WP25

THE COMBINED APPLICATION OF PHOTOGRAPHIC IDENTIFICATION AND GENETIC SAMPLING TO UNDERSTANDING POPULATION STRUCTURE AND DYNAMICS OF SMALL CETACEANS

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INTRODUCTION

One of the more challenging aspects of small cetacean conservation research is the identification of biologically-meaningful population units within continuous distributions of species. Population identification is crucial for evaluation of exposure to, and impacts of, threats from a variety of natural and anthropogenic sources. Within recent years, research on a variety of small cetaceans in coastal waters, such as bottlenose dolphins (*Tursiops truncatus*) at several sites in the Atlantic and Gulf of Mexico waters of the United States, has shown that population structure exists on a finer scale than previously believed. Conservation plans that do not take into account fine scale structuring may not adequately protect small population units that are in fact distinct, functional elements of their ecosystem. Localized threats may impact discrete population units, with only minimal potential for recruitment from adjacent units. Thus, the precautionary principle dictates exploring the possibility of fine scale population structuring in continuously-distributed species. Knowledge of population structure facilitates determination of trends in abundance estimates and vital rates, leading to more effective risk identification and assessment.

A variety of tools has been developed over the last four decades for elucidating the structure of dolphin populations. These tools can be applied across a variety of field situations, including observations and remote sampling of free-ranging animals, capture-release, and strandings. The most frequently used techniques include photographic identification and genetic analyses, but a number of other approaches have been developed that have great value for distinguishing between population units. Each of the techniques is of utility by itself, but in combination the techniques become much more powerful (Wells 1994; Hohn 1997). Often the effectiveness of these techniques is enhanced through collaborative application over broader geographical ranges. We will briefly describe the available techniques, and then describe how they have been applied in one long-term study of bottlenose dolphins along the central west coast of Florida. Many of these techniques should be equally applicable to coastal *Sotalia*.

The senior author was very disappointed about having to change his plans to attend this workshop, but he was required to participate in a concurrent workshop in Chicago, on increasing conservation capacity in Latin America. He will have access to e-mail and would be happy to address any questions that arise from this presentation or subsequent discussion during the workshop (rwells@mote.org).

APPROACHES TO UNDERSTANDING POPULATION STRUCTURE

Coastal small cetacean population structure often seems to be related to geography. Thus, sampling techniques that match samples with an animal's typical range may facilitate defining population units.

Morphometrics -- One of the earliest and most basic approaches to distinguishing between population units is through comparison of ratios of standard anatomical measurements. Units with sufficient genetic separation should exhibit significant phenotypic differences as evidenced in morphometrics differences. Some of these differences may be evident in external measurements as can be obtained during capture-release operations or strandings, but the most diagnostic measures come from measurements of skeletal specimens, especially skulls.

Photographic Identification -- Individual identification through photographs of distinctive dorsal fin markings has become a standard technique for field studies of many dolphins over the past 30 years (Würsig and Jefferson 1990; Scott *et al.* 1990a; Wells 2002). Many dolphins, including *Tursiops* and *Sotalia* (Flores 1999), acquire distinctive nicks and notches in their dorsal fins that allow reliable identification of individuals from photographs over periods of years. Re-sightings of individuals provide documentation of ranging and social association patterns. The power of this approach is greatly enhanced when networks of collaborating independent scientists are established along a coastline, such that the potential exists to obtain re-sightings at distances well beyond the operating range of a single investigator, should such movements occur (Würsig and Harris 1990; Wells *et al*. 1990; Urian and Wells 1996). Photo-ID data can provide the basis for mark-recapture analyses of abundance and vital rates.

Genetic Techniques – Genetic analyses provide a powerful means of distinguishing between population units, especially through the examination of chromosomes, mitochondrial DNA and microsatellites (Duffield and Wells 1991, 2002; Taylor 1997). Samples may be obtained from free-swimming dolphins through several techniques, including biopsy darting via a rifle or crossbow system (*e.g*., Krützen *et al.* 2002; Sellas *et al.* 2005), or from swabs rubbed on bow-riding dolphins (Harlin *et al.* 1999). Genetic samples can also be obtained during capture-release operations (Duffield and Wells 1991, 2002) or from stranded carcasses or skeletal specimens in museum collections. Genetic analyses are of greatest utility when the ranging patterns of the sampled individuals are known; samples from stranded individuals of unknown origin are of less value.

