DUSKY DOLPHINS OF KAIKOURA, NEW ZEALAND: BEHAVIORAL EFFECTS OF GENETIC SAMPLING AND ANALYSIS OF POPULATION STRUCTURE

A Thesis

by

APRIL DAWN HARLIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 1999

Major Subject: Wildlife and Fisheries Sciences

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May 1999

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ABSTRACT

Dusky Dolphins of Kaikoura, New Zealand: Behavioral Effects of Genetic Sampling and Analysis of Population Structure. (May 1999)

April Dawn Harlin, B.S., University of California, Davis

Chair of Advisory Committee: Dr. Bernd Würsig

Seasonal differences in group size, behavior, distribution, and coloration patterns of dusky dolphins (Lagenorhynchus obscurus) in Kaikoura, New Zealand, have led researchers to question whether "winter" and "summer" groups are temporally and behaviorally segregated into genetically distinct populations. Exfoliated skin samples were collected in Kaikoura from July 1997 to May 1998 for genetic analysis of 40 "winter" and 40 "summer" individuals via skin swab. A 473 base pair section of the mitochondrial DNA control region was amplified and sequenced for the 80 samples. Nucleotide and haplotype diversity were 0.16 and 0.98, respectively. AMOVA and phylogenetic analyses indicate "winter" and "summer" groups are not subdivided with respect to maternal lineages. Lack of subdivision between seasonal populations is further supported by: (1) demographic patterns determined from mismatch distribution analysis suggest New Zealand dusky dolphins underwent a population expansion in the Pleistocene; (2) current levels of diversity suggest the long-term effective population size has been large; (3) preliminary analysis of photo-identification data indicate individuals are present in Kaikoura both winter and summer; (4) comparison of 80

samples from Kaikoura to eight beach-cast samples from locations throughout New Zealand reveal shared haplotypes between regions.

Behavioral responses to sampling were recorded for 315 contacts and 48 controls. The number of pre- and post- contact bowriders and sample time were used as indicators of group-level response to sampling. The behavioral state of dolphins prior to sampling or time of day did not affect responses to sampling. Small groups were found to be more sensitive to sampling. Dolphin groups appeared to habituate to sampling activities after the first hour spent sampling. Responses to sampling were mild with 18% showing no response to contact. The most frequent response was to move right or left of the bow. Thirty-three percent of dolphins returned to the bow within 10.8 ± 0.73 seconds. There was no significant difference between proportion of responses between treatment and control groups, suggesting a proportion of responses to sampling can be explained by normal behavior in the presence of a vessel.

DEDICATION

To my life partner and best friend, Tim.

I love you always.

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To my mom, thanks for being there for me whenever I needed to chat and for having faith in me when I doubted myself. And, to my husband Tim, I owe the utmost thanks for his patience and love.

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CHAPTER I

INTRODUCTION

Problem definition: winter vs. summer populations

Dolphin activities such as feeding, socializing and resting are all incorporated into daily and seasonal patterns; and are often correlated with change in temperature, prey distribution and cyclic reproductive activities (Gaskin 1968; Würsig and Würsig 1980; Shane 1990; Cipriano 1992; Black 1994; Würsig et al. 1994, 1997). Spinner dolphins (Stenella longirostris) in Kealake'akua Bay, Hawaii, spend much of the morning resting close to shore, moving to open water and increasing afternoon activity levels in preparation for feeding (Würsig et al. 1994). Diurnal and seasonal patterns of behavior have also been observed for bottlenose dolphins (Tursiops truncatus; Shane et al. 1986; Scott et al. 1990). Shane (1990) found bottlenose dolphins around Sanibal Island, Florida, feeding more in the morning, with increasing social activity in the evenings. Würsig and Würsig (1980) found a similar increase in social activity in the evening for bottlenose dolphins in Golfo San José, Argentina. In Galveston Bay, Texas, bottlenose dolphin groups decreased time feeding and increased time spent socializing from morning to late afternoon in summer (Bräger 1993). In higher latitudes, bottlenose groups tend to migrate seasonally as water temperature changes (Shane et al. 1986).

This thesis follows the style of Marine Mammal Science.

Daily movements in and offshore in response to feeding also have been observed for dusky dolphins (Lagenorhynchus obscurus) in Golfo San José, Argentina. Here, movement of dusky groups follow seasonal changes in water temperature correlated with availability of anchovy (Engraulis anchoita; Würsig 1982; Würsig and Würsig 1980).

Dusky dolphin groups off the coast of the Kaikoura Peninsula on the eastern shore of New Zealand's South Island also make pronounced changes in behavior, group size, and distance from shore both diurnally and seasonally (Cipriano 1992; Würsig et al., 1997). Historical sightings from locations throughout New Zealand suggest dusky dolphin groups generally shift their distribution north in winter and south in summer in response to changes in water temperature which may alter the distribution and abundance of prey (Gaskin 1968, 1972; Leatherwood 1991; Würsig et al. 1991; Cipriano 1992). In Kaikoura, groups rest close to shore during the morning and early afternoon hours (Barr 1997; Würsig et al. 1997), and move to deeper water in the afternoon and evening to begin feeding on squid and myctophid fishes which rise with the Deep Scattering Layer (DSL, Cipriano 1992). Summers are spent close to shore in groups of 5 to 250, where calving and mating activities are at their peak. In winter these activities shift to rapid travel along the coastline in groups of up to 1500 or more individuals 5-12 kilometers from shore (Cipriano 1992; Würsig et al. 1997).

In addition to seasonal changes in distribution and behavior, both researchers and dolphin tourist companies that have operated in Kaikoura for the past 10 years have observed white, blotchy pigmentation anomolies in winter dolphins that are rarely seen in summer. Marked seasonal differences in behavior, group size, distribution, and

pigmentation patterns of dusky dolphins in Kaikoura have led researchers to question whether winter and summer groups are temporally segregated into two, genetically subdivided populations.

It is likely that localized movements of dusky dolphin groups in Kaikoura are not totally independent of broad-scale changes along the New Zealand coast. Patterns of seasonal shift in location of groups in Kaikoura may, in fact, be indicative of the changes that are occurring throughout New Zealand. For example, like humpback whales (Megaptera novaeangliae; Baker et al. 1993, Palsbøll et al. 1995) and bottlenose dolphins (Shane et al. 1986, Duffield and Wells 1991), female dusky dolphins may exhibit philopatry; and seasonal changes in group size and movement in and out of Kaikoura represent seasonal addition of male groups on a broad scale. However, the details of seasonal population movements, amount of gene flow, and extent of site fidelity in Kaikoura, cannot be determined without identification of population structure and boundaries.

Genetic analysis and population structure

Recent advances in genetic technology have provided unparalleled power to determine population structure and genetic variation of natural populations. Genetic analysis using the polymerase chain reaction (PCR; Saiki et al. 1988), for example, can clarify aspects of population structure from minute amounts of DNA found in hair (Morin et al. 1993) and feces (Höss et al. 1992). In studies of cetacean populations, difficulties in observing group movements and social interactions have made molecular techniques a particularly valuable tool (e.g., Baker et al. 1990, 1993; Hoelzel and Dover

1991; Amos et al. 1993; Dowling and Brown 1993; Palumbi and Baker 1994). Questions concerning population structure are commonly approached through mitochondrial DNA (mtDNA) analysis; there are numerous examples of studies where behavioral inferences were made from mtDNA analysis of population structure and genetic diversity. Maldonado et al. (1995) determined female philopatry of California sea lions (Zalophus californianus) using sequences of the cytochrome b region of the mtDNA genome. Other examples include social unit and population structure of bottlenose dolphins (Duffield and Wells 1991; Dowling and Brown 1993) and population structure and female migration site fidelity of humpback whales (Baker et al. 1990, 1993; Palsbøll et al. 1995). Genetic analysis of tissue samples has been used to address an array of questions regarding site fidelity (Baker et al. 1990, 1993; Palsboll et al. 1995), structure and phylogeography (Baker et al. 1993; Dowling and Brown 1993; Palumbi and Baker 1994; Patenaude et al. 1994; Palsboll et al. 1995; Curry and Smith 1997; Lux et al. 1997; Valsechhi et al. 1997; Hoelzel et al. 1998a; Pichler et al. 1998), and evolutionary history (Rooney 1998) of cetacean populations.

Skin swabbing: a new tissue sampling technique

Several methods have been developed for collecting tissue for genetic analyses of wild populations. The most common is the use of a biopsy dart shot from a crossbow or modified capture gun to collect small "plugs" of tissue (Lambertsen 1987). The impact of this technique has been investigated to determine behavioral responses of sampled animals (Brown et al. 1991; Weinrich et al. 1991, 1992; Clapham and Mattila 1993; Brown et al. 1994; Weller et al. 1997), and the physical damage inflicted by darts

(Patenaude and White 1995; Weller et al. 1997). These investigations have shown that biopsy darting of cetaceans generally produces low-level behavioral responses and only minor wounding if done properly (Brown et al. 1991; Weinrich et al. 1991, 1992; Brown et al. 1994; Weller et al. 1997).

The least invasive techniques for collection of tissue require no physical contact with the sampled animal. Such non-invasive techniques developed for the chimpanzee (Pan troglodytes) used hairs collected from nest sites to obtain DNA for genetic analysis of community structure and paternity (Morin et al. 1993). Fecal samples also have become a source of genetic information (Höss et al. 1992) and have been used to verify species of canids (Paxinos et al. 1997) and gender of seals (Reed et al. 1997). Similarly, several cetacean researchers have used skin that sloughs naturally from large whales for genetic analysis (Whitehead et al. 1990; Baker et al. 1991; Amos et al. 1992; Clapham et al. 1993; Richard et al. 1996; Valsecchi et al. 1998).

Unlike whales, small cetaceans do not shed their skin in large patches. "Non-injurious" techniques, i.e., those which require contact but do not leave an open wound, are a potentially less invasive alternative to biopsy darting of small cetaceans. Sufficient DNA has been obtained from epidermal cells scraped from the backs of captive delphinids (Milinkovitch et al. 1994). Building on this work, Harlin et al. (in press) developed a "skin swab" technique to collect bits of epidermal tissue from the backs of free-living dusky dolphins.

Skin swabs were collected from dusky dolphin populations in Kaikoura, New Zealand, from July 1997 through May 1998. Responses of dolphins to skin swabbing

were recorded to determine the effects of the sampling technique on dolphin behavior. A portion of the control region of the mtDNA genome was sequenced to determine baseline genetic diversity and investigate the relationship between winter and summer dusky dolphin populations in Kaikoura. A preliminary comparison between dusky groups in Kaikoura and other localities in New Zealand was made to examine the relationship between dusky groups on a New Zealand-wide scale. Such information on population structure is especially valuable to conservation agencies attempting to manage habitat and population numbers while maintaining genetic diversity.

Summary of objectives

The broad-scale objectives of this research were (1) to quantify the behavioral responses of dusky dolphins to skin swabbing, and (2) to use molecular genetic techniques to examine the relationships of winter and summer dolphin groups in Kaikoura, New Zealand. Further detail of the scope of each section is presented in the following chapters.

CHAPTER II

GENETIC SAMPLING: BEHAVIORAL EFFECTS OF A SKIN SWABBING TECHNIQUE

INTRODUCTION

Several methods for collecting genetic material from free-ranging cetaceans have been developed, with the most common being the use of a biopsy dart shot from a crossbow or modified capture gun to collect small "plugs" of tissue (Lambertsen 1987). Even less invasive sampling methods have been developed that attempt to (1) minimize contact with the animals sampled, and (2) avoid puncturing the skin and leaving an open wound (Whitehead et al. 1990; Baker et al. 1991; Amos et al. 1992; Clapham et al. 1993; Richard et al. 1996;). Investigations of the impact of such techniques (Brown et al. 1991; Weinrich et al. 1991, 1992; Clapham and Mattila 1993; Brown et al. 1994; Weller et al. 1997; Harlin et al. in press) have shown that behavioral responses of cetaceans to sampling are generally mild.

For large-bodied cetaceans, researchers have investigated how other factors, such as sex, age, social class and the manner of vessel approach, can subsequently increase the response to sampling. For example, an aggressive approach of a vessel toward humpback whales greatly increased the probability of eliciting a negative behavioral response to biopsy darting (Clapham and Mattila 1993). Barrett-Lennard et al. (1993) found no significant effect of age class or sex on behavioral response of killer whales (Orcinus orca) to sampling. However, female humpback whales were more likely to respond to sampling than were males (Brown et al. 1994). Humpback calves in the West Indies reacted more often than members of other social classes including mothers,

escorts, and competitive males (Clapham and Mattila 1993). However, the behavioral state of humpback whale pods prior to sampling had no effect on responses of whales to sampling (Brown et al. 1994).

Like their larger relatives, dolphins may respond differently to contact depending upon several factors, such as the time spent in contact with pods, time of day, season, group size and behavioral state prior to sampling. There are, however, no studies that have investigated detailed responses of small-bodied cetaceans to sampling. Harlin et al. (in press) introduced an alternative to biopsy darting, the "skin swab", and examined the behavioral responses of dusky dolphins to this technique in Kaikoura, New Zealand, from July-September 1997. Here, with additional data from July 1997 through May 1998, I further examine the responses of dusky dolphins to skin swabbing, emphasizing the effects of group size, season, time of day, behavioral state, and total time spent in sampling activities.

