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**EXAMINATION OF DIET OF HARBOR PORPOISE (*PHOCOENA PHOCOENA*)
FROM CENTRAL CALIFORNIA USING STOMACH CONTENT AND STABLE
ISOTOPE ANALYSIS FROM MULTIPLE TISSUES**

**A Thesis Presented to the Faculty of
Moss Landing Marine Laboratories
and
San Jose State University**

**In partial Fulfillment
Of the Requirements for the Degree
Master of Science**

**By
Amanda Kate Toperoff**

August 2002

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Abstract

Dietary fractionation of carbon and nitrogen stable isotope ratios in bone collagen, muscle, and skin tissue were examined from harbor porpoise (*Phocoena phocoena*) from Monterey Bay. Fractionation was calculated as the difference between the estimated isotopic composition of the diet and each tissue. Fractionation factors are necessary to interpret tissue values indicative of trophic level and habitat. Understanding skin will allow the gathering of information through non-lethal methods. Comparisons of stomach contents between stranded and by-catch animals showed differences in the proportion of prey species consumed. The $\delta^{13}\text{C}$ values of all tissues indicated that animals from both groups shared similar habitats. The $\delta^{15}\text{N}$ values were similar between groups for bone collagen, indicative of trophic similarities over long time scales. The $\delta^{15}\text{N}$ values of skin tissue were greater for stranded animals than by-catch, reflective of recent trophic differences and may be related to starvation. Additionally, aspects of life history were examined.

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Table of Contents

Copyright.....	ii
Signature Page.....	iii
Abstract.....	iv
Acknowledgments.....	v
Table of Contents.....	vii
List of Tables.....	ix
List of Figures.....	xi
Appendices.....	xiii
Chapter 1 Examination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ Dietary Fractionation within Three Tissues of Harbor Porpoises (<i>Phocoena phocoena</i>) from Monterey Bay	1
Introduction.....	1
Methods.....	4
Results.....	6
Discussion.....	8
Chapter 2 Food Habits of Stranded and By-Catch Harbor Porpoise (<i>Phocoena phocoena</i>) from Monterey Bay, 1997 – 2000: Analysis of Stomach Contents and Stable Isotope Ratios	16
Introduction.....	16
Methods.....	21
Results.....	24
Discussion.....	27
Chapter 3 Age, Growth, and Reproduction of Harbor Porpoise (<i>Phocoena phocoena</i>) from central California	36
Introduction.....	36
Methods.....	39
Results.....	44

Discussion.....	48
Literature Cited.....	55
Tables.....	69
Figures.....	80
Appendices.....	102

List of Tables

Table	Page
1. Mean nitrogen and carbon stable isotope values for three tissues from 29 harbor porpoise and porpoise prey collected in Monterey Bay.	69
2. Repeated measures ANOVA table for comparisons of harbor porpoise tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values	70
3. Calculation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation within skin, muscle, and bone collagen of 29 harbor porpoise.	71
4. Mean percent number (%N), mean percent mass (%M), percent frequency of occurrence (%FO), mean index of relative importance (IRI), mean percent index of relative importance, and standard error for each prey species identified in stomachs of 14 stranded harbor porpoise.	72
5. Mean percent number (%N), mean percent mass (%M), percent frequency of occurrence (%FO), mean index of relative importance (IRI), mean percent index of relative importance, and standard error for each prey species identified in stomachs of 18 by-caught harbor porpoise.	73
6. Comparison of past and present diet data based on ranks of IRI values reported for stranded harbor porpoise from Monterey Bay.	74
7. Symbol, mean, and standard error of prey array indices from stranded and by-caught harbor porpoise from Monterey Bay.	75
8. Stable carbon and nitrogen isotopic compositions of bone collagen, muscle, and skin from porpoise from Monterey Bay.	76
9a. T-test table for comparisons of stable carbon isotope values for stranded and by-catch harbor porpoise from Monterey Bay.	77
9b. T-test table for comparisons of stable nitrogen isotope values for stranded and by-catch harbor porpoise from Monterey Bay.	77
10. Fitted growth curve equations for length-at-age data. Equations represent Gompertz, Laird-Gompertz, and von Bertalanffy growth models for male and female harbor porpoises.	78

11. Catalog number, sex, month, year of collection, and standard length of neonates used to calculate length at birth. 79

List of Figures

1.	Monthly averaged $\delta^{15}\text{N}$ values of skin and muscle tissues of 29 harbor porpoise collected between 1997 and 1999.	80
2.	Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of harbor porpoise prey species.	81
3.	Locations of harbor porpoise collected in Monterey Bay as beach strandings and by-catch.	82
4.	Distribution of stranded and by-catch harbor porpoise collected from Monterey Bay between 1997 and 2000 and used in stomach content analysis.	83
5.	Cumulative number of species for stranded harbor porpoise stomach samples from Monterey Bay used to determine the adequacy of sample size.	84
6.	Cumulative number of species for by-catch harbor porpoise stomach samples from Monterey Bay used to determine the adequacy of sample size.	84
7.	Cumulative mean %M (Mass) and %N (Number) of prey species (<i>L. opalescens</i> , <i>E. mordax</i> , <i>P. notatus</i>) in stomach contents of stranded harbor porpoise from Monterey Bay.	85
8.	Cumulative mean %M (Mass) and %N (Number) of prey species (<i>L. opalescens</i> , <i>E. mordax</i> , <i>M. productus</i>) in stomach contents of by-catch harbor porpoise from Monterey Bay.	86
9.	Standard length of main prey species (<i>L. opalescens</i> , <i>S. sagax</i> , <i>E. mordax</i> , <i>M. productus</i> , <i>P. notatus</i>) consumed by stranded and by-catch harbor porpoise in Monterey Bay.	87
10.	Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for bone collagen of harbor porpoises and harbor seals from central California.	88
11.	Monthly occurrence of harbor porpoise samples examined in this study.	89
12.	Distribution of ages of male harbor porpoise collected from central California between 1970 and 2000.	90
13.	Distribution of ages of female harbor porpoise collected from central California between 1970 and 2000.	90

14.	Length at age of male harbor porpoise from Central California from 1970-2000 using the Gompertz growth function.	91
15.	Length at age of female harbor porpoise from Central California from 1970-2000 using the Gompertz growth function	92
16.	Length at age of male harbor porpoise from Central California from 1970-2000 using the von Bertalanffy growth function.	93
17.	Length at age of female harbor porpoise from Central California from 1970-2000 using the von Bertalanffy growth function.	94
18.	Seasonal distribution and standard length of neonatal and fetal harbor porpoise from central California.	95
19.	Mean testes tubule diameter at length for immature, pubertal, and mature male harbor porpoise from central California.	96
20.	Age at sexual maturity of 25 male harbor porpoise from central California fit to a logistic model.	97
21.	Scatterplot of age and left ovarian corpora (CA) count for 20 female harbor porpoise from central California between 1970 and 2000.	98
22.	Scatterplot of body length and left ovarian corpora (CA) count for 20 female harbor porpoise from central California between 1970 and 2000.	99
23a.	Mean tubule diameter from 30 harbor porpoise collected in central California between 1970 and 2000 for each month.	100
23b.	Mean testes mass from 30 harbor porpoise collected in central California between 1970 and 2000 for each month.	101

List of Appendices

A1.	Collection data and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from 29 harbor porpoises collected in Monterey Bay between March 1997 and September 1999	102
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Chapter I

Examination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ Dietary Fractionation within Three Tissues of Harbor Porpoise (*Phocoena phocoena*) from Monterey Bay, California

INTRODUCTION

Cetacean ecology is often difficult to study because of the wide distribution of individuals, long migration routes, vastness of suitable habitat, and long durations of animal submergence. Although data regarding movement and habitat use has increased greatly by the use of satellite tags, temperature loggers, and time-depth recorders, determining feeding ecology remains difficult for many live marine mammals. Measurements of stable isotope ratios of nitrogen and carbon in marine mammal tissues have proven useful for determining several key aspects of feeding ecology. Most researchers have studied isotopic compositions of bone collagen or muscle tissue from dead animals (Schoeninger and DeNiro 1984, Ostrom et al. 1992). However, I describe the relationship between stable isotope ratios of nitrogen ($^{15}\text{N}/^{14}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$) in skin, muscle, and bone collagen of harbor porpoise, and examine their relationship to the isotopic composition of porpoise diet. Understanding the relationship between the isotopic composition of skin and prey will allow the interpretation of food habits based on skin biopsies of live animals.

Stable isotope ratios of nitrogen and carbon in animal tissues can reveal trophic relationships (DeNiro and Epstein 1978; Rau 1983), the relative importance of certain prey species to a consumer (Harrigan et al. 1989), location (onshore vs. offshore) of food consumption (Burton and Koch 1999), and they can explicate details of migration

patterns (Schell et al. 1989, Walker et al. 1999). The isotope ratios of nitrogen and carbon in animal tissues reflect those of the diet, but with a consistent deviation (DeNiro and Epstein 1978, 1981). This deviation results from the loss of the atomically lighter isotopes or the retention of the atomically heavier isotopes of these elements. As a result a consumer becomes more enriched with the heavier isotope with each increase in trophic level. This change in ratios, termed “fractionation”, is the result of several factors such as metabolism, physiological condition (e.g. pregnant or starving), or variations in diet (Cormie and Schwarz 1996).

An additional factor that affects stable isotope ratios within an animal's tissues is turnover time. Turnover time is the rate that a tissue incorporates the isotopes derived from the consumer's diet. This time is determined primarily by the metabolic activity of the tissue, and ranges from days (for blood) to years (for bone collagen; Tieszen et al. 1983, Hobson and Clark 1992a). The isotopic composition of blood, therefore, represents the isotopic composition of an animal's more recent diet whereas bone collagen represents an average of the animal's diet during a greater period of time.

Nitrogen isotope ratios are strongly affected by trophic level. The $^{15}\text{N}/^{14}\text{N}$ ratio of an animal's tissues increases consistently with each level of the food web (Fry 1988, Hobson and Clark 1992b, Gould et al. 1997). In the ocean, dissolved inorganic nitrogen (DIN) enters the base of the foodweb through the activities of nitrogen-fixing microorganisms, and atmospheric and riverine inputs. Although there may be geographic variations in the isotopic composition of DIN, once nitrogen enters the foodweb, the fractionation between trophic levels is relatively consistent. In general, nitrogen isotope

ratios of whole organisms increase by ~3‰ with each trophic step (DeNiro and Epstein 1978, Minigawa and Wada 1984, Michener and Schell 1994). This fractionation is the result of the balance of nitrogen isotopes absorbed from the diet versus those excreted as nitrogenous waste (Macko et al. 1987, Eckert et al. 1988). The ^{14}N is preferentially incorporated into urea, uric acid, and ammonia, which leaves the organism's tissues enriched in ^{15}N , after waste excretion (Minigawa and Wada 1984).

Although carbon isotope ratios in animal tissue also are affected by trophic level, these ratios are more sensitive to external factors such as sources of production or geographic variations. In a marine ecosystem, foraging locations can be identified by the distinctive carbon ratios of primary producers in different oceanographic regions (Rau et al. 1982). These differences may be attributed to: 1) variations in the amount of available nutrients, 2) dissolved CO_2 concentration (Rau et al. 1990), 3) variation in the composition of dissolved inorganic carbon, 4) morphology and growth rate of individual plankton species (Rau et al. 1990, Fry and Wainright 1991), and 5) effects of benthic macrophyte carbon input (Duggins et al. 1989).

The carbon ratios of an entire animal are reflective of the bulk diet (Michener and Schell 1994). Therefore, the carbon isotope ratios of primary producers can be identified throughout the foodweb. Within the foodweb there tends to be a slight and consistent enrichment (~1‰) in carbon isotope ratios between prey and the consumer tissues (Rau et al. 1983). This slight ^{13}C -enrichment is caused mainly by preferential loss of $^{12}\text{CO}_2$ through respiration. These ratios also may be affected by preferential loss of ^{12}C during digestion, or distinctive metabolic processes of individual tissue types (Tieszen et al.

1983, Michener and Schell 1994). Carbon fractionation between diet and bone collagen is ~5‰ (Koch et. al. 1994) and is ~1‰ for muscle tissue (DeNiro and Epstein 1978).

In most cases where researchers have examined the isotopic composition of cetaceans, they examined muscle or bone from dead animals, because these tissues were readily accessible when individuals stranded. Although the isotopic information from these tissues is useful for obtaining food habit information, exclusive use of tissues from dead animals limits our ability to draw comparisons with live animals. Skin samples can be obtained from live animals with biopsy darts (Winn et al. 1973, Patenaude and White 1995) or can be collected as sloughed skin (Ames et al. 1996). Isotopic fractionation within cetacean skin tissue, however, has not been widely studied. Here I report the results of a study of the nitrogen and carbon stable isotope ratios of bone collagen, muscle, and skin from 29 harbor porpoise (*Phocoena phocoena*). These values, along with the isotopic compositions of known prey items obtained from their stomachs, were used to estimate fractionation of nitrogen and carbon within harbor porpoise bone collagen, muscle, and skin.

METHODS

Beachcast animals were collected by personnel of the marine mammal stranding network from Moss Landing Marine Laboratories and Long Marine Laboratory, in Monterey and Santa Cruz counties, California between March 1997 and December 2000 (n = 14). Harbor porpoises incidentally taken in the local halibut fishery were collected by the fishery observer program of National Marine Fisheries Service and delivered to

the Southwest Fisheries Science Center for examination between April 1999 and December 1999 ($n = 15$). All animals underwent complete necropsies including the determination of sex, standard length ($\pm 0.5\text{cm}$), and mass ($\pm 1\text{g}$). A portion of rib bone, lateral epaxial muscle, and skin tissue was collected from each animal for isotopic analysis. Skin samples were removed from the animal's side, caudal to the dorsal fin (the target area for retrieving biopsy samples). All tissue samples were stored frozen prior to analysis. Fifty percent of all samples were analyzed a second time to estimate within-sample variability.

