NUTRITIONAL CONSTRAINTS ON THE SOUTHERN SEA OTTER IN THE MONTEREY BAY NATIONAL MARINE SANCTUARY

and a comparison to sea otter populations at San Nicolas Island, California and Glacier Bay, Alaska.

Joint Final Report to

Monterey Bay National Marine Sanctuary

(and **Monterey Bay Sanctuary Foundation**)

and the **Marine Mammal Commission**.

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Executive Summary

The Monterey Bay National Marine Sanctuary in California, which extends from Marin in the north to Cambria in the south, encompasses much of the range of the threatened southern sea otter, *Enhydra lutris nereis.* The growth of this otter population has slowed and/or stalled due to increased mortality, and many otters die of disease. We reasoned that nutrition might constrain this population if availability of high quality food resources was limited. Sea otters are well known for their ability to locally reduce the abundance of preferred prey such as abalone and sea urchins. Nutritional deficits might affect body condition, reproductive performance, immune function, susceptibility to disease and ability to withstand contaminant or toxin loads, and may ultimately contribute to reduced survival. However, sea otters in MBNMS feed exclusively on large marine invertebrates, and the nutritional consequences of this diet had not been studied prior to the work described in this report.

This project examined the nutritional constraints faced by southern sea otters in and adjacent to the sanctuary via a combined analysis of foraging data and nutritional assessment of all major prey species. These two components involved extensive synergy, as the foraging observations indicated which prey taxa to target for detailed nutritional analysis, while assessment of the edible portion, biomass, and energy content of prey allowed more accurate assessment of the energy returns per unit of foraging time. Estimated nutritional quality of diets was compared among otters in MBNMS, and between otter populations in MBNMS, San Nicolas Island, CA and Glacier Bay, AK. The latter two were selected as examples of expanding populations in apparently good condition. We also compared prey and diet composition to recommended nutrient levels for domestic carnivores, the closest available model for sea otter nutrient needs.

From the comparison to the San Nicolas and Glacier Bay populations, we concluded that sea otters in MBNMS are faced with food and/or nutritional shortages. Sea otters at San Nicolas and Glacier Bay have access to abundant prey resources and are characterized by large body size and good body condition, higher rates of energy gain when foraging, reduced foraging times per 24-hour day, and low dietary diversity at the population level. The central California sea otter population, in an environment where populations of some preferred prey items have been reduced, is characterized by smaller body size, poor body condition, lower rates of energy gain when foraging, increased foraging times per 24-hour day, and high dietary diversity at the population level.

Diets at the population level are composed of the diets of many individuals. Although dietary specialization is always advantageous to sea otters due to increased efficiency in handling prey, individuals within a population may tend to specialize on the same or different suites of prey. Theory predicts that individuals within a population will tend to specialize on different suites of prey as resources become less abundant. At San Nicolas, all individuals (n = 11) fed predominantly on urchins, with smaller amounts of crab, lobster and snails. Although the otters in Glacier Bay were not individually identifiable, the population-level diet consisted largely of clams, suggesting that there was relatively little dietary specialization among individuals. In contrast, individual otters in MBNMS (n = 63) had extremely varied diet that could be grouped into several distinct types. These diet types could be categorized by 3 prey sizes (large = 1, medium =2, small = 3) and 6 predominant prey types: abalone and crabs (Type 1a), *Cancer* crabs (Type 1b), kelp crabs and rocky bottom prey (Type 2a), urchins and mussels (Type 2b), clams and sandy bottom prey (Type 2c) and trochid snails (Type 3a). While these types could be clearly distinguished statistically, some prey (such as crabs and urchins) were found in most diet types.

A survey of prey nutrient composition in California and Alaska (more than 700 samples of 76 taxa) revealed that prey energy content (on a dry mass basis) was primarily determined by two factors: the amounts of mineral matter (ash) and fat in the edible portion. Across a wide range of bivalves and gastropods, fat content was uniformly low, so that for these taxa ash was the primary determinant of energy, with a strong negative correlation between ash and energy. Some bivalves had remarkably high ash content,

apparently due to accumulation of sand or sediment; these species were low in calcium. Among gastropods, high ash was largely due to the inclusion of shell fragments when the prey were crushed; such samples had very high calcium content. Decapods were also high in calcium and ash, presumably due to calcification of the exoskeleton, but some species (such as sand crabs) accumulated modest amounts of fat at least seasonally. The edible portion (gonads, ceca, viscera) of echinoderms (such as urchins and sea stars) was typically high in fat, regardless of season.

Our analysis indicated that fat may be a limiting resource for sea otters for several reasons. Many prey taxa are very low in fat, resulting in low energy content and the need for otters to consume large amounts of prey to meet energy requirements. Diets high in snails (3a) were particularly low in fat and energy on a dry basis. Low fat is also associated with low levels of fat-soluble vitamin A and of essential fatty acids of the omega6 family. Estimated levels of vitamin A and omega6 fatty acids were so low in some MBNMS diet types that one would predict that these could produce nutritional deficiency. MBNMS diets were also apparently lower in vitamin A than diets at Glacier Bay. The possible consequences of low dietary vitamin A in sea otter diets warrant investigation given the importance of vitamin A for proper immune system function. However, carotenoids such as β- carotene and γ-carotene were abundant in most sea otter prey, and it is not known whether sea otters can utilize carotenoids as a source of vitamin A.

Other nutrients were also of concern in MBNMS diets. The B vitamin thiamin was low to marginal in all MBNMS diet types, although further study is needed because vitamin losses may have occurred during analysis. Estimated thiamin levels were higher in San Nicolas and Glacier Bay diets. High levels of calcium were prevalent in many prey types (especially gastropods and decapods). The high calcium levels found in the diets of MBMNS otters (from 4.8% in the urchin and mussel diet type to 19% in the snail diet type) could produce malabsorption problems for other minerals such as phosphorus and zinc. This is of particular concern in MBNMS diet types that were deemed marginal or low in phosphorus and/or zinc, such as the kelp crab (2a), urchin and mussel (2b) and snail (3a) diets. Otters at MBNMS consumed significantly more calcium, on average, than otters at San Nicolas, and the Glacier Bay diet was lower still (1.9% calcium). The

normal calcium:phosphorus ratio (2:1) at Glacier Bay was in stark contrast to the MBNMS ratio that averaged 8:1 but was as high as 40:1 in the snail diet (3a).

Our analysis suggests that the six specialized diets adopted by MBNMS otters are not all of equal nutritional value. In particular, the snail diet (3a) adopted by some otters appeared to be low, marginal or excessive in many nutrients, including fat, omega6 fatty acids, calcium, phosphorus, iron, zinc, thiamin, vitamin B6, and vitamin A. Any one or a combination of these nutritional imbalances could impair reproductive performance, immune function, resistance to disease, or ability to deal with toxin or contaminant loads.

We believe that additional research is needed on the health status and fate of otters of differing diet histories. Such an analysis may be able to take advantage of new indicators of the diet history of individual animals, such as stable isotope analysis and fatty acid analysis, in addition to or instead of traditional observational and radiotelemetry techniques. We have demonstrated differences in stable isotope profiles of sea otters in different populations and in the estimated fatty acid composition of different otter diet types. These techniques offer the promise of being able to indicate diet type of otters captured live or obtained postmortem and warrant further study as means of monitoring sea otter populations in the MBNMS.

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Part 1. Introduction and background to study

Sea otter life history

The sea otter, *Enhydra lutris,* is one of the largest otters but one of the smallest marine mammals. It is an important predator in its nearshore ecosystem, where it eats many species of large marine invertebrates, such as abalones, urchins, and crabs. Due to its small body size (averaging 45-65 pounds in California for females and males, respectively), lack of insulating blubber, and elevated metabolic rate, the sea otter must capture a substantial amount of invertebrate prey (about 25% of its body weight each day), to keep warm and remain healthy in its cold marine environment. Sea otters are known to reduce populations of their preferred prey species, and their ability to limit the abundance of dominant invertebrate herbivores make them a classic keystone species with major effects on the ecology of nearshore ecosystems. A detailed account of their biology can be found in Riedman and Estes (1990).

Sea otters once ranged across the northern Pacific from Japan to Baja California but populations were drastically reduced by 18th and 19th century fur traders. After protection in 1911, many remnant populations increased and re-colonized large parts of their former range, but these populations have recovered at different rates. Three subspecies of sea otters are currently recognized (Wilson et al. 1991), based on geographical variation in cranial morphology. The southern sea otter (*Enhydra lutris nereis*) also has several unique mitochondrial DNA haplotypes not found in the two northern subspecies (Sanchez 1992; Cronin et al. 1996). Much of the southern sea otter population occurs within the Monterey Bay National Marine Sanctuary (MBNMS). It is considered threatened under the Endangered Species Act because of its small size, limited distribution, and vulnerability to oil spills (U.S. Fish and Wildlife Service 2003). This population has recovered more slowly than expected after receiving protection. Its growth rate has never exceeded 5%, although other sea otter populations in Washington and Alaska have increased at higher rates ranging from about 9% to near the theoretical maximum rate of 20% (Estes 1990). The causes of the California population's historically slow growth rate are unclear. In the late 1990s, the population declined to a low of about 2000. More recently, it has increased to around 3000 otters but growth has

all but ceased in the center of the range, and in MBNMS there has been essentially no overall increase in population size over the last 12 years (USGS unpublished survey data). Reproductive rates in the population are similar to those in other sea otter populations, so lack of population growth is due to elevated mortality rates, even among prime-aged adults (Estes et al. 2003a).

Judging from necropsied carcasses that wash ashore, the population suffers from chronically high levels of mortality due to infectious disease, particularly parasitic diseases for which the sea otter is not the normal host (Thomas and Cole 1996; Kreuder et al. 2003; Mayer et al. 2003; Jessup et al. 2004). These diseases are not transmitted between otters, so are not clearly linked to population density but have been assumed to result largely from contamination of nearshore habitats with infectious products from the normal hosts of these parasites, such as cats and opossums (Kreuder et al. 2003; Jessup et al. 2004). However, a high proportion of deaths from disease could be a symptom of inadequate nutrition as nutritional deficiencies exert profound effects on immune function (Calder 2002) and other physiological processes. The effects of poor nutrition could be widespread, affecting not only body condition, but also reproductive success, disease resistance, and ultimately survival.

The extent to which sea otter diets have changed along the California coast since the arrival of Europeans is not known, but it may be substantial. In addition to the impacts of human fisheries, sea otter predation itself can greatly reduce the abundance and/or size of preferred prey in both rocky and sandy habitats (Kvitek et al. 1988; Reidman and Estes 1990; Estes and Duggins 1995; Jolly 1997; Fanshawe et al. 2003). Food resources ultimately limit population growth in northern sea otter populations (Garshelis et al. 1986; Estes 1990; Estes et al. 1996; Bodkin et al. 2000) and there is accumulating evidence that food limitation contributes to the problems of the mainland California sea otter population (Tinker 2004; Bentall 2005).

Body condition is declining as shown by decreasing mass to length ratios over the last 31 years (Estes et al. 2003a). Since the 1980s, foraging time per day has increased (Ralls and Siniff 1990; Tinker 2004) while female survival has decreased (Siniff and Ralls 1991; Tinker 2004). Sea otters in the population at San Nicolas Island, where food resources are much more abundant than on the mainland coast, are larger and in better

body condition than those along the mainland coast, forage fewer hours per day and have higher survival rates (Tinker 2004; Bentall 2005).

The extent to which individual otters specialize on particular species of prey is thought to be a function of prey availability, increasing as sea otter populations become more crowded and reduce the abundance of their preferred prey (Estes et al. 2003b; Tinker 2004). Although the mainland population as a whole consumes many species of prey, individual sea otters usually specialize on only 1- 4 prey types (Riedman and Estes 1990; Ralls et al. 1995, Estes et al. 2003b; Tinker 2004). These individual differences in prey consumption do not appear to be based on genetic or morphological differences among individuals (Tinker 2004), nor can they be attributed to passive responses to environmental variation, as individuals with completely overlapping home ranges can have very different diets (Tinker et al. 2007). Rather, this phenomenon appears to represent behaviorally-mediated diet specialization, with prey preferences likely transmitted culturally from mother to pup (Estes et al. 2003b). However, individuals can learn to eat new types of prey, as demonstrated by the diets of sea otters at San Nicolas Island, California, which no longer resemble those of their ancestors captured along the central coast of California in the late 1980s and taken to San Nicolas to found a new population (Bentall 2005).

Using foraging data from 60 radio-tagged sea otters captured in and near MBNMS, Tinker (2004) found that individual diets were exceedingly varied, but could be grouped into three general types. The estimated rate of energy gain while foraging was low for the population as a whole but showed a high degree of variation. Although the three foraging strategies had different mean rates of energy gain, the probability of exceeding a critical rate of energy gain on any given day was similar for the three strategies because the mean and intra-individual variation in rate of gain were positively correlated. Unlike the mainland otters, the 11 radio-tagged otters studied at San Nicolas Island had similar diets (Bentall 2005).

Are sea otters potentially prone to nutritional inadequacies?

Sea otters have a long history of specialization on marine benthic invertebrates (Riedman and Estes 1990). Obligate terrestrial carnivores, such as domestic cats, have

evolved a suite of metabolic and biochemical traits that reflect the nutritional properties of their vertebrate prey (Morris and Rogers 1983; National Research Council 1986; Allen et al. 1996b), and it is possible that sea otters have similarly adapted to the nutritional properties of marine benthic invertebrates. This could involve loss of some biosynthetic abilities, unusual nutrient requirements, and, as in cats, an inability to adapt to diets much different in composition than typical prey.

A parallel situation exists among carnivores that depend on insect prey. The low calcium content of terrestrial invertebrates (Allen and Oftedal 1989) may limit reproduction in both bats and birds, and require such insectivores to seek out prey with high calcium in their gut contents (Bilby and Widdowson 1971; Barclay 1994, 1995; Hood 2001). Thus the assumption that dietary energy is the only limiting nutrient in sea otters (e.g., Costa 1978) may not be correct for a species that feeds entirely on invertebrates (Barclay 1994, 1995).

Little is known about the nutrient composition of benthic invertebrates. Although benthic invertebrates often have a calcareous shell or exoskeleton, the soft parts eaten by humans (and otters) are low in calcium, have an inverse calcium:phosphorus ratio, and may be low in manganese (USDA Agricultural Research Service 2003). Bivalves appear to be low in vitamin E, while gastropods and crustaceans may be low in vitamin A. Some species are not only low in thiamine, but contain thiaminases that destroy this vitamin (Allen et al. 1996b). Prey species that are low in lipids are likely to be low in essential fatty acids and fat-soluble vitamins. Differences in enzyme systems, anatomical structures and metabolic pathways among invertebrates may produce quite different tissue levels of trace elements, amino acids and water-soluble vitamins, such that differences in the diets consumed by different individuals may have substantial impacts on otter nutrition.

Comparative nutritionists recognize that animals ingesting foods dissimilar in composition to their own tissues have a greater likelihood of suffering from nutritional problems (Allen et al. 1996b). Such problems are most often seen during periods of intensive nutrient investment in tissue or product synthesis, such as during early growth or lactation. One would predict nutritional difficulties to be most prevalent at these life stages. In fact, sea otters in California appear to have high pre-weaning mortality

(Riedman et al. 1994; Siniff and Ralls 1991) as well as significant mortality among reproductively mature females (Estes et al. 2003a). Female mortality is greatest in summer when a large proportion of females are at the end of lactation.

Nutrient deficits may be evident from frank deficiency signs, such as myopathies, neurologic disorders, anemias or bone abnormalities, or they may cause more generalized impairment (such as of immune or reproductive function). The discovery that pathologic deterioration of cardiac musculature is a primary cause of death in adult sea otters in MBNMS (Kreuder et al. 2003) is particularly disturbing. Although the etiology of this cardiomyopathy in sea otters is poorly understood, cardiac and other myopathies can be produced by vitamin E and selenium deficiency in a wide range of species, including marine carnivores (Machlin 1980; Oftedal and Boness 1983; McDowell 1989). Vitamin E requirements are increased by intake of polyunsaturated fatty acids (as are typical of marine prey [Iverson 1993, Iverson et al. 1997a,b]) and by exposure to natural or artificial (contaminant) oxidants; the tissues affected may be influenced by other nutrients, such as sulfur amino acids (Machlin 1980). Given that such otter prey types as mussels and clams are reported to be low (<30 IU/kg) in vitamin E (USDA Agricultural Research Service 2003), the possibility that otter cardiomyopathy is correlated to vitamin E deficiency seemed plausible; unfortunately, the available data on vitamin E in sea otter prey is limited.

In both animals and humans nutritional stress associated with marginal nutrient intakes is much more common than obvious clinical deficiency syndromes (such as scurvy from vitamin C deficiency or rickets from calcium or vitamin D deficiency), and may produce impaired immune function, increased susceptibility to disease and increased mortality (e.g., Cunningham-Rundles 2002; Tompkins 2002). Nutrients are primary factors in the regulation of the immune response and nutrient shortage may compromise tissue, cellular and humoral defenses (Calder et al. 2002). Both innate and adaptive immune systems are affected. The nutrients known to be important to immune function in humans and animals include protein, specific amino acids (such as arginine and sulfur amino acids), essential and other polyunsaturated fatty acids, vitamin A, antioxidant nutrients (e.g., vitamin E, vitamin C, carotenoids) and trace minerals, especially those involved in protective enzyme systems, such as iron [catalase], selenium [glutathione

peroxidase] and manganese, copper and zinc [all in superoxide dismutase]) (for references see review chapters in Calder et al. 2002).

Unfortunately, the recent debate on potential causes of morbidity and mortality in sea otter populations (e.g., Reeves 2002; Estes et al. 2003a; U. S. Fish and Wildlife Service 2003) has largely overlooked nutrition, despite the passing acknowledgement that "unrecognized factors, such as inadequate nutrition, may be undermining the health and productivity of southern sea otters" (Reeves 2002). Thomas and Cole (1996) warned that sea otters appear to be unusually vulnerable to mortality from a wide variety of diseases that would not be expected to cause more than sporadic mortality in other species. A similar situation is manifest in another threatened species on which one of us (OTO) has worked, the desert tortoise. A horrific series of population crashes were initially associated with a respiratory disease that it was thought had been introduced into the populations (Jacobsen et al. 1991; Brown et al. 1999). However, it now appears that tortoises are nutritionally constrained due to a shortage of plants of high nutritional quality, forcing them to switch to less profitable foods. A consequent reduction in nutritional status may underlie the vulnerability of tortoise populations to epidemic outbreaks of upper respiratory and other infectious diseases (Oftedal 2002a,b; Oftedal et al. 2002). If anthropogenic effects (harvesting, invasive species, sedimentation, pollution, etc.) have reduced favored otter prey, sea otters could be in a parallel situation.

In conclusion, sea otters appear potentially prone to nutritional inadequacies given the unusual specialization on invertebrate prey, the changing densities of prey over time, and the variability in the prey species consumed by individual otters. Available data on mortality associated with reproduction, adult condition (mass to length ratio), prey composition, susceptibility to infectious disease, and prevalence of specific pathologic syndromes are all consistent with nutritional inadequacy. However, in the absence of any research in this area, we can only speculate about the importance of nutrition to recovery of sea otter populations.

Objectives of the Research

Our understanding of the population-level consequences of dietary specialization and of the apparent low mean rate of energy gain by southern sea otters was hampered by

a lack of detailed information about the variation in nutritional and energetic composition among and within prey species. To address this shortcoming, we initiated a study, with support from the Monterey Bay National Marine Sanctuary (MBNMS) and the Monterey Bay Sanctuary Foundation (MBSF), to assess the nutrient composition of prey species consumed by sea otters along the coast of mainland California. Our overall goal was to determine if southern sea otters are subject to nutritional constraints, either on individual, seasonal or population levels, which could help explain patterns of reproduction, morbidity, mortality and population growth. This work had four specific objectives: 1) to examine geographical and seasonal variation in proximate composition (water, fat, protein) and major minerals (calcium, phosphorus, magnesium) in the 10 major prey types consumed by mainland southern sea otters; 2) to measure additional nutrients (vitamin E and other vitamins, trace minerals, amino acids, and fatty acids) in these 10 major prey items in the summer, which is the season of maximal reproductive stress and adult sea otter mortality; 3) to estimate the nutrient composition of individual otter diets, based on our multiyear observations of foraging by individually known, radio-tagged otters and to correlate nutrient composition to age/sex differences, foraging location, reproductive history and, where available, data on morbidity and mortality; and 4) to compare the nutritional data obtained in Objective 3 with data for an expanding otter population in Glacier Bay, Alaska, where otters appear to have adequate food resources, the population is expanding and otters are larger and in better body condition than those in mainland California.

In the course of working towards these objectives, we found that processing of the various prey species to separate out the edible portions (from a sea otter's point of view) before nutritional analyses was much more time-consuming than we had anticipated. This part of the work necessitated devising protocols for a wide variety of invertebrate species so we could handle them as similarly as possible to the way in which an otter would handle them. Due to the large amount of technician time required for this work, it became apparent that the initial budget from MBSF would be insufficient to allow us to complete all the work we had planned, particularly the work in Alaska. In addition, the USGS, under the leadership of Jim Estes, began radiotelemetry work on sea otters at San Nicolas Island, where otters were translocated in the mid-1980s. The otters at San Nicolas were

thought to have abundant food resources and would provide an ideal contrast with the mainland California population. Finally, Tim Tinker, as part of his dissertation work (Tinker 2004), did a comprehensive review of the available information on the energy content of various sea otter prey species and the allometric relationships between the size of a prey species in terms of linear dimensions (which is all that can be observed in the field) and its edible biomass. He found that information on these topics was not available for many of the prey species on which sea otters were feeding in MBNMS, and it became apparent we would have to generate the missing information to accomplish our research goals.

Accordingly, in 2004 we sought and received funding from the Marine Mammal Commission (MMC) for three projects that would extend and complement the work already funded by MBSF: 1) fieldwork in Glacier Bay, Alaska, to standardize and enhance prey collection efforts there so that field protocols in Alaska would match those used in California; 2) collection and laboratory analyses of sea otter prey at San Nicolas Island, so we could add this population to our study; 3) additional laboratory work to fill the major data gaps in our knowledge of the biomass and energy content of prey species consumed by sea otters.

Because the work under both grants was tightly integrated and directed towards our overall goal of understanding the importance of nutritional constraints for the recovery of the sea otter population along the central California coast, this report presents the results of the work funded by the MBNMS and the MMC.

Part 2. Nutritional evaluation of sea otter prey. I. Energetic constituents

INTRODUCTION

It has long been recognized that sea otters have high energy requirements, stemming from a high metabolic rate, a lifestyle involving virtually continuous immersion in cold water, low levels of subcutaneous body fat that could provide thermal insulation and surface barrier (air trapped in very dense hair) that becomes compressed during diving and thus loses its value as insulation (e.g., Costa 1978). Otters therefore must expend substantial energy for basal metabolism, activity and thermoregulation, and this energy must be obtained from invertebrate prey that are generally low in fat (USDA Agricultural Research Service 2003).

A primary objective of this study was to obtain a much broader database on the proximate constituents of otter prey that influence energy content, such as water, fat, carbon (a measure of organic matter), nitrogen (a measure of crude protein) and ash (aggregate mineral matter devoid of combustible energy). Of these, water and ash dilute energetic constituents, increasing prey mass without providing usable energy. Carbon is a reflection of total organic matter and thus is, in a sense, the alternative to the inorganic constituent, ash. Protein is a good source of gross energy (ca. 5.6 kcal/g dry matter [DM]) although approximately 1.6 kcal/g is lost during digestion and excretion of nitrogenous waste as urea (Kleiber 1975). However, some nitrogen in crustaceans and other prey may be in the form of chitin, which is likely indigestible in sea otters as it is in some, but not all, insectivorous mammals (Allen 1989). Chitin may be assessed by an acid-detergent fiber analytic system, but this needs further validation prior to broad scale use across the diverse array of invertebrates (Allen 1989), and thus we chose not to analyze chitin content. Fat is typically the most important determinant of energy content of prey, as the gross energy content of fat (ca. 9 kcal/g) is much greater than that of carbohydrate (ca. 4 kcal/g) or protein. Fat is also highly digestible, and is completely combustible to carbon dioxide and water, so unlike protein it does not create an energetic excretory burden. The total amount of energy available upon combustion is determined by bomb calorimetry and is termed the gross energy. Note that the gross energy is an overestimate of the amount of energy actually available to a predator, as it does not account for energy losses

during incomplete digestion of prey or metabolic losses such as the energy loss associated with urea excretion (Kleiber 1975). However, the soft tissues of invertebrate prey are undoubtedly highly digestible, as are vertebrate muscle and internal organs (Allen et al. 1996; Robbins 1993), and thus the errors are much smaller than in diet items with large indigestible fractions, such as most plant materials.

The final result of all components in prey is that a certain amount of energy is available to the predator once prey are captured, ingested, digested and metabolized. Each of these steps requires time as well as energy expenditure by the predator. We will discuss the time requirements of otter foraging based on behavioral observations (Part 5), and calculate the energy returns (in terms of ingested energy) per unit time expenditure. It is unlikely that corrections for digestibility (digestible energy) or metabolic losses (metabolizable energy) would alter conclusions about the energetic consequences of foraging on different types of prey, although it would be useful to obtain more data via digestibility trials with captive sea otters.

Dietary fat is also important as a primary determinant of the intake of essential fatty acids, that is fatty acids that cannot be synthesized by mammalian carnivores but are required for cell membranes, immune function, and other functions (Part 4). Fat also has an impact on the amounts and absorption of fat-soluble vitamins (Gershoff 1957; Jalal 1998; Jeanes et al. 2000). Thus diets that are low in fat may pose additional problems to a carnivore besides the effect on dietary energy intake.

Otters also require protein in the form of essential amino acids and non-essential amino acid nitrogen, which will be discussed in Part 4. For practical purposes, however, sea otter prey are sufficiently rich in protein that it is unlikely that sea otters ever face a protein deficit. Even rapidly growing puppies and kittens, and lactating bitches and queens, do not require more than about 22.5% crude protein in the diet (as per recommended levels by the Committee on Animal Nutrition of the National Research Council of the National Academies [National Research Council 2006]), and otter prey contain levels far above this (see below).

METHODS

Prey collection

Species selection and collection sites: A primary objective of this project was to examine the nutrient composition of the major prey types of sea otters in and near the Monterey Bay National Marine Sanctuary (MBNMS), including, for some constituents, the potential effect of seasonal and geographic variation. A first step was to define the "Top Ten" prey types consumed by sea otters in MBNMS. Tinker (2004) aggregated otter prey into prey groups that varied in frequency of occurrence in sea otter diets in the northern and southern parts of MBNMS (Table 2.1). However, from a nutritional perspective prey importance is better characterized by the proportion of overall biomass contributed by each prey group. Based on data summarized by Tinker (2004), the major prey categories (on a fresh biomass basis) for sea otters in central California are: 1. cancer crabs (21%), 2. clams (16%), 3. abalone (13%), 4. innkeeper worm (8%), 5. purple urchin (8%), 6. mussels (8%), 7. turban snails (7%), 8. kelp crab (7%), 9. other crabs (5%), 10. octopus (4%), and 11. sea stars (3%). Since the diets of individual otters involve differing combinations of prey, some prey types (such as clams and snails) are particularly important for individual otters. We defined our Top Ten list for geographic and seasonal sampling as:

Crabs
1. Large cancer crabs (*Cancer magister/ C. antennarius*)
2. Kelp crabs (*Pugettia producta*)

Bivalves
3. Mussels (*Mytilus californianus*)
4. Large clams (*Saxidomus nuttalli/Tresus nuttallii*)
5. Small clams (*Protothaca staminea, Macoma* spp.)

Gastropods
6. Abalone (*Haliotis rufescens/H. cracherodii*)
7. Turban snails (*Tegula funebralis/T. montereyi/T. brunnea/T. pulligo*)

Echinoderms
8. Sea stars (*Pisaster ochraceus/P. giganteus*)
9. Urchins (*Strongylocentrotus purpuratus*)

Echiurid worms
10. Innkeeper worms (*Urechis caupo*)

Table 2. 1. Prey groups in sea otter diets, their constituent taxa and frequency of occurrence in sea otter diets in southern and northern MBMNS.[1]

Prey Group	Common Name	Latin Name or Taxonomic group	San Simeon % occurrence	Monterey Bay % occurrence
1. Abalone	Abalone	*Haliotis cracherodii, H. rufescens,* and other spp.	0.54	1.94
2.Cancer crab	Cancer crabs	*Cancer spp.*	10.43	9
3. Kelp crab	Kelp crab	*Pugettia producta (*and *P. richii)*	20.05	8.4
4. Crab (un-id)	Crabs, unidentified	Various decapods	8.89	5.97
5. Urchin	Purple urchin	*Strongylocentrotus purpuratus*	9.11	16.11
	Red urchin	*Strongylocentrotus franciscanus*	0.02	0.21
6. Clam/bivalve	Clams, unidentified	Various pelecypods	14.25	10.57
	Giant rock-scallop	*Crassadoma gigantea*	0.11	0.21
	Nuttall cockle	*Clinocardium nuttalli*	0.01	0.28
	Pacific gaper clam	*Tresus nuttalli*	0.24	0.03
7. Mussel	California mussel	*Mytilus californianus*	8.51	17.76
8. Sea star	Sea stars	*Pisaster spp*	3.94	0.82
9. Snail	Tegula snails	*Tegula spp.*	10.97	17.3
10. Sand dollar	Eccentric sand dollar	*Dendraster excentricus*	0.91	1.58
11. Sand crab	Sand and mole crabs	*Emerita analoga, Blepharipoda occidentalis*	0.42	2.09
12. Worm	Fat innkeeper worm	*Urechis caupo*	3.38	4.6
	Worm, unidentified	various polychaetes	0.21	0.72
13. Cephalapod	Octopus	*Octopus spp.*	0.36	0.71
	Market squid	*Loligo opalescens*	0.06	0.12
14. Small rocky	Small kelp fauna	Various small invertebrates	7.2	0.22
	Chitons	*Mopalia, Tonicella* and other	0.07	0.58
	Limpets	*Diodora aspera* and other taxa	0.01	0.39
	Isopod	Various isopod species	0.13	0.01
		Total, all taxa > 0.01%	99.82	99.62

1. From Tinker (2004).

Our goal was to collect species in each of these 10 categories in 4 seasons (winter, spring, summer and fall) in both northern (Monterey Bay and vicinity) and southern (Piedras Blancas to southern Estero Bay) portions of the range of sea otters in central California. Most of this area falls within the Monterey Bay National Marine Sanctuary, although areas south of San Simeon fall outside Sanctuary boundaries. As these prey species are distributed among different habitat types (intertidal pools, flats and beaches; subtidal muddy, sandy and rocky habitats with or without a kelp forest) and must therefore be collected by a variety of procedures, obtaining prey from all 10 categories each season in the north and south required an intensive effort up and down the central coast. The actual collection areas, and seasons in which they were visited, are presented in Table 2.2.

A second important objective was to compare diets of otters in the Monterey Bay National Marine Sanctuary and vicinity to diets of otters in recently colonized areas with expanding populations. Two areas were chosen for comparison: San Nicolas Island, California, where southern sea otters (*Enhydra lutris nereis*) were introduced in the 1980s and Glacier Bay National Park, Alaska, which Alaskan sea otters (*Enhydra lutris kenyoni*) colonized about 1995. The current population at San Nicolas is less than 50 individuals, is quite small in relation to the available habitat, and is growing at approximately 9% per year (Bentall 2005). The population in Glacier Bay has increased from near zero in 1995 to around 2,300 animals in 2004 , although some of the increase is due to otters moving into the area rather than reproduction. This population is thought to be below carrying capacity (Bodkin et al. 2007b)). Due to the remoteness of these sites, and difficulties of access in certain seasons (e.g., winter in Glacier Bay), coverage of all seasons was not attempted for either San Nicolas or Glacier Bay. However we did attempt to collect all of the primary prey of sea otters at these locations and obtained foraging data from Bentall (2005) for San Nicolas and from J. Bodkin and colleagues for Glacier Bay.

A third objective was to sample additional prey items that may be important in individual sea otter diets in an attempt to develop a catalogue of the nutritional resources available to sea otters and other predators on invertebrates. This objective was inherently opportunistic rather than targeted, and the analysis of samples for this purpose was a

Table 2.2. Areas and seasons of sea otter prey collections.

Region	Collection Areas	Habitat type	Season
MBNMS, North	Half Moon Bay	Subtidal (crab trapping)	Summer and fall 2004
	Elkhorn Slough	Mud flats, beach	Summer and fall 2004, Winter and spring 2005
	Monterey Bay, area along shoreline	Subtidal (diving)	Summer and fall 2004, Winter 2005
	Monterey Bay, offshore area	Subtidal (diving)	Summer and fall 2004, Winter and spring 2005
	Pebble Beach, China Rock / Point Joe area	Tide pools	Summer and fall 2004, Winter and spring 2005
	Stillwater Cove	Subtidal (diving)	Summer 2004
MBNMS and vicinity, South	San Simeon to Piedras Blancas area	Tide pools, subtidal kelp (diving)	Summer and fall 2004, Winter and spring 2005
	Rancho Marino	Tide pools	Summer and fall 2004, Winter and spring 2005
	Cayucos Point	Tide pools, beach	Summer and Fall 2004
	Estero Bay (from Pt. Buchon to Pt. Estero)	Subtidal kelp (diving; crab trapping)	Summer 2004, Winter 2005
	Avila	Beach	Spring 2005
	Morro Bay harbor and beach area	Mud flats, beach, subtidal mud (diving)	Summer and fall 2004, Winter and spring 2005
SNI	San Nicolas Island	Tide pools Subtidal kelp (diving; crab trapping)	Winter, spring and fall 2005
SE Alaska	Glacier Bay National Park, Alaska	Tide pools, intertidal beaches Subtidal (diving, crab trapping)	Summer 2004 Spring 2005

lower priority. More than 110 species of invertebrates have been collected from all sites (Appendix I), but not all have been analyzed to date, due to limitations on sample mass, technician time and funding.

Species were identified via descriptions, illustrations and keys in a variety of sources, including Morris et al. (1980), Kozloff (1996) and Coan et al (2000).

Collection Methods: The necessity of obtaining a wide range of prey types, whether for Top Ten collections, for comparisons to other areas or for the nutritional resource catalogue, resulted in a wide range of collection methods in diverse habitats. We can identify seven primary sources of prey samples:

i. Tide pools – periods of seasonal low tides were identified, and areas of known tide pools were visited for collections by hand. Collectors waded through tide pools seeking snails, sea stars, urchins, black abalone, limpets, chitons, shore crabs, hermit crabs and occasional kelp crabs. Prey were hand captured, using a knife or flat blade to pry loose prey that were firmly attached to the substrate.

ii. Sand beaches – collectors dug in exposed sand flats at seasonal low tides seeking sand crabs, mole crabs, cockles and Pismo clams.

iii. Mud flats – collectors dug in mud flats at seasonal low tides seeking small and large clams, fat innkeeper worms, and ghost shrimp. Sand dollars and moon snails were also found in mixed sand/mud flats at low tide.

iv. Kelp beds – scuba divers would deploy from a boat and search the kelp forest and surrounding rocky or sandy areas for snails, Cancer crabs, sea stars, urchins, red abalone, limpets, chitons, sea cucumbers and miscellaneous taxa. Kelp crabs were located both around kelp holdfasts and in the kelp canopy, including removal from kelp that was being harvested by the Monterey Abalone Co. as food for captive abalone.

v. Subtidal mud/other substrates – divers excavated fat innkeeper worms and large clams in subtidal mud in Morro Bay by injecting a stream of pumped water into a cylinder placed on the mud bottom. The mud would become

suspended, leaving the clams exposed. Fat innkeeper worms were dislodged and found on the surface of the mud bottom in the vicinity of the excavation site. In Alaska, intertidal clams were obtained by excavating sediments are varying size from silt to cobble/boulder. A suction dredge was used for subtidal clams and subtidal mussels were hand-picked by divers.

vi. Crab traps – California Department of Fish and Game employees and commercial fisherman deployed crab traps to obtain Cancer crabs and sheep crabs. Crabs in Glacier Bay were obtained both by trapping and by divers.

vii. Commercial products – farmed red abalone and wild red octopus were purchased from a commercial supplier who raised red abalone in pens beneath a pier in Monterey and where octopus were also incidentally trapped; commercially harvested market squid were obtained live from fishermen in Monterey.

Collections in California were made either by employees of the California Department of Fish and Game, or by others under the authority of scientific research permits for collection of invertebrates issued to Alice Green, Olav Oftedal and Tim Tinker by the California Department of Fish and Game.

Sample Processing and Analysis

Terminology: For purposes of this project, invertebrates collected in the field that were stored in individually inventoried bags are termed "collections". For purposes of analysis, it was often necessary to pool these collections to generate "samples", the basic unit of analysis. These samples were homogenized prior to analysis, and the small subsets of the material actually used for analysis are termed "subsamples". Note that a collection often contained multiple individual prey items, that a sample may have derived from multiple collections, and that for each sample multiple subsamples were actually analyzed.

Processing Prior to Analysis: In the field, collected invertebrates were placed in tubs or buckets containing cool, fresh seawater until they could be sorted by species, counted, weighed and frozen. Collections were initially frozen at –20° C (if access to freezers was available) or in a cooler filled with dry ice (during extended boat trips, as in diving trips to San Nicolas Island). A few collections destined for vitamin analysis were anesthetized

by chilling to near freezing in a CO_2 enriched atmosphere (generated by dry ice) and then processed into edible vs. inedible fractions prior to freezing. This step eliminated the need to thaw samples for processing, which can create conditions in which labile nutrients are degraded and lost (see Part 3). Collections destined for vitamin analysis were stored at –80° C prior to analysis. Otherwise collected material was kept frozen at –20 C.

A subset of each species was used to develop edible mass:length allometric relationships by weighing individual specimens and measuring their longest dimension(s) prior to further processing. Generally up to 50 individuals of a species were individually weighed and measured. Total mass of a prey item was corrected to an edible mass basis by subtracting the proportion of the mass removed during processing (see below). For each prey species power functions were fit to our empirical data of wet edible biomass (g) vs. maximum linear dimension (Table 2.3; Figure 2.1): these species-specific allometric relationships were used to estimate biomass consumption of otters from data on size of prey (see Part 5).

Collections were allowed to thaw at room temperature prior to processing. Any fluid in the bag derived from ice was considered extra-corporal water and discarded. However, any exudates subsequently produced were considered part of the animal and included in the fresh mass. In the event that material needed to be rinsed of debris or otherwise cleaned, artificial seawater was prepared and used so that we would not alter the inorganic contributions of adhering seawater, as otters eat prey that have been immersed in seawater prior to ingestion at the surface.

A processing protocol was developed for each prey type in order to separate the parts considered inedible (i.e. discarded by a foraging otter) vs. edible (i.e. chewed and swallowed). The decision as to what parts were edible was based on observer records and recollections from field observations of tens of thousands of foraging dives (as reported by field observers to Tim Tinker). Note that whatever otters were observed to eat was considered edible, regardless of its expected digestive or nutritional value; thus the edible fraction sometimes included parts of the exoskeleton or fragments of shell. For most bivalves and gastropods the shells (valves) were removed and the remainder considered edible. However since otters crush the shells of snails before eating them, we assumed

Table 2.3 Species-specific conversion functions for deriving wet edible biomass from maximum linear dimension of sea otter prey.

			Parameter a (Coefficient)			Parameter b (Exponent)		
Prey Species	**Taxonomic Group**	**Prey Group**	**Predicted**	**95% CI Lower**	**95% CI Upper**	**Predicted**	**95% CI Lower**	**95% CI Upper**
Cancer antennarius	Decapod	CancerCrab	0.00886	-0.03158	0.04930	2.1694	1.2275	3.1114
Cancer magister	Decapod	CancerCrab	0.00531	-0.00118	0.01180	2.1949	1.9551	2.4346
Cancer productus	Decapod	CancerCrab	0.00142	-0.00781	0.01064	2.4625	1.2347	3.6904
Hemigrapsis nudis	Decapod	Crab	0.00043	-0.00039	0.00125	3.0004	2.4976	3.5032
Pachygrapsus crassipes	Decapod	Crab	0.00088	-0.00056	0.00233	2.7998	2.3569	3.2427
Pugettia producta	Decapod	KelpCrab	0.00882	0.00146	0.01617	2.1525	1.9381	2.3669
Blepharipoda occidentalis	Decapod	Sandcrab	0.00044	-0.00010	0.00098	2.9221	2.5951	3.2491
Emerita analoga	Decapod	Sandcrab	0.00008	0.00001	0.00015	3.4326	3.1702	3.6949
Chlamys sp	Bivalve	Clam	0.00070	-0.00205	0.00345	2.4455	1.5230	3.3681
Clinocardium nuttalli	Bivalve	Clam	0.00017	-0.00009	0.00043	2.9868	2.5785	3.3950
Crassadoma gigantea	Bivalve	Clam	0.00024	-0.00099	0.00148	2.9071	1.7902	4.0240
Macoma nasuta	Bivalve	Clam	0.00046	-0.00031	0.00123	2.5212	2.0637	2.9788
Macoma secta	Bivalve	Clam	0.00012	-0.00007	0.00031	2.8563	2.4644	3.2482
Protothaca staminea	Bivalve	Clam	0.00046	0.00014	0.00077	2.7080	2.5248	2.8912
Tresus nuttalli	Bivalve	Clam	0.00000	0.00000	0.00000	4.3284	3.3803	5.2764
Mytilus californianus	Bivalve	Mussel	0.00341	-0.00354	0.01036	2.0464	1.5551	2.5377
Haliotis cracherodii	Gastropod	Abalone	0.00016	-0.00008	0.00039	2.9269	2.6105	3.2434
Haliotis rufescens	Gastropod	Abalone	0.00011	-0.00005	0.00027	2.9747	2.6664	3.2830
Lottia gigantea	Gastropod	Limpet	0.00025	-0.00003	0.00052	2.6971	2.4284	2.9657
Acanthina spirata	Gastropod	Snail	0.00078	-0.00008	0.00165	2.4938	2.1534	2.8341
Lithopoma gibberosum	Gastropod	Snail	0.00019	0.00011	0.00026	2.9687	2.8606	3.0769
Tegula brunnea	Gastropod	Snail	0.00026	-0.00002	0.00054	3.1304	2.7886	3.4722
Tegula eiseni	Gastropod	Snail	0.00722	-0.01388	0.02832	2.0711	1.1144	3.0278
Tegula funebralis	Gastropod	Snail	0.00187	-0.00076	0.00450	2.4998	2.0300	2.9695
Tegula montereyi	Gastropod	Snail	0.00016	0.00006	0.00027	3.1511	2.9625	3.3396
Tegula pulligo	Gastropod	Snail	0.00024	0.00004	0.00043	3.0425	2.7820	3.3030
Dendraster excentricus	Echinoderm	Sanddollar	0.00046	0.00008	0.00083	2.3715	2.1630	2.5800
Pisaster ochraceus	Echinoderm	Star	0.07764	-0.23596	0.39123	1.4003	0.5206	2.2800
Stronglyocentrotus franciscanus	Echinoderm	Urchin	0.00000	0.00000	0.00001	4.0647	3.5055	4.6239
Strongylocentrus purpuratus	Echinoderm	Urchin	0.00051	0.00031	0.00071	2.9035	2.7934	3.0136
Loligo opalescens	Cephalapod	Squid	0.00014	-0.00033	0.00062	2.6306	1.9554	3.3058
Nereis vexillosa	Annelid	Worm	0.00003	-0.00010	0.00016	2.2252	1.4609	2.9896
Urechis caupo	Annelid	Worm	0.05166	-0.04394	0.14727	1.2873	0.9491	1.6255

Note: Functional form used to convert linear dimension to biomass: $[\text{biomass}] = a \cdot [\text{diameter}]^{b}$

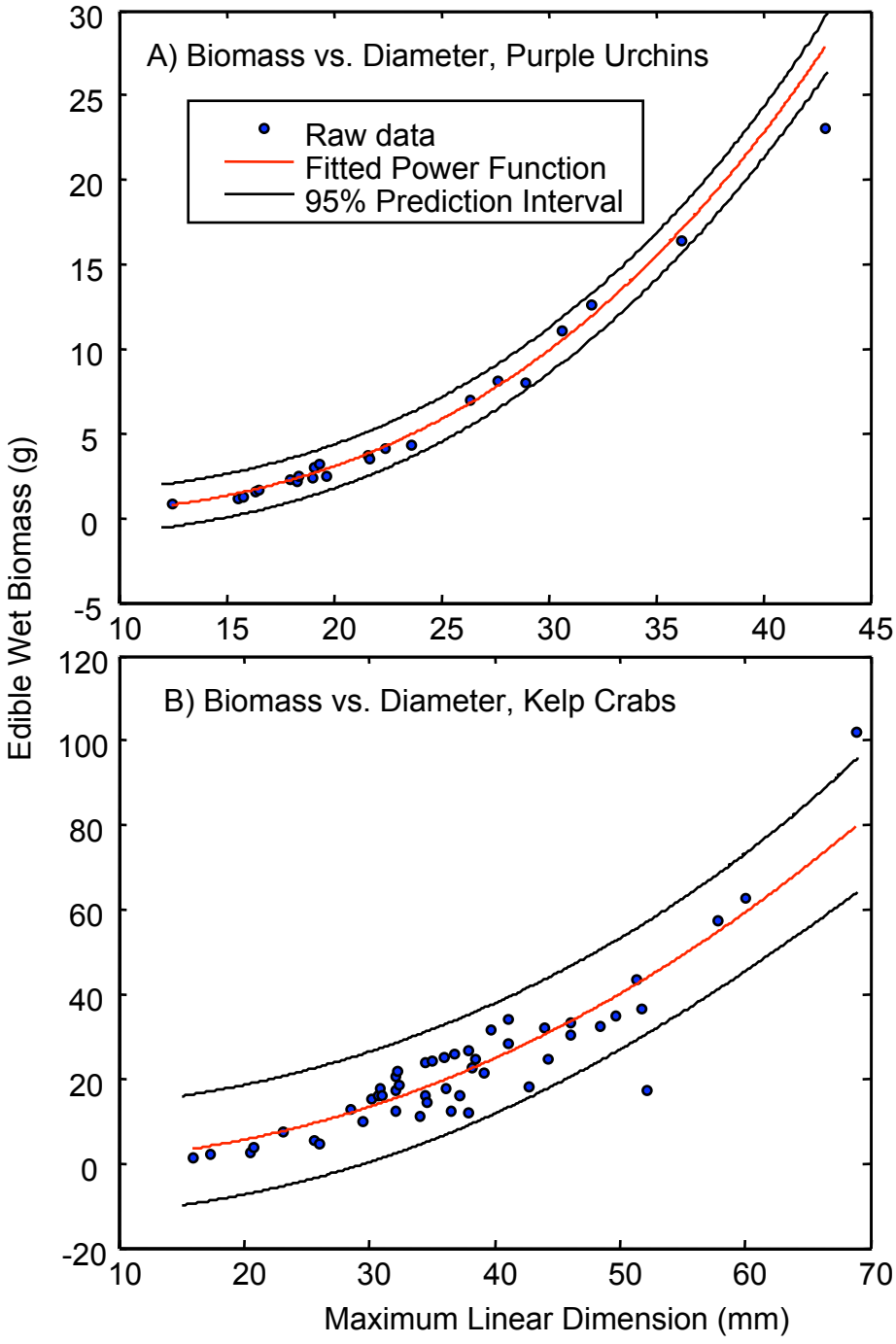

Figure 2.1 Species-specific power functions used to estimate wet edible biomass from maximum linear diameter of sea otter prey items. Functions are shown (for illustrative purposes) for A) purple urchins (*Strongylocentrotus purpuratus*) and B) kelp crabs (*Pugettia producta*).

that they would ingest small shell pieces. When crushing snails in the laboratory only large to medium sized pieces of shell were removed, and small shell fragments were considered part of the edible fraction. In large crabs (> 4 cm) the hard carapace (after extracting the soft parts) and large claws were removed as inedible, whereas the remainder, including the additional appendages, was considered edible. For small crabs, the entire animal was considered edible (see crab protocol, Appendix II). For urchins and sand dollars the test and spines were considered inedible, but the remaining soft visceral material was considered edible. For large stars (*Pisaster* spp.), otters have been observed to bite off arms and suck out the contents. In processing stars, the arms were cut off and the soft material (mostly ceca and gonadal material) was scooped out of the arms and body cavity for analysis (see star protocol, Appendix III). For fat innkeeper worms and other soft-bodied invertebrates the entire organism was considered edible.

