal migratory population, including some of the estuaries in the south that contain year-round residents, then moving toward the center of the distribution. Results are sufficiently preliminary for many of the methods that estimating sample sizes, *a priori*, required to detect differences (at $\alpha=0.05$ and $\beta=0.20$) is not feasible. Hence, adaptive sampling will be adopted. Each year a sample will be analyzed from each of the geographic sites chosen with each of the appropriate methods, and those results used to determine the optimal way to proceed. Samples and data are available from various sources. Matched samples will be collected from live-captures and specimens killed in fishery interactions. Additional samples will be collected through biopsy sampling. Photo-identification based at five core sites along the coast and satellite transmitters deployed on animals caught during live-captures will provide critical movement data. A central photo-identification catalogue for the entire coast is being developed for use in matching between sites and identification of known individuals for biopsy sampling. The scope of the question will require a multi-year effort; a 3-yr field plan has been developed.

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INTRODUCTION

From June 1987 through March 1988, a greater than 10-fold increase occurred in the number of bottlenose dolphins stranded along the Atlantic coast of the United States from New Jersey to central Florida (Scott, Burn, and Hansen 1988). This die-off event resulted in two significant decisions. First, it was estimated that the die-off reduced the population below optimum sustainable population (OSP) and, hence, the population was listed as depleted under the MMPA (58 CFR 17789, 6 April 1993). Second, the Advanced Notice of Proposed Rulemaking (ANPR) for the depletion listing (54 FR 41654, October 11, 1989) defined the population boundaries on the basis of the temporal and spatial pattern of the die-off (Fig. 1, from Scott *et al.* 1988). The event began along the coast of North Carolina, Virginia, and Maryland then moved north toward New Jersey as summer progressed then tracked sequentially southward toward Florida throughout the late summer, fall, and early winter. This pattern suggested that the die-off was "lodged" in a single population that was migrating along the Atlantic coast, more or less moving northward in summer and southward in winter:

> "...The stranding data collected during 1987 and 1988, and the observed density distribution patterns along the U.S. coast, support a ***single coastal-migratory stock of bottlenose dolphins*** that ranges seasonally as far north as Long Island, NY, and as far south as central Florida..." (Scott *et al.* 1988; 54 FR 41654, October 11, 1989) [emphasis added].

The ANPR and final rule on the listing (58 CFR 17789, 6 April 1993) acknowledged the existence of two other stocks of bottlenose dolphin in the western Atlantic: an offshore stock and a resident coastal population. These stocks were specifically excluded from the listing action. Evidence available at that time supporting the distinctness of the offshore form was summarized. No evidence was provided for how the coastal migratory stock was separate from the resident coastal population except the presumed migratory pattern of the former.

A requirement to better define the stocks of bottlenose dolphins in the western Atlantic now exists because the 1994 amendments to the Marine Mammal Protection Act (MMPA) changed the way the National Marine Fisheries Service approaches management of marine mammal populations. These amendments specify that the maximum number of marine mammals that may be removed from a population due to human causes (Potential Biological Removal, PBR) be determined on the basis of its abundance, maximum rate of increase, and a recovery factor. Necessarily, therefore, populations must be defined so that abundance can be accurately estimated and removals can be correctly attributed.

The current population delineations for bottlenose dolphins in the western Atlantic have not yet achieved this level of precision. An imperative to do so now flows from three directions: the need to calculate PBR, the relatively high rate of human-caused removals of inshore bottlenose dolphins, and the need to better define the stock or stocks affected by the die-off so that a Conservation Plan can be developed that includes criteria for determining when the stock(s) has recovered.

Fortunately, more information on stock structure exists now than following the die-off. Multiple methods that successfully distinguish individuals from the offshore and inshore stocks have been developed: morphometrics, parasite loads, isotope ratios, genetics, and even feeding habits. Given a specimen or sample in hand, the ability to separate these stocks is no longer in doubt. What remains a question is the spatial and temporal distribution of the offshore stock relative to the inshore stock(s). Aerial surveys during the CETAP program showed two distinct distributions north of North Carolina, with the offshore form found between the 200m and 2000m isobaths and the inshore form seen hugging the coast (Kenney