MATERIALS AND METHODS

Tissue collection

This research was conducted in the waters off the Kaikoura Peninsula, South Island, New Zealand (42°S Lat. 173°E Long.) from July 20, 1997 to May 5, 1998, and in the Marlborough Sounds area north of Kaikoura on May 14 and 15, 1998. Figure 1 illustrates the assembled sampling device and its components. One end of a wooden dowel 62 cm. long and 1 cm. in diameter was sanded to a smooth, rounded tip. A shallow groove was cut around the dowel, about 1.5 cm from the rounded end.

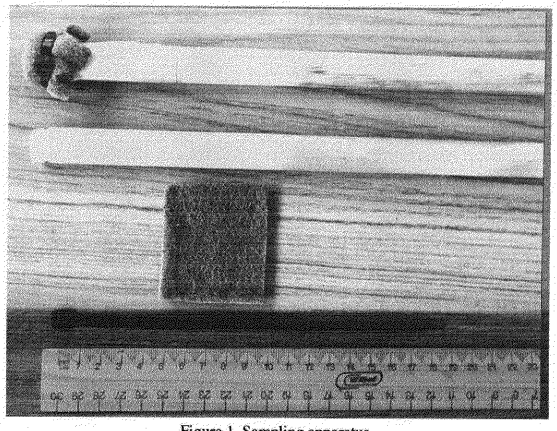

Figure 1. Sampling apparatus.

New nylon scrub pads (i.e. ScotchBrite, 3M Corporation) were cut into 4 cm. x 4 cm. squares, individually wrapped in aluminum foil, and autoclaved.

A finger cut from a clean latex glove was taped to the rounded end of the dowel to protect the wood from water and contamination from repeated use. Over the latex covering, a sterile scrub pad square was attached by tightening a plastic cable fastener over the scrub pad at the groove. Finally, a finger was cut from a latex glove and placed over the scrub pad to prevent contamination. Dowels were prepared in advance of each boat trip, and were easily re-fitted in the field for additional sampling.

A 4.3 m Zodiac inflatable boat with a 25 Hp outboard engine was used to collect samples. To minimize potential disturbance to the animals, a steady speed and course were maintained during sampling, and care was taken to avoid approaching or entering the group too quickly, following the guidelines of Constantine and Baker (1997). The sampler held the dowel raised above the water surface while leaning over the bow. As bowriding dolphins approached the surface of the water, epidermal cells were swabbed from their dorsal or lateral surfaces by quickly and decidedly making contact between the sterile pad and skin. Best contact was obtained when animals were close to the surface, and the boat was traveling at no-wake speed. Contact with dolphins was brief, with only one contact per scrub pad. A sample was considered successful if skin was visible on the sampling pad. Vessel position with respect to the group was changed regularly in an attempt to prevent repeated sampling of the same individuals. General observations of scars, pigmentation patterns, and dorsal fin notches suggest that the composition of bow riding individuals was fluid, as dolphins frequently replaced one another from the larger, overall group.

After sample collection, the plastic cable tie was cut to remove the pad. To avoid sample contamination, the sampler wore gloves during the entire sampling bout, and

changed gloves between samples. Successful samples were stored at the field site in sterile 30 ml vials with 20% dimethylsulfoxide (DMSO) saturated in NaCl (Amos and Hoelzel 1991) at ambient temperature.

Behavioral data

The general behavioral state of dolphin groups was classified as "mill", "travel", or "rest", following Shane (1990). Instantaneous scan samples were collected at two minute intervals for a minimum of 40 minutes prior to sample collection for 31 sampling sessions. The proportion of time spent traveling was the most frequent behavioral state, and was used as a measure of the "mood" of dolphin groups prior to sampling. Group size was categorized as "small" (50), "medium" (100-250), "large" (251-500), and "Texas-sized" (>500). The behavior of sampled individuals immediately following contact was recorded as a "post contact response".

A "sampling bout" was defined as the time beginning when the sampler leaned out over the bow of the boat, until 30 seconds post contact. This 30 second period was defined as a "post contact observation period". "Sample time" was a measure of effort required to obtain a sample, and was defined as the time from initiation of the sampling bout until contact was made with an individual dolphin. "Total Elapsed Treatment Time"(TETT) was measured at the initiation of each sampling bout and was defined as the total time spent in sampling effort since the onset of sampling. If contact resulted in the movement of the dolphin from the bow, the sampler continued to monitor the dolphin's movements for 30 seconds post sample. If the dolphin returned to the boat within 30 seconds post contact, the time until the individual returned was noted as "return time". If the dolphin did not return by the end of thirty seconds, "no return" was noted. If the dolphin moved out of sight, or if the sampler could not keep track of the

dolphin as it moved into the group, "return unknown" was noted. Behavioral responses were judged by either of the two designated observers. Responses were defined a priori as follows:

Move right/Move left --- Dolphin moved from position at the bow by making a lateral move to the right or starboard (MR), or to the left or port (ML) side of boat after contact.

Dive ---Dolphin dove directly under the bow, or to the right or left of bow. "Dive" was defined as a rapid vertical or near-vertical move from the surface.

Startle ---Dolphin flinched in response to contact, similar to the startle response defined for humpback whales by Brown et al. (1994)

Flight ---Dolphin fled from the boat in a prolonged "slicing" behavior, indicating a flight response to sampling.

Tail slap ---Dolphin flexed its caudal region and brought it forcefully down on the water, making a large splash and loud slap. This behavior is thought to be an indicator of aggression in at least some cetacean species (Shane 1990).

Increased speed ---Dolphin increased speed of travel for a short duration, generally for no more than a few seconds. Movement from the bow, as defined above, and a quick return to previous travel speed usually followed.

No response ---Contact did not elicit any visible behavioral response. The dolphin continued to ride the bow of the vessel with no change in behavior, similar to that defined by Brown et al. (1994).

All behaviors except "no response" were considered a potential response to contact. In addition to manual notation of behavioral response, video footage was recorded with a Sony Hi-8 camcorder for 226 of 315 samples in which behavioral responses were recorded. Video footage was used in frame-by-frame post hoc analysis

for detailed observation of behavioral responses of dolphins to sampling and to confirm manually-recorded data. If the sampled dolphin was not in view during filming, the sample was noted as "dolphin not visible". This occurred on 54 occasions and consequently, these were not used and written responses were not verified.

In addition to the behavioral responses of sampled individuals, the number of bowriders pre- and post-sample and the sample time were considered as indicators of general response of the dolphin group to sampling activity. Effects of time of day, season, group size, and total treatment time were evaluated for each of these response indicators. General behavior of groups ("mood" Würsig et al. 1989) also was evaluated for effects on sample time, i.e. time required to make contact with an individual.

Behavioral controls

From October 22, 1997 to April 28, 1998, data were collected for 48 behavioral controls. Controls were designed to test responses of dolphins to the presence of a person holding a sampling device over the bow of the boat. The control procedure was similar to the sampling treatment except no contact was made with dolphins. The "control bout" began when the person holding the sampling device leaned over the boat. Constant speed and direction were maintained for 2 minutes, the median sampling time for sampling treatment. After 2 minutes, a bowriding dolphin that could have potentially been sampled was chosen, and "sampled" without contact and its behavior was noted at that instant. Behavioral response categories were identical to those defined for treatment.

Statistics

Data were tested for normality using a Kolmogorov-Smirnov test, and parametric tests were performed only on data not differing significantly from a normal distribution. All tests were evaluated at a 0.05 significance level, and results are reported with standard errors unless otherwise noted.

A Wilcoxon matched pairs test was used to compare pre- and post-contact number of bowriders across all samples. Paired t-tests were used to evaluate differences between pre- and post-contact number of bowriders for three of four group size categories ("small", "medium", and "Texas-sized"), and a Wilcoxon matched pairs test examined pre- and post-contact number of bowriders for "Large" groups. The average number of bowriders present during sampling was tested across all four group sizes with a Kruskal-Wallis ANOVA, and planned linear comparisons were done with Mann Whitney *U* tests to determine the pattern of differences within pod size categories. A chi-square test of independence was used to determine whether the probability of a particular behavioral response by a particular individual was independent of pod size.

The number of pre- and post-contact bowriders was divided into blocks by austral season ("winter", "spring", "summer", and "fall"). Mean number of pre- and post- contact bowriders was compared using paired *t*-tests for "fall", "summer", and "spring", and a Wilcoxon matched pairs test was used for "winter" samples. Spearman's ranked correlation analysis was used to determine if the mean number of bowriders per sampling day was related to the "mood" of dolphin groups prior to initiation of sampling.

Time of day was divided into three blocks "morning", 8-11AM; "early afternoon", 12-2PM; and "late afternoon", 3PM+. A Kruskal-Wallis ANOVA was used to determine if sample time was significantly different across all three time categories.

TETT was measured for each sample and Spearman's rank correlation was calculated to investigate the relationship of total treatment time and sample time. TETT was then divided into 5 categories: "0-15 min.", "16-30 min", "31-45 min", "46-60 min", and ">60 min". Sample time and mean number of bowriders were each compared across all five treatment time categories with Kruskal-Wallis ANOVA's. Separate single Mann-Whitney U tests were used to examine the differences for sample time between time categories.

A Kruskal-Wallis ANOVA was used to test if group size affected "return time". Chi-square goodness-of-fit tests were used to determine significant differences from expected values for "return" and post-contact behavior categories. A chi square test for homogeneity of proportions was used to investigate if the probability of "return", "no return", or "return unknown" was equal across all post contact behavior categories.

RESULTS

Tissue collection

From July 20, 1997 to May 15, 1998, 321 contacts were made with bowriding dolphins on 50 sample days. Of the 321 contacts made, behavioral responses were recorded for 315. All but two contacts were with dusky dolphins, the other 2 were with offshore common dolphins (Delphinus delphis). Eighty percent (n=257) of contacts resulted in tissue samples with visible pieces of skin.

Mean sampling time once dolphins initiated bowriding was 148.4 ± 8.49 sec., with a range of 2-780 sec. and a median of 94.5 sec. Spearman's rank correlation analysis yielded no significant relationship between sample time and either general behavioral state of the group (r=-0.18, P=0.36), or number of bowriders (r=-0.36, P=0.69). Likewise, length of time to acquire samples did not vary between "small",

"medium", "large", and "Texas-sized" dolphin groups (Kruskal-Wallis, $H_{3,304} = 1.24$, $P=0.74$) nor between "morning", "early afternoon", and "late afternoon" time periods (Kruskal-Wallis ANOVA, $H_{2,316}=2.37$, $P=0.31$). There was, however, a weak positive relationship between the TETT and sample time (Spearman's rank correlation, $r=0.15$, $P=0.007$), suggesting a slight increase in the time required to obtain a sample as the sampling period grew longer. The effect of TETT and sample time becomes more clear when examined by treatment time blocks. The Kruskal-Wallis ANOVA showed a difference among the five treatment time blocks (0-15 min, 16-30 min, 31-45 min, 46-60 min, >60 min) for effects on sample time ($H_{4,317}=23.69$, $P=0.001$). Comparisons between treatment time blocks indicated no difference between 0-15 min and >60 min blocks (Mann-Whitney $U=3825.0$, $P=0.69$), but a significantly greater sample time across 16-30 min, 31-45 min, and 46-60 min blocks ($P < 0.04$; Fig. 2).

Behavioral response

The most frequent response to contact was MR and ML (Table 1, Fig. 3), and the proportion of dolphins that responded with ML/MR, D, or IS differed significantly from random ($\chi^2_2=71.42$, $P \leq 0.001$). The same was true for proportion of individuals that fell into the categories "return", "no return", and "return unknown" ($\chi^2_2=3.43$, $P<0.05$; Table 1). A chi-square test for homogeneity of proportions indicated that the post-contact response to sampling was independent of pod size ($\chi^2_6=4.68$, $P>0.05$), but post-contact response affected whether or not an individual returned, did not return, or passed from sight within the 30 second post-contact observation period ($\chi^2_6=17.38$, $P=0.01$, Fig. 3). An individual that responded to contact by diving (D) was more likely to fall

Table 1. Summary of behavioral responses of dusky dolphins to sampling.

Responses	**Treatment (n=315)**	**Control (n=48)**
Move Right/Left	47%	29%
Dive	12%	17%
Increase Speed	21%	8%
Tail Slap	<1%	0%
Startle	2%	0%
No Response	18%	46%
Return Behaviors	**Treatment (n=259)**	**Control (n=26)**
Return	33%	42%
Return Unknown	37%	38%
No Return	29%	19%
Return time(sec)	10.8±0.73	8.8±1.70

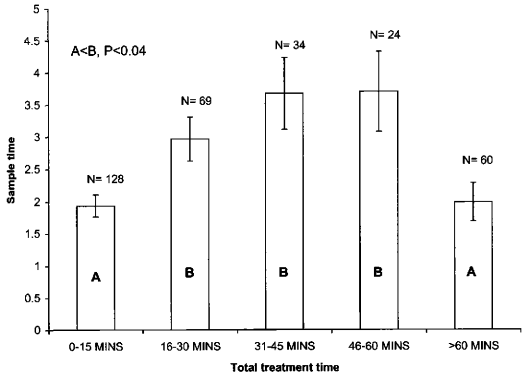

Figure 2. Relationship between the total time spent in contact with dolphin groups and the time required to obtain a sample. Values on y-axis are mean sample time; whiskers are +/- standard errors.

into the "return unknown" category (Fig. 3), as it often moved quickly out of sight and could not be reliably re-identified as it resurfaced. Mean return time was 10.8±0.83 (n=85) seconds. There was no relationship between group size and return time (Kruskal-Wallis ANOVA, $H_{3,77}$=3.69, P=0.30).