For each pooled sample for analysis, the edible portion was calculated as edible mass / fresh mass. Individual prey samples were combined into pooled samples to give sufficient edible mass (typically > 40 g) for all analyses. Number of prey per pooled sample ranged from 1 to 200, depending on prey size and expected edible mass yield. These pooled samples are the fundamental unit used for analysis, and are referred to hereafter simply as samples.

Laboratory analysis at the NZP Nutrition Laboratory: The extracted and weighed edible material was refrozen on a plastic weigh boat and then placed in a lyophilizer (freeze-drier) for 2-3 weeks. The lyophilized material was weighed, cut into pieces (if necessary) and ground through a Wiley Food Mill with a 0.5 mm pore screen, or with a mortar and pestle, depending on prey type and ease of grinding.

All samples were analyzed for each constituent in duplicate; if the coefficient of variation attributable to the duplicates was greater than 5% for any constituent (10% for calcium; see Part 4), this analysis was rerun in duplicate. The value reported for each sample was the average of all replicate analyses unless one or more of the results was an unequivocal outlier and thus appeared to be erroneous.

Duplicate subsamples of 5-10 mg ground material were placed in tin vials for CHN gas analysis and dried at 100° C. These subsamples were rolled into compact balls, and combusted in a CHN elemental gas analyzer (Model 2400, series 2, Perkin Elmer

Co., Norwalk, Conn.) at a combustion temperature of 950° C with a supplemental oxygen boost of 2 sec prior to ignition to ensure complete combustion. In our laboratory, this procedure has been demonstrated to produce nitrogen values equivalent to those of the standard macro-Kjeldahl method. Carbon and total nitrogen (TN) content were expressed on a dry matter basis and the ratio of C:N calculated. Crude protein was calculated as TN x 6.25.

Duplicate subsamples of 1.0-1.2 g were dried at 55° C in a forced convection oven and placed in an alundum thimble in a Soxhlet fat extraction apparatus. These were extracted overnight (16-18 hr) with petroleum ether. The ether extract was collected and dried; the mass of extracted material was weighed, and reported as %fat (dry matter [DM] basis).

Duplicate subsamples of 0.5-0.7 g were compressed in a pellet press and dried at 55° C in a forced convection oven. Pellets were combusted in an adiabatic bomb calorimeter (Model 1241, Parr Instruments, Moline, Illinois), yielding energy content (cal/g DM). Calculated energy content was corrected for the energy produced in the calorimeter by burning of the fuse wire and by production of sulfuric acid (quantitated by titration). The ash residue remaining after combustion was weighed and is reported as a percentage of initial DM. To determine if ashing was complete, a subset of bomb ash samples (n=17) was combusted overnight in a muffle furnace at 550° C. As these only lost 4.4% ± 0.8 additional mass, we concluded that that bomb ash is a useful indication of the total inorganic ash in subsamples.

Sample dry matter content as reported was calculated from the mass loss during lyophilization plus the average mass loss during subsequent oven drying steps; after lyophilization samples were typically 96-98% dry.

Laboratory analysis by collaborating and commercial laboratories: Samples were sent to the following collaborating and commercial laboratories for additional analyses: Amino acids – University of California, Davis; Trace minerals –University of California, Davis; Vitamin E and carotenoids – University of Illinois, Chicago; water soluble and fat soluble vitamins – Eurofins Scientific Inc, Memphis, TN; vitamin D – Boston University Medical School; and fatty acids – University of Central Florida. The methods used for these assays are discussed in Parts 3 (vitamins) and 4 (other constituents).

Data presentation and statistical analysis

Summary tables - Sea otters consume a wide array of invertebrates, including soft-bodied taxa, taxa with an external calcareous shell, taxa armored with spines, and taxa with a chitinous exoskeleton. These different body plans primarily represent different taxonomic groups, including decapods (such as crabs and lobsters), bivalves (such as clams and mussels), gastropods (such as abalone, limpets and snails) and echinoderms (such as stars and urchins). Sea otters handle and process these prey body types differently, removing and/or breaking protective structures, and ingesting soft parts along with those hard parts that are difficult to remove. Therefore, it is to be expected that the nutrient composition of the edible portion will differ more among than within taxonomic groups. We therefore organize and discuss our prey data by taxonomic group. For each species, data are presented in summary tables as means, standard errors of the means and numbers of samples analyzed after pooling (as described above). As the numbers of samples analyzed for each species often differed according to constituent, the numbers of samples analyzed for each constituent are presented.

In the summary tables, region refers to either 1. the northern part of the MBNMS (N), particularly Monterey Bay, Elkhorn Slough and Pebble Beach; 2. the southern part of the MBNMS (S), from Piedras Blancas to south of Morro Bay; 3. both northern and southern parts of MBMNS (B); 4. San Nicolas Island in the Channel Islands (CI); or 5. Glacier Bay National Park in Alaska (AK). Season refers to winter (W, Dec-Feb), spring (Sp, Mar-May), summer (S, June-Aug) or fall (F, Sept-Nov). "Individual prey" refers to the aggregate number of all prey that were included in all samples that were processed for analysis. The edible portion refers to the proportion of the original wet mass of the prey that was retained for analysis. The individual edible mass is the average mass of the edible portion for the individuals in a given pooled sample, obtained by dividing the total edible mass of the sample by the numbers of individuals in that sample. The standard error of the mean refers to the variation among pooled samples, not among individuals (unless there was only one individual per sample); note that as only the average value for all replicates was used for each sample, this estimate does not include any variation among replicates which would be a measure of laboratory error rather than error among

samples. Dry matter is expressed as a percentage of wet edible mass, but other constituents are expressed as a percentage of the dry matter (DM) in the edible mass. Energy, however, is expressed in two ways: as calories per g dry matter (as measured by bomb calorimetry) and as kcal per g wet edible mass (calculated from assayed energy per g dry matter multiplied by the proportion of dry matter in the wet edible mass). Although the latter is influenced by dilution by both ash and water in the edible portion, it represents the actual prey biomass as consumed and is used for energy intake calculations (Part 5).

Seasonal and regional variation within MBNMS: To evaluate the potential importance of seasonal or regional variation in nutritional composition of prey for sea otters, we focused our attention on energy and fat contents, reasoning that these two parameters would be the most likely to be impacted by nutrient storage and gamete production associated with seasonal reproductive cycles of the prey species. Although it is probable that other nutrients vary spatially and temporally as well, fat and energy content are likely to have the greatest impact on individual fitness of otters, given that otters expend great amounts of energy which must be recovered via high levels of prey consumption (Costa 1978). Fat may also be important as a vehicle for storage of fat-soluble vitamins in prey and may play a role in vitamin uptake following prey ingestion (see Part 3). Limitations in our database precluded direct examination of seasonal or regional variation in most trace nutrients and vitamins (but see Part 3 for discussion of vitamin E and carotenoids), and the significance to otters of variation in abundant nutrients (such as dry matter, carbon, protein, and ash), other than their effects on energy content, is unclear. Moreover, we expected that spatial/temporal variation in the nutrient profiles within any one prey species would be considerably less than the variation between different prey species and thus between individual otter diets, a subject which we discuss elsewhere (Part 5).

Within the MBNMS, Tinker (2004) categorized prey species into 9 major prey types that account for the preponderance of invertebrate biomass consumed by sea otters (Tinker 2004): crabs of the genus *Cancer*, kelp crabs, urchins, clams, mussels, abalone, turban snails, sea stars and fat innkeeper worms. Accordingly, we focused collecting efforts and evaluation of spatial and seasonal differences on these prey types, as represented by 12 different species (see Results, below). For each of these species we

compared energy content (kcal/gram of edible fresh biomass) and fat content (% of total dry matter) of samples collected in four seasons (winter 2004-2005; spring 2005; summer 2004 and 2005; fall 2004) and at each of two locations (the north and south ends of MBNMS: see Figure 2.2). We used two-way ANOVA to test for regional and seasonal variation in energy and fat content, as well as for interactions between seasonal and regional effects. For species showing seasonal variation in energy content, we assessed a) which seasons corresponded to the highest and lowest energy contents, and b) whether increased energy density reflected a corresponding increase in fat content, as might be expected if the underlying cause was increased investment in reproductive tissue. In the case of significant regional effects or regional-seasonal interactions, we assessed seasonal effects separately for each region. We then compared seasonal patterns of energy and fat content in prey taxa to seasonal variation in sea otter diets (see Part 5 for methods of measuring biomass intake by otters) to evaluate the degree to which sea otters adjust prey selection to take advantage of seasonal peaks in prey energy value. We also reviewed the literature to determine whether seasonal patterns we detected for a particular species corresponded to published information on seasonal reproductive cycles.

We were able to reliably determine sex for only one prey type, decapods. Males and females were distinguished by the width and shape of the abdomen or characteristics of the appendages (pleopods) (see Appendix II). Mean values for males and females for each species were compared by t-tests. The potential interacting effects of sex and season were examined by two-way ANOVA for those species (*Cancer antennarius, C. magister, Pugettia producta*) with sufficient numbers of samples for this analysis. Post hoc comparisons among means were made by the Holm-Sidak method.

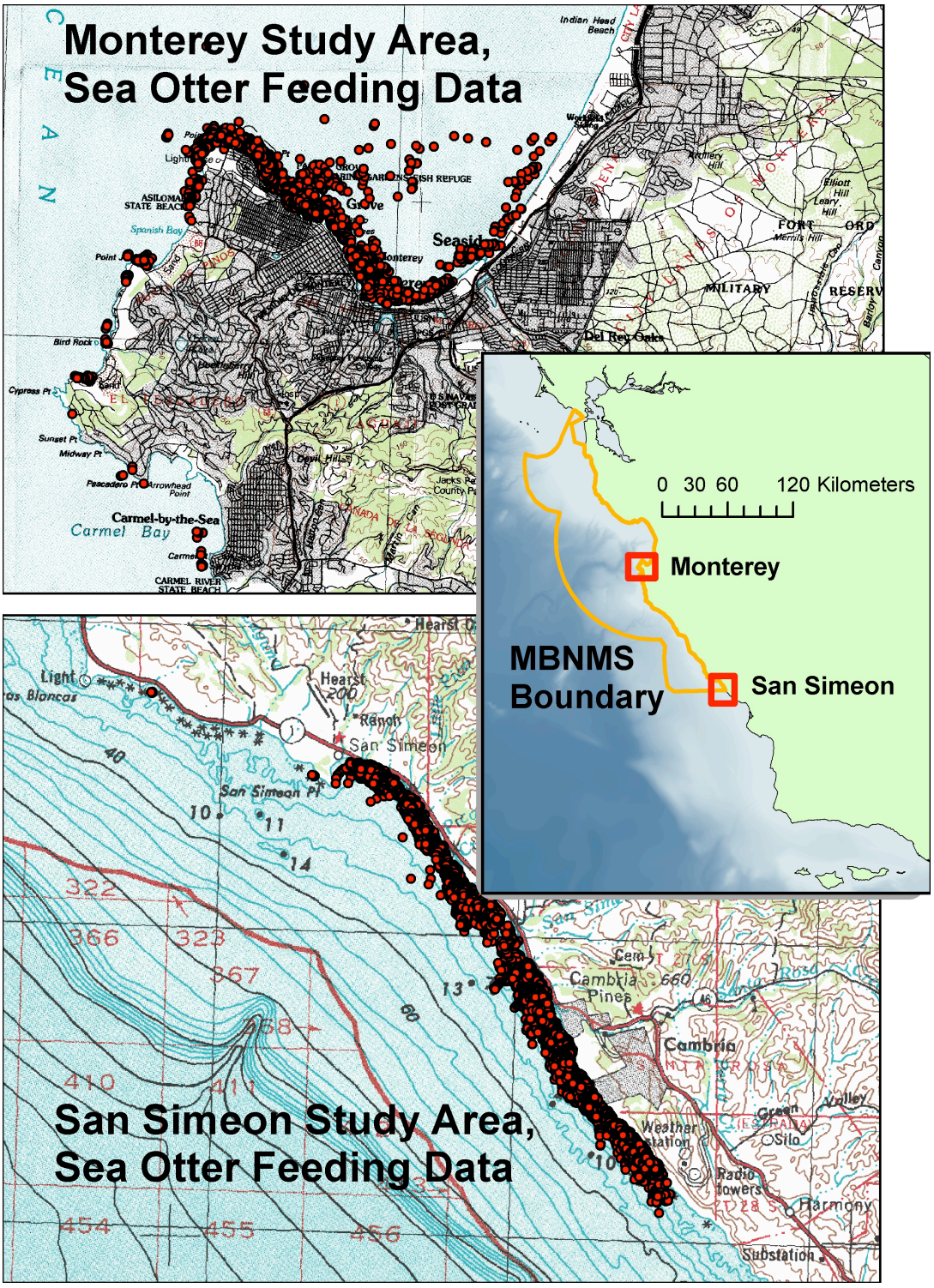

Figure 2.2. Northern and southern study areas for foraging observations in Monterey Bay National Marine Sanctuary and its southern border area. Prey collection areas extended in the north to Elkhorn Slough (and, for Cancer crabs, to Half Moon Bay), and in the south to Morro Bay and Point Buchon.

RESULTS

Analytic results are primarily presented on a dry matter basis, removing the diluting effect of water on other constituents. Water is a problematic constituent for marine invertebrates that have been frozen and thawed because it is not possible during processing to distinguish between sea water escaping from body cavities and exudates derived from tissue damage; hence all water measurements may include some error. However, it was necessary to express energy density of otter prey on a fresh weight basis in order to calculate energy intakes of otters from observed consumption of fresh biomass (see Part 5), and hence energy is presented herein on both a dry matter and fresh weight basis.

Ash is an additional constituent that dilutes rather than increases concentrations of energy and most nutrients in sea otter prey. Ash is the sum of the oxides of all major and many trace elements, and as such is particularly pertinent to mineral nutrition of sea otters (Part 4). However, ash concentrations are so variable among sea otter prey as to explain much of the variation in energy density (kcal/g dry matter) and therefore ash will be presented in this part of the report. Unlike water, ash cannot be assumed to be merely a diluent since ash may contain large amounts of compounds such as silica that are abrasive and refractory to digestion as well as calcium compounds that may impact mineral uptake and balance (Part 4).

Decapods – Crabs and their relatives

Analytic data were obtained for 19 species of crabs and lobsters, including 14 species from the MBNMS (200 pooled samples), five species from San Nicolas Island (21 pooled samples), and five species from Glacier Bay, Alaska (23 pooled samples) (Table 2.4). These included five species of the genus *Cancer*, two large bodied Alaskan crabs (*Chionoectes*, *Paralithodes*), spiny lobsters (*Panulirus*), three species of burrowing sand or mole crabs (*Emerita*, *Blepharipoda, Lepidoma*), two species of semi-terrestrial shore crabs (*Hemigrapsus, Pachygrapsus*), two species of hermit crabs, a decorator crab and the northern kelp crab (Table 2.4). Thus the large-bodied decapod taxa of the intertidal and shallow subtidal zones in sea otter habitat are well-represented, although small-bodied species (which are probably of minor importance in otter diets) are not.

Table 2.4. Major energetic constituents of decapods.

	Scientific Name	Common Name	Season	Region	Indiv. Prey	Edible portion	Indiv. Edible biomass (g)	DM (%)	Fat (% DM)	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash (% DM)	Energy (cal/g DM)	Energy (kcal/g WM)
I. Monterey Bay National Marine Sanctuary & vicinity, CA															
D1	*Blepharipoda occidentalis*	spiny mole crab	WS	B	59	0.983	24.39	26.23	2.81	34.50	7.88	49.23	28.82	3094	0.766
						0.007	2.41	0.80	0.28	0.96	0.27	1.68	0.97	89	0.048
						22	22	22	21	12	12	12	12	12	12
D2	*Cancer antennarius*	Pacific rock crab	all	B	38	0.739	224.45	22.88	2.33	35.26	8.63	53.94	28.23	3065	0.752
						0.013	20.96	2.24	0.36	0.80	0.22	1.37	1.10	97	0.049
						29	38	38	30	30	30	30	26	27	27
D3	*Cancer anthonyi*	yellow rock crab	S	S	6		212.75	24.01	5.03	39.43	9.17	57.32	19.76	3702	0.885
							43.42	3.21	0.47	2.08	0.68	4.26	1.82	207	0.113
							3	3	3	3	3	3	3	3	3
D4	*Cancer gracilis*	slender crab	WF	S	7	0.901	60.83	29.62	2.76	36.17	8.19	51.2	28.6	3061	0.909
						0.010	8.30	1.21	0.56	1.48	0.26	1.6	2.3	184	0.075
						3	5	5	5	5	5	5	5	5	5
D5	*Cancer magister*	Dungeness crab	all	B	36	0.828	490.76	23.00	3.44	38.70	9.70	60.18	19.65	3752	0.860
						0.011	29.68	0.39	0.45	0.70	0.17	1.12	1.12	92	0.033
						32	35	34	33	25	25	25	24	25	25
D6	*Cancer productus*	red rock crab	S	B	10	0.957	171.71	22.16	1.67	35.53	9.07	56.71	30.79	2875	0.598
						0.043	52.48	1.63	0.39	0.59	0.31	1.93	4.09	265	0.049
						2	6	6	6	4	4	4	5	5	5
D7	*Emerita analoga*	Pacific sand crab	WS	B	525	0.999	4.81	26.14	11.59	36.58	6.99	41.60	22.18	3812	0.947
						0.000	0.85	1.44	1.84	1.01	0.20	2.26	1.35	169	0.088
						14	14	14	14	11	11	11	11	11	11

D8	*Hemigrapsus nudus*	purple shore crab	S	N	10	0.977	32.97	32.92	1.71	27.31	5.45	34.07	43.27	1929	0.625
						0.014	7.28	3.51	0.33	2.58	0.78	4.89	3.68	148	0.202
						5	5	5	5	3	3	3	2	2	2
D9	*Lepidopa californica*	California mole crab	S	S	1	1.000	3.143	28.36	9.30						
D10	*Loxorhynchus crispatus*	moss crab	S	B	5	0.784	266.62	26.32	1.38	28.62	6.32	39.51	40.58	2143	0.561
						0.038	62.92	2.05	0.88	2.92	0.76	4.74	5.75	386	0.146
						5	5	5	5	4	4	4	4	4	4
D11	*Pachygrapsus crassipes*	striped shore crab	S	B	22	0.970	23.59	36.29	1.38	30.27	6.63	40.88	38.89	2126	0.777
						0.013	2.58	1.73	0.22	1.50	0.48	2.83	2.15	132	0.040
						9.000	9	9	9	5	5	5	5	5	5
D12	*Pagurus hemphilli*	maroon hermit	W	B	16	0.225	1.73	28.94	1.52	28.75	5.98	37.37	35.24	2555	0.749
						0.063	0.41	5.47	0.78	3.13	0.95	5.93	2.04	175	0.190
						2	2	2	2	2	2	2	2	2	2
D13	*Pugettia producta*	northern kelp crab	all	B	110	0.942	51.30	27.68	1.77	31.39	6.98	43.56	30.94	2669	0.730
						0.009	4.71	0.79	0.17	0.70	0.19	1.19	0.90	72	0.033
						40	54	51	38	31	31	31	29	29	29
D14	*Pugettia richii*	cryptic kelp crab	S	B	4	1.000	1.29	28.41	1.91						
						0.000	0.06	0.77	1.00						
						2	2	2	2						
II. San Nicolas Island, CA															
D2	*Cancer antennarius*	Pacific rock crab	F	CI	4	0.732	375.68	27.19	2.57	35.28	8.65	54.08	31.37	2967	0.806
						0.019	65.82	0.40	1.22	1.20	0.34	2.14	2.66	192	0.049
						4	4	4	4	4	4	4	4	4	4
D6	*Cancer productus*	red rock crab	F	CI	4	0.759	640.02	20.57	0.44	30.83	8.09	50.54	37.29	2400	0.490
						0.024	83.97	1.56	0.07	1.15	0.45	2.78	1.91	122	0.027
						4	4	4	4	4	4	4	4	4	4

D11	*Pachygrapsus crassipes*	striped shore crab	W	CI	4	1.000	19.47	21.20	3.19	34.09	6.92	43.28	33.31	2771	0.560
						0.000	9.46	9.17	1.51	2.98	0.43	2.71	1.52	295	0.192
						2	2	2	2	2	2	2	2	2	2
D15	*Panulirus interruptus*	california spiny lobster	F	CI	6	0.802	481.11	24.93	5.95	43.18	11.37	71.05	12.74	4423	1.106
						0.039	42.41	1.27	1.07	0.83	0.29	1.83	1.10	95	0.070
						6	6	6	2	6	6	6	6	6	6
D13	*Pugettia producta*	northern kelp crab	SpF	CI	8	0.924	127.46	28.96	1.32	34.73	7.64	47.72	30.50	2773	0.795
						0.003	24.44	1.61	0.36	1.39	0.35	2.21	3.04	217	0.052
						5	5	5	5	5	5	5	5	5	5
III. Glacier Bay National Park, AK															
D5	*Cancer magister*	Dungenes s crab	SpS	AK	10	0.871	559.0	29.62	2.22	36.14	9.24	57.77	22.02	3552	1.223
						0.000	72.1	7.78	0.39	0.75	0.08	0.52	1.59	84	0.441
						2	10	10	10	4	4	4	6	6	6
D16	*Chionoecetes bairdi*	tanner crab	Sp	AK	9	0.901	400.8	23.91	1.84	37.43	9.68	60.48	34.95	3537	0.851
						0.016	130.2	0.75	0.40	1.95	0.66	4.13	14.42	180	0.064
						6	6	6	6	6	6	6	6	6	6
D17	*Hyas lyratus*	Pacific lyre crab	Sp	AK	3	0.906	40.9	19.82	1.03	28.07	6.45	40.28	39.28	2241	0.434
						0.004	6.4	4.26	0.60	2.30	1.22	7.60	1.93	241	0.048
						2	2	2	2	2	2	2	2	2	2
D18	*Pagurus ochotensis*	Alaskan hermit crab	Sp	AK	2	0.659	53.2	34.13	3.08	36.28	8.46	52.88	27.54	3113	1.060
						0.341	21.9	0.75	0.60	0.81	0.04	0.23	2.66	272	0.070
						2	2	2	2	2	2	2	2	2	2
D19	*Paralithodes camtschaticus*	red king crab	Sp	AK	3	0.932	763.6	18.70	1.59	34.30	8.73	54.59	24.65	3308	0.635
						0.012	99.4	4.99	0.26	1.62	0.51	3.16	2.73	190	0.196
						3	3	3	3	3	3	3	3	3	3

Many large decapods are preferred prey of foraging sea otters, including *Cancer* crabs (*C. antennarius, C. magister, C. productus*), sand crabs (*Emerita, Blepharipoda*), the northern kelp crab (*Pugettia producta*) in MBNMS and vicinity and Dungeness, tanner and king crabs in Alaska (*C. magister*, *Chionoectes*, *Paralithodes*, respectively) (Bodkin et al. 2001, 2002, 2003; Tinker 2004; Bentall 2005). We attempted to collect and analyze *Cancer* crabs (*C. antennarius, C. magister*) from both the north and south of MBNMS and vicinity in all seasons, but to do so we had to rely on crab trappers in Half Moon Bay as well as in Monterey Bay and Estero Bay. Kelp crabs were another target species for seasonal and regional comparisons, but we were unable to find kelp crabs in the south following winter storms that devastated the kelp canopy. Seasonal and regional variation will be detailed in a subsequent section of this Part.

The edible portion of the crabs ranged from 0.73 to 1.00 (i.e. 73-100%), except for hermit crabs in which the inedible portion is larger as it included the host gastropod shell. The carapace was removed from large crabs (carapace width > 4 cm) but was assumed to be eaten in small crabs (see Appendix II for processing protocol). In general, the edible portions of crabs were of low to moderate dry matter (19-36%) content, and the dry matter was mostly protein (40-70%) and ash (20-40%) with low to moderate amounts of fat (1-3%), such that the energy content of the dry matter was typically less than 3.5 kcal/g. However, there were some exceptions. Sand crabs (*Emerita*) and spiny lobsters (*Panulirus*) were both relatively high in fat (6-12%) and low in ash (13-22%) with a consequent 3.8-4.4 kcal/g in dry matter. *Cancer* crabs ranged from 0.4 to 5% fat and from 20 to 37% ash, with an energy content of 2.4 to 3.8 kcal/g DM. On a fresh edible mass basis, the highest energy values (> 0.9 kcal/g) were observed in some species of *Cancer* crabs, sand crabs, spiny lobsters and Alaskan hermit crabs.

The variation in ash content was a major determinant of crab energy density on a dry matter basis (Figure 2.3, $r^2 = 0.904$). To examine the effects of the various major constituents on energy (dry mass basis), we performed stepwise regression on E_D (dependent variable) using ash, carbon, dry matter, fat, nitrogen and hydrogen as candidate independent variables. Ash by itself (step 1) explained 91.2% of the variance, fat (step 2) explained an additional 3.1%, whereas the other significant variables (steps 3, 4) explained an additional 1.3%. Nitrogen and hydrogen did not significantly add to the

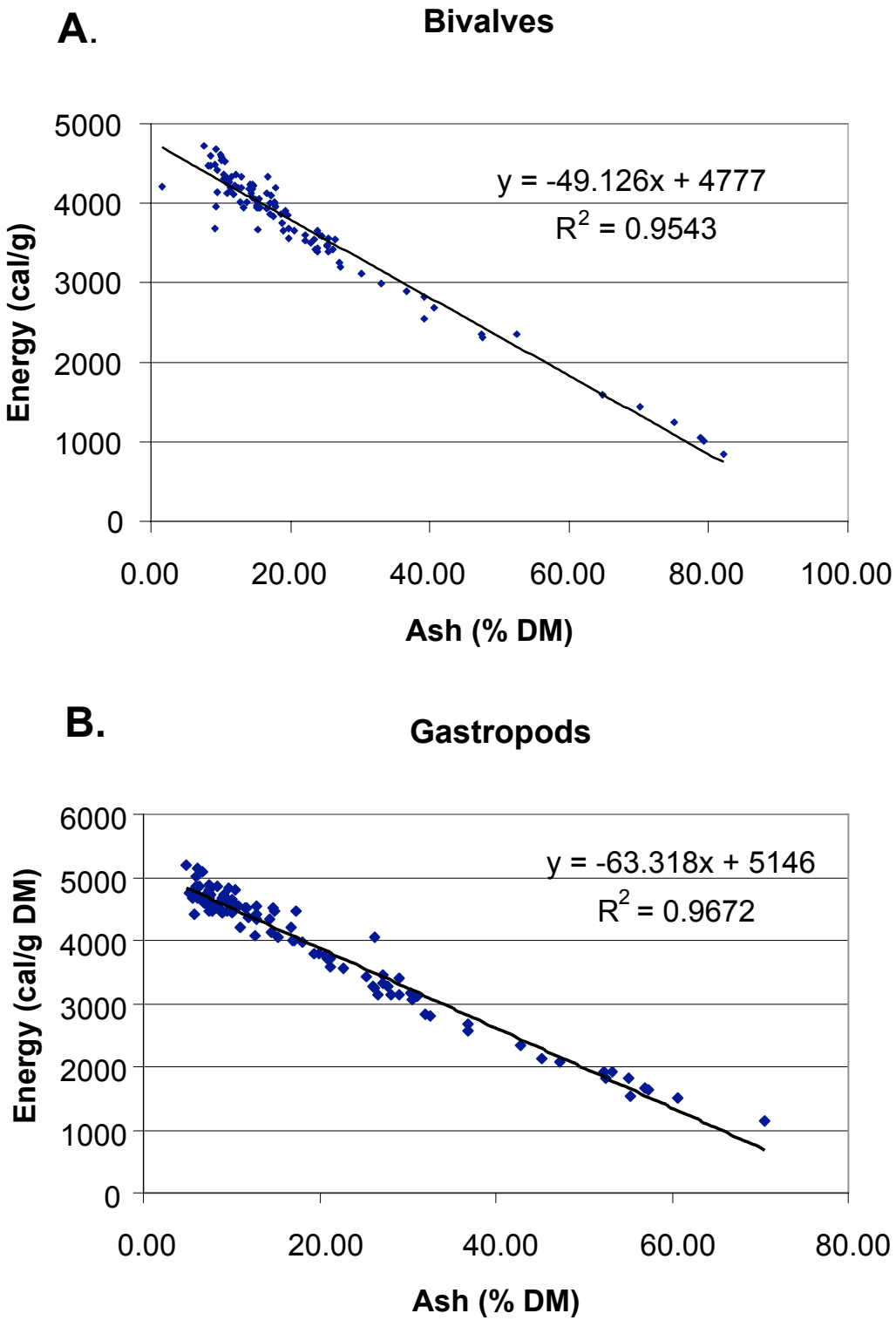

Figure 2.3. Linear regression of prey energy content (cal/g DM) versus ash (% DM) for: A) bivalves and B) gastropods.

ability of the equation to predict energy and were not included in the final equation: E_D = 3757 – 55.889 ash + 53.889 fat + 28.358 carbon - 9.918 dry matter ($F_{4,144}$ = 792.242, $p < 0.001$, $r^2 = 0.957$).

Since the crabs contain considerable amounts of calcium (Part 4), much of the ash is likely due to calcification of the exoskeleton. The high ash contents (39-43% DM) of shore (*Hemigrapsus, Pachygrapsus*), lyre (*Hyas lyratus*) and moss (*Loxorhynchus crispatus*) crabs probably reflect calcium accumulation in well-developed protective exoskeletons; none of these taxa appear to be particularly important as sea otter prey.

In all seven MBNMS species for which data were available, females tended to be higher in mean fat and energy content than males (Table 2.5), although given the low sample sizes, statistically significant differences were rare. The fat content of females averaged 110-280% that of males (interspecies average 168%), while energy content of females, on a fresh edible basis, averaged 103-122% that of males (interspecies average 113%). In many species females also tended to be lower in ash, although this difference was significant ($p<0.05$) only in *Cancer magister*, *Pachygrapsus crassipes* and *Pugettia producta*. This suggests that the calcified exoskeleton may be a greater proportion of mass in males than females, or that the exoskeleton is more highly calcified in the latter; we did not have any data on molting stage of these crabs, which might also affect results.

Bivalves – Clams, mussels and scallops

Assays were conducted on 19 species of bivalves, including 12 species (98 samples) from MBNMS and vicinity, 2 species (7 samples) from San Nicolas Island and 10 species (39 samples) from Glacier Bay (Table 2.6). Our data set includes 4 species of mussels (Order Mytiloida, Family Mytilidae), 2 species of scallops (Order Pterioida, Family Pectinidae) and 13 species of clams of the Orders Veneroida (Families Tellinidae, Solenidae, Cardiidae, Veneridae) and Myoida (Families Myidae, Hiatellidae). Two taxa from MBNMS (gaper clams, *Tresus nuttallii* and California mussels, *Mytilus californianus*) were collected in both the northern (Elkhorn Slough and Pebble Beach, respectively) and southern (Morro Bay) regions in all four seasons. Two other MBNMS taxa (littleneck clams, *Protothaca staminea* and California butterclams, *Saxidomus gigantea*) were collected in all four seasons but only in the north (Elkhorn Slough).

Table 2.5. Energetic constituents of decapods, compared by sex

Scientific Name	Common Name	Sex	Season	Region	Indiv. Prey		Indiv. Edible biomass (g)	DM % fresh	Fat % DM	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash % DM	Energy (cal/g DM)	Energy (kcal/g edible)
Blepharipoda occidentalis	spiny mole crab	F	S	B	17	x	21.36	28.63	3.47	***37.59***	8.11	50.70	26.72	3300	0.942
						sem	4.52	0.91	0.27	***0.61***	0.29	1.80	2.44	227	0.101
						n	8	8	8	***2***	2	2	3	3	3
		M	S	B	17	x	24.51	27.66	3.16	***32.41***	7.06	44.15	28.08	3152	0.812
						sem	3.64	1.00	0.38	***0.88***	0.19	1.22	1.12	136	0.049
						n	7	7	7	***3***	3	3	3	3	3
Cancer antennarius	Pacific rock crab	F	all	B	13	x	226.41	25.48	3.01	36.58	8.76	54.74	25.42	3284	0.815
						sem	31.08	1.56	0.65	0.97	0.26	1.64	1.58	132	0.084
						n	13	13	12	13	13	13	9	11	11
		M	all	B	24	x	240.36	24.41	1.87	33.95	8.45	52.80	29.54	2915	0.707
						sem	23.63	1.09	0.40	1.19	0.33	2.09	1.47	126	0.060
						n	24	21	18	17	17	17	16	16	16
Cancer magister	Dungeness crab	F	SpSW	B	11	x	360.94	22.82	***6.17***	40.44	***9.30***	***58.15***	***16.79***	***4098***	***0.942***
						sem	13.62	0.88	***0.89***	1.15	***0.20***	***1.28***	***0.89***	***121***	***0.058***
						n	11	10	***10***	9	***9***	***9***	***10***	***10***	***10***
		M	all	B	17	x	516.93	23.00	***2.18***	37.93	***9.95***	***62.18***	***21.69***	***3522***	***0.806***
						sem	41.51	0.57	***0.33***	0.78	***0.23***	***1.44***	***1.62***	***91***	***0.034***
						n	16	16	***15***	16	***16***	***16***	***14***	***15***	***15***
Cancer productus	red rock crab	F	S	B	5	x	146.40	21.95	2.09	35.84	8.89	55.53	32.12	2861	0.608
						sem	60.21	2.24	0.68	0.12	0.60	3.77	6.91	466	0.052
						n	3	3	3	2	2	2	3	3	3
		M	S	S	5	x	197.02	22.36	1.25	35.23	9.26	57.89	28.79	2896	0.583
						sem	97.50	2.88	0.35	1.37	0.37	2.33	4.14	231	0.124
						n	3	3	3	2	2	2	2	2	2

Emerita analoga	Pacific sand crab	F	S	B	441	x	4.23	30.20	17.78	40.05	6.58	41.15	17.70	4419	1.242
						sem	0.71	1.10	1.30	1.34	0.51	3.16	0.31	53	0.046
						n	6	6	6	3	3	3	3	3	3
		M	S	S	90	x	0.71	28.67	13.17	38.46	6.99	43.70	18.32	4216	1.209
						sem	0.04	0.22	0.03	0.50	0.21	1.28	0.27	45	0.022
						n	2	2	2	2	2	2	2	2	2
Pachygrapsus crassipes	striped shore crab	F	S	B	3	AVE	20.65	35.39	***2.16***	33.66	7.47	46.68	***34.19***	2376	0.839
						SEM	4.20	1.00	***0.14***	2.20	0.68	4.24	***0.61***	130	0.022
						N	2	2	***2***	2	2	2	***2***	2	2
		M	S	B	19	AVE	24.44	36.55	***1.16***	28.10	5.95	37.17	***42.46***	1960	0.736
						SEM	3.17	2.24	***0.21***	0.39	0.33	2.04	***1.46***	134	0.056
						N	7	7	***7***	3	3	3	***3***	3	3
Pugettia producta	northern kelp crab	F	all	B	37		47.38	26.72	1.99	32.95	7.09	44.33	***28.23***	***2881***	***0.811***
							6.77	1.24	0.22	1.10	0.30	1.87	***1.01***	***87***	***0.058***
							21	18	17	13	13	13	***13***	***13***	***13***
		M	all	B	57		62.98	23.47	1.50	30.61	6.86	42.90	***33.52***	***2475***	***0.664***
							6.81	2.14	0.26	0.89	0.29	1.84	***1.31***	***104***	***0.039***
							28	21	19	17	17	17	***14***	***14***	***14***

Table 2.6. Major energetic constituents of bivalves

	Scientific Name	Common Name	Season	Region	Indiv. Prey	Edible portion	Indiv. Edible biomass (g)	DM (% fresh)	Fat % DM	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash % DM	Energy (cal/g DM)	Energy (kcal/g WM)
I. Monterey Bay National Marine Sanctuary & vicinity, CA															
B1	*Clinocardium nuttallii*	Nuttall cockle	S	B	25	0.469	14.27	12.24	1.34	33.90	9.15	57.2	22.0	3663	0.464
						0.021	4.29	0.54	0.45	1.59	0.44	2.7	2.5	218	0.053
						5	5	5	3	3	3	3	3	3	3
B2	*Crassodoma gigantea*	Rock scallop	W	S	5	0.358	37.48	18.18	4.11	42.20	11.48	71.74	10.07	4602	0.836
						0.025	14.43	0.45	0.01	0.18	0.00	0.00	0.07	11	0.019
						2	2	2	2	2	2	2	2	2	2
B3	*Macoma nasuta*	Bent-nose macoma	S	B	26	0.504	2.89	14.02	0.94	27.19	6.46	40.36	34.70	2973	0.415
						0.069	0.34	1.08	0.05	3.87	1.00	6.23	4.51	144	0.012
						2	2	2	2	2	2	2	2	2	2
B4	*Macoma secta*	White-sand macoma	S	B	109	0.635	7.88	24.05	0.36	16.35	3.66	22.87	75.02	1197	0.272
						0.030	1.33	1.31	0.05	1.00	0.33	2.06	2.66	115	0.028
						8	8	8	8	8	8	8	6	6	6
B5	*Mytilus californianus*	California mussel	all	B	316	0.362	11.62	10.36	2.57	36.126	8.90	55.7	15.5	3941	0.547
						0.013	1.994	3.404	0.157	0.703	0.15	0.9	1.3	84	0.041
						16	22	22	19	19	19	19	19	20	20
B6	*Mytilus edulis*	Bay mussel	W	S	40	0.558	12.04	12.42	3.40	35.50	7.43	46.42	11.10	3988	0.495
						0.006	4.12	0.04	0.03	0.07	0.37	2.30	1.75	31	0.002
						2	2	2	2	2	2	2	2	2	2
B7	*Panopea generosa*	Geoduck clam	WSp	S	3	0.860	909.61	16.41	1.13	38.53	8.74	54.60	7.97	4249	0.698
						0.002	391.45	1.05	0.24	0.53	0.39	2.45	3.27	28	0.049
						3	3	3	3	3	3	3	3	3	3

B8	*Protothaca staminea*	Pacific littleneck clam	all	N	337	0.430	8.63	13.26	2.00	36.45	8.20	51.24	14.47	4013	0.530
						0.017	1.83	0.39	0.19	0.76	0.18	1.11	1.60	64	0.026
						14	14	14	13	13	13	13	9	11	11
B9	*Saxidomus nuttalli*	California butterclam	all	N	52	0.562	58.38	18.56	2.65	39.16	9.75	60.92	13.07	4175	0.774
						0.061	7.55	0.79	0.28	0.60	0.21	1.31	1.02	55	0.061
						10	11	11	9	10	10	10	7	7	7
B10	*Siliqua patula*	Pacific razor clam	S	S	3	0.760	16.20	26.38							
						1	1	1							
B11	*Tivela stultorum*	Pismo clam	S	B	10	0.242	18.67	21.45	4.12	41.34	8.78	54.8	10.9	4376	0.920
						0.016	2.53	1.31	0.42	0.46	0.27	1.7	2.3	166	0.054
						4	4	4	4	4	4	4	3	3	3
B12	*Tresus nuttallii*	Pacific gaper	all	B	62	0.682	282.52	18.69	1.73	34.33	9.02	56.20	23.38	3708	0.681
						0.022	26.41	0.67	0.12	0.94	0.24	1.63	2.88	121	0.042
						22	24	24	20	22	22	22	13	16	16
II. San Nicolas Island, CA															
B2	*Crossodoma gigantea*	Giant rock scallop	F	CI	16	0.302	87.93	17.63	3.11	41.98	11.93	74.55	9.37	4621	0.816
						0.020	24.03	0.69	0.42	0.38	0.22	1.38	0.64	50	0.040
						4	4	4	4	4	4	4	4	4	4
B5	*Mytilus californianus*	California mussel	W	CI	72	0.406	4.75	9.36	2.14	31.60	8.52	53.26	25.77	3438	0.286
						0.097	1.41	1.82	0.39	4.30	1.11	6.91	7.15	488	0.155
						3	2	3	2	3	3	3	3	3	3
III. Glacier Bay National Park, AK															
B13	*Chlamys rubida*	Scallop	Sp	AK	22	0.551	21.2	12.65	2.52	37.98	10.65	66.55	15.38	4167	0.530
						0.061	4.5	1.51	0.16	1.50	0.55	3.44	2.35	175	0.085
						2	2	2	2	2	2	2	2	2	2
B1	*Clinocardium nuttallii*	Nuttall's cockle	Sp	AK	6	0.509	111.0	11.66	3.61	36.21	9.18	57.39	16.84	3964	0.462
						0.015	10.4	0.55	0.02	0.52	0.27	1.70	0.25	37	0.018
						2	2	2	2	2	2	2	2	2	2

B14	*Hiatella arctica*	Arctic hiatella	Sp	AK	47	0.363	0.88	18.63	2.13	37.90	10.32	64.51	17.32	4264	0.794
						0.003	0.13	0.44	0.11	0.90	0.27	1.71	0.58	67	0.006
						2	2	2	2	2	2	2	2	2	2
B15	*Macoma balthica*	Baltic macoma	Sp	AK	35	0.375	0.27	18.42		40.25	9.25	57.84			
						0.020	0.07	0.03		0.04	0.14	0.85			
						2	2	2		2	2	2			
B3	*Macoma nasuta*	Bent-nosed macoma	Sp	AK	113	0.509	3.6	9.34	1.11	33.01	8.08	50.52	23.75	3472	0.324
						0.006	0.1	0.38	0.14	0.38	0.18	1.09	0.83	24	0.015
						4	4	4	2	4	4	4	4	4	4
B16	*Mya truncata*	Truncated softshell-clam	Sp	AK	69	0.643	12.4	14.87	0.56	23.28	6.59	41.22	43.06	2559	0.379
						0.018	3.2	0.40	0.07	0.97	0.26	1.62	2.66	140	0.011
						4	4	4	4	4	4	4	4	4	4
B17	*Modiolus modiolus*	Horse mussel	S	AK	14		61.1	13.10	2.80	39.11	9.55	59.67	12.49	4271	0.498
							7.7	0.86	0.32	0.50	0.32	2.01	0.81	49	0.024
							7	7	7	3	3	3	3	3	3
B18	*Mytilus trossulus*	Foolish mussel	SpS	AK	122	0.558	11.9	12.32	3.39	34.78	8.29	51.80	22.37	3637	0.329
						0.009	3.0	1.75	0.50	2.08	0.30	1.90	2.30	187	0.057
						4	8	8	8	4	4	4	4	4	4
B8	*Protothaca staminea*	Pacific littleneck clam	Sp	AK	30	0.412	7.9	9.34	1.72	35.21	9.12	56.97	16.97	3848	0.389
						0.016	2.3	0.49		0.43	0.02	0.13	1.78	98	0.003
						5	5	5	1	5	5	5	3	3	3
B19	*Saxidomus gigantea*	Butter clam	SpS	AK	10	0.486	56.5	12.94	2.23	36.76	9.30	58.13	15.96	3905	0.505
						0.030	20.7	1.28	0.91	0.63	0.21	1.30	1.92	153	0.052
						3	3	3	2	3	3	3	3	3	3

The edible portion, which excluded the paired valves (shell), but included all soft tissues, comprised 40-60% of total mass in most taxa, but was lower in species with exceptionally thick valves (24% in Pismo clams, *Tivela stultorum*; 30-35% in giant rock scallops, *Crassadoma gigantea*) and higher in those taxa with thinner valves (76% in Pacific razor clams, *Siliqua patula*) or smaller valves relative to the mantle and siphon (86% in geoduck clams, *Panopea generosa*).