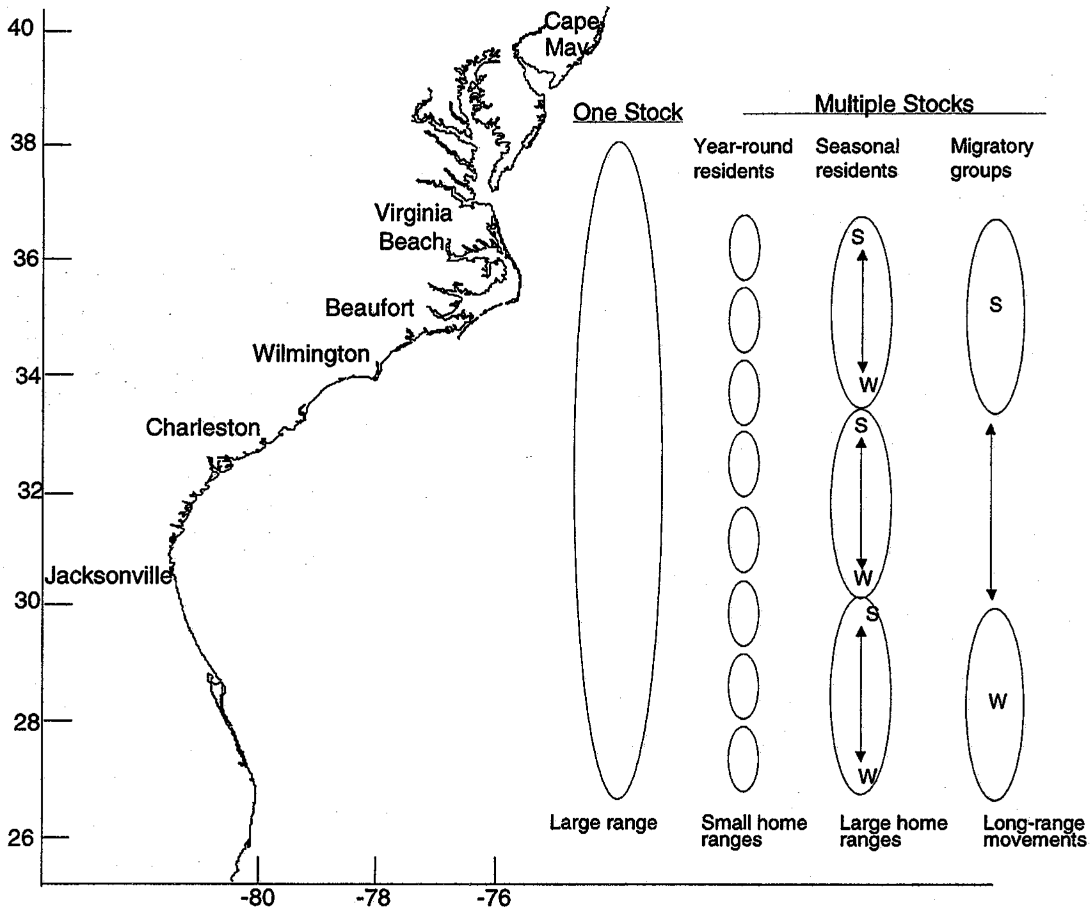

Figure 1. Illustration of stock structure hypotheses of Atlantic coastal bottlenose dolphins: one stock ranging from New Jersey to Florida or multiple stocks which may include 1) year-round residents with small home ranges, 2) multiple, contiguous, seasonally resident groups with relatively large home ranges, and 3) groups with long-range migratory pattern. [Figure by L. Hansen]

1990). Relatively few bottlenose dolphins were seen between these two high-density areas north of North Carolina. At Cape Hatteras, NC, the continental shelf is very narrow and the 200m isobath is close to the coast. South of North Carolina, the continental shelf is wide and surveys have shown a continuous distribution of bottlenose dolphins from the coast to the shelf edge (Blaylock *et al.* 1995).

Shipboard surveys planned for 1998, primarily to estimate the abundance of pelagic cetaceans, will be designed to collect biopsy samples across the shelf to determine the distribution of inshore and offshore stocks. Smaller-vessel surveys will be planned to provide complementary coverage from the coast out to the depth safely traversed by the large ship. With samples collected throughout the likely range of overlap, we should be able to define the spatial distribution of the two stocks.

The more difficult question is resolution of the structure of the inshore stock. Do the coastal animals comprise a single coastal migratory stock from New Jersey to Florida as defined in the depletion listing? Are the estuarine forms reproductively isolated from the coastal form? Do they mix but at a rate of dispersal of several percent per year or less indicating the need for managing the units separately (Taylor 1995)? Some of the patterns emerging indicate a possible combination of year-round resident groups,

groups with relatively small home-ranges but with different summer and winter areas, and possible migratory groups with larger ranges (Figure 1).

The primary purposes of this workshop were to (1) review new information that contributes to interpreting the stock structure of the inshore form of bottlenose dolphins and (2) use that information as the basis for determining an optimal means of proceeding to resolve stock structure. The success of this approach hinges on the concurrent application of complementary methods for dolphins along the mid-Atlantic coast. To date, the methods that have contributed to our understanding include photo-identification, analysis of isotope ratios, traditional morphological traits, feeding habits, genetics, and freeze brands applied during live captures. One critical aspect contributing to success will be the analysis of matched samples (various tissues analyzed from an individual) and then the merging of the disassociated results. Methods for resolving stock structure were chosen for inclusion because they had been shown to be successful, some in preliminary but promising studies, or were accepted as important tools for addressing stock questions. The matched-sample analysis also allows us to evaluate which method(s) will succeed in defining stock structure. The scope of the problem requires a multi-year effort; the experimental design was formulated assuming a 3-yr field project.

In developing the experimental design, the intent was to estimate sample sizes required for each method to ensure an adequate power to detect differences between sites and to select the number and location of sites from which samples would be collected. The latter goal was relatively easily achieved following careful consideration of existing information and the questions being addressed as part of this project. The ability to estimate sample sizes was hampered by insufficient data, in part due to previous studies focusing more on differences between inshore and offshore forms rather than the more subtle differences expected among the inshore forms. As a result, an alternative approach is the use of adaptive sampling in which "the procedure for selecting units to include in the sample may depend on values of the variable of interest observed during the survey" (Thompson and Seber 1996, pg. ix). With this approach, optimal sample sizes and sampling locations can be determined from each year's progress so that, at the end of the project, the chance of detecting stock differences, if they exist, is greatest.

SUMMARY OF INFORMATION ON STOCK IDENTIFICATION IN THE MID-ATLANTIC SINCE THE DEPLETION LISTING

The likelihood of resolving stock structure is much higher now than any time previously because of some important preliminary and recent results from a number of studies.