Stomach Contents – Diets of marine mammals often vary with habitat, and stomach contents can provide a clue to ranges of stranded individuals (Barros and Odell 1990; Barros and Wells 1998).

Stable Isotope Ratios – Because dolphin food from different habitats is represented by differences in stable isotopes of carbon, nitrogen, and sulfur in dolphin tissues, it is possible to use ratios of stable isotopes to assign putative habitats to dolphins when tissue samples can be obtained (strandings, biopsy darting, capture-release). Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotopic signatures in different tissues provide insights into temporal feeding patterns, depending on stability of the tissue. For example, signatures obtained from teeth reflect longer-term patterns than signatures from skin or muscle, where cell turn-over is more frequent. Large-scale geographical variations in feeding can also be identified with stable isotopes.

Environmental Contaminant Ratios – Ratios of concentrations of environmental contaminants such as organochlorines in blubber (*e.g*., PCBs, DDT and metabolites) can reflect geographical variation based on pollutant source sites or deposition patterns (Westgate and Tolley 1999).

Similarities in ratios of congeners among individuals can suggest congruent ranges. Blubber samples can be obtained from carcasses, biopsy darting, or during capture-release operations.

Parasite loads – Dolphins living in different habitats can be exposed to different ecto- and endo-parasites. Data from photographs of free-swimming animals or from examination of stranded carcasses or live animals during capture-release operations can provide supplemental information for distinguishing between populations, but the level of precision of parasite burden information for this purpose is low.

Tagging and Tracking -- Resightings of marked animals or tracking of dolphins tagged with radio transmitters can provide very important information on ranging patterns that can be used to define population units. A wide range of tagging techniques has been developed for dolphins, including freeze-branding, attachment tags such as cattle ear tags (roto tags), small VHF radio transmitters for direct line-of-sight tracking, and satellite-linked radio systems for remote tracking and data collection (Irvine *et al.* 1982; Scott *et al.* 1990a; Read 2002; Wells 2002). Currently, each of these techniques must be conducted during capture-release operations, but techniques for deploying VHF tags on free-swimming dolphins are currently under development.

Integration – The discriminatory power for identifying population units increases when more than one technique is applied. For example, a quantitative approach has been developed to define bottlenose dolphin communities based on ranging and social association patterns (Urian 2002). Similarly, statistical techniques are being developed to relate genetic data and ranging patterns to objectively define population units through a geographically constrained, hierarchical clustering algorithm, referred to as Boundary Rank (Martien *et al.* 2003).

CASE STUDY: BOTTLENOSE DOLPHINS IN SARASOTA BAY, FLORIDA

Bottlenose dolphins along the central west coast of Florida are distributed more or less continuously. Tagging studies conducted in the 1970s began to show fine scale structuring in the form of residency within this continuous distribution (Irvine *et al*. 1981), leading to a long-term program of research to investigate population structure and dynamics (Scott *et al*. 1990b; Wells 1991, 2003).

Experimental tagging studies initiated in Sarasota Bay in 1970 and continuing through 1976 provided the first hints of residency of bottlenose dolphins along the central west coast of Florida (Irvine and Wells 1972; Irvine *et al*. 1981). Tagged dolphins were resighted repeatedly in the same area over periods of years. Radio-tracking during the mid-1970s confirmed the patterns of residency suggested by earlier tagging, and led to the definition of a utilization area for a resident group of about 120 bottlenose dolphins, extending from southern Tampa Bay to southern Sarasota Bay and out several kilometers into the Gulf of Mexico (Wells *et al.* 1980, Figure 1).