The constituent that displayed most variation among taxa was ash content, which varied from 8% of dry mass in geoduck clams (*Panopea generosa*) to 75% in white-sand clams (*Macoma secta*). This variation was surprising, as the valves had been removed, and must reflect differences in ingestion of sand and mud by clam species. It is not clear how *Macoma secta* in central California manages to accumulate 75% inorganic material in its soft tissues but the finding was consistent: the 6 pooled samples, containing 5-20 clams each, varied from 64.7 to 82.1% ash. Two other clam species also accumulated substantial amounts of ash: bent-nose macoma (*Macoma nasuta,* 35%) from central California and the truncated soft-shell clam (*Mya truncata*, 43%) from Glacier Bay, Alaska.

One would expect large amounts of ash to dilute out the concentrations of organic constituents, such as carbon, fat and protein, and this is evident in *Macoma secta*. It should also have a large effect on energy content, especially as all bivalves are relatively low in fat (≤4.1 % of dry matter). We regressed energy content, on a dry matter basis, against ash for all bivalve samples, and found a tight linear relationship with an $r^2 = 0.954$ (Figure 2.3). To examine the effects of the various major constituents on energy (dry mass basis), we performed stepwise regression on E_D (dependent variable) using ash, carbon, dry matter, fat, nitrogen and hydrogen as candidate independent variables. Ash by itself (step 1) explained 95.6% of the variance, while other significant variables (Steps 2-5) explained an additional 2.27%. Fat, carbon and hydrogen did not significantly add to the ability of the equation to predict energy and were not included in the final equation: $E_D = 3647 - 43.471$ ash $+ 80.479$ nitrogen $+ 21.004$ dry matter ($F_{3,90} = 999.999$, $p < 0.001$, $r^2 = 0.987$). Thus ash content is the most important variable in explaining variation in the energy and nutrient composition of bivalves. Bivalves of low to intermediate ash

content (<20%) contained 1-4% fat, 35-42% carbon and 3800-4200 cal/g on a dry matter basis, whereas bivalves of > 20% ash contained 0.4-3.4% fat, 16-34% carbon and 1200 to 3700 cal/g.

Clearly there would be an advantage to sea otters in feeding on low ash bivalves, both in terms of the higher fat and energy density of low ash bivalves, and because the presence of ash may interfere with mineral uptake and balance (Part 4). On this basis, the most nutritious bivalves in MBNMS and vicinity are geoduck clams (*Panopea generosa*, only found at Morro Bay in central California), giant rock scallops (*Crassadoma gigantea*), Pismo clams (*Tivela stultorum*) and California butterclams (*Saxidoma nuttalli*). These bivalves also have the advantage of being relatively large (20-900 g edible biomass per bivalve) such that the total nutrient and energy amounts per clam are high. The only other large clam that we collected in MBNMS, the Pacific gaper clam (*Tresus nuttallii*), was somewhat higher in ash (23%), and lower in fat (1.7%), carbon (34%) and energy (3700 cal/ g DM). On a fresh weight basis, the bivalves highest in energy in MBNMS and vicinity were Pismo clams (0.92 kcal/g), giant rock scallops (0.84 kcal/g), California butterclams (0.77 kcal/g), geoduck clams (0.70 kcal/g and Pacific gapers (0.68 kcal/g).

In Glacier Bay, Alaska, four large bivalves (horse mussels, *Modiolus modiolus*; scallops, *Chlamys rubida*; Nuttall's cockles *Clinocardium nuttallii*; butter clams, *Saxidomus gigantea*) and one small bivalve (arctic hiatella, *Hiatella arctica*) were relatively low in ash (12-17%) and relatively high in carbon (36-39%) and energy (3900-4300 cal/g) on a dry matter basis. As eaten by otters, fresh material would provide 0.46-0.79 kcal/g. The other four species analyzed (Pacific littleneck clams, *Protothaca staminea*; foolish mussels, *Mytillus trossulus*; bent-nosed macomas, *Macoma nasuta*; and softshell clams, *Mya truncata*) were higher in ash and lower in energy, whether on a dry matter (2600-3800 cal/g) or fresh edible (0.32-0.39 kcal/g) basis.

Among all bivalves, the scallops (*Crassadoma, Chlamys*) contained the highest nitrogen and crude protein, but whether this is of any significance for foraging otters is not known.

Gastropods – Abalone, limpets, and snails

We analyzed major constituents for 19 species of gastropods, including 99 samples of 13 species from MBNMS and vicinity, 26 samples of seven species from San Nicolas Island, and 5 samples of three species from Glacier Bay, Alaska (Table 2.7). This data set includes representative of the Archaeogastropoda (abalone, Haliotidae; limpets, Lottidae; keyhole limpets Fissurellidae; top snails, Trochidae; turban snails, Turbinidae), Mesogastropoda (moon snails, Naticidae; tritons, Cymatiidae) and Neogastropoda (dogwhelks, Thaisidae; whelks, Buccinidae; miters, Mitridae; neptunes, Neptuneidae). Although we collected additional taxa of small body size (Appendix I), the large numbers of small snails required to provide sufficient edible mass (up to 200 individual snails per sample), and the laborious effort required to crush the shells and extract the edible portion, limited the numbers of taxa we analyzed. Particular attention was directed at top and turban snails as these are known to be important in sea otter diets.

Three gastropod taxa (black abalone, *Haliotis cracherodii*; red abalone, *Haliotis rufescens*; black turban, *Tegula funebralis*) were collected in the northern and southern regions of the MBNMS and vicinity in all four seasons to examine seasonal and regional variation (see section below). Black abalone were obtained from the intertidal zone at Pebble Beach and Rancho Marino, red abalone from Monterey Bay (both farmed under a pier and wild, collected by diving) and Estero Bay, and black turban snails from Pebble Beach and Rancho Marino. The farmed red abalone were assumed to be representative as they were only fed kelp harvested from Monterey Bay, but this assumption warrants further study. Unfortunately our sample design did not include matched seasonal samples from the wild and from the abalone farm that would allow a detailed comparison.

The edible portion (all soft tissues, including the foot) varied widely among taxa as a percentage of total body mass, reflecting variation in the size, shape and thickness of the shell. The edible portion ranged from 18 to 52% of total body mass in snails, from 49 to 92% in limpets, and the edible portion of abalone was 60.6-61.0%. For abalone and limpets, the soft tissues were easily removed with a scalpel, but for snails, crushing of the shell resulted in various-sized shell fragments, and a decision had to be made about which fragments to remove and which would be consumed incidentally by an otter. We

Table 2.7. Major energetic constituents of gastropods.

	Scientific Name	Common Name	Season	Region	Indiv. Prey	Edible portion	Indiv. Edible biomass (g)	DM % fresh	Fat % DM	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash % DM	Energy (cal/g DM)	Energy (kcal/g WM)
I. Monterey Bay National Marine Sanctuary & vicinity, CA															
G1	*Acanthina spirata*	Angular unicorn	S	S	187	0.260	0.85	48.98	3.33	35.11	7.97	46.4	51.8	1934	0.966
						0.008	0.08	1.36	0.26	0.42	0.24	0.9	2.3	78	0.016
						4	4	4	4	3	3	3	3	3	3
G2	*Calliostoma ligatum*	Blue top snail	WS	B	120	0.299	0.89	27.90	3.98	35.66	9.14	57.12	24.13	3599	1.005
						0.034	0.16	1.18	1.17	1.99	0.53	3.28	3.94	315	0.101
						2	3	3	2	3	3	3	3	3	3
G3	*Haliotis cracherodii*	Black abalone	all	B	31	0.610	121.40	21.85	3.58	41.41	11.22	70.10	7.93	4609	0.972
						0.013	13.27	0.96	0.70	0.63	0.22	1.38	0.58	68	0.061
						19	21	21	18	17	17	17	15	16	16
G4	*Haliotis rufescens*	Red abalone	all	B	38	0.606	142.38	21.97	3.95	40.88	10.90	68.15	7.44	4634	0.996
						0.013	20.96	0.60	0.50	0.46	0.21	1.32	0.39	38	0.040
						27	29	27	23	21	21	21	20	21	21
G5	*Kelletia kelletii*	Kellet's whelk	SpF	B	3	0.315	37.45	41.28							
							8.23	2.26							
						1	2	2							
G6	*Lithopoma gibberosa*	Red turban snail	S	N	55	0.369	2.12	47.19	2.34	32.34	7.54	44.28	39.80	2459	1.162
						0.014	0.19	1.55	0.40	1.26	0.21	1.56	3.00	123	0.096
						2	2	2	2	2	2	2	2	2	2
G7	*Lottia gigantea*	Owl limpet	W	B	20	0.494	10.73	18.17	2.87	41.22	11.59	72.41	10.08	4662	0.849
						0.023	3.07	0.97	0.62	0.81	0.15	0.93	0.40	82	0.059
						3	3	3	3	3	3	3	3	3	3

G8	*Mitra idea*	Ida's miter	WS	B	8	0.185	0.95	30.37								
							0.20	0.01								
						1	2	2								
G9	*Polinices lewisii*	Lewis's moon snail	WS	S	8	0.432	118.75	15.25	1.28	32.91	8.99	56.21	23.89	3707	0.631
						0.145	87.35	3.09	0.24	2.92	0.88	5.48	2.30	228	0.182
						2	4	4	3	2	2	2	3	3	3
G10	*Tegula brunnea*	Brown tegula	S	B	76	0.347	1.58	41.01	3.63	38.95	9.81	61.34	32.27	2825	1.159
						0.056	0.10	3.20	0.65	1.06	0.49	3.09	0.28	16	0.097
						2	2	2	2	2	2	2	2	2	2
G11	*Tegula funebralis*	Black tegula	all	B	2033	0.327	1.12	33.59	3.17	36.29	8.67	54.16	30.02	3260	0.909
						0.027	0.06	3.44	0.41	1.41	0.49	3.07	4.45	220	0.061
						12	19	19	19	18	18	18	14	17	17
G12	*Tegula montereyi*	Monterey tegula	S	B	99	0.417	2.48	44.41	3.01	36.30	9.09	55.3	45.1	2265	0.997
						0.022	0.50	0.77	0.48	2.61	0.73	6.1	7.3	401	0.166
						4	4	4	4	3	3	3	3	3	3
G13	*Tegula pulligo*	Dusky tegula	S	B	262	0.281	1.20	48.96	2.28	32.23	7.83	47.1	58.3	1604	0.775
						0.004	0.03	2.09	0.19	3.29	1.07	8.5	1.2	48	0.033
						4	4	4	4	3	3	3	3	3	3
II. San Nicolas Island, CA																
	Kelletia kelletii	Kellett's Whelk	Sp	CI	6	0.323	43.42	24.01	3.09	39.20	9.87	61.67	7.73	4749	1.138
						0.036	6.91	1.83	0.35	2.87	0.74	4.63	0.80	53	0.077
						4	4	4	4	4	4	4	4	4	4
G7	*Lottia gigantea*	Owl limpet	W	CI	35	0.506	7.87	16.88	3.19	39.49	11.07	69.17	11.43	4472	0.754
						0.009	1.96	0.85	0.10	0.75	0.22	1.39	2.29	28	0.034
						4	4	4	4	4	4	4	4	4	4
G5	*Megastraea undosa*	Wavy turbansnail	SpF	CI	15	0.320	108.97	20.61	2.82	33.20	8.66	54.10	21.59	3744	0.769
						0.016	25.18	0.74	0.39	1.88	0.56	3.53	2.32	119	0.025
						6	6	6	6	6	6	6	6	6	6

G14	*Megathura crenulata*	Giant keyhole limpet	Sp	CI	6	0.915	238.19	11.59	1.12	28.56	8.01	50.08	26.41	3195	0.370
						0.001	12.85	0.10	0.00	0.02	0.02	0.14	0.23	57	0.010
						2	2	2	2	2	2	2	2	2	2
G15	*Norrisia norrisi*	Norris' topsnail	Sp	CI	13	0.296	15.72	25.37	5.68	41.77	11.01	68.80	9.61	4683	1.193
						0.022	0.95	2.36	0.61	0.78	0.29	1.81	0.54	71	0.121
						6	6	6	6	6	6	6	6	6	6
G16	*Tegula eiseni*	Banded turban snail	W	CI	57	0.177	0.92	29.13	3.01	37.30	9.30	58.15	29.13	3211	0.938
						0.018	0.09	2.43	0.22	0.01	0.08	0.53	1.91	102	0.108
						2	2	2	2	2	2	2	2	2	2
G11	*Tegula funebralis*	Black tegula	W	CI	200	0.277	1.27	24.96	3.82	41.59	10.23	63.95	11.56	4444	1.109
						0.002	0.06	0.07	0.08	0.92	0.33	2.07	1.28	100	0.022
						2	2	2	2	2	2	2	2	2	2
III. Glacier Bay National Park, AK															
G17	*Fusitriton oregonensis*	Hairy triton	SpS	AK	8	0.515	33.2	22.92	4.84	43.39	9.70	60.63	9.07	4807	1.111
						0.024	2.7	3.35	1.07	2.16	0.20	1.26	2.45	284	0.226
						2	2	2	2	2	2	2	2	2	2
G18	*Neptunea lyrata*	Ridged whelk	Sp	AK	2	0.313	28.2	24.29	2.85	43.70	10.57	66.09	9.33	4678	1.136
G19	*Tectura scutum*	Plate limpet	Sp	AK	30	0.559	2.9	20.57	6.23	40.38	9.99	62.43	14.85	4489	0.924
						0.010	0.3	0.70	0.24	0.34	0.07	0.41	0.09	24	0.036
						2	2	2	2	2	2	2	2	2	2

removed all large to medium pieces, assuming an otter would do the same, but retained small fragments in the edible portion.

A number of the smaller snails (dogwhelks, *Acanthina spirata*; red turban snails, *Lithopoma gibberosa*; turban snails, *Tegula brunnea*, *T. eiseni*, *T. funebralis* (MBNMS), *T. montereyi*, and *T. pulligo*) were similar in being high in ash (29-58% total body mass) with correspondingly low levels of fat (2.3-3.6%), carbon (33-38%), nitrogen (7.5-9.8%) and energy (1900-3300 cal/g) on a dry matter basis. In gastropods, the effect of ash content on energy content is highly linear and profound, with a r^2 of 0.967 (Figure 2.3). To examine the effects of the various major constituents on energy (dry mass basis), we performed stepwise regression on E_D (dependent variable) using ash, carbon, dry matter, fat, nitrogen and hydrogen as candidate independent variables. Ash explained 97% of the variance, while fat (step 2) explained an additional 0.57%. Dry matter, nitrogen, carbon and hydrogen did not significantly add to the ability of the equation to predict energy and were not included in the final equation: $E_D = 4968 - 61.992$ ash $+ 41.293$ fat ($F_{2,102} = 999.999$, $p < 0.001$, $r^2 = 0.976$).

One reason that fat did not contribute more to energy content was that values were uniformly low; only 3 species (none from MBNMS and vicinity) exceeded 4% fat: Norris' topsnail (*Norrisia norrisi*, 5.7%), hairy tritons (*Fusitriton oregonensis*, 4.8%) and plate limpets (*Tectura scutum*, 6.2%). The highest value obtained for any gastropod was 10.9% fat for a red abalone (*Haliotis rufescens*). Apparently gastropods do not normally store much energy in the form of lipid.

Intraspecific variation in ash content undoubtedly reflects variation in the inclusion of shell fragments, and this in turn affects measured energy density . Lewis' moon snails (*Euspira lewisii*), whelks (*Kelletia kelletii, Neptunea lyrata*), wavy turbans (*Megastraea undosa*), tritons (*Fusitriton oregonensis*) and Norris' topsnails were sufficiently large that included shell fragments were a small proportion of edible mass. Ash was correspondingly low (8-23%) and these taxa typically contained 3700-4800 cal/g DM. Similarly no shell fragments were included in processing abalone, which contained only 7-8% ash, or limpets, which averaged 10-15% ash in smaller species and 26% in giant keyhole limpets (*Megathura crenulata*). These taxa averaged 4400-4700 cal/g DM, except for giant keyhole limpets that were only 3200 cal/g DM. Otters feeding

on abalone and limpets have the advantage that they can easily discard inert shell material, assuming they are able to pry these taxa lose from the substrate in the first place. To obtain the same energy intake, otters feeding on snails would need to consume more dry matter to compensate for the low energy in the dry matter.

Echinoderms – urchins, stars and sand dollars

The major constituents were analyzed for the edible portion of 12 echinoderm species, including 9 species (112 samples) from MBNMS and vicinity, 4 species (30 samples) from San Nicolas Island and 3 species (9 samples) from Glacier Bay, Alaska (Table 2.8). These included sea stars of two orders (Forcipulatida: Asteridae, 5 spp.; Spinulosida: Poraniidae, 1 sp.), sea urchins (Echinoida: Strongylocentrotidae, 4 spp.), a sand dollar (Clypeasteroida: Dendrasteridae) and a sea cucumber (Aspidochirotida: Stichopodidae). Two taxa, ochre stars (*Pisaster ochraceus*) and purple urchins (*Strongylocentrotus purpuratus*), were collected in or near MBNMS in all seasons and in both northern and southern regions. Ochre stars and purple urchins were collected at Monterey Bay and Pebble Beach in the north and at San Simeon, Rancho Marino and Morro Bay in the south.

Echinoderms include a calcareous skeleton and surface materials that otters typically do not consume. In medium to large sea urchins, the edible portion, which was considered to be the soft inner material (gonads, gastrointestinal tract and other visceral organs) ranged from 32 to 52% of body mass. However, otters have been observed to eat most of the body in the smallest urchins (< 2cm), so for this size class of purple urchins (*S. purpuratus*) the calcareous skeleton (test) was included. The arms of stars were cut off at the central disc and the soft inner material of the arms and core scooped out to yield an edible portion of 7-24% in California stars. However, sunflower stars (*Pycnopodia helianthoides*), which appeared less calcified than other species, were assayed whole. The tests of sand dollars were cut along the outermost rim, allowing dorsal and ventral halves to be separated and the soft inner material (14% of the total) to be scraped off. We considered the entire sea cucumber to be edible, including the calcareous spicules that consitute a reduced skeleton.

The edible portions of many echinoderms were remarkably different from other marine invertebrates in that they contained quite high concentrations of fat. For example,

Table 2.8. Major energetic constituents of echinoderms

	Scientific Name	Common Name	Season	Region	Indiv. Prey	Edible portion	Indiv. Edible biomass (g)	DM (% fresh)	Fat (% DM)	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash % DM	Energy (cal/g DM)	Energy (kcal/g WM)
I. Monterey Bay National Marine Sanctuary & vicinity, CA															
E1	*Dermasterea imbicata*	Leather star	S	N	2		102.21	11.06	11.55	37.60	7.24	45.25	20.06	4373	0.483
							25.84	0.18	3.34	0.29	0.59	3.71	0.23	161	0.010
							2	2	2	2	2	2	2	2	2
E2	*Dendraster excentricus*	Eccentric sand dollar	WS	S	85	0.139435	1.79	8.77	1.99	23.86	4.86	30.37	44.23	2387	0.210
							0.61	1.04	0.47	0.36	0.17	1.07	2.16	86	0.032
						1	2	2	2	2	2	2	2	2	2
E3	*Parastichopus californicus*	CA sea cucumber	S	S	4	1.000	174.32	6.49	0.43	16.14	3.87	24.20	53.60	1744	0.113
						0.000	27.25	0.00	0.03	0.13	0.03	0.19	1.11	27	0.002
						2	2	2	2	2	2	2	2	2	2
E4	*Pisaster brevispinus*	Pink sea star	Sp	B	7	0.196896	61.00	10.82	16.63	42.42	7.02	43.85	15.78	4877	0.529
						0.056352	36.55	1.26	6.84	2.09	0.72	4.52	1.88	248	0.072
						3	3	3	3	3	3	3	3	3	3
E5	*Pisaster giganteus*	Giant spined star	S	B	22	0.070518	12.18	26.14	34.65	50.02	6.25	39.04	6.95	6028	1.544
						0.006937	2.83	0.55	1.03	0.84	0.11	0.69	0.16	64	0.040
						4	8	8	7	7	7	7	5	5	5
E6	*Pisaster ochraceus*	Ochre star	all	B	72	0.169321	45.72	24.31	24.81	48.80	7.37	46.09	6.78	5779	1.378
						0.017291	4.46	0.91	1.71	0.63	0.19	1.18	0.46	93	0.094
						26	36	36	28	31	31	31	16	17	17
E7	*Pycnopodia helianthoides*	Sunflower star	S	S	3	1.000	290.68	19.11	1.68	28.67	6.94	43.38	36.91	2411	0.460
						0.000	180.01	0.55	0.49	1.67	0.64	4.01	1.77	179	0.021
						2	2	2	2	2	2	2	2	2	2
E8	*Strongylocentrotus franciscanus*	Red urchin	WSpS	B	30	0.318867	29.42	18.68	17.96	30.90	4.93	30.78	30.74	3606	0.513
						0.069595	10.28	2.31	4.93	3.50	0.59	3.71	5.43	465	0.184
						18	18	18	6	11	11	11	8	8	8

E9	*Strongylocentrotus purpuratus*	Purple urchin >2 cm	all	B	361	0.355054	14.43	13.71	6.01	30.68	5.78	36.14	37.17	3008	0.393
						0.025157	3.17	0.81	0.63	1.08	0.31	1.92	4.36	216	0.036
						29	33	33	26	29	29	29	15	18	18
		Purple urchin <2 cm	SpS	B	36	0.854285	2.27	39.22	0.84	15.92	1.59	9.97	82.01	632	0.236
						0.037879	0.27	0.96	0.11	0.44	0.13	0.82		28	0.013
						6	6	6	4	5	5	5	1	2	2
II. San Nicolas Island, CA															
E5	*Pisaster giganteus*	Giant spiny star	SpF	CI	12	0.191	63.00	13.04	13.34	42.20	7.73	48.32	14.30	4769	0.657
						0.039	19.82	2.66	3.73	3.40	0.21	1.33	3.63	357	0.177
						5	5	5	5	5	5	5	5	5	5
E6	*Pisaster ochraceus*	Ochre star	W	CI	9	0.242	71.73	20.00	18.12	49.36	8.44	52.76	8.12	5576	1.115
						0.021	14.83	0.41	1.51	0.40	0.30	1.87	0.74	44	0.020
						4	4	4	4	4	4	4	4	4	4
E8	*Strongylocentrotus franciscanus*	Red urchin	WSpF	CI	30	0.478	157.91	10.22	17.31	35.86	4.34	27.10	24.49	3975	0.420
						0.008	20.27	0.56	1.50	1.83	0.36	2.25	3.92	243	0.042
						16	16	16	16	16	16	16	16	16	16
E9	*Strongylocentrotus purpuratus*	Purple urchin	Sp	CI	44	0.380	16.48	17.94	7.80	31.78	6.16	38.53	34.84	3281	0.657
						0.072	5.01	6.92	1.28	4.58	1.17	7.31	9.72	577	0.299
						5	5	5	5	5	5	5	5	5	5
III. Glacier Bay National Park, AK															
E10	*Evasterias troschelii*	Mottled sea star	Sp	AK	5	0.425	186.6	16.63	10.86	45.74	8.97	56.06	11.19	5169	0.860
						0.019	40.5	0.40	1.36	0.54	0.32	1.98	0.66	16	0.023
						3	3	3	3	3	3	3	3	3	3
E11	*Strongylocentrotus droebachiensis*	Green urchin	Sp	AK	29	0.517	20.0	10.04	4.50	21.91	4.82	30.15	50.63	2313	0.236
						0.029	6.7	0.52	0.86	2.25	0.75	4.66	5.93	295	0.041
						4	4	4	4	4	4	4	4	4	4
E12	*Strongylocentrotus pallidus*	White urchin	Sp	AK	22	0.470	7.8	10.81	3.34	25.80	5.74	35.86	51.76	2209	0.239
						0.008	0.4	0.14	0.29	1.53	0.17	1.05	1.46	117	0.016
						2	2	2	2	2	2	2	2	2	2

red urchins (*S. franciscanus*), pink sea stars (*P. brevispinus*), giant spiny stars (*P. giganteus*) and ochre stars (*P. ochraceus*) averaged more than 15% body fat, with individual values as high as 29%, 30%, 37% and 44% in these four taxa, respectively. Leather stars (*Dermasteria imbricata*), mottled stars (*Evasterias troschelii*) and purple urchins had intermediate fat levels (6-11%) while sunflower stars, green and white urchins and sand dollars were low in fat (2-4%). Such extreme variation in fat was not observed in other invertebrate groups in our study. Ash content was also highly variable, ranging from 7% in the edible portion of some stars to 54% in entire sea cucumbers (*Parastichopus californicus*); even higher levels were observed in small purple urchins but as these included the ash-rich test the edible fraction assayed differed from many of the other echinoderm samples. To examine the effects of the various major constituents on energy (dry mass basis), we performed stepwise regression on E_D (dependent variable) using ash, carbon, dry matter, fat, nitrogen and hydrogen as candidate independent variables. In contrast to the other taxonomic groups, carbon explained most (96.5%) of the variance (step 1), followed by ash (1.02% and fat (0.69%). If carbon was excluded, ash explained 92.6%, fat 4.35% and nitrogen 0.091% of the variance. The latter model was $E_D = 3390 - 40.825$ ash $+ 59.456$ fat $+ 138.036$ nitrogen ($F_{3,85} = 999.999$, $p < 0.001$, $r^2 = 0.979$). The stepwise procedure indicated that hydrogen and dry matter provided significant additional stepwise contributions, but as these contributed so little to the variance (0.13% and 0.11%, respectively) they were omitted.

With such variation in energy-dense fat and energy-free ash, energy content differed 3-4 fold, even among related taxa. For example, ochre stars and giant spined stars contained more than 3 times the energy (1.4-1.6 kcal/g), on an edible basis, than leather and sunflower stars (about 0.5 kcal/g). Nitrogen varied from 4 to 7%, equivalent to 25 to 45% crude protein.

Other taxa

Other taxa collected and assayed from the MBNMS included sea cucumbers, fat innkeeper worms, gumboot chitons, tunicates, squid and octopus. We also analyzed spoolworms and pile worms from Alaska (Table 2.9). As most of these are soft-bodied we considered the entire organism to be edible for otters, except the dorsal plates of the

Table 2.9. Major energetic constituents of miscellaneous other invertebrates

	Scientific Name	Common Name	Season	Region	Indiv. Prey	Edible portion	Indiv. Edible biomass (g)	DM % fresh	Fat % DM	Carbon % DM	Nitrogen % DM	Crude Protein % DM	Ash % DM	Energy cal/g DM	Energy (kcal/g WM)
I. Monterey Bay National Marine Sanctuary & vicinity, CA															
O1	*Cryptochiton stelleri*	Gumboot chiton	W	N	2	0.520 0.040 2	367.53 24.93 2	14.28 0.55 2	3.05 0.93 2	33.47 0.40 2	8.20 0.60 2	51.22 3.76 2	17.92 0.35 2	3806 47 2	0.543 0.014 2
O2	*Loligo opalescens*	California market squid	S	N	21	1.000 0.000 5	49.96 6.75 5	21.94 0.55 5	12.57 6.63 5	45.86 0.31 3	12.05 0.13 3	75.32 0.81 3	7.29 0.22 3	5268 22 3	1.167 0.046 3
O3	*Octopus rubescens*	Red octopus	SpW	N	5	1.000 0.000 5	122.52 14.81 5	19.87 0.27 5	2.82 0.21 4	43.75 0.22 5	11.97 0.08 5	74.82 0.50 5	7.86 0.18 4	4914 19 4	0.975 0.020 4
O4	*Styela montereyensis*	Stalked tunicate	S	S	34	1.000 0.000 2	5.38 0.58 2	11.93 1.60 2	1.38 0.16 2	28.27 0.06 2	5.03 0.19 2	31.41 1.18 2	28.63 5.56 2	2902 221 2	0.343 0.020 2
O5	*Urechis caupo*	Fat innkeeper worm	all	B	156	1.000 0.000 20	61.24 6.43 25	14.02 0.62 25	2.27 0.17 20	34.10 1.31 21	7.79 0.25 21	48.66 1.58 21	-41.96 63.22 17	3706 123 19	0.502 0.033 19
II. Glacier Bay National Park, AK															
O6	*Echiurus echiurus alaskanus*	Alaskan spoonworm	Sp	AK	14	1.000 0.000 3	20.11 12.92 3	10.39 1.16 3	0.98 0.13 2	20.02 0.64 3	4.78 0.24 3	29.85 1.49 3	54.50 4.40 3	1872 182 3	0.193 0.025 3
O7	*Nereis vexillosa*	Pile worm	Sp	AK	8	0.989 0.011 2	2.72 0.20 2	15.70 0.31 2		42.38 0.24 2	9.78 0.28 2	61.12 1.73 2	14.70 0.67 2	4685 57 2	0.736 0.023 2

chitons that were removed. Relatively few samples were assayed for these taxa, except fat innkeeper worms that were one of the "Top Ten" prey types and thus were collected in all four seasons in both the north (Elkhorn Slough) and in the south (Morro Bay).

Most of these taxa were low in fat (3% or less) and energy (0.6 kcal/g or less), with the exception of market squid that contained 12.5% fat and 1.2 kcal/g; octopus also had moderately high energy (0.98 kcal/g). Sea cucumbers were both very low in dry matter (6.5%) and high in ash (54% of dry matter) and thus were extremely low in energy content (0.1 kcal/g). Nitrogen and calculated crude protein varied 3-fold among taxa, with the highest levels (75% protein) in squid and octopus.

Seasonal and Regional Variation

In the sections above, analyses focused on energy content on a dry mass basis (E_D, cal/g DM) as we were attempting to understand which constituents of the dry mass had the greatest impact on prey energy density. In the following section, we consider variation in energy on a wet basis (E_W, kcal/g WM) as well, as this is the currency in which otters obtain food. If a diving otter is limited to retrieving a set amount of food per dive, or is selecting among prey items of comparable size, its energetic harvest will depend on the energy content on a wet, rather than dry basis. Our field foraging estimates are for biomass per unit time, and the energy return per unit time will depend on E_W (kcal/g WM).

Based on two-way ANOVA of region and seasonal effects, there was very little difference between the north end and south end of the MBNMS with respect to energy (E_W) and fat content (% DM) for most species (Table 2.10). For the two species showing significant regional differences, *Mytilus californianus* and *Tresus nuttallii*, the pattern in both cases was that winter energy values were higher in the southern sampling site (San Simeon – Morro Bay). We also found regional-seasonal interactions for two species: in the case of *Haliotis rufescens*, the peak energy values occurred in the fall at the southern site but occurred slightly earlier (summer/fall) in the north, while in the case of *Urechis caupo*, the peak energy values occurred in the fall at the southern site but slightly later (fall/winter) in the north. Overall, our results suggest that energy content (kcal/g WM) is relatively consistent across the main regions of the MBNMS for most prey species, but

Table 2.10. Statistical significance of regional and seasonal variation in 12 major otter prey species from MBNMS and vicinity.[1]

Prey Type	Species	N	Regional Effect	Seasonal Effect	Season x Region	Peak Season	Low Season	Seasonal % change	Seasonal Variation in Fat?	Comment/Description of Interaction
cancer crab	Cancer antennarius	37	0.813	**0.001**	0.717	F	W	0.51	**0.000**	Strong fall peak
cancer crab	Cancer magister	26	0.553	0.839	0.722				0.346	No significant variation
kelp crab	Pugettia producta	53	0.896	0.235	0.220				**0.001**	Fat content peaks in summer, lowest in fall
clam	Protothaca staminea	14	-	0.216	-				**0.021**	Only collected in North: spring peak in fat content
clam	Tresus nuttallii	23	**0.007**	**0.001**	**0.010**	F	W/Sp	0.39	0.456	Winter energy values higher in south
mussel	Mytilus californianus	21	**0.002**	**0.001**	0.076	S	F	0.51	**0.062***	Winter energy values higher in south
abalone	Haliotis cracherodii	21	0.677	**0.05***	0.317	F	Sp	0.39	**0.083***	Slight trend towards peak in fall
abalone	Haliotis rufescens	29	0.577	**0.001**	**0.001**	F	Sp	0.34	0.599	Fall peak in south, summer/fall peak in north
snail	Tegula funebralis	19	0.380	0.563	0.855				**0.006**	Fat content higher in fall/winter
star	Pisaster ochraceus	36	0.175	0.134	0.206				0.363	No significant variation
urchin	Strongylocentrotus purpuratus	39	0.273	**0.006**	0.199	S/F	Sp	0.41	**0.074**	Energy and fat content drops sharply in spring
worm	Urechis caupo	13	0.311	**0.002**	**0.001**	F/W	Sp/S	0.39	**0.003**	Fall peak in south, fall/winter peak in north

1. Based on 2-way ANOVA except for *Protothaca staminea* , which was only collected in the north. Significant effects indicated in bold. Seasonal % change is the magnitude of change as a proportion of mean.

where energetic differences do occur, they are likely due to variation in the timing of seasonal peaks, possibly reflecting asynchrony of reproductive cycles at larger spatial scales.

In contrast to the limited regional variation, many species showed considerable seasonal variation in energy density and/or fat content, with a 34-51% difference in energy density between the highest and lowest measurements (Table 2.10; Figs. 2.4, 2.5). In most cases, the seasonal peak in energy values reflected a corresponding peak in fat content, although for two species (*Haliotis rufescens* and *Tresus nuttallii*) there was no significant difference in fat content, and the variation in energy density largely reflected variation in the relative water content (a lower percent of dry matter results in less energy per g of wet biomass due to a dilution effect).

Ash is another important constituent that dilutes energy content of prey and that varied by region or season. The variation was most notable in California mussels, in which ash varied both by season ($p<0.001$) and region ($p=0.001$), but there were no significant interactions (2-way ANOVA). It was remarkable that ash content increased from 10.9% in summer to 23.5% in fall; ash content in fall was significantly different from all other three seasons ($p<0.05$, Holm-Sidak), and summer also was significantly lower than spring (15.9%). Mussels in the north (Pebble Beach, 17.8%) were significantly higher in ash than mussels in the south (Morro Bay, 13.7%). Littleneck clams at Elkhorn Slough varied seasonally in ash (Kruskal-Wallis 1-way ANOVA on Ranks, $p=0.008$). There were no regional differences in any other taxa, and seasonal differences in ash were otherwise only observed in *Cancer antennarius* ($p=0.023$; 2-way ANOVA), *Pisaster ochraceus* ($p=0.049$, 2-Way ANOVA) and *Urechis caupo* ($p=0.023$; 2-way ANOVA).

Sex and Season Interactions in Crabs

If seasonal variation in nutrient composition of crabs reflects seasonality of reproduction, it is likely that patterns of nutrient deposition and depletion may differ between females and males. We were able to assess the combined effects of season and sex by two-way analysis of variance of energy (E_W) and fat concentrations in several crab species. In *Cancer magister*, the pattern of empty cells did not allow assessment of

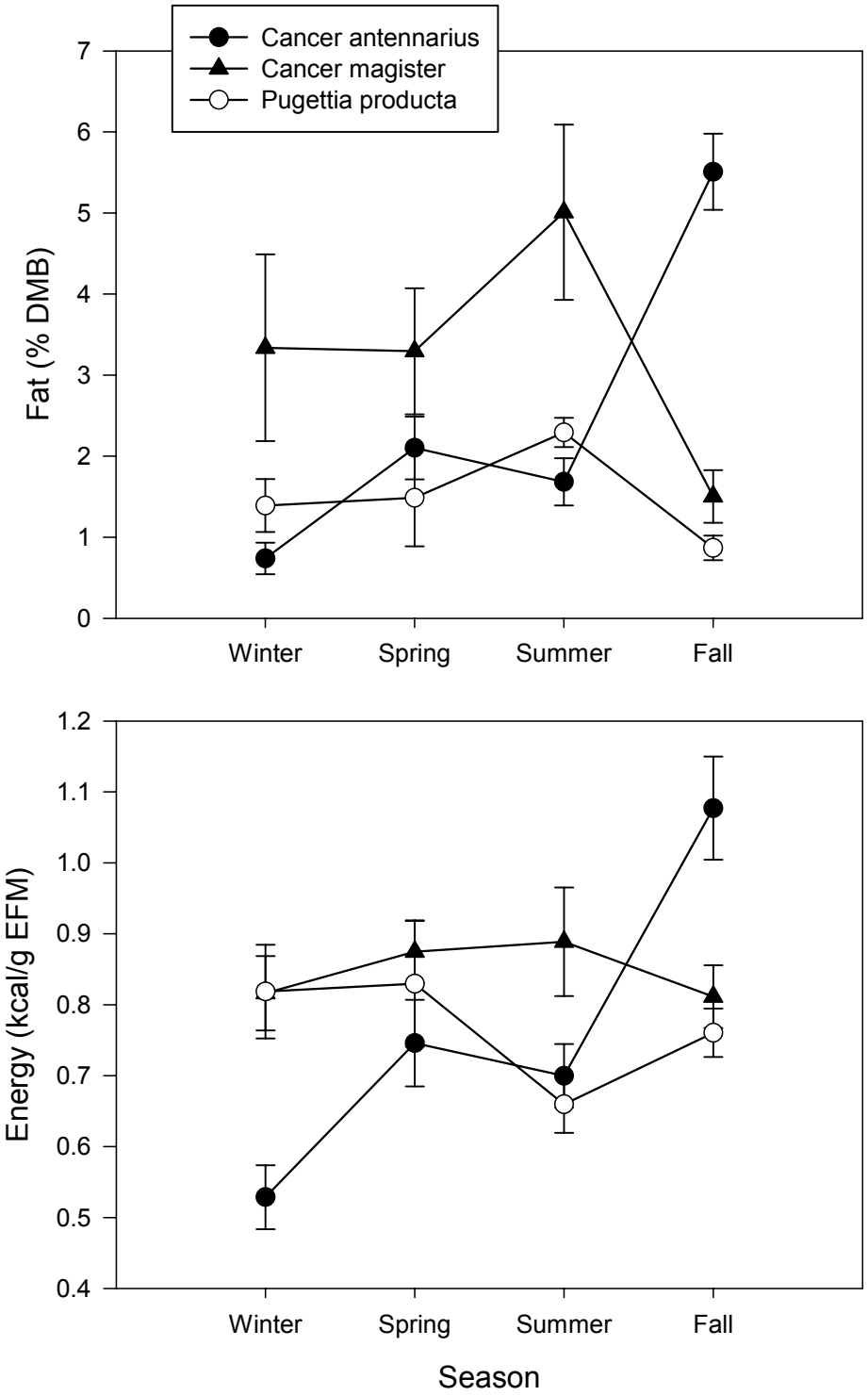

Figure 2.4. Seasonal variation in fat (top panel) and energy (bottom panel) in 3 decapods, *Cancer magister* (solid triangles), *Cancer antennarius* (solid circles) and *Pugettia prodcta* (open circles). Error bars indicate ± 1 standard error.

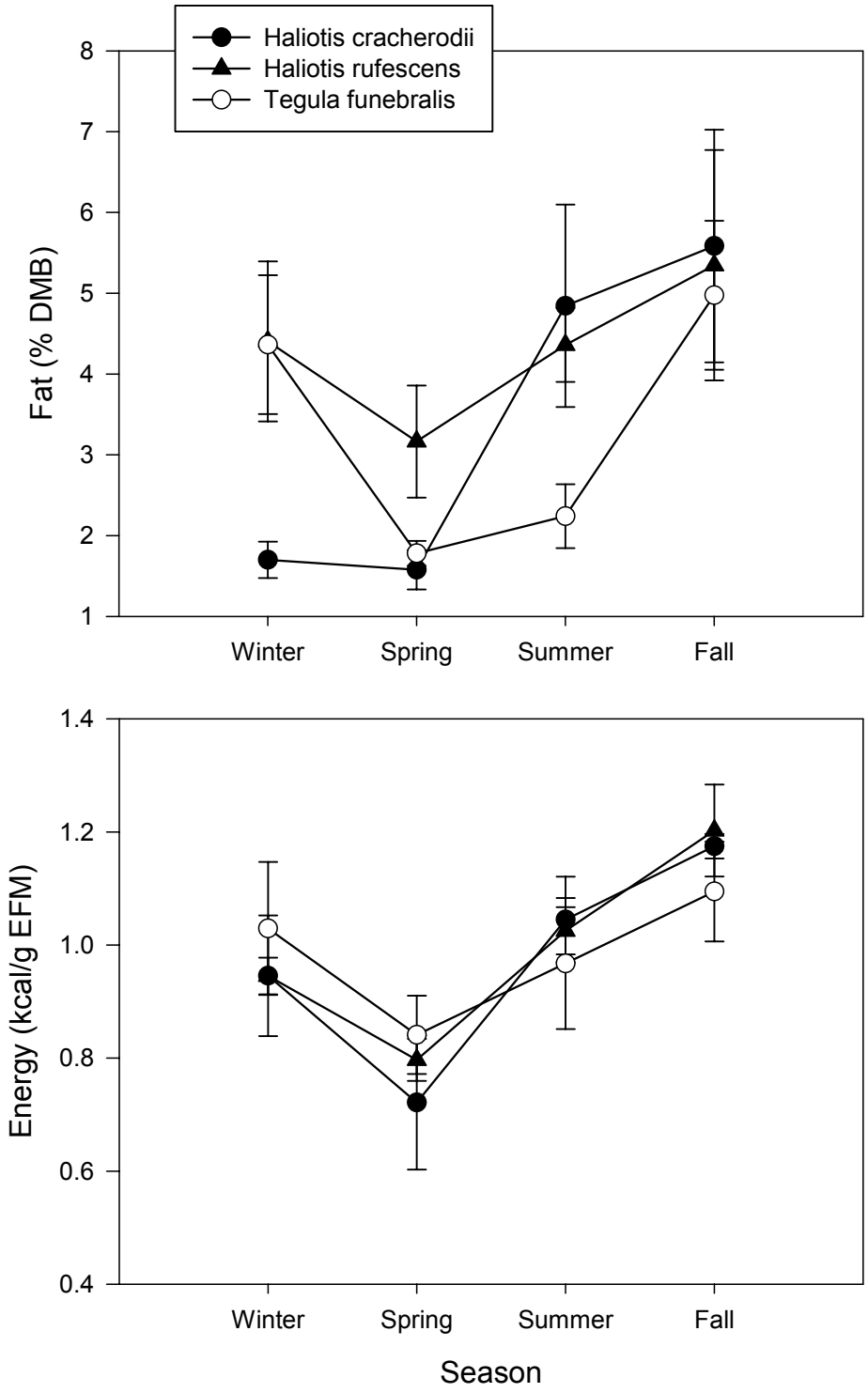

Figure 2.5. Seasonal variation in fat (top panel) and energy (bottom panel) in 3 gastropods, *Haliotis rufescens* (solid triangles), *Haliotis cracherodii* (solid circles) and *Tegula funebralis* (open circles). Error bars indicate ± 1 standard error.

interaction of season and sex, and season had no effect on either energy or fat. In *Cancer antennarius*, season had a significant effect on energy and fat ($p<0.001$ for both constituents, 2-way ANOVAs), but sex had no effect for either of these constituents ($p=0.132$, 0.434, for energy and fat, respectively); there were no significant interactions between sex and season.. In *Pugettia producta*, energy content was not significantly influenced by sex, season or the interaction between them, but fat did differ by season ($p=0.002$) and the interaction between season and sex was highly significant ($p=0.007$). Overall, females were not significantly higher in fat (1.79% ± 0.210) than males (1.62% ± 0.247), but they were significantly higher in fat in fall (females 1.66% vs males 0.53%) and in winter (2.07% vs 0.37%; $p<0.05$ for both comparisons, Holm-Sidak method). By contrast, females were lower in fat in spring (1.19% in females vs. 3.29% in males, $p<0.05$, Holm-Sidak method). We also examined the pattern in energy on a dry mass basis which showed a similar pattern as fat. In this case, females were overall higher in E_D (2881 ± 89 cal/g DM) than males (2570 ± 95 cal/g DM), and this difference was significant in fall and winter ($p<0.05$) but not in spring and summer.