Aerial surveys: During July 1994, in a series of coastal aerial surveys, dolphins were sighted along the Atlantic coast from central Florida to New Jersey in each of 3-4 passes (Blaylock *et al.* 1995). However, in the area between Cape Fear, NC, and Cape Romain, SC, and to a lesser extent between Cape Fear and Cape Lookout, NC, there were consistently very few sightings compared to other areas (NMFS, SEFSC data reported in Blaylock *et al* and re-analyzed for this workshop by L. Hansen). The coastline in this area is relatively featureless and may be less preferred habitat than areas with river mouths, sounds, embayments, and other estuarine habitat, as seen to the north and to the south. Dolphins were also sighted in the two estuarine areas surveyed: the mouth of the Chesapeake Bay and estuaries in Georgia and South Carolina.

Freeze brands: During live captures in July, 1995, (Hansen and Wells 1996) 31 bottlenose dolphins in the estuaries around Beaufort, NC, were marked with freeze brands. These easily identified specimens provided almost immediate information on movement patterns of some of the estuarine animals seen in Beaufort (unpublished resights by NMFS-Beaufort Laboratory, North Carolina Maritime Museum, Duke University, and Univ. of North Carolina in Wilmington). Furthermore, the size, sex, reproductive condition, and age is known for these individuals so it is possible to monitor habitat use and movement patterns as a function of life history.

Photo-identification: The most interesting and comprehensive results on movement and residency patterns have come from a series of photo-identification sites along the Atlantic coast between Cape May, NJ, and Jacksonville, FL (summarized in Urian and Wells 1996) (Fig. 2).

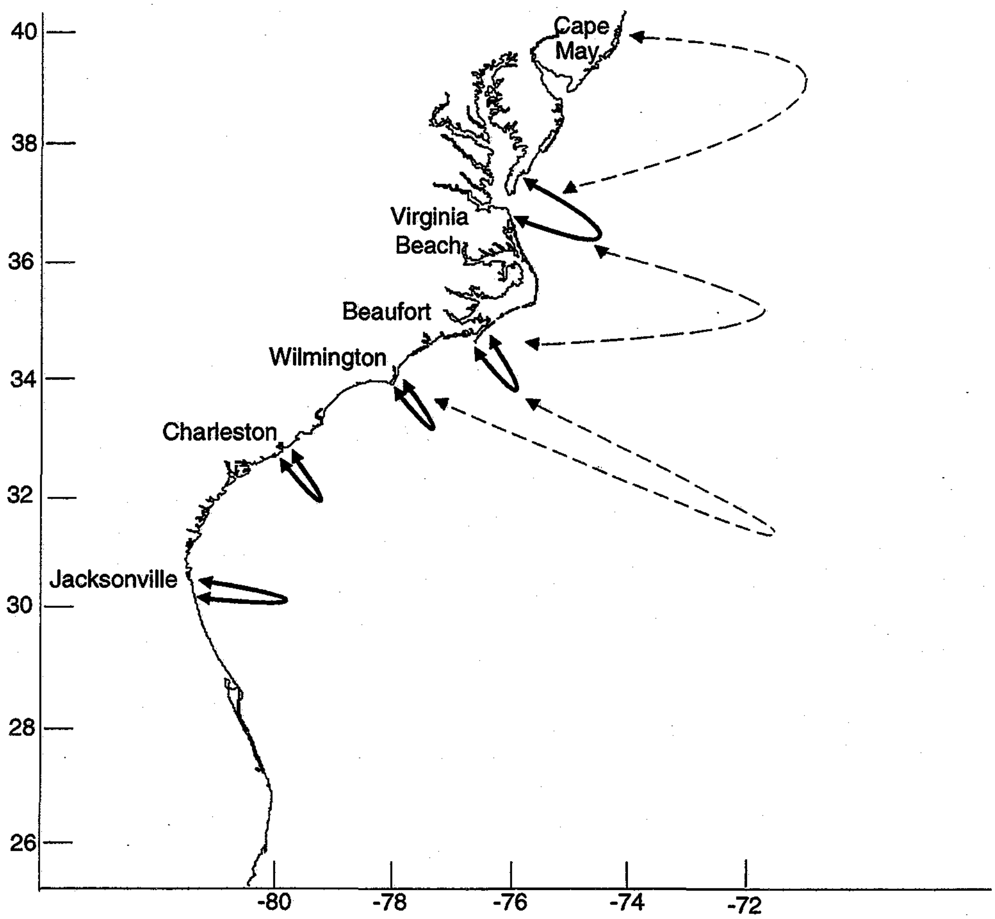

Fig. 2. Sites included in the photo-identification component of the stock identification project. Solid arrows indicate matches of individuals within sites between seasons or years; dashed arrows represent matches between sites (Barco and Swingle 1996, Caldwell 1996, Rittmaster and Thayer 1996, Sayigh *et al.* 1997, Zolman 1996).

Spatial and temporal patterns identified from photo-identification suggest a combination of year-round residents, seasonal residents (returning the same season each year but not present during other seasons), migrants or transients seen infrequently, and possibly groups with large home ranges (Table 1). Year-round residents have been reported only in the south (South Carolina: Zolman 1996, Petricig 1995; Florida: Caldwell 1996, Odell and Asper 1990); in the same areas seasonal residents and migratory or transient animals also occur. The northernmost documented year-round residency occurs in the Beaufort-Wilmington area of North Carolina (Sayigh *et al.* 1997); in North Carolina, both "coastal migratory" (winter) and estuarine (summer) animals are found (Rittmaster *et al.* 1997). Further north, the patterns are somewhat different (summarized in Wang, Payne and Thayer 1994). Bottlenose dolphins are rare north of North Carolina from October through May; animals migrate to the coast of Virginia through New Jersey in summer.

Table 1. Residency and movements patterns of bottlenose dolphins documented from photo-identification.