As appreciation for the natural variability of bottlenose dolphin dorsal fin features developed in the mid-1970s, photographic identification became an increasingly important tool in Sarasota Bay. By 1980, it became the primary technique for individual identification, leading to the development of a current identification catalog of more than 3,000 distinctive dolphins from the central west coast of Florida.

Ranging patterns for many of the identifiable dolphins have remained relatively stable over time, suggesting a geographical basis to population structure. Some of the individuals in the catalog have been re-identified in Sarasota Bay more than 1,000 times over more than three decades. Through these repeated identifications, long-term, multi-generational residency has been defined for the bottlenose dolphins of Sarasota Bay and adjacent waters. About one third of the dolphins first tagged in 1970-71 were still observed in the region during 2005, as were their calves, grand-calves, and great-grand-calves. During 2005, the first instance of a five-generation span of living residents was documented in Sarasota Bay.

In order to place the patterns of residency in Sarasota Bay into appropriate perspective, photographic identification surveys were conducted through adjacent waters in Tampa Bay, Charlotte Harbor, Pine Island Sound, and the coastal Gulf of Mexico in the 1980s, 1990s and 2000s. In addition, collaborative efforts with Eckerd College in St. Petersburg, Florida involve ongoing comparisons of catalogs to document occurrence of dolphins in Tampa Bay and vicinity. Patterns of residency similar to those demonstrated for Sarasota Bay dolphins were documented in each of the bays, sounds, and estuaries, forming a mosaic of slightly overlapping ranges along the coast. A few individuals were observed to move between areas, but residency was the more dominant pattern. Clear distinctions were apparent between dolphins primarily using Sarasota Bay vs. Gulf of Mexico waters, based on sighing patterns (Fazioli *et al.* in press). Sarasota Bay residents remained disproportionately near passes between barrier islands, while Gulf residents were distributed uniformly along the coast. Few Sarasota Bay dolphins were observed in adjacent waters to the north, south, or west, with the primary exception being movements by adult males. These findings provided further support to the concept of a geographically-based population unit.

Analyses of social association patterns during the 1970s and 1980s found a relationship between social and ranging patterns, such that assemblages of dolphins that inhabited similar ranges and interacted socially more with each other than with adjacent assemblages were considered members of a "community" (Wells 1986; Wells *et al.* 1987). Urian (2002) developed a more objective, quantitative approach to defining communities through analyses of the photographic identification survey data on sighting locations and social associates in Tampa Bay. Her putative communities corresponded well to those proposed by Wells (1986) where their study areas overlapped. Thus, behavioral features of ranging patterns and social associations have been found to be useful in distinguishing between potential population units.

The advent of the application of genetic techniques to dolphin population questions beginning in the 1980s provided a means for testing the biological basis of the community designations. Samples for genetic analyses were collected during capture-release operations for life history studies and health assessment (Wells *et al*. 2004) and through biopsy darting. Early analyses indicated genetic differentiation between geographical regions corresponding to the patterns of communities identified behaviorally (Duffield and Wells 1991, 2002). Subsequent analyses have provided further confirmation of the validity of the community designations. Sellas *et al.* (2005) provided additional support for distinctions between inshore communities and Gulf of Mexico dolphins as proposed by Fazioli *et al*. (in press) based on behavioral patterns. Martien *et al.* (2003) found strong correspondence between current community designations and mtDNA data through Boundary Rank analyses, suggesting a genetic basis to the designations, reinforcing the demographic and behavioral bases. However, genetic paternity testing showed a high level of genetic exchange between communities, indicating that they are not closed reproductive units, and therefore are not populations in the strictest sense (Duffield and Wells 1991, 2002).