Of 259 individuals that responded to contact, we were able to monitor 161 (63%) of them for 30 seconds post contact; the other 98 (37%) were considered as "return unknown" (Table 1). We found that 53% (n=85) of these 161 dolphins that could be followed for the entirety of the 30 second post contact observation returned to bowride in 30 seconds or less post contact. This, however, does not preclude the possibility that a proportion of the dolphins returned to the bow after 30 seconds. The size of the dolphin groups, especially the large "super pods" found in the winter, made it difficult to track sampled individuals for much more than 30 seconds, so data on long-term returns to the bow were not available.

The average number of bowriders decreased significantly after contact (pre-contact mean = 5.1±0.1; post-contact mean = 4.0±0.22; Wilcoxon matched pairs, T_{306}=8063.5, P<0.001). When analyzed by group size, there was a significantly lower number of pre- vs. post-contact bowriders for "small" (t_{46}=6.43, P=0.001), "medium" (t_{39}=2.83, P=0.007), and "large" (Wilcoxon matched pairs, T_{137}=1551.50, P<0.001) groups (Fig. 4). There was, however, no significant change in number of bowriders for "Texas-sized" groups (t_{78}=1.66, P=0.10). Larger groups also tended to have more bowriders as a rule (Kruskal-Wallis ANOVA, $H_{3,610}$=96.27, P<0.001). There was a

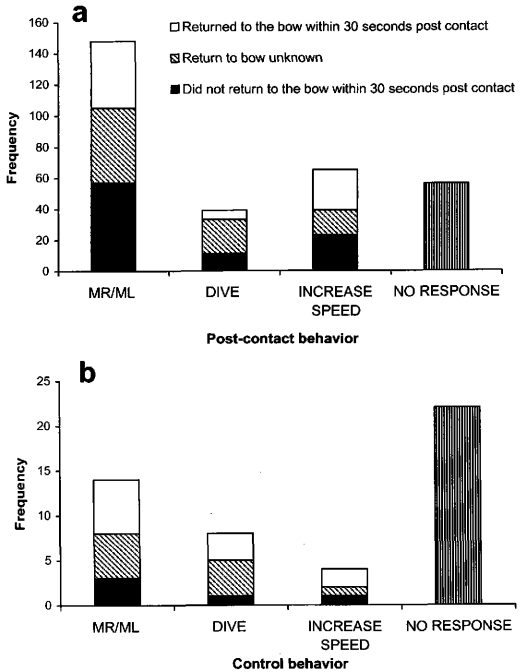

Figure 3. Frequency of responses to contact for (a) treatment and (b) controls.

significant difference in the number of bowriders across seasons (Kruskal-Wallis ANOVA, $H_{3, 307}$=58.12, P<0.001), with winter having more bowriders than other seasons (Fig. 4). There was no significant difference between bowriders before and after contact in winter (Wilcoxon matched pairs, T_{108}=1567.50, P=0.80), but a significant decrease for spring (t_{65}=4.27, P<0.001), summer (t_{62}=5.56, P<0.006), and fall (t_{43}=4.09, P<0.002) months. Spearman's rank correlation analysis showed no relationship between the "mood" of dolphin groups and the number of bowriders (r=-0.18, P=0.36).

Behavioral controls

Of the 48 individuals selected during control bouts, 26 (54%) showed a change in behavior (Table 1, Fig. 3). Forty-six percent exhibited "no response". Thirty-eight percent of dolphins that responded to "contact" moved out of sight during the 30 second observation period and were classified as "return unknown". Of those individuals we were able to follow for 30 seconds post "contact" (n=16), 11 (69%) returned to ride the bow within 30 seconds with a mean return time of 8.8±1.70 sec. The most common response of the 35 control individuals was ML/MR (n=14), followed by D (n=8) and IS (n=4). Proportions of behavioral responses for controls were similar in many cases to those in which actual contact was made (Fig. 3). The proportion of individuals in each behavioral response category was not significantly different between treatment and controls (χ^2_2=4.69, P>0.05). This also was true for "return", "no return" and "return unknown" categories (χ^2_2=1.46, P>0.05); however, the proportion of individuals which showed no response to contact was significantly greater for controls than treatments (two population proportion; Z=3.5, P<0.05).

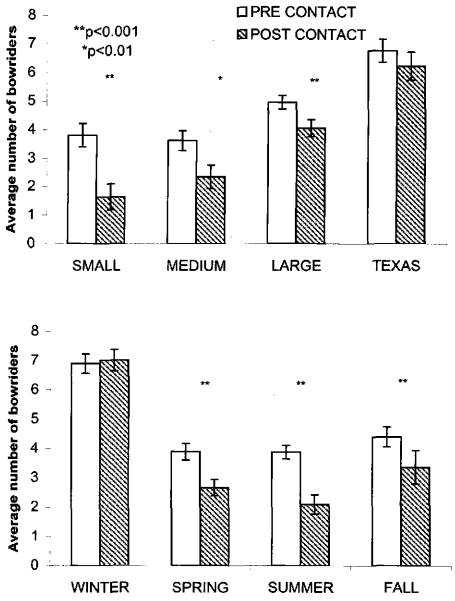

Figure 4. The relationship between season, group size and the number of bowriding dolphins during sampling. Numbers on y-axis represent mean number of bowriders, whiskers are +/- standard errors.

DISCUSSION

In general, dusky dolphins showed little or no aversion to the sampling conducted in this study. If only those contacts in which an individual could be monitored for the 30 second post contact observation period are considered (n=219), 65% (n=141) of these dolphins either showed no response or returned to bowride in 30 seconds or less. During the 315 sampling bouts, "flight" was never observed and only five "startle" responses were recorded. While sampled animals were commonly observed to move away from the bow after skin swabbing, these responses were nearly always mild. Likewise, data from sampling controls indicate no significant difference in frequency of behavioral response categories between the 48 control bouts and the sampling bouts in which contact was made, although control sample size is comparatively smaller than treatment (Fig. 3).

Our results show that responses of dolphin groups to sampling, with sample time as a measure of response intensity, are not affected by time of day, season, or behavioral state of the group prior to initiation of sampling. However, data suggest that after the first 15 minutes of the first hour of sampling may be a sensitive period for dolphins, so that a group is generally less approachable within the first 60 minutes. After this initial period, the behavior of the dolphins towards the boat, measured by a decrease in sample time, becomes more affiliative, suggesting a habituation-type response to sampling activities.

The number of bowriders pre- vs. post-contact may be a slightly better indicator of responses to contact, especially when compared among different sized groups.

Würsig (pers. com.) noticed that smaller groups of Argentine dusky dolphins responded dramatically more to hand lance tagging and handling during radio transmitter attachment than larger groups. Smaller groups of New Zealand duskies found in spring, summer, and fall tended to have fewer bowriders post contact than larger winter groups. The fact that there was no significant difference in the number of bowriders pre- and post-contact in the largest, winter groups may be more an artifact of group size than of behavioral responses to sampling.

It is likely that only a proportion of a dolphin group is prone to bowride at any given time. Casual observation of bowriding groups indicate that the composition of groups at the bow is fluid, with a constant influx and efflux of dolphins from the bow. The bow of a small boat is large enough for only a limited number of dolphins. A larger group has a greater cohort of bowriders, and positions at the bow are continuously being filled by new members from this cohort. In smaller groups, however, there are fewer bowriders, and fewer replacements to fill positions at the bow. Therefore, the significant decrease in the number of bowriders in smaller groups may be a result of a group that (1) generally shows less interest in bowriding, and/or (2) may represent the entire cohort of bowriders within the main group. Because only the largest groups (>500) showed no difference between pre- vs. post- contact bowriders perhaps any change in the number of bowriders at the bow was undetectable due to the constant influx of new individuals that filled the positions of those leaving the bow in response to contact. If so, responses of the bowriding group to sampling would be masked by the total number of bowriding individuals. Even though the manner in which an individual dolphin responds to contact

is independent of group size, there was a slight tendency for fewer bowriders post contact in all group size categories.

Because behavioral responses of dolphins to treatment were not significantly different from control data, responses of dolphins to treatment are more likely a general indicator of behavior in relation to the presence of a vessel, rather than to sampling activities. Dolphin groups have been shown to change their behavior in response to the presence of vessels up to 6 miles or more away (Au and Perryman 1982). The presence of boats has been correlated with tightening of groups for Hector's dolphins (Cephalorhynchus hectori; Bejder 1997) and pan-tropical spotted dolphins (Stenella attenuata; Pryor and Shallenberger 1991). Upon approach of a vessel, killer whales (Kruse 1991) and Stenella spp. (Au and Perryman 1982) increased their travel speed. Length of time spent in contact with Hector's dolphin groups caused changes in group formation (Bejder 1997). The responses of dolphin groups to human activity may be affected by seasonal and diurnal shifts in distribution, behavior, and group size. For example, dusky dolphin groups engage in rest during morning and early afternoon hours (Würsig et al. 1997) and are more likely to show a change in pod dispersion, speed of travel and number of directional changes in response to tourist boats during these times (Barr 1997). Therefore, any boating activity is a potential source of disturbance for dolphin groups. Time of day and behavioral state of dolphin groups did not have an effect on how dolphins responded to our sampling procedure. This suggests that our sampling procedure is less of a disturbance to dusky dolphins than are boating activities. In fact, because responses of dolphins to sampling were not significantly different from

behavioral controls, it is difficult to separate responses to sampling from alterations in behavior due to the presence of a vessel around groups. Our vessel was one of three vessels in proximity to a dolphin group; the other two being larger dolphin tour vessels. Impacts of boats on dolphin behavior can be minimized if proper boat-handling guidelines are followed (Constantine and Baker 1997). Therefore, the potential effects of tissue collection, however minimal, can be tempered even further by proper boating practices.

We were not able to test for more subtle longer-term reactions by dolphins to these sampling efforts. For example, it is possible that dolphin groups that are repeatedly sampled over a season learn to avoid the sampling vessels, and perhaps other vessels. Unfortunately, the same characteristics which facilitated tissue collection from many dusky dolphins in a small period of time (i.e., large group size), made it impossible to maintain visual contact with the dolphins in all instances, especially when they dove or increased speed following contact. For this reason, in roughly two out of every five trials it was impossible to determine if the dolphins returned to bowriding within 30 seconds of moving away from the bow. Although our technique is potentially less invasive than methods of tissue collection, such as dart-propelled biopsy sampling, any boat approach with even a minor negative stimulus (e.g., motor noise) may have some level of effect on behavior.

CHAPTER III

POPULATION STRUCTURE AND GENETIC DIVERSITY OF DUSKY DOLPHINS IN NEW ZEALAND

INTRODUCTION

Mitochondrial DNA genealogies have become a valuable tool for discerning population structure, distribution, and geography (Avise et al. 1987) for numerous taxa (e.g., Encalada et al. 1996; Baker et al. 1990). In cetacean populations, mtDNA differentiation has been correlated with differences in behavioral patterns (Hoelzel et al. 1998a), distribution in relation to shore (Rosel et al. 1994; Curry and Goodwin 1997; Hoelzel 1998b), female migration site fidelity (Baker et al. 1990), and temporal segregation of sympatric populations (Hoelzel and Dover 1991). The study of intraspecific mtDNA lineages, in combination with behavioral ecology, permits the testing of hypotheses about relationships within and between groups in separate geographic regions (i.e., phylogeography, Avise et al. 1987), and addresses questions of dispersal, site fidelity, and demography of populations.

Dusky dolphins are discontinuously distributed in the Southern Hemisphere around the coasts of South Africa, New Zealand and South America (Gaskin 1968; Leatherwood and Reeves 1983, Leatherwood 1991; Würsig et al., 1997) in waters less than 2000 meters deep (Würsig et al., 1997). Groups also have been seen around the

Campbell and Falkland Islands, and in the Magellan Straits (Leatherwood and Reeves 1983). Historical records of dusky dolphin sightings from locations throughout New Zealand suggest that groups make broad-scale changes in group size and distribution in response to seasonal changes in water temperature, which alter the availability and distribution of prey (Gaskin 1968, 1972; Leatherwood 1991; Würsig et al. 1991; Cipriano 1992). There is evidence for such seasonal changes in distribution of dusky dolphins in Argentina where movement of groups is in accordance with seasonal changes in water temperature correlated with availability of anchovy (Würsig and Würsig 1980).

On a fine geographical scale, dusky dolphin groups in Kaikoura make pronounced changes in behavior, group size and distribution between winter and summer months (Würsig, Cipriano and Würsig 1991; Cipriano 1992; Würsig et al. 1997). Peak calving season for dusky dolphins is during the austral summer from December to February. During this period, individuals aggregate in groups of 250-350 (Markowitz, Harlin and Würsig, unpublished data), and spend most of the daylight hours close to shore (Cipriano 1992; Würsig et al. 1997). Mothers often form nursery groups and spend the day at rest with newborn calves, while more active groups spend time engaged in other activities (e.g., mating). In winter, groups of up to 1500 or more dolphins are common, and much of the day is spent 5 kilometers or more from shore, traveling north and south along the coastline. Interestingly, dolphin tourist companies operating in Kaikoura for the last 10 years often have commented on the differences in pigmentation of "winter" and "summer" dolphins. Dolphins observed in the winter often

havepigment anomalies (Fig. 5), while these patterns of coloration are infrequent in summer (D.Buurman, R. Buurman, I. Bradshaw, pers. com.). Do changes in population size, behavior, distribution, and coloration patterns of dusky dolphins indicate that "winter" and "summer" groups in Kaikoura are, like killer whales, temporally and behaviorally segregated into genetically distinct populations (Würsig et al. 1997)?