DISCUSSION

Taxonomic variation in composition

The fact that compositional differences were found among the major taxonomic groups is not surprising: the common ancestor (Bilateria) of these taxa lived in the Precambrian (more than 600 million years ago), and the early split of the protosomes (including Crustaceans, and the two mollusk groups, Gastropoda and Bivalvia) and the deuterostomes (including Echiodermata and Chordata) was well-established by the Cambrian (Lecointre and Le Guyader 2006). These two taxonomic lineages differed in body plan in a variety of ways, including whether the skeleton was external (protosomes) or internal (deuterostomes). Much greater taxonomic sampling will be needed to determine whether the compositional patterns we observed correspond to particular evolutionary branch points, but it is clear that feeding on different marine invertebrate taxa involves differing nutritional consequences.

The different body plans themselves have nutritional consequences. For example, otters can separate the valves of large bivalves to gain access to the soft tissues, but must

crush snail shells, leading to inclusion of high-ash shell fragments in the edible portion. We did not address whether variation in the ash content (and calcium content, see Part 4) in decapods is a consequence of differing degrees of exoskeletal calcification associated with the molting cycle, but clearly any predator feeding on prey that undergo ecdysis and shedding of an exoskeleton will face different nutritional challenges at different times in the molt cycle. In terrestrial insects, for example, the potential digestibility of chitin and protein in the exoskeleton (cuticle) depends on the extent of polyphenolic crosslinkages associated with post-molt hardening (sclerotization) of the cuticle (Allen 1989). When feeding on echinoderms, sea otters usually discard the outer part (including the internal skeleton), and thus the proportion that is edible is reduced.

The most remarkable and perhaps biologically significant difference was that among the taxa we examined only one group, the echinoderms, routinely accumulated substantial amounts of fat in the portion eaten by sea otters, with more modest levels of fat in some decapod crustaceans, such as sand crabs (*Emerita analoga*). The gastropods and bivalves that figure predominantly in sea otter diets in some locations (such as abalone at San Nicolas and clams in Glacier Bay; see Part 6) or in some specialized diet types in MBNMS (see Part 5), were uniformly low in fat. This is important not only to energy content but also to the amounts of essential fatty acids available to sea otters (Part 4), and may correlate to amounts and availability of fat-soluble vitamins (Part 3). Thus our observed taxonomic variation in fat content may impact the daily fat intakes of sea otters (Part 5) and thus have major consequences to sea otter nutrition.

Seasonal variation in composition

Within major prey species there was substantial seasonal variation in both fat content and energy content (on a fresh edible basis) (Table 2.10; Figs. 2.4, 2.5). These trends were of particular interest as they may explain seasonal variation in feeding intensity by otters on certain prey species. Presumably seasonal variation in fat and energy content is partly due to an accumulation of nutrients by the prey species in preparation of forming and releasing gametes. As seasonal reproduction is apt to be timed to resource availability and nutrient stores, these two seasonal trends (feeding intensity by

otters and reproductive patterns of prey) may be strongly correlated and difficult to tease apart without intensive study.

The lack of a seasonal trend in fat content for *Haliotis rufescens* was not surprising considering the lack of a distinct reproductive cycle in this species (high gonad and hepatic indices are apparently maintained throughout the year), but interestingly the seasonal pattern of energy content (peak in the fall and low value in the spring) corresponded exactly to the reported annual pattern of hepatic tissue growth (Boolootian et al. 1962). In most of the other species for which we found temporal variation in energy content, the seasonal pattern in our data – energy value increasing to a peak, followed by a low value the next season – was consistent with published data on reproductive cycles and probably corresponds to a build-up of gonad tissue over part of the year, followed by a shedding of gametes during the spawning season (and thus a loss of energy content). In the case of *Mytilus californianus*, for example, spawning generally occurs between October and March (Young 1946), and thus gonads should be developing in spring and summer, with a late summer peak corresponding to the peak in energy density and fat content seen in our data (Table 2.10). Similarly, spawning in *Strongylocentrotus purpuratus* occurs in April and May (Kenner and Lares 1991), and thus gonads would be building up in the summer and fall with a peak in late fall and early winter, closely matching our data on fat and energy content. In the fat innkeeper worm, *Urechis caupo*, the energy stored in gametes has been found to increase gradually between summer and winter, decrease sharply after reaching a winter peak, increased slightly again to a second, smaller peak in the spring, and then decline to low summer values (Suer 1984), again consistent with our data (Table 2.10). The one species for which published reproductive cycles do not seem to match up with our data on energy content is the Pacific rock crab, *Cancer antennarius*: spawning is reported to begin in early March and to be finished by the end of June (Shanks and Eckert 2005). This would suggest that reproductive tissues should be at their maximum in the late winter, somewhat later than our measured peak in energy content in the fall.

Effect of seasonality on sea otter diets

Because many of the key prey species vary substantially in energy content as a result of reproductive or hepatic cycles, we might expect sea otters to respond by adjusting their seasonal patterns of prey selection to take advantage of these trends. Figure 2.6 summarizes the seasonal energy content data for 8 prey species, and superimposed over these box plots are trend lines showing the average relative frequency with which each prey type was captured by 63 study animals in MBNMS between 2001 and 2004 (Tinker 2004). Given that the foraging data were collected during different years than the nutritional data, and that the foraging data are averaged across so many study animals, we would not expect a close correspondence. Nonetheless, for some species (*Mytilus californianus*, *Haliotis rufescens*, *Strongylocentrotus purpuratus*, *Urechis caupo*, and to a lesser extent *Cancer antennarius*) the annual variation in prey capture rates shows a good match to the seasonal trends in energy content. When we examine individual otter diets, this pattern becomes clearer: there were 23 sea otters from which we had a sufficient quantity of foraging data across all seasons that we could reliably evaluate seasonal variation in prey selection. Of these 23 animals, 5 showed strong patterns of seasonal diet variation: Figure 2.7 shows the relative dietary frequency of the most preferred prey type for each of these otters as measured during different months of the year, with the season of peak energy content for that prey type indicated by shading. In each case, it appears that these individual otters are consuming a higher frequency of their preferred prey when the energy content of that prey is highest, and lowering their consumption of those species when energy content is lowest. While such a pattern would be predicted, it is somewhat puzzling that only 5 of the 23 study animals showed a substantial degree of seasonal diet variation, while the other animals had much more constant diets throughout the year. Whether this lack of response to variation in prey nutritional content reflects some other constraint, such as patterns of prey availability, or is a consequence of the limited temporal resolution of our foraging data set, is not entirely clear.

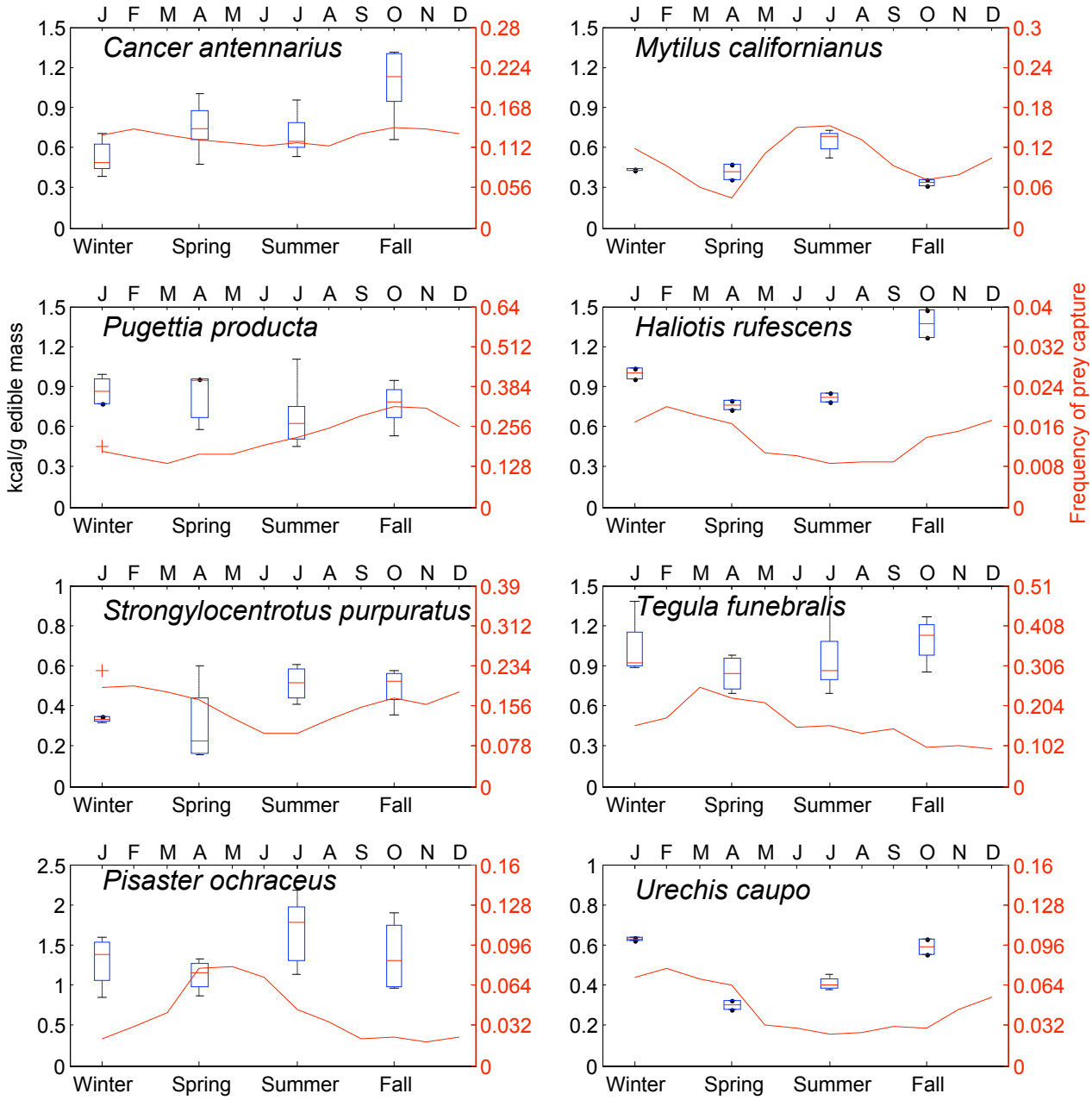

Figure 2.6 Seasonal variation in energy density (kcal per g edible wet mass) of prey (box plots, left axes) and frequency of occurrence of that prey species in the diet (red solid lines, right axes).

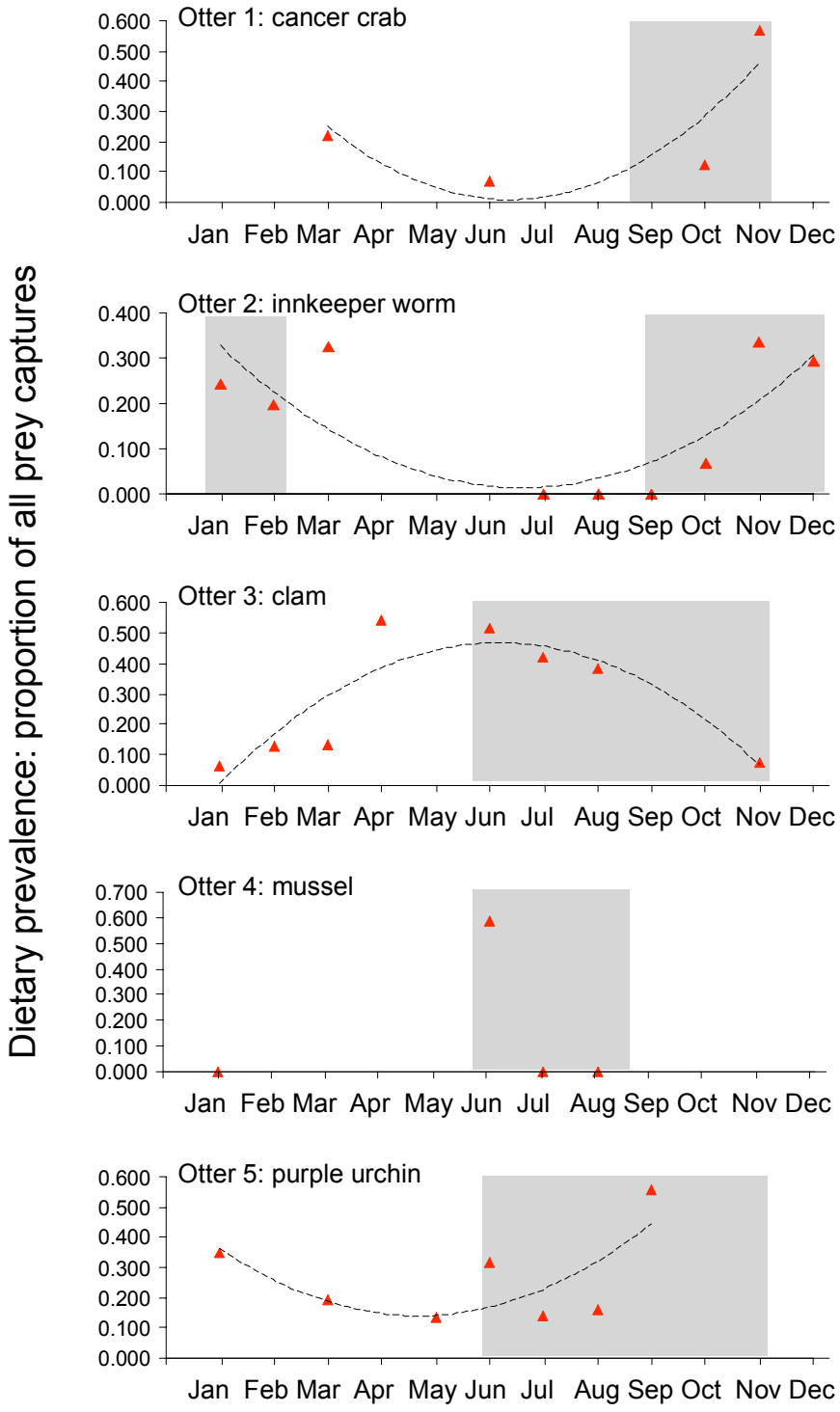

Figure 2.7. Dietary frequency (proportion of all prey captures) of the most preferred prey type for each of 5 otters in MBNMS that showed strong seasonal diet shifts (see text, page 58). For each plot, the season of peak energy content (on a wet edible basis) for the relevant prey type is indicated by gray shading (see Table 2.10).

CONCLUSIONS AND RECOMMENDATIONS

Sea otter prey show considerable taxonomic variation in energetic constituents, and thus it is reasonable to use taxonomic groups to characterize sea otter diets. One would expect diets high in gastropods and bivalves, for example, to differ in energy constituents from diets high in echinoderms such as urchins and stars (see Parts 5, 6).

Since most sea otter prey are low in fat but high in inorganic constituents (ash), the proportion of ash has a major effect on the energy density of prey on a dry mass basis. More research is needed on the causes of the large variation in ash content between and within species. The composition of the ash is discussed in Part 4.

Although fat appears to be important to sea otter nutrition, fat content was low in all gastropods and bivalves studied. Although some of these species demonstrated seasonal variation, the highest fat levels accumulated were still comparatively low.

We found little evidence of regional variation in the energetic constituents of sea otter prey, at least within the MBNMS and vicinity. Regional variation might be more likely to occur in constituents that prey obtain directly from their diets and store in their tissues with minimal modification, such as trace elements, vitamins and essential fatty acids (Parts 3, 4).

Seasonal variation in fat and energy content appeared to be related to reproductive cycles in many of the prey we studied. It would be informative in future studies to dissect out and analyze gonads and egg masses separately so that a direct correlation to body composition can be established. In addition, it would be useful to conduct studies in which sex of all individuals was confirmed via gonadal dissection and/or direct examination of gametes; we were only able to determine sex reliably in decapods as these have distinctive sexual characteristics of the exoskeleton (abdomen and appendages). In decapods, females tended to be higher in fat and energy density than males.

Another potential source of variation in nutrient composition of sea otter prey is size and age of the prey. For example, red urchins demonstrated a positive correlation between body size and percentage of fat in the edible portion. Sea otters may prefer large prey not only because the handling time per g edible biomass is reduced, but also because larger prey are higher in key nutrients. This possibility warrants further investigation. If otters that expand into new foraging grounds (as in Glacier Bay, AK; Part 6) remove

larger, nutrient-rich individual prey first, the prey remaining may differ in nutrient composition. Thus there could be a subsequent decline in nutrient intakes even without a change in the prey species being consumed.

There is undoubtedly a fitness benefit for sea otters to detect and prefer prey of higher nutrient and energy densities. That at least some otters appear to have this capability was suggested by the finding that in at least some individual otters seasonal patterns of prey consumption appeared to track seasonal patterns in fat and energy density. We recommend that such analyses comparing seasonality in prey composition and otter foraging should be extended to other potentially important nutrients in prey, including vitamin A, thiamin, trace minerals and essential fatty acids (Parts 3 and 4).

Part 3. Nutritional evaluation of sea otter prey. II. Vitamins

INTRODUCTION

In addition to energy and protein nitrogen, mammals require about 13-14 vitamins, 22 mineral elements, 10-12 amino acids and several essential fatty acids (Allen and Oftedal 1996). Estimation of exact requirements is complicated by differences in bioavailability of different compounds, factors that affect digestion and absorption of nutrients, and differing nutritional needs associated with activity, growth, reproduction and disease. Some species with highly specialized diets, such as cats, may also have metabolic peculiarities that influence requirements (Allen et al. 1996b). Although the precise nutritional requirements of any wild mammal are unknown and may be influenced by a variety of dietary, digestive and environmental factors, the similarity in biochemical needs at a cellular level among vertebrates makes it possible to extrapolate at a general level across species, at least those of similar diet and related by phylogeny.

This is the basis of the science of comparative nutrition, and is widely used in the practical formulation and assessment of the diets of wild mammals in captivity (Allen and Oftedal 1996; Oftedal and Allen 1996). Our working hypothesis is that the best estimate of sea otter nutrient requirements can be made by comparison to domestic carnivores such as dogs, cats and mink (National Research Council 1982, 2006). As determination of nutrient requirements requires both careful control over experimental diets and detailed study of the biochemical and physiologic responses of the experimental animals, more precise estimates for sea otters will require intensive studies of captive animals. A primary goal of the present study was to examine nutrient levels in otter prey and in the overall diets of individual otters in an attempt to determine if any specific nutrients are likely to be limiting due to low levels, or if some nutrients at high levels could have adverse effects. As the precise requirements of sea otters are not known, the best that can be done at present is to develop a hypothesis about sea otter nutrient limitations or toxicities that may be examined and tested in future research.

Vitamins are dietary compounds with a wide variety of chemical structures and physiological roles, each one an essential nutrient for the normal biochemical functions and health of most vertebrate species. Although present in only trace amounts in foods, a

deficiency of any essential vitamin in the diet may cause specific metabolic defects, characteristic disease states (deficiency syndromes) or more commonly result in impairments of growth, reproductive performance or immune competence.

The vitamins are commonly divided into two groups: fat-soluble and water-soluble. We targeted for analysis those vitamins that have been implicated in deficiency states in other mammals, can be assayed by commercial laboratories at reasonable cost, or that are known to be important in diet formulation for captive animals. A secondary outcome of this research was to provide reference data on prey species in the wild that can be used in assessment of diets of captive sea otters (Allen et al. 1996). Relatively few data are available for marine invertebrates eaten by sea otters, and these are often biased by the fact that the assayed portions are what would be eaten by humans, not sea otters.

Fat-soluble vitamins analyzed in this study include vitamins A, D and E. Water-soluble vitamins analyzed include ascorbic acid (vitamin C), thiamin, riboflavin, niacin and vitamin B_6. In addition, the carotenoid composition of prey species was examined. A few comments on physiological functions and consequences of deficiency of each vitamin are summarized below.

Vitamin A is actually a group of related molecules called retinoids, all having the biological activity of retinol (the form measured in this study). Vitamin A plays an active role in the regulation of gene expression via its nuclear receptors and is essential for normal vision, growth, immune function, morphogenesis and cellular differentiation (Ross 1999). Vitamin A is required for the generation of mucosal barriers and the function of neutrophils, macrophages and natural killer cells, in addition to its role in the adaptive immune response (Stephensen 2001; Chew and Park 2004). Thus vitamin A deficiency could result in impaired immune function and increased susceptibility to infection, a matter of potential importance to sea otters faced with infectious agents such as the parasitic protozoan *Toxoplasma gondii*. Clinical manifestations of acute vitamin A deficiency include night blindness, corneal ulcerations, conjunctivitis, anorexia, weight loss, ataxia, lesions of the skin, metaplasia of the bronchiolar epithelium and increased susceptibility to infections (McDowell 2000). Vitamin A is also toxic at high levels; both deficiencies and toxicities of vitamin A have been diagnosed in captive carnivores (Allen et al. 1996).

Carotenoids do not have established requirements in animals, but many have provitamin A activity. β-carotene appears to be the most important vitamin A precursor but is poorly utilized for vitamin A by obligate carnivores such as the cat and ferret, so that these species have a high requirement for preformed vitamin A in the diet (Lederman 1998; Schweigert 2002; National Research Council 2006). Dogs can convert β-carotene to vitamin A, but it is unknown if sea otters can do so. Other carotenoids measured in sea otter prey in this study were α-carotene, β-cryptoxanthin, echinenone and lutein + zeaxanthin (measured together at co-eluting peaks). In addition to some potential provitamin A activity in α-carotene and β-cryptoxanthin, these carotenoids have been shown to scavenge free radicals and enhance the immune response in many species, including cats and dogs (Rühl 2007; Stahl and Sies 2005; Chew and Park 2004) and thus could be beneficial to sea otters. The oxygenated carotenoids lutein and zeaxanthin have a role in the protection of eye tissues (Landrum and Bone 2001).

Vitamin E is a group of tocopherol and tocotrienol derivatives having the biological activity of α-tocopherol, the most potent of the group. As an antioxidant, vitamin E scavenges free radicals, thereby protecting polyunsaturated fatty acids (PUFAs) and nucleic acids (in DNA) from oxidative damage. Vitamin E also protects macrophages and neutrophils involved in immunity (McDowell 2000; Azzi 2004). The requirement for vitamin E is increased by higher dietary PUFA concentrations, as in many marine oils (Muggli 1994), and by increased oxidative stress, such as can occur from heavy metal exposure, toxin accumulation and various diseases (Traber 1999). Given the challenges sea otters face due to exposure to domoic acid, novel infectious agents and contaminants from surface runoff, their vitamin E status may be of particular importance. Particular signs of vitamin E deficiency include reproductive failure (e.g., fetal resorption), muscle weakness and myocarditis (including cardiomyopathies), yellow fat disease, peripheral neuropathy, hemolytic anemia and red cell fragility (National Research Council 1982; National Research Council 2006). In this study, both α- and γ-tocopherol were measured in sea otter prey. While γ-tocopherol has less than 20% of the antioxidant activity of α-tocopherol, it has several protective functions, including the attenuation of inflammatory damage (Jiang 2003) and the trapping of reactive oxygen species (Christen 1997).

Vitamin D is a group of steroid molecules required for calcium and phosphorus homeostasis. Vitamin D also functions as an active immune modulator and is effective at reducing autoimmune responses, though its interaction with infectious diseases is poorly understood (Manolagas 1985; Cantorna 2004; Holick 2003, 2006). Although many mammals synthesize vitamin D in the skin upon exposure to UV radiation, dogs and cats exposed to UV light are not effective at vitamin D synthesis, making them entirely dependent on dietary sources (How 1994; Morris 1999; National Research Council 2006). A dietary vitamin D requirement for mink has not been established (National Research Council 1982). Some marine oils (e.g., cod liver oil) are rich sources of cholecalciferol (vitamin D_3), but little is known about marine invertebrates. As we do not know if sea otters can synthesize vitamin D, we assayed otter prey for both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). The classic manifestations of vitamin D deficiency are rickets (deformed bones) in growing young and osteomalacia (abnormal bone calcification) in adult animals; vitamin D and/or calcium deficiency is a major nutritional problem for captive insectivores that do not get UV exposure since insects are typically low in calcium and vitamin D (Allen et al. 1996). Vitamin D toxicity can also occur due to excessive vitamin D intakes.

Vitamin C (ascorbic acid) – Ascorbic acid functions as an intracellular antioxidant and is involved in synthesis of collagen and other tissue constituents (Jacob 1999). The classic symptoms of vitamin C deficiency in humans (scurvy) include: impaired collagen synthesis resulting in skin lesions, bleeding gums, poor wound healing, and impaired bone formation. Most animals, including dogs, cats and mink, synthesize ascorbic acid and thus have no dietary requirement for it (National Research Council 1982, 1996; Smirnoff et al. 2004). However, clinical symptoms suggestive of ascorbic acid deficiency have been observed in marine mammals (captive bottlenose dolphins), which responded to ascorbic acid therapy (Miller and Ridgeway 1963). We examined the concentrations of vitamin C in sea otter prey to determine if sea otters receive enough dietary vitamin C to make synthesis unnecessary. Sea otters apparently have the ability to synthesize this compound, as judged by L-gulonolactone oxidase activity (Barck Moore 1980).

Thiamin is an essential enzyme cofactor that is important in reactions of carbohydrate and energy metabolism (McDowell 2000). Thiamin is one of the few vitamins in which

destructive enzymes (thiaminase) or anti-thiamin factors in foods can produce thiamin deficiency. For example, thiamin deficiency occurs in zoo animals consuming raw or fermented fish that contains thiaminases, such that thiamin supplementation of fish-eating animals is now a common practice (Allen 1996; Worthy 2001). Thiaminases may occur in clams and other marine invertebrates (Jacobsohn and Azevedo 1947; Scardi and Magri 1957; National Research Council 1982), so sea otters likely encounter them. Thiamin storage in the body is limited, so deficiency can develop rapidly with consumption of a thiamin-deficient diet. Chronic thiamin deficiency causes cardiac abnormalities such as decreased heart rate, dilation, edema and eventual congestive heart failure. This may be relevant for sea otters, as both endomyocarditis and cardiomyopathy have recently been identified as a significant source of mortality (Kreuder et al. 2005). Acute thiamin deficiency is associated with severe neurological symptoms causing lesions to the brain, convulsions, paralysis and sometimes sudden death (National Research Council 2006). Thiamin deficiency has been induced in harp seals, resulting in anorexia and mild to severe neurological tremors (Geraci 1972).

Riboflavin is a component of two essential metabolic cofactors (FAD and FMN) required for energy metabolism and the regeneration of cellular antioxidants. Riboflavin deficiency has been reported to cause anorexia, weight loss, hypothermia, weakness, ataxia, coma, cataracts and fatty liver (National Research Council 2006).

Niacin serves as an important constituent of coenzymes (NAD and NADP) important to a wide variety of metabolic reactions (Kirkland and Rawlings 2000). In some mammals, dietary intake of niacin may be supplemented by microbial synthesis in the intestine, or niacin may be synthesized from the amino acid tryptophan. However, the domestic cat, an obligate carnivore, does not synthesize measurable amounts of niacin from tryptophan. This is explained by the high activity of a competing metabolic pathway that presumably evolved due to an ample supply of niacin in tissues of vertebrate prey and the high rate of amino acid oxidation for energy in carnivores consuming high protein, low carbohydrate diets. Whether this occurs in an obligate invertebrate predator such as the sea otter is not known. Niacin deficiency results in a broad range of symptoms such as anorexia, fever, diarrhea, inflammation and ulceration of the oral cavity, weakness, confusion, aggression and eventual dementia (National Research Council 2006).

Vitamin B_6 serves as an essential cofactor in many reactions involved in the metabolism of protein, carbohydrate and fat. Animals consuming high protein diets have an increased requirement for vitamin B_6 (Bai 1991). A deficiency in vitamin B_6 in animals can present as decreased growth, defects in amino acid metabolism, neurological symptoms, microcytic hypochromic anemia, cardiac dilation (National Research Council 2006) and decreased immune response (Rall and Meydani 1993).

METHODS

Sample collection and processing: Samples of 11 major sea otter prey species, representing the major prey groups were collected from the central CA coast in the spring of 2005 for vitamin analysis: Cancer crabs (*Cancer antennarius, C. magister*), abalone (*Haliotis rufescens, H. cracherodii*), sea stars (*Pisaster ochraceus*), urchins (*Strongylocentrotus purpuratus*), clams (*Protothaca staminea, Tresus nuttallii*), mussels (*Mytilus californianus*), marine snails (*Tegula funebralis*) and innkeeper worms (*Urechis caupo*). To minimize potential vitamin losses during freezing and thawing, most samples were processed to remove the inedible fraction the same day as samples were collected. Prey were kept alive in cool seawater during holding and transport. They were initially chilled in a refrigerator, and then asphyxiated and chilled to near freezing in a sealed container containing dry ice. Inedible portions were then removed by dissection. The edible portion was immediately frozen on dry ice.

We collected samples of these 11 prey species, as well as kelp crabs (*Pugettia producta*) and California butterclam (*Saxidomus nuttalli*), from all four seasons (fall 2004 – summer 2005) for vitamin E and carotenoid analyses. All samples for vitamin E analyses were frozen on dry ice as soon as possible in the field. Samples were shipped on dry ice overnight to NZP, where they were kept at -80°C until shipment to individual service laboratories.

Samples of red urchins (*Strongylocentrotus franciscanus*), wavy turban snails (*Megastraea undosa*) and kelp crabs (*Pugettia producta*) were collected for vitamin analysis on San Nicolas Island in the fall of 2005. As the collections were made by boat-based scuba divers, it was not feasible to process these prey prior to freezing. These were frozen on dry ice the day of collection and transferred to a –80 C freezer for storage. Prior

to shipping to laboratories for analysis, they were partially thawed to allow removal of test and spines, shell, and carapace, and then immediately refrozen at –80 C.

A commercial laboratory (Eurofins Scientific, Inc., Memphis, TN) conducted the analyses for retinol (vitamin A), ascorbic acid (vitamin C), thiamin, riboflavin, niacin and vitamin B_6. The samples were thawed, homogenized in a blender and then refrigerated overnight prior to analysis. This procedure proved to be inadequate, as indicted by not detectable levels of thiamin and vitamin A; substantial losses had apparently occurred during overnight refrigeration. Therefore a second set of samples was collected in central California, processed as above, and stored frozen at –80 C until thawed at Eurofins. These samples were stabilized with added antioxidant and sampled for analysis the same day as thawing occurred. We only utilized the second set of analytic results for the vitamin analyses that were rerun. The San Nicolas Island samples were reported to be handled at Eurofins by the same protocols as the second set of central California samples.

Retinol was measured using reverse phase high performance liquid chromatography (HPLC) coupled with colorimetric detection (Association of Official Analytical Chemists (AOAC) 1990, Method 974.29, Quackenbush and Smallidge 1986, Rettenmaier and Schuep 1992). Ascorbic acid was measured fluorometrically after reaction with *o*-phenylenediamine (AOAC 1990, Method 967.22 modified). Both niacin and vitamin B_6 were measured by microbiological assay following acid hydrolysis (AOAC1990, Method 944.13 modified, and AOAC1990, Method 961.15 modified, respectively). Riboflavin was assayed by fluorometric measurement following acid hydrolysis (AOAC 1990, Method 970.65 modified). The assay for thiamin was based on alkaline conversion to and measurement of fluorescent thiochrome (AOAC 1990, Method 942.23 modified).

Samples from central California (but not San Nicolas Island) were analyzed for vitamin D at the Vitamin D, Skin and Bone Research Laboratory at the Boston University School of Medicine. After homogenization, saponification with potassium hydroxide and lipid extraction with hexane, the vitamin D fraction was collected using normal phase HPLC. Vitamins D_2 and D_3 were then separated and quantified using reverse phase HPLC coupled with UV spectroscopy.

Vitamin E and carotenoids were measured for central California and San Nicolas Island samples by Dr. Maria Sapuntzakis at the Department of Human Nutrition at the University of Illinois at Chicago. Samples were extracted as described for vitamin D, and α- and γ-tocopherols and carotenoids were then separated using HPLC and quantified by UV absorption. Results for each species are reported as micrograms (μg) or milligrams (mg) per kilogram (kg) on a dry matter (DM) basis.

The published recommended allowances (RA) for nutrients at different life stages of the cat, mink and dog (National Research Council 1982; National Research Council 2006) are used herein as yardsticks with which to assess the adequacy of vitamin levels in sea otter prey. These recommendations are based on a diet of 4.0 kcal metabolizable energy per g. Although we do not know the metabolizability of energy in sea otter diets, the fact that otter prey are high in crude protein (Part 2), and that a proportion of the gross energy in protein is lost as urea (Kleiber 1975), makes it unlikely that otter diets are typically this high in energy (see Parts 5, 6). We did not introduce a correction for the metabolizability of energy in sea otter diets because we believed that any correction would be of arbitrary magnitude and because using nutrient concentrations for a somewhat more energy dense diet would provide a margin of safety for interspecific extrapolation. We considered any nutrient that was at a concentration below the target level to be potentially deficient, although of course otters could complement low levels in one prey type by consumption of other prey with higher vitamin levels.

The RA values are based on a minimum requirement demonstrated to support health and metabolic function in controlled experimental trials using standardized diets and usually include a safety factor for nutrients with variable or unknown bioavailability. Minimal requirements are typically determined with high quality diets and in the absence of stress, parasites or disease, and thus may not cover nutrient requirements under more rigorous conditions, as may occur with wild animals. If a minimum requirement for a given vitamin has not been determined, then the recommended allowance is based on an "adequate intake," or an amount known to sustain the animal in a given life stage, although this amount may not be the minimum required.

RESULTS

Results for fat-soluble and water-soluble vitamins, and comparisons to published RA's for domestic carnivores, are presented in Tables 3.1 and 3.2.

Fat-soluble vitamins

Vitamin A concentrations measured in prey were highly variable, with a high of 106 mg/kg DM measured in a Dungeness crab (*Cancer magister*) and a low of <0.45 mg/kg DM for kelp crabs (*Pugettia producta)*. The vitamin A concentration in *C. magister* was an outlier, being extremely high relative to expected values and to other prey samples, and presumably represents either contamination or laboratory error. Many prey species were low to marginal in vitamin A compared to target RA concentrations of 1500-2000 μg /kg DM for reproduction of domestic carnivores. Of the 14 species analyzed, six (*P. producta*, *Haliotis rufescens*, *Megastraea undosa, Tegula funebralis*, *Pisaster ochraceus* and *Strongylocentrotus franciscanus*) contained substantially less than 1500 μg /kg DM indicating that vitamin A may be limiting in sea otter diets that are based heavily on these taxa.

Carotenoids were detected in all prey species, and appeared to differ among taxa and by season. The results for α-carotene and β-carotene analyses of sea otter prey are presented by season in Table 3.3 and the concentrations of echinenone, β-cryptoxanthin and lutein + zeaxanthin are presented in Table 3.4. The major carotenoids present in sea otter prey are β-carotene and lutein + zeaxanthin, while the other carotenoids are present in much smaller amounts. Echinoderms had the highest concentration of β-carotene; 26 mg β-carotene/kg DM was measured in both *P. ochraceus* and *S. purpuratus*. β-carotene was not detectable in the clams *T. nuttallii* and *P. staminea* and was low (4 mg/kg) in the crabs *C. antennarius* and *C. magister*. Of all the prey species, echinoderms appear to be the richest in other carotenoids as well, though gastropods are also quite high and the California mussel *M. californianus* is particularly high in lutein + zeaxanthin.

Vitamin D_2 was not detectable in all prey species, and vitamin D_3 was below the detection limit for a crab (*C. antennarius*), purple urchin (*S. purpuratus*) and innkeeper worm (*Urechis caupo*) (Table 3.1). The remaining species ranged in vitamin D_3 concentration from 4 to 65 μg/kg DM (*P. ochraceus* and *C. magister*, respectively). The only prey species that appeared to be good sources of vitamin D by comparison to

Table 3.1. Results of fat-soluble vitamin analyses of sea otter prey species in central CA with recommended allowances for the growing mink (NRC, 1982) and cat and dog at various life stages (NRC, 2006) for reference. nd = nondetectable. n/a = not applicable. Samples from central California unless marked by *, indicating San Nicolas Island sample.

		Vitamin A (retinol)	**Vitamin D_2 (ergocalciferol)**	**Vitamin D_3 (cholecalciferol)**		**Vitamin E (α-tocopherol)**
	N (n)	ug/kg DM	ug/kg DM	ug/kg DM	N (n)	mg/kg DM
Decapods						
Cancer antennarius	1 (1)	1560	ND	<4	4 (4)	74
Cancer magister	1 (1)	??	ND	65	4 (4)	157
*Pugettia producta**		<450	n/a	n/a	4 (6)	69
Gastropods						
Haliotis cracherodii	1 (6)	1510	ND	9	4 (4)	68
Haliotis rufescens	1 (1)	1000	ND	5	3 (3)	97
*Megastraea undosa**	1	<640	n/a	n/a	1	138
Tegula funebralis	1 (10)	544	ND	15	4 (65)	49
Bivalves						
Mytilus californianus	1 (5)	3490	ND	58	4 (39)	47
Protothaca staminea	1 (3)	2100	ND	15	4 (12)	24
Saxidomus nuttallii		N/a	n/a	n/a	3 (3)	19
Tresus nuttallii	1 (1)	1980	ND	5	4 (4)	15
Echinoderms						
Pisaster ochraceus	1 (1)	790	ND	4	4 (8)	174
Strongylocentrotus purpuratus	1 (1)	1460	ND	<7	4 (16)	47
*Strongylocentrotus franciscanus**	1 (1)	<1300		n/a	1	244
Echiura						
Urechis caupo	1 (15)	1561	ND	<7	4 (13)	13
Mean						**82.3**
Standard error						**17.5**
Recommended Allowances for domestic animals						
Mink, weaning to 13 wks		1918	n/a	n/a		29
Dog, all life stages		1515	n/a	13.8		30
Kitten, growth after weaning		1000	n/a	5.6		38-120
Cat, adult maintenance		1000	n/a	7.0		38-120
Cat, gestation and lactation		2000	n/a	7.0		31-120

Table 3.2. Results of water-soluble vitamin analyses of sea otter prey species in central CA with recommended allowances for the growing mink (NRC, 1982) and cat and dog at various life stages (NRC, 2006) for reference. nd = nondetectable. n/a = not applicable.

	N (n)	Ascorbic Acid (mg/kg)	Thiamin (mg/kg)	Riboflavin (mg/kg)	Niacin (mg/kg)	Vitamin B_6 (mg/kg)
Decapods						
Cancer antennarius	1 (1)	<20	1.44	52.3	91	2.3
Cancer magister	1 (1)	<20	1.55	227.2	194	5.2
*Pugettia producta**		53	0.73	9.5	112	5.6
Gastropods						
Haliotis cracherodii	1 (6)	119	9.17	31.0	117	2.1
Haliotis rufescens	1 (1)	<20	1.54	25.4	105	1.8
*Megastraea undosa**	1	1135	5.00	10.0	84	2.7
Tegula funebralis	1 (10)	26	1.44	16.5	73	2.4
Bivalves						
Mytilus californianus	1 (5)	<40	1.74	16.8	154	1.9
Protothaca staminea	1 (3)	<30	1.26	19.1	135	1.9
Tresus nuttallii	1 (1)	<20	1.00	6.3	95	1.3
Echinoderms						
Pisaster ochraceus	1 (1)	100	1.80	73.4	119	2.1
Strongylocentrotus purpuratus	1 (1)	<30	1.69	86.2	88	1.9
*Strongylocentrotus franciscanus**	1 (1)	<40	13.11	31.9	98	2.9
Echiura						
Urechis caupo	1 (15)	<30	2.37	56.0	126	2.8
Mean			**3.13**	**47.6**	**114**	**2.6**
Standard error			**0.968**	**15.35**	**8.5**	**0.33**
Recommended Allowances for domestic animals						
Mink, weaning to 13 wks		n/a	1.46	1.76	22	1.8
Puppy, growth after weaning		n/a	1.38	5.25	17	1.5
Dog, adult maintenance & reproduction		n/a	2.25	5.30	17	1.5
Kitten, growth after weaning		n/a	5.50	4.00	32	2.5
Cat, adult maintenance		n/a	5.60	4.00	40	2.5
Cat, late gestation and lactation		n/a	6.30	4.00	40	2.5

Table 3.3. Results of seasonal analyses of α- and β-carotenes in sea otter prey in central CA, expressed as mg/kg DM. nd = nondetectable. n/a = not applicable.

	Winter			Spring			Summer			Fall	
	N	**α-carot.**	**β-carot.**	**N**	**α-carot.**	**β-carot.**	**N**	**α-carot.**	**β-carot.**	**N**	**α-carot.**	**β-carot.**
Decapods - crabs												
Cancer antennarius	1	nd	1.66	1	0.15	2.78	1	0.20	3.09	1	0.05	3.78
Cancer magister	1	nd	2.26	1	nd	3.16	1	0.23	20.60	1	0.11	2.50
Pugettia producta	1	nd	1.75	2	0.58	19.54	1	4.39	70.70	2	0.95	18.57
Mean		nd	1.89		0.36	8.50		1.60	31.46		0.37	8.28
Gastropods												
Haliotis cracherodii	1	3.97	28.12	1	3.28	47.44	1	2.85	40.60	1	3.21	14.62
Haliotis rufescens	1	3.70	21.23	1	15.34	15.45	1	4.69	32.02		n/a	n/a
Tegula funebralis	19	5.38	40.38	10	3.90	28.95	27	1.27	18.31	9	2.42	36.57
Mean		4.35	29.91		7.51	30.61		2.94	30.31		2.82	25.60
Bivalves												
Mytilus californianus	3	0.27	0.68	5	0.45	15.30	16	0.44	5.88	10	0.95	4.77
Protothaca staminea	3	0.09	0.52	3	0.09	1.34	3	0.85	3.09	3	0.13	1.36
Saxidomus nuttallii	1	0.04	0.22	1	0.11	2.06	1	0.38	1.07		n/a	n/a
Tresus nuttallii	1	0.07	0.51	1	0.21	4.60	1	0.43	1.68	1	Nd	0.25
Mean		0.12	0.48		0.22	5.83		0.53	2.93		0.54	2.13
Echinoderms												
S. purpuratus	2	9.63	22.08	1	15.19	15.05	9	7.45	37.72	4	7.49	16.03
Pisaster ochraceus	3	0.25	12.87	1	0.68	9.51	3	1.03	10.91	1	9.08	49.38
Mean		4.94	17.48		7.93	12.28		4.24	24.31		8.28	32.70
Echiura												
Urechis caupo	4	0.16	8.60	3	0.27	18.24	1	1.59	12.95	5	0.79	25.61
Mean		**2.36**	**10.84**		**3.35**	**14.11**		**1.98**	**19.89**		**2.52**	**15.77**
Standard deviation		**3.19**	**12.79**		**5.61**	**12.57**		**2.24**	**19.75**		**3.52**	**16.01**

Table 3.4. Results of seasonal analyses of β-cryptoxanthin (β-cry.), Echinenone (Ech.) and Lutein + Zeaxanthin (Lut/Zea) in sea otter prey in central CA, expressed as mg/kg DM. nd = nondetectable. n/a = not applicable.