Additional information on large scale geographical variation in feeding as well as fine scale distinctions between communities has been possible through examination of dolphin diets. The stomach contents of stranded Sarasota Bay residents indicate that they feed primarily on fishes associated with sea grasses, and only rarely on squid, while dolphins from adjacent Gulf of Mexico waters are more commonly found with squid in their stomachs (Barros and Wells 1998). Further support was derived from stable isotope analyses. Teeth obtained from dolphins stranded in Sarasota Bay (resident animals of known feeding history), the adjacent Gulf of Mexico, and from Charlotte Harbor were analyzed for their carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotopic signatures. Selected fish prey collected in Sarasota Bay were also analyzed for C and N isotopes (ranges of $\delta^{13}C$: -16.2 to -10.2‰ and $\delta^{15}N$: 2.9 to 8.6‰). Sarasota Bay dolphins (n= 31) had significantly higher $\delta^{13}C$ and lower $\delta^{15}N$ values (ranging from -15.2 to -8.2‰ and 9.7 to 15.2‰, respectively) than Gulf and Charlotte Harbor animals. Isotope data for Sarasota Bay are consistent with preference for seagrass-associated fish prey, as determined from stomach content analyses and behavioral observations. Ontogenetic trends for carbon enrichment ($\delta^{13}C$: $r^2 = 0.27$, $p < 0.01$) and nitrogen depletion ($\delta^{15}N$: $r^2 = 0.32$, $p < 0.01$) in Sarasota Bay dolphins suggest shifts in prey preference, prey movement between habitats (*e.g*., seagrass meadows, mangroves) and/or dolphin habitat use. Preliminary analysis of sulfur isotope data suggests nearly non-overlapping ranges in the few specimens analyzed, where estuarine < Gulf < offshore dolphins. Thus, animals inhabiting the Charlotte Harbor, an estuary influenced by three major rivers, had significantly lower $\delta^{34}S$ values (7.2 ‰ ± 2.1 SD, n= 5) compared to other populations (inshore animals stranded on Gulf beaches: 10.3 ‰ ± 1.5 SD, n= 5; animals from the offshore ecotype: 17.3 ‰ ± 1.1 SD, n= 4), likely reflecting their freshwater signature.

In summary, consistent evidence from ranging patterns, social associations, genetics, and diets supports the existence of distinct, geographically-based, long-term, multi-generational communities of bottlenose dolphins along the central west coast of Florida. Defining these population units, and in particular the Sarasota Bay community, has allowed the determination of abundance estimates and vital rates, and trends in these parameters (Wells and Scott 1990; Wells 2003). Over the past decade, the numbers of dolphins using Sarasota Bay on a regular basis has increased to about 150. In spite of this increase, the community exhibits a disturbing trend of high first-born calf mortality within the first year of life. Research on concentrations of environmental contaminants in Sarasota Bay dolphins offers a potential explanation for at least some of this poor calf survivorship. Primiparous females have significantly higher concentrations of organochlorine pollutants in their tissues than do multiparous females, and their body burdens decline during lactation, indicating transfer of potentially lethal quantities of contaminants to some of their calves through milk. Surviving first-born calves have higher concentrations of organochlorines than do subsequent calves. As can be seen from this example, defining population units facilitates evaluating risks to the units. When population units have a geographical basis, it becomes easier to evaluate the exposure of the animals to the risks. In at least some places in South America, *Sotalia* exhibits comparable long-term residency. Thus, some of the approaches described here may be applicable to the conservation of *Sotalia*.

LITERATURE CITED

Barros, N.B. and D.K. Odell. 1990. Food habits of bottlenose dolphins in the southeastern United States. Pp. 309-328 *In*: Leatherwood, S. and R.R. Reeves (eds). The Bottlenose Dolphin. Academic Press: San Diego.

Barros, N.B. and R.S. Wells. 1998. Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. J. Mammalogy 79(3):1045-1059.

Duffield, D.A. and R.S. Wells. 1991. The combined application of chromosome, protein and molecular data for the investigation of social unit structure and dynamics in *Tursiops truncatus*. Pp. 155-169 *In:* A.R. Hoelzel (ed.) Genetic Ecology of Whales and Dolphins. Report of the International Whaling Commission, Special Issue 13, Cambridge, U.K.

Duffield, D.A. and R.S. Wells. 2002. The molecular profile of a resident community of bottlenose dolphins, *Tursiops truncatus*. Pp. 3-11 *In*: C.J. Pfeiffer, ed., Molecular and Cell Biology of Marine Mammals. Krieger Publishing Company, Melbourne, FL.