Nitrogen and carbon isotope ratios were determined for known prey items. Prey were identified based on stomach contents from 22 of the 29 porpoises used for tissue analysis, from 17 additional animals, and from previous harbor porpoise diet studies (Chapter 2, Sekiguchi 1987, Dorfman 1990, Byrd 2001). Fish muscle or cephalopod mantle obtained from porpoise stomachs or from fishing docks were analyzed, including market squid (*Loligo opalescens*), northern anchovy (*Engraulis mordax*), plainfin midshipman (*Porichthys notatus*), Pacific sardine (*Sardinops sagax*), rockfish species (*Sebastes* spp.), white croaker (*Genyonemus lineatus*), surfsmelt (*Hypomesus pretiosus*) and shiner perch (*Cymatogaster aggregata*). Isotopic composition of the bulk diet was estimated from values obtained from prey that comprised approximately 85 - 90% of porpoise diet (Chapter 2, Dorfman 1990). From this estimate of the isotopic composition of bulk diet, the fractionation of carbon and nitrogen isotope ratios was determined for each porpoise tissue examined.

Lipids were removed from all samples using a chloroform/methanol solution.

After extraction, samples were dried, placed in Sn foil boats, and analyzed using a Carlo-Erba elemental analyzer interfaced with a Micromass Optima gas source stable isotope ratio mass spectrometer. Analyses were performed in the Department of Earth and Ocean Sciences, University of California, Santa Cruz. The results are expressed as:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

R_{sample} and R_{standard} are the corresponding ratios of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ for the sample and the standard. The standards are atmospheric N_2 for nitrogen and Pee Dee Belemnite limestone for carbon.

Stable isotope ratios of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were compared for each tissue type (bone, muscle, and skin). The mean isotope ratios for each tissue were compared using a repeated-measures ANOVA (SYSTAT 9.0) and Ryan's Q post hoc tests. All measurements from animals collected in the same month were averaged for each tissue type and plotted in chronological order.

RESULTS

Mean isotope values for bone collagen, muscle, and skin tissue from 29 harbor porpoise and muscle or mantle tissue from their main prey items are presented in Table 1 (Table A1). Mean $\delta^{15}\text{N}$ values did not differ between the sexes for bone collagen ($P = 0.16$), muscle ($P = 0.42$), or skin ($P = 0.95$). Similarly, no differences occurred in the mean $\delta^{13}\text{C}$ values between the sexes for any tissue (bone: $P = 0.48$; muscle: $P = 0.76$; skin: $P = 0.25$), therefore, all animals were combined for analyses.

The $\delta^{15}\text{N}$ values were significantly different among the three tissues examined (Table 2). Porpoise mean bone collagen values were ^{15}N -enriched by 0.5‰ more than muscle $\delta^{15}\text{N}$ values. Mean skin $\delta^{15}\text{N}$ values were more ^{15}N -enriched than muscle values in every case but one, with a mean difference of 0.8‰. Muscle and skin isotope values of animals collected during each month indicated similar patterns of enrichment and depletion of ^{15}N through time (Fig. 1).

Mean $\delta^{13}\text{C}$ values differed among the three tissues (Table 2). Mean $\delta^{13}\text{C}$ values were similar for muscle and skin, but differed significantly for bone collagen, which was ^{13}C -enriched by 2.8‰ relative to the other tissues (Table 2).

The four primary porpoise prey items were *L. opalescens*, *E. mordax*, *P. notatus*, and *S. sagax*. These prey had mean $\delta^{15}\text{N}$ values ranging from -13.2‰ to 13.9‰, and $\delta^{13}\text{C}$ values between -17.7‰ and -16.7‰. There was no significant difference in the isotope compositions among any of these common prey species' for nitrogen or carbon (Nitrogen ANOVA: $F = 1.5$, $n = 26$, $P = 0.24$; Carbon ANOVA: $F = 1.27$, $n = 26$, $P = 0.31$; Fig. 2). All distributions were normal (one-sample K-S tests on residuals). The isotope values of porpoise bulk diet, based on the four common prey species, were $\delta^{15}\text{N} = 13.5‰$ and $\delta^{13}\text{C} = -17.2‰$.

Using the bulk diet values, I estimated nitrogen and carbon isotope fractionation for bone collagen, muscle, and skin tissue (Table 3). The least nitrogen fractionation occurred for muscle tissue, and the greatest carbon fractionation occurred for bone collagen.

DISCUSSION

Nitrogen and carbon isotopic compositions of animal tissues are effected by the organism's diet (DeNiro and Epstein 1978, 1981), habitat use (Burton and Koch 1999), physiology, and health (Cormie and Schwarcz 1996). Small cetaceans have unique diving physiology (Kooyman 1989, Noren and Williams 2000), increased metabolic rate (Worthy et al. 1987), diverse diet, and large ranges. To accurately interpret isotopic compositions, it is essential that fractionation factors be established that are specific to small cetacean tissues. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of harbor porpoise tissues reported here were consistent with values for these tissues from other upper trophic level marine carnivores (Schoeninger and DeNiro 1984, Abend and Smith 1997, Burton and Koch 1999, Kurle and Worthy 2001). Additionally, estimates of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation were comparable with those determined for other animals (Schoeninger and DeNiro 1984, Hobson and Clark 1992b, Hobson et al. 1996, Abend and Smith 1997).

Nitrogen isotope compositions of most animal tissues are between 2‰ and 4‰ greater than the isotopic composition of the animal's bulk diet (Schoeninger and DeNiro 1984, Hobson et al. 1996). Results from harbor porpoise indicated that isotopic composition of muscle tissue fell in this range relative to the diet but was consistently less enriched than the other tissues examined. Blood and individual components of blood (red blood cells and plasma) fractionate less than skin, muscle, or bone tissues (Hobson et al. 1996, Lesage et al. 2002). Blood was not specifically removed from muscle samples and might have contributed to less ^{15}N -enrichment within muscle tissue. However, after repeated rinsing and treatment for lipids, no blood was present upon visible inspection.

Each of the amino acids forming the main tissue proteins uniquely fractionates nitrogen isotopes (Tieszen and Fagre 1993). If fractionation in proteins forming muscle tissue is less than in those that form skin and bone collagen, then muscle tissue $\delta^{15}\text{N}$ values would be lower than the other tissues. Increased levels of myoglobin, specialized metabolic activities, or other unique features of muscle tissue of diving marine mammals (Kooyman 1989, Noren and Williams 2000), also could contribute to reduced levels of nitrogen fractionation within muscle tissue. Low $\delta^{15}\text{N}$ muscle values relative to other tissues are a common trend in marine mammals, however, the causes have not been extensively addressed (Abend and Smith 1997).

Differences in $\delta^{13}\text{C}$ values between bone collagen, and muscle and skin tissues, likely were due to the variation of metabolic routing of dietary components, turn-over times, and fractionation factors associated with each tissue (Hobson and Clark 1992a, Ambrose and Knorr 1993, Tieszen et al. 1993). Ratios of carbon isotopes within bone collagen are determined primarily by the protein component of the diet (Ambrose and Knorr 1993) and are integrated into the tissue over a period of one to several years (Hobson and Clark 1992a). The ^{13}C -enrichment of bone collagen relative to diet is mainly due to the high proportion of glycine amino acid residues. Glycine comprises approximately 33% of the amino acid residues of bone collagen (Lehninger et al. 1993), and has $\delta^{13}\text{C}$ values that are 8‰ enriched over diet (Hare et al. 1991). Additionally, there is fractionation associated with catabolism and synthesis of glutamate and aspartate, which are ^{13}C -enriched relative to diet by 6‰ and 3‰, respectively (Tieszen and Fagre 1993). Based on the dietary sources of carbon for each tissue type, tissue fractionation,

and the respective estimates of integration time, mean differences in $\delta^{13}\text{C}$ among porpoise bone collagen and the other tissues, were expected.

The four main porpoise prey species (*L. opalescens*, *E. mordax*, *P. notatus* and *S. sagax*) had similar isotopic compositions, which reflects the similarity or overlap of these species' habitats and trophic levels. *Loligo opalescens*, *E. mordax*, and *S. sagax* are pelagic, schooling organisms that move throughout the water column. All three occur within Monterey Bay throughout the year, however, each have seasonal increases in abundances (Recksiek and Frey 1978, R. Parrish pers. comm.). *Loligo opalescens* eats mainly euphausiids, copepods, small fish, small cephalopods, and megalops larvae (Karpov and Cailliet 1978). *Engraulis mordax* and *S. sagax* are mainly zooplankton feeders (Koslow 1981). *Porichthys notatus* is a demersal fish that exhibits diel migration, which spends daylight hours buried in sediments and feeds at night within the water column on the deep scattering layer (Echmeyer et al. 1993). Diet of *Porichthys notatus* varies by age, as juveniles they are zooplanktivores and as adults they supplement their diets with small crustaceans and fishes (Garrison and Miller 1982, Love 1996, Cailliet et al. 2000). Because all these species fed mainly on a variety of zooplankton species and supplemented their diets with crustaceans or small fish, they all foraged at similar trophic levels, which was reflected in their isotopic compositions.

Estimates of fractionation based on the isotopic composition of harbor porpoise bone collagen ($\delta^{15}\text{N}$: 2.2‰ and $\delta^{13}\text{C}$: 3.8‰) are within the ranges reported by other researchers (DeNiro and Epstein 1978, Schoeninger and DeNiro 1984). Nitrogen fractionation within all tissues ranges from 2 to 5‰ (DeNiro and Epstein 1981,

Minagawa and Wada 1984, Schoeninger and DeNiro 1984). Carbon fractionation within bone collagen is approximately 3 to 6‰ (Vogel 1978, Schoeninger and DeNiro 1984, Ambrose and Knorr 1993). The precise relationship between nitrogen and carbon isotopic composition of diet and bone collagen has been studied in laboratory/captive settings using gerbils, mice, ungulates, and birds (DeNiro and Epstein 1981, Hobson and Clark 1992b, Hobson et al. 1996), but has not been reported for cetaceans. It is extremely difficult to precisely measure fractionation within the bone collagen of large mammals (Gannes et al. 1997) because it may take years for a particular diet to be reflected in the tissue, and sampling generally requires sacrificing an animal.

In marine mammal muscle tissue, fractionation of nitrogen isotopes was less than 3‰, and was consistently less in muscle than in other tissues (Hobson et al. 1996, Abend and Smith 1997). I also observed this trend in the fractionation of nitrogen isotopes for porpoise muscle tissue. Nitrogen fractionation was 1.7‰, and was less than the estimates derived from bone collagen (2.2‰) and skin (2.5‰) values from the same individuals. Hobson et al. (1996) found that $\delta^{15}\text{N}$ values in muscle tissue from captive seals ($n = 2$) were 2.4‰ greater than the $\delta^{15}\text{N}$ values of their diet. Abend and Smith (1997) measured the nitrogen isotope compositions of muscle tissue of long-finned pilot whales (*Globicephala melas*), and of their main prey species including long-finned squid (*Loligo pealei*) and Atlantic mackerel (*Scomber scombrus*). They estimated nitrogen isotopic fractionation within the pilot whale muscle tissue was 1.0‰. Although the $\delta^{15}\text{N}$ values reported for pilot whale muscle tissue was within the range expected for pelagic marine mammals, their estimate of $\delta^{15}\text{N}$ fractionation was less than values reported for other

animal groups (DeNiro and Epstein 1981).

My estimates of fractionation of carbon isotopes for muscle tissue (1‰), was similar to values reported by previous researchers (DeNiro and Epstein 1978, Tieszen et al. 1983, Mitzutani et al. 1991, Hobson and Clark 1992a, Hobson et al 1996). Tieszen and others (1983) showed that carbon isotopic values of gerbil (*Meriones unguiculatus*) muscle were ^{13}C -enriched by approximately 1‰ more than their diet. Hobson and Clark (1992b) fed chickens (*Gallus gallus*), quails (*Coturnix japonica*), and gulls (*Larus delawarensis*) consistent diets, and determined that the mean ($\pm 1\text{SD}$) $\delta^{13}\text{C}$ values of their muscle were $0.3\text{‰} \pm 0.3$, $1.1\text{‰} \pm 0.5$, and $0.3\text{‰} \pm 0.4$ greater than their diets, respectively. Carbon isotope compositions of three seal species raised in captivity (*Pagophilus groenlandicus*, *Phoca vitulina*, and *Phoca hispida*) were greater than their diet by a mean of +1.3‰ (Hobson et al. 1996). All of these examples support the concept that muscle carbon isotope ratios reflect the isotopic composition of the bulk diet, and fractionation of carbon within muscle tissue is approximately 1.0‰ (DeNiro and Epstein 1978, Tieszen et al. 1983, Mitzutani et al. 1991, Hobson and Clark 1992a, Hobson et al 1996).

Estimates of nitrogen isotope fractionation for skin tissue of cetaceans are limited (Abend and Smith 1997). Nitrogen fractionation for skin tissue of harbor porpoise (2.5‰) was slightly greater than the value reported for pilot whales (1.7‰; Abend and Smith 1997). The $\delta^{15}\text{N}$ fractionation estimated in my research was based on the isotopic values of prey obtained directly from stomachs of harbor porpoises, and supported the conclusion that the accepted nitrogen fractionation estimate of 3‰ (DeNiro and Epstein

1981, Schoeninger and DeNiro 1984, Minagawa and Wada 1984) may be too high for correctly interpreting isotope values of cetacean skin tissue.