	Winter				Spring				Summer				Fall			
	N	β-cry.	Ech.	Lut/Zea.	N	β-cry.	Ech.	Lut/Zea.	N	β-cry.	Ech.	Lut/Zea.	N	β-cry.	Ech.	Lut/Zea.
Decapods																
C. antennarius	1	0.44	nd	Nd	1	0.09	0.13	2.23	1	0.19	nd	0.56	1	0.58	nd	0.69
C. magister	1	0.07	0.08	0.24	1	0.20	0.30	0.65	1	0.43	nd	8.94	1	0.20	nd	0.27
P. producta	1	nd	1.03	2.82	2	0.17	1.16	1.08	1	nd	16.57	10.5	2	nd	nd	5.56
Mean		**0.17**	**0.37**	**1.02**		**0.15**	**0.53**	**1.32**		**0.21**	**5.52**	**6.65**		**0.26**	**0.00**	**2.17**
Gastropods																
H. cracherodii	1	1.04	nd	53.4	1	1.14	0.30	65.7	1	0.76	0.21	81.2	1	0.42	0.10	22.9
H. rufescens	1	0.37	nd	24.3	1	0.72	0.23	31.6	1	0.63	nd	60.1		n/a	n/a	n/a
T. funebralis	19	1.52	4.80	66.6	10	1.38	2.01	71.4	27	0.51	1.00	22.6	9	1.13	1.81	41.7
Mean		**0.98**	**1.60**	**48.15**		**1.08**	**0.85**	**56.3**		**0.63**	**0.40**	**54.7**		**0.52**	**0.64**	**21.5**
Bivalves																
M. californianus	3	0.07	0.19	0.09	5	nd	nd	95.0	16	0.14	0.30	120	10	0.27	0.50	147.2
P. staminea	3	nd	nd	2.62	3	nd	nd	0.66	3	0.11	nd	3.23	3	0.05	nd	3.94
S. nuttallii	1	nd	nd	0.28	1	0.04	nd	1.08	1	nd	nd	1.10		n/a	n/a	n/a
T. nuttallii	1	nd	nd	0.98	1	nd	nd	3.18	1	nd	nd	6.85	1	Nd	nd	0.61
Mean		**0.02**	**0.05**	**0.99**		**0.01**	**0.00**	**25.0**		**0.06**	**0.08**	**32.8**		**0.08**	**0.13**	**37.9**
Echinoderms																
S. purpuratus	2	1.79	42.52	60.2	1	2.11	8.33	51.2	9	4.66	37.72	42.0	4	4.76	45.07	53.3
P. ochraceus	3	4.09	nd	11.4	1	7.16	2.37	276.6	3	2.37	nd	66.2	1	Nd	29.43	0.71
Mean		**2.94**	**21.26**	**35.8**		**4.64**	**5.35**	**164**		**3.52**	**18.86**	**54.1**		**2.38**	**37.25**	**27.0**
Echiura																
U. caupo	4	0.21	0.17	20.9	3	0.20	0.36	43.6	1	0.27	0.55	79.7	5	0.28	0.59	37.9
Mean - all species		**0.74**	**3.75**	**18.8**		**1.02**	**1.17**	**49.5**		**0.78**	**4.33**	**38.7**		**0.59**	**5.96**	**24.2**
Std. deviation		**1.23**	**11.75**	**23.6**		**2.04**	**2.38**	**75.9**		**1.42**	**10.86**	**36.5**		**1.28**	**15.46**	**38.4**

recommended levels for domestic carnivore diets were Dungeness crabs (*C. magister*) and mussels (*Mytilus californianus*).

The α-tocopherol (vitamin E) concentrations of prey species varied more than 10-fold among species, from low levels (<30 mg/kg DM) in three clam species and innkeeper worms (*U. caupo*) to more than 150 mg/kg DM in a crab (*C. magister)*, a star (*P. ochraceus*), and an urchin (*S. franciscanus*). When the results are presented by season (Table 3.5, Figure 3.1), several trends are apparent: 1. bivalves are consistently low in vitamin E, compared to crabs, gastropods and echinoderms, and 2. apparent seasonal variation includes an increase of vitamin E in the crabs in summer and in echinoderms in fall. However, these seasonal trends are based on a single sample per prey species per season and thus require confirmation by further testing. There were also large differences between species in γ-tocopherol concentrations (Fig 3.5). Crabs and *P. ochraceus* contain very little γ-tocopherol, while *M. californianus*, *S. purpuratus*, and *U. caupo* contain much higher levels, sometimes exceeding the concentration of α-tocopherol. A regression analysis showed no significant association between α- and γ-tocopherol concentrations (data not shown).

Water-soluble vitamins

Ascorbic acid or vitamin C was below detection limits (20-40 mg/kg) in 9 prey species, barely detectable (26 mg/kg) in black turban snails (*T. funebralis*) and of moderate concentration (50-120 mg/kg) in kelp crabs (*P. producta*), black abalone (*H. cracherodii*) and ochre stars (*P. ochraceus*); only wavy turban snails (*M. undosa*) were high in ascorbic acid (Table 3.2). Dogs, cats and mink do not require a dietary source of vitamin C and hence have no recommended dietary levels.

Thiamin concentrations ranged from less than 1 mg/kg DM in kelp crabs (*P. producta*) up to 13 mg/kg DM in red urchins (*S. franciscanus*), with most species containing such low levels (1-2 mg/kg DM) that they would not meet recommended dietary allowances for domestic carnivores. These data suggest sea otters may be prone to thiamin deficiency.

By contrast both riboflavin and niacin concentrations were high in sea otter prey, averaging 47.6 and 114 mg/kg DM, respectively (Table 3.2). Despite variation in assayed

Table 3.5. Results of seasonal analyses of α- and γ-tocopherols (vitamin E) in sea otter prey in central CA, expressed as mg/kg DM. nd = nondetectable. n/a = not applicable.

	Winter			Spring			Summer			Fall	
	N	**α-toc.**	**γ-toc.**	**N**	**α-toc.**	**γ-toc.**	**N**	**α-toc.**	**γ-toc.**	**N**	**α-toc.**	**γ-toc.**
Decapods												
Cancer antennarius	1	64.9	2.2	1	47.2	0.8	1	101.7	2.2	1	83.6	0.3
Cancer magister	1	134.0	0.0	1	143.8	0.0	1	240.8	1.5	1	107.7	0.6
Pugettia producta	1	52.9	4.4	2	61.6	8.5	1	112.8	2.7	2	49.2	3.6
Mean		**83.9**	**2.2**		**84.2**	**3.1**		**151.8**	**2.1**		**80.2**	**1.5**
Gastropods												
Haliotis cracherodii	1	69.9	9.5	1	84.6	15.4	1	60.6	11.2	1	57.0	6.0
Haliotis rufescens	1	106.5	4.2	1	53.8	3.8	1	130.6	4.2		n/a	n/a
Tegula funebralis	19	74.7	7.6	10	51.9	3.0	27	23.2	2.5	9	46.3	5.0
Mean		**83.7**	**7.1**		**63.4**	**7.4**		**71.5**	**5.9**		**51.6**	**5.5**
Bivalves												
Mytilus californianus	3	35.1	57.3	5	36.6	16.5	16	38.8	29.0	10	78.3	46.5
Protothaca staminea	3	21.3	2.3	3	33.8	3.7	3	16.2	11.8	3	23.6	4.0
Saxidomus nuttallii	1	14.8	3.2	1	26.4	7.3	1	15.1	6.6		n/a	n/a
Tresus nuttallii	1	0.0	6.4	1	34.2	18.8	1	0.0	10.9	1	24.0	6.5
Mean		**17.8**	**17.3**		**32.8**	**11.6**		**17.5**	**14.6**		**42.0**	**19.0**
Echinoderms												
S. purpuratus	2	56.4	7.9	1	15.6	15.6	9	50.9	76.6	4	63.4	74.0
Pisaster ochraceus	3	165.3	1.3	1	246.7	2.0	3	140.6	1.1	1	284.5	2.7
Mean		**110.8**	**4.6**		**131.2**	**8.8**		**95.8**	**38.8**		**173.9**	**38.3**
Echiura												
Urechis caupo	4	10.3	21.7	3	8.6	14.4	1	6.5	13.9	5	24.5	34.0
Mean - all species		**64.8**	**9.4**		**68.0**	**8.3**		**75.0**	**13.8**		**79.3**	**16.5**
Standard deviation		**46.4**	**13.6**		**59.0**	**6.1**		**65.6**	**19.1**		**68.6**	**21.9**

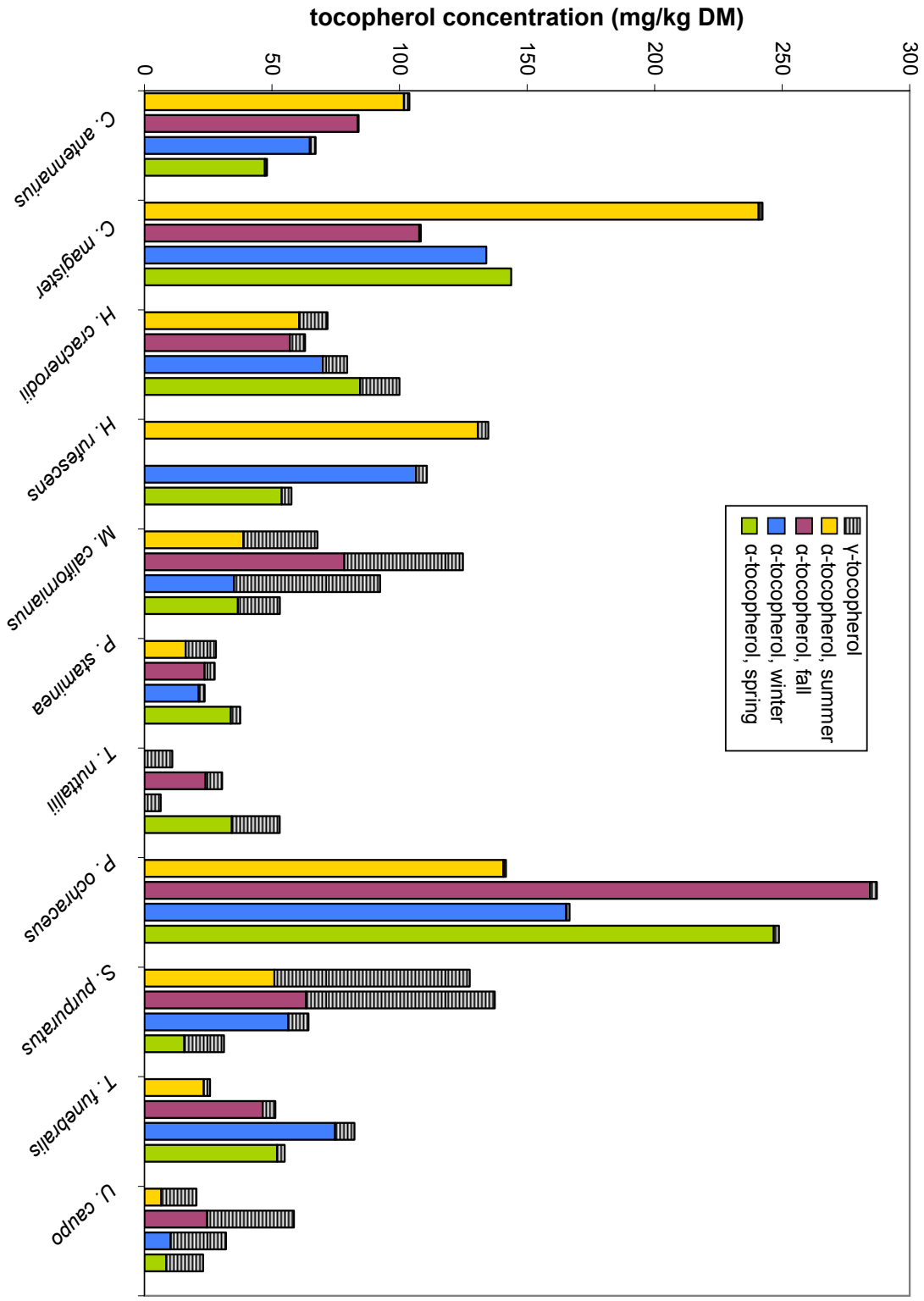

Figure 3.1. Seasonal concentrations of α-tocopherol and γ-tocopherol in sea otter prey species in central California. Bars representing γ-tocopherol are added above bars representing α-tocopherol for each species and season.

concentrations among taxa, all values were above or greatly above the recommended dietary levels for domestic carnivores, and thus are not likely to be limiting in sea otter diets.

Vitamin B_6 concentration averaged 2.6 mg/kg DM in the species analyzed. Most otter prey contained B_6 levels in the range (1.5-2.5 mg/kg DM) of the recommended dietary allowance for domestic carnivores, although a few taxa were above and one below (Table 3.2).

DISCUSSION

Assessment of vitamin levels in prey

Assessment of the nutritional adequacy of prey items is constrained by our limited knowledge of the nutrient requirements of carnivores in the wild and the need to rely on recommended nutrient levels for diets of domestic carnivores such as dogs, cats and mink. Minimal nutrient requirements are usually determined under controlled conditions with highly digestible diets containing nutrients in highly bioavailable forms. The fact that otters consume prey in large quantities, equivalent to 17-33% of body mass daily (Reidman and Estes 1990), and pass this material through the digestive tract quickly (Kirkpatrick 1955; Kenyon 1969) may limit the availability of vitamins and other nutrients. Total digestibility of prey has been shown to be variable in sea otters depending on the individual and on the prey species, ranging from 66% (crab diet) to 95% (abalone diet) (Fausett 1976). Absorption of fat-soluble vitamins and carotenoids is affected by dietary fat concentration (National Research Council 2006), yet most sea otter prey species (other than echinoderms) are low or very low in fat. In addition, sea otters are exposed to a variety of environmental stressors such as cold temperatures, contaminants and diseases which could increase their nutrient requirements (Allen et al. 1996). Oxidative stress from disease or contaminant exposure may increase requirements for antioxidant vitamins. Furthermore, the high metabolic rate of sea otters relative to their body size (Costa 1978) may influence their requirements for vitamins involved in substrate metabolism and oxidative release of energy. Thus our use of recommended nutrient allowances for domestic carnivores as a means of assessing prey nutrient levels may underestimate true otter requirements. On the other hand, if otters have evolved a

life history pattern that entails use of prey of particularly low nutrient levels, it is possible that physiologic and/or biochemical adaptations have evolved that maximize the efficiency of nutrient use and permit otters to survive on diets that might be inadequate for other carnivores. For example, many of the peculiarities of the nutritional physiology of the domestic cat are thought to represent consequences of a prolonged evolutionary history of obligate carnivory (Allen et al. 1996).

Several other limitations of this study should be considered in the interpretation of the results of vitamin analyses. Due to analytic cost, most vitamin analyses were completed on only one sample per species (though, in many cases, containing multiple individuals), collected in one season of the year. Thus we have not accounted for seasonal or geographic variation in vitamin concentrations. The exceptions are vitamin E and carotenoid analyses, which were completed for one sample from each of four seasons. These results demonstrate substantial seasonal fluctuations of uncertain origin. One possibility is that prey composition is affected by seasonal fluxes in the vitamin content of the prey's food resources.

Many of these vitamins are labile, being susceptible to degradation or change due to interactions with heat, light, oxygen or reactive compounds released during physical disruption of cellular matrixes (which occurs during homogenization of samples prior to analysis). We made a dedicated effort to reduce losses during handling and storage. For example, for prey that required extensive processing to remove inedible portions, we completed this on the same day as collection, prior to freezing. Prey were kept alive in cool seawater until they were killed for processing. After processing the samples were immediately frozen on dry ice; they were shipped on dry ice, and they were stored in a – 80 C freezer until submitted to a laboratory for analysis. When the laboratory conducting vitamin analyses initially reported no detectable levels of vitamin A in 7 of 11 samples, we were informed that the samples had been stored overnight in a refrigerator after homogenization, potentially permitting losses. We therefore collected, processed and shipped a second set of samples to the laboratory which upon analysis had higher levels of vitamin A, thiamin and, in some cases, vitamin C (Table 3.6). Although some of this discrepancy may result from inter-sample variation (the second samples were collected later in spring than the first samples), the consistent increase from not detectable to

Table 3.6. Analytic results for labile vitamins, before (sample 1) and after (sample 2) modification of processing procedure.[1]

Sea Otter Prey Species		DM	Retinol (Vit A)			Thiamin (Vit B_1)			Ascorbate (Vit C)		
			sample 1	sample 2	Diff.	sample 1	sample 2	Diff.	sample 1	sample 2	Diff.
		%	ug/kg DM		%	mg/kg DM		%	mg/kg DM		%
Cancer antennarius	Pacific rock crab	22.9	< *577*	**1564**	171	**1.89**	**1.44**	-24	< *19.2*	< *19.2*	0
Cancer magister	Dungenes crab	23.0	< *574*	NA		**1.58**	**1.55**	-2	< *19.1*	< *19.1*	0
Haliotis cracherodii	Black abalone	21.9	< *604*	**1505**	149	**1.08**	**9.17**	750	< *20.1*	**119.1**	491
Haliotis rufescens	Red abalone	22.0	< *601*	**1000**	66	**1.29**	**1.54**	19	< *20.0*	< *20.0*	0
Tegula funebralis	Black tegulas	33.6	**599**	**573**	-4	**0.64**	**1.44**	123	< *13.1*	**25.7**	96
Mytilus californianus	California mussels	10.4	**1864**	**3486**	87	**2.26**	**1.74**	-23	< *42.5*	< *42.5*	0
Protothaca staminea	Littleneck clams	13.3	**1487**	**2095**	41	**1.10**	**1.26**	15	< *33.2*	< *33.2*	0
Tresus nuttallii	Gaper clams	18.7	< *706*	**1982**	181	**0.88**	**1.00**	14	< *23.5*	< *23.5*	0
Pisaster ochraceus	Ochre star	24.3	**1023**	**792**	-23	**0.91**	**1.80**	98	**100.7**	**100.7**	0
Strongylocentrotus purpuratus	Purple urchin	13.7	< *963*	**1472**	53	**1.01**	**1.69**	67	< *32.1*	< *32.1*	0
Urechis caupo	Fat innkeeper	14.0	< *942*	**1548**	64	**1.67**	**2.37**	42	< *31.4*	< *31.4*	0
	average				79			98			53
	sem				22			67			45
	n				10			11			11

1. Values given in italics are the maximum value (detection limit), based on sample dry matter (DM) content. Values in bold are actual values. The difference between samples 1 and 2 (expressed as a percent of sample 1) are minimum values if sample 1 was below the detection limit. Only sample 2 is included in vitamin tables.

detectable levels in most prey species suggest the first samples had deteriorated, with loss of vitamins. For example, thiamin content of frozen fish (alewife) is also highly labile after thawing, with substantial losses in just a few hours, even at cold temperatures (Wright et al. 2005). Great care must be exercised in handling of samples for vitamin analysis. In the absence of a dedicated effort to investigate potential analytic losses, we are unable to rule out the possibility that some of our reported vitamin concentrations may be lower than what otters eating live prey would ingest. It is some comfort that most of our values are similar to those reported for marine invertebrates elsewhere (Sidwell 1978; Sikorski 1990; USDA 2003; Table 3.7), but further investigation of the accuracy and recovery of these processing and analytic methods is needed.

Are Vitamin A Concentrations in Sea Otter Prey Adequate?

A major goal of the analysis of sea otter prey was to survey for nutrients that might be in such low concentrations as to potentially have a limiting role in growth, reproduction or health of sea otters in central California. Although our analyses and the scope of the current study do not allow us to prove such a limitation, two nutrients appear to be consistently low in otter prey: vitamin A and thiamin (see below).

Domestic carnivores require 1500-2000 mg/kg vitamin A in the diet during pregnancy and lactation, but most otter prey contain substantially less than this amount. The only prey type we analyzed that contained 2000 or more mg/kg vitamin A was bivalve mollusks (Table 3.2). Available data on vitamin A levels in other invertebrates confirm that bivalves tend to have higher vitamin A concentrations than other invertebrate taxa, except perhaps octopus and cuttlefish (Table 3.7). If most marine invertebrates are low in vitamin A, otters may be at risk of vitamin A deficiency. An exception would be areas where bivalves predominate in the diet, such as in Glacier Bay, Alaska where clams may constitute 70% of otter diets (Bodkin et al. 2001, 2002, 2003).

Otter prey are also typically low in fat, which may impair vitamin A absorption (Gershoff 1957; Jalal 1998). Additional factors may also affect vitamin A absorption and metabolism in sea otters. For example, lipophilic, persistent organochlorine pollutants (POPs) can decrease vitamin A status in exposed animals. POPs have been shown to decrease hepatic vitamin A stores, disrupt vitamin A transport, and increase glomerular

Table 3.7. Vitamin A (retinol) and thiamin concentrations in marine invertebrates[1]

Species	Common name	Location/season or reference no.	Retinol mcg/kg DM	Thiamin mg/kg DM
Decapods				
Cancer antennarius	**Pacific rock crab**	**North/spring**	**1564**	**1.44**
Cancer magister	Dungeness crab	NDB No: 15143	1297	2.26
		North/spring		**1.55**
Pugettia producta	**Kelp crab**	**SNI/fall**	**<456**	**0.73**
Paralithodes camtschatica	Alaska king crab	NDB No: 15136	343	2.10
Callinectes sapidus	Blue crab	NDB No: 15139	95	3.81
Chionoectes opilio	Queen crab	NDB No: 15144	**2317**	4.12
Astacus, Orconectes, and Procambarus spp.	Crayfish, mixed species	NDB No: 15145	901	3.94
Homarus americanus	Northern lobster	NDB No: 15147	904	0.26
Jasus spp. and Panulirus spp.	Spiny lobster	NDB No: 15154	193	0.27
Bivalves				
Mytilus californianus	**California mussels**	**North/spring**	**3486**	**1.74**
Mytilus edulis	Blue mussel	NDB No: 15164	**2472**	8.24
Crassostrea gigas	Pacific oyster	NDB No: 15171	**4515**	3.73
Pectinidae	Scallop, mixed species	NDB No: 15172	700	0.56
Protothaca staminea	**Littleneck clams**	**North/spring**	**2095**	**1.26**
Tresus nuttallii	**Gaper clams**	**South/spring**	**1982**	**1.00**
Lamellibranchia	Clam, mixed species	NDB No: 15157	**4950**	4.40
Gastropods				
Haliotis cracherodii	**Black abalone**	**North/spring**	**1505**	**9.17**
Haliotis rufescens	**Red abalone**	**North/spring**	**1000**	**1.54**
Haliotis spp.	Abalone, mixed species	NDB No: 15155	79	7.47
Megastraea undosa	**Wavy turbansnail**	**SNI/fall**	**<640**	**5.00**
Tegula funebralis	**Black tegulas**	**North/spring**	**573**	**1.44**
	Snail, unspecified species	NDB No: 90560	1442	0.48
Buccinidae	Whelk, unspecified species	NDB No: 15177	765	0.76
Echinoderms				
Pisaster ochraceus	**Ochre star**	**South/spring**	**792**	**1.80**
Strongylocentrotus	**Red urchin**	**SNI/fall**	**<1290**	**13.11**
Strongylocentrotus	**Purple urchin**	**South/spring**	**1472**	**1.69**
Other taxa				
Octopus vulgaris	Common octopus	NDB No: 15166	**2278**	1.52
Sepiidae	Cuttlefish, mixed species	NDB No: 15163	**5813**	0.46
Urechis caupo	**Fat innkeeper**	**South/spring**	**1548**	**2.37**

1. Data for the edible portion eaten by sea otter (in bold, current study) or human (not bolded, from USDA 2004), presented on a dry matter basis. North and south refer to northern and southern collection areas in MBNMS: SNI = San Nicolas Island.

filtration and excretion of vitamin A metabolites (Simms and Ross 2000). A study of California sea lions at Año Nuevo Island, within the sea otter range, found that serum vitamin A concentrations were negatively correlated to serum PCB toxic equivalent levels (Debier 2005). Significant amounts of POPs, including PCBs and DDT, have been found in sea otter tissues in California (Nakata et al. 1998; Bacon et al. 1999; Kannan et al. 2004). If the vitamin A status of sea otters is already marginal because of limited dietary supply, exposure to POPs could push these animals into a state of vitamin A deficiency.

Vitamin A is critical to so many physiological functions that a deficiency can manifest in myriad ways, sometimes with very specific clinical symptoms and other times with more general, nonspecific signs such as decreased immune response and fertility (McDowell 2000). The multifaceted roles of vitamin A in many aspects of immunity has recently been reviewed, and includes participation in maintenance of mucosal surfaces, in the generation of antibody responses, in the function of lymphocytes, natural killer cells and neutrophils, and as modulators of gene transcription (Semba 2002). Thus vitamin A deficiency may increase susceptibility to infectious disease via a variety of mechanisms. The southern sea otter population in central CA suffers from an unusually high incidence of infectious disease (Estes et al 2003a; Kreuder et al. 2003), and it is possible that this phenomenon is related to a marginal dietary supply of vitamin A. In domestic cats, a dietary level of 600-1200 μg/kg vitamin A, similar to that in *Tegula* snails, stars and kelp crabs, produces an array of reproductive and developmental disorders, including fetal resorption, abortion, premature birth and deformed neonates (J. G. Morris and Q.R. Rogers, cited in National Research Council 2006). Although adult mortality rather than reproductive failure is usually identified as a constraint on population growth in sea otters, it is possible that the high incidence of individual diet specialization may leave a subset of otters at particular risk of the reproductive and health risks associated with inadequate vitamin A intake.

Two alternative explanations to inadequacy of vitamin A in sea otter prey must be considered, however: vitamin losses in samples and potential conversion of carotenoids to vitamin A by sea otters. Vitamin A levels may be underestimated if losses occur during sample handling, storage and processing, or if analytic problems arise in the separation

and identification of retinol and related compounds. We addressed this issue via requesting a change in laboratory handling procedures (Table 3.6; see above), but further testing and validation of the impacts of sample handling and processing on vitamin A should be undertaken. Another possibility is that sea otters can utilize carotenoids as a source of retinol or vitamin A even though many other carnivores are limited in this respect. In humans β-carotene can be cleaved to produce retinol, with a conversion rate of about 21 μg dietary β-carotene required to form 1 μg retinol activity. In contrast to preformed vitamin A, β-carotene was found in relatively high concentrations (i.e., mg rather than μg per kg DM) in many sea otter prey species, especially gastropods and echinoderms (Table 3.3). If sea otters are able to convert β-carotene to vitamin A, this additional source of vitamin A may reduce the likelihood of deficiency. Among domestic carnivores, dogs have some, ferrets very little, and cats virtually no ability to utilize β-carotene as a source of vitamin A (Lederman et al. 1998; Schweigert et al. 2002; National Research Council 2006). However, if sea otters evolved on a diet rich in β-carotene and other carotenoids while low in preformed vitamin A, there may have been a selective advantage in enzymatic alterations that increased efficiency of conversion of β-carotene to vitamin A. If sea otters differ from most other carnivores in ability to utilize carotenoids as a source of vitamin A, the low preformed vitamin A concentrations in sea otter prey may not be of such concern.

Other Fat-soluble Vitamins

In the design of this study, particular attention was directed towards vitamin E. The discovery that pathologic deterioration of cardiac musculature was a primary cause of death in adult sea otters in MBNMS (Kreuder et al. 2003) suggested a possible nutritional deficiency. Although the etiology of this cardiomyopathy in sea otters is poorly understood, cardiac and other myopathies can be produced by vitamin E and selenium deficiency in a wide range of species, including marine carnivores (Machlin 1980; Oftedal and Boness 1983; McDowell 1989). Vitamin E requirements are increased by intake of polyunsaturated fatty acids (as are typical of marine prey [Iverson 1993, Iverson et al. 1997a,b]) and by exposure to natural or artificial (contaminant) oxidants; the tissues affected may be influenced by other nutrients, such as sulfur amino acids (Machlin 1980). Given that some otter prey types such as mussels and clams had been

reported to be low in vitamin E (<30 mg/kg DM, USDA Agricultural Research Service 2004), we opted to examine the prevalence of vitamin E not just in single prey samples, but rather over the course of the year (Table 3.5). Our results confirmed that low levels (<25 mg/kg DM) of the primary form of vitamin E, α-tocopherol, are characteristic of clams and fat innkeeper worms, although even clams have moderate levels (26-34 mg/kg DM) in spring. Other otter prey have high levels (>50 mg/kg) of α-tocopherol, with some species reaching seasonal levels of 100 mg/kg or more (Table 3.5). It was surprising that some species, particularly purple urchins (*S. purpuratus*), California mussels (*M. californianus*) and fat innkeeper worms (*U. caupo*) contained greater amounts of γ-tocopherol than α-tocopherol, at least at certain times of the year (Table 3.5). γ-tocopherol has only about $1/10^{th}$ the biological activity of α-tocopherol, but at these high concentrations may contribute to the vitamin E status of sea otters. Given that the recommended dietary allowance for vitamin E in domestic carnivore diets is about 30-40 mg/kg DM α-tocopherol, sea otter prey are generally well-supplied with this vitamin. Cats fed high-fat fish rich in partially oxidized polyunsaturated lipids may have a much higher vitamin E requirement (up to 120 mg/kg DM; National Research Council 2006), but sea otters in the wild are mostly feeding on fresh, low-fat prey, and are unlikely to require such high levels. However, a low-fat diet may result in reduced vitamin E absorption (Jeanes et al. 2000).

A dietary supply of vitamin D is essential for carnivores such as dogs and cats that are unable to synthesize vitamin D upon exposure to UV light (National Research Council 2006), but it is not known if sea otters are subject to this constraint. Ergosterol, the predominant form of vitamin D in plants (vitamin D_2), was not detected in sea otter prey, and is not found in marine fish either (Holick 2003). However cholecalciferol, or vitamin D_3 was present at moderately high levels (>50 μg/kg DM) in some prey (e.g., *C. magister, M. californianus*), intermediate levels (5-15 μg/kg DM) in most prey, and not detectable levels in Pacific rock crabs (*C. antennarius*), purple urchins (*S. purpuratus*) and fat innkeeper worm (*U. caupo*). Thus otter intake of vitamin D will depend on type of diet; the recommended dietary level for carnivores is about 6-14 μg/kg DM. The significance of low vitamin D intake will depend on synthetic ability; to our knowledge there have been no reports of bone demineralization or deformities that might suggest

vitamin D deficiency in wild otters. Whether subclinical deficiencies could exist in otters, as suspected in human populations (Holick 2006), is unknown.

Water-soluble vitamins

Among the water-soluble vitamins, both niacin and riboflavin were found in concentrations well above estimated requirements of domestic carnivores (Table 3.2). While ascorbic acid (vitamin C) was not detectable in most prey, this nutrient is probably not required by sea otters as they have been shown to be capable of ascorbic acid synthesis (Barck Moore 1980; Smirnoff et al. 2004). Thus the only two water-soluble vitamins that we assayed that could be limiting to sea otters are thiamin and vitamin B_6.

Most sea otter prey species were considerably below the recommended dietary thiamin levels of 5.5-6.3 mg/kg DM for cats, with only red urchins (*S. franciscanus,* 13.1 mg/kg) and black abalone (*H. cracherodii*, 9.1 mg/kg) having higher concentrations. Of 14 prey species, 10 (71%) contained less than 2.0 mg/kg DM and 12 (86%) less than 5.5 mg/kg (Table 3.2). A prior study of 15 marine invertebrate taxa found similar thiamin results: 8 taxa (53%) less than 2 mg/kg and 13 (87%) less than 5 mg/kg DM (USDA 2004), even though only one species was common to both studies (*C.magister*: 1.6 g/kg in present study vs. 2.3 g/kg in USDA dataset; Table 3.7). There were no obvious differences among taxonomic groups (Table 3.7). Thus sea otters confront food resources that are very low in thiamin, but if their requirements are closer to those reported for mink and dogs (1.4-2.3 g/kg DM) than cats, a mixed diet of various prey species may still contain enough thiamin to meet needs (see Part 5). Note that losses during processing and analysis can affect thiamin results (Tale 3.6), so great care is needed in sample handling. Wright et al. (2005) demonstrated that thiamin concentrations declined by 30-55% in 2 hours after thawing of frozen fish, depending on temperature. Any sea otter demonstrating behavioral or neurologic abnormalities should be considered suspect for thiamin deficiency; other signs of deficiency in domestic carnivores include slow growth, failure of appetite, ataxia, CNS depression, impaired reflexes, convulsions, profound muscle weakness, bradycardia, and pathologic changes in the brain, peripheral nerves and myocardium (National Research Council 2006). Whether the abnormal haul-out behavior of some adult females following the weaning of pups (T. Tinker, pers. obs.), the high incidence of mortality due to cardiomyopathy, or the positive correlation between

cardiomyopathy and emaciation (Kreuder et al. 2003) have anything to do with thiamin status is unknown, but certainly warrants further study.

The situation with regards to vitamin B_6 is worth noting, but does not appear to be of practical concern. The concentrations of vitamin B_6 in all prey except gaper clams (*T. nuttallii*) are in the range of, or slightly higher than, the recommended dietary levels for domestic carnivores (1.5-2.5 mg/kg DM). The overall average (2.6 ± 0.33 se mg/kg DM; range 1.3-5.6, n=14) for marine invertebrates in our study is somewhat lower than in the USDA database (6.6 ± 3.66 se mg/kg DM; range 2.6-18.2, n = 15), but not low enough to warrant concern about vitamin B_6 in sea otter diets. The vitamin B_6 requirement of cats increases with dietary protein (Bai 1991), and the recommended allowance for cats is designed for diets up to 50% crude protein. As many sea otter prey contain even higher crude protein concentrations (up to 75% of dry matter), it is possible that sea otter requirements could exceed those of cats, but this has not been studied.

CONCLUSIONS AND RECOMMENDATIONS

This broad survey of vitamin concentrations in sea otter prey has revealed that at least two vitamins may be sufficiently low in sea otter diets to be a matter of practical concern: vitamin A and thiamin. The vitamin A status of sea otters is of particular importance because of the role of vitamin A in protection against infection as well as its susceptibility to adverse impacts from contaminants such as persistent organic pollutants. One can visualize a scenario in which sea otters constrained to marginal vitamin A intakes via dietary specialization develop impaired vitamin A status in response to environmental contaminants and thus develop increased susceptibility to infectious disease. There may be synergies amongst these three threats (malnutrition, contaminants, and diseases) that contribute to increased mortality, especially among females stressed by the great nutritional demands of lactation. Thus we recommend that high priority be given to in depth study of the vitamin A nutrition of sea otters in the Monterey Bay National Marine Sanctuary. This should include 1. assessment of circulating and storage levels of vitamin A in captive and free-ranging otters consuming different prey types and at different stages of life and reproduction; 2. evaluation of past and future necropsy data for any evidence of deficiency signs; 3. assessment of the potential errors in sample

storage, processing and analysis of vitamin A in prey; 4. a broader survey of vitamin A concentrations in sea otter prey, including seasonal variation, and 5. studies of captive sea otters to measure the efficiency of uptake and utilization of β-carotene as a source of vitamin A. More controlled studies examining the effects of different levels of dietary vitamin A and/or carotenoids on health and reproduction would likely be too invasive to conduct on otters but could be conducted using a model species such as mink.

The possibility of thiamin deficiency seems less likely, given the absence of neurologic signs, although it is possible that thiamin deficiency and domoic acid poisoning could have overlapping symptoms. Whether deficiencies of thiamin or other nutrients contribute to the cardiomyopathies observed in sea otters is impossible to say at this point. Nonetheless, it would be valuable to undertake studies of the thiamin status of sea otters, particularly with captive animals that could be used in supplementation studies. In zoos and aquaria it is common to supplement marine fish and invertebrates used to feed marine mammals with added thiamin out of concern that thiaminases in seafood may destroy thiamin in stored foods, leading to overt deficiency (Allen et al. 2006).

Data obtained on vitamin E and carotenoids in sea otter prey indicate that substantial seasonal variation may occur in vitamin concentrations. This is not surprising, as the vitamins in sea otter prey derive from the foods the prey species eat, and both seasonality in food availability and seasonal storage of nutrients prior to spawning may contribute to seasonal changes in prey vitamin concentrations. It will be important to assess seasonality in potentially limiting vitamins, such as vitamin A and thiamin, to understand which prey species are most important to otter health. We also recommend that research be conducted on the ability of sea otters to synthesize vitamin D from provitamin D precursors in the skin. Prey vitamin D levels were low, but this is of concern only if otters are unable to synthesize vitamin D upon UV exposure.

Finally the database generated herein may be particularly valuable to nutritionists and veterinarians facing the challenge of feeding otters in captivity. Based on our results, it appears that sea otters in captivity should be routinely supplemented with vitamin A and thiamin, and that there may be benefits to supplementation with vitamin D and vitamin B_6. We also recommend supplementation with vitamin E because it may deteriorate rapidly during storage and thawing of frozen prey.

Part 4. Nutritional evaluation of sea otter prey. III. Minerals, fatty acids and amino acids

Section A. Major minerals

INTRODUCTION

Sea otters have a long evolutionary history of feeding on marine benthic invertebrates (Riedman and Estes 1990). Obligate terrestrial carnivores, such as domestic cats, have evolved a suite of metabolic and biochemical traits that reflect the nutritional properties of their vertebrate prey (Morris and Rogers 1983; Allen et al. 1996b; National Research Council 2006), and it is reasonable to suppose that sea otters have similarly adapted to the nutritional properties of marine benthic invertebrates. A parallel situation exists among carnivores that depend on insect prey. The low calcium content of terrestrial invertebrates (Allen and Oftedal 1989) may limit reproduction in both bats and birds, and require such insectivores to seek out prey with high calcium in their gut contents (Bilby and Widdowson 1971; Barclay 1994, 1995; Hood 2001). Thus the assumption that dietary energy rather than nutrients is the limiting factor in foraging (e.g., Costa 1978 for sea otters) may not be correct in species that specialize on invertebrate diets (Barclay 1994, 1995).

However, little is known about the nutrient composition of benthic invertebrates. Although benthic invertebrates often have a calcareous shell or exoskeleton, the soft parts eaten by humans (and otters) appear, from a limited data set, to be low in calcium, to have an inverse calcium:phosphorus ratio and to be low in manganese (n= 17; USDA Agricultural Research Service 2003). Differences in enzyme systems, anatomical structures, and metabolic pathways among invertebrates may produce quite different tissue levels of trace elements and other constituents, such that diet shifts from one prey type to another may have substantial impacts on otter nutrition. In this section, we examine the concentrations of macrominerals (calcium, phosphorus, potassium and magnesium) in sea otter prey; trace elements will be discussed in a subsequent section. Sodium analysis was not conducted as it was deemed inaccurate given the sodium contamination from sea water adhering to, or within body cavities of, sea otter prey.

Although the macromineral requirements of sea otters have not been studied directly, one can assume that sea otters require the same macrominerals as dogs, cats and mink, and presumably in similar proportions. Mammals require dietary **calcium** for tissue growth (especially bone deposition), fetal development, milk production, and replacement of endogenous losses. About 98% of the calcium in the body is deposited as bone mineral, and this supplies a reserve that can buffer short-term changes in calcium intake and loss. For example, in humans calcium is mobilized from bone during pregnancy and lactation, even if dietary intake is adequate (Prentice 2000). Chronic calcium deficiency results in excessive bone mineral loss, bone abnormalities, pathologic fractures and excessive parathyroid hormone secretion (National Research Council 2005).

Phosphorus is also required for bone growth, with as much as 86% of body phosphorus being deposited in bone mineral, but phosphorus is also a key constituent of many tissues and compounds, including DNA, RNA and high-energy phosphate compounds such as ATP. Phosphorus deficiency also results in bone mineral loss, as well as growth retardation, metabolic acidosis, locomotor disturbances and hemolytic anemia (National Research Council 2006).

Potassium is a major electrolyte, and in fact 90% of the potassium in the body is present in intracellular fluid. Potassium is critically involved in acid-base regulation, nerve impulse transmission, enzyme reactions, and membrane transport, and hence its concentration in different fluids is tightly regulated in the body. In cats, potassium deficiency results in anorexia, growth retardation, neurological disorders, muscle weakness and ataxia (National Research Council 2006).

Magnesium is involved in a wide array of biochemical functions in the body including enzyme function, oxidative phosphorylation, DNA and RNA metabolism, protein synthesis, membrane stability and immune function responses. In carnivores, magnesium deficiency produces anorexia, lameness, joint hyperextension, convulsions and paralysis (National Research Council 2006). We are not aware of reports of deficiencies of any of these macrominerals in wild sea otters.

Mammals also require **sodium** in their diets, but as sea otters live and feed in a medium (sea water) containing 10.8 g sodium chloride per liter, and inevitably ingest some sea water when consuming prey, it is highly unlikely that sea otters are limited by

dietary sodium. It is also difficult to obtain accurate measurements of the sodium content in sea otter prey due to the presence of sea water on respiratory and external surfaces of prey, as well as in internal compartments (such as the digestive system and cavities inside shells or exoskeletons). To make sure that we did not alter the composition of prey during any rinsing steps, we used artifical seawater as the rinse solution (Part 2). Under these circumstances, it was our decision not to analyze prey for sodium concentrations.

METHODS

The methods of collection and processing of sea otter prey into pooled samples have been described in Part 2. A subset of the samples collected in the Monterey Bay National Marine Sanctuary and vicinity (MBNMS), at San Nicolas Island, CA, and in Glacier Bay National Park, AK was used for macromineral analyses, with particular attention to species that represented the top ten prey types.

Subsamples of about 0.25 g of lyophilized material were oven dried to determine dry mass, and then digested in concentrated nitric acid under high pressure in a computer-controlled microwave digestion system (MARS 5, CEM Corporation, Matthews, NC). Digests were ramped to 220° C over 15 minutes and held at this temperature for 15 minutes. Digests were diluted with distilled, deionized water and used for macromineral analysis. Phosphorus was assayed by the Gomori molybo-vanadate spectroscopic method (Horwitz 1980) in an ultraviolet-visible spectrophotometer (Model DU640, Beckman Instruments, Fullerton, CA) equipped with a flow-through cell. The remaining macrominerals were assayed by flame atomic absorption spectroscopy (Perkin-Elmer AAnalyst 800, Norwalk, CN) using either a nitrous oxide-acetylene flame (calcium) or an air-acetylene flame (potassium, magnesium) at an appropriate wavelength (calcium, 422.7 nm; potassium, 766.5 nm; magnesium, 202.5 nm). Modifiers were added to sample aliquots to reduce interferences, including lanthanum chloride to a final concentration of 4000 ppm lanthanum for calcium analysis and 1000 ppm lanthanum for magnesium analysis, and cesium chloride to a final concentration of 1000 ppm cesium for potassium analysis. All analyses were performed using duplicate subsamples from the pooled samples, but if the CV of the duplicates was greater than 5% (phosphorus, potassium, magnesium) or 10% (calcium), additional duplicates were run. Reference tissue materials

supplied by the National Institute of Standards and Technology (Gaithersburg, MD) were used to validate that N, Ca, P, Mg and K assays fell within specified ranges. Mineral concentrations were expressed on a dry matter (DM) basis.

The evaluation of macromineral levels in sea otter prey requires reference values for comparison. Although the nutrient requirements of sea otters are not known, they are likely similar to those of well-studied domestic carnivores, given similarities in digestive tract morphology, a common phylogenetic origin, and the fact that all of these species have evolved to feed on digestible animal tissues. The recommended dietary levels (relative to diet dry matter) of calcium, phosphorus, potassium and magnesium for dogs, cats and mink are summarized in Table 4.1. These recommendations include a modest safety factor over the lowest possible safe levels (the minimum requirements), which is appropriate given uncertainties about mineral bioavailability in sea otter prey. These recommendations are based on a diet of 4.0 kcal metabolizable energy per g. Although we do not know the metabolizability of energy in sea otter diets, the fact that otter prey are high in crude protein (Part 2), and that a proportion of the gross energy in protein is lost as urea (Kleiber 1975), it is unlikely that otter diets are typically this high in energy (see Parts 5, 6). We did not introduce a correction for the metabolizability of energy in sea otter diets because we believed that any correction would be of arbitrary magnitude and because using nutrient concentrations for a somewhat more energy dense diet provides a margin of safety for interspecific extrapolation.

Maximum tolerable levels of macrominerals are also presented in Table 4.1; these are the maximal levels that can be fed to domestic animals without an adverse effect on performance (including adverse effects on absorption or utilization of other nutrients). As data for domestic carnivores are limited, we also include the range of maximal tolerable levels for rodents and livestock (pigs, horses, sheep and cattle). Dietary levels above these may reduce feed intake, diet digestibility and mineral absorption, or may lead to physiologic abnormalities (National Research Council 2005).

We compare the concentrations in sea otter prey to the recommended minimal levels for growth and reproduction, as a diet that only provided for maintenance needs would not support reproduction or recruitment of young animals. Thus a prey species that contains at least 0.6-1.2% calcium, 0.6-1.0% phosphorus, 0.30-0.52% potassium and

Table 4.1 Recommended levels of macrominerals for domestic carnivores[1]

Species	Stage	Calcium [2]	Phosphorus[2]	Potassium	Magnesium
		%DM	%DM	%DM	%DM
Recommended dietary levels					
Mink	growing kits after weaning	0.40	0.40	0.30	0.044
	adult mink, maintenance	0.30	0.30	0.30	0.044
	lactation	0.60	0.60	0.30	0.044
Dog	growing puppies after weaning	1.20	1.00	0.44	0.040
	adult dog, maintenance	0.40	0.30	0.40	0.060
	late gestation, lactation	0.80	0.50	0.36	0.060
Cat	growing kittens after weaning	0.80	0.72	0.40	0.040
	adult cat, maintenance	0.29	0.26	0.52	0.040
	late gestation, lactation	1.08	0.76	0.52	0.050
Maximum tolerable levels					
Livestock and rodents		1.0-2.0	0.6-1.0	1.0-2.0	0.24-0.80
Dogs		1.80	NA	NA	>0.17
Cats		1.04-1.84	1.0-1.4	NA	>0.10

1. From National Research Council (1982, 2005, 2006), assuming a dietary metabolizable energy density of 4 kcal/g DM. NA = not available

2. Both reqauirements and tolerances for calcium (Ca) and phosphorus (P) are affected by the ratio of Ca:P in the diet; ratios outside of the limits of 1.0-2.0 may lead to increased requirements or changed tolerance. In dogs, most breeds can tolerate a Ca:P ratio up to 3.0 without adverse effect (National Research Countil 2005).