Fazioli, K.L., S. Hofmann, and R.S. Wells. In press. Use of coastal Gulf of Mexico waters by distinct assemblages of bottlenose dolphins, *Tursiops truncatus.* Aquatic Mammals.

Flores, P.A.C. 1999. Preliminary results of a photoidentification study of the marine tucuxi *Sotalia fluviatilis* in southern Brazil**.** Marine Mammal Science, 15:840-847.

Harlin, A.D., B. Würsig, C.S. Baker, and T.M. Markowitz. 1999. Skin swabbing for genetic analysis: Application to dusky dolphins (*Lagenorhynchus obscurus*). Marine mammal Science 15:409-425.

Hohn, A.A. 1997. Design for a multiple-method approach to determine stock structure of bottlenose dolphins in the mid-Atlantic. NOAA Tech. Memo. NMFS-SEFSC-401. 22 pp.

Irvine, B. and R.S. Wells. 1972. Results of attempts to tag Atlantic bottlenose dolphins (*Tursiops truncatus*). Cetology 13:1-5.

Irvine, A.B., M.D. Scott, R.S. Wells and J.H. Kaufmann. 1981. Movements and activities of the Atlantic bottlenose dolphin, *Tursiops truncatus*, near Sarasota, Florida. Fish. Bull. U.S.79:671-688.

Irvine, A.B., R.S. Wells and M.D. Scott. 1982. An evaluation of techniques for tagging small odontocete cetaceans. Fish. Bull. U.S. 80:135-143.

Krützen, M., L.M. Barre, L.M. Möller, M.R. Heithaus, C. Simms, and W.B. Sherwin. 2002. A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. Marine Mammal Science 18:863-878.

Martien, K., A. Sellas, P. Rosel, B. Taylor, and R. Wells. 2003. Integration of genetic and behavioral data for bottlenose dolphin population unit definition. 15th Biennial Conference on the Biology of Marine Mammals. December 14-19, 2003, Greensboro, NC.

Read, A.J. 2002. Telemetry. Pp.1232-1235 *In:* W.F. Perrin, B. Würsig, and J.G.M. Thewissen, eds., Encyclopedia of Marine Mammals. Academic Press, San Diego, CA.

Scott, M.D., R.S. Wells, A.B. Irvine and B.R. Mate. 1990a. Tagging and marking studies on small cetaceans. Pp. 489-514 *In*: S. Leatherwood and R.R. Reeves (eds.), The Bottlenose Dolphin. Academic Press, San Diego. 653 pp.

Scott, M.D., R.S. Wells and A.B. Irvine. 1990b. A long-term study of bottlenose dolphins on the west coast of Florida. Pp. 235-244 *In*: S. Leatherwood and R.R. Reeves (eds.), The Bottlenose Dolphin. Academic Press, San Diego. 653 pp.

Sellas, A.B., R.S. Wells, and P.E. Rosel. 2005. Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. Conservation Genetics 6:715-728.

Taylor B.L. 1997. Defining "populations" to meet management objectives for marine mammals. pp. 347-364. *In*: A.E.Dizon, S.J.Chivers and W.F.Perrin (eds). *Molecular Genetics of Marine Mammals*. Society for Marine Mammalogy, Special Publication 3, Lawrence, Kansas.

Urian, K.W. 2002. Community structure of bottlenose dolphins (*Tursiops truncatus*) in Tampa Bay, Florida, USA. M.Sc. thesis, University of North Carolina, Wilmington. 26 pp.

Urian, K.W. and R.S. Wells. 1996. Bottlenose dolphin photo-identification workshop. Final contract report to the National Marine Fisheries Service, Southeast Fisheries Science Center, Charleston, SC. Contract No. 40-EUNF-500587. NMFS Technical Memo. NMFS-SEFSC-393. 91 pp.

Wells, R.S. 1986. Structural aspects of dolphin societies. PhD dissertation, University of California, Santa Cruz. 213 pp.