Location	Year-round Residents	Seasonal Residents	Migratory
Virginia Beach, VA	No	Jun-Sept	Jun-Sept
Beaufort, NC, "coastal"		Oct-Apr	?
Beaufort, NC, "estuarine"	Possible large home range		
Wilmington, NC	Possible large home range		
Charleston, SC	✓	fall-winter	spring, fall
Bull Creek, SC	✓	✓	
Jacksonville, FL	✓	Mar-Sept	all year (?)
Indian River, FL	✓	✓	?

Morphometrics: Morphometrics, parasite loads, and feeding habits positively separate individuals, of all age classes, into offshore and inshore forms (Mead and Potter 1995). In addition, variation in skull morphology within the inshore form suggests that the sample is derived from more than one population (Mead and Potter 1995).

Isotope ratios: Isotope ratios of carbon and nitrogen in teeth have been shown to be diagnostic for separating inshore and offshore *Tursiops* along the Atlantic and indicate the possibility of two inshore forms (Walker, Potter, and Macko in press, Walker and Macko in press). Analyses of specimens collected in the last century during the shore-based net fishery for *Tursiops* along the Outer Banks suggest that the feeding habits of inshore *Tursiops* may have changed over the past 100 years (Walker *et al.* in press).

Genetics: One published study (Dowling and Brown 1992) detected differences between *Tursiops* from (1) near Miami, (2) the mid-Florida coast and Georgia, and (3) Roanoke Island, NC. The small sample sizes (only one from some locations) suggest that these results be considered tentative. No other studies have examined differences within the inshore stock. However, in a comparison of inshore and offshore *Tursiops* in the Atlantic and Gulf of Mexico, greater genetic divergence was found in Atlantic inshore animals than in the Gulf inshore animals (Woodley and Curry 1996).

These preliminary results, taken together, provide a reasonable foundation on which to develop a field study and experimental design to resolve the stock structure of bottlenose dolphins in inshore waters of the mid-Atlantic.

EXPERIMENTAL DESIGN CONSIDERATIONS

Samples and data will be collected through a variety of means for a number of different analyses (Fig. 3). Each methodology or technique will be run independently, then the dissociated results will be merged to maximize the likelihood of defining stocks and identifying the best techniques for doing so. Collection of data and samples will be influenced by geographic and temporal factors which must be anticipated in developing the design.

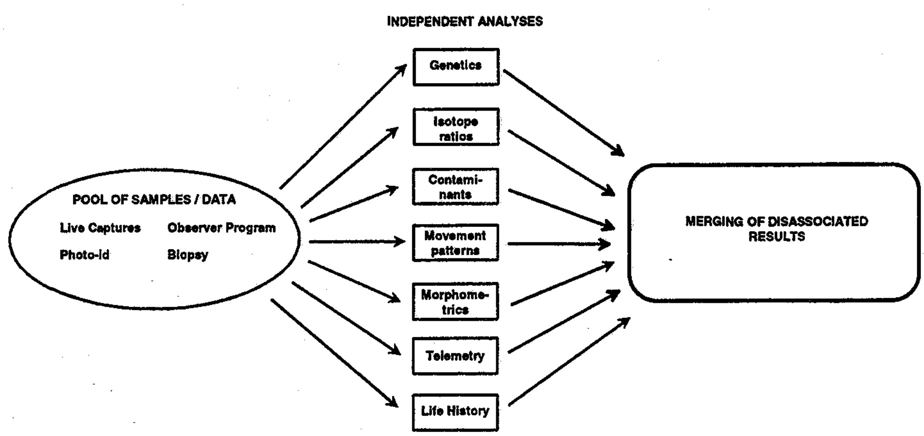

Figure 3

ANALYSES

Overall Approach

The experimental design has been developed using the following guidelines.

❖ First year sample sizes and geographic coverage to be determined from existing data and information, which in some cases are limited.

❖ After the first year, adaptive sampling will be instituted, i.e., site selection and estimates of sample sizes required will be modified on the basis of new data each year.

❖ Sample sizes for each method will be appropriate, not excessive (due to funding constraints) or insufficient, but still ensure an adequate power to detect differences among stocks.

❖ It will be assumed that if the sample size is adequate for each method independently, then the sample size when compiling data from matched samples will be adequate because covariation among variables will increase discrimination through multivariate analyses.

❖ All analyses will be predicated on detection at a significance level of $\alpha=0.05$ and $\beta=0.20$.

Matched Samples

A key component of the stock identification project is to identify which method(s) most readily separates stocks and which method(s) is the most efficient at doing so. Addressing these questions requires obtaining an adequate number of matched samples from each of the identified sites. There are assumptions and requirements pertaining to the collection and analysis of matched samples over and above those relevant to individual analyses.

❖ Overall minimum sample size is based on the technique requiring the most samples (to conduct a balanced multivariate analysis).

❖ For some areas, the most likely source of matched samples will be live captures. For other areas, the most likely source of matched samples will be entangled animals. The difference in the source of the samples is unlikely to bias the outcome because entangled specimens are essentially live-capture specimens that died. This approach assumes that stranded specimens not attributed to human interactions are excluded from the sample chosen to develop the methods to differentiate stocks because sick animals may have wandered before they ultimately beached. An exception is that stranded individuals with a photo-id history will be valuable. It also assumes inclusion of specimens only if collected in recent years in order to avoid confounding of results due to long-term changes in environmental or oceanographic conditions that might influence stock movements.