In 1989, a "swim-with-dolphin" tourism industry was introduced in Kaikoura. As a result of this, as well as tourism activity primarily geared to sperm whale watching, the last ten years have seen a strong increase in the amount of boat traffic around dolphins in the Kaikoura area (Barr 1997; Würsig et al. 1997). Tourist boats come in contact with dolphin groups several times daily, and provide some of these people the opportunity to swim with dolphins in the open sea. In Argentina, dusky dolphin mothers and newborn calves are easily disturbed, and approaching boats can alter social behavior (Würsig and Würsig 1980). Data from theodolite tracking studies in Kaikoura indicate that approach of boats may alter group behavior, especially during periods of rest (Barr 1997; Würsig et al. 1997). Groups have been observed to split into smaller subgroups, change direction, scatter into different directions, or stop travel when approached by a vessel (Barr 1997; Würsig et al. 1997). Such information provides us with some insight into the short-term impacts of vessel traffic on dolphin behavior in general; however, little is known about the potentially long-term impacts this boating activity may have on the dolphins (Würsig et al. 1997), especially during the calving season.

Groups interested in management, research and commercial utilization are

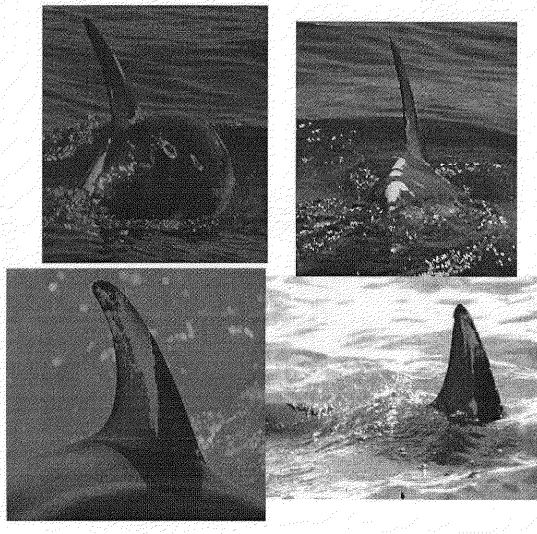

Figure 5. Pigmentation patterns of dusky dolphins in Kaikoura, New Zealand.

concerned with the short- and potentially long-term effects of vessel traffic on dolphin populations in Kaikoura, but very little is known about how these populations are defined. The genetic segregation and diversity of populations are perhaps the most fundamental pieces of information for management (Baverstock and Moritz 1990). With increasing effects of human encroachment on habitat, knowledge of population structure is especially valuable to conservation agencies attempting to manage habitat and population numbers while maintaining genetic diversity. This is especially true in Kaikoura, where seasonal shifts in population distribution and behavior may be indicative of temporal subdivision of populations on a local scale.

Cetaceans, like many other marine species (Palumbi 1992), do not usually show marked genetic divergence over short geographic distances. Harbor porpoises (Phocoena phocoena) from along the North Pacific Coast from Alaska to California share several haplotypes suggesting high levels of gene flow between regions (Rosel et al. 1995). Likewise, Garcia-Martinez et al. (1995) found no evidence of genetic differentiation between striped dolphin (Stenella coeruleoalba) populations along the Spanish Mediterranean coast. Even over moderate distances, the mobility of cetaceans provides opportunity for exchange of effective migrants between populations. For example, there is no evidence for subdivision between minke whale (Balaenoptera acutorostrata) management units in the North Atlantic, spanning distances of over 1600 kilometers (Bakke et al. 1996). Similarly, Lux et al. (1997) found populations of Pacific white-sided dolphin (Lagenorhynchus obliquidens) of coastal California, Oregon and Washington to be genetically undifferentiated from groups found over 1500 km from

shore. On a larger geographic scale, humpback whale populations are genetically subdivided worldwide; however, although separated by thousands of kilometers, a few mtDNA haplotypes are shared between oceans (Baker et al. 1993). Therefore, for some species, there exists a potential for gene flow between populations even when separated by very long distances.

There are, however, incidences when cetacean populations show marked differentiation without geographic separation. Studies of killer whale populations of the Pacific Northwest indicate genetic differentiation between sympatric "resident" and "transient" killer whale populations (Hoelzel and Dover 1991), as well as between foraging specialists (Hoelzel et al. 1998a). Here, I use mtDNA control region sequence data to assess genetic diversity and investigate the relationship between "winter" and "summer" populations of dusky dolphins in Kaikoura. The control region was chosen because of its rapid rate of evolution (Brown et al. 1979; Brown 1985), high variability (e.g., Vigilant et al. 1991; Baker et al. 1993) and sensitivity to demographic changes in populations (Avise et al. 1984, 1987). Populations that are decreasing in size have a greater probability of becoming genetically differentiated due to a reduction in effective population size and range overlap (Avise et al.1984). Therefore demographic change, such as bottlenecks or expansions, can affect levels of differentiation between populations (e.g., Harpending et al. 1993; Rogers 1995; Bonatto and Salzano 1998). In particular, I ask the following questions: (1) Are dusky dolphin groups in Kaikoura temporally segregated into genetically distinct populations? (2) What do historical and current diversity levels tell us about the evolutionary demography of New Zealand dusky

dolphins as a whole? (3) What does this suggest for management of dolphin groups in Kaikoura? Such information can be used to make informed management decisions in a geographic area important to dusky dolphin reproduction and development.

MATERIALS AND METHODS

Sample collection

Skin swab samples were collected following the protocol of Harlin et al. (in press) and as described in Chapter I of this volume. From July 1997 to May 1998, samples were collected in the waters off the Kaikoura Peninsula of New Zealand's South Island (42°S 174°E). Of these samples, 40 were chosen from winter (July-Aug) and 40 from summer (Dec-Jan) for comparison of seasonal populations. Six individuals from spring (Sep-Nov) and other beach-cast or net-caught animals from Kaikoura provided additional tissue samples for calculation of baseline genetic diversity indices. Tissue from beach-cast or by-catch specimens from other locations in New Zealand also were used to begin a comparison of the relationships between dusky dolphins in Kaikoura and other areas of New Zealand (Fig. 6). Table 2 lists the source, locality, and numbers of samples collected for analysis.

Table 2. Summary of samples used for genetic analysis (n=100). W=Winter, S=Summer, SP=Spring. See Figure 6 for geographic location.

New Zealand Locality	Collection Method	Source	Number Analyzed
Kaikoura	Skin swab	A. Harlin	86 (40 W, 40 S, 6 SP)
	Beach-cast	F. Cipriano, unpub. data	5
Otago Peninsula	Beach cast	Cetacean Tissue Bank, University of Auckland, New Zealand	2
	Beach-cast	F. Cipriano, unpub. data	1
Marlborough Sounds region	Beach cast	Cetacean Tissue Bank, University of Auckland, New Zealand	2
West Coast	Beach-cast	F. Cipriano, unpub. data	1
Northland	Beach cast	Cetacean Tissue Bank, University of Auckland, New Zealand	1
	Beach-cast	F. Cipriano, unpub. data	1
Unknown	Net-caught	F. Cipriano, unpub. data	1

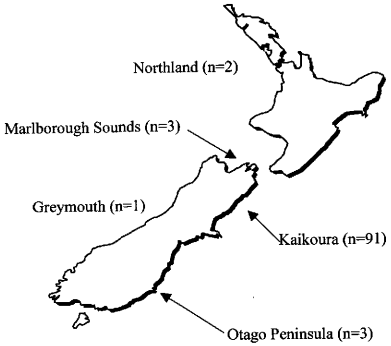

Figure 6. Geographical origin of dusky dolphin tissue samples in New Zealand. Values in parentheses indicate sample size.

DNA was extracted from beach-cast or by-catch specimens listed in Table 2 using a standard phenol/chloroform protocol (Sambrook, et al. 1989). Skin swab samples were extracted with a protocol modified for accommodation of the sampling pad (Harlin et al. in press). DNA was suspended in 100mL of 1X TE (10mM Tris, 1mM EDTA), pH 8.0. Controls were included in all extractions to detect possible contamination.

Amplification and sequencing

An approximately 473 base pair region of the 5' end of the mtDNA control region (positions 16036-16549 of the human mitochondrial genome; Anderson et al. 1981) was amplified by the polymerase chain reaction (PCR; Saiki et al. 1988). PCR was performed in 50 µl reaction volume containing 10X Tris-HCl (pH 8.8), 2.5 mM $MgCl_2$, 200M dNTP, 0.2 M of each oligonucleotide primers, and 1 unit of Taq DNA polymerase. PCR primers were those of Baker et al. (1996), tPro (5'-TCACCCAAAGCTGRATRRCTA-3') and Dlp5 (5'-CCATCGWGATTTCTTATTTAAGRGGAA-3'). Amplification procedures were 94°C for 2 min followed by 35 cycles at 92°C for 30 sec, annealing at 52° C for 30 sec, and extension at 72° for 30 sec. Blank PCR controls were included with all amplification reactions to detect possible cross-contamination. PCR products were visualized on 1.6% agarose/Tris-borate EDTA (TBE).

Excess primers and dNTP's were removed from PCR reactions following either High Pure (Boehringer Mannheim) or QIAquick (QIAGEN) spin columm protocols.

Amplified PCR products were then sequenced on an ABI Biosystems automated sequencer following manufacturer protocols. Initial sequencing was done on an ABI Biosystems 373 sequencer with dye-terminator chemistry. Sequence quality improved considerably when products were sequenced on an ABI 377 sequencer with big dye chemistry (see Appendix for sample electropherograms); therefore, all but 6 sequences were done in this manner. Hypervariable region I and flanking regions were sequenced using t-PRO as the initiating primer. Sequences were aligned using Clustal W (version 1.6, Thompson et al. 1994), with adjustments made by eye using MacClade data editor (version 3.06, Maddison and Maddision 1996). All of the 16 non-unique haplotypes were verified by sequencing both strands for at least one sample per haplotype using the Dlp5 primer. Nineteen of the 28 unique haplotypes were also verified in this manner. In some cases, problems with DNA degradation and lack of additional tissue prevented verification of all sequences; however, there were no conflicts between forward and reverse sequence alignment in those cases where sequencing on both strands was successful.

In order to test for potential bias due to resampling, microsatellite loci were amplified for selected individuals that shared mtDNA haplotypes. Three microsatellite loci, EV94 (Valsecchi and Amos 1996), 31 (Palsbøll et al. 1997a) and 415/416 (Schlotterer et al. 1991) were used to amplify alleles via PCR for 25 individuals representing 8 haplotypes. PCR protocols followed those outlined by authors. Fluorescent dye-labeled primers were used for amplification of all three loci. The diluted PCR products were run on an ABI Biosystems automated sequencer, and allele

sizes were determined by comparison to size standards with the program GeneScan (ABI Systems Software, v. 2.1). None of the individuals that shared mtDNA haplotypes had the same microsatellite allele profile (Table 3). These results indicate the probability of resampling of individuals was low.

Genetic analyses

Standard diversity indices, such as nucleotide (π) and haplotype diversity (Nei 1987), haplotype frequencies, and number of segregating sites, were calculated using the program ARLEQUIN. (Schneider et al. 1997). Although relationships among 48 control region haplotypes from winter and summer populations (table on p. 47) were initially investigated by maximum parsimony analysis, the large number of haplotypes and many most parsimonious trees (>4000) rendered a full parsimony search impractical. Alternatively, a Neighbor-Joining tree (NJ, Saitou and Nei 1987) was constructed with the program PAUP* (Swofford, unpub. data) using Tamura-Nei distances (Tamura and Nei 1993) with a gamma correction (α, Wakeley 1993) of 0.10 estimated by the parsimony-based procedure of Yang and Kumar (1996). Table 4 lists the species and source of outgroups used for analysis. Support for groups identified by NJ analysis were evaluated with bootstrap analysis (500 replicates).

The partitioning of variation between winter and summer dolphin groups was investigated with an Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) to determine the degree of subdivision, if any, between the two groups. AMOVA is specified for molecular data and investigates the significance of population subdivision

for different levels of a hierarchy (within individuals, between individuals within populations, between populations, etc.). The test statistic ϕ_{sr} is analogous to Wright's F_{sr}

Table 3. Microsatellite allele profile for individuals which share mtDNA haplotypes. Numbers listed in columns are length of alleles in base pairs.

LOCUS	EV94 (TC)n...(AC)n	31 TAA(n)	415/416 GT(n)	mtDNA HAPLOTYPE
K897010	-	107/107	-	
K897074	258/258	107/110	-	
K897104	254/264	101/101	-	A
K1297184	246/262	-	-	
K1297185	248/248	107/110	-	
K0298216	242/242	107/107	-	
K797013	252/256	110/110	212/222	C
K797016	264/264	-	212/222	
K797017	258/272	106/106	-	
K1297205	262/268	106/109	-	
K1297212	258/258	-	-	D
K1297229	254/260	-	-	
K897092	256/270	-	212/230	
K1297181	258/260	-	-	H
K1297190	252/266	-	-	
K897050	242/242	107/110	-	
K897090	258/266	-	-	L
K1297180	248/264	-	212/214	
K1297170	254/256	-	222/226	
K1297189	258/264	-	-	N
K1197169	258/260	-	-	
K897091	260/266	-	212/228	P
K1297175	254/258	-	-	
K897101	260/262	-	-	Q
K1297204	258/262	-	-	

Table 4. Species used as outgroups.