Carbon isotope fractionation for cetacean skin tissue, have not been reported previously, however, mean $\delta^{13}\text{C}$ values have been compared between cetacean muscle and skin tissues (Todd et al. 1997). Results reported by Todd and others (1997) for humpback whale (*Megaptera novaeangliae*) indicated no differences between $\delta^{15}\text{C}$ values of muscle and skin tissues. If muscle and skin $\delta^{13}\text{C}$ values are similar, and the two tissues reflect the same bulk diet (see below), then the carbon isotopes within them must undergo the same degree of fractionation. Additionally, carbon isotopic fractionation of 1‰ is common in animal muscle tissues (Tieszen et al. 1983, Hobson et al. 1996), therefore, a 1‰ fractionation within porpoise muscle and skin is not unexpected.

Tissue turnover rates have been examined in small and/or captive mammals but no estimates have been reported for cetaceans. Values of $\delta^{15}\text{N}$ in porpoise skin and muscle through time indicated that muscle and skin tissues may have had similar turnover rates (Fig.1). Averaged monthly skin and muscle $\delta^{15}\text{N}$ values were consistently offset from each other, although the actual values varied considerably. This suggested that $\delta^{15}\text{N}$ values for these tissues reflected a similarity in the processes through which dietary compounds were incorporated into these two tissues.

Tieszen et al. (1983) examined carbon turnover rates in muscle of gerbils and estimated a half-life of 27.6 days. Hobson and Clark (1992a) determined that carbon had a half-life of 12.4 days in quail muscle tissue. Kurle and Worthy (2001) estimated turnover rates of skin and muscle of northern fur seals (*Callorhinus ursinus*), by coupling

carbon isotopic turnover rates determined for gerbils tissues (Tieszen et al. 1983) and rates of protein synthesis determined from dog (*Canis familiaris*) tissues (Biolo et al. 1994 in Welle 1999). Kurle and Worthy (2001) determined an average half-life of residency for carbon of 12-13 days in muscle tissue and 7-8 days in skin. Additional studies of rat (*Rattus norvegicus*) and lamb (Family Bovidae) tissues also have shown that muscle and skin had very similar rates of protein synthesis (Attaix et al. 1988, Cherel et al. 1991, Mays et al. 1991, summarized in Welle 1999). Based on these comparisons, muscle and skin from harbor porpoise likely incorporated dietary components into skin and muscle on the order of days to weeks, thus, represented the animal's diet over a relatively short time period.

Although skin nitrogen and carbon isotope values and some estimates of fractionation have been reported for cetaceans, these studies included small sample sizes, and interpretation was based on fractionation estimates derived from feeding experiments on small, terrestrial mammals. In this study, I examined these three separate tissues (skin, muscle, bone collagen) because bone collagen and muscle tissues are relatively well studied, and changes in carbon and nitrogen ratios within them are fairly well understood (DeNiro and Epstein 1981, Tieszen et al. 1983, Schoeninger and DeNiro 1984, Ambrose and Knorr 1993). Skin, although less often studied, may be the most useful in the application of stable isotope analyses to the study of live marine mammals. The use of stable isotope ratios of carbon and nitrogen can effectively address large scale, long-term questions related to where an animal feeds and its trophic position in the ecosystem (Rau et al. 1983). Elucidating the relationship between diet and isotope

compositions of skin tissue provides the groundwork necessary for gathering information on habitat use and trophic level from live marine mammals through the use of non-lethal biopsy sampling. This research is an important step in establishing isotopic fractionation estimates for skin tissue obtained from small cetaceans.

Chapter 2

Food Habits of Stranded and By-Catch Harbor Porpoise (*Phocoena phocoena*) from Monterey Bay, California, 1997 – 2000: Analysis of Stomach Contents and Stable Isotope Ratios

INTRODUCTION

Harbor porpoise (*Phocoena phocoena*) are small cetaceans that inhabit temperate and sub-polar coastal environments in the northern hemisphere (Gaskin 1984). The abundance and distribution of prey is likely the most influential factor determining porpoise use of coastal waters (Wursig and Wursig 1980, Croll et al. 1998). Their use of nearshore habitat, however, results in incidental mortalities via fishery interactions, throughout most of their range (Jefferson and Curry 1994, Forney et al. In press). Understanding the distribution of food resources used by this species, therefore, is essential for accurately assessing the potential impacts that conflicts with inshore fisheries represent (Murie 1987).

One method used to determine prey resources is the characterization of stomach contents (Sekiguchi 1987, Dorfman 1990, Byrd 2001). Stomach contents can reveal an individual's most recently ingested food items, and the examination of fish otoliths and cephalopod beaks can be used to identify, enumerate, and determined the frequency, size, and relative importance of prey species in a consumer's diet. This method is, however, limited to recent time scales and requires the availability and use of dead animals. This method also is biased by factors such as differential digestion of prey items, secondary ingestion, non-random or opportunistic sampling, and small sample sizes (Prime and Hammond 1987, Ostrom et al. 1992, Antonelis et al. 1987).

Information from dead animals may be subject to additional biases related to the source and timing of animal collection. Cetacean carcasses usually are available as beach strandings or as by-catch from fisheries. Samples from strandings are subject to differential post-mortem food degradation, and the time between death and sample recovery is usually unknown. Unhealthy individuals that strand might have been physiologically stressed or consuming easily caught prey, which may not represent the diet of normal, healthy animals (Pierce and Boyle 1991). Further problems include opportunistic sampling, and limited sample sizes. Specimens collected as by-catch in fisheries may be limited because certain age classes or genders are more prone to net entanglement (Hohn and Brownell 1990). The results also may be affected by limitations of observer programs, and subjected to sampling biases relating to fishing effort influenced by market demand, weather, and target species abundance.

Although both sample sources have biases, information from animals collected through fishery interactions may be more suitable for accurately determining the diet of a healthy population. An animal killed during fishery interactions likely was healthy and feeding normally at the time of death, and the specimens were immediately collected and preserved. Alternatively, a stranded animal may have come ashore for numerous reasons, which could include disorientation of healthy animals, human impacts, or weakened condition of animals in poor health (Walsh et al. 1991). Because harbor porpoise are coastal, non-migratory animals, stranding due to disorientation of healthy animals is unlikely. Regardless of these biases, the analysis of stomach contents of fishery by-catch

and stranded animals can only reveal the animal's diet during the course of several meals, and may not reflect longer term foraging patterns (Stroud 1981).

Fecal analysis (Prime and Hammond 1987), identification of fatty acids from prey (Iverson et al. 1997, Kirsch et al. 1998), and stable isotope compositions (Ostrom et al. 1992) can provide additional insight into the diets of animals. These methods may ameliorate some of the disadvantages associated with stomach content analysis, and can be applied to live animals.

Porpoise fecal samples are difficult to collect and generally cannot be associated with specific individuals. Identification of prey fatty acids in consumer tissue can reveal specific prey species, but large sample sizes are difficult to obtain and the analytical techniques are expensive and labor intensive. Analysis of stable carbon and nitrogen isotope compositions in animal tissues can reveal the relative importance of certain items within the diet (Harrigan et al. 1989) and can provide detailed information regarding an animal's trophic level over a range of timescales (DeNiro and Epstein 1978; Tieszen et al. 1983, Rau 1983, Todd et al. 1997). Analyses of stable isotopes also can reflect habitat use (Schell et al. 1989, Hobson et al. 1996, Burton et al. 2001) and an animal's physiological condition (e.g. starving, dehydrated; Hobson and Clark 1992b, Cornie and Schwarcz 1996). Sample preparation and isotopic analysis is relatively simple and inexpensive, can be applied to a variety of tissue types, and allows for the study of large sample sizes.

The interpretation of carbon and nitrogen stable isotopes is based on the observation that these elements are incorporated into a consumer's tissue from the diet,

and the isotopes of these elements are differentially discriminated against through biochemical pathways (Wada and Hattori 1976, DeNiro and Epstein 1981, Hobson et al. 1996). The time that a tissue integrates the different isotopes from the organism's diet, 'turnover time', is determined primarily by the metabolic activity of the tissue, which may range from days to years (Tieszen et al. 1983, Hobson and Clark 1992a). For example, bone collagen turns over at a much slower rate than muscle or skin tissue (Hobson and Clark 1992a). These differences in fractionation and turnover rates allows for the interpretation of diet from the isotopic composition of different tissues, over a range of time scales (Tieszen et al. 1983, Hobson and Clark 1992a).

In the marine setting, carbon isotope ratios in animal tissues also can provide information about where an animal feeds based on the unique isotopic ratios of primary producers at the base of a food web (macrophytes vs. phytoplankton) or on variations related to different oceanographic regions (Duggins et al. 1989, Rau et al. 1982). Throughout the food web, the carbon isotopic composition of the primary producer is conserved, however, consumer tissues are slightly enriched in the heavier isotope (by $\leq 1\text{‰}$ in most tissues, 3 - 5‰ in bone collagen; DeNiro and Epstein 1978, Rau et al. 1983, Tieszen et al. 1983). Carbon fractionation between diet and harbor porpoise bone collagen is 3.9‰, and is 1‰ for muscle and skin tissues (Chapter 1).

Nitrogen isotopic compositions are strongly affected by trophic level (Rau 1983, Fry 1988, Gould et al. 1997). The $\delta^{15}\text{N}$ values of whole organisms increase by $\sim 3\text{‰}$ with each trophic step (DeNiro and Epstein 1981, Minigawa and Wada 1984, Michener and Schell 1994). Nitrogen fractionation occurs through the balance of nitrogen isotopes

retained from the diet and those excreted as waste (Macko et al. 1987, Eckert et al. 1988). Nitrogen fractionation between diet and harbor porpoise bone collagen is 2.2‰, for muscle it is 1.9‰, and it is 2.5‰ for skin tissue (Chapter 1).

Animals suffering from nutritional stresses (i.e. starvation, dehydration) may have elevated $\delta^{15}\text{N}$ values in response to nitrogen recycling of the heavier isotope which is used during amino acid synthesis (Hobson and Clark 1992b, Hobson et al. 1993). When an animal is starving, it essentially begins to feed off of its own fat and protein stores until other nourishment is available (Castellini and Rea 1992), which causes a rise in $\delta^{15}\text{N}$ values over those values expected for a healthy animal.

Due to the predictable patterns exhibited by stable isotope ratios of carbon (identifying feeding areas), and nitrogen (trophic level, nutritional status), isotope compositions of harbor porpoise tissue can supplement the food habit information obtained from stomach contents, observations of habitat use, and other data, over multiple time scales.

The objectives of this research were to investigate food habits by characterizing recent diet through stomach content analysis and prey array indices, and longer-term diet, through measurements of stable carbon and nitrogen isotope ratios from a variety of tissues. These data, collected from stranded and net-caught animals, also were compared to evaluate the accuracy of dietary information derived from stranded animals and those obtained as fishery by-catch.

METHODS

Stranded animals were collected from beaches in Monterey and Santa Cruz counties, California, by California Marine Mammal Stranding Network personnel from Moss Landing Marine Laboratories and Long Marine Laboratory at the University of California Santa Cruz. All stranded animals were collected between March 1997 and 31 December 2000. Porpoises caught incidentally in fisheries were collected by National Marine Fisheries Service observers between April 1999 and September 2000, and were subsequently examined at the Southwest Fisheries Science Center. Complete necropsies were performed on all animals. Sex, standard length (± 0.5 cm), and total mass (± 1 g) were determined. Skin, muscle, and bone tissue samples also were collected. Stomachs were removed intact and contents of all three stomach chambers from each individual were examined. Stomachs were weighed full and then the contents were rinsed through 0.5 mm sieves. Fish otoliths, squid beaks, and other skeletal prey remains were collected from the sieves, examined under a dissecting microscope, and identified to the lowest possible taxon. Taxonomic identifications were made using the comparative otolith reference collection at Moss Landing Marine Laboratories and available literature (Clarke 1986, Harvey et al. 2001). Both right and left otoliths were separated and counted, and the greatest count of either side was used as the minimum number of individuals of that species. Fish otoliths and cephalopod beaks were measured using Scion Imaging System software (1998). To compensate for digestive degradation, correction factors were applied to otolith length measurements when appropriate (Harvey 1989). For species not reported in the literature, an average correction factor (27.5%) was applied (Harvey

1989). For broken otoliths, the average length of all whole otoliths of that species was used. Regression equations and ratios of standard length to otolith length were used to convert otolith and beak measurements to prey length and mass (Wolfe 1982, Harvey et al. 2001). For species for which linear regressions have not been calculated, a general equation was applied (Harvey 1989) or an estimate of mass was obtained from other studies (Orr unpublished data, Watanabe et al. 1999). All remaining stomach contents were dehydrated in petri-dishes and examined for missed remaining hard parts. Samples were divided by the method of collection (stranding vs. fishery by-catch), and statistical analyses were performed separately for each group. Several animals were collected from beaches but possessed evidence of possible fishery interactions (e.g. presence of net marks). These animals were placed in the 'stranded animal' category because actual cause of death could not be confirmed, and several of the biases associated with beach-cast animals (e.g. post mortem degradation, unknown time between death and animal recovery) also applied for these animals.

Cumulative prey species curves were formulated by randomizing samples five times and plotting the cumulative mean number of prey species for stranded and fishery by-catch animals, separately. Formation of a curve plateau was used to determine whether sufficient numbers of samples were collected to represent the spectrum of prey in harbor porpoise diet (Ferry and Cailliet 1996). For each prey type, the percentage abundance within a sample (%N), percentage mass (%M), and percentage frequency of occurrence (%FO) were calculated. To determine if sample sizes were adequate for describing prey species in porpoise diet, cumulative mean %N and %M were plotted

against the number of sampled stomachs (Byrd 2001). Percent mass and %N for each animal were sampled one to 14 times with 1000 iterations using Resampling Statistics 5.0.2 software. The significance of each prey item was determined using the Index of Relative Importance (IRI; Pinkas et al. 1971; Hyslop 1980) as follows:

$$\text{Mean IRI} = (\text{mean \%number} + \text{mean \%mass}) * \% \text{ frequency of occurrence}$$

Spearman's rank correlation was used to statistically compare diets between the two sources (Zar 1996). The percent of diet overlap between sources was determined using the percent similarities index (PSI, Krebs 1999) as follows:

$$\text{PSI} = \sum \text{minimum} (P_{si}, P_{bi})$$

The PSI is the sum of the minimum relative abundance of each species i in the diet of either the stranded group (s) or by-catch group (b).