INDIVIDUAL ANALYSES

a. Genetics

In addition to samples collected through live captures and from entanglements, this analysis will have access to biopsy-dart samples of skin (these samples may also be useful for isotope ratio analyses). One goal is to biopsy individuals with sighting histories from photo-identification. The spatial and temporal distribution of biopsy-sampling effort will be determined using the adaptive sampling approach so that coverage is directed as needed each year in order to complement or supplement current data and information needs. Biopsy samples (n=31) currently exist for the animals live-captured in Beaufort, NC, in the summer of 1995; some biopsy samples exist for resident bottlenose dolphins near Charleston, SC; and additional samples will obtained during 1997 from resident dolphins near Charleston, from resident dolphins near Jacksonville, and from dolphins north of the mouth of the Chesapeake Bay during the late summer.

Inasmuch as insufficient data are currently available to estimate sample size requirements, and it is overly and unnecessarily presumptive to extrapolate results from other species, it was agreed that for genetics 20 specimens would likely be a conservative number from each of the first sites chosen.

Genetic, isotope ratio, and morphometric analyses provide the opportunity for historical evaluation of stock identification using material from museum collections. Genetic and isotope ratio analyses also provide the opportunity of detecting changes in population size or habits.

Provided the necessary facilities and equipment are available, the number of samples that can be run each year is not a limiting factor for the purposes of this study. Therefore, samples can be analyzed sufficiently in real time to use each year's results to modify the experimental design using adaptive sampling methods.

b. Isotope ratios

Sample sizes necessary to separate the inshore and offshore forms are about 10 per site (for an 80% power as determined from simulations using existing data sets, analysis by D. Colby, NMFS Beaufort Lab). Resolution of multiple inshore stocks of *Tursiops* using carbon and nitrogen would require larger sample sizes than are feasible to collect from each site (n=40 for an 80% power as determined from simulations using existing data sets, D. Colby, NMFS Beaufort Lab). As a result, use of other elements, such as ^{18}O, deuterium or sulfur, and other tissues, such as blood, bone, or muscle, is advised. Sample sizes necessary to detect differences within the inshore form can be determined after initial analyses have been conducted on these other elements and tissues. Samples are available from the live capture in Beaufort, NC, and from entangled specimens from along the mid-Atlantic coast. Skin samples taken by biopsy dart may also be useful.

The number of samples that can be run each year is not a limiting factor for the purposes of this study. Therefore, samples can be analyzed sufficiently in real time to use each year's results to modify the experimental design using adaptive sampling methods.

c. Contaminants

Differences in contaminant loads are detectable between *Tursiops* from coastal Texas and those from Beaufort, NC, sampled during live captures without correcting for age class or reproductive history (L. Hansen, SEFSC Charleston Laboratory, unpubl. data). Differences between stocks along the mid-Atlantic coast have not been analyzed and may be more difficult to detect because differences in contaminant burdens along the mid-Atlantic coast is expected to be less than that between Texas and Beaufort. Because contaminants loads are contingent upon age, sex, and reproductive history (for females), this analysis is difficult to incorporate as part of the matched sample series. One possibility would be to limit matched samples to males and juvenile females to remove the influence of reproductive history. Limiting matched samples to males only would prevent confounding of contaminant results (and the necessity for additional strata) but would hinder acquisition of a sufficient number of samples for contaminant and other analyses, and may bias the results. Final decisions about this method are pending until more information is available.

The number of samples that can be run each year is not a limiting factor. Therefore, samples can be analyzed sufficiently in real time to use each year's results to modify the experimental design using adaptive sampling methods.

d. Morphometrics

Morphometric differences indicate more than one inshore form (Mead and Potter 1995), particularly when complementary data on length-at-age (Hohn, Potter, and Mead, unpub data) and isotope ratios (Walker *et al.* in press) are evaluated concurrently for the same specimens. Larger sample sizes and samples from targeted areas are needed. Actual sample sizes required from each inshore area will be estimated from analyses conducted during the first year.

Because many of the criteria pertain to cranial morphology, samples must come from dead animals. It may be possible to use specimens already in museum collections if criteria for inclusion are carefully defined. For some geographic areas, samples are currently available.

The number of samples that can be run each year is not a limiting factor. Therefore, samples can be analyzed sufficiently in real time to use each year's results to modify the experimental design using adaptive sampling methods.

e. Satellite tracking

This technique is likely to provide invaluable information on movement patterns not readily obtainable through other means. Previous satellite transmitters on *Tursiops* suggest that two months of data might be certain but longer periods of time cannot be guaranteed at this time. However, a tag used successfully for longer periods of time on harbor porpoise might also be successful, with some modifications, for longer than two months on *Tursiops*. Ideally, transmitters need to function for one year before they contribute significantly to stock identification and movement patterns, *per se*, although shorter periods of time are likely to provide valuable information.

The number of animals that would need to be tracked from each site has not been determined. The number of transmitters that can be deployed each year is limited by the number of animals that can be live-captured and by the cost of the hardware.

f. Photo-identification

Photo-identification effort supported by NMFS is currently focussed at five sites: Virginia Beach, Beaufort, Wilmington (NC), Charleston, and Jacksonville (FL). These sites appear, for the time being, to be the most appropriate for detecting movement and residency patterns as determined from photo-id results to date and stranding patterns. More effort to cover other sites would be useful. For example, sightings from coastal aerial surveys in 1994 showed a lower density and abundance of dolphins between Cape Fear, NC, (near Wilmington) and just north of Cape Romain, SC, in summer. If this represents a "break" in distribution between "northerly" and "southerly" *Tursiops*, photo-id effort should result in lack of matches between these two sites. At this time, Wilmington is a photo-id site but no effort occurs near Cape Romain. It might be possible for some redirection of effort from Charleston or Wilmington, although this would be at the expense of important information emanating from those two sites. Other "gaps" include the eastern shore of Maryland and Virginia, the Outer Banks of North Carolina and much of the coast of Georgia.