Common name	Species name	Source
Long-nosed common dolphin	Delphinus delphis	GenBank U02639; Rosel et al. (1994)
Bottlenose dolphin	Tursiops truncatus	GenBank U20919; Siemann (1994)
Hector's dolphin	Cephalorhynchus hectori	GenBank AF057997; Pichler et al. (1998)
Hector's dolphin	Cephalorhynchus hectori	GenBank AF057996; Pichler et al. (1998)
Dusky dolphin (Peru)	Lagenorhynchus obscurus	F.Cipriano, unpub. data
Pacific white-sided dolphin	Lagenorhynchus obliquidens	C.S. Baker, unpub. data

(Wright 1951), but is a more powerful sequence based statistic. AMOVA calculations were performed with the ARLEQUIN software package (Schneider et al. 1997). 1000 non-parametric permutations were performed to test significance of ϕ_{st} value. In addition to AMOVA, an exact test of population subdivision (Raymond and Rousset 1995) was performed as an additional method for testing subdivision between populations.

The distribution of pairwise differences, or the mismatch distribution (MMD), was generated to gain insight into the demographic history of populations (Slatkin and Hudson 1991; Rogers and Harpending 1992; Harpending et al. 1993; Rogers 1995). The shape of the mismatch distribution presumably provides evidence of either population expansion or stationarity over evolutionary time (Rogers 1995). The parameters which determine the shape of the distribution also give insight into demographic patterns, including historical female effective population size before the hypothetical expansion (N_0), and the time since the expansion measured in units of mutational time (τ, Rogers and Harpending 1992).

If a population has undergone a recent expansion, the distribution of pairwise differences is expected to take the smooth, wave-like shape of a Poisson distribution (Slatkin and Hudson 1991; Rogers and Harpending 1992; Rogers and Jorde 1995). The smoothness of the mismatch distribution curve, measured by the "raggedness index" (r, Harpending 1994), also can give information about the historical demography of a population. A high (r) is indicative of a multimodal distribution typical of a stationary population.

Rogers (1995) derived a method of moments formula for calculating the expected distribution of pairwise differences under an expansion model based on the parameters θ_0 and τ. N_0, the effective female population size at time zero, is estimated by:

$$\theta_0 = 2N_0u \quad (1)$$

where θ is the overall genetic variation in a population at time 0 and $u = 2\mu k$, where μ is the mutation rate per site per generation and k is the length of the sequence. Watterson (1975) has shown that:

$$\theta = 4N_e\mu \quad (2)$$

where μ is the mutation rate per generation for a particular nucleotide sequence. Since nucleotide diversity (π; Nei 1987) is equal to θ (Nei and Li 1979), it holds true that at equilibrium:

$$E(\pi) = \theta = 4N_e\mu \quad (3)$$

for nuclear markers (Rooney 1998). It follows that:

$$E(\pi) = \theta = 2N_f\mu \quad (4)$$

for mitochondrial DNA. The current female effective population size can be estimated with equation 4 by plugging in current estimates of π and μ, and then solving for N_f. If we correct estimates of θ_0 for sequence length, substitute this corrected value for θ in equation 4 and solve for N_f, the historical female effective population size also can be estimated. If a population has undergone a bottleneck followed by expansion, the historical effective female population size calculated from θ_0 should be much less than that of estimates of N_f from current nucleotide diversity estimates. Estimates of

historical diversity (θ_0) from the mismatch distribution analysis (Rogers 1995) and current nucleotide diversity (π) are used to calculate and compare historical and current effective female population sizes from mtDNA sequences under the assumption of a 1:1 sex ratio. The minimum number of units of mutational time since the potential population expansion was measured from $\tau = 2\mu t$, where $\mu = 2uk$ as above. If τ is corrected for sequence length and u is the mutation rate per site per generation, solving for t gives the minimum number of generations since the time of expansion.

Hoelzel et al. (1991) estimated the area of the mtDNA D-loop, outside the 740 bp conserved central region, has a substitution rate that ranges from 5.2×10^{-9} and 10.4×10^{-9} substitutions per site per year. These estimates were based on sequence divergence between the basal member of the Delphinidae, the killer whale, and other dolphins. Rooney (1998) suggested that the mutation rate of the hypervariable control region I of baleen whales is very close to that calculated by Vigilant et al. (1991) for humans, ranging between 5 and 8×10^{-8} substitutions per site per year. These calculations are based on a per-lineage estimate (Nei 1992) incorporating the phylogenetic approach of Lynch and Jarrell (1993). If this rate for mysticetes is assumed to mirror that of delphinids, the mutation rate as estimated by Hoelzel et al. (1991) is an order of magnitude less than that calculated for humans. I estimated a mutation rate for hypervariable region I and flanking region for the dusky dolphin lineage by comparison to the killer whale (GenBank Accession M60409). Calculations based on those of Nei (1992) indicate a within-lineage mutation rate of 5.8×10^{-8} substitutions per site per year. This estimate, approximately that of the lower estimate for humans (Vigilant et al.

1991), is almost 6 times faster than that estimated for baleen whales (Hoelzel et al. 1991; Baker et al. 1993). For purposes of analysis, I calculated a range of historical demographic statistics with minimum estimates of mutation rates per site as estimated here for dusky dolphins (D) and Hoelzel (H) et al. (1991). Dusky dolphins are known to live up to 25 years or more. Females reach sexual maturity around 6-7 years and give birth about every 2 years (Würsig et al. 1997). A conservative generation time of 10 years was used to estimate minimum substitution rate for the control region of 5.2×10^{-8} and 5.8×10^{-7} substitutions per site per generation from H and D estimations, respectively.

Mismatch distribution analysis was carried out with the software program ARLEQUIN (Schneider et al. 1997). MMD parameters, including θ_0 (historical nucleotide diversity, Nei and Li 1979), and τ (units of evolutionary time since expansion) were calculated with the moment estimators of Rogers (1995). A chi-square goodness of fit test was performed to judge the fit of the frequency of pairwise differences to the expansion model (Poisson distribution of pairwise differences). A range of historical and current effective population sizes were calculated with both H and D mutation rates.

RESULTS

Diversity indices

Sequencing analysis of a 473 bp consensus of 80 individuals from Kaikoura (winter n=40, summer n=40) generated 44 haplotypes defined by 49 segregating sites (Table 5). Transitions were the most common base substitution with only one

transversion and one indel observed in all sequences. Haplotype diversity was high (0.97±0.008; Nei 1987), with 28 unique haplotypes. The most common haplotype (H) was shared by only 9 individuals (see Table 6 for list of haplotypes and relative frequencies). Nucleotide diversity was 0.017±0.009 with a mean number of pairwise differences of 8.0±3.77 and a range of 1-12. Nucleotide composition was typical for cetacean control region sequences (Hoelzel et al. 1991), with A and T represented in greatest proportion (30.62% and 34.76%, respectively), followed by C (21.49%) and G (13.13%).

An additional 20 sequences from locations throughout New Zealand were sequenced and diversity indices were calculated for 100 total samples (Table 2). Haplotype diversity and nucleotide diversity were 0.97±0.01 and 0.017±0.01, respectively, and did not differ much from indices calculated from winter and summer sequences alone. These additional sequences added 8 new haplotypes and 6 segregating sites to the sequence data. Relative frequencies of haplotypes were low, ranging from 0.01 for unique haplotypes to 0.13 for the most frequent haplotype (H) shared by 13 individuals (Table 6). The high frequency of unique haplotypes, as shown by high haplotype diversity and low relative frequencies, provides support for high levels of diversity within the New Zealand population(s). Haplotypes of these 20 additional individuals are not shown here, and were used for comparative purposes only.

Table 5. 49 segregating sites in the 44 haplotypes of dusky dolphins from winter (n=40) and summer (n=40) populations in Kaikoura. Numbered sites at the head of each column represent the relative position number within the control region following the end of the tPRO RNA gene. Sites that are identical to the reference sequence (WINTER 1) are shown by a '.', and gaps inserted to improve alignment are shown by a dash.

		11111	1122222222	2222222233	3333333333	3344444444
		45882366	9901444456	7788889900	0012225689	9900555555
		7390665670	2334747808	2312792301	2455686990	2378234567
1	WINTER 1	ACGATTGTTG	ATCCCTGCCC	ACTTTGCCTC	GTATCCTTAC	TCAATTAAT
2	A	..A.C.....	...T.C....	GT....T...	.C........	....C....
3	WINTER 2	..A.CC....	...T......	GT....T...	.C........	....C....
4	B	....	......A...	......T...	.........T	.T...CG..
5	C	....	....	C	....T.....	....
6	WINTER 3	.T..C.A...	.C....A..T	......T.C.	...CT....T	....CC...
7	D	....	....	....	.........T	....
8	E	..A.C.....	...T......	GT....T...	.CG..T....	..G.C....
9	WINTER 12	-..G......	...T....T.	......T...	.....T.C..	....
10	G	..A.C.....	...T......	GT....T...	....	....C....
11	H	..A.C.....	...T......	GT....T...	.C........	....C....
12	I	.T..C.....	.C.......T	......T.C.	....T....T	....CC.G.
13	J	-..........	...T......	.....T..T	......C...	....
14	WINTER 13	....	...T......	.CC..T...	A........T	C..G.....
15	L	....	.....CA...	......T...	........GT	C.........
16	WINTER 4	..A.C.....	...T......	GT....T...	.C........	....C...C
17	WINTER 5	..A.C..C..	...T......	G.....T...	.C........	....C....
18	WINTER 6	..A.C.....	...T.CA...	GT....T...	AC........	....CC...
19	WINTER 7	....C.....	...T......	GT....TT..	.C.....C..	....
20	WINTER 8	..A.C.....	...T......	.T....T...	.C........	....C....
21	WINTER 9	..A.C.....	...T......	GTC...T...	......C...	....C....
22	N	....	...T..A...	......T...	.........T	.T...CG..
23	WINTER 10	-..........	...T......	......TT..	.......C.T	....
24	P	....C.....	...T......	GT....T...	.C....C...	....
25	WINTER 11	-..G......	...T....T.	......T...	.C...T.C..	....
26	Q	..A.C.....	.....C....	GT....T...	....	....C....
27	R	..A.CC....	...T......	GT....T...	....	....C....
28	S	..A.C.....	...T...T..	GT...AT...	.........T	....C....
29	SUMMER 1	-..........	...T......	......T...	......C...	....
30	SUMMER 2	....C.....	....T.....	GT....T...	.C........	....C....
31	SUMMER 3	....	..T..CA...	......T...	........GT	C.........
32	SUMMER 4	....	....	......T...	.........T	..G.....
33	SUMMER 5	..A.C.....	...T.C....	GT.C..T...	.C........	C.....
34	SUMMER 6	..A.C.....	...T.CA...	GT.C..T...	....	....C....
35	SUMMER 7	..A.C.....	...T......	GT..C.T...	.C........	....C....
36	SUMMER 8	......A.CA	.....C....	......T.C.	A........T	....
37	SUMMER 9	-..........	G..T....TT	......T...	......C...	....
38	SUMMER 10	....	....	....	.....T....	....
39	SUMMER 11	..A.C.....	..T.CA...	GT....T...	.C........	....CC...
40	T	....	....	......T...	......C..T	C.....
41	SUMMER 12	....	....	......T...	....	C.....
42	SUMMER 13	....C.....	...T......	GT....T...	......C...	.....C...
43	SUMMER 14	..A.C.....	...T.C....	GT....T...	.C.....T	....C....
44	SUMMER 15	..A.C.....	.....C....	GT....T...	A.........	....C....

Table 6. Relative frequencies of mtDNA haplotypes of individuals from New Zealand. Winter and summer frequencies include only those individuals sampled in Kaikoura during 1997/98. Total of all samples includes beach-cast specimens from locations throughout New Zealand and individuals sampled in Kaikoura during spring.

Haplotype	Total frequency all samples (n=100)	Beach cast and Spring (n=20)	Winter frequency (n=40)	Summer frequency (n=40)	Relative frequency all samples
WINTER 1	1	0	1	0	0.01
A	8	1	3	4	0.08
WINTER 2	1	0	1	0	0.01
B	2	0	2	0	0.02
C	3	0	3	0	0.03
WINTER 3	1	0	1	0	0.01
D	5	1	1	3	0.05
E	2	0	1	1	0.02
WINTER 12	1	0	1	0	0.01
G	2	0	2	0	0.02
H	13	5	4	4	0.13
I	4	1	2	1	0.04
J	2	0	2	0	0.02
WINTER 13	1	0	1	0	0.01
L	4	1	2	1	0.04
WINTER 4	1	0	1	0	0.01
WINTER 5	1	0	1	0	0.01
WINTER 6	1	0	1	0	0.01
WINTER 7	1	0	1	0	0.01
WINTER 8	1	0	1	0	0.01
WINTER 9	1	0	1	0	0.01
N	5	2	1	2	0.05
WINTER 10	1	0	1	0	0.01
P	4	1	1	2	0.04
WINTER 11	1	0	1	0	0.01
Q	4	0	1	3	0.04
R	2	0	1	1	0.02
S	2	0	1	1	0.02
SUMMER 1	1	0	0	1	0.01
SUMMER 2	1	0	0	1	0.01
SUMMER 3	1	0	0	1	0.01
SUMMER 4	1	0	0	1	0.01
SUMMER 5	1	0	0	1	0.01
SUMMER 6	1	0	0	1	0.01
SUMMER 7	1	0	0	1	0.01
SUMMER 8	1	0	0	1	0.01
SUMMER 9	1	0	0	1	0.01
SUMMER 10	1	0	0	1	0.01
SUMMER 11	1	0	0	1	0.01
T	2	0	0	2	0.02

Table 6. Continued.