Kendall's coefficient of correlation (Zar 1996) was used to compare the diets of stranded animals in this study with animals from previous studies (Sekiguchi 1987, Dorfman 1990, Byrd 2001). Because the other studies did not use animals obtained directly from fishery interactions, no comparisons were made of harbor porpoise caught as by-catch.

Several indices were used to describe aspects of the prey consumed by harbor porpoise and to characterize differences between stranded and net caught animals. These prey array indices included:

$$1) \text{ Shannon-Weiner diversity index: } H' = \left(\sum p_i \ln p_i \right),$$

$$2) \text{ Species richness: } S = \text{total \# of prey species present in sample,}$$

$$3) \text{ Prey evenness: } J = H'/H'_{\max}, \text{ where } H'_{\max} = \ln S, \text{ and}$$

4) Simpson's index (prey dominance): $D = \sum p_i^2$;

where: p_i = proportion of total sample belonging to i th species (Krebs 1999).

During necropsies of 29 individuals, a portion of rib bone, lateral epaxial muscle, and skin tissue were collected from each animal and carbon and nitrogen isotopic compositions were measured (See Chapter 1 for methods of isotopic analysis). Of those animals, all individuals one year or less in age and animals with standard lengths less than 125.0 cm were removed from comparisons because these young animals may have ingested ^{15}N -enriched milk from their mothers, which would may have elevated nitrogen isotope values (Hobson et al. 1997). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of all animals that met the age and length criteria (12 by-caught, 9 stranded) were compared between collection methods for each tissue type.

RESULTS

The stomach contents of 36 harbor porpoises were examined. Of these 36 animals, 19 were obtained from fishery interactions and 17 were collected after they stranded on beaches in Monterey and Santa Cruz counties (Fig 3). From 1997 to 2000, the occurrence of strandings was fairly evenly distributed throughout each year (Fig. 4). Samples from fisheries were collected between April 1999 and May 2000. Eight of 19 specimens were recovered during the month of September (Fig. 4). Stomachs from both groups contained 580 fish otoliths and 1,256 cephalopod beaks. Eighteen fish species and two cephalopod species were identified, representing 18 families. All cephalopod beaks were identified to species, 94% (543) of all otoliths were identified to species, 3%

(18) were identifiable to family, and 3% (19) of the otoliths were not identifiable. Of the 19 fishery by-catch animals, only one stomach did not contain hard parts. Stomachs from three of the 17 stranded animals did not contain otoliths or beaks or contained parts that could not be identified. These specimens were excluded from the stomach content analyses.

The stranded animals consisted of 8 males and 9 females, between one and 12 years old, and standard lengths from 110 cm to 171 cm. The fishery by-catch animals included 13 males and 6 females, from one year to 11 years old, with standard lengths of 118.0 cm to 155.5 cm. Three animals from each group were not aged and two individuals from the fishery by-catch group were not measured because they were missing their flukes.

Cumulative species curves of stomach contents from both groups reached asymptotes and error bars were even (Figs. 5 and 6). Sample sizes were adequate for characterizing the importance of the main prey items as indicated by the consistency of error values in all plots of cumulative mean %N and %M for the three highest ranked prey species in both collection categories (Figs. 7 and 8).

Plots of cumulative mean %M and %N of *Loligo opalescens* and *Engraulis mordax* indicated that less than 10 samples were sufficient for characterizing their importance in porpoise diet for both stranded and fishery by-catch porpoises (Figs. 7 and 8). *Merluccius productus*, the third highest ranked species in the fishery by-catch sample, required less than 13 animals to accurately characterize the %M and %N in harbor porpoise diet. Seven samples sufficed for characterizing the %M and %N of *Porichthys*

notatus in the diet of stranded animals.

Diets were significantly different between animals obtained from the two sources, based on mean IRI values (Spearman's rank correlation, $n = 19$, $P < 0.05$). Diet of stranded animals was composed of 73% *Loligo opalescens* and 3% *Engraulis mordax*, whereas, diet of net-caught harbor porpoise consisted of 32% *L. opalescens* and 49% *E. mordax* (Table 4 and 5). The mean size of main prey items, however, was similar between the groups (Fig. 9). The PSI indicated a 44.2% diet overlap between the two sources. Sample sizes were too small to make dietary comparisons among years or seasons. Harbor porpoise diet identified by Sekiguchi (1987), Dorfman (1990), and Byrd (2001) was similar to the diet determined for stranded animals in this study (Table 6; Kendall's coefficient of concordance: $\chi_{calc} = 44.795$, $n = 2$, $P = 0.05$).

Although prey IRI values were different for fishery by-catch and stranded porpoise, prey indices indicated no significant differences in species richness, diversity, evenness, or dominance (Table 7). Distributions of Species Richness (S), Shannon-Weiner diversity Index (H'), Species Evenness (J) and Dominance (D) data measuring characteristics of each sample were normally distributed for all data sets (Kolmogorov-Smirnov tests, $P > 0.05$).

Carbon and nitrogen isotopic compositions of bone collagen, muscle, and skin were compared between fishery and stranded animals that were older than one year or greater than 125.0 cm in standard length (Table 8). No significant differences were detected in carbon isotope values between stranded and fishery by-catch specimens for any tissues (Table 9a). Nitrogen isotope compositions of skin tissue differed between net

caught (15.5‰) and stranded animals (16.1‰). Muscle and bone collagen $\delta^{15}\text{N}$ values were similar between the two groups (Table 9b).

DISCUSSION

Ninety-seven percent of harbor porpoise diet was composed of 5 prey species including *L. opalescens*, *M. productus*, *E. mordax*, *S. sagax*, and *P. notatus*. Four of these five species (*L. opalescens*, *M. productus*, *E. mordax*, *S. sagax*) are pelagic schooling fish/cephalopods that occur in nearshore waters either year round or seasonally. The fifth (*P. notatus*) is a nearshore neritic species that exhibits diel migration (Love 1996, Cailliet et al. 2000).

Market squid (*L. opalescens*) are an extremely important source of food, comprising about 64% of the porpoise diet. Squid were found in porpoise stomachs during every month that animals were collected, which included all but December and January. This is likely due to a combination of squid having a high caloric content (85 kcal/100 g), therefore providing a valuable source of energy (Dorfman 1990), and their tendency to form large, predictable patches. In nearshore waters, spawning squid form aggregations that can span up to 300m in diameter and 40m thick (Karpov and Cailliet 1978, Recksiek and Frey 1978). In Monterey Bay, spawning occurs from April through November with peaks in June and sometimes again in November (Recksiek and Fry 1978). The size and regularity of these occurrences make squid an important predictable food source for harbor porpoise in Monterey Bay (Recksiek and Frey 1978).

Northern anchovy (*E. mordax*) also are a major porpoise prey item and have a high caloric content (152 kcal/100g; Dorfman 1990). They are present year-round in Monterey Bay but exhibit small-scale seasonal variations. The greatest abundance occurs in fall (September – November) during the relaxation of upwelling and the onset of warmer, more saline oceanic conditions. The warmer water results in a higher concentration of fish in water farther from shore, whereas during other times of the year they are found adjacent to the beach (Richard Parrish pers. comm.).

Diets of porpoises obtained as fishery by-catch and as stranded animals had similar species compositions, but had differences in the percentages that each prey species contributed to the total diet. These differences may be attributed to differences in preferences for certain prey species, which may be based on protein or caloric content, access to prey, artifacts related to timing of sample collection, or to post mortem biases associated with each prey species (Harvey 1989, Dorfman 1990). Obvious dietary differences occurred between mean IRI values of squid and anchovy for netted and stranded animals. Data from stranded animals indicated a reliance on market squid (%IRI: 73.4), whereas fishery by-catch animals consumed more anchovy (%IRI: 53.8). Both squid and anchovy have high caloric contents providing the energy necessary for small, metabolically active cetaceans such as porpoises (Worthy et al. 1987). Squid aggregations are dense, predictable, and long lasting (Karpov and Cailliet 1978, Recksiek and Fry 1978). This may make squid easy to catch and, a more readily available prey item for unhealthy animals. In contrast, anchovies form schools that may be large, but these are also ephemeral in space and time. The majority of by-catch animals were

obtained in 1999, therefore, if anchovy abundance was abnormally high during that period, by-catch results may have been influenced. Recent anchovy biomass in Monterey Bay has been stable (Starr et al. In prep). Using landing data as a proxy for biomass (PacFIN), there is no indication that there was a greater abundance of anchovy in Monterey Bay during 1999 relative to the other years of this study. However, these data are based on pounds landed per year and not standardized for fishing effort. Cephalopod beaks are extremely rigid and much less susceptible to degradation during digestion than fish otoliths (Bigg and Fawcett 1985, Tollit et al. 1997). Stranded harbor porpoise may float in the water and remain on beaches for many days before recovery, during which time only prey hard structures remain in the stomach. Fish otoliths are composed of relatively fragile calcium carbonate, which may continue to be digested, eroded, and crushed before the carcass is collected. Therefore, fish may be under represented, and cephalopods may be over represented, in beach cast specimens.

Contrary to the differences that emerged through Spearman rank correlations of IRI values, no differences were detected between collection sources for estimates developed using the prey array indices. The prey indices, however, used only the percentage number of each prey species in a sample, and did not take into account the frequency of species in diet or the mass of individual prey items as did the IRI values.

There are several biases associated with stomach content analysis regardless of collection method. To determine prey, I only used fish otoliths and cephalopod beaks. Invertebrates, some cartilaginous fish species, and juvenile fish or cephalopods may not be apparent in stomach contents because they possess no or few hard structures or they

are digested due to their small size and fragility (Everitt and Gearin 1981, Harvey 1989). Digestive passage rates may differ for different species (Bigg and Fawcett 1985, Harvey 1989, Tollit et al. 1997). In pinnipeds and some cetaceans, cephalopod beaks are amassed in the stomach rugae (Bigg and Fawcett 1985, Gales and Pemberton 1992). If beaks gather in the stomach rugae of porpoise, then estimates of the importance of cephalopods could be inflated. Additionally, there is natural variation in fish length/weight to otolith size relationships, making it difficult to achieve accurate estimates of fish mass and species importance. Degradation of hard parts varies by size of otolith and by species (Harvey 1989, Tollit et al. 1997). Estimates of the effect of partial digestion on otolith length/ mass relationships have been calculated for some species (Harvey 1989, Harvey et al. 2001). For the species that have not been examined for digestion effects, a general equation was applied, that may misrepresent the length and mass of the prey and led to erroneous IRI values (Harvey 1989).

Stomach contents from both groups contained species that were largely consistent with those found in previous diet studies of porpoises from Monterey Bay. However, eight new taxa were present in stomachs that have not been previously reported as part of the diet of local harbor porpoises (Table 6). Of those eight new taxa, five were present only in net caught specimens, one (the mesopelagic lantern fish, *Diaphus theta*) was only present in the stranded animal group and in only one individual. Two species that were found in both stranded and net caught animals, but were not previously seen in porpoise diet, were *S. sagax* and *C. sordidus* (Sekiguchi 1987, Dorfman 1990, Byrd 2001). One of these, *S. sagax*, was identified as an important prey item in this study. Reasons for the

emergence of *S. sagax* as an important prey species may be related to the recent resurgence of the species following their decline in the 1950s (CalCOFI 1999). During years of cool water, *S. sagax* spawn off of Mexico and in warmer years they spawn off Point Conception, California, and then migrate north through Monterey Bay (Richard Parrish pers. comm., Checkley et al. 2000). *Sardinops sagax* are present in Monterey Bay throughout the year, but schools are mainly composed of young animals (2-3 yrs old). Locally, they increase in abundance during August through November. Sardine oil content is the greatest during their southern fall migration, indicating they may be of greater caloric value and, thus, more attractive to predators during this period (Richard Parrish pers. comm.). Sardines were present in porpoise diet, however, during spring, summer, and fall with the greatest masses occurring in June.

Part of this study was conducted during an extreme El Niño Southern Oscillation (ENSO) event, which may have influenced the abundances of prey species. These events are characterized by low primary productivity and a rise in surface water temperatures (Lalli and Parsons 1997), resulting in large-scale anomalies in oceanographic conditions, thus affecting normal distribution patterns of fish and cephalopod species (CalCOFI 1999). During that time, the ENSO event may have caused greater concentrations of certain species (e.g. sardine), and decreased levels of others (e.g. squid), affecting the diet of porpoises examined in this study. Furthermore, the El Niño event was followed by a period of cold water that may have further affected prey species abundance and distributions (CalCOFI 1999). Additionally, these conditions may have effects on species abundance and distribution that were not apparent until after a considerable lag

time. The effects of El Niño and/or season, on diet, were not examined because of limited sample sizes, however, each of the main prey species consumed exhibits seasonal behaviors (Hayward et al. 1999). The disproportionate numbers of samples during years and seasons, combined with seasonal prey changes may have influenced the results of this study.

Statistical comparisons of previous dietary data reported for harbor porpoise from Monterey Bay, and the results of this study support the conclusion that collection method may influence results obtained from stomach contents. Previous studies of harbor porpoise diets from the central California coastal region, were all based on stomach contents of stranded harbor porpoise (Seckiguchi 1985, Dorfman 1990, and Byrd 2001), and the results were similar to the results reported here for stranded porpoise.