The centralized photo-id catalogue is essential to facilitate matching among the many sites along the mid-Atlantic (Urian, Hohn and Hansen 1997). Early effort (summer 1997) will focus on quality assurance and incorporating photos from northern areas (New Jersey through North Carolina), those sites identified as highest priority in the experimental design of the overall project. The catalogue is unlikely to be completed until the third year of the project but will be accessible in the interim for those sites for which photos have been entered. Interpreting matching patterns resulting from the central catalogue will require standardizing definitions and use of some commonly used terms, such as "residents", "migrants", and "transients", that describe sighting intervals of individuals. Those definitions may also include specifications of effort required before an acceptable level of certainty in designations can be assured. A separate meeting of photo-id principles will be held for this purpose.

g. Life history

Reproductive season and location may contribute to stock separation. The Smithsonian's stranding database can be evaluated for indications of patterns in these parameters.

SAMPLES AND DATA

SOURCES OF SAMPLES AND DATA

Multiple means of obtaining samples and data are available (Table 2). Two of these, live captures and entanglements, allow for collection of matched samples. Live capture has the further advantages of sampling animals with resight histories, obtaining relatively large sample sizes in a very discrete period of time, and allowing deployment of transmitters for telemetry. In areas where live captures would be prohibitive due to expense or logistical difficulty and where there are few entangled specimens, biopsy samples will be invaluable. The flexibility in site selection offered with biopsy sampling will allow for "filling in the gaps" for areas where additional samples are needed. The samples would be limited to analyses for genetic and isotope ratios differences, *i.e.*, no matched samples could be obtained, and significant effort would be required to obtain sufficient sample sizes using this method alone.

Table 2. The various means of collecting samples and data for the various methods of testing for stock discreteness.

METHOD, TECHNIQUE, APPLICATION	PRIMARY ACTIVITY				
	Free-Ranging Animals				Stranded/ entangled[1]
	Photo-id[2]	Live Captures[3]	Coastal Biopsy	Pelagic Biopsy[4]	Observer Program[3]
Genetics[5]		✓	✓	✓	✓
Isotope Ratios[5]		✓	✓	✓	✓
Life History	✓	✓			✓
Contaminants		✓		✓	✓
Morphometrics		✓			✓
Movement Patterns	✓	✓			
Tagging	✓	✓			
Central Catalogue	✓	✓	✓	✓	✓

[1] stranded specimens would be appropriate for developing a stock model only if they are due to human interactions and are reported in good condition
[2] including photo-id central catalogue
[3] will provide "matched" samples
[4] pelagic vessel survey scheduled for 1998
[5] allows for historical testing

Photo-identification has been one the most informative techniques to date. It is likely to continue to form the foundation for some of the decision-making because it tracks individual animals. Given what may be an inshore stock pattern that reflects a small level of mixing between management units (*e.g.*, see Taylor 1995), photo-identification and satellite tracking are likely to be instrumental in initially identifying sites for biological sampling and eventually in elucidating fine-scale stock structure. They will not, however, provide a means for identifying, for example, the stock of an entangled animal unless that specimen has been individually identified. The central photo-identification catalogue is essential to facilitate matching among the various photo-id sites and identifying animals from which biopsy samples are collected.

MOST LIKELY SOURCES OF SAMPLES AND DATA FOR EACH SITE

The availability of samples by various means of collecting is not equally distributed along the coast (Table 3). From North Carolina through Virginia and Maryland, samples from specimens taken in fishery interactions can serve as a reliable source for matched samples. Net bans from South Carolina and south result in relatively few carcasses for the purposes of obtaining adequate sample sizes from restricted geographic areas. Gear restrictions, the seasonal presence of bottlenose dolphins, and less extensive nearshore fishing effort along the coasts of Maryland, Delaware and New Jersey will result in fewer fisheries samples from those regions. Live captures can be conducted where capture conditions are appropriate, in reality limiting the number of sites for this important source of obtaining samples. Small-vessel biopsy sampling is adaptable and is likely to be successful along much of the coast.

Photo-id sites can be established almost anywhere along the mid-Atlantic. The current sites are well-spaced and are producing matches that already are revealing patterns. More sites and more effort would decrease the time required to define the overall inshore pattern of movements and residency.

The various means of obtaining sources of samples and data are complementary and their use must be determined, in part, on geographic location.

Table 3. Most likely means of collecting samples from various sites along the mid-Atlantic coast.

Site	Live Capture	Biopsy	Photo-id	Entangle-ments
north of the mouth of the Chesapeake Bay	✓	✓	✓	✓
Virginia Beach, VA		✓	✓	✓
Beaufort, NC	✓	✓	✓	✓
Wilmington, NC		✓	✓	✓
Cape Romain, SC		✓	✓	
Charleston, SC	✓	✓	✓	
Georgia (site TBD)	✓	✓	✓	
Jacksonville, FL	✓	✓	✓	

Anticipated Sample Sizes Obtainable from Each Source

The largest single sample available from one site at one time (thereby eliminating temporal variability) is from live captures. A single live capture is expected to yield matched samples from 30 animals, and allow the deployment of satellite transmitters on as many of those animals as is supported. Human interactions will provide matched samples from northern areas, but are unlikely to provide sufficient numbers from the south.

Biopsy samples of skin (and possibly small quantities of blubber) can be collected in relatively large numbers if a significant amount of field time is devoted to obtaining the samples. Realistically, with existing staff, it is estimated that about 20 individuals known from photo-identification can be sampled from up to three sites each year (a site may be the same geographic location during a different season).