0.044-0.060% magnesium should provide sufficient macrominerals to meet otter requirements, if this species is the sole dietary item. Of course otters that combine disparate prey items can achieve these target levels by combining species with lower and higher concentrations, as will be discussed in our assessment of otter diet types (part III). However, a bigger concern with some macrominerals is mineral excess (see below).

RESULTS

The mineral concentrations of sea otter prey are presented according to the four primary taxonomic groups: decapod crustaceans (Table 4.2), bivalve mollusks (Table 4.3), gastropod mollusks (Table 4.4) and echinoderms (Table 4.5). Results for a few additional species in other taxonomic groups are presented in Table 4.6.

Calcium – Calcium was the most variable mineral in sea otter prey, ranging from about 0.1% in abalone and squid up to about 30% in some turban snails and small urchins. Some sea otter prey contain very low levels of calcium, including market squid (*Loligo*) at 0.09%, abalone (*Haliotis*) at 0.1-0.2%, the edible material in stars (*Pisaster*) at 0.2-0.3%, and several species of bivalves such as cockles (*Clinocardium*), scallops (*Crassadoma*) and horse mussels (*Modiolus*) at 0.3-0.4%. On the other hand, decapod crustaceans (*Cancer* and 8 other genera, 4-13%), top and turban snails (*Calliostoma*, *Tegula*, 3-30%) and sea urchins (*Stronglyocentrotus*, 2.5-30%) are very high in calcium. Note that the very high value of 30% for purple urchins (*S. purpuratus*) refers to small urchins (<2 cm width) in which the entire animal, including the test, was ground and analyzed; for larger purple urchins, the test and spines were removed as inedible, and the remaining tissue contained on average 2.5 to 3.5% calcium. The very high calcium in snails undoubtedly reflects the inclusion of some shell fragments in the edible portion, while that in decapods reflects the inclusion of most of the calcified exoskeleton (except the carapace in larger crabs) in the edible portion. Data on the mass of the edible portions of these prey species are presented in Part 2.

Phosphorus – Most sea otter prey appear to be good sources of phosphorus, providing 0.6-1.5% on a dry matter basis. The low phosphorus levels (0.2-0.5%) in thin-shelled burrowing clams *Macoma nasuta*, *Macoma secta* and *Mya truncata* (Table 4.3) appear to be a result of dilution by inorganic matter ingested by the clams: the ash

Table 4.2. Macrominerals in sea otter prey. I. Decapods[1]

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Monterey Bay National Marine Sanctuary, CA								
Blepharipoda occidentalis	spiny mole crab	21	WS	B	8.94	1.11	0.86	1.02
					0.38	0.20		
					4	2	1	1
Cancer antennarius	Pacific rock crab	16	SF	B	10.43	0.90	0.83	0.74
					0.86	0.04	0.04	0.03
					12	12	14	12
Cancer anthonyi	yellow rock crab	6	S	S	7.49	1.04	0.96	0.57
					1.12	0.07	0.08	0.04
					3	3	3	3
Cancer gracilis	slender crab	2	W	S	9.18	0.96		
					1.01	0.01		
					2	2		
Cancer magister	Dungeness crab	8	SF	N	7.08	1.15	1.01	0.56
					0.64	0.03	0.03	0.06
					8	8	8	8
Cancer productus	red rock crab	8	S	S	8.89	0.86	1.01	0.70
					0.26	0.03	0.03	0.02
					4	4	4	4
Emerita analoga	Pacific sand crab	64	S	B	7.94	0.99	0.78	0.99
					0.24	0.01		0.02
					2	2	1	2
Pugettia producta	northern kelp crab	46	SF	B	13.07	0.87	0.77	0.91
					0.54	0.02	0.04	0.02
					17	17	12	13
San Nicolas Island, CA								
Cancer antennarius	Pacific rock crab	4	F	CI	11.46	0.98	0.87	0.83
					1.04	0.08	0.04	0.06
					4	4	4	4

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Cancer productus	red rock crab	4	F	CI	12.57	1.02	0.91	0.98
					0.70	0.02	0.02	0.02
					4	4	4	4
Panulirus interruptus	California spiny lobster	4	F	CI	4.14	0.98	1.13	0.41
					0.92	0.04	0.05	0.04
					4	4	4	4
Pugettia producta	northern kelp	12	SpF	CI	11.29	1.02	0.74	0.95
					1.16	0.11	0.04	0.04
					5	5	5	5
Glacier Bay National Park, AK								
Cancer magister	Dungeness crab	5	SpS	AK	7.46	1.25	1.08	0.50
					0.29	0.04	0.02	0.04
					5	5	5	5
Chionoecetes bairdi	tanner crab	6	Sp	AK	9.26	1.50	1.10	0.76
					1.62	0.14	0.05	0.05
					4	4	4	4
Hyas lyratus	Pacific lyre crab	3	Sp	AK	12.82	0.90	0.73	1.07
					1.40	0.04	0.01	0.02
					2	2	2	2
Pagurus ochotensis	Alaskan hermit crab	2	Sp	AK	11.48	1.33	0.78	0.73
					0.55	0.09	0.04	0.04
					2	2	2	2
Paralithodes camtschaticus	red king crab	2	Sp	AK	6.68	1.14	0.99	0.70
					1.52	0.21	0.02	0.09
					2	2	2	2

1. For each species and nutrient, mean, standeard error of mean, and number of samples analysed listed in sequential rows.

Table 4.3. Macrominerals in sea otter prey. II. Bivalves[1]

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Monterey Bay National Marine Sanctuary, CA								
Clinocardium nuttallii	Nuttall cockle	14	S	B	0.36	0.89	0.92	0.93
					0.02	0.07		0.17
					2	2	1	2
Macoma nasuta	bent-nose macoma	26	S	B	1.06	0.46	0.79	1.07
					0.30	0.00	0.05	0.07
					2	2	2	2
Macoma secta	white-sand macoma	41	S	B	0.99	0.23	0.49	0.54
					0.34	0.03	0.04	0.05
					3	3	3	3
Mytilus californianus	California mussel	73	SF	B	0.77	0.62	1.06	0.65
					0.13	0.04	0.05	0.09
					8	8	6	6
Protothaca staminea	Pacific littleneck clam	217	WSF	N	0.63	0.71	0.98	0.70
					0.08	0.04	0.02	0.05
					10	10	6	6
Saxidomus nuttalli	California butterclam	38			0.54	0.69	1.09	0.51
					0.13	0.02	0.07	0.04
					7	7	7	7
Tresus nuttallii	Pacific gaper	25	SF	B	0.73	0.56	0.96	0.49
					0.08	0.02	0.04	0.03
					10	10	6	7
San Nicolas Island, CA								
Crassadoma gigantea	rock scallop	16	F	CI	0.40	0.75	1.43	0.42
					0.03	0.05	0.02	0.01
					4	2	2	2
Mytilus californianus	California mussel	71	W	CI	0.71	0.87	1.05	0.97
					0.16	0.11	0.14	0.17
					2	2	2	2

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Glacier Bay National Park, AK								
Chlamys rubida	scallop	22	Sp	AK	0.30	0.96	1.46	0.66
					0.04	0.03	0.08	0.06
					2	2	2	2
Clinocardium nuttallii	Nuttall's cockle	6	Sp	AK	0.39	1.07	1.58	0.71
					0.00	0.01	0.04	0.03
					2	2	2	2
Macoma balthica	Baltic macoma	35	Sp	AK	0.73	1.12	1.19	0.48
					0.22	0.01	0.05	0.04
					2	2	2	2
Macoma nasuta	bent-nosed macoma	113	Sp	AK	1.22	0.73	1.43	0.97
					0.09	0.01	0.03	0.07
					4	4	4	4
Mya truncata	truncated softshell-clam	39	Sp	AK	2.58	0.45	0.95	1.05
					0.43	0.01	0.02	0.03
					2	2	2	2
Mytilus modiolus	horse mussel	4	S	AK	0.31	0.80	1.14	0.56
					0.05	0.20	0.10	0.01
					2	2	2	2
Mytilus trossulus	foolish mussel	88	SpS	AK	1.62	0.83	1.38	0.90
					0.27	0.06	0.08	0.13
					5	5	5	5
Protothaca staminea	Pacific littleneck clam	26	Sp	AK	0.72	0.82	1.25	0.84
					0.06	0.04	0.02	0.05
					4	4	4	4
Saxidomus gigantea	butter clam	4	Sp	AK	0.91	0.82	1.39	0.66
					0.46	0.01	0.01	0.02
					2	2	2	2

1. For each species and nutrient, mean, standeard error of mean, and number of samples analysed listed in sequential rows.

Table 4.4. Macrominerals in sea otter prey. III. Gastropods[1]

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
III. Gastropods								
Monterey Bay National Marine Sanctuary, CA								
Calliostoma ligatum	blue top snail	120	WS	B	6.02	0.64		
					2.65	0.05		
					2	2		
Haliotis cracherodii	black abalone	18	WSF	B	0.12	0.58	1.00	0.32
					0.01	0.01	0.04	0.02
					10	10	6	6
Haliotis rufescens	red abalone	13	SF	B	0.21	0.64	1.09	0.31
					0.04	0.01	0.03	0.03
					10	10	6	6
Polinices lewisii	Lewis' moon snail	2	WS	S	3.56	0.38		
					0.09	0.03		
					2	2		
Tegula brunnea	brown tegula	76	S	B	18.51	0.44	0.62	
					4.84	0.13	0.14	
					2	2	2	
Tegula funebralis	black tegula	982	SF	B	10.10	0.47	0.72	0.35
					3.68	0.07	0.08	0.05
					8	8	7	7
Tegula montereyi	Monterey tegula	99	S	B	21.99	0.40	0.43	0.22
					4.41	0.02	0.02	0.02
					4	4	4	4
Tegula pulligo	dusky tegula	262	S	B	30.22	0.22	0.31	0.16
					0.95	0.01	0.04	0.03
					4	4	4	4
San Nicolas Island, CA								
Kelletia kelletii	Kellett's whelk	6	Sp	CI	1.11	0.55	1.05	0.64
					0.20	0.02	0.04	0.09
					4	2	4	2

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Lottia gigantea	owl limpet	26	W	CI	0.73	0.52	0.98	0.47
					0.35	0.02	0.03	0.06
					3	2	3	2
Megastraea undosa	wavy turbansnail	14	SpF	CI	2.33	0.80	0.92	0.54
					0.40	0.06	0.01	0.02
					5	2	3	2
Megathura crenulata	giant keyhole limpet	6	Sp	CI	1.46			
					0.18			
					2			
Norrisia norrisi	Norris' topsnail	13	SpF	CI	0.89	0.70	0.98	0.50
					0.20	0.10	0.02	0.05
					6	2	3	2
Tegula eiseni	banded turban snail	57	W	CI	7.90	0.65	0.74	0.45
					0.56	0.02	0.01	0.02
					2	2	2	2
Tegula funebralis	black tegula	200	W	CI	2.97	0.64	0.94	0.43
					1.17	0.03	0.04	0.02
					2	2	2	2
Glacier Bay National Park, AK								
Fusitriton oregonensis	hairy triton	8	SpS	AK	0.62	0.56	1.12	0.65
					0.15	0.02	0.04	0.07
					2	2	2	2
Neptunea lyrata	ridged whelk	2	Sp	AK	1.39	0.64	1.00	0.52

1. For each species and nutrient, mean, standeard error of mean, and number of samples analysed listed in sequential rows.

Table 4.5. Macrominerals in sea otter prey. IV. Echinoderms[1]

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Monterey Bay National Marine Sanctuary, CA								
Pisaster giganteus	giant spined star	8	S	S	0.32	0.51	0.61	0.40
					0.05	0.01	0.09	0.03
					3	3	3	3
Pisaster ochraceus	ochre star	13	SF	B	0.20	0.54	0.67	0.29
					0.03	0.03	0.09	0.02
					11	11	7	6
Strongylocentrotus franciscanus	red urchin	7	WS	N	3.93	0.94	0.47	0.60
					2.34	0.23		0.16
					3	4	1	2
Strongylocentrotus purpuratus	purple urchin	21 (small, entire)	S	B	29.64	0.10	0.18	1.53
					0.56	0.01		
					4	4	1	1
	purple urchin	81 (med-large,edible)	SF	B	3.47	0.78	0.94	0.88
					0.55	0.11	0.10	0.08
					7	7	5	6
San Nicolas Island, CA								
Pisaster giganteus	giant spiny star	2	F	CI	0.18	0.48	0.95	0.43
					0.07	0.01	0.07	0.10
					2	2	2	2
Pisaster ochraceus	ochre star	5	W	CI	0.18	0.70	1.05	0.35
					0.02	0.11	0.10	0.01
					2	2	2	2
Strongylocentrotus franciscanus	red urchin	20	WSp	CI	2.42	0.46	1.33	0.82
					0.64	0.15	0.07	0.08
					7	6	6	2
Strongylocentrotus purpuratus	purple urchin	31	WSp	CI	2.52	0.29	1.33	0.97
					0.52	0.00	0.01	0.41
					3	2	2	2

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
Glacier Bay National Park, AK								
Strongylocentrotus droebachiensis	green urchin	10	Sp	AK	5.32	1.75	1.43	1.12
					2.66	0.50	0.09	0.04
					2	2	2	2
Strongylocentrotus pallidus	white urchin	22	Sp	AK	7.70	0.64	1.30	0.97
					0.75	0.01	0.05	0.06
					2	2	2	2

1. For each species and nutrient, mean, standeard error of mean, and number of samples analysed listed in sequential rows.

Table 4.6. Macrominerals in sea otter prey. V. Other Taxa[1]

Scientific Name	Common Name	n	Season	Region	Calcium	Phosphorus	Potassium	Magnesium
					%DM	%DM	%DM	%DM
V. Other taxa								
Monterey Bay National Marine Sanctuary, CA								
Loligo opalescens	Market squid	16	S	N	0.089	1.20	1.54	0.22
					0.006	0.13	0.09	
					2	2	4	1
Octopus rubescens	Octopus	1	W	N		0.86		
Urechis caupo	Fat innkeeper worm	22	SF	B	0.37	0.60	1.35	0.85
					0.06	0.02	0.08	0.11
					6	6	6	6
Glacier Bay National Park, AK								
Echiurus echiurus alaskanus	Alaskan spoonworm	14	Sp	AK	3.35	0.48	1.20	1.45
					0.10	0.04	0.04	0.08
					3	3	3	3
Nereis vexillosa	Pile worm	8	Sp	AK	2.24	0.75	1.30	0.43
					0.01	0.01	0.11	0.04
					2	2	2	2

1. For each species and nutrient, mean, standeard error of mean, and number of samples analysed listed in sequential rows.

contents of these taxa comprised 35-75% of dry matter (Table 2.6) even though the totals of the measured macrominerals were only 2.5-5% of dry matter. The difference is probably mostly silica due to ingested particulate matter. We removed sand or visible particles adhering to the outside of these clams prior to analysis but left any particulate matter mixed with internal tissues on the assumption that otters would ingest this particulate matter while eating clam soft tissues. Dilution by shell fragments or urchin test may also explain low phosphorus in the edible portion of *Tegula* snails and purple urchins (*S. purpuratus*).

Potassium – Virtually all prey contained concentrations of potassium that were above the recommended diet levels of 0.45-0.52%. Thus sea otters consume an excess of potassium which must be excreted in urine.

Magnesium – All otter prey contained large amounts of magnesium (0.2-1.5%) relative to the recommended diet level of 0.05-0.06%, and thus variation in magnesium among prey appears to be unimportant to otters.

DISCUSSION

Terrestrial carnivores feeding on vertebrate prey obtain large amounts of highly available phosphorus from organ meat and muscle tissue, as well as substantial amounts of calcium from the endoskeleton (bone) (Allen et al. 1996b). Calcium deficiencies in zoo animals can result from the feeding of meat and organs without bone, but it is unlikely that this ever occurs in the wild, especially when entire vertebrate prey are consumed. By contrast, terrestrial insectivores consume prey which is very low in calcium, as insects lack a calcified skeleton (the exoskeleton is comprised primarily of chitin and protein, with little mineral matter). It has been shown that much of the calcium intake of terrestrial insectivores, including insectivorous birds, may derive from the gut contents of the prey (Bilby and Widdowson 1971; Allen and Oftedal 1989; Allen et al. 1996).

Our data indicate that the marine invertebrates present quite a different challenge than either vertebrate prey or terrestrial invertebrates. Many marine organisms take advantage of the availability of dissolved calcium in sea water to construct a protective calcified structure around vulnerable soft tissues, including the shell of gastropods, the

valves of bivalves, the test of echinoderms and the calcified exoskeleton of crustaceans. Thus sea otters must break through these structures to gain access to the energy-containing soft tissues and in the process obtain substantial amounts of calcium, either as shell/valve/test fragments or as crushed exoskeletal parts. Our processing of marine invertebrates attempted to duplicate otter feeding behavior, and thus when we crushed snail shells we only removed large fragments, when we removed a crab carapace we left other exoskeletal parts, and when we invaded urchin tests we no doubt included small bits of test in the edible fraction. As a consequence, there was substantial variation in calcium content among duplicate samples, and it was common to have CV values for calcium analyses that were in excess of 10%, even when multiple duplicates were assayed. Thus the calcium values we report are not as precise as other analytical results, and may include error due to differences in how we processed prey as compared to sea otters foraging in the wild.

Nonetheless, our data indicate that the soft parts of certain types of marine invertebrate prey are indeed low in calcium. It is unlikely that otters could develop a normal skeleton or produce milk on a diet consisting entirely of the edible portions of abalone or stars, as these contain only 0.1-0.3% calcium, far below requirements for growth and reproduction. We predict that otters feeding heavily on either abalone or stars need to complement them with calcium-rich prey items such as crabs or urchins. It is not known if otters have a physiological mechanism to sense calcium status and thus develop a calcium appetite if calcium-depleted, although some other mammals and egg-laying birds do (Schulkin 2001). We will return to the issue of diets of multiple prey types in Part 5.

Many otter prey contain less than the recommended levels for domestic carnivores of 0.8-1.0% phosphorus on a dry matter basis, but in this instance the recommended levels are probably too high for sea otters. Dietary recommendations for domestic carnivores must include compensation for the low bioavailability of phosphorus in plant-based diets (such as dry dog and cat food) in which much of the phosphorus is present in the form of phytate (National Research Council 2006). Phosphorus availability on such diets may be as low as 35-50%, depending on phytate concentration, calcium:phosphorus ratio, level of food intake and other factors. One would expect the

phosphorus in the soft tissues of marine invertebrates to be highly available, and hence it is likely that 0.4-0.5% phosphorus will be sufficient even during growth and reproduction. Sea otters also have much smaller litters than dogs, cats or mink, and presumably have a corresponding reduction in milk output (Oftedal 1984a, b; Oftedal and Gittleman 1989). Thus the mineral demands of lactation are probably considerably lower in sea otters rearing a single pup than the demands faced by a dog, cat or mink rearing a litter of 4 or more. It is the need to mobilize so much calcium and phosphorus into milk that results in the doubling or tripling of calcium and phosphorus requirements of the lactating bitch or queen as compared to an adult animal at maintenance.

As neither potassium nor magnesium appears to be limiting to sea otters, future studies may ignore these constituents in assessment of the nutritional quality of sea otter diets.

Another uncertainty revolves about the impact of very high calcium intakes associated with consumption of crabs, snails and other taxa in which calcified structures are ingested. Very high calcium intakes in terrestrial mammals can lead to impaired absorption of phosphorus, magnesium and zinc (Allen and Oftedal 1996; National Research Council 2006), presumably due to formation of insoluble complexes in the digestive tract. Much of the calcium in shell and other prey structures is in the form of calcium carbonate, which should dissolve readily in the acid pH of the sea otter's stomach contents, but whether this will result in any reduction in absorption of other minerals is not certain. However, if dietary phosphorus levels are adequate and dietary magnesium levels are excessive, the practical consequences of a reduction in absorption of these minerals may be insignificant.

CONCLUSIONS AND RECOMMENDATIONS

Although marine invertebrates typically have calcium-rich protective structures, sea otters feeding on certain types of prey, such as abalone and stars, may only consume prey parts that are deficient in calcium. It would be valuable to determine if otters demonstrate a behavioral calcium appetite when fed low-calcium prey, as this could explain some of the mixed diets (e.g., "abs and crabs") otters ingest in the wild.

Otherwise it appears that marine invertebrate prey species have adequate levels of phosphorus and excessive levels of potassium and magnesium.

In feeding sea otters in captivity, it is prudent to use a mixture of prey, including calcium-rich prey, to avoid any potential for calcium deficiency that can occur in carnivores fed on diets with an inverse calcium-phosphorus ratio (Allen et al. 1996b). This is preferred to calcium supplementation, which may be difficult to control given that otters feed in the water.

Section B. Trace Elements

INTRODUCTION

Trace elements or trace minerals are inorganic elements that are required in the diets of animals at parts per million (ppm or mg/kg) concentrations (Table 4.7). In this study, 12 sea otter prey species from mainland California were subjected to analyses to determine the concentration of several trace minerals most likely to impact the health of sea otters: copper, iron, manganese, selenium and zinc. Each of these serves a variety of functions and should be present in the diet within an acceptable range of concentration, as both deficiency and toxicity of trace minerals can have serious consequences. Trace mineral nutrition is also complicated by high variability in bioavailability depending on the mineral source and interactions with other dietary minerals.

Iron is a component of hemoglobin and myoglobin and thus is essential to the transport and binding of oxygen. In addition, it is a part of many enzyme systems central to metabolism, including aconitase of the Krebs cycle and the cytochromes of the electron transport chain. Iron deficiency causes anemia, decreased cell proliferation and impaired immune function (Fairbanks 1999). Iron toxicity can be equally detrimental, resulting in oxidative damage of tissues as a consequence of iron's ability to catalyze free radical reactions. Iron is also an essential nutrient to many pathogens, so excess iron can increase the success of some pathogens (Doherty 2007).

Copper serves as a cofactor or allosteric component of many enzyme systems with diverse functions such as cellular respiration, connective tissue formation, catecholamine production and antioxidant defense. Copper deficiency causes neurological symptoms, anemia, impaired keratin formation, depigmentation of hair, bone and connective tissue abnormalities and cardiomyopathy (Turnland 1999). Copper toxicity causes oxidative stress resulting in damage to tissues, particularly the liver and kidney (Bremner 1998).

Zinc is essential to many enzyme systems, in which it can have catalytic, structural or regulatory roles. It is concentrated in biological membranes, where it likely serves to protect and stabilize membrane structure and function. In addition, zinc plays an important role in protein structure by forming the zinc finger motif. Zinc can also impact gene expression by interacting with the metal response element present in the promoter

Table 4.7 Recommended levels of trace elements for domestic carnivores[1]

Species	Stage	Iron	Copper	Zinc	Manganese	Selenium
		mg/kg DM	mg/kg DM	mg/kg DM	mg/kg DM	ug/kg DM
Recommended dietary levels						
Dog	growing puppies after weaning	88	11.0	100	5.6	350
	adult dog, maintenance	30	6.0	60	4.8	350
	late gestation, lactation	70	12.4	96	7.2	350
Cat	growing kittens after weaning	80	8.4	75	4.8	300
	adult cat, maintenance	80	5.0	74	4.8	300
	late gestation, lactation	80	8.8	60	7.2	300
Maximum tolerable levels						
Rodents		500	500	500	2000	5000
Livestock		500-3000	15-250 [2]	300-1000	400-2000	4000-5000

1. From National Research Council (1982, 2005, 2006). Data for mink are insufficient to warrant inclusion
2. Sheep are particularly sensitive to copper toxicity and have maximum tolerable levels of only 15 mg/kg, compared to 40 mg/kg for cattle, and 250-500 mg/kg for rodents, swine and horses.

region of some genes, such as that for metallothionein and retinol binding protein (King and Keen 1999). Deficiency of zinc causes skin lesions, impaired wound healing, reproductive failure, skeletal abnormalities and depressed immune function (Nielson 2002). Zinc toxicity is rare but does cause anemia, depressed growth, copper deficiency and impaired immune response (National Research Council 2005).

Manganese is part of the structure of several enzymes (arginase, pyruvate carboxylase and manganese superoxide dismutase) and an essential activator of many others important to cellular metabolism. Manganese deficiency results in decreased growth, skeletal abnormalities, hypocholesterolemia, ataxia and reproductive failure (Nielsen 2002). Manganese is one of the least toxic trace minerals, with a range of several hundred-fold difference between the dietary requirement and toxic concentration. When manganese toxicity does occur, it causes decreased iron status and hematological changes (National Research Council 2005).

Selenium is a component of oxidation-reduction enzymes, such as glutathione peroxidases and thioredoxin reductase, giving it an antioxidant role. Selenium is also distinguished as the only mineral known to be directly encoded in DNA, and a process called “co-translation” incorporates selenium into proteins as part of the amino acid selenocysteine. Selenium deficiency can result in muscle pain and wasting, cardiomyopathy and increased susceptibility to viral infections (Beck et al. 2003). Selenium toxicity causes decreased growth, impaired reproduction and neurological signs (National Research Council 2005).

METHODS

Samples of 12 species of marine invertebrates were assayed for trace elements by standard methods. The species, locations and month of collection are presented in Table 4.8. Duplicate subsamples of ca. 0.50 g were digested in nitric acid and perchloric acid at 210°C according to modified AOAC Method 996.16 (1990). These digests were diluted with distilled, deionized water (Milli-Q, Millipore, Billerica, MA, USA) and the following trace minerals were assayed using flame atomic absorption spectroscopy (AAnalyst 800, Perkin Elmer, Wellesley, MA, USA) at predetermined wavelengths: copper (wavelength 324.8 nm), iron (wavelength 248.3 nm), manganese (wavelength

Table 4.8 Trace element composition of sea otter prey in comparison to reference data.[1]

Species name	Common name	Region	Collection site/ USDA Ref. No.	Season	Fe mg/kg DM	Cu mg/kg DM	Zn mg/kg DM	Mn mg/kg DM	Se ug/kg DM
Decapods									
Cancer antennarius	Pacific rock crab	***S***	Morro Bay	Apr-05	81	92.2	153	7.1	5373
Cancer magister	Dungeness crab	***S***	Morro Bay	Apr-05	239	40.8	116	6.7	2141
Pugettia producta	northern kelp crab	***S***	Pt Estero	Sep-04	35	5.2	40	4.0	679
Cancer magister	Dungeness crab		**NDB No:** 15143		18	*32.4*	*205*	*3.8*	*1800*
Paralithodes camtschatica	Alaska king crab		**NDB No:** 15136		29	*45.1*	*291*	*1.7*	*1800*
Callinectes sapidus	Blue crab		**NDB No:** 15139		35	*31.9*	*169*	*7.1*	*1800*
Chionoectes opilio	Queen crab		**NDB No:** 15144		129	*29.4*	*144*	*1.5*	*1800*
Homarus americanus	Northern lobster		**NDB No:** 15147		13	*71.6*	*130*	*2.4*	*1800*
Bivalves									
Protothaca staminea	littleneck clam	***N***	Elkhorn Slough	May-05	138	6.0	125	1.9	2085
Tresus nuttallii	gaper clam	***S***	Morro Bay	Apr-05	2269	6.1	55	117.3	1165
Mytilus californianus	California mussel	***N***	Pebble Beach	Apr-05	83	4.4	131	2.8	1933
Mytilus edulis	edible mussel		**NDB No:** 15164		203	*4.8*	*82*	*175.1*	*2307*
Lamellibranchia	clam, mixed spp.		**NDB No:** 15157		769	*18.9*	*75*	*27.5*	*1340*
Pectinidae	scallop, mixed spp.		**NDB No:** 15172		14	*2.5*	*44*	*4.2*	*1040*
Gastropods									
Haliotis cracherodii	black abalone	***N***	Pebble Beach	Apr-05	172	11.1	111	1.8	284
Haliotis rufescens	red abalone	***N***	Monterey	Apr-05	508	3.5	112	3.9	387
Haliotis spp.	abalone, mixed spp.		**NDB No:** 15155		125	*7.7*	*32*	*1.6*	*1761*
Tegula funebralis	black Tegula	***S***	Rancho Marino	Sep-04	1791	54.5	78	17.6	1600
	snail, mixed spp.		**NDB No:** 90560		168	*19.2*	*48*	*n/a*	*1320*
Buccinidae	whelk, unspecified		**NDB No:** 15177		148	*30.3*	*48*	*13.1*	*1320*
Echinoderms									
Pisaster ochraceus	ochre star	***S***	Morro Bay	Jun-04	94	6.6	64	1.8	1896
Strongylocentrotus	purple urchin	***S***	San Simeon	Jun-04	378	4.4	57	10.8	353
Other taxa									
Urechis caupo	fat innkeeper	***S***	Morro Bay	Apr-05	2167	6.1	266	25.1	1845
Octopus vulgaris	common octopus		**NDB No:** 15166		268	22.0	85	1.3	2700
Sepiidae	cuttlefish, mixed		**NDB No:** 15163		310	30.2	89	5.7	2300

1. USDA reference data from USDA (2004) are highlighted.

279.5 nm, 0.2% $CaCl_2$ added to samples to overcome interferences from silica) and zinc (wavelength 213.9 nm, 0.2% $CaCl_2$ added to samples to overcome interferences from silica). For the selenium assay, 1 ml of 12M hydrochloric acid was added to 4 ml digest solution and heated for 30 min at 120°C to fully reduce all selenium to Se^{4+}. Selenium was measured using a flow injection mercury hydride system (FIAS 100, Perkin Elmer, Wellesley, MA, USA) with 10% HCl as carrier solution and 0.2% $NaBH_4$ in 0.05% NaOH as the reducing agent. Samples were atomized at 2000°C and absorption measured at 196 nm. All trace mineral assays were calibrated with standards made from pure metals dissolved in nitric or hydrochloric acid and brought to volume with distilled, deionized water (Milli-Q, Millipore, Billerica, MA, USA). All data and recommended levels are expressed in mg/kg on a dry matter basis.

As for macrominerals, we compare trace mineral concentrations in sea otter prey to the recommended dietary levels (expressed as mg/kg on a dry matter basis) established for well-studied domestic carnivores during growth and reproductive states (Table 4.7). These recommendations are based on a diet of 4.0 kcal metabolizable energy per g. Although we do not know the metabolizability of energy in sea otter diets, the fact that otter prey are low in fat and high in crude protein (Part 2), and that a proportion of the gross energy in protein is lost as urea (Kleiber 1975), it is unlikely that otter diets are quite this high in energy (see Parts 5, 6). We did not introduce a correction for metabolizability because any correction would be of arbitrary magnitude and use of nutrient concentrations for a somewhat more energy dense diet provides a margin of safety for interspecific extrapolation.

In addition, because trace mineral toxicities may be a concern, we also refer to the "maximum tolerable levels" for each mineral, supported by experimental evidence in rodents, poultry and swine (National Research Council 2005). The maximum tolerable level is defined as "the dietary level that, when fed for a defined period of time, will not impair animal health and performance" (National Research Council 2005). When animals are fed diets with minerals at concentrations above the maximum tolerable level, signs of reduced performance or toxicity begin to develop. The existing data on trace mineral toxicities in domestic carnivores are considered insufficient to establish toxicity levels in these species (National Research Council 2005, National Research Council 2006).

RESULTS

Trace mineral concentrations of 12 sea otter prey species from Central CA are given in Table 4.8, with reference data from USDA (2004) provided for comparison. Across species, the trace mineral concentrations were variable, but evaluation is complicated by the lack of variance estimates given that only single samples were assayed.

Iron – Most prey species are quite high in iron. Turban snails (*Tegula*, 1791 ppm), *T. nuttallii* (2269 mg/kg) and fat innkeeper worms (*Urechis*, 2167 mg/kg) contain excessively high iron levels, within the maximum tolerable range of 500-3000 mg/kg. Only kelp crabs are low in iron (34.6 mg/kg) compared to the recommended level of 70-88 mg/kg. The remaining species contain adequate to high iron concentrations.

Copper – Most species analyzed contain low concentrations of copper (3.5-6.6 mg/kg) compared to recommended dietary levels of 8.4-12.4 mg/kg for growth and reproduction (Table 4.7). Only *Cancer* crabs (40.8-92.2 mg/kg) and turban snails (*Tegula*, 54.5 mg/kg) are above the recommended range, and black abalone (*H. cracherodii*, 11.1 mg/kg) is likely adequate. All prey species are below the maximum tolerable level for copper for rodents, pigs, and horses (250 –500 mg/kg) but crabs and turban snails contain levels that would be toxic to sheep (maximum tolerable level 15 mg/kg) and perhaps cattle (maximum tolerable level 40 mg/kg).

Zinc – Five prey species contain zinc concentrations at or below the recommended level of 75-100 mg/kg for growth and reproduction and may be considered low to marginal (Table 4.8). Particularly low levels were observed in kelp crabs (*Pugettia*, 40.0 mg/kg), gaper clams (*Tresus*, 55.2 mg/kg) and purple urchins (*Strongylocentrotus*, 56.8 mg/kg). Fat innkeeper worms (*Urechis*, 265.6 mg/kg) contain the highest concentration of zinc, but this is below the maximum tolerable level of 300-1000 mg/kg so is not considered to be excessive.

Manganese – Several species are below the recommended range for manganese (4.8-7.2 mg/kg), including abalone (*Haliotis*, 1.8-3.9 mg/kg), kelp crabs (*Pugettia*, 4 mg/kg), mussels (*Mytilus*, 2.8 mg/kg), cockles (*Protothaca*, 1.9 mg/kg) and ochre stars (*Pisaster*, 1.8 mg/kg). The remaining species are adequate to high in manganese. Gaper

clams (*Tresus*) contain the highest concentration of manganese at 119.3 mg/kg, but this is still well below the maximum tolerable level and is not considered excessive.

Selenium – Most sea otter prey species are adequate to high in selenium compared to the recommended level of 0.3-0.35 mg/kg. There are two exceptions: black abalone (*H. cracherodii*, 0.28 mg/kg) may be marginal, while rock crabs (*C. antennarius*, 5.37 mg/kg) are just above the maximum tolerable level range of 3-5 mg/kg.

DISCUSSION

The results of our trace element analyses demonstrate a high degree of variability and the potential for both deficiencies and toxicities. For all five trace minerals assessed, at least one of the 12 prey species had low to marginal levels. In addition, several prey species contain excessive levels of iron and perhaps copper, and one prey species may contain excessive selenium. However, individual otter diets are generally made up of at least several prey species, and so the mix of species selected determines the final dietary concentration of each mineral. These will be discussed in Part 5.

Our data indicate that many otter prey species are low in copper, manganese and zinc. A recent study reported concentrations of many trace elements in 80 California sea otter livers from necropsy and found that copper was quite high compared to published data on other marine mammals (Kannan et al. 2006), making a copper deficiency seem unlikely in this population. A manganese deficiency in sea otters would likely result in skeletal abnormalities, ataxia and neonatal death. To our knowledge, such signs have not been reported in this population, making a manganese deficiency inconsistent with necropsy and observational data. Selenium is also not likely to be a problem, as only two prey species were outside the desirable concentration range (one high and one low). Our data on copper, manganese and selenium concentrations in sea otter diet types (see Part 5) also support the conclusion that these minerals are likely adequate in most if not all diets eaten by California sea otters.

The low levels of zinc seen in some important prey, and the consequent marginal zinc levels in certain diet types (Part 5) is of greater concern, especially given that the very high calcium levels in many otter prey could depress zinc absorption and thus increase required dietary levels (see above). Zinc deficiency results in impaired immune

function, including decreased production and maturation of leukocytes and natural killer cell function (Rink and Gabriel 2000). When pregnant mice are made moderately zinc deficient, their offspring and the two following generations demonstrate reduced immunocompetence, even when all offspring are fed zinc-adequate diets (Beach et al. 1982). Zinc deficiency may also impact vitamin A metabolism by decreasing the synthesis of retinol binding protein, the vitamin A transporter. Zinc interacts with the DNA metal response element to inhibit the transcription of the retinol binding protein gene. In addition, the conversion of retinol to retinal (the form of vitamin A important to the vision cycle and nuclear transporter interactions) is catalyzed by zinc-dependent retinol dehydrogenase (Christian and West 1998). Given that vitamin A concentrations in many prey species appear to be marginal, it is possible that a zinc deficiency may further compound the problem.

Several prey species contain iron levels in the range of the maximum tolerable levels for domestic animals, making iron toxicity a potential concern for sea otters in California. This may be particularly important for sea otters that feed predominantly on turban snails (Part 5). Excessive dietary iron causes oxidative damage to tissues and an impaired immune response to some pathogens. Bacterial pathogens have evolved methods of sequestering iron from transferrin, ferritin and heme of the host, despite counter efforts by the host to make iron unavailable during an inflammatory response (Doherty 2007). There is also a known interaction between iron status and infection with protozoal parasites. Iron supplementation of children in areas plagued by a high prevalence of malaria has been shown to increase both morbidity and mortality (Oppenheimer 2001; Sazawal et al. 2006). In California sea otters, it is not known if the high incidence of infectious disease caused by exposure to pathogens of terrestrial origin, including the protozoal parasites *Toxoplasma gondii* and *Sarcocystis neurona* (Miller et al. 2002; Hanni et al 2003; Conrad et al. 2005), is correlated to iron status. Kannan et al. (2006) did not include iron among the trace minerals measured in California sea otter liver samples. The issue of potential dietary iron excess and infectious disease in sea otters deserves study.

Interpretation of the nutritional consequences of trace mineral data is difficult in the absence of knowledge about the bioavailability of the minerals in sea otter prey.

Mineral-mineral interactions further complicate the picture. For example, high calcium could inhibit iron absorption, protecting an animal from iron toxicity, but also inhibit the absorption of minerals that are low or marginal, such as zinc, leading to deficiency. Sorting out the nutritional significance of these complex interactions will require direct measurements of nutrient uptake via digestibility trials that quantitate both intake and fecal excretion. Such work would need to be done with captive otters fed either single prey species or planned combinations of species, and would benefit from tracer studies that could validate and extend measurements.

The variability we observed in trace mineral concentrations among prey species likely reflects variation due to differing diets, environmental exposure, mineral uptake rates, biochemical pathways and structural components (Schipp and Hevert 1978; Howard and Brown 1983; Weeks et al. 1993; Rainbow 1995; Abdennour 1997; Baden and Neil 1998). For example, in some taxa such as decapods, cephalopods and some gastropods the principal respiratory pigment in the hemolymph is the copper-containing protein homocyanin, rather than iron-containing hemoglobin (Prosser 1973; Depledge and Bjerregaard 1989). Hemocyanin contains 1700-2500 mg copper per kg in anthropods and mollusks; hemolymph copper levels, on a wet weight basis, are about 40-90 mg per kg in crustaceans, up to 200 mg per kg in some marine gastropods, and 250 mg per kg in cephalopods (Betzer and Pilson 1974; Prosser 1973). In decapods, more than 50% of the whole body copper load is stored in the hemolymph (Depledge and Bjerregaard 1989). Marine gastropods also store substantial copper in tissues, with whole body (soft tissue) copper concentrations averaging 76mg/kg fresh mass in whelks (*Busycon canaliculatum*) in the Atlantic (Betzer and Pilson 1974). Thus the high copper concentrations we observed in *Cancer magister, C. antennarius*, and *Tegula funebralis* reflect the predominance of hemocyanin in these taxa. High copper levels have also been reported in other crustaceans, snails and cephalopods (e.g., Table 4.8) suggesting that high whole-body copper concentrations may be characteristic of these taxa. The lower copper levels that we and USDA found in abalone (Table 4.8) are consistent with an earlier report (cited in Betzer and Pilson 1974), even though abalone contain varying concentrations of hemocyanin (Pilson 1965). By contrast, the innkeeper worm (*Urechis*) utilizes iron-containing hemoglobin as a respiratory pigment in both coelomic corpuscles and the body

wall (Prosser 1973); *Urechis* is high in iron but low in copper (Table 4.8). Some taxa contain both hemocyanin in blood and iron-containing myoglobin in muscles, such as the gumboot chiton (*Cryptochiton stelleri*), but we did not analyze this species for trace elements.

The present study involved only single samples of twelve prey species, but the findings are sufficiently provocative to warrant a broader study involving analysis of more taxa. It is also important to examine seasonal and geographic variation in trace element concentrations, including cycles associated with reproduction and moulting (Beltzer and Pilson 1974; Howard and Brown 1983; Depledge and Bjerregaard 1989; Abdennour 1997; Taylor and Anstiss 1989). The wide range of concentrations we measured in all elements is intriguing. Trace element concentrations also likely reflect the elemental composition of the sediment of the collection sites. For example, the Mussel Watch Project has found differences in trends in copper and zinc concentrations in mussels at different sites along the California coast (O'Connor and Lauenstein 2006). Thus, deficiencies or toxicities of trace minerals could involve site-specific, as well as taxon-specific, concerns.

CONCLUSIONS AND RECOMMENDATIONS

Our trace mineral analyses of sea otter prey show that many prey species contain low levels of copper, manganese and zinc, while iron is present in excessive concentrations in several prey species. Our analysis of sea otter diet types in Part 5 will evaluate concentrations of these minerals in whole diets, allowing for a more accurate picture of the potential for deficiency or toxicity.

Further work on trace mineral nutrition of sea otters is required to determine the physiological relevance of the findings of this report. We recommend that future studies focus on the following:

1. A broad survey of trace elements in prey species, covering a wide phylogenetic, geographic and seasonal distribution, to assess the phylogenetic, geographic and seasonal factors that underlie the large variation that we observed in such potentially important elements as iron, copper and zinc. The sample material that we have already collected, processed and stored could be used for this purpose.

2. Determination of trace mineral concentrations and functional indices (i.e. metalloenzyme activity) in blood and tissues of sea otters in relationship to diet type, reproductive status and geographic location.
3. Digestibility trials and tracer studies to quantify the bioavailability of important trace elements in the prey eaten by sea otter diet.

Section C. Fatty Acids

INTRODUCTION

Fat is an important energy source for carnivores, including sea otters (Part 2), but it is also a source of essential fatty acids. Essential fatty acids are those fatty acids that cannot be synthesized, or cannot be synthesized at a sufficient rate, to meet the requirements of an animal. Fatty acids are usually characterized by their chain length (number of carbons) and the number and location of double bonds, if any. The longer chain fatty acids with multiple double bonds (so-called long-chain polyunsaturated fatty acids, or long-chain PUFAs) are essential constituents of a wide variety of cell membranes (National Research Council 2006). Some of these long chain PUFAs are also required for synthesis of important bioactive metabolites, known as eicosanoids (including prostaglandins, leucotrienes, thromboxanes) that are involved in modulation of inflammatory and immune responses (Calder and Field 2002).

Considerable research has been devoted in the past two decades to defining the functions, health implications and requirements of essential fatty acids in animals and humans. Mammals require fatty acids from two different fatty acid families, the omega3 and omega6 families (also designated herein as w3 and w6). The w- (or ω-) designation refers to the distance (in number of carbons) of the terminal double bond from the methyl end of a fatty acid; omega3 fatty acids have a double bond three carbons from the methyl end, and omega6 fatty acids have fatty acids with a double bond six carbons from the methyl end. Because vertebrates are unable to insert a double bond (desaturate) at either of these positions in long chain fatty acids, they must ingest PUFAs with these double bonds already formed. However, once ingested the fatty acids themselves can be modified, either via chain elongation, or addition of double bonds in other locations. The parent fatty acid in terrestrial communities is usually linoleic acid (18:2w6) for the omega6 family, and α-linolenic acid (18:3w3) for the omega3 family, but in marine systems longer chain PUFAs (often with 20 or 22 carbons) are quite common among plants and animals.

There is good evidence that cats, in particular, have a low activity of the enzyme Δ6-desaturase that is required to synthesize some of the long chain PUFAs from 18:2w6

and 18:3w3 fatty acids, such that cats require preformed arachidonic acid (20:4w6) and eicosapentaenoic acid (20:5w3) or docosahexaenoic acid (22:6w3) (Table 4.9). The most recent National Research Council (2006) Committee on Dog and Cat Nutrition recommended that dogs also receive these longer chain PUFAs in the diet, particularly during early growth when neural tissue is actively developing. Sea otters presumably have fatty acid requirements comparable to those of dogs and cats, although this has not been studied.

A lack of essential fatty acids is known to produce a variety of deficiency signs in dogs, cats and other mammals, including skin lesions, reproductive abnormalities, growth retardation, immunologic abnormalities, increased bruising and decreased wound healing, decreased visual acuity and other neurologic impairments (National Research Council 2006). Marine foods are well known as good sources of omega3 fatty acids, and thus an omega3 deficiency in sea otters might seem unlikely. However, otter prey are mostly very low in total fat (Part 2) such that the daily supply of all fatty acids (including essential fatty acids) to otters will be correspondingly low.

Another important issue in dietary studies is whether fatty acids can be used to determine diet of marine predators (Iverson 1993). This would seem promising for two reasons: 1. carnivores typically deposit fatty acids in milk adipose tissue stores without much modification, such that tissue fatty acids should reflect dietary fatty acids (Iverson et al. 1995); and 2. there are a very large number of long-chain fatty acids of varying degrees of unsaturation in omega3, omega6 and other fatty acid families in marine organisms, such that there is potential for fatty acid signatures to differ substantially among prey (e.g., Kharlamenko et al. 1995; Iverson et al. 1997a,b; Iverson et al. 2002). Although this approach to diet assessment has been applied to a variety of pinnipeds and cetaceans, the extent to which sea otter diets vary in fatty acid composition has not been examined. However, if the diet type of a sea otter could be determined from fatty acid analysis of a biopsy sample (whether during live capture or in postmortem exam), it would be possible to examine correlations of diet to morbidity, mortality and reproductive status without the need for prolonged behavioral studies (Part 5). Thus we sought to examine the extent to which different types of sea otter diets generated differing patterns of fatty acid intake.