Haplotype	Total frequency all samples (n=100)	Beach cast and Spring (n=20)	Winter frequency (n=40)	Summer frequency (n=40)	Relative frequency all samples
SUMMER 12	1	0	0	1	0.01
SUMMER 13	1	0	0	1	0.01
SUMMER 14	1	0	0	1	0.01
SUMMER 15	1	0	0	1	0.01
SPRING 1	1	1	N/A	N/A	0.01
SPRING 2	1	1	N/A	N/A	0.01
SPRING 3	1	1	N/A	N/A	0.01
NZ 5	1	1	N/A	N/A	0.01
NZ 8	1	1	N/A	N/A	0.01
NZ 1	1	1	N/A	N/A	0.01
NZ 3	1	1	N/A	N/A	0.01
LOB 3 RIPIRO	1	1	N/A	N/A	0.01

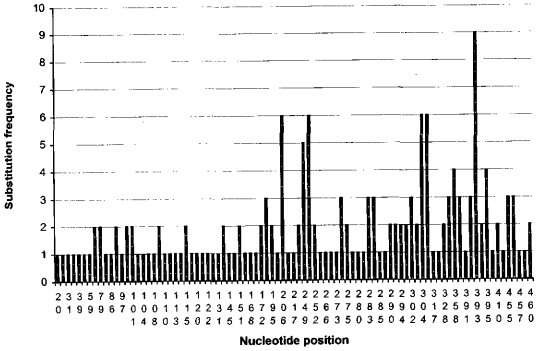

Figure 7. Frequency of multiple base substitutions at positions in the control region of dusky dolphins. Nucleotide positions are relative to the end of the tPRO RNA gene. Substitution frequencies were obtained from one of 1000 most parsimonious trees and are counts of character changes within this tree topology (R.I.=0.76; C.I.=0.55).

Population structure and phylogeny

The rate of substitution is unequal among sites (Fig. 7). Neighbor-joining analysis of haplotypes using the distance method of Tamura and Nei (1993) with a gamma correction of 0.1 (Wakeley 1993), produced a phylogeny with short internodes, small branch lengths, and little topological resolution between winter and summer haplotypes (Figs. 8, 9). A 50% bootstrap consensus (500 iterations) collapsed the majority of branches (Fig. 9) providing almost no support for relationships between haplotypes. All but one shared haplotype contains members from both seasonal groups. This tree topology suggests that (1) there is no separation of mtDNA haplotypes between winter and summer dolphin groups, and (2) there has been a radiation of unique haplotypes that could indicate a population expansion some time in the past (Slatkin and Hudson 1991).

There was no significant difference between winter and summer dolphins (Table 7, AMOVA, ϕ_{sr}=-0.0073, P=0.68, 1023 permutations). A negative ϕ_{st} value is essentially zero, suggesting that the separation of populations into winter and summer groups is not supported. An exact test of population differentiation (Raymond and Rousset 1995) indicates that the probability of non-differentiation is 0.57±0.0316, further supporting lack of subdivision between groups.

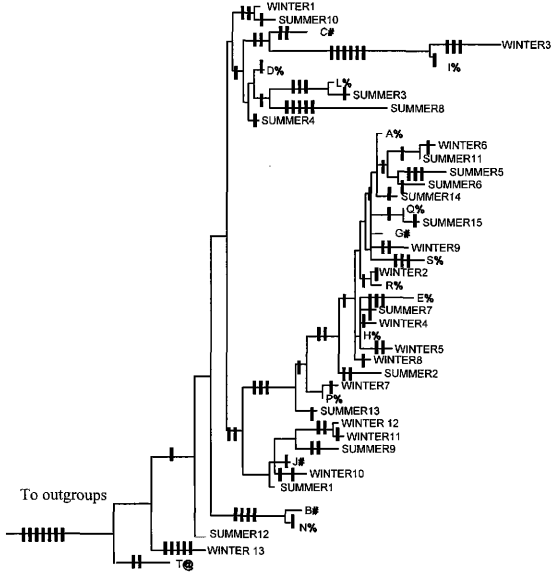

Figure 8. Neighbor-joining tree of haplotypes from 40 winter and 40 summer individuals from Kaikoura, New Zealand. Shared haplotypes are noted by letter. A '%' indicates a haplotype found in both Winter and Summer; '#' and '@' indicate haplotypes found only in Winter and Summer, respectively. Tree was generated with distance algorithm of Tamura and Nei (1993) with a parsimony-based estimate of the likelihood gamma shape parameter (α=0.1). Substitutions are indicated by hatch marks. See Table 3 for outgroups.

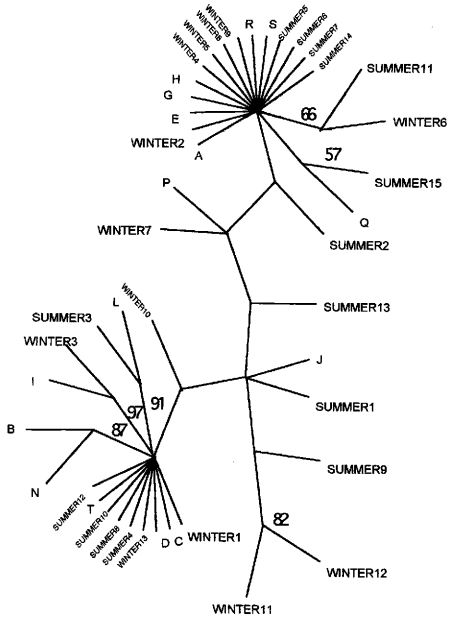

Figure 9. Unrooted phylogram of 44 haplotypes from winter and summer groups in Kaikoura, New Zealand. Tree represents consensus of 1000 most parsimonious trees generated by an heuristic parsimony search with tree bisection-reconnection (TBR) and random addition. Characters were unweighted. Bootstrap values were generated with 500 "fast" stepwise replicates.

Table 7. Results of AMOVA analysis.

Source of variation	d.f	Sum of Squares	Variance components	Percentage of variation
Among populations	1	2.830	-0.02918 V_a	-0.73
Within populations	79	316.893	4.01131 V_b	100.73
Total	80	319.723	3.98213	

Fixation index ϕ_{st} = -0.00733

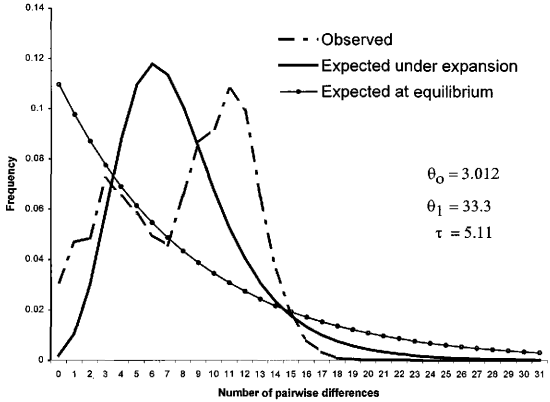

Figure 10. Frequency distribution of pairwise differences for 473 base pairs of the mtDNA control region of 100 dusky dolphins from New Zealand. Observed distribution compared to those expected under expansion and equilibrium.

Mismatch distribution

Figure 10 compares the observed distribution of pairwise differences for 100 dusky dolphin sequences from New Zealand to the distributions expected under equilibrium and expansion. Observed frequency of pairwise differences deviated significantly from a Poisson curve representative of a rapid expansion (χ_{26}^2=50004.9, P<0.001); however, the lack of fit of the observed frequencies to a Poisson curve does not in itself reject the hypothesis of rapid expansion. Simulated expansion populations often are statistically different from a Poisson distribution despite obvious similarity to the shape of the expected distribution under expansion (Rogers and Harpending 1992). Therefore, if Poisson-like in form, the observed distribution is consistent with the hypothesis of population expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992). The shape of the observed frequency distribution of dusky dolphins is more similar to the Poisson curve than to that expected of a population at equilibruim (Fig. 10), and resembles the bimodal shaped curves generated in the expansion simulations of Rogers and Harpending (1992). Harpending's raggedness index was low at 0.005, suggesting a smooth distribution typical of a population having undergone expansion.

Analysis of the distribution of pairwise differences gave values τ=5.11, θ_0=3.012. Correcting these values for the sequence length (473bp) yielded values of θ_0=0.00636 and τ=0.11. Using these values and current nucleotide diversity estimates, Equation 4 was used to calculate the historical (N_0) and current (N_f) female effective female population size for each mutation rate (R=Rooney 1998, H=Hoelzel et al. 1991). N_0 was estimated to be between 6,630 (R) and 61,154 (H). Current female effective population

size was estimated as a range between 17,000 (R) and 163,461 (H) individuals. This hypothetical expansion in population size occurred somewhere between 11,000 (R) and 105,769 (H) generations ago. If generation time for dusky dolphins is assumed to be 10 years, this puts the date of expansion between 110,000 and 1,057,690 years ago. Preliminary analysis of photo-identification work suggests that the population of dusky dolphins that passes through Kaikoura is roughly 10,000 (Markowitz, unpub. data). If this represents a relatively large portion of the entire New Zealand population, which is suspected, I would suggest that the estimation of effective population size based on the mutation used by Rooney (1998) produces values much closer to realistic estimates of population size. Therefore, only the estimates based on this mutation rate will be considered further.

DISCUSSION

Population subdivision

Gene flow between cetacean populations becomes increasingly limited in cases where large geographic distances or barriers exist, as can be seen for the separation of bottlenose dolphins by the Florida peninsula into genetically distinct Gulf of Mexico and Atlantic Ocean groups (Dowling and Brown 1993). Populations of New Zealand's Hector's dolphin have highly localized distributions, show no evidence of seasonal along-shore migrations, and little movement of individuals between groups (Dawson and Slooten 1993; Bräger 1998). As a result, there are high levels of genetic differentiation

between populations of the east and west coasts of the South Island and North Island (Pichler et al. 1998).

If dusky dolphins of New Zealand were like Hector's dolphins one would expect to see similar patterns of differentiation and phylogenetic resolution of mtDNA haplotypes by region. Nevertheless, tests for geographic subdivision, and the lack of resolution among haplotypes and the mixing of individual lineages collected in winter and summer, suggest that the Kaikoura population is one large, mating population. Furthermore, when the additional 20 samples from locations throughout New Zealand are included in NJ analysis, the resulting topology suggests that not only are the dusky dolphins of Kaikoura one, randomly breeding population, but groups found throughout New Zealand are part of one genetic stock (Fig. 11). Samples collected from dolphins found up to 270 kilometers from Kaikoura are not only closely related to individuals from Kaikoura, but 7 share haplotypes with Kaikoura dolphins.

These results are not unexpected considering evidence for moderate to large scale shifts in distribution of dusky dolphin groups along the coasts of New Zealand and South America as determined by photo identification and radio tracking studies. In New Zealand, recent photo-identification results indicate that individuals are in Kaikoura during winter and summer. Additionally, a pair of individual dolphins that were in Kaikoura during summer were sighted some 250 km north in spring. A pair of dolphins off the coast of Argentina was resighted 780 km southwest of their original location, eight years after initial sighting (Würsig and Bastida 1986). Therefore, there is no evidence that dusky dolphins, a coastal and highly mobile species, are genetically

Sample	Location	Source
NZ1	Unknown	F. Cipriano, unpub data
NZ3	Otago Peninsula	F. Cipriano, unpub data
NZ5	Kaikoura, 1995	F. Cipriano, unpub data
NZ6	Kaikoura, 1994	F. Cipriano, unpub data
NZ7	Kaikoura, 1994	F. Cipriano, unpub data
NZ8	Kaikoura, 1994	F. Cipriano, unpub data
NZ10	Greymouth	F. Cipriano, unpub data
NZ11	Kaikoura, 1995	F. Cipriano, unpub data
NZ14	Auckland	F. Cipriano, unpub data
LOB1	Otago Peninsula	U. of Auckland
LOB2	Otago Peninsula	U. of Auckland
LOB 3	Ripiro Beach	U. of Auckland
LOB 4	Motueka	U. of Auckland
LOB6	Abel Tasman	U. of Auckland
LOB7	Nelson	U. of Auckland

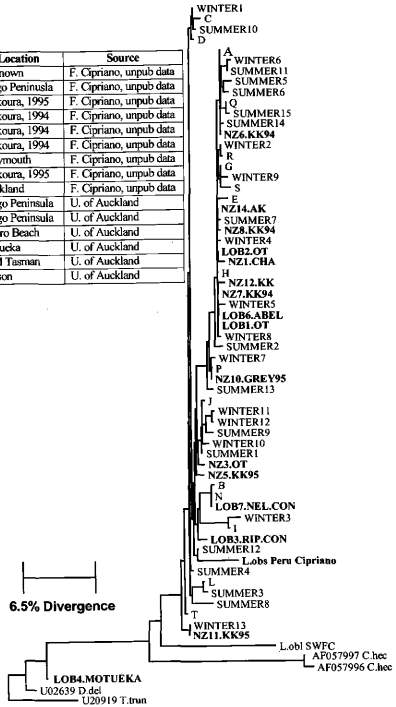

Figure 11. Relationships between winter and summer haplotypes and beach-cast animals from different locations around New Zealand. Distances were calculated with the method of *Tamura-Nei* with a parsimony derived gamma correction ($\alpha = 0.13$) as estimated from the data. Individuals in bold type are from outside Kaikoura. See insert for information on sample origin.

differentiated within a small geographical area like Kaikoura on the basis of temporal isolation, despite observed differences in seasonal distribution and behavior

Current and historical population demography

The MMD analyses (i.e., low raggedness index and lack of fit of MMD to equilibrium model) suggest a population expansion for New Zealand dusky dolphins around 110,000 years ago. The potential for a threshold effect in the magnitude of change in population size that can be detected by MMD analysis limits its ability to detect changes in demography on a fine temporal scale (Lavery et al. 1996). Therefore, the robustness of the mismatch distribution to subsequent bottleneck and expansion events could mask more recent population demographic change. The phylogeny of dusky dolphin haplotypes provide additional evidence for recent population expansion (Slatkin and Hudson 1991). However, because mutations accumulate slowly over thousands of generations, an unresolved and star-like phylogeny may indicate change over a broad evolutionary time scale, but has nothing to do with recent bottleneck events (Rooney 1998). Comparison of historical and current levels of diversity, as measured by θ_0 and π, may be a better means to assess recent changes in population demography. A population that has undergone a bottleneck and subsequent expansion thousands of generations in the past, will demonstrate an increase in genetic diversity post-expansion (Rooney 1998), and a rapid accumulation of new mtDNA haplotypes as the lineage extinction rate is actuely reduced (Avise et al. 1984). Therefore, populations that have had a low long-term effective population size, or have recently gone through a severe

genetic bottleneck, will likely have low current levels of genetic diversity due to the rapid elimination of mtDNA lineages (Avise et al. 1984).