Few researchers have reported comparisons of diets of beach-cast and fishery by-catch individuals of the same species. Studies of local common dolphin (*Delphinus delphis* and *Delphinus capensis*) diet through samples recovered from strandings and through fishery by-catch, have yielded mixed results (Osnes-Erie 1999). Osnes-Erie (1999) concluded that the diet of common dolphin (*D. delphis*) determined from stranded animals was different from incidentally taken animals. Additionally, animals that inhabited different geographic locations (*D. delphis* and *D. capensis*) had similar diets. Diet of stranded and by-catch *D. capensis* were similar regarding their important prey species (Osnes-Erie 1999).

Specimens recovered from fishery observer programs are subject to fewer biases regarding prey determination than stranded animals. The financial responsibilities,

logistical challenges, and public relation issues result in limited implementation of observer programs and fewer specimens from fisheries. The research value of such programs in any fishery known to incidentally take cetaceans would greatly improve our ability to develop effective and realistic management measures for these animals.

All tissue carbon compositions were consistent with those expected for middle latitude ($\sim 36^\circ$ N), non-migratory, nearshore inhabitants. Harbor porpoise in Monterey Bay have relatively isolated ranges (Hanson et al. 1999, Chivers et al. In press, Chivers pers. comm.), thus, their carbon ratios should not be influenced by latitudinal isotopic gradients. Similarities between carbon isotopic compositions of animals collected from beaches and as by-catch, indicated that the two groups used the same oceanographic region on both short and long time scales.

Carbon isotope values indicated that stranded and by-catch animals were exploiting resources from the same habitats (i.e. nearshore Monterey Bay) and are not shifting their foraging areas seasonally. Bone collagen incorporates diet over long time periods (on the order of years; Hobson et al. 1992a), whereas, muscle and skin tissue represents diet over a shorter duration and more recent time period (weeks to months).

The main prey species consumed by porpoises feed at similar trophic levels therefore, fine scale diet evaluations could not be made via nitrogen stable isotope ratios. *Loligo opalescens*, *E. mordax*, *P. notatus*, and *S. sagax* comprised 85% of porpoise diet but were isotopically indistinct (Chapter 1). Comparisons of isotope values of harbor porpoise tissues from fishery by-catch and stranded individuals yielded data that were useful for interpreting the feeding ecology of each group.

Skin and muscle nitrogen isotopic ratios were reflective of the more recent diet, and differences between stranded and by-catch animals reflect possible malnutrition prior to stranding. Mean nitrogen values of muscle from stranded animals had a 0.5 ‰ enrichment over values for animals killed as by-catch. Though values were not significantly different, the results may be biologically meaningful. Mean nitrogen isotopic values of skin from stranded animals indicated a significant enrichment (0.6 ‰) compared with net caught animals. The statistical insignificance in the muscle comparison could be due to the variability in muscle values (muscle SD: strand = 0.4, net = 0.9; skin SD: strand = 0.4, net = 0.7).

Muscle and skin tissues are very metabolically active, and isotopic values may have been affected by the poor health of stranded individuals. The body's natural response to nutritional stress is to recycle nitrogen for the synthesis of amino acids (Castellini and Rea 1992, Adams and Costa 1993). Because the pool of recycled nitrogen is enriched in ^{15}N (Jackson 1998), the tissues comprised of amino acids derived from the nitrogen pool exhibit ^{15}N -enriched nitrogen ratios (Ambrose 1991, Cormie and Schwarcz 1996). If any of the animals within the stranded group were suffering from starvation, an increase in $\delta^{15}\text{N}$ would be expected (Sealy et al. 1987).

The similarity of nitrogen isotopic ratios in bone collagen nitrogen between stranded and fishery by-catch harbor porpoise samples, and variations in nitrogen isotopic compositions of muscle and skin tissues indicated that the recent diets of stranded animals were different than their long-term diets, and were different than the short-term diets of healthy animals that died as a result of fishery interactions. This corroborates

evidence that stranded animals may not be feeding normally before stranding events (Pierce and Boyle 1991), and provides further evidence that stomach contents of stranded animals are not representative of healthy individuals.

Isotopic values obtained from bone collagen of harbor seal (*Phoca vitulina*) by Burton and Koch (1999) from the Monterey Bay area, were compared with harbor porpoise values collected in this research (Fig. 10). Average $\delta^{15}\text{N}$ values of harbor seal collagen are 3‰ greater than values for porpoise collagen, indicative of a one trophic level difference between the species. Similarly, there was a 1‰ difference in $\delta^{13}\text{C}$ values between species, further indicating that harbor seals are feeding in local nearshore areas similar to harbor porpoises, but at a higher trophic level. These findings are consistent with what is known (from scat and gut contents) about both species. Both are nearshore feeders but porpoise rely more heavily on planktivorous species (Sekiguchi 1987, Dorfman 1990, Byrd 2001) whereas seals consume greater quantities of piscivorous fish species (Harvey et al. 1995, Oxman 1995).

Knowledge of harbor porpoise food habits is essential for understanding the species' movement and abundance patterns, and to effectively manage these populations. The method of specimen collection may affect the data obtained from stomach contents. Stable isotope ratios of carbon and nitrogen are reflective of dietary and habitat use patterns, and nutritional status, over a range of time periods. Isotopic information in this research supports the conclusion that stomach content data from stranded animals may not necessarily represent the diet of healthy animals, and must be interpreted with caution.

Chapter 3

Age, growth, and reproduction of harbor porpoise (*Phocoena phocoena*) from central California

INTRODUCTION

Harbor porpoise (*Phocoena phocoena*) are small cetaceans endemic to northern hemisphere, temperate, coastal regions (Leatherwood and Reeves 1983, Gaskin 1984). Within the North Pacific, subpopulations occur in the Bering Sea, Sea of Okhotsk, coastal waters of northern Japan, and the western North American coast from Point Conception, California to northern Alaska (Gaskin 1984). Several stocks have been identified along the coast of North America. Stocks have been designated based on limited latitudinal movements of eastern Pacific harbor porpoise in comparison with animals in other locations (Gaskin 1984). Barlow and Hanan (1995) recommended that harbor porpoise off central California (e.g. Point Conception to the Russian River) be considered a separate stock. Harbor porpoise from California, Oregon, and Washington had significantly different pollutant loads (Calambokidis and Barlow 1991) reflective of harbor porpoise inhabiting waters with differing pollutant concentrations and consistent with separate stocks. Additionally, recent genetic analyses indicated that harbor porpoises resident in Monterey Bay may be a discrete population isolated from their nearest neighbors in San Francisco Bay (Chivers et al. In press).

If porpoise in Monterey Bay are reproductively isolated, the population may be more susceptible to decline during dramatic environmental change or increased anthropogenic mortalities. Knowledge about life history parameters, therefore, is

essential to effectively manage the population. Harbor porpoise are caught incidentally in gillnet fisheries throughout their range (Jefferson and Curry 1994). Mortality occurs mainly in large-mesh, set gillnets, and by-catch is often unreported making accurate estimates of mortality difficult. Off California, gillnets are used in the California halibut (*Paralichthys californicus*) and angel shark (*Squatina californica*) fisheries (Diamond and Hanan 1986, Wild 1990). Despite the paucity of mortality estimates, gillnet fishing has a potentially important impact on California populations of harbor porpoise. From 1980 to 1991, annual mortality fluctuated between zero and 38 individuals ($\bar{x} = 11$, $SE = 4.1$; Wild 1990). Unfortunately, the percentage of fishery observer coverage was not calculated and fishing effort was unknown. Between 1995 and 1998, estimates of local porpoise mortality due to gillnet interactions were between 42 ($CV = 0.19$) and 80 ($CV = 0.19$) individuals per year (Forney et al. 2000). Additionally, fishing effort nearly doubled after 1995 (Forney 1998, Forney et al. In press), and since 1997, the fishery has shifted its activity within Monterey Bay, into areas of greater porpoise densities (Forney et al. In press). Accompanying this shift in fishing effort and location was an increase in the occurrence of harbor porpoise strandings on Monterey Bay beaches (Forney et al. In press). Between January and September of 1999, approximately 123 harbor porpoise were killed in the fishery (Forney et al. 2000). Subsequently, the use of gillnets in water less than 60 fathoms was prohibited. The estimated number of annual mortalities in recent years exceeded the potential biological removal (the number of animals that can be removed from the population without effectively causing a decline), calculated for the central California harbor porpoise population (Forney et al. 2000). Determining the true

impact of net catches on the species is difficult (Gaskin 1984), however, and can be confounded by changes in stranding rates and abundance estimates; increased natural mortality associated with predation, disease, prey abundance (Forney 1995); and movements related to long-term ocean warming (Roemmich and McGowan 1995).

To assess the impacts of anthropogenic porpoise mortalities on the population, data are needed regarding growth and reproduction (Eberhardt and Siniff 1977). Life history parameters of harbor porpoise off California are not well understood (Hohn and Brownell 1990). Most information on harbor porpoise growth and reproduction is from populations in the North Atlantic (Fisher and Harrison 1970, Read 1990a, 1990b, Read and Gaskin 1990, Read and Hohn 1995, Lockyer 1995a, Lockyer et al. 2001). The asymptotic length estimates for male porpoises were between 143 cm and 146 cm in the Atlantic (Read and Gaskin 1990, Read and Tolley 1997, Lockyer et al. 2001). Females reached greater sizes than males, and had asymptotic lengths between 154 cm and 163 cm (Read and Gaskin 1990, Read and Tolley 1997, Lockyer et al. 2001). Data from central California indicated that, in general, animals from this area were larger than animals from the Atlantic (Hohn and Brownell 1990). Growth curves, however, were not constructed to describe the growth of animals from this region (Hohn and Brownell 1990).

There are few data regarding reproduction for harbor porpoise in the eastern Pacific (Hohn and Brownell 1990). In the North Atlantic, average age at sexual maturity of females was between 3 and 4 years (Read and Gaskin 1990, Lockyer 1995a, Lockyer et al. 2001). Females in the Gulf of Maine and Bay of Fundy had synchronized

reproductive schedules (simultaneous pregnancy and lactation) resulting in one calf each year (Read and Hohn 1995). Hohn and Brownell (1990) determined that females off the west coast of North America became sexually mature at 4.6 years, which was slightly greater than estimates for harbor porpoise in the north Atlantic. Furthermore, these authors suggested that female harbor porpoise in the Pacific Ocean had a two-year reproductive cycle consisting of disjunct pregnancy and lactation periods (Hohn and Brownell 1990).

The use of gillnets in waters occupied by harbor porpoise, underscores the need to understand this species' life history characteristics, so that managers and policy makers can affectively predict the effects of fishing on the porpoise populations. Because of the differences in growth and reproduction, movements, and genetic isolation between porpoise from central California and other parts of the world, life history parameters of harbor porpoise in other locations may not represent California populations. Therefore, life history information must be established directly from each population.

I estimate life history parameters including sex ratios, age structure, age at sexual maturity, reproductive seasonality, and postnatal growth, for harbor porpoise off central California. This should provide essential information for evaluating the potential impacts of inshore gillnet fisheries and natural mortalities on local porpoise populations.

METHODS

Stranded animals were collected from beaches in Monterey, Santa Cruz, and San Mateo Counties, California by Marine Mammal Stranding Network personnel from Moss

Landing Marine Laboratories, Long Marine Laboratory, California Academy of Science, and the University of California - Berkeley. Harbor porpoises incidentally taken in the local halibut fishery were collected by National Marine Fisheries Service and delivered to the Southwest Fisheries Science Center for examination. In addition, archived teeth and gonads were examined from several institutions including California Academy of Sciences, Humboldt State University, Los Angeles County Museum of Natural History, Moss Landing Marine Laboratories, Santa Barbara Museum of Natural History, and University of California - Berkeley.

Sex, standard length (± 0.5 cm), and mass (± 1 g), were determined during complete necropsies of all whole individuals collected. Reproductive tracts were removed and stored frozen, in formalin, or alcohol. Approximately three to five middle, mandibular teeth were extracted from each animal, stored in 70% isopropyl alcohol, and then used for age determination. Sex ratios were estimated from animals collected from beaches and as by-catch combined. Deviations from the expected 1:1 ratio were assessed using a Chi-squared test (Zar 1996).

Harbor porpoise ages were estimated from teeth that were prepared following the techniques summarized in the NMFS protocol for cetacean teeth (Myrick et al. 1983, Lockyer and Calzada 1992). Teeth were extracted from the middle of either side of the lower jaw. After extraction, teeth were cleaned, placed in 70% isopropyl alcohol, and decalcified in RDO (a commercial decalcifying agent) for several hours (depending on tooth size). Teeth were then sectioned using a freezing microtome into 25 μm sections, with the long axis placed vertically on the stage. Sections were stained using

Hematoxylin stain, mounted on glass slides, and examined under a compound microscope.

Age of individual animals was determined using methods of Myrick et al. (1983). Layers of dark and light bands, termed growth layer groups (GLG), were counted within the dentin of each tooth from the neonatal line inward to the pulp cavity. Each pair of bands was estimated to represent one year (Neilsen 1972, Gaskin and Blair 1977). Teeth from each animal were read multiple times by two independent readers (A. Toperoff (AT) and K. Robertson (KR)). Each reader made three age estimates for each animal with at least 7 days between readings. If estimates within or between readers differed by more than one year for an individual animal, the sections were examined by both readers together, discussed, and a final age determined. If no agreement was reached, samples were discarded.

The precision of age determinations was measured for each reader using the Average percent error index (Eq.1, Beamish and Fournier 1981), coefficient of variation (Eq.2, Chang 1982), and the index of precision (Eq. 3, Chang 1982).