Table 4.9. Recommended dietary levels for lipid components in carnivore diets[1]

			Dog			Cat		
Fatty Acid	Name		puppy after weaning	adult maintenance	gestation lactation	kitten after weaning	adult maintenance	gestation lactation
Dietary fat		% DM	8.5	5.5	8.5	9	9	9
w6 family								
18:2w6	linoleic acid	g/kg DM	13	11	13	5.5	5.5	5.5
20:4w6	arachidonic acid	g/kg DM	0.3			0.2	0.06	0.2
	Subtotal w6		**13.3**	**11**	**13**	**5.7**	**5.56**	**5.7**
w3 family								
18:3w3	a-linolenic acid	g/kg DM	0.8	0.44	0.8	0.2		0.2
20:5w3	eicosapentaenoic acid	g/kg DM						
22:6w3	+ docosahexaenoic acid	g/kg DM	0.5	0.44	0.5	0.1	0.1	0.1
	Subtotal w3		**1.3**	**0.88**	**1.3**	**0.3**	**0.1**	**0.3**

1. From *Nutrient Requirements of Dogs and Cats* (National Research Council 2006).

In this section we examine the fatty acid profiles for samples of 11 major prey species: two crabs (*Cancer antenarrius, C. magister*), three gastropods (*Haliotis cracherodii, H. rufescens, Tegula funebralis*), three bivalves (*Mytilus californianus, Protothaca staminea, Tresus nuttallii*), two echinoderms (*Pisaster ochraceus, Stronglyocentrotus purpuratus*) and fat innkeeper worms (*Urechis caupo*). We also examine the fatty acid composition of discrete diet types, using diet data from Part 5.

METHODS

Prey samples were lyophilized and ground as described in Part 2. Samples were shipped to the Physiological Ecology and Bioenergetics Lab of the University of Central Florida for fatty acid analysis using the methods described by Samuel (2000) and Samuel and Worthy (2004).

Lipids were extracted in duplicate from each of the prey samples with a solution of 2:1 chloroform–methanol using a modified version of Folch et al.'s (1957) method. Esterification was performed by adding 8% boron trifluoride in methanol to the extracted lipids followed by a series of hexane extractions to isolate and purify the resultant fatty acid methyl esters (FAMEs) (see Iverson et al. 1997b; Samuel 2000). FAMEs were stored at –20 °C until further analysis.

Gas–liquid chromatography was performed on FAMEs using a Perkin–Elmer Autosystem XL gas chromatograph fitted with a 30 m × 0.25 mm i.d. column coated with a 0.25 μm thick 50% cyanopropyl polysiloxane film (DB-23, J&W/Agilent, Folsom, California). The gas chromatograph was connected to a computerized integration system using the software package Turbochrome Workstation® (versions 4 and 6.1.2.0.1; Perkin–Elmer Instruments LLC 2000). Helium was used as the carrier gas. The injector temperature was held at 250 °C and the detector temperature remained at 270 °C. The initial oven temperature, 153 °C, was maintained for 2 min, then ramped at 2.3 °C/min to 174 °C, held for 0.2 min, then ramped at 2.5 °C/min to a final temperature of 220 °C and held for 3 min (S.J. Iverson, personal communication). Total program duration was 32.73 min.

Fatty acids in the sample were identified from known standard mixtures (68B, 68D, 87, 463, Nu-Chek Prep, Inc., Elysian, Minnesota) and secondary reference

mixtures. Fatty acids were named using standard nomenclature (number of carbons: number of double bonds, where **ω** or **w** indicates the position of the first double bond in relation to the terminal methyl end of the fatty acid) and were converted to percent amount of the total fatty acids. All unknown peaks within the sample were quantified but not used for further analysis, since it was not possible to determine their origin.

The nutritional significance of fatty acid levels in sea otter prey was judged by comparison to recommended levels of essential fatty acids of the omega6 and omega3 families for the diets of dogs and cats, as summarized by the National Research Council (2006). For this comparison, the percentage of fatty acids of each family was summed, and multiplied by 0.9 times the fat content of the prey species to provide an estimate of mg/kg DM fatty acids of that family. The 0.9 correction takes into account the fact that the glycerol backbone of triacylglycerols accounts for about 10% of the mass when these are composed of long-chain fatty acids (range 8-12% for carbon chain lengths of 14 to 22). We have opted to compare the total amounts of omega6 and omega3 fatty acids rather than levels of specific fatty acids as the relative efficacy of individual fatty acids in meeting sea otter requirements is not known (cf. National Research Council 2006).

RESULTS

A total of 63 fatty acids was found in sea otter prey, including 14 saturated fatty acids (SFA, from 12:0 through 20:0, including iso and anti forms), 19 monounsaturated fatty acids (MUFA, from 14:1 through 24:1, with the double bond in the w5, w7, w9, w11 and w13 positions) and 30 polyunsaturated fatty acids (PUFA, from 16:2 to 22:6, with the terminal double bond in the w1, w3, w4, w6 and w7 positions). Although there was variation among prey species, PUFAs were generally the most abundant fatty acid class, representing 42-60% of all fatty acids in 9 of the 11 prey species (Table 4.10). However, in Dungeness crabs (*Cancer magister*) MUFAs predominated, while in purple urchins (*Strongylocentrotus purpuratus*) there were more saturated fatty acids than MUFAs or PUFAs. Identified fatty acids accounted for 91-97% of the fatty acids.

Thirty-one fatty acids were present at 1.0% or more of fatty acids in at least one species of sea otter prey (Tables 4.11, 4.12). The most abundant fatty acids differed

Table 4.10. Fatty acids in sea otter prey. I. Major categories[1]

z1	*Haliotis cracherodii*	*Haliotis rufescens*	*Tegula funebralis*	*Mytilus californicus*	*Protothaca staminea*	*Tresus nuttallii*	*Cancer antennarius*	*Cancer magister*	*Pisaster ochraceus*	*Strongylo-centrotus purpuratus*	*Urechis caupo*
No. individuals	1	1	10	5	3	1	1	1	1	1	3
Sample Mass, g	87	48	11	87	33	350	129	396	47	13	204
Sat. FA %	27.865	30.6	26.345	22.925	23.08	32.43	16.65	23.09	27.13	36.46	29.49
MUFA %	18.09	17.63	16.74	16.03	10.055	10.16	32.78	44.33	24.62	28.14	11.53
PUFA %	44.745	44.46	50.26	53.98	59.725	51.46	46.525	29.265	41.885	30.095	52.785
All FA	90.7	92.69	93.345	92.935	92.86	94.05	95.955	96.685	93.635	94.695	93.805

1. Major categories are as follows: Sat. FA = Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 4.11 Major fatty acids in crabs, echinoderms and innkeeper worms.[1]

z2 Fatty acid	*Cancer antennarius*		*Cancer magister*		*Pisaster ochraceus*		*Strongylocentrotus purpuratus*		*Urechis caupo*	
	mean %	sem	mean %	sem	mean %	sem	mean %	sem	mean %	sem
14:00	**1.60**	0.010	**5.36**	0.715	**5.85**	0.550	**13.33**	0.205	**4.24**	0.015
14:1w9	0.34	0.050	0.17	0.040	**1.21**	0.095	0.26	0.030	0.03	0.005
14:1w5	0.02	0.000	0.11	0.015	0.04	0.000	**1.97**	0.075	0.01	0.005
iso15	0.11	0.010	0.21	0.025	**1.00**	0.075	0.13	0.015	0.13	0.005
15:00	0.35	0.005	0.36	0.010	**1.44**	0.105	0.44	0.000	0.57	0.025
15:1w6	0.34	0.050	0.27	0.005	**1.12**	0.040	0.13	0.005	0.33	0.045
16:00	**10.60**	0.170	**14.55**	1.695	**11.08**	0.475	**16.09**	0.395	**18.75**	0.400
16:1w7	**4.88**	0.095	**8.66**	1.155	**1.48**	0.120	**3.45**	0.220	**3.55**	0.030
7Me16:0	0.06	0.000	0.15	0.040	0.11	0.015	**3.76**	0.090	0.20	0.000
16:2w6	0.41	0.005	0.16	0.025	0.48	0.030	0.06	0.010	0.28	0.110
17:00	0.29	0.050	0.12	0.000	0.80	0.095	0.08	0.010	0.13	0.005
16:4w1	0.99	0.120	0.37	0.005	0.86	0.275	0.40	0.045	**2.81**	0.695
18:00	**3.10**	0.215	**1.88**	0.345	**6.00**	0.015	**2.08**	0.030	**5.12**	0.240
18:1w9	**20.70**	0.450	**15.96**	0.225	**2.98**	0.080	**3.05**	0.055	**2.50**	0.070
18:1w7	**4.83**	0.075	**5.09**	0.755	**4.17**	0.230	**2.47**	0.390	**2.21**	0.135
18:2w6	**5.31**	0.115	**1.22**	0.035	0.25	0.015	0.59	0.075	0.57	0.005
18:3w3	0.74	0.005	0.42	0.030	0.13	0.010	0.79	0.020	0.44	0.095
18:4w3	0.32	0.000	**1.13**	0.155	0.33	0.025	**2.67**	0.040	**1.22**	0.035
20:1w11	0.21	0.000	**5.38**	1.690	**4.94**	0.735	**5.10**	0.070	**1.28**	0.020
20:1w9	0.72	0.050	**1.58**	0.345	**6.37**	1.075	**4.45**	0.140	0.78	0.080
20:1w7	0.08	0.000	0.08	0.035	0.71	0.125	**5.48**	0.840	0.11	0.025
20:2w6	0.97	0.065	0.43	0.040	1.58	0.205	**1.83**	0.010	0.51	0.090
20:4w6	**3.75**	0.200	**2.20**	0.150	**16.05**	0.120	**9.31**	0.570	0.64	0.010
20:3w3	0.17	0.020	0.15	0.035	0.80	0.050	**1.38**	0.100	0.15	0.015
20:4w3	0.50	0.010	0.47	0.090	0.36	0.020	**1.06**	0.020	0.28	0.025
20:5w3	**17.27**	0.020	**10.54**	1.395	**13.26**	0.385	**7.50**	0.130	**17.91**	0.675
22:1w11	0.08	0.010	**4.68**	2.105	0.33	0.055	0.11	0.020	0.13	0.035
21:5w3	0.35	0.020	0.36	0.025	0.34	0.045	0.04	0.005	0.84	0.030
22:4w6	0.26	0.055	0.26	0.035	**1.23**	0.215	0.35	0.025	0.23	0.000
22:5w3	**2.62**	0.055	1.77	0.215	**3.77**	0.020	**1.64**	1.350	**2.52**	0.525
22:6w3	**11.11**	0.060	**7.92**	0.750	**1.51**	0.140	**1.08**	0.000	**23.31**	0.770
Total major fatty acids	93.03		91.91		90.49		91.03		91.69	

1. Fatty acids designated by chain length, number of double bonds, and position (w) of terminal double bond; sem refers to variation between replicate analyses. Highlighted cells indicate concentrations of 1% or more.

Table 4.12 Major fatty acids in gastropods and bivalves[1]

z3	*Haliotis cracherodii*		*Haliotis rufescens*		*Tegula funebralis*		*Mytilus californicus*		*Protothaca staminea*		*Tresus nuttallii*	
	mean %	sem	mean %	sem	mean %	sem	mean %	sem	mean %	sem	mean %	sem
14:00	**4.42**	0.210	**6.04**	0.340	**1.33**	0.075	**4.45**	0.050	0.83	0.015	**2.53**	0.020
14:1w9	**1.95**	0.035	**2.73**	0.285	**2.71**	0.135	**2.27**	0.025	0.75	0.250	0.58	0.005
14:1w5	0.07	0.000	0.20	0.010	0.02	0.000	0.04	0.005	0.00	0.000	0.00	0.000
iso15	0.05	0.010	0.02	0.015	0.19	0.025	0.04	0.000	0.11	0.000	0.01	0.000
15:00	**1.32**	0.080	0.52	0.010	0.79	0.010	0.36	0.005	0.39	0.030	0.68	0.015
15:1w6	0.43	0.430	0.25	0.100	0.77	0.030	0.25	0.090	1.01	0.035	0.36	0.105
16:00	**17.13**	0.355	**19.16**	0.780	**18.38**	0.485	**14.34**	0.150	**16.26**	0.010	**21.56**	0.105
16:1w7	**1.02**	0.000	**1.53**	0.045	**1.17**	0.220	**8.16**	0.120	**2.42**	0.050	**2.49**	0.100
7Me16:0	0.21	0.000	0.15	0.010	0.16	0.010	0.16	0.000	0.13	0.005	0.21	0.005
16:2w6	0.28	0.190	0.14	0.040	**1.01**	0.020	0.50	0.030	**1.61**	0.025	0.29	0.080
17:00	0.88	0.050	0.44	0.010	**1.25**	0.090	0.17	0.050	0.14	0.005	0.21	0.005
16:4w1	0.81	0.805	0.49	0.215	**2.25**	**1.09**	**1.62**	**1.50**	**2.64**	0.720	**1.57**	0.455
18:00	**3.74**	0.825	**4.13**	0.090	**3.71**	0.225	**3.18**	0.005	**4.84**	0.560	**6.95**	0.190
18:1w9	**4.18**	0.285	**4.03**	0.215	**5.63**	0.085	**1.02**	0.015	**1.87**	0.140	**2.30**	0.015
18:1w7	**6.98**	0.915	**6.18**	0.535	**4.12**	0.500	**1.81**	0.005	**2.34**	0.325	**1.75**	0.025
18:2w6	0.99	0.000	**2.10**	0.040	**2.08**	0.130	**1.11**	0.060	0.29	0.005	0.33	0.005
18:3w3	**1.04**	0.050	**3.17**	0.040	**1.42**	0.045	0.59	0.085	0.24	0.020	0.33	0.055
18:4w3	0.74	0.025	**2.00**	0.060	0.55	0.160	**1.94**	0.040	0.64	0.030	0.94	0.080
20:1w11	**2.03**	0.300	**1.48**	0.005	**1.45**	0.095	0.35	0.035	0.46	0.025	0.36	0.030
20:1w9	0.85	0.065	0.76	0.240	0.00	0.000	**1.64**	0.010	0.60	0.145	**1.16**	0.005
20:1w7	0.00	0.000	0.02	0.020	0.28	0.015	0.15	0.025	0.37	0.010	0.86	0.100
20:2w6	0.29	0.005	0.47	0.010	0.30	0.015	0.26	0.035	0.71	0.060	**1.08**	0.185
20:4w6	**13.70**	0.665	**12.37**	0.435	**18.55**	0.390	**3.71**	0.025	**1.35**	0.185	**1.22**	0.075
20:3w3	0.00	0.000	0.29	0.000	0.12	0.010	0.00	0.000	0.05	0.045	0.09	0.005
20:4w3	0.27	0.020	0.78	0.035	0.37	0.050	0.35	0.005	0.34	0.205	0.31	0.000
20:5w3	**12.14**	0.485	**10.36**	0.065	**9.90**	0.210	**26.21**	0.540	**11.05**	0.060	**14.81**	0.095
22:1w11	0.00	0.000	0.00	0.000	0.16	0.005	0.00	0.000	**0.00**	0.000	0.03	0.010
21:5w3	0.24	0.060	0.21	0.020	0.43	0.075	**1.33**	0.045	0.92	0.110	**1.44**	0.845
22:4w6	**2.88**	0.110	**1.90**	0.030	**3.65**	0.085	0.55	0.045	0.33	0.040	0.32	0.095
22:5w3	**10.15**	0.510	**9.28**	0.385	**7.50**	0.195	**1.17**	0.050	**2.19**	**1.66**	**5.47**	0.050
22:6w3	0.36	0.140	0.20	0.055	**1.05**	0.035	**11.95**	0.445	**36.45**	**1.47**	**22.05**	0.380
Total major fatty acids	89.10		91.34		91.22		89.61		91.26		92.20	

1. Fatty acids designated by chain length, numbr of double bonds, and position (w) of terminal double bond.

Highlighted cells indicate concentrations of 1% or more.

among species. In Cancer crabs (*C. antennarius, C. magister*) the four most abundant fatty acids (16:0, 18:1w9, 20:5w3, 22:6w3) accounted collectively for 49-60% of all fatty acids (Table 4.z2). In ochre stars (*Pisaster ochraceus*) the four most abundant were 16:0, 20:1w9, 20:4w6, and 20:5w3 (collectively 47%). In purple urchins the four most abundant were 14:0, 16:0, 20:4w6, and 20:5w3 (46%). In fat innkeeper worms (*Urechis caupo*) the four most abundant were 16:0, 18:0, 20:5w3 and 22:6w3 (65%) (Table 4.11). Among the gastropods, the four most abundant were 16:0, 20:4w6, 20:5w3, and 22:5w3, collectively accounting for 51%, 53% and 54% of fatty acids in red abalone (*Haliotis rufescens*), black abalone (*Haliotis cracherodii*) and black turban snails (*Tegula funebralis*), respectively (Table 4.12). In bivalves, three fatty acids were characteristically very high, 16:0, 20:5w3, and 22:6w3 and accounted for 53%, 58% and 64% of all fatty acids in California mussels (*Mytilus californianus*), gaper clams (*Tresus nuttallii*) and Pacific littleneck clams (*Protothaca staminea*) (Table 4.12).

Thus the fatty acids 16:0 and 20:5w3 were among the 3-4 most abundant fatty acids in every prey species studied, but other fatty acids were more variable. For example, 18:1w9 ranged from 1.0% in California mussels to 21% in Pacific rock crabs (*Cancer antennarius*), 20:4w6 from 1.2% in gaper clams to 18.6% in black turban snails and 22:6w3 from 0.2% in red abalone to 36% in littleneck clams (Tables 4.11, 4.12).

In general, sea otter prey were excellent sources of essential fatty acids of the omega3 family (18:3w3 or α-linolenic acid and its derivatives) (Table 4.13). Each prey species provided 6 or more g/kg DM of omega3 fatty acids, well above the recommended dietary levels of 0.1-1.3 g/kg DM for carnivores (Table 4.9). Essential fatty acids of the omega6 family were more variable, with high percentages in gastropods and ochre stars, but low percentages in bivalves and fat innkeeper worms. The absolute amounts of omega6 fatty acids were therefore variable (from 0.5 to 44 g/kg DM), with many prey containing less than the recommended dietary levels of about 6-13 g/kg DM. However, ochre stars were particularly rich in both omega3 and omega6 fatty acids (46 and 44 g/kg DM, respectively), thanks in large part to the high fat content measured in the edible portion of this species.

Table 4.13. Calculated fatty acid composition of sea otter diet types[1]

z4	Abalone and Cancer Crabs	Cancer Crabs	Kelp Crabs & misc. rocky	Urchins and Mussels	Clams & misc. sandy	Turban Snails
	1a	1b	2a	2b	2c	3a
Diet fat content (%)						
	5.1	5.2	5.3	10.2	3.8	3.8
Proportion of fat from prey type (%)						
Abalone	28.2	1.3	0.4	0.6	0.2	0.0
Crabs[2]	26.5	43.0	38.3	5.4	55.1	11.4
Urchins	31.3	25.3	38.7	77.7	9.9	16.7
Stars	10.6	25.3	16.2	6.6	1.6	15.1
Bivalves	0.9	1.6	4.5	6.8	17.0	0.6
Snails	0.5	2.2	0.1	0.9	9.2	55.9
Principal fatty acids (%)						
14:0	7.32	6.55	7.70	**11.35**	4.50	4.28
14:1w9	0.99	0.60	0.50	0.49	0.86	**1.78**
14:1w5	0.68	0.54	0.80	**1.54**	0.30	0.35
iso15	0.20	**0.36**	0.28	0.18	0.17	0.29
15:0	0.66	0.67	0.58	0.51	0.55	**0.78**
15:1w6	0.35	0.48	0.38	0.23	0.45	**0.66**
16:0	15.21	13.41	14.00	15.52	15.69	**16.22**
16:1w7	3.53	4.31	**4.46**	3.73	4.13	2.26
7Me16:0	1.28	1.04	1.53	**2.96**	0.63	0.75
16:2w6	0.22	0.30	0.25	0.15	0.49	**0.69**
17:0	0.37	0.35	0.26	0.16	0.35	**0.86**
16:4w1	0.63	0.72	0.68	0.57	1.33	**1.54**
18:0	3.19	3.38	3.04	2.53	**3.71**	3.65
18:1w9	7.43	**9.65**	8.71	3.81	8.08	6.22
18:1w7	**4.49**	4.06	3.69	2.74	3.60	3.93
18:2w6	1.55	**1.70**	1.56	0.77	1.64	1.68
18:3w3	**1.03**	0.55	0.59	0.73	0.68	1.01
18:4w3	1.49	1.13	1.46	**2.29**	1.08	0.90
20:1w11	3.41	3.83	3.90	**4.53**	2.31	2.73
20:1w9	2.65	3.28	3.29	**4.09**	1.65	1.85
20:1w7	1.84	1.62	2.30	**4.34**	0.97	1.19
20:2w6	1.05	1.19	1.27	**1.61**	0.83	0.79
20:4w6	9.35	8.40	7.68	9.09	6.42	**14.72**
20:3w3	0.61	0.63	0.73	**1.14**	0.32	0.44
20:4w3	0.66	0.59	0.68	**0.91**	0.49	0.50
20:5w3	11.14	12.06	11.64	9.54	**13.18**	10.51
22:1w11	0.71	**1.14**	0.99	0.25	0.84	0.43
21:5w3	0.23	0.29	0.28	0.17	**0.56**	0.34
22:4w6	1.02	0.64	0.49	0.47	0.86	**2.31**
22:5w3	4.36	2.70	2.32	1.95	3.44	**5.31**
22:6w3	3.47	5.32	5.45	2.64	**11.45**	2.24

1. See Chapter 5 for diet types; highest fatty acid values in bold (highlight).
2. Crab species differed among diet types, with a preponderance of Cancer crabs in diets 1a and 1b, kelp and cancer crabs in diet 2a, and sand and cancer crabs in diet 2c; however, fatty acid data are only for cancer crabs.

DISCUSSION

The large number (63) and diversity of fatty acids we encountered in sea otter prey was not surprising. In a study encompassing 15 invertebrate taxa (including polychaetes, bivalves, gastropods, an echinoderm and a crustacean) from a shallow water hydrothermal ecosystem in the Kurile Islands, Kharlamenko et al. (1995) reported detection of about 60 fatty acids. Iverson et al. (2002) measured 66 fatty acids among the fish, shrimp, squid and octopus of Prince William Sound, Alaska. Given this diversity, it is common for investigators to report only on those fatty acids that are relatively abundant. Fatty acids that are present in only trace amounts in prey will have little effect on predators, whether as a source of essential fatty acids or in leaving an imprint in the predator's fatty acid pattern or signature following consumption.

Sea otter prey species were good sources of omega3 fatty acids, as are most foods of marine origin. Thus despite the low levels of fat in most prey, sea otters are unlikely to experience omega3 deficiencies. If sea otters are like cats in having low levels of the Δ6-desaturase enzyme (National Research Council 2006), they may require up to 0.5 g/kg DM of long chain omega3 PUFAs, such as eicosapentaenoic acid (20:4w3) and docosahexaenoic acid (22:6w3) (Table 4.9), but as these are typically among the most abundant w3 fatty acids in sea otter prey, this should present no problem.

On the other hand, many sea otter prey appear to be low in total amounts of omega6 fatty acids. Of all the prey investigated, only ochre stars had absolute concentrations of omega6 fatty acids that exceeded the recommended levels for dogs (11-13 g/kg DM); the gastropods (abalone and snails) and purple urchins exceeded the recommended levels for cats (5.6-5.7 g/kg DM) but were below the recommended levels for dogs. The very low levels of omega6 levels in clams and fat innkeeper worms (0.5-0.6 g/kg DM) suggest that otters feeding by excavating prey in sandy bottom areas may be susceptible to omega6 fatty acid deficiencies. Although our fatty acid data are based on only single samples per species, the fact that all bivalves appear to be low in fat, regardless of season or region (Part 2) suggests that this may be a general nutritional constraint of feeding on clams. Of course, an otter could alleviate such a constraint by feeding on occasional prey that are higher in fat such as ochre stars, red urchins or (on a seasonal basis) crabs.

The fact that fatty acid patterns were so different among different taxa suggests that fatty acids in sea otter milk or in sea otter adipose tissue may provide useful information on prey consumption of individual animals (Iverson 1993; Iverson and Oftedal 1995). Our small data set suggests similarities among related taxa (such as among gastropods and among bivalves) that may make it difficult to distinguish the particular species eaten, but we expect that fatty acid data would be sufficiently robust to allow determination of major diet types. Some fatty acids appear to provide indicators for certain types of prey:

- Cancer crabs were high in 18:1w9 (16-21% vs. 1-6% in other taxa);
- Purple urchins were high in 14:0 (13% vs. 1-6%), 14:1w5 (2.0% vs. 0-0.2%), 7Me16:0 (3.8% vs. 0.1-0.2%) and 20:1w7 (5.5% vs. 0.0-0.8%);
- Ochre stars were high in iso15 (1.0% vs. 0.0-0.2%);
- Black turban snails were high in 17:0 (1.3% vs. 0.1-0.8%) and 22:4w6 (3.7% vs. 0.2-2.9%)
- California mussels were high in 20:5w3 (26% vs. 8-18%)
- Clams and innkeeper worms were high in 22:6w3 (22-36% vs. 0-12%)

The magnitude of the fatty acid signal due to particular prey species depends not only on the amount of prey eaten and the fatty acid composition of the prey, but also on the amount of fat in the prey, as it is the proportion of dietary fat contributed by different prey items that determine the fatty acid composition of the overall diet. We illustrate this by calculating the fatty acid composition of the different diet types that MBNMS otters consume, as described in Part 5 (Table 4.14). Note that a substantial amount of fat in all diets comes from urchins and stars, except diet 2c that contains little fat from stars. Crabs are also a predominant source of fat in 4 of the 6 diets, although there is some variation in the types of crabs (*Cancer* vs. *Pugettia* vs. *Emerita*) in different diets, which our calculations do not reflect as we used fatty acid data for Cancer crabs for all diet types. Obviously the shared components across diets will diminish the fatty acid differences among diets. Differences are nonetheless apparent, especially in the indicator fatty acids listed above. The four high crab diets (1a, 1b, 2a, 2c) were high in 18:1w9; the urchin and

Table 4.14 Essential fatty acids in sea otter diet types[1]

z5	Tyoe	Abalone and Cancer Crabs 1a	Cancer Crabs 1b	Kelp Crabs & misc. rocky 2a	Urchins and Mussels 2b	Clams & misc. sandy 2c	Turban Snails 3a
Diet fat content (%)							
		5.1	5.2	5.3	10.2	3.8	3.8
Major w6 fatty acids							
18:2w6	%	1.55	1.70	1.56	0.77	1.64	1.68
20:2w6	%	1.05	1.19	1.27	1.61	0.83	0.79
20:4w6	%	9.35	8.40	7.68	9.09	6.42	14.72
22:4w6	%	1.02	0.64	0.49	0.47	0.86	2.31
w6 total	%	13.0	11.9	11.0	11.9	9.7	19.5
	g/kg DM	6.0	5.6	5.2	11.0	3.3	6.6
Major w3 fatty acids							
18:3w3	%	1.03	0.55	0.59	0.73	0.68	1.01
18:4w3	%	1.49	1.13	1.46	2.29	1.08	0.90
20:3w3	%	0.61	0.63	0.73	1.14	0.32	0.44
20:4w3	%	0.66	0.59	0.68	0.91	0.49	0.50
20:5w3	%	11.14	12.06	11.64	9.54	13.18	10.51
21:5w3	%	0.23	0.29	0.28	0.17	0.56	0.34
22:5w3	%	4.36	2.70	2.32	1.95	3.44	5.31
22:6w3	%	3.47	5.32	5.45	2.64	11.45	2.24
w3 total	%	23.0	23.3	23.1	19.4	31.2	21.3
	g/kg DM	10.6	10.9	11.0	17.8	10.7	7.2

1. See Chapter 5 for discussion of diet types.

mussel diet was high in the 4 indicator fatty acids for purple urchins (14:0, 14:1w5, 7Me16:0, 20:1w7); the clam and sandy bottom diet was high in 22:6w3; and the snail diet was high in 17:0 and 22:4w6.

Although more research needs to be done on the variation in the fatty acid composition of sea otter prey and on the extent to which fatty acids in otter milk or tissues reflect diet fatty acids, our results suggest that fatty acids may provide a useful tool for diet identification in sea otters, as it has in a wide range of marine taxa, including marine mammals (Kharlamenko et al. 1995, 2001; Iverson 1993; Iverson et al. 1997a,b; 2002, 2004; Kirsch et al. 1998; Bachok et al. 2003; Abed-Navandi et al. 2005; Hughes et al. 2005).

CONCLUSIONS AND RECOMMENDATIONS

Sea otters consume diets that are typically low in fat, and thus the supply of fatty acids is limited. This may pose a problem with respect to w6 essential fatty acids, which are required by carnivores in substantial amounts. Of the prey eaten by sea otters, clams and fat innkeeper worms are particularly low in w6 fatty acids, suggesting that otters may be at risk of fatty acid deficiency when feeding predominantly on such prey. This warrants further investigation, with special attention to the sources of variation in the w6 fatty acids in bivalves and other prey. In contrast, w3 fatty acids are at high enough concentrations in all sea otter prey examined to meet estimated sea otter needs.

To avoid w6 fatty acid deficiency in the feeding of captive sea otters, a diet containing clams should also contain other prey types, including, if possible, fat-rich echinoderms.

The substantial variation in fatty acid composition of prey indicates that fatty acid composition of sea otter milk or sea otter adipose tissue may provide valuable information on the diet histories of individual animals. Storage lipids in recently weaned young may also provide information on the fatty acid composition of maternal milk, which in turn should reflect maternal diet (Iverson et al. 1995). However, validation of the use of fatty acids as diet indicators requires further research to determine:

1. the extent of within-species variation in fatty acid composition of prey;

2. the extent of similarity in fatty acid composition among related prey taxa (such as among decapods, gastropods, bivalves and echinoderms);
3. via captive feeding trials, whether sea otters deposit fatty acids in milk or adipose tissue in direct proportion to diet, and if not, what correction factors need to be applied to individual fatty acids,
4. the extent of variation in the fatty acid composition in sea otter milk or adipose tissue in different regions, different seasons, and different conditions (such as acute or chronic illness).

Finally, we predict that there will be greater heterogeneity in milk or adipose tissue lipids among otters in areas with substantial individual diet specialization, such as MBNMS. Such a pattern could prove useful because it may allow for the measurement of dietary specialization in a given population by fatty acid analysis: this would not only provide a useful index of population status, but also provide a means of correlating particular mortality risk factors (disease, contaminant exposure) with particular prey types.

Section D. Amino Acids

INTRODUCTION

Dietary protein is made up of amino acids, which are nitrogen-containing molecules linked together in sequence by peptide bonds and other interactions to form the three-dimensional structure of proteins. There are two types of amino acids: dispensable and essential amino acids. Dispensable amino acids can be synthesized *in vivo* by the animal so are not required in the diet, though they serve many important physiological functions. Dispensable amino acids are important as a source of nitrogen for the formation of other dispensable amino acids and other essential compounds such as neurotransmitters, hormones, purines and pyrimidines. In addition, dispensable amino acids provide carbon for gluconeogenesis, an anabolic process particularly important to carnivores, which consume little preformed glucose.

On the other hand, essential amino acids cannot be synthesized, or cannot be synthesized in sufficient quantities to meet animal needs, and so must be derived from the diet. A dietary deficiency of any essential amino acid results in decreased growth or reproduction or other more specific deficiency signs associated with the functions of each amino acid (see Table 4.15). Several essential amino acids are especially important to the health and growth of carnivores. For example, arginine is an essential amino acid for some carnivores because the enzymes of the synthetic pathway have low activity in these species. Arginine serves as an intermediate in the urea cycle, which is necessary for the detoxification of ammonia. Because of their high protein intakes, carnivores are especially sensitive to arginine deficiencies, which result in the rapid development of hyperammonemia with consequences as severe as death. Methionine and cysteine, sulfur-containing amino acids, are among the most frequently limiting amino acids for carnivores. In addition to many metabolic functions, methionine and cysteine are important for the growth of hair and fur (Glem-Hansen 1982). Symptoms of methionine deficiency include weight loss, dermatitis, abnormal eye secretions and lethargy. Taurine is a sulfur-containing β-amino acid that is essential for carnivores, but it is dispensable for herbivores and omnivores. Taurine has many functions, but the conjugation of bile acids is the most quantitatively important. Taurine deficiency in cats results in central retinal degeneration and dilated cardiomyopathy (Hayes et al. 1975; Pion et al. 1978).

Table 4.15. Amino acids that have been shown to be essential in carnivorous species, listed with some examples of their functions. Adapted from NRC, 2006.

Essential Amino Acid (abbreviation)	Example Functions (in addition to formation of proteins)
Arginine (Arg)	• Urea cycle intermediate • Precursor of nitric oxide • Precursor of biogenic amines
Histidine (His)	• Component of hemoglobin • Precursor of neurotransmitters (ex. histimine)
Isoleucine (Ile)	• No known specific functions
Leucine (Leu)	• Regulation of catabolism of branched-chain amino acids (Ile, Leu, Val) • Regulation of metabolism in concert with insulin
Lysine (Lys)	• Formation of cross-linkages of collagen
Methionine (Met)	• Metabolic methyl group donor • Synthesis of cysteine, a dispensable amino acid important for hair formation and glutathione
Phenylalanine (Phe)	• Synthesis of melanin (important for hair color), thyroid hormones, catecholamines
Taurine (Tau)	• Conjugation of bile acids • Osmoregulation
Threonine (Thr)	• No known specific functions
Tryptophan (Trp)	• Synthesis of niacin in most animals (limited in carnivores) • Precursor of neurotransmitters (ex. serotonin)

In this study, the amino acid composition of 15 sea otter prey species from central California was determined.

METHODS

Sample collection and processing procedures for amino acid analysis was the same as for macronutrient analysis. Lyophilized and ground samples were shipped to the amino acid laboratory at the University of California, Davis. Duplicate 5 mg samples were hydrolyzed in hydrochloric acid at 110°C for 24 hours. After drying under nitrogen, samples were re-dissolved in lithium citrate loading buffer and analyzed with an automated amino acid analyzer (Biochrom 30, Cambridge, England) using norleucine as an internal standard. The analysis was repeated on separate subsamples for quantification of methionine and cysteine, with the addition of oxidation in performic acid on ice for 16 hours prior to hydrolysis. Results are reported as the mean of the duplicate samples in mg/kg DM. Recommended allowances of the domestic cat for growth are used to assess the adequacy of sea otter prey, and amino acids essential for the cat (National Research Council 2006) are assumed to also be essential for the otter. The amino acid requirements of cats for maintenance and reproduction have not been determined, but it is generally assumed that these are not greater than the requirements for growth.

RESULTS

The essential amino acid composition of 15 sea otter prey species along with the recommended allowances for amino acids for growing kittens are presented in Table 4.16. The dispensable amino acid concentrations are given in Table 4.17. Amino acid concentrations in all sea otter prey analyzed are high relative to the requirements of growing kittens, as shown in Figure 4.1. Species are quite similar in amino acid composition and much of the variation observed is related to the large indigestible fraction present in some prey species. Taurine is the most variable amino acid, being lowest in concentration in *T. funebralis* (3.74 mg/kg) and highest in *H. rufescens* (69.76 mg/kg), but it is present in excess of the feline requirement in all species.

Table 4.16. Essential amino acid composition of sea otter prey species (n=15).

	Amino acid concentration (mg/kg DM)									
Species	**Arg**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Tau**	**Thr**	**Val**
Decapods										
Cancer antennarius	32.23	7.95	10.28	24.59	24.15	9.98	15.33	8.92	16.56	11.94
Cancer magister	32.14	6.05	6.12	19.21	19.35	7.82	13.35	12.85	12.31	8.24
Pugettia producta	29.25	7.51	13.80	26.37	20.13	7.74	16.00	12.93	17.74	17.90
Emerita analoga	18.83	4.27	4.71	14.62	15.34	5.81	8.90	3.74	10.78	6.94
Gastropods										
Haliotis cracherodii	54.57	5.47	8.56	33.96	26.21	11.45	14.82	68.54	17.20	11.84
Haliotis rufescens	61.77	7.27	17.75	41.45	32.40	13.68	18.63	69.76	26.05	21.23
Tegula funebralis	40.04	8.94	10.11	29.40	26.24	10.09	17.04	44.37	19.98	13.99
Tegula montereyii	15.39	3.25	3.90	11.43	10.97	4.22	6.53	19.91	8.14	5.23
Bivalves										
Clinocardium nuttallii	32.20	10.23	15.12	30.69	29.47	10.88	15.53	26.44	21.00	17.62
Mytilus californianus	26.35	5.55	8.80	23.88	22.32	8.40	10.94	27.03	16.83	10.20
Protothaca staminea	31.51	11.34	14.10	28.77	30.86	9.61	14.32	40.74	19.39	15.83
Tresus nuttallii	40.34	8.81	12.55	35.98	32.33	12.62	16.86	37.04	21.40	13.99
Echinoderms										
Pisaster ochraceus	21.49	6.56	11.84	24.98	24.11	8.92	14.77	10.88	20.94	14.21
Strongylocentrotus purpuratus	20.40	4.94	6.20	17.42	18.62	6.63	10.95	5.68	12.66	9.07
Echiura										
Urechis caupo	24.65	6.93	9.87	27.65	25.40	11.39	14.56	16.43	17.92	13.16
Mean	32.08	7.00	10.25	26.03	23.86	9.28	13.90	27.02	17.26	12.76
Standard deviation	12.87	2.22	4.01	8.08	6.22	2.58	3.27	21.19	4.68	4.38
Feline recommended allowance (growth)	7.7	2.6	4.3	10.2	6.8	7.0	4.0	1.5	5.2	5.1

Abbreviations are as follows: Arginine (Arg), Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Taurine (Tau), Threonine (Thr), Tryptophan (Trp).

Table 4.17. Dispensable amino acid composition of sea otter prey species (n=15).

Species	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
Decapods								
Cancer antennarius	22.05	33.13	5.01	50.85	30.07	11.98	17.30	12.87
Cancer magister	21.86	29.91	4.62	44.35	28.23	13.67	16.79	11.21
Pugettia producta	22.81	31.94	5.05	46.16	20.26	16.64	19.63	14.85
Emerita analoga	16.29	21.67	3.43	32.06	20.84	11.81	12.53	7.55
Gastropods								
Haliotis cracherodii	36.41	48.78	15.20	75.89	42.94	19.91	28.57	13.25
Haliotis rufescens	37.67	55.08	11.73	86.90	45.26	23.37	30.68	16.52
Tegula funebralis	25.46	44.30	9.26	55.05	35.91	15.73	22.93	16.16
Tegula montereyii	11.39	17.11	4.55	22.61	14.71	5.98	10.42	6.40
Bivalves								
Clinocardium nuttallii	27.91	42.45	9.08	60.59	34.20	13.28	22.61	15.27
Mytilus californianus	24.43	35.63	9.54	53.96	32.74	12.90	19.69	13.25
Protothaca staminea	24.85	40.05	8.95	59.07	29.07	11.61	20.16	13.81
Tresus nuttallii	54.54	49.81	7.84	73.85	47.11	15.75	25.83	21.58
Echinoderms								
Pisaster ochraceus	19.78	32.74	7.57	41.60	31.67	10.59	18.61	13.04
Strongylocentrotus purpuratus	18.20	24.54	6.46	30.70	37.12	8.90	14.53	10.41
Echiura								
Urechis caupo	51.26	39.53	7.35	53.86	85.24	15.28	23.99	12.57
Mean	27.66	36.45	7.71	52.50	35.69	13.83	20.28	13.25
Standard deviation	12.29	10.76	3.11	17.57	16.45	4.27	5.66	3.66

Abbreviations are as follows: Alanine (Ala), Aspartic Acid (Asp), Cystine (Cys), Glutamic Acid (Glu), Glycine (Gly), Proline (Pro), Serine (Ser), Tyrosine (Tyr).

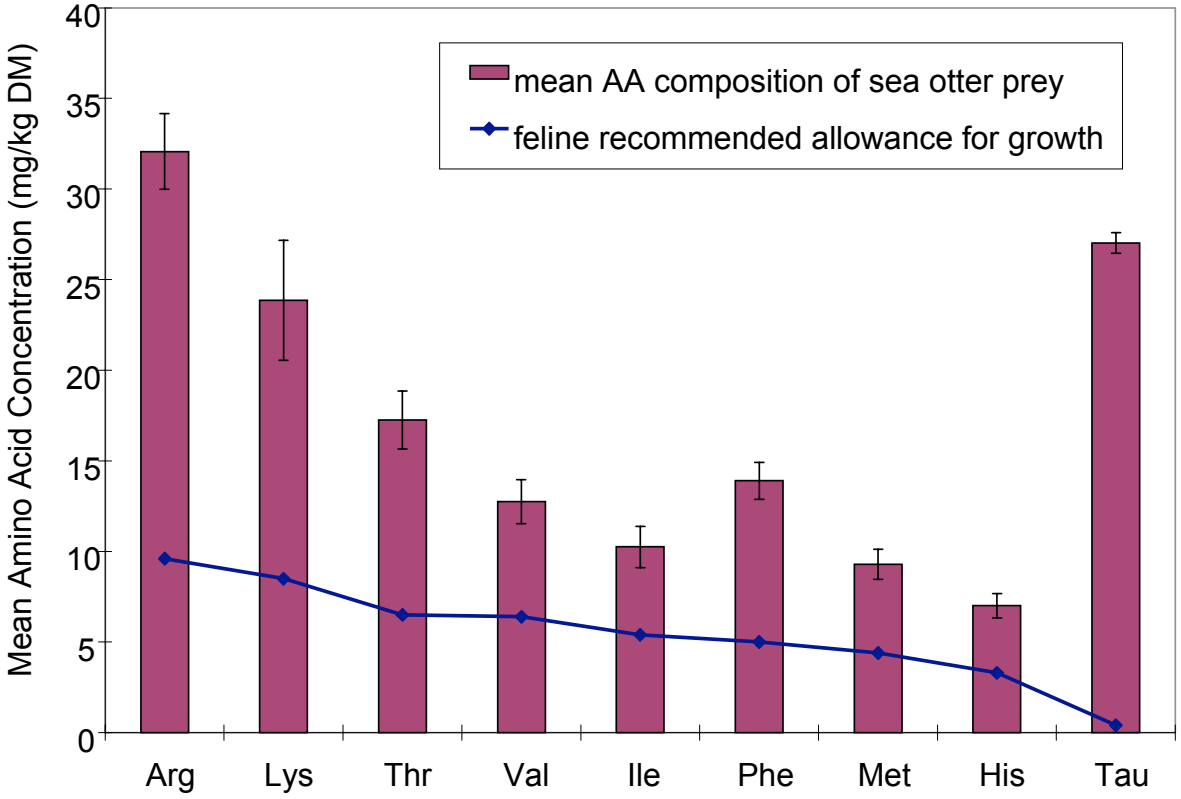

Figure 4.1. Mean essential amino acid composition of sea otter prey species (n=15) compared to the feline recommended allowance for growth.

DISCUSSION

Amino acid concentrations in sea otter prey exceed the requirements of the cat and dog so are unlikely to limit this population. These results are not surprising, given the high nitrogen levels measured in prey samples (see Part 2). Animal tissue has generally been found to supply well-balanced, high quality protein, and our data show that marine invertebrates are no different. Taurine, though variable, is high in all prey samples relative to the feline requirement. Thus taurine deficiency is not likely to be a cause of the dilated cardiomyopathy observed in this population.

Part 5. Diets of sea otters in the Monterey Bay National Marine Sanctuary

INTRODUCTION

Past research on sea otter physiology has shown that sea otters require a high rate of food intake to meet energetic requirements (Costa 1982) and, as a population, consume a wide variety of subtidal and intertidal invertebrate species (e.g. Ostfeld 1982; Ralls et al. 1995). Although at the population level sea otters in the MBNMS may be considered dietary generalists, the diets, and hence energy and nutrient intake rates, of individual sea otters can differ greatly from one another (Reidman and Estes 1990; Ralls et al. 1995; Estes et al. 2003b; Tinker 2004), with a high proportion of the typical individual's diet consisting of just 3 to 4 prey species (Tinker 2004). These individual differences in prey consumption do not appear to be based on genetic or morphological differences among individuals (Tinker 2004), nor can they be attributed to passive responses to environmental variation, as individuals with completely overlapping home ranges can have very different diets (Tinker et al. 2007). Rather, this phenomenon appears to represent behaviorally-mediated diet specialization, with prey preferences likely transmitted culturally from mother to pup (Estes et al. 2003b).