Wells, R.S. 1991. The role of long-term study in understanding the social structure of a bottlenose dolphin community. Pp. 199-225*In:* K. Pryor and K.S. Norris (eds.), Dolphin Societies: Discoveries and Puzzles. Univ. of California Press, Berkeley. 397 pp.

Wells, R.S. 1994. Determination of bottlenose dolphin stock discreteness: Application of a combined behavioral and genetic approach. Pp. 16-20 *In*: K.R. Wang, P.M. Payne, and V.G. Thayer (compilers), Coastal Stock(s) of Atlantic Bottlenose Dolphin: Status Review and Management. Proceedings and Recommendations from a Workshop held in Beaufort, NC, 13-14 September 1993. NOAA Technical Memorandum NMFS-OPR-4. 120 pp.

Wells, R.S. 2002. Identification methods. Pp.601-608 *In:* W.F. Perrin, B. Würsig, and J.G.M. Thewissen, eds., Encyclopedia of Marine Mammals. Academic Press, San Diego, CA.

Wells, R.S. 2003. Dolphin social complexity: Lessons from long-term study and life history. Pp. 32-56 *In:* F.B.M. de Waal and P.L. Tyack, eds., Animal Social Complexity: Intelligence, Culture, and Individualized Societies. Harvard University Press, Cambridge, MA.

Wells, R.S. and M.D. Scott. 1990. Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. Pp. 407-415 *In:* P.S. Hammond, S.A. Mizroch and G.P. Donovan (eds.) Individual Recognition of Cetaceans: Use of Photo-Identification and Other Techniques to Estimate Population Parameters. Report of the International Whaling Commission, Special Issue 12, Cambridge, U.K. 440 pp.

Wells, R.S., A.B. Irvine and M.D. Scott. 1980. The social ecology of inshore odontocetes. Pp. 263-317 *In*: Herman, L.M. (ed.) Cetacean Behavior: Mechanisms and Functions. New York: J. Wiley & Sons, 463 pp.

Wells, R.S., M.D. Scott and A.B. Irvine. 1987. The social structure of free-ranging bottlenose dolphins. Pp. 247-305 *In*: Genoways, H. (ed.), Current Mammalogy, Vol. 1. New York: Plenum Press.

Wells, R.S., L.J. Hansen, A. Baldridge, T.P. Dohl, D.L. Kelly and R.H. Defran. 1990. Northward extension of the range of bottlenose dolphins along the California coast. Pp. 421-431 *In*: S. Leatherwood and R.R. Reeves (eds.) The Bottlenose Dolphin. Academic Press, San Diego. 653 pp.

Wells, R.S., H.L. Rhinehart, L.J. Hansen, J.C. Sweeney, F.I. Townsend, R. Stone, D. Casper, M.D. Scott, A.A. Hohn, and T.K. Rowles. 2004. Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system. EcoHealth 1:246-254.

Wells, R.S., V. Tornero, A. Borrell, A. Aguilar, T.K. Rowles, H.L. Rhinehart, S. Hofmann, W.M. Jarman, A.A. Hohn, and J.C. Sweeney. 2005. Integrating life history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Science of the Total Environment 349:106-119.

Westgate, A.J. and K.A. Tolley. 1999. Regional differences in organochlorine contaminants in harbour porpoises (*Phocoena phocoena*) from the western North Atlantic. Marine Ecology Progress Series 177: 255-268.

Würsig, B. and G. Harris. 1990. Site and association fidelity in bottlenose dolphins off Argentina. In The bottlenose dolphin (ed. S. Leatherwood and R.R. Reeves), pp. 361-365. San Diego: Academic Press.

Würsig, B. and T.A. Jefferson. 1990. Methods of photo-identification for small cetaceans. *In*: Individual Recognition of Cetaceans: Use of Photo-Identification and Other Techniques to Estimate Population Parameters (P.S. Hammond, S.A. Mizroch and G.P. Donovan, eds.) pp. 43-55, Report of the International Whaling Commission, Special Issue 12, Cambridge, U.K. 440 pp.