Low levels of nucleotide diversity in cetacean species have been correlated with (1) bottleneck events due to overexploitation (e.g., northern elephant seal (Mirounga angustirostris, Hoelzel et al. 1993); (2) massive die-offs due to disease (e.g., striped dolphins of the Spanish Mediterranean, Garcia-Martinez et al. 1993); or (3) matrilineal kin structure (e.g., sperm whales (Physeter macrocephalus, Whitehead 1998). Nucleotide diversity estimates from these populations are usually less than 0.01 (Table 8) in contrast to higher levels of diversity for other populations with larger long-term effective population sizes (e.g., Pacific white-sided dolphins, π=0.021. Lux et al. 1997) or populations which suffered a demographic reduction but not a genetic bottleneck (e.g., bowhead whales (Balaena mysticetus), π=0.016, Rooney 1998; humpback whales, π=0.020, Baker et al. 1993).

The current level nucleotide diversity (π = 0.017) in the dusky dolphin population suggests that the species in New Zealand has not experienced a recent genetic bottleneck, but has had a relatively large long-term effective population size. Comparison of historical and current female effective population size indicates merely a three-fold increase in population numbers over the last 110,000 years. Compared to the 100-fold expansion of the human population over the last 150,000 years (Rogers and Jorde 1995; Rogers 1995), this increase is relatively small. Aris-Brosou and Excoffier (1996) showed that molecular markers with high substitution rate heterogeneity (a low α

Table 8. Some nucleotide diversities for mtDNA control region of marine mammals. Values in bold type indicate species that have low levels of diversity. See text for further discussion.

Species	Region	Nucleotide Diversity	Haplotype Diversity	Source
Dusky dolphin (Lagenorhynchus obscurus)	New Zealand	0.0170	0.98	Harlin, present study
Pacific white-sided dolphins (Lagenorhynchus obliquidens)	NW Pacific	0.0211	N/A	Lux et al.1997
Striped dolphins (Stenella coeruleoalba)	W Mediterranean	**0.0023**	0.79	Garcia-Martinez et al. 1995
Hector's dolphin (Cephalorhynchus hectori)	New Zealand	**0.0061**	0.75	Pichler, et al. 1998
Short beaked common dolphins (Delphinus capensis)	World	0.0180	0.97	Rosel et al. 1994
Long-beaked common dolphins (Delphinus delphis)	Pacific	0.0120	0.94	Rosel et al.1994
Harbour porpoise (Phocoena phocoena)	N Pacific, N Atlantic Black Sea	**0.0325**	0.94	Rosel et al. 1995
Sperm whale (Physeter macrocephalus)	World	**0.0038**	0.74	Lyrholm et al. 1996
Minke whale (Balaenoptera acutorostrata)	N Atlantic	**0.0064**	0.85	Bakke et al. 1996
	Antarctic	0.0159	0.96	Bakke et al. 1996
Humpback whales (Megaptera novaeangliae)	World	0.0257	0.88	Baker et al. 1993
Bowhead whale (Balaena mysticetus)	N Pacific	0.0160	0.98	Rooney 1998
Steller sea lion (Eumetopias jubatus)	E Pacific	0.0120	0.93	Bickham et al. 1996
	W Pacific	**0.0090**	0.90	Bickham et al. 1996
Harbor seal (Phoca vitulina)	Pacific	0.0120	0.89	Stanley et al. 1996
Northern elephant seal (Mirounga angustirostris)	Pacific	**0.0043**	0.41	Hoelzel et al. 1993

value for the gamma distribution) may cause a population under equilibrium to exhibit a smooth MMD even when at equilibrium. They also suggest that large population expansions are indicated by shifts of Tajima's D (Tajima 1989) to significantly negative values where rate heterogeneity has the opposite effect, shifting Tajima's D to more positive values. A negative but non-significant Tajima's D for the dusky dolphin population ($p=0.21$) is expected for a historical expansion event estimated from a gene region with rate heterogeneity. The star-like phylogeny of dusky dolphin haplotypes, and the smoothness of the MMD could be indicative of a Pleistocene expansion, but, because of a potential to mask more recent bottleneck and expansion events (e.g., Lavery et al. 1996), the MMD does not represent current demographic changes.

A similar pattern of historical and current demographic changes, as measured by the MMD and levels of diversity, exists for Steller sea lion (Eumetopias jubatus) populations of the northeastern Pacific (including coasts of California, British Columbia, and southeast Alaska). MMD analysis of the hypervariable region of mtDNA D-loop (Bickham et al. 1998, Bickham unpub. data) suggests the population has undergone expansion in the past. The unimodal distribution of pairwise differences is significantly different from a Poisson distribution ($\chi^2_8 = 23.0$, $P=0.003$), but the observed curve is very close in shape to the Poisson, even more so than for the dusky dolphin distribution (Fig. 12). The raggedness index is low ($r=0.022$) reflecting the smooth, wave-like MMD curve (Fig. 12). Calculation of current and historical effective population sizes based on a generation time of 4 years and a mutation rate of 10% per

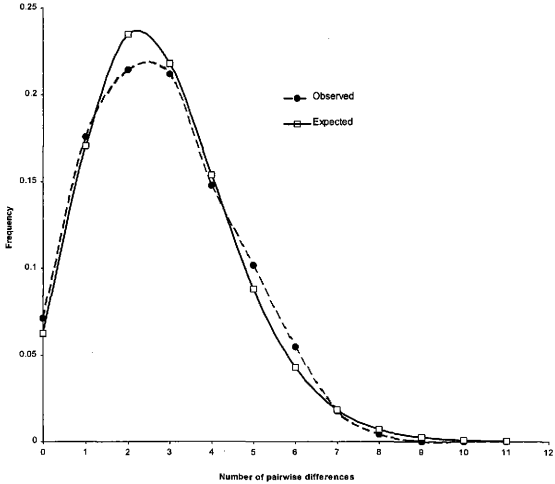

Figure 12. Frequency distribution of observed pairwise differences for 238 base pairs of the mtDNA control region of 59 Steller sea lions from the Northeastern Pacific. Observed distribution is compared to that expected of an exponentially growing population.

million years (Bickham et al. 1996) indicate an eight-fold expansion in female effective population size (from 1000 to 8125 individuals) 12,899 generations or 52,000 years ago.

Surveys of sea lion populations since the 1960's indicate numbers have remained fairly stable in this area at least since this time despite severe reduction in numbers in neighboring localities (Bickham et al. 1998). The estimate of current female effective population size might be slightly underestimated, but not unreasonable considering a census in 1994 that estimated total population size of around 23,000 (National Marine Fisheries Service, unpublished data). These results are similar to those for dusky dolphins where the shape of the Steller sea lion MMD indicates historical expansion while current levels of nucleotide diversity ($\pi = 0.012$), along with census data, provide evidence for a stable population size over recent evolutionary time. Consequently, a combination of MMD analyses and comparison of historical and contemporary genetic diversity can be used to investigate population demography on coarse and fine evolutionary scales. In any case, the relatively high levels of diversity for dusky dolphin and sea lion populations suggest that long-term effective population size of these species has been fairly large (Avise et al. 1984). Therefore, large population size, along with the relatively small area of New Zealand coastline, further reduces the probability that "winter" and "summer" dusky dolphin groups are genetically differentiated.

Statistical power and the demographic unit

Taylor et al. (1997) address the issue of power analysis as a necessary component of population studies for management. Power, or Type II error, is the probability of failing to reject the null hypothesis when the alternative is true, and in this case

determines the ability of a given data set to define demographic units for management. Managers faced with regulating human-induced mortality of populations, are interested in knowing the minimum rate of dispersal needed to maintain viable populations (critical dispersal rate). However, geneticists, when they fail to reject the null hypothesis of no subdivision, rarely report statistical power of their results. This is important since failure to reject the null hypothesis with no statistical power to do so could result in improper grouping of populations into one management unit. The greater the dispersal rate and abundance of populations, the more samples are needed to reduce variability and thus increase power.

Taylor et al. (1997) calculated that for two simulated harbor seal populations of mean weighted abundance of 1300, a dispersal rate of 3% per year, and a sample size of 40 from each population, power was less than 0.2 when the probability of a Type I error (α) was 0.05. Because dusky dolphin populations are larger and may be connected by greater dispersal rates, these would be conservative estimates for winter and summer populations. Preliminary results from photo identification data suggest that between 7,000 and 10,000 dusky dolphins are present in Kaikoura over the course of a year (T. Markowitz, unpublished data). If I consider the alternative hypothesis that the winter and summer dolphin populations are subdivided, but 3% per year were dispersing from one population to another, what power would there be to detect a difference? If we consider a population of at least 7,000 individuals, the mean weighted abundance for winter and summer groups is three times larger than the harbor seal population mean of 1400. This suggests that the ability of the dusky dolphin data set to detect a difference

between "winter" and "summer" populations is much less than 0.20. From a phylogenetic perspective, issues of statistical power are irrelevant; populations that have such high levels of haplotypic diversity are most certainly members of one breeding unit. However, from a demographic perspective, it is important to note that more subtle phylogeographically cohesive units may exist in dusky populations in Kaikoura or around New Zealand, for which the sampling regime is inadequate to detect. Therefore, there are limits to how the results here should be interpreted for management purposes.

However, for Kaikoura, the combined evidence of photo-identification and radio tracking studies, along with negative ϕ_{st} values, and results of the MMD analysis, suggest that there is little probability of subdivision between seasonal populations. From a management perspective, this suggests that commercial and recreational vessels operating in Kaikoura are essentially interacting with the same population of dolphins year round. In fact, this study suggests that the dolphins in Kaikoura represent a portion of the entire mating population of New Zealand. It might be important to monitor closely tourism activities in Kaikoura as a means to regulate the potential impact on dusky dolphin groups throughout the country.

Future research

Mt DNA, because of it's maternal inheritance (Giles et al. 1980), is useful for investigating demographic patterns for conservation since successful recruitment depends heavily on the demography of females in many species (Avise 1995). Analysis of samples collected throughout the dolphins' range in New Zealand might reveal structure of populations on a larger geographic scale. The decrease in population size in

Kaikoura from winter to the summer breeding and calving season suggests that Kaikoura is not the only breeding location for these dolphins. If this is the case, the agglomerations of dolphins in the Kaikoura area during winter might be the result of a joining of multiple breeding groups from other sites throughout New Zealand. Collection of samples from around both the North and South Islands during both the summer and winter might reveal structuring of female populations during the breeding season as has been seen with humpback whales (Baker et al. 1990; Palsbøll et al. 1997b).

However, studies of demography based on a single marker can be misleading (Moritz 1994; Rand 1995). It is a possibility that there is a division between winter and summer groups that is too recent in time to detect with mtDNA data. Therefore, analysis of dusky dolphin populations with both mtDNA and nuclear markers might reveal demographic patterns not detectable by mtDNA analysis alone. Microsatellites, short, tandem repeat sequences of the nuclear genome, can also be used to investigate social structure of species. Analyses of microsatellite markers have determined male chimpanzees within populations have coefficients of relatedness of half-siblings on average (Morin et al. 1994). This pattern of male relatedness is expected in a species with female dispersal, and behavioral data supports this pattern (Morin et al. 1994). Similar analyses of grey-sided voles (Clethrionomys rufocanus) revealed female relatedness is negatively correlated with geographic distance (Ishibashi et al. 1997). For pilot whales (Globicephala macrorhynchus), Amos et al. (1993) found evidence for lifetime pod membership for both males and females, suggesting strong familial bonds and lack of dispersal from the natal group for both sexes. There have also been several

studies which have used microsatellite markers to investigate relationships between humpback whale populations in winter calving and summer feeding grounds (Larsen et al. 1996; Palsbøll et al. 1997; Valsecchi et al. 1997). There are, however, few, if any, studies on the social structure of small delphinids which occur in large groups. Analyses of pod structure, or grouping of individuals based on familial relationships, would provide new and valuable insight into dolphin societies.