Eq. 1. Average percent error (APE; Beamish and Fournier 1981):

$$\left[\frac{1}{R} \left(\frac{|X_{ij} - X_j|}{X_j} \right) \right] \quad \text{where: } R = \text{number of times each animal is aged}$$

X_{ij} = ith age determination for the jth animal
 X_j = average age calculated for the jth animal

Eq. 2. Coefficient of Variation (CV; Chang 1982):

$$CV = \frac{SD_j}{X_j} \quad \text{where: } SD_j = \text{standard deviation of ages for jth animal}$$

X_j = average age calculated for the jth animal

Eq. 3. Index of precision (D; Chang 1982)

$$D = \frac{CV}{\sqrt{R}} \quad \text{where: } CV = \text{coefficient of variation (standard dev/ mean)}$$

$$R = \text{number of times each animal is aged}$$

Growth curves were fitted to sex-specific length-at-age data using a non-linear least-squares approach. The Laird-Gompertz (Eq. 4), Gompertz (Eq. 5), and von Bertalanffy (Eq. 6) formulas (Gompertz 1825, von Bertalanffy 1938, Laird 1969, Ricker 1975) were used to fit the data using SYSTAT 9.0 and IGOR (Cope 2000). The Laird-Gompertz was anchored by the length at birth estimated from neonates. Length at birth was defined as the mean length of neonates (82 cm) and was determined using data from several institutions including California Academy of Sciences, University of California-Berkeley, Humboldt State University, and the Ray Bandar private collection, in addition to data collected during this research.

Eq. 4. Laird-Gompertz model (Laird 1969):

$$\text{Length} = L_0 \exp \{a[1 - \exp(\alpha - (\alpha - \text{age}))]\}$$

where: L_0 = length at birth
 a = specific rate of exponential growth
 α = rate of decay of exponential growth

Eq. 5. Gompertz model (Gompertz 1825):

$$\text{Length} = L_\infty \exp(-b \exp(-k \cdot \text{age}))$$

where: L_∞ = asymptotic length
 k = growth rate
 b = growth constant

Eq 6. von Bertalanffy model (von Bertalanffy 1938, Ricker 1975):

$$\text{Length} = L_\infty [1 - b \exp(-k \cdot \text{age})]$$

where: L_∞ = asymptotic length
 k = exponential coefficient
 b = growth constant

These are convenient models because they are comparable with other porpoise growth studies which is useful for management purposes (Moreau 1987, Read and Tolley 1997, Ferrero and Walker 1999, Lockyer et al. 2001). The Gompertz and von Bertalanffy models are favorable because they also estimate the asymptotic length of individuals in the population.

Age of attainment of sexual maturity (ASM) for a population was defined as the average age males undergo spermatogenesis and females ovulate for the first time (DeMaster 1984). Reproductive tracts were examined for indications of maturity following methods of Perrin et al. (1976). Testes were weighed and prepared histologically (cross-sectioned, stained, and mounted on slides). Tubules were examined for indications of spermatogenesis. Additionally, tubule size, tubule shape, lumen size, amount of interstitial tissue, and stages of spermatogenesis were considered in the determination of male reproductive status. Males were classified as mature, immature, or pubertal. Females with a fetus or gonads containing corpora albicantia (CA) or corpora lutea (CL) were considered mature.

Sample sizes were too small to reliably estimate ASM for males using the sum of fraction immature method. Therefore, the ASM for male porpoises was calculated as the age at which 50% of porpoises were mature based on a logistic function (Eq. 8, DeMaster 1984, Hohn 1989).

Eq. 8. ASM - Logistic function (DeMaster 1984, Hohn 1989):

$$L_t = L_{inf} / 1 + (b * \exp(-g * t))$$

where: L_t = Value calculated from equation

L_{inf} = Maximum size

b = Growth constant

g = Instantaneous growth rate

t = Age

t_0 = Inflection Point (50% mature)

The age of sexual maturity was calculated for female porpoises using the 50% mature logistic method and the sum of fraction immature formula (Eq. 9, DeMaster 1978, 1984, Hohn 1989).

Eq. 9. ASM, sum of fraction immature (DeMaster 1978, 1984, Hohn 1989):

$$ASM = x + \sum_{i=x}^j p_i$$

where: x = age of the youngest mature animal

j = age of the oldest immature animal,

$$S^2 = \sum_{i=x}^j \{ [p_i * (1 - p_i)] / (n_i - 1) \}$$

p_i = proportion immature in age class i ,

n_i = sample size for age class i

RESULTS

Specimens were beachcast ($n = 22$), collected from fishery interactions ($n = 16$), or borrowed archives from museum collections ($n = 73$). From 1993 through 2000 the distribution of stranded animals from Monterey and Santa Cruz counties, was somewhat even throughout the years (Fig. 11). The fishery's seasonal activity and the lack of takes during winter months, and time constraints of the research resulted in by-catch samples only available from April through September 1999 (Fig. 11). Museum specimens were collected from Point Conception to the Russian River, California between 1970 and 2000,

and were recovered during all months except December, with noticeable peaks in June and July (Fig. 11).

To increase sample size, sex ratios were determined for the combination of recent stranded and by-catch animals (years 1997 – 2000), assuming there are no gender biases in either collection method. Sex ratios did not significantly differ from the expected 1:1 ratio of males to females ($n = 43$, $X^2 = 0.2093$, $P > 0.50$). Archived material was excluded from this analysis to avoid representing several generations.

The average percent error of porpoise age estimates was 9.5%, the coefficient of variation was 12.8%, and the precision was 7.4% for reader 1 (AT). Reader 2 (KR) age estimates resulted in an average percent error of 8.3%, a coefficient of variation of 11.0%, and a precision of 6.3%.

Male porpoises from all collection sources had a mean age of 4.7 years ($SD = 4.06$) and ranged from 0 to 14 years. Samples were unevenly distributed with a modal age of 0-1 year (Fig. 12). Females had a mean age of 6 ($SD = 3.61$) and ranged from 0 to 13 years old. A modal age class was evident for females between 9 and 10 years, but distribution was even among all younger ages, and declined after age 10 (Fig. 13).

The Laird-Gompertz, Gompertz, and von Bertalanffy growth formulas adequately represented the data for both sexes, demonstrated by similar and high r^2 values (Table 10). The Gompertz and von Bertalanffy curves of male growth appeared indistinguishable and exhibited the steepest slopes between birth and age 1. Animals grew more slowly with each additional year until reaching asymptotic lengths at 145.0 and 145.8 cm, respectively (Figs. 14 and 16, Table 10).

Female growth was comparable among the models (Figs. 15 and 17). Female porpoise asymptotic length was 169.1 cm using the Gompertz growth model, and was 169.4 cm using the von Bertalanffy model. Growth was fastest between birth and age 1, however, animals reached asymptote at a slower rate than males. Females obtained a larger size and grew at a slightly slower rate than males.

The average standard length of all neonates collected during this research and from other institutions, resulted in a mean length at birth estimate of 82 cm ($n = 24$, $SD = 6.1$; Table 11), which was used to anchor the Laird-Gompertz growth model. These data indicated birthing occurred May through August, with the greatest numbers in July (Fig. 18). Additionally, three fetuses collected during this research fit well into the seasonal pattern (Fig. 18).

Thirty males were examined and classified as mature (5), pubertal (immature/verging on maturity; 7), or sexually immature (18). Testes from mature males were 625 to 846 g with an average mass of 739.6 g ($SE = 44.3$) and testes of immature males were 1.9 to 68.9 g with a mean mass of 20.4 g ($SE = 4.5$). Pubertal animals had a mean testes mass of 83.6 g ($SE = 12.2$; range = 52.9 - 133.4 g). Mean tubule diameters were 79.1 μm ($SE = 3.9$) for mature animals, 34.1 μm ($SE = 1.4$) for pubertal, and 19.9 μm ($SE = 0.56$) for immature porpoises. Diameters differed significantly among the three classes (ANOVA, $F = 383.47$, $P < 0.001$; Fig. 19).

Twenty-five male harbor porpoises (no age data for 5 individuals) were examined to determine ASM. The age at which 50% of the animals were sexually mature based on a logistic regression yielded an estimated ASM of 7.0, however the corresponding r^2

value was 0.57 (Fig. 20). This estimate must be interpreted with caution due to the extremely low sample size and poor fit of the logistic function. All males older than 6 years were mature and one male of 3 years also was mature. All mature males were greater than 140 cm (SL), however, length data were only collected for three of the five mature individuals.

Of 22 female animals examined, 10 were classified as mature and 12 as immature. Two animals were considered mature based on the presence of a fetus. Corpora albicantia (CA) were present on one right ovary, whereas the remainder of mature females had CAs only on the left ovaries. The number of CAs present on mature ovaries increased with age and length of the individual animals (Figs. 21 and 22). The individual with the greatest number of CAs (23) was 12 years old. The corpora counts for mature females were not normally distributed, and the number of CAs regressed on age had an r^2 value of 0.69. The resulting equation was: Number of CAs = $2.5274x - 14.378$ (where x = age in years).

The estimated average age at sexual maturity for females was 5.5 years (SD = 0.35) based on the sum of the fraction of immature animals (DeMaster 1978, Hohn 1989). Fitting a logistic curve to the age and sexual maturity data predicted an estimated ASM of 4.9 years ($r^2 = 87$). The youngest mature female was 5 years old, the same age as the oldest immature animal.

DISCUSSION

Correct estimates of growth and reproductive parameters rely on precise and accurate age estimates. It is essential that proper methods and criteria are used for establishing age estimates using the teeth of harbor porpoise (Beamish and McFarlane 1983). Nielsen (1972) reported one growth layer group is deposited each year in porpoise tooth dentin, based on one captive, known-aged animal. Although this assumption has not been examined in free-ranging porpoise, Hohn et al. (1989) determined that one GLG corresponds to one year in wild bottlenose dolphin (*Tursiops truncatus*) based on teeth collected from 16 known-aged individuals. I have, therefore, assumed that this relationship is similar for wild harbor porpoise.

Misidentification of GLGs in the teeth of my samples may have biased the age data. The identification of GLGs in the teeth of harbor porpoise is particularly challenging. The small size and natural curvature of whole teeth makes sectioning difficult, and bands are hard to discern because GLGs narrow with age, and because of tooth abnormalities (i.e. marker lines, cemental disturbance; Gaskin and Blair 1977, Lockyer 1995b). Average percent error, coefficient of variation, and index of precision demonstrated, however, that age estimates could be reliably duplicated.

The harbor porpoise examined from both groups had short life spans (maximum age of 14 years), which is consistent with other reports of maximum age of animals that died from anthropogenic and natural causes (Hohn and Brownell 1990, Lockyer 1995a, Read and Hohn 1995, Lockyer et al. 2001). Hohn and Brownell (1990) examined one female that died of natural causes at age 24, however, 98% of 89 females and 97% of 100

males, did not live more than 9 years (Hohn and Brownell 1990). Maximum age of harbor porpoise from West Greenland was 14 years for females and 17 yrs for males (Lockyer et al. 2001). Harbor porpoise in British waters had age distributions heavily skewed toward animals in the 0 - 1 age class with a maximum age of 24 years (Lockyer 1995a). Read and Hohn (1995) examined porpoise from the Gulf of Maine, and reported a maximum age of 17 years, and modes of one year for males and two years for females.

The length at birth determined for harbor porpoise from central California was comparable with estimates from populations in other locations (Fisher and Harrison 1970, Smith and Gaskin 1974, Van Utrecht 1978). Length at birth of animals from the North Sea were 67 to 80 cm (Van Utrecht 1978), in the North Atlantic 80 to 90 cm (Fisher and Harrison 1970), and 78 cm in the Bay of Fundy, specifically (Smith and Gaskin 1974), similar to the length (82 cm) determined in this study.

Harbor porpoise from California had dramatic seasonal patterns of parturition, similar to patterns of other harbor porpoise populations (Fisher and Harrison 1970, Smith and Gaskin 1974, Read and Hohn 1995, Lockyer et al. 2001). Neonates only occurred during summer, most frequently occurring in July (Fig. 18). Harbor porpoise from other locations also had dramatic calving seasonality with maximum occurrences of neonates between May and August (Fisher and Harrison 1970, Smith and Gaskin 1974, Read and Hohn 1995, Lockyer et al. 2001).

All of the growth curve models employed in this research fit the data well. Therefore, one is not inherently better than the other, and all should be useful for making comparisons with growth curves of other species, or similar species from different areas.

Growth curves indicated that females were slightly larger than males, similar to trends in harbor porpoise growth from other areas (Read and Gaskin 1990, Sorensen and Kinze 1994, Lockyer 1995a, Lockyer et al. 2001). Read and Tolley (1997) discussed sexual dimorphism in porpoises from the Bay of Fundy. They suggested that the large size of the family Kentriodontidae, the precursors of modern Phocoenidae, indicated that natural selection has resulted in the success of smaller sized individuals. Female size reduction may have been limited by size requirements for bearing offspring and surviving in a cold environment (Read and Tolley 1995). Males are not affected by constraints relating to birthing young, hence males evolved to be smaller than females. Additionally, the mating system may be driven primarily by sperm competition, therefore, there is no reproductive advantage for an animal to be larger than other members of the population (Read and Tolley 1995, Fontaine and Barrette 1997).

Female harbor porpoise from California were larger than females from other areas. Females off central California had an estimated asymptotic length 9 to 15 cm greater than estimates of animals from the Atlantic (154 -160 cm; Read and Gaskin 1990, Sorensen and Kinze 1994, Lockyer 1995a, Lockyer et al. 2001). The range of water temperatures in harbor porpoise habitats on the Pacific and Atlantic coasts of North America are comparable (Lolli and Parsons 1997) during some parts of the year, and reach lower temperatures in the North Atlantic than in Monterey Bay during other times, therefore, it is unlikely that limitations on body size that result from calving in cold water, are influencing the size differences observed between locations. An alternative, explanation is that their body size is related to prey quality and availability, in

conjunction with the demands of bearing young (Brodie 1995). However, prey identification, distribution, and availability must be studied in detail to assess if there is any relationship to body size.