Matched Samples Already Collected

Samples have been collected from stranded and entangled animals for at least a couple of decades. Optimally, however, only samples collected in recent years will be included to minimize temporal variability in stock structure. Once stock differences have been identified and defined, the previously collected samples can be evaluated to determine from which stock specimens, particularly entangled specimens or those from the die-off, came. An initial inventory of available matched samples collected in recent years showed variability in samples by geographic location and the source of those samples (Table 4).

Table 4. Samples available for use in the stock identification project. Human interaction and stranding samples are from 1992-96. The live capture in North Carolina occurred in 1995, during which time the biopsy samples were collected. Samples may be available for some of the other areas (those noted with a "?").

	NJ	MD/ DE	VA	NC	SC	GA	FL
Live capture	0	0	0	31	0	0	0
Human interactions	?	?	14	19	8	?	?
Strandings (not HI)	?	?	36	14	38	?	?
Biopsy	0	0	0	31	0	0	0

HYPOTHESES AND HYPOTHESIS TESTING FOR RESOLVING STOCK STRUCTURE OF INSHORE BOTTLENOSE DOLPHINS

Null and alternative hypotheses:

H_0: One inshore "coastal migratory stock"

H_A: More than one inshore stock

Related questions

Existing data suggest a number of questions pertaining to the structure of the inshore stock of bottlenose dolphins and questions to be considered:

- Do dolphins seen off the coast of New Jersey in summer travel as far south as central Florida in winter, as defined for the "coastal migratory stock"?
- Can separate groups that represent management units be identified?
- Can the rate of mixing be quantified?
- Are there differences between "resident" groups at different sites (*e.g.*, Charleston, Jacksonville, Indian River)?
- What are relationships among various seasonal groups and individuals at different sites?
- Within a site, are there differences between year-round residents, seasonal residents, and transients?
- What is the spatial pattern of overlap between the inshore and offshore forms?

Furthermore, the design is structured to address the question of means of resolving stock differences:
- What sample sizes are necessary for each of the various techniques to support conclusions under various hypotheses of stock structure?
- How many methods are needed to resolve stock structure?
- Which method(s) is most efficient in resolving stock structure questions?

YEAR 1 ACTIVITIES

Year 1 will focus on using samples on hand or readily collected during Year 1, such as biopsy samples. Emphasis will be on detecting differences between the clearly migratory animals that are north of the mouth of the Chesapeake Bay in summer and animals that are found off of South Carolina, Georgia, and Florida in summer and winter. If this is one stock, no differences will be detectable. Some emphasis will also be placed on developing techniques, such as cloning microsatellites and testing satellite transmitter configuration and attachment.

Genetics:
- Isolate and clone *Tursiops* microsatellites.
- Analyze the Beaufort live capture samples to provide information on variability within a site.
- Analyze biopsy samples (to be collected in Year 1) from north of the mouth of the Chesapeake Bay (summer) and South Carolina (year-round residents and seasonal residents with peak abundance in November); where possible biopsy animals with sighting histories verified through photo-id.
- Estimate sample sizes needed.

Isotope Ratios:
- Obtain blood from Beaufort (1995 live captures) for ^{18}O and deuterium.

- Collect and run blood, teeth and muscle from stranded animals from north of the mouth of the Chesapeake Bay and from South Carolina for comparison of isotope ratios using, in some combination, carbon, nitrogen, sulfur, ^{18}O, and deuterium.
- Analyze isotope ratios of muscle from fish recovered in the stomachs of entangled dolphins from which tissues are analyzed for isotope ratios.
- Estimate sample sizes needed.

Telemetry:
- Field test satellite tags developed for *Phocoena* and modified for *Tursiops* preferably by live captures along the mid-Atlantic or alternatively during Sarasota live captures.

Life History:
- Analyze data from strandings of neonates for spatial and temporal patterns.

Contaminants:
- Analyze samples from entangled animals north of the mouth of the Chesapeake Bay in summer and from South Carolina in summer and winter.
- Analyze the small set of sample from biopsies already collected from Charleston.
- Estimate sample sizes needed.

Morphometrics:
- Run growth curves using cranial analyses as determinant of sample stratification.
- Evaluate cranial morphology for animals from north of the mouth of the Chesapeake Bay in summer and Charleston, Georgia, and Florida in summer and winter using existing prepared crania.
- Prepare (clean, catalogue, etc.) crania collected in recent years, particularly those collected as part of the matched-sample set.
- Estimate sample sizes needed to differentiate inshore forms.

Central Photo-identification Catalogue:
- Begin effort on the central catalogue by consulting with the managers of well-established central catalogues (*e.g.*, manatees, right whales, humpback whales) to query about the most successful approaches to establishing and operating these important tools.
- Develop catalogue by archiving the best images of each identified dolphin from each of the photo-id sites, beginning in the north (New Jersey, Virginia Beach, Cape Charles, and Beaufort winter animals) where early biopsy effort is scheduled to begin.
- Meet with photo-id collaborators to develop and establish a consistent protocol/experimental design for use at each site.
- Begin or continue photo-id effort at each of the five sites identified for high-effort sampling.

YEAR 2 ACTIVITIES

Year 2 will focus on obtaining samples in a more targeted manner, by means of live capture and increased biopsy sampling, and to obtain large sample sizes collected at the same point in time. Live captures are needed to deploy satellite transmitters, an important component of Year 2 activities. Choice of sites will be finalized following analysis of results from Year 1. Sample sizes for each method will also be determined by adaptive sampling techniques applied to Year 1 results.

Coordination:

- Convene collaborators in the study to compile and discuss results from Year 1, choose sites for sampling, and determine sample sizes necessary.