On a global scale, very little is known about the relationship between New Zealand dusky dolphins and their conspecifics in South Africa and South America. Preliminary phylogenetic results presented here (Fig. 11) suggest that the separation of New Zealand dusky dolphins from conspecifics in South America was relatively recent. Avise et al. (1984) investigated the influences of demography on mtDNA lineage survivorship and found that a stable-sized population can expect to trace all mtDNA lineages to a single female after 4n generations since a founding event (where n is the number of founding females). If we consider the historical female effective population size of 6360 as the founding population of dusky dolphins in New Zealand, we would expect there to be no mtDNA lineages in the New Zealand population that predate separation from Peruvian or South African populations after 25,440 generations. However, this estimate of historical female effective population size is based on a coalescence time (τ) of only 11,000 generations as calculated from the MMD; consequently, not enough time has elapsed for lineage sorting to eliminate lineages that predate the founding of the New Zealand dusky dolphin population. This could explain why we see close relationship between the Peruvian mtDNA sequence and those from

New Zealand (Fig. 11). However, these estimates assume that (1) the New Zealand population has been stable since the founding event, and (2) estimation of coalescence time from the MMD corresponds to the time of the founding event. MMD analyses suggest the New Zealand dusky dolphin population underwent expansion in the Pleistocene, but this is does not necessarily correspond to the time when the New Zealand population was founded. Therefore, comparison of molecular diversity and phylogenetic relationships between Peruvian, South African, and New Zealand dusky dolphins would give insight into the timing and location of dusky dolphin origins in New Zealand. If the separation from South Africa and South America were relatively recent, we'd predict similar levels of diversity and degree of divergence between all three regions.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Genetic sampling

Although I believe that the sampling technique presented here will prove effective for many bowriding cetaceans in a variety of situations, different species could prove more difficult to sample and/or more easily disturbed by the sampling process. For this reason, we recommend that responses to sampling be documented and carefully evaluated on a case-by-case basis. The large sizes of dusky dolphin groups (Cipriano 1992) and their highly interactive nature (Würsig et al. 1997) make them ideally suited to this type of sampling. It seems likely that this technique will have greatest utility for sampling small cetaceans with similar gregarious tendencies, such as spinner dolphins, common dolphins, and others.

Several researchers have found minimal or "mild" reactions to invasive tagging and biopsies (Würsig 1982, for hand-lancing tags into dusky dolphins; Weinrich et al., 1991, Clapham and Mattila 1993, for biopsy darting of humpback whales; Brown et al. for biopsy darting of Atlantic right whales, Eubalaena glacialis; Barrett-Lennard et al. 1996, Hoelzel et al. 1998a, for biopsy darting killer whales). Dolphin groups have been shown to change their behavior in response to the presence of vessels up to 6 miles or more away (Au and Perryman 1982). Therefore, any sort of boating activity is a potential source of disturbance for dolphin groups. Since time of day and behavioral state of dolphin groups did not affect how dolphins responded to sampling, the impact of

sampling is likely less of a disturbance than boating activities. In fact, since responses of dolphins to sampling were not significantly different from behavioral controls, it is difficult to separate responses to sampling from alterations in behavior due to the presence of a vessel around groups. Most times, our vessel was one of three vessels in proximity to a dolphin group, the other two being larger dolphin tour vessels. The impacts of boats on dolphin behavior can be minimized if "dolphin-friendly" boating guidelines are followed (Constantine and Baker 1997) e.g. parallel approach with minimal interference with group directional heading. Therefore the potential effects of tissue collection, however minimal, can thus be tempered even further by proper boating practices.

I have not yet been able to test for more subtle longer-term reactions by dolphins to these sampling efforts. For example, it is possible, although we consider it unlikely, that dolphins that are repeatedly sampled over a season learn to avoid the sampling vessels, and perhaps other vessels. Although the present technique is potentially less invasive than other methods of tissue collection, such as dart-propelled biopsy sampling, it is possible that any boat approach with an even minor negative stimulus may have some level of long term effect.

Genetic analysis

Populations that are decreasing in size have a greater probability of becoming genetically differentiated just by the random effects of drift due to a reduction in effective population size and number of effective migrants due to spatial or temporal

separation (Avise et al.1984). This is likely not the case for dusky dolphins. Mismatch distribution analysis suggests that the dusky dolphin population underwent expansion during the Pleistocene, but has been relatively stable since this time. Comparison of historical and current levels of diversity suggest that there has been little net increase in female effective population size over the last 110,000 years. These estimates, based on unreliable evolutionary rates, could be gross underestimates; however, even if the scale of these estimates are off by 10-fold or more, the pattern of the MMD would remain the same. Therefore, New Zealand dusky dolphin populations have been at equilibrium since the Pleistocene, over a period of 110,000 years.

Analysis of population structure does not support segregation of winter and summer dolphin groups into genetically distinct populations. Yet on a broader geographic scale, female dusky dolphins may, like humpback whales (Baker et al. 1993, Palsbøll et al. 1995) and bottlenose dolphins (Shane et al. 1986, Duffield and Wells 1991), exhibit philopatry, and seasonal changes in group size and proximity to shore in Kaikoura reflect a pattern of calving site fidelity in females. However, the lack of haplotypic structure by geographic regions throughout New Zealand suggests that female dusky dolphins are not philopatric. Preliminary analysis of photo-identification data indicates some level of movement between groups. It could be that females disperse in this species, with males remaining closer to natal territories; however, such questions require the analysis of nuclear DNA markers that tell about sex-specific demographic patterns.

The next phase of this research in New Zealand will address questions of population subdivision and movement patterns in relation to ecological and behavioral factors. Near-shore shallow areas, such as those found in Kaikoura, are likely important to mothers and offspring during the early phases of offspring development following parturition (Cipriano 1992; Würsig et al. 1997). Proximity to the shoreline may serve as a means to avoid predation on both calves and adults by limiting the directions from which a predator can approach, and by giving dolphins the chance to hide in the turbulent surf zone when potential predators are present (Würsig and Würsig 1980, Cipriano 1992).

If places such as Kaikoura, where dolphins gather for mating, calving and nursing young, are rare, it might be important to implement management regulations that protect the dolphins at times when they are particularly vulnerable. What proportion of the New Zealand dusky dolphin population passes through Kaikoura during the different seasons? Are the dolphins we see in Kaikoura, members of one large, New Zealand-wide panmictic population; or are there distinct divisions between groups, for example, between the west and east coasts? Do females show fidelity to breeding/calving sites? Is Kaikoura the only place in New Zealand where dolphins gather during the breeding season? On a global scale, how related are dusky dolphins of New Zealand to their conspecifics in South Africa and South America? These questions are critical to development of management strategies for these dolphins both within New Zealand, as well as on a global scale.

Mt DNA, because of it's maternal inheritance (Giles et al. 1980), is useful for investigating demographic patterns for conservation; however, studies of demography based on a single marker can be misleading (Moritz 1994; Rand 1995). Relationships between taxa or populations based on single markers are "gene trees" or locus specific patterns (Rand 1995). Therefore, studies based on multiple markers are likely to give more accurate representations of population demographic patterns. Analysis of populations with multiple nuclear loci (i.e., microsatellites) and mtDNA are needed before definitive statements can be made regarding relationships between populations. There is the possibility that there is a nested matrilineal structure within the large dolphin pods. If this is the case, the sampling regime in Kaikoura that focused on structure between seasons, was inadequate for testing hypotheses of fine-scale intragroup structure. The large size of the groups (>1000) and inability to distinguish cohorts within the overall large group, would make such detailed sampling nearly impossible. Yet, intensive sampling of small groups outside the main pod might reveal matrilineal substructure. Similarly, allelic markers, like microsatellites, also can be used to investigate relatedness of dolphins within and between pods. Such analysis can give insight into pod structure or grouping of individuals based on familial relationships. Results such as these would provide never-before-seen details of dusky dolphin social structure and society.

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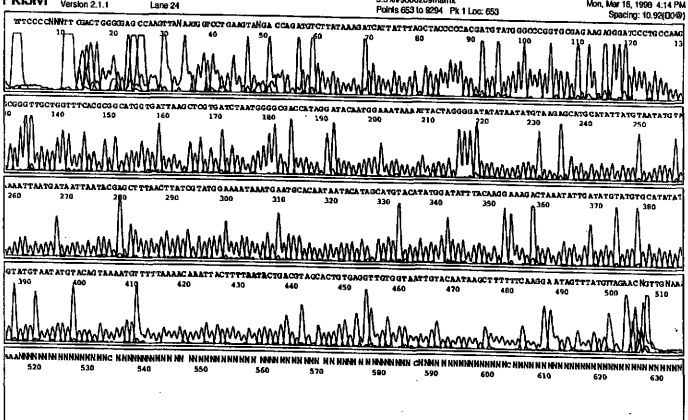
APPENDIX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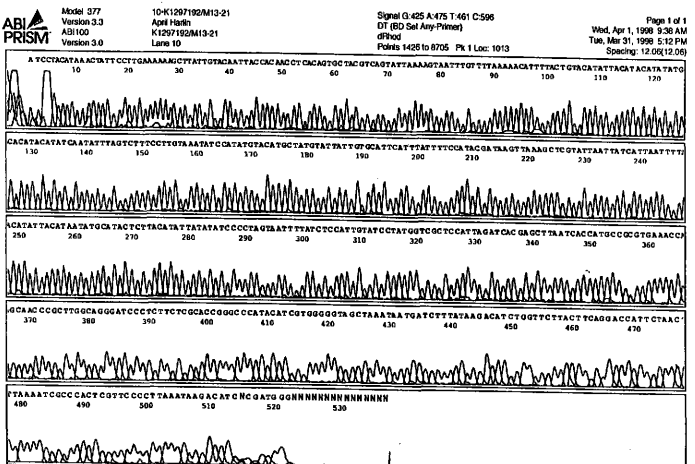
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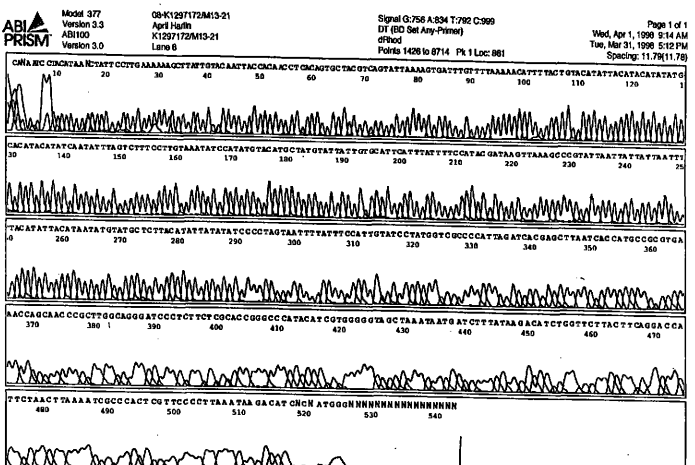
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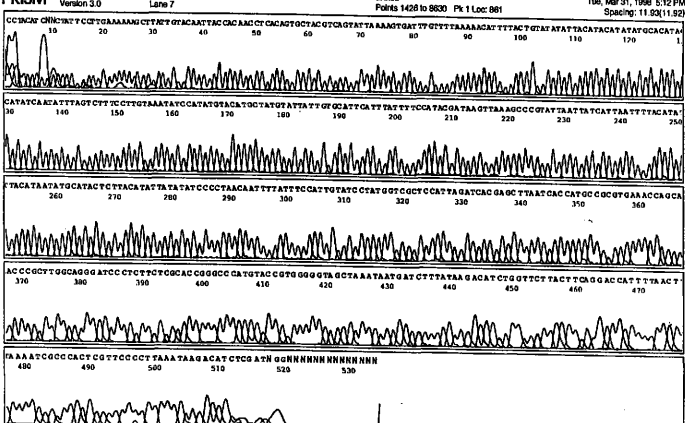
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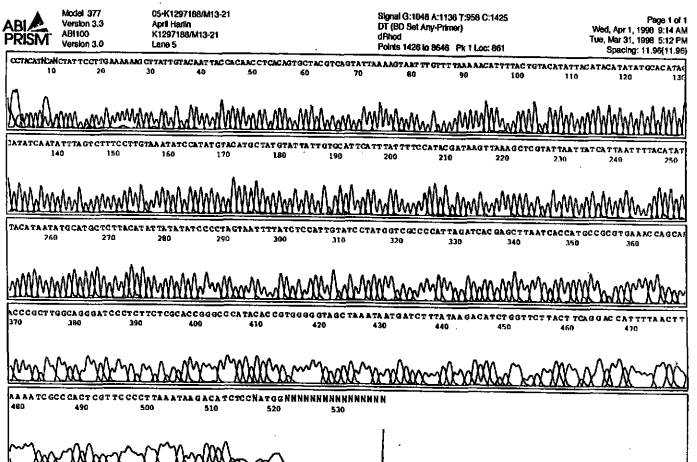
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VITA

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I. Personal Information

Born October 19, 1970, St. Louis, Missouri.

II. Education

University of California, Davis. B.S. Biological Anthropology
Graduated with honors, June 1996

Texas A&M University, College Station, Texas. M.S. Wildlife and Fisheries Sciences, May 1999, Dr. Bernd Würsig, major advisor

III. Professional Experience

Co-Principal Investigator Dusky Dolphin Research Project, Earthwatch Center for Field Research. October 1997 to present

Population structure of the New Zealand dusky dolphin (Lagenorhynchus obscurus). M.S. thesis, Texas A&M University. August 1997-May 1999

Distribution and habitat utilization of the dusky titi monkey in Yasuni Reserve, Ecuador. Dr. Peter Rodman, U. C. Davis Department of Anthropology. June-August 1994

Isozyme analysis of population divergence and diversity of native Californian wildflowers, Dr. Leslie Gottlieb, UC Davis Department of Molecular and Cellular Biology. January-June 1995

IV. Scholarships, Fellowships

President's Undergraduate Fellowship, University of California, Davis, 1995
Willie May Harris Graduate FellowshipTexas A&M University, 1996

V. Publications

Harlin, A. D., B. Würsig, C. S. Baker, and T. M. Markowitz. 1999. Skin swabbing for genetic analysis: Application on dusky dolphins (Lagenorhynchus obscurus). Marine Mammal Science, in press.