The greater age at sexual maturity for males in this study compared with other data, may be biased due to a relatively small sample size ($n = 25$). Few reproductive samples were available in age classes surrounding the observed transition from immaturity to maturity. My estimate of ≈ 7 years for ASM is greater than the average ASM of ≈ 3 yrs for animals in the Atlantic, however, estimates from the Atlantic are based on greater sample sizes (Read 1990b, Read and Gaskin 1990, Lockyer 1995a, Sorensen and Kinze 1994, Lockyer et al. 2001).

Sexual maturity was evaluated through the examination of histologically prepared testes. The presence and development of spermatozoa, lumen size, amount of interstitial tissue, and seminiferous tubule diameter were considered in the determination of sexual maturity status. Graphs of tubule diameter indicated clear groupings of each maturity state (Fig. 19), however, diameter is one of the characteristics used for indicating maturity, therefore, the two variables are not independent. An additional confounding factor is the extreme seasonality of porpoise mating (Fontaine and Barrette 1997). Male harbor porpoise greater than four years old, examined by other researchers, indicated a decline in testes mass (Read 1990a, Fontaine and Barrette 1997) and seminiferous tubule diameter (Fontaine and Barrette 1997) through the summer months. These conclusions were based only on animals collected between June and August, so no comments could be made about characteristics of testes during other times of the year (Fontaine and

Barrette 1997). All animals in the mature category occurred in June and July. The assignment of these testes to the mature category could be attributed to increased tubule diameter and testes mass in response to reproductive seasonality of porpoises off California (Figs. 23a and b). If seasonality has a dramatic effect on testes characteristics, animals collected during non-summer months may be mature, however, their tubule diameter and testes mass may be decreased leading to erroneous conclusions that they are pubertal. Additionally, samples were originally stored frozen which caused considerable tissue damage to testes, and made the identification of mature sperm difficult. Further research using fresh specimens and larger sample sizes during all times of year is necessary to adequately assess the effect of seasonality on testes characteristics and sexual maturity criteria.

Although I did not consider testes mass in the evaluation of male sexual maturity, the results of this research correspond well with the 200 g mass criterion often used in the reproductive assessment of male cetaceans (Hohn and Brownell 1990, Lockyer 1995a, Lockyer et al. 2001). The testes of all mature males had masses greater than 200 g, and the testes of all immature and pubertal animals had masses less than 200 g (Fig. 23b). Therefore, if animals were categorized based on testis mass, the results would be similar.

The ASM of female harbor porpoise off California was greater than porpoises in most other locations (Hohn and Brownell 1990, Read and Gaskin 1990, Lockyer 1995a, Lockyer et al. 2001). In several areas of the Atlantic, the average age at sexual maturity was between 3 and 4 years. Van Utrecht (1978) determined that animals matured at age 6 in the North Sea. Hohn and Brownell (1990) examined harbor porpoise off California

and determined that the average ASM was 4.6 years, similar to the ASM of females calculated in this study (5.5 years). If prey resources are not limiting and/ or there are lesser rates of mortality in populations off Central California, then an elevated ASM is expected (Fowler 1984, 1990).

Evidence of ovulation was apparent almost exclusively on the left ovary, which is a frequently observed pattern in harbor porpoise (Sorensen and Kinze 1994). Of 54 mature females examined by Sorensen and Kinze (1994), CAs were only present on the right ovary of one individual. Read (1990a) determined that of 55 mature females from the Bay of Fundy, only one individual had corpora albicantia on her right ovary, and in that case it was only one. The left ovary matures first and most often produces offspring. Read (1990a) suggested that the pattern of repeated implantation on the left side was because of inactivity of the right ovaries.

I could not determine ovulation rate and calving interval, however, previous studies of harbor porpoise off central California indicated long calving intervals in comparison with Atlantic porpoises. Hohn and Brownell (1990) suggested that porpoise off California likely reproduce every other year. This was based on the observation that of 16 pregnant and 13 lactating females, none were pregnant and lactating simultaneously. Because gestation period is approximately 11 months (Read 1990a), they concluded that porpoise off California likely reproduce every two or more years (Hohn and Brownell 1990). In contrast, most populations in the Atlantic calve every year (Read and Hohn 1995).

Animal size, age at sexual maturity, and calving interval apparently were different

between animals off California and those in the North Atlantic. Reasons for these differences are not well understood but may relate to prey resource availability in each location. Harbor porpoise in the Bay of Fundy show seasonal movements attributed to the distribution and abundance of herring (*Clupea pallasii*), their main prey species (Smith and Gaskin 1974, Brodie 1995). Herring constitute a large proportion of porpoise diet (Rae 1965, Smith and Gaskin 1974, Recchia and Read 1989), and herring distribution, abundance, and caloric content may change on seasonal and yearly scales (Stoddard 1968). Dependence on ephemeral prey resources may partially explain the smaller size, therefore reduced food requirements, of individual porpoises in the Atlantic Ocean. Producing yearly offspring is energetically costly, however, it may also be an effective strategy for taking advantage of all years when prey are abundant.

These differences in size and reproductive parameters among locations may be attributed to density-dependent factors reflective of resources availability in each area (Smith and Gaskin 1974, Fowler 1984, 1990).

Baseline data on these animals is crucial to adequately evaluate the status and viability of local populations. Clear differences occur among populations, therefore, estimates of life history traits of one population may not represent traits of harbor porpoise in other areas. Management plans based on the life history characteristics of population of harbor porpoise other than the target population could be devastating. With the increase in human activities in all of the country's coastal areas, it is important that life history data be collected in each area so policies and management plans can efficiently and effectively protect this species.

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Table 1. Mean nitrogen and carbon stable isotope values for three tissues from 29 harbor porpoises and porpoise prey collected in Monterey Bay.

Sample Type	mean $\delta^{15}\text{N}$ ($\pm$ SD)	mean $\delta^{13}\text{C}$ ($\pm$ SD)	n
Porpoise tissue			
bone collagen	15.7 (0.7)	-13.4 (0.5)	29
muscle	15.2 (0.8)	-16.2 (0.5)	29
skin	16.0 (0.7)	-16.2 (0.6)	29
Prey			
<i>L. opalescens</i>	13.3 (0.7)	-17.2 (0.5)	10
<i>P. notatus</i>	13.9 (0.4)	-17.1 (0.6)	7
<i>E. mordax</i>	13.7 (0.9)	-16.9 (0.8)	5
<i>S. sagax</i>	13.2 (0.4)	-17.7 (0.5)	4
<i>H. pretiosus</i>	13.4 (0.1)	-18.6 (0.6)	2
<i>Sebastes spp.</i>	13.3 (0.3)	-16.9 (0.1)	2
<i>T. symmetricus</i>	15.0 (0.1)	-17.2 (0.5)	2
<i>G. lineatus</i>	16.27	-16.54	1
<i>C. aggregata</i>	15.99	-16.21	1

Table 2. Repeated measures ANOVA table for comparisons of harbor porpoise tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values.

Nitrogen	SS	df	MS	F	P
Tissue	9.53	2	4.77	2.84E+01	0.00*
Error	9.39	56	0.17		

Carbon	SS	df	MS	F	P
Tissue	154.45	2	77.23	3.78E+02	0.00†
Error	11.44	56	0.20		

*Significant differences occurred between all three tissues (Ryan's Q post-hoc test).

†Significant differences occurred between bone collagen and the other two tissues (Ryan's Q post-hoc test).

Table 3. Calculation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation within skin, muscle, and bone collagen of harbor porpoise ($n = 29$).

Tissue	Carbon			Nitrogen		
	Bone			Bone		
	Collagen.	Muscle	Skin	Collagen	Muscle	Skin
Mean	-13.4 (0.5)	-16.2 (0.4)	-16.2 (0.6)	15.7 (0.7)	15.2 (0.7)	16.0 (0.7)
Bulk diet*	-17.2	-17.2	-17.2	13.5	13.5	13.5
Fractionation	3.8	1.0	1.0	2.2	1.7	2.5

*Bulk diet was calculated as the average of values obtained from market squid mantle and northern anchovy, plainfin midshipman, and Pacific sardine muscle tissue.
Note: Numbers in parentheses represent ± 1 standard deviation.

Table 4. Mean percent number (%N), mean percent mass (%M), percent frequency of occurrence (%FO), mean index of relative importance (IRI), mean percent index of relative importance, and standard error for each prey species identified in stomachs of stranded harbor porpoise (n = 14).

Prey species	Mean %N	SE	Mean %M	SE	%FO	Mean IRI	%IRI
<i>Loligo opalescens</i>	54.76	5.65	47.71	5.95	85.71	8783.61	73.34
<i>Merluccius productus</i>	10.13	8.39	20.42	8.60	42.86	1309.50	10.93
<i>Porichthys notatus</i>	16.08	7.46	14.88	7.33	35.71	1105.71	9.23
<i>Engraulis mordax</i>	4.57	4.14	3.70	4.11	50.00	413.72	3.45
<i>Sebastes spp.</i>	3.72	4.96	3.26	5.39	21.43	149.52	1.25
<i>Trachurus symmetricus</i>	2.56	5.03	5.91	6.56	14.29	120.97	1.01
<i>Phanerodon furcatus</i>	6.43	9.49	0.13	1.37	7.14	46.88	0.39
<i>Sardinops sagax</i>	0.24	1.83	1.81	5.03	7.14	14.62	0.12
<i>Octopus rubescens</i>	0.52	1.84	0.22	1.27	14.29	10.52	0.09
<i>Clupea pallasii</i>	0.21	1.71	0.70	3.13	7.14	6.49	0.05
<i>Genyonemus lineatus</i>	0.18	1.58	0.71	3.16	7.14	6.37	0.05
<i>Diaphus theta</i>	0.20	1.67	0.23	1.80	7.14	3.07	0.03
<i>Chilara taylori</i>	0.20	1.67	0.17	1.54	7.14	2.62	0.02
<i>Citharichthys sordidus</i>	0.21	1.71	0.14	1.38	7.14	2.47	0.02

Table 5. Mean percent number (%N), mean percent mass (%M), percent frequency of occurrence (%FO), mean index of relative importance (IRI), mean percent index of relative importance, and standard error for each prey species identified in stomachs of by-caught harbor porpoise (n = 18).

Prey species	Mean %N	SE	Mean %M	SE	%FO	Mean IRI	%IRI
<i>Engraulis mordax</i>	44.39	6.55	33.71	9.06	66.67	5207.00	53.78
<i>Loligo opalescens</i>	33.28	10.00	27.67	10.00	55.56	3386.07	34.97
<i>Merluccius productus</i>	5.23	4.65	8.54	7.01	31.58	435.09	4.49
<i>Sardinops sagax</i>	3.02	3.78	11.03	0.18	27.78	390.23	4.03
<i>Clupea pallasii</i>	1.74	3.16	5.66	0.46	11.11	82.21	0.85
<i>Sebastes spp.</i>	5.56	4.39	5.56	6.58	5.56	61.73	0.64
<i>Porichthys notatus</i>	1.28	0.96	2.47	0.33	11.11	41.68	0.43
<i>Spirinchus spp.</i>	2.38	6.27	4.56	7.77	5.56	38.55	0.40
<i>Chilara taylori</i>	1.11	1.49	0.26	1.93	16.67	22.93	0.24
<i>Citharichthys sordidus</i>	0.41	6.88	0.15	7.27	11.11	6.26	0.06
<i>Pleuronectes spp.</i>	0.79	1.15	0.00	1.68	5.56	4.42	0.05
<i>Pleuronectes vetulus</i>	0.56	6.27	0.01	6.89	5.56	3.15	0.03
<i>Lycodes spp. (Zoarcidae)</i>	0.12	3.25	0.21	1.39	5.56	1.83	0.02
<i>Leptocottus armatus</i>	0.07	2.10	0.16	1.49	5.56	1.28	0.01
<i>Phanerodon furcatus</i>	0.05	5.10	0.01	8.19	5.56	0.32	0.00

Table 6. Comparison of past and present diet data based on ranks of IRI values reported for stranded harbor porpoise from Monterey Bay.

	Dorfman (1990)	Sekiguchi (1995)	Byrd (2001)	Toperoff (this study)
	n = 19	n = 9	n = 9	n = 14
Species	Rank	Rank*	Rank	Rank
<i>Engraulis mordax</i>	23	24	21	21
<i>Loligo opalescens</i>	24	20	24	24
<i>Porichthys notatus</i>	8	21	20	22
<i>Merluccius productus</i>	8	17	22	23
<i>Citharichthys sordidus</i>	8	2.5	9	11
<i>Clupea pallasii</i>	18	16	9	15
<i>Sebastes spp.</i>	20	22	9	20
<i>Sardinops sagax</i>	8	2.5	9	17
<i>Chilara taylori</i>	21	23	23	12
<i>Phanerodon furcatus</i>	8	14	9	18
<i>Trachurus symmetricus</i>	8	19	9	19
<i>Octopus rubescens spp.</i>	8	9	9	16
<i>Diaphus theta</i>	8	2.5	9	13
<i>Genyonemus lineatus</i>	19	2.5	9	14
<i>Citharichthys stigmaeus</i>	8	2.5	19	5
<i>Cymatogaster aggregata</i>	8	9	9	5
<i>Hyperprosopon anale</i>	8	9	9	5
<i>Lepidogobius lepidus</i>	8	9	9	5
<i>Scomber japonicus</i>	8	9	9	5
<i>Spirinchus starksi</i>	17	15	9	5
<i>Sciaenidae spp.</i>	8	18	9	5
Embiotocidae	22	13	9	5
Ophidiidae	8	9	9	5
Osmeridae	8	9	9	5

*These data are based on a modified IRI = %N * FO.

Table 7. Symbol, mean, and standard error of prey array indices from stranded and by-caught harbor porpoise from Monterey Bay.