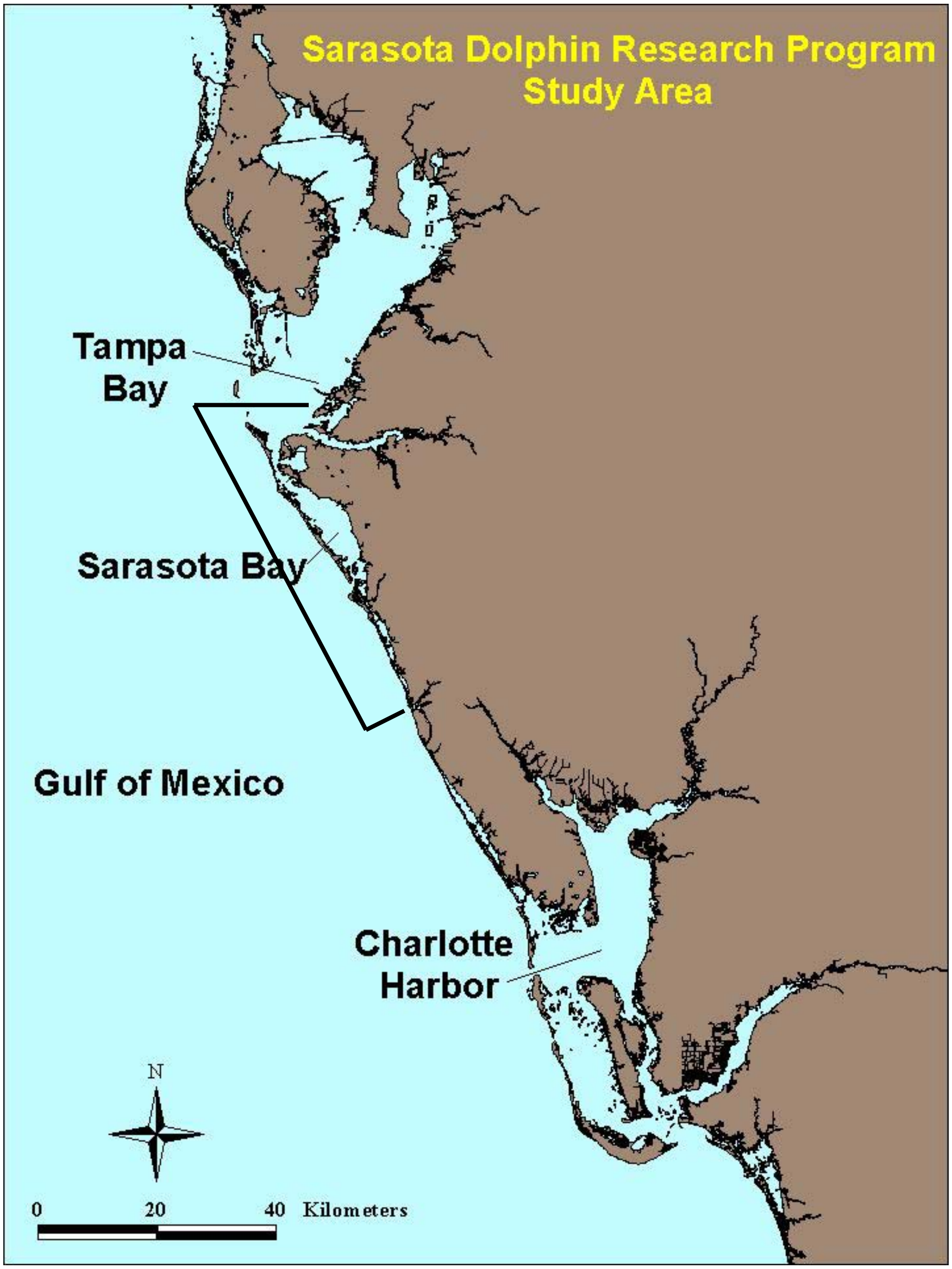

Figure 1. Approximate utilization area of the Sarasota Bay bottlenose dolphin community, depicted by brackets.