Live capture:

- Two sites, tentatively identified as north of the mouth of the Chesapeake Bay in mid-late summer and near Charleston in winter (focusses on the purported coastal migratory stock).
- Obtain 30 full sets of samples from each site.
- Deploy satellite transmitters.

Analyses:

- Use all techniques appropriate for samples collected, compare results from the sites and to the Year 1 results.
- Run genetic and isotope analyses on biopsy samples from pelagic surveys.

YEAR 3 ACTIVITIES

Emphasis will be on detecting differences between groups of animals not at the extremes of the distribution of the "coastal migratory stock", assuming this question has been resolved during Years 1 and 2. Particular areas of emphasis will be the southern limit of the presumed northern migratory form, the relationship between different forms inhabiting the same area (*e.g.*, residents v. transients), and groups with large home ranges that might comprise a separate management unit.

Coordination:

- Convene collaborators in the study to compile and discuss results from Years 1 and 2, choose sites for sampling, and determine sample sizes necessary.

Live capture:

- Two sites, to be determined on the basis of results from Year 1 and 2.
- Obtain 30 full sets of samples from each site.
- Deploy satellite transmitters.

Analyses:

- Use all techniques appropriate for samples collected, compare results from the sites and to results from Years 1 and 2.

YEAR 4 ACTIVITIES

Coordination:

- Convene collaborators to discuss the results from each methodology and those from the analysis of matched samples to develop a stock model.

Draft report.

ACKNOWLEDGMENTS

The workshop was held at the North Carolina Maritime Museum, which turned out to be an excellent venue for a productive meeting. Our thanks to the Director and staff of the NCMM, particularly Keith Rittmaster, and to the Friends of the Museum for assistance and cooperation. Caterina D'Agrosa and Katie Touhey helped with many aspects of preparation for of the workshop, and helped ensure a minimal number of unanticipated glitches. Caterina also assisted with the figures in this report. Most of the participants were requested to present summaries current research and results and explain the techniques relevant to the stock identification project. Dr. David Colby, NMFS Beaufort Lab, helped with statistical advice and simulations prior to the workshop, offering insights into experimental design that proved very helpful during workshop discussions.

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APPENDICES

Tuesday, February 11

0900	Introductions	Hohn
0930	Review of the purpose of this workshop and overview of stock proposal	Hohn
1000	Coffee break	
1020	Review of results from 1994 aerial surveys from Florida to New Jersey: the distribution of coastal bottlenose dolphins	Hansen
1045	Informal presentations on each technique and activity involved in the stock identification project: description of methods and results to date*	
	Morphometrics	Potter
	Isotope ratios	Macko
	Genetics	Rosel
noon	Lunch	
1330	Biopsy sampling (coastal and pelagic)	Hansen
	Live captures	Hansen
	Satellite telemetry technique	Read
	Photo-id sites and results	Hohn
	Photo-id catalogue	Urian
1500	Coffee Break	
1520	Observer program	McLellan
	Sample dissemination	Touhey
1550	Development of Experimental Design a. Number and location of sites to sample b. Number of samples required from each site for each method	Hohn/all
1800	*Informal reception for participants and the local marine mammal community at the NCMM*	

Wednesday, 12 February

0900-1600	Continue development of experimental design until complete

*Where possible to include sites sampled, sample sizes, similarities and differences found

Appendix II: WORKSHOP PARTICIPANTS

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Appendix III: LETTER TO PARTICIPANTS

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aleta.hohn@noaa.gov

14 January 1997

[Participant name
and address]

Dear [participant],

Thank you for agreeing to participate in the stock identification workshop. The purpose of the workshop is to develop an experimental design that will maximize the possibility of determining the stock structure of bottlenose dolphins along the mid-Atlantic. The workshop is scheduled for February 11-12, 1997, in Beaufort, N.C. In planning your travel, please assume that the workshop will convene at 9:00am and strive to adjourn no later than 4:00pm on the 12th so that participants preferring to leave that afternoon may easily do so. A list of participants is attached.

The workshop will be a working session with the specific goals of:

(1) introducing the proposed approach of concurrent application of complementary methods towards defining stocks while ensuring that each participant is familiar with each technique and with the overall purpose of the stock identification project;

(2) evaluating existing information about the distribution and movement patterns of bottlenose dolphins to determine sites from which samples should be collected; and

(3) estimating necessary sample sizes from each site for each methodology.

To accomplish the first goal, I would appreciate having each principal provide a brief (15 min) informal summary of their methodology and results to date. If you need more time, please let me know. Overhead and slide projectors will be available. Handouts are welcome. In addition, prior to the workshop I would like to distribute background material that includes reprints or other information on each technique to each participant. I will be contacting you about donating material but also would appreciate if you would send information when you receive this letter.

Achieving the second goal will require some imagination. Only limited data are available on distribution and movements of bottlenose dolphins along the Atlantic coast from New Jersey through northern Florida; these data will be summarized. To the extent possible, it will be important to use that information to select the number and location of sites from which it is desirable to collect samples.

Estimating sample sizes, the third goal, will require that each principal bring information from previous studies that suggest the type of variation that might be expected. Using standard techniques and with help from an analyst we will try to determine the number of samples needed from each site. In addition, some of the techniques may be able to use other samples that will contribute to the overall stock question. For example, specimens from the dolphin fishery on the Outer Banks during the end of the last century and beginning of this century might be accessed from the Smithsonian collection for isotope ratio analyses and morphology. Biopsy samples are expected to be available for genetic analyses.

Following the workshop, I will compile the results into a report. Our approach and outcome then will be reflected in future proposals.

If you have any questions, please do not hesitate to ask at any time.

Sincerely,

Aleta A. Hohn, Ph.D.
Leader, Marine Mammal Project

Enclosure