Prey Array Index	Symbol	Fishery	SE	Stranded	SE	P-value	Signif.
Species Richness	S	15	0.411	15	0.533	0.530	No
Dominance	D	0.28	0.061	0.28	0.069	0.910	No
Shannon-Weiner Diversity	H'	-1.491	0.109	-1.393	0.133	0.818	No
Species Evenness	J	-0.551	0.079	-0.515	0.080	0.336	No

Table 8. Stable carbon and nitrogen isotopic compositions of bone collagen, muscle, and skin from porpoise from Monterey Bay (n = 21). Mean and standard error of values are included.

Date	Collect	Sample #	CARBON			NITROGEN		
			Bone Coll.	Muscle	Skin	Bone Coll.	Muscle	Skin
4/5/99	By-catch	JPL052	-13.4	-16.6	-16.1	15.7	14.8	15.3
4/5/99	By-catch	JPL054	-13.1	-16.1	-15.1	14.5	14.5	15.8
4/9/99	By-catch	STL002	-13.4	-15.8	-16.7	15.8	15.5	16.1
5/19/99	By-catch	JPL055	-13.2	-15.9	-15.6	15.4	14.8	15.5
6/18/99	By-catch	JPL063	-13.3	-16.2	-16.2	15.2	15.0	15.3
6/30/99	By-catch	STL008	-13.7	-16.7	-16.0	15.1	14.3	15.2
6/30/99	By-catch	STL007	-13.1	-16.4	-15.3	15.1	14.3	16.1
7/6/99	By-catch	JPL072	-13.5	-15.6	-16.6	14.9	15.3	15.3
7/6/99	By-catch	JPL071	-13.0	-16.0	-15.8	15.2	14.9	15.0
9/15/99	By-catch	JPL218	-13.3	-16.4	-16.2	15.0	14.7	15.6
9/18/99	By-catch	STL113	-13.8	-17.1	-17.4	15.5	14.5	15.1
9/30/99	By-catch	JPL251	-13.8	-16.3	-16.6	15.9	14.9	15.3
3/24/97	Strand	MLML440	-13.4	-16.1	-16.8	15.8	15.1	15.4
4/11/97	Strand	MLML446	-12.9	-15.9	-16.5	16.4	14.8	15.7
4/12/97	Strand	MLML445	-13.1	-15.9	-16.5	15.1	14.5	15.7
9/22/97	Strand	MLML524	-13.1	-16.1	-16.8	16.8	15.3	16.1
4/16/98	Strand	MLML624	-12.6	-15.3	-16.1	15.3	16.2	17.4
11/17/98	Strand	MLML820	-12.9	-16.4	-15.7	15.7	14.7	15.8
2/27/99	Strand	MLML547	-14.0	-16.3	-17.0	15.4	15.5	16.7
4/1/99	Strand	MLML557	-13.3	-15.9	-15.5	15.1	14.6	15.9
6/17/99	Strand	UCSC01	-12.8	-15.5	-15.6	15.8	17.4	16.3
Mean			-13.3	-16.1	-16.2	15.5	15.0	15.7
SE			0.1	0.1	0.1	0.1	0.1	0.1

Table 9a. T-test table for comparisons of stable carbon isotope values for stranded and by-catch harbor porpoise from Monterey Bay.

Tissue	DF	Diff. of means	P- value
Bone	19	0.31	0.057
Muscle	19	0.32	0.082
Skin	19	0.14	0.623

Table 9b. T-test table for comparisons of stable nitrogen isotope values for stranded values for stranded and by-catch harbor porpoise from Monterey Bay.

Tissue	DF	Diff. of means	P- value
Bone	19	0.35	0.060
Muscle	19	0.54	0.079
Skin	19	0.62	0.013

Table 10. Fitted growth curve equations for length-at-age data. Equations represent Gompertz, Laird-Gompertz, and von Bertalanffy growth models for male and female harbor porpoises.

Gompertz		r^2
Male	Length = $145.0 * \exp(-0.493(1 - \exp(-0.982 * \text{age})))$	0.85
Female	Length = $169.1 * \exp(-0.493(1 - \exp(-0.514 * \text{age})))$	0.83
Laird-Gompertz		r^2
Male	Length = $82.00 * \exp(0.565(1 - \exp(1.182 * \text{age})))$	0.82
Female	Length = $82.00 * \exp(0.716(1 - \exp(0.606 * \text{age})))$	0.83
von Bertalanffy		r^2
Male	Length = $145.8 * (1 - \exp(-0.795(\text{age} - (-1.171))))$	0.85
Female	Length = $169.4 * (1 - \exp(-0.635(\text{age} - (-0.894))))$	0.84

Table 11. Catalog number, sex, month, year of collection, and standard length of neonates used to calculate length at birth.

Source*	Number	Sex	Month	Year	Length (cm)
HUMB	630	Unkown	7	75	67.0
CAS	4353	Unkown	7	98	71.0
MVZ	172149	M	6	70	76.0
CAS	21707	F	6	81	77.0
HUMB	601	Unkown	7	78	77.0
HUMB	459	M	7	76	78.5
MVZ	172154	F	7	75	79.0
MVZ	172157	F	6	77	81.0
HUMB	1094	F	7	85	81.0
HUMB	625	Unkown	7	75	81.0
MLML	404	M	6	94	82.0
CAS	23156	M	5	90	82.5
HUMB	488	Unkown	7	78	83.0
HUMB	1698	M	7	90	83.0
HUMB	5133b	F	6	89	84.0
CAS	4302	Unkown	5	98	84.0
REJ	1464	F	6	91	85.0
HUMB	920	M	8	85	85.0
REJ	1508	M	6	94	85.2
REJ	1476	M	6	92	87.0
HUMB	2035	M	7	94	87.0
HUMB	SAS 51	M	7	80	89.0
HUMB	RMS 333	M	7	77	92.0
REJ	1509	M	6	94	95.1

* Source codes: CAS: California Academy of Science, HUMB: Humboldt State University, MVZ and REJ: Museum of Vertebrate Zoology- UC Berkeley, MLML: Moss Landing Marine Laboratories

Fig. 1. Monthly averaged $\delta^{15}\text{N}$ values of skin and muscle tissues of harbor porpoise collected between 1997 and 1999 ($N = 29$). Vertical error bars represent $\pm 1\text{SD}$.

Fig. 2. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of harbor porpoise prey species. Square symbols indicate values used to estimate bulk diet. Error bars represent ± 1 SD.

Fig. 4. Distribution of stranded and by-catch harbor porpoise collected from Monterey Bay between 1997 and 2000 and used in stomach content analysis.

Fig. 5. Cumulative number of species for stranded harbor porpoise stomach samples from Monterey Bay ($n = 14$) used to determine the adequacy of sample size. Samples were chosen randomly during 5 iterations. Y-axis error bars represent 1 standard deviation.

Fig. 6. Cumulative number of species for by-catch harbor porpoise stomach samples from Monterey Bay ($n = 18$) used to determine the adequacy of sample size. Samples were chosen randomly during 5 iterations. Y-axis error bars represent 1 standard deviation.

Fig. 7. Cumulative mean %M (Mass) and %N (Number) of prey species (*L. opalescens*, *E. mordax*, *P. notatus*) in stomach contents of stranded harbor porpoise from Monterey Bay. Samples were assigned randomly, and 1000 iterations were performed. Error bars represent one standard deviation.

Fig. 8. Cumulative mean %M (Mass) and %N (Number) of prey species (*L. opalescens*, *E. mordax*, *M. productus*) in stomach contents of by-catch harbor porpoise from Monterey Bay. Samples were assigned randomly, and 1000 iterations were performed. Error bars represent one standard deviation.

Fig. 9. Standard length of main prey species (*L. opalecscens*, *S. sagax*, *E. mordax*, *M. productus*, *P. notatus*) consumed by stranded and by-catch harbor porpoise in Monterey Bay. Dashed bars represent stranded porpoise prey and solid bars represent by-catch prey and indicate ± 1 standard deviation.

Fig. 10. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for bone collagen of harbor porpoises and harbor seals from central California (Burton and Koch 1999).

Fig. 11. Monthly occurrence of harbor porpoise samples examined in this study. Bars represent multiple years combined.

Fig. 12. Distribution of ages of male harbor porpoise collected from central California between 1970 and 2000 ($n = 56$).

Fig. 13. Distribution of ages of female harbor porpoise collected from central California between 1970 and 2000 ($n = 52$).

Fig. 14. Length at age of male harbor porpoise from Central California from 1970-2000 using the Gompertz growth function ($n = 54$). Circles represent data points and error bars represent one standard deviation (Cope 2000).

Fig. 15. Length at age of female harbor porpoise from Central California from 1970-2000 using the Gompertz growth function ($n = 51$). Circles represent data points and error bars represent one standard deviation (Cope 2000).

Fig. 16. Length at age of male harbor porpoise from Central California from 1970-2000 using the von Bertalanffy growth function ($n = 54$). Circles represent data points and error bars represent one standard deviation (Cope 2000).

Fig. 17. Length at age of female harbor porpoise from Central California from 1970-2000 using the von Bertalanffy growth function ($n = 51$). Circles represent data points and error bars represent one standard deviation (Cope 2000).

Fig. 18. Seasonal distribution and standard length of neonatal and fetal harbor porpoise from central California. Data collected by California Academy of Sciences, Humboldt State University, Moss Landing Marine Laboratories, National Marine Fisheries Service, and University of California- Berkeley.

Fig. 19. Mean testes tubule diameter at length for immature, pubertal, and mature male harbor porpoise from central California.

Fig. 20. Age at sexual maturity of 25 male harbor porpoise from central California fit to a logistic model. The age at which 50% were mature was nearly seven years. Numbers in parenthesis indicate sample sizes greater than one.

Fig. 21. Scatterplot of age and left ovarian corpora (CA) count for 20 female harbor porpoise from central California between 1970 and 2000. Multiple animals aged 1 through 3 had zero CAs.

Fig. 22. Scatterplot of body length and left ovarian corpora (CA) count for 20 female harbor porpoise from central California between 1970 and 2000.

Fig. 23a. Mean tubule diameter from 30 harbor porpoise collected in central California between 1970 and 2000 for each month.

Fig. 23b. Mean testes mass from 30 harbor porpoise collected in central California between 1970 and 2000 for each month.

Appendix 1. Collection data and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from 29 harbor porpoises collected in Monterey Bay between March 1997 and September 1999.

Date	Spec. #	Carbon			Nitrogen			Sex	Age (Years)	Length (cm)
		Bone			Bone					
		collagen	Muscle	Skin	collagen	Muscle	Skin			
3/24/97	MLML440	-13.4	-16.1	-16.8	15.8	15.1	15.4	M	4	144.0
4/11/97	MLML446	-12.9	-15.9	-16.5	16.4	14.8	15.7	M	5.5	145.0
4/12/97	MLML445	-13.1	-15.9	-16.5	15.1	14.5	15.7	M	4	142.0
9/22/97	MLML524	-13.1	-16.1	-16.8	16.8	15.3	16.1	F	8	164.5
3/19/98	MLML604	-13.1	-15.9	-16.8	16.3	16.1	17.0	F	1	123.0
3/19/98	MLML606	-12.8	-16.0	-16.4	16.3	16.0	17.0	M	1	117.5
4/16/98	MLML624	-12.6	-15.3	-16.1	15.3	16.2	17.4	M	5	142.5
11/17/98	MLML820	-12.9	-16.4	-15.7	15.7	14.7	15.8	F	?	155.0
2/27/99	MLML547	-14.0	-16.3	-17.0	15.4	15.5	16.7	M	?	132.0
4/1/99	MLML557	-13.3	-15.9	-15.5	15.1	14.6	15.9	F	11	173.0
4/5/99	JPL052	-13.4	-16.6	-16.1	15.7	14.8	15.3	M	4	129.5
4/5/99	JPL054	-13.1	-16.1	-15.1	14.5	14.5	15.8	M	4.5	135.5
4/9/99	STL002	-13.4	-15.8	-16.7	15.8	15.5	16.1	F	3	140.5
5/5/99	MLML636	-12.9	-15.9	-16.6	16.8	16.0	16.9	F	1	118.0
5/19/99	JPL055	-13.2	-15.9	-15.6	15.4	14.8	15.5	F	5	155.5
6/17/99	UCSC01	-12.8	-15.5	-15.6	15.8	17.4	16.3	F	5	159.0
6/18/99	JPL063	-13.3	-16.2	-16.2	15.2	15.0	15.3	F	3	151.0
6/18/99	JPL062	-13.7	-16.0	-15.7	14.9	14.6	16.0	M	1	128.5
6/29/99	JPL064	-13.8	-16.7	-17.0	16.2	14.8	16.0	F	1	?
6/30/99	STL008	-13.7	-16.7	-16.0	15.1	14.3	15.2	M	7	145.5
6/30/99	STL007	-13.1	-16.4	-15.3	15.1	14.3	16.1	M	11	141.0
7/6/99	JPL072	-13.5	-15.6	-16.6	14.9	15.3	15.3	M	3	?

Appendix 1 (continued)

Date	Spec. #	Carbon			Nitrogen			Sex	Age (Years)	Length (cm)
		Bone			Bone					
		collagen	Muscle	Skin	collagen	Muscle	Skin			
7/6/99	JPL071	-13.0	-16.0	-15.8	15.2	14.9	15.0	M	10	?
8/5/99	JPL091	-14.8	-16.9	-15.5	16.8	15.9	16.7	M	1	111.0
8/22/99	MLML623	-13.3	-17.2	-16.7	15.4	14.3	15.5	M	?	121.5
8/23/99	MLML625	-14.4	-15.9	-15.7	17.0	16.4	16.9	M	?	112.0
9/15/99	JPL218	-13.3	-16.4	-16.2	15.0	14.7	15.6	M	2	138.5
9/18/99	STL113	-13.8	-17.1	-17.4	15.5	14.5	15.1	F	2	133.0
9/30/99	JPL251	-13.8	-16.3	-16.6	15.9	14.9	15.3	F	2	131.5