Theory predicts that individual dietary specialization will increase as intra-specific competition increases due to declining availability of preferred prey species (Glasser 1982; Schindler et al. 1997). The abundance of some preferred prey types within MBNMS is known to be greatly reduced compared to historical levels (Fanshawe et al. 2003; Bentall 2005 -- see Part 6). Thus, the dietary specialization seen in MBNMS sea otters suggests that competition for food is intense in this population.

The most recent analysis of the diets consumed by sea otters in the MBNMS is provided by Tinker (2004). Multivariate analyses (Tinker 2004) showed that the diets of individuals could be clustered into three more or less distinct groups, corresponding to otters specializing on large, medium, and small prey species. Estimated rates of energy gain during feeding bouts varied greatly, both between and within individuals, but were low compared to those reported for other sea otter populations. Specialists on large prey had the highest mean rates of energy acquisition but also had the highest between-bout

variation. All three specialist types had similar probabilities of exceeding a critical rate of energy gain on a given day because the mean and degree of inter-bout variation in the rate of energy gain were positively correlated. Tinker (2004) concluded that the individual dietary specialization of otters in MBNMS and the generally low rates of energy acquisition suggest that the population there is becoming increasingly food-limited.

Tinker's (2004) analyses were necessarily based on available published information on the allometric relationships between the size of prey items belonging to various species as judged by their greatest linear dimension (which is all that can be observed when collecting foraging data) and the edible wet mass, and on the energetic content of the edible wet mass. However, information on these matters was sparse or lacking for many prey species consumed by otters in the MBNMS. Our extensive collections, measurements, and energetic analyses of sea otter prey in MBNMS allowed us to repeat Tinker's analyses based on a much stronger empirical data set on the energetic and nutritional attributes of MBNMS prey species (Part II).

METHODS

Collection of foraging data

All MBNMS foraging data were collected between January 2001 and April 2004 from sea otters, captured in either the Monterey Bay or San Simeon area, that were tagged and instrumented with radio transmitters as part of a range-wide population study (Tinker et al. 2006). We systematically collected observational foraging data from tagged and instrumented otters using standard protocols (e.g. Ralls et al. 1995; Watt et al. 2000; Estes et al. 2003b). Field observations were collected 3-7 days per week throughout the study period: otters were initially located by radio signal using standard telemetric techniques and then visually monitored from shore using a 30× spotting scope (Questar Inc., Isanti, MN). Foraging bouts (defined as unbroken sequences of feeding dives) typically lasted 1-4 hours, and data were recorded throughout the entire bout or for as many dives as possible. The information recorded included date and time, precise location of each dive (determined by visual triangulation using GPS, compass and laser range-finder), duration of the subsurface dive interval ("DT") and the post-dive surface

interval ("ST") for each feeding dive (in seconds), success of each dive (i.e. whether or not prey was captured), species of prey captured, number and size of prey items, and handling time per prey item. Prey size was recorded as the estimated diameter of the shell or maximum body dimension (excluding appendages), categorized into 1.5 cm size-classes, using the fore-paw width (about 50 mm) as a relative gauge. For many observations, prey could not be identified to species; in such cases we classified prey to the lowest possible taxonomic unit, and we listed as "unknown" any prey items that could not be reliably categorized. Any prey items that were stolen by or from the focal animal were also recorded (and in the case of females with dependant pups, the number of items that were shared with the pups).

Diet composition

To avoid potential biases due small sample size, we limited our analyses of the MBNMS data to those otters from Monterey and San Simeon for which we recorded a minimum of 300 observed feeding dives over a minimum of 1 year and across all seasons: this resulted in a sample size of 63 otters, from which we recorded 1,620 feeding bouts comprising 58,319 feeding dives (Table 5.1). From these data we calculated two indices of diet composition for each individual study animal: the relative frequency of occurrence of each prey type, and the edible wet mass contribution of each prey type to the diet. The first index was calculated as the proportion of all recorded dives that prey type was observed, and provided a measure of the likelihood of observing a particular prey species at a given place and time. We used this index to evaluate spatial and temporal differences in diet composition at the level of the population. The second index of diet composition, prevalence of each prey type by mass, accounts for the actual consumption rate of each prey type (measured as grams of wet mass per minute of foraging time). To estimate this rate, we first needed a way of estimating the edible wet mass that could be obtained from 1 item of a given prey type of a given size class. Accordingly, for each prey species we fit power functions to our empirical data on wet mass (g) vs. maximum linear dimension: these functions represented species-specific allometric relationships between wet-weight and diameter, and the underlying function parameters and their variances were used to create log-normal sampling distributions for

Table 5.1. Summary of morphometric and foraging data available for 63 individual sea otters in the Monterey Bay National Marine Sanctuary

Location	ID number	Sex	Weight (kg)	Length (cm)	Number dives	Number Bouts
Monterey	637	female	23.7	120.2	388	12
Monterey	688	female	20.9	153.3	741	16
Monterey	743	male	26.6	124.4	899	31
Monterey	760	female	28.2	121.8	1132	23
Monterey	767	male	30.0	124.8	618	22
Monterey	779	female	20.0	116.2	386	22
Monterey	780	male	27.7	126.0	667	15
Monterey	783	female	20.4	115.2	1178	24
Monterey	823	female	16.5	111.3	517	16
Monterey	824	female	19.3	117.2	959	29
Monterey	826	female	17.7	110.5	345	10
Monterey	828	female	20.5	116.8	402	17
Monterey	829	female	20.0	118.3	538	17
Monterey	835	female	19.8	113.2	375	21
Monterey	877	female	29.5	122.8	447	16
Monterey	879	female	21.4	122.9	704	29
Monterey	880	female	18.7	113.5	824	21
Monterey	881	female	18.0	116.5	1255	34
Monterey	885	female	29.1	119.9	371	17
Monterey	886	male	43.9	122.3	443	18
Monterey	899	female	19.5	116.8	1222	30
Monterey	942	female	18.8	111.3	1336	24
Monterey	943	female	18.1	112.8	1089	34
Monterey	946	female	18.8	115.5	711	25
Monterey	952	female	21.1	117.3	596	17
Monterey	953	female	18.5	112.8	677	21
Monterey	956	female	19.9	116.6	690	28
Monterey	2787	female	19.0	110.0	1138	26
San Simeon	787	female	18.4	114.0	2291	50
San Simeon	788	female	19.7	121.4	1821	41
San Simeon	789	female	18.6	113.0	300	7
San Simeon	790	female	18.9	121.1	1185	41
San Simeon	791	female	14.3	106.0	839	17
San Simeon	792	female	23.0	127.0	1342	34
San Simeon	796	female	14.9	107.5	1714	29
San Simeon	802	female	19.9	118.0	1128	22
San Simeon	803	female	16.8	137.0	1813	49
San Simeon	804	male	24.9	125.3	2033	49
San Simeon	805	male	29.7	132.0	418	10
San Simeon	809	male	28.4	124.0	923	24
San Simeon	838	female	15.4	115.0	2045	34
San Simeon	839	female	20.9	122.7	639	24
San Simeon	840	female	19.2	111.5	985	24
San Simeon	842	male	26.3	123.0	497	18
San Simeon	843	male	30.6	126.0	328	11
San Simeon	844	female	15.3	110.2	853	22

Location	ID number	Sex	Weight (kg)	Length (cm)	Number dives	Number Bouts
San Simeon	845	female	15.0	110.3	799	25
San Simeon	846	female	17.0	115.5	982	22
San Simeon	847	female	21.6	117.0	970	32
San Simeon	850	female	22.0	119.0	574	24
San Simeon	851	female	14.5	109.4	941	35
San Simeon	854	female	17.3	115.6	1626	41
San Simeon	867	female	16.6	109.0	555	26
San Simeon	868	female	20.6	119.0	699	25
San Simeon	870	female	17.0	110.0	692	25
San Simeon	887	female	19.6	119.0	1046	32
San Simeon	888	male	24.8	123.0	910	34
San Simeon	889	male	22.0	116.0	473	24
San Simeon	890	female	19.8	117.5	1526	32
San Simeon	891	female	17.7	115.0	1624	36
San Simeon	895	female	19.3	117.0	1678	46
San Simeon	897	male	28.5	128.0	736	22
San Simeon	898	female	20.0	120.0	686	18

each size class of each prey type (see Table 2.3 and Figure 2.1). For a few prey species there were insufficient data available to fit mass-length functions, however these corresponded to relatively unimportant prey species (i.e. they occurred just a few times in the foraging database), and so for these species we simply took the mean wet mass values of all collected specimens as an estimate of wet mass, irrespective of prey size.

Mean and variance of wet mass and energy consumed per feeding bout

We next utilized a re-sampling approach (see Tinker 2004) to estimate mean and variance in the per-bout intake rate of each prey type for each individual. Our re-sampling method was designed to account for known biases in the observational data set (i.e. large prey species are easier to identify at distance and thus more likely to be recorded than smaller species; prey types captured on dives with short surface intervals are less likely to be recorded as such dives are often associated with small prey items), and to explicitly incorporate uncertainty into the estimates of feeding rates. Our general approach was to "boot-strap" foraging bouts (draw bouts randomly with replacement) from the database for each animal, and then calculate prey intake rates on a dive-by-dive basis for each of these bouts. Prey consumption was summed for all the dives in the bout and then divided by the total bout duration to create estimates of prey-specific consumption rates (g/min) for each bout. From these wet mass intake rates we also calculated energy intake rates, by multiplying the consumed wet mass for each prey species by the mean measured caloric density (on a fresh edible basis) (Tables 2.4–2.8). In the case of dives with no missing information, the calculations were straightforward: the estimated wet mass of each captured prey item was drawn at random from the species- and size-specific log-normal sampling distributions, and multiplied by the number of items of each prey type. Appropriate adjustments were then made for prey sharing or stealing: any prey items shared with a pup or stolen by another otter were subtracted, while any additional prey items stolen from another otter were added.

In the case of dives with one or more unrecorded parameters (e.g. unknown dive success, unknown prey, unknown prey size or unknown number of items), an appropriate estimate for the parameter in question was assigned based on the characteristics of the dive (following Tinker 2004). In particular, because the post-dive surface interval (ST)

was strongly correlated with dive success rate and the number/size of prey items, this information was used to restrict the range of possible values for each unrecorded parameter. For example, dive outcome can be modeled as a binomial variable (successful = 1, unsuccessful= 0) that is a function of ST: the probability of dive success is low for dives with small ST values and high for dives with long ST values. Accordingly, for each individual otter a logit function (the inverse of the familiar logistic function) was fit to all dives with known outcome, and this function was used to estimate the probability of success for any dives with unknown outcome. In the case of successful dives where the prey type was known but the number of items or size of prey was unrecorded, an appropriate value was drawn (with replacement) from the empirical distributions of size class and number of items for all dives of that prey type recorded for that otter. To minimize sampling bias, these empirical distributions were stratified by ST (short ST < 45s; medium ST ≥ 45s and < 90s; long ST ≥ 90s; this classification scheme was somewhat arbitrary, but provided adequate sample sizes for short, medium and long surface intervals). Finally, in the case of successful dives where the prey type was unknown, the prey type was drawn randomly from the sample of all known prey items recorded for the individual otter, but stratified by ST (i.e. to avoid prey types with long required handling times being assigned to successful dives with short surface times) and weighted by observed frequency: in this way we accounted for the bias against recording smaller prey items with faster handling times.

The boot-strap analysis described above was repeated 1000 times for each individual, to create distributions of the mean intake rates and between-bout variance in these rates for each individual animal (see Table 5.2). These rates were used for all further analyses of diet composition and nutrient intake rates. Because rates for wet mass and energy intake were log-normally distributed, all statistical tests were conducted using log-transformed values.

Estimating Individual Daily Required Foraging Time

We calculated the number of kilocalories per day each study animal would require by combining published data on sea otter field metabolic rates (average maintenance requirements = 240 kcal/kg/day; Costa 1982; Dean et al 2002) with body weight

Table 5.2 Summary of the results of boot-strap analysis of foraging success for 63 sea otters in MBNMS: Monterey peninsula (north end) and the coastal region around San Simeon (south end).

Location	ID number	Sex	Mean intake rate (g/min)	Between-bout variance (SD)	Energy intake rate (kcal/min)	Expected kcal/day	Expected hours feeding	Expected % time feeding
Monterey	637	female	16.74	3.03	11.88	5191.20	7.28	30.33
Monterey	688	female	12.35	1.19	9.38	6620.40	11.76	48.99
Monterey	743	male	9.39	1.32	8.45	5371.92	10.59	44.14
Monterey	760	female	18.14	1.69	16.18	5259.60	5.42	22.58
Monterey	767	male	22.41	2.84	18.30	5389.20	4.91	20.45
Monterey	779	female	8.08	1.04	7.30	5017.68	11.46	47.74
Monterey	780	male	8.79	1.21	8.04	5443.20	11.28	47.01
Monterey	783	female	13.37	1.70	11.67	4975.20	7.11	29.60
Monterey	823	female	12.06	1.36	7.68	4806.00	10.43	43.45
Monterey	824	female	13.86	1.31	12.40	5061.60	6.80	28.35
Monterey	826	female	12.65	1.24	9.23	4773.60	8.62	35.91
Monterey	828	female	18.79	2.39	11.19	5043.60	7.51	31.30
Monterey	829	female	10.35	0.93	10.16	5108.40	8.38	34.92
Monterey	835	female	7.25	0.83	5.86	4891.32	13.90	57.93
Monterey	877	female	11.05	0.78	7.72	5306.40	11.45	47.73
Monterey	879	female	14.79	2.60	11.53	5307.12	7.67	31.96
Monterey	880	female	14.86	2.17	12.92	4903.20	6.32	26.35
Monterey	881	female	10.86	1.40	8.81	5032.80	9.53	39.69
Monterey	885	female	8.89	1.68	9.79	5177.52	8.82	36.73
Monterey	886	male	11.54	1.49	11.64	5281.20	7.56	31.51
Monterey	899	female	11.78	1.26	9.29	5043.60	9.05	37.70
Monterey	942	female	12.12	1.53	10.24	4806.00	7.82	32.58
Monterey	943	female	7.75	1.09	6.04	4870.80	13.44	56.01
Monterey	946	female	5.67	0.91	4.60	4989.60	18.08	75.31
Monterey	952	female	8.29	1.48	6.47	5065.20	13.04	54.35
Monterey	953	female	4.01	0.66	3.96	4870.80	20.48	85.34
Monterey	956	female	13.67	1.70	11.77	5037.12	7.13	29.72
Monterey	2787	female	5.92	0.63	5.21	4752.00	15.21	63.37
San Simeon	787	female	9.89	0.96	9.45	4651.20	8.20	34.17
San Simeon	788	female	9.71	0.96	8.91	4953.12	9.27	38.62
San Simeon	789	female	9.68	0.59	6.86	4610.40	11.20	46.65
San Simeon	790	female	15.43	1.88	11.87	4940.88	6.94	28.91
San Simeon	791	female	14.08	1.38	11.89	4324.80	6.06	25.26
San Simeon	792	female	6.90	0.90	6.91	5181.60	12.49	52.04
San Simeon	796	female	10.71	1.10	9.74	4386.00	7.51	31.28
San Simeon	802	female	9.62	0.66	8.89	4814.40	9.02	37.60
San Simeon	803	female	10.06	1.30	8.06	5589.60	11.56	48.18
San Simeon	804	male	12.92	1.38	11.44	5110.20	7.44	31.01
San Simeon	805	male	23.55	1.69	10.63	5385.60	8.44	35.18
San Simeon	809	male	8.81	0.87	7.46	5059.20	11.30	47.10
San Simeon	838	female	7.68	0.86	6.77	4692.00	11.56	48.15
San Simeon	839	female	9.24	0.59	9.75	5006.16	8.56	35.67
San Simeon	840	female	4.88	0.58	4.35	4549.20	17.42	72.59
San Simeon	842	male	6.01	0.63	5.31	5018.40	15.76	65.67
San Simeon	843	male	7.46	0.33	9.73	5140.80	8.80	36.68
San Simeon	844	female	5.46	0.76	5.19	4496.16	14.44	60.17
San Simeon	845	female	11.66	1.53	11.54	4500.24	6.50	27.08
San Simeon	846	female	8.51	1.25	8.53	4712.40	9.21	38.36
San Simeon	847	female	9.67	1.02	10.94	4773.60	7.27	30.30
San Simeon	850	female	6.77	0.83	6.30	4855.20	12.85	53.55

Location	ID number	Sex	Mean intake rate (g/min)	Between-bout variance (SD)	Energy intake rate (kcal/min)	Expected kcal/day	Expected hours feeding	Expected % time feeding
San Simeon	851	female	8.68	0.98	7.76	4463.52	9.59	39.96
San Simeon	854	female	7.06	0.95	6.26	4716.48	12.56	52.35
San Simeon	867	female	6.37	0.72	6.81	4447.20	10.88	45.34
San Simeon	868	female	7.71	0.88	7.86	4855.20	10.30	42.90
San Simeon	870	female	10.67	0.88	8.95	4488.00	8.35	34.81
San Simeon	887	female	7.11	0.78	6.57	4855.20	12.32	51.33
San Simeon	888	male	14.48	1.60	10.60	5018.40	7.89	32.87
San Simeon	889	male	6.40	1.00	4.45	4732.80	17.73	73.89
San Simeon	890	female	10.94	1.48	8.97	4794.00	8.90	37.09
San Simeon	891	female	4.83	0.53	4.89	4692.00	16.00	66.66
San Simeon	895	female	10.38	0.87	9.79	4773.60	8.12	33.85
San Simeon	897	male	8.37	1.03	7.91	5222.40	11.00	45.83
San Simeon	898	female	9.34	0.82	8.15	4896.00	10.02	41.74
Average			**10.42**	**1.21**	**8.84**	**4954.00**	**10.26**	**42.76**

measurements (Table 5.1). Note that this calculation did not take into account energy losses due to incomplete digestion or energy losses due to excretion of excess nitrogen as urea, and thus represents a minimal estimate of daily gross energy requirements. Using these predicted energy requirements, we estimated the number of hours each otter would have to forage each day, given its rate of energy intake, to acquire the necessary number of kilocalories.

Identification of diet types

To simplify further analyses and interpretation of results, we combined similar prey species together to form prey categories or functional groups (see Table 2.1). The raw data analyzed were $p_{i,k}$, the prevalence (by mass) of prey type i in the diet of individual k: thus individual otters represent sample units ($N = 63$) and diet types represent the variables of interest. We used hierarchical cluster analysis to detect discontinuous groupings or "clumps" of data points in multidimensional space (McGarigal et al. 2000). The distance measure used was the standardized Euclidean distance, and we used Ward's minimum variance method to link similar points. The number of significant clusters (if any) was determined by graphical examination of the resulting dendrogram and scree plot of inter-cluster distance vs. number of clusters (McGarigal et al. 2000). After classifying each otter by cluster membership, we used linear discriminant analysis to evaluate the effectiveness of the classification (the frequency with which otters were correctly assigned cluster membership, using a "jack-knife" re-sampling test procedure) and to determine the key prey variables that contributed most to the classification. Assuming that distinct clusters could be identified, they were described in terms of the relative frequency of the main prey functional groups.

Nutrient composition of sea otter diets

We estimated the nutrient composition of individual sea otter diets by combining the observational data on diet wet mass composition and laboratory results for the nutrient composition of sea otter prey species. Specifically, we conducted the following four steps:

1. We generated an average, representative value for each nutrient for each major prey type as recorded in the observational database. Unless otherwise stated, the prey data are from MBNMS collections. The assumptions made for this step are as follows:

- Abalone – We used average values for red abalone (*Haliotis rufescens*) and black abalone (*H. cracherodii*). As we had data on macronutrients, macrominerals, trace minerals and vitamins for both species, this was straightforward.
- Cancer crabs – We used average values for Pacific (*Cancer antennarius*) and Dungeness (*C. magister*) crabs. Again we had full data for both species. The lab result for vitamin A for Dungeness crabs appeared erroneous (see part II), so we substituted the published value from the on-line USDA human food database, which was similar to our results for *C. antennarius*.
- Kelp crabs – We used macronutrient, macromineral, trace mineral and vitamin E data from our samples of *Pugettia producta* from MBNMS; we did not have other vitamin data from MBNMS but used values assayed for kelp crabs from San Nicolas Island.
- Crabs un-ID – We used average values for the two *Cancer* species and for *Pugettia*.
- Urchins – For macronutrients and macrominerals we used the average of purple urchins (*Strongylocentrotus purpuratus*) and red urchins (*S. franciscanus*). Trace mineral and vitamin data were only available for purple urchins from MBNMS.
- Clams – For macronutrients and macrominerals we used the average of three species: Pacific littleneck clams (*Protothaca staminea*), California butterclams (*Saxidomus nuttalli*) and gaper clams (*Tresus nuttallii*). For trace minerals and vitamins, we used average values for littleneck and gaper clams.
- Mussels – For all nutrients we used data for California mussels (*Mytilus californianus*).
- Stars – For macronutrients and macrominerals we used the average of giant spined (*Pisaster giganteus*) and ochre stars (*P. ochraceus*). For trace minerals and vitamins, we only had data for ochre stars.

- Snails – For macronutrients and macrominerals we used average values from three turban snail species: black (*Tegula funebralis*), dusky (*T. pulligo*) and Monterey (*T. montereyi*). For trace minerals and vitamins we only had data for black turban snails.
- Sand dollars – For macronutrients and macrominerals we used data for sand dollars (*Dendraster excentricus*), but as we lacked trace mineral and vitamin data we substituted values from another echinoderm, the purple urchin.
- Sand crabs – We used average values for mole crabs (*Blepharipoda occidentalis*) and sand crabs (*Emerita analoga*) for macronutrients and macrominerals. We did not have trace minerals or vitamins for these species, so we used average trace mineral values for *Cancer* spp. and *Pugettia* and vitamin data from *Cancer antennarius*.
- Worms – For all nutrients we used data from fat innkeeper worms (*Urechis caupo*), as this species comprised the majority of all recorded observations of worm consumption.
- Cephalopods – For macronutrients and macrominerals, we used average values for red octopus (*Octopus rubescens*) and market squid (*Loligo opalescens*). For trace minerals and vitamins, we used average values for all other taxa.
- Small rocky – for macronutrients and macrominerals, we used average values from a snail species (angular unicorn), a chiton (gumboot) and a tunicate, three taxa that were believed to represent some proportion of the "small rocky-bottom prey" category. For trace minerals and vitamins, we used average values for all other taxa.

2. All data were converted to a wet mass basis, to allow matching with the observational database.

3. We used the proportion of wet mass comprised by each prey type in each individual otter's diet to calculate the nutrient contribution of each diet item.

4. We summed these diet item contributions to get the nutrient composition of the individual otter diet on a wet mass basis, and then divided by dry mass to get the nutrient composition on a dry matter basis.

For each nutrient, we calculated the degree of variability between individuals as the coefficient of variation (CV). We used variance component analysis to determine the proportion of this variability that could be attributed to differences between the 6 diet-type groupings. We also used multivariate discriminant analysis to evaluate the efficacy of the diet-type classification for capturing variation across all nutrients. Finally, we calculated average nutrient profiles for each of the 6 diet-types in order to interpret patterns of variation.

A difference in nutrient composition among diet types is of interest primarily if it has significance to the nutritional status of sea otters. To permit this assessment, we compared nutrient levels on a dry matter basis to those recommended for growing or reproducing dogs and cats (as discussed in Parts 3 and 4). Our assessment assigned diet levels to one of five categories: 'low', 'marginal', 'adequate', 'high', and 'excessive'. 'Low' indicates a diet well below the recommended levels for dogs and cats and serves as a "red flag" indicating that nutrient deficiency is a possibility. 'Marginal' indicates that levels are below recommended levels, but not greatly so, or for nutrients where dog and cat recommended levels differ, levels may be lower than that of one species but not the other. 'Marginal' levels are suspect, but of less concern than 'low' levels. 'Adequate' levels are very close to or above recommended levels, and are likely sufficient for animal growth, reproduction and overall health. 'High' levels are somewhat to greatly in excess of recommended levels, but there is no suspicion that these levels are high enough to cause adverse effects. Finally, 'excessive' indicates a potential for adverse effects, such as toxicity (as in the case of nutrients with a narrow range between requirement and toxicity, such as copper and vitamin A) or impairment in the absorption of other nutrients (as in the case of calcium, which can reduce absorption of phosphorus, magnesium and zinc; see Part 4).

Although these assignments were based on informed judgment, it would be misleading to assign a rank of 'marginal' to one diet and 'adequate' to another if the nutrient concentrations of the two diet types are not significantly different. Therefore, for all nutrients where rankings differed among diet types, we conducted ANOVA tests (with diet type as a grouping factor) using Sidak's adjusted P-values to account for multiple contrasts (significance was set at alpha of 0.05). In the case of significant ANOVA

results, post-hoc pairwise comparisons were made using Tukey's test. If a diet type of one assessment rank was significantly different in nutrient concentration from diet types of a second rank, it is indicated by an asterisk in Table 5.6. Note that we do not report comparisons among diets of the same rank, which may or may not differ significantly from each other.

RESULTS

Diet composition at the population level

The sea otter population along the central California coast consumes a wide variety of macroinvertebrates, including cancer crabs, sea urchins, kelp crabs, clams, mussels, abalones, turban snails, sea stars, sand dollars and fat innkeeper worms. The most frequently captured prey were kelp crabs, snails, clams, cancer crabs and mussels (Figure 5.1a, Table 5.3). The composition of the diet varies seasonally, with increased proportions of kelp crabs in the fall, snails and sea stars in the spring, urchins in the fall and winter, and clams and mussels in the summer (Figure 5.2). The type of prey captured by females does not appear to vary in a consistent manner with reproductive condition, as neither females with small pups nor females with large pups ate more sea stars, which are high in fat – see Part 2 – or cancer crabs, which provide a substantial amount of wet mass due to their large size (Figure 5.3). Nonetheless, although there were no obvious or consistent dietary trends related to otter reproductive status, a few individual females did consume prey types differentially depending on whether they were feeding with or without pups (Figure 5.3).

The composition of the population's diet, when measured in terms of wet mass consumed, is quite different from its composition by frequency of occurrence (Figure 5.1b, Table 5.3). Cancer crabs, abalone, and other large-bodied invertebrates comprise a much larger proportion of the diet, and the most important prey are cancer crabs, urchins, kelp crabs, and clams.

However, diet composition at the population level provides only limited insight into the diets consumed by individual otters, as these are highly specialized, with each otter typically consuming only a few of the many prey types consumed by the population as a whole (Estes et al. 2003b, Tinker 2004).

Table 5.3. Population-level diet composition of sea otters in MBNMS, California categorized in terms of major ecological functional groups of prey species.

	Number Recorded Occurences	Proportion of all occurences	Mean Intake (g/min)	Standard deviation	Proportion of total biomass
Cancer Crab	1871	0.113	2.957	0.3005	0.284
Urchin	1568	0.095	1.458	0.1759	0.140
Kelp Crab	3258	0.197	1.431	0.1331	0.137
Clam	2138	0.129	1.092	0.1413	0.105
Mussel	1648	0.100	0.710	0.0835	0.068
Snail	2206	0.134	0.701	0.0788	0.067
Abalone	204	0.012	0.642	0.0791	0.062
Crab un-ID	1370	0.083	0.573	0.0668	0.055
Worm	685	0.041	0.252	0.0415	0.024
Sea Star	620	0.038	0.237	0.0332	0.023
Cephalopod	87	0.005	0.069	0.0220	0.007
All Other (sandy habitat)	114	0.007	0.218	0.0282	0.021
All Other (rocky habitat)	751	0.045	0.080	0.0231	0.008
All Prey Types	**16520**		**10.420**	(± 1.207)	
Energy Intake (kcal/min)			**8.840**	(± 2.769)	

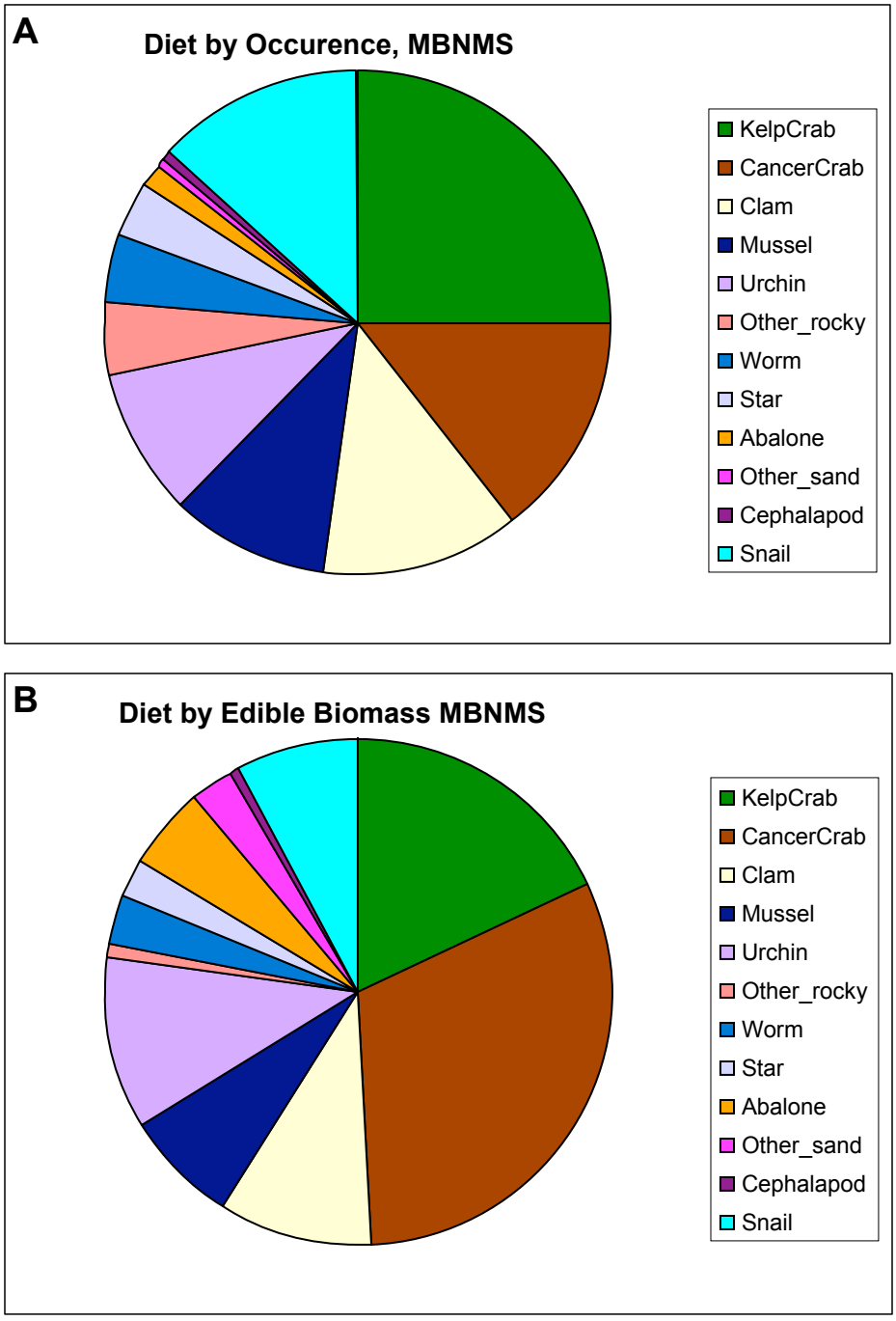

Figure 5.1. Population-level diet composition of sea otters in the MBNMS, categorized in terms of major ecological functional groups of prey species. Diet composition is shown in terms of A) the frequency of occurrence (i.e. the proportion of all recorded prey captures), and B) the proportional contribution to total edible wet biomass consumed.

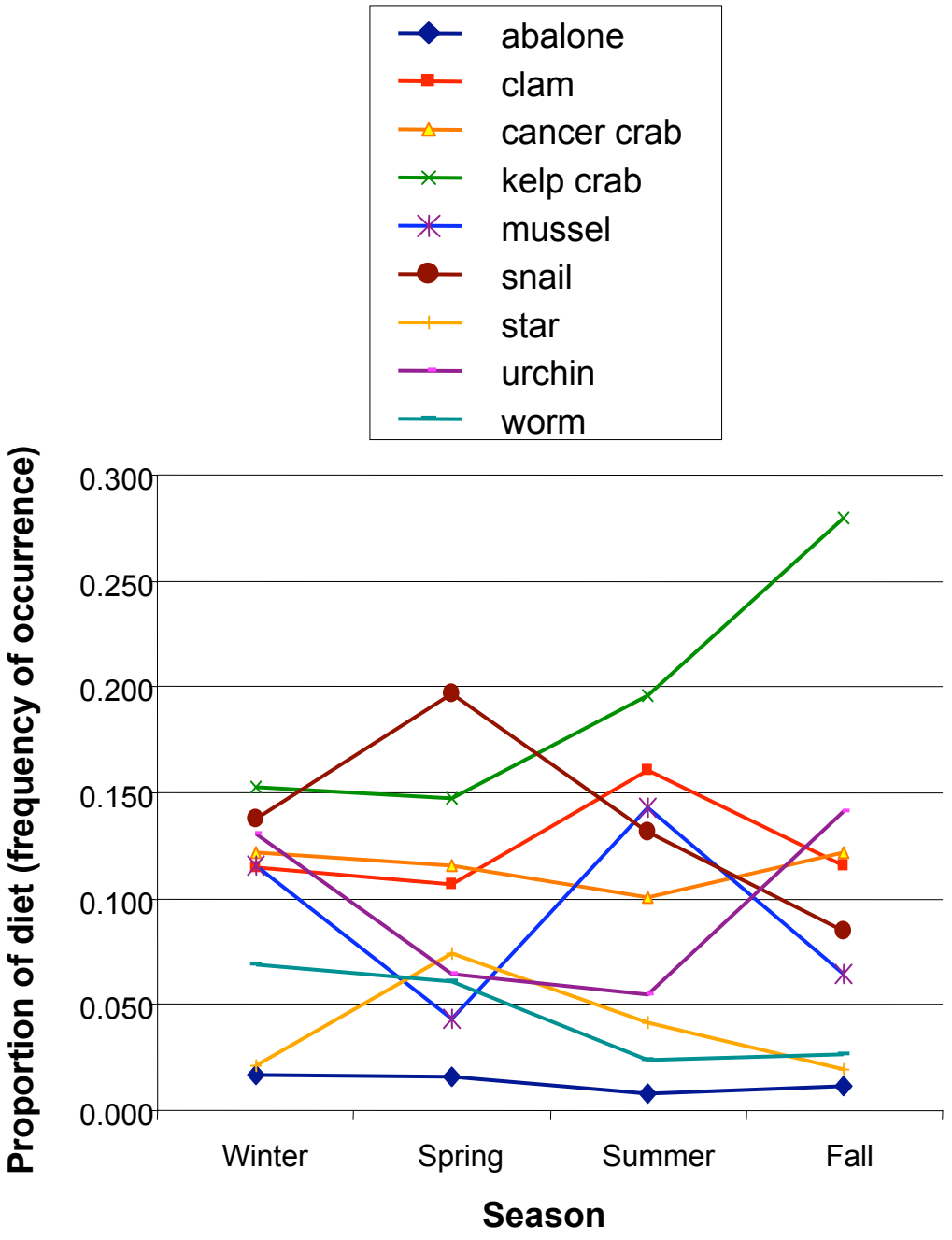

Figure 5.2. Seasonal variation in the relative frequency of 9 major prey functional groups in the diet of sea otters in MBNMS.

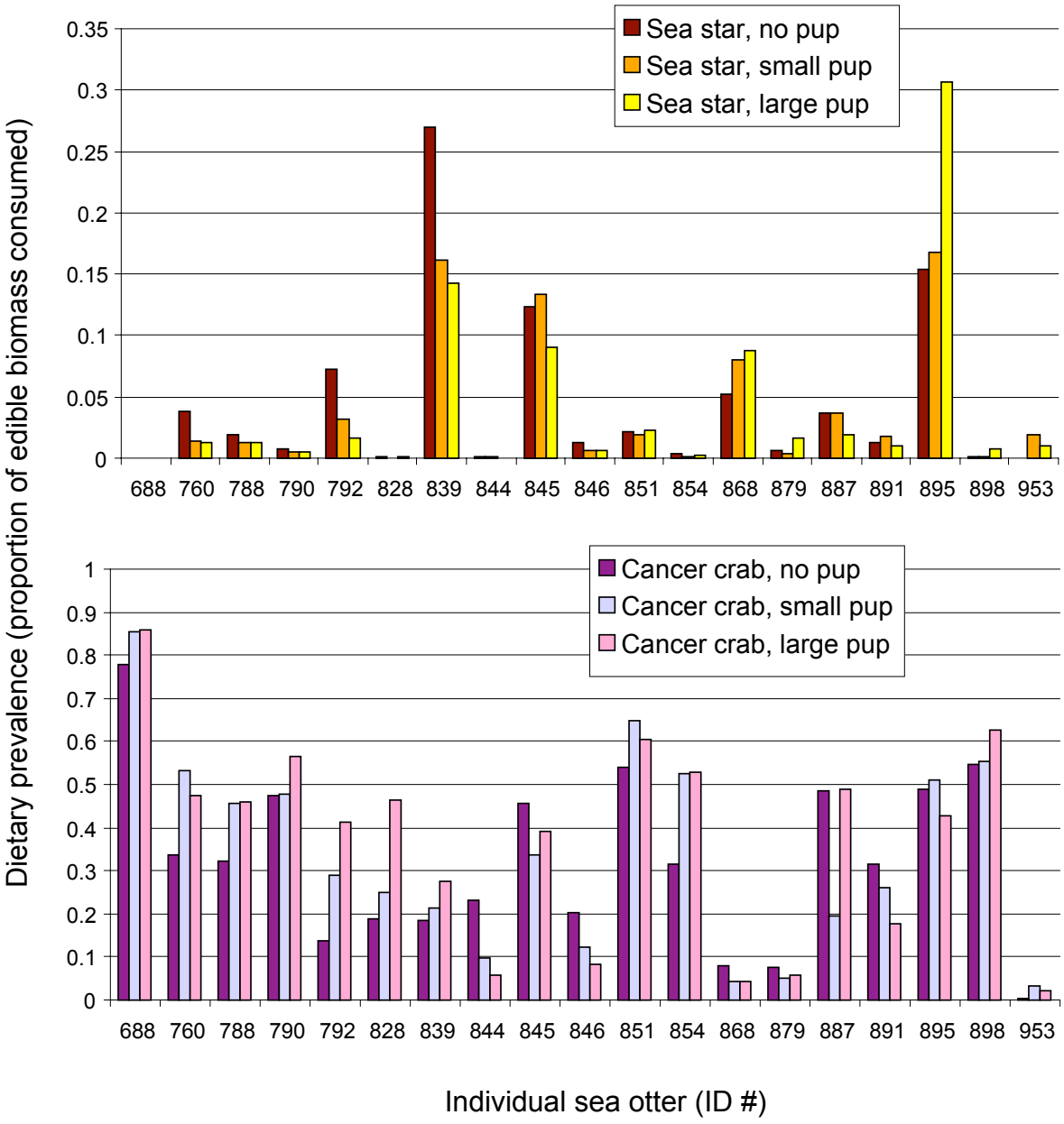

Figure 5.3. Relative dietary prevalence of two prey types, sea stars (*Pisaster* sp.) and cancer crabs (*Cancer sp*.), in the diets of individual adult female sea otters in MBNMS, shown as a function of reproductive state. All foraging data were classified based on whether females were feeding alone, with a dependant small pup (≤10 weeks), or with a large pup (> 10 weeks). Dietary prevalence is measured in terms of estimated proportion of total wet edible biomass consumed.

Foraging specialization/diet types and the composition of their diets

Although individual diets are highly varied (e.g., see Figure 5.3), they can be classified into a small number of groups based on the main types of prey being consumed. A cluster analysis of the diets of 63 sea otters (Figures 5.4, 5.5) shows two hierarchical levels of classification. At the highest level, we found that individuals could be grouped into one of three broad diet types, as reported by Tinker (2004). Individuals with a Type 1 diet eat mainly larger prey (mean wet mass per prey item = 60.4 g, ±26.97), those with a Type 2 diet eat medium-sized prey (23.7 g/item, ± 15.73), and those with a Type 3 diet eat small prey (8.8 g/item, ± 4.07). The distinct nature of these groupings was apparent in the discriminant analysis canonical scores plot (Figure 5.6). However, a closer inspection of the cluster analysis results (Figure 5.5) revealed that two of these major clusters could be further subdivided, resulting in six distinct diet types (Table 5.4). Large prey specialists were divided into abalone/crab specialists (Type 1a) and cancer crab specialists (Type 1b). Medium prey specialists were divided into those that feed in the kelp forest, with a relatively varied diet including kelp crabs (Type 2a), those that specialized on urchins and/or mussels (Type 2b), and those that fed in soft-bottom habitats, consuming mainly clams, worms, and other sand-dwelling benthic invertebrates (Type 2c). Otters that specialized on very small prey (Type 3) consumed primarily turban snails, but also fed on kelp crabs and sea stars.

The disparities in typical diet composition (on the basis of proportion of total wet mass consumed) for each of the six foraging specialist/diet types is illustrated in Figure 5.7, where the high proportion of particular prey eaten by some foraging specialists – such as snails by Type 3a and mussels and urchins by 2b – is strikingly apparent.

Rates of edible wet mass and energy intake

Rates of wet mass intake (g/minute of foraging time) did not vary by either sex or area (north vs. south) within MBNMS (two-way ANOVA), either in terms of gross intake rates (area effect $p = 0.094$, sex effect $p = 0.166$) or in terms of mass-specific intake rates (area effect $p = 0.615$, sex effect $p = 0.067$). Mean individual rates of energy intake were

Table 5.4. Average diet composition of sea otters according to diet type.

Prey Size	Diet type	# otters	Description based on principal prey	abalone	cancer crab	kelp crab	crab, un-ID	urchin	clam	mussel	star	snail	sand dollar	sand crab	worm	cephalopod	small rocky
Large	1a	7	**Abalone and Cancer Crabs**	0.398	0.335	0.069	0.027	0.092	0.011	0.020	0.016	0.005	0.000	0.000	0.001	0.006	0.020
Large	1b	16	**Cancer Crabs**	0.019	0.526	0.173	0.055	0.079	0.037	0.017	0.042	0.022	0.008	0.001	0.012	0.005	0.005
Medium	2a	15	**Kelp Crabs & misc. rocky**	0.006	0.236	0.309	0.108	0.118	0.065	0.090	0.026	0.001	0.000	0.004	0.024	0.004	0.008
Medium	2b	7	**Urchins and Mussels**	0.015	0.069	0.046	0.029	0.391	0.053	0.325	0.018	0.014	0.000	0.000	0.005	0.015	0.020
Medium	2c	12	**Clams & misc. sandy**	0.002	0.179	0.053	0.060	0.020	0.338	0.041	0.002	0.060	0.057	0.071	0.107	0.004	0.006
Small	3	6	**Turban Snails**	0.001	0.061	0.183	0.021	0.057	0.016	0.008	0.027	0.623	0.000	0.000	0.001	0.000	0.001

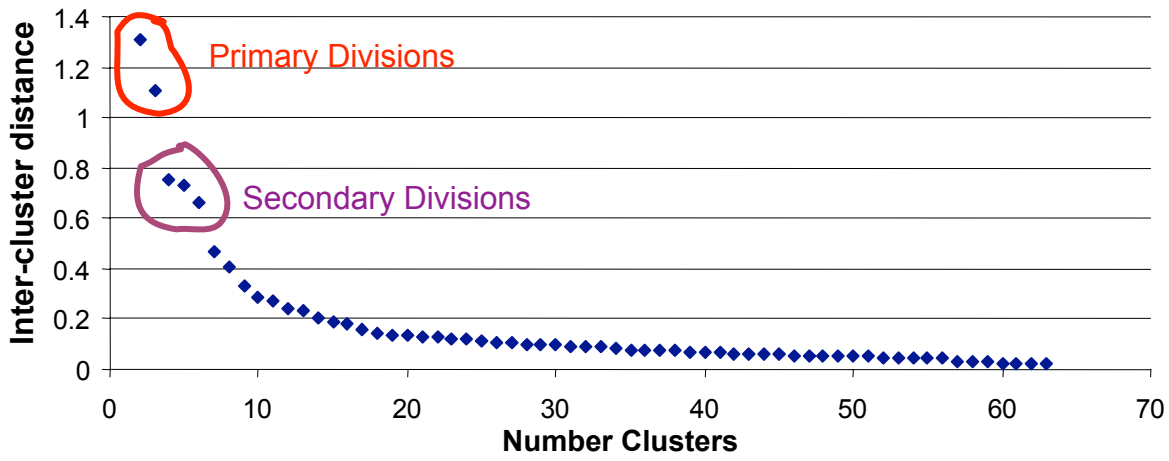

Figure 5.4. Graphical summaries of the results of a multivariate, hierarchical cluster analysis of diet composition for individual study animals in MBNMS. Part I. The distance metric used is the standardized Euclidean distance; see text for more explanation of methods (page 151). The scree-plot of inter-cluster distances indicates two breakpoints, and thus two hierarchical levels of classification: a primary division into 3 main groups, and a secondary division of these into 6 sub-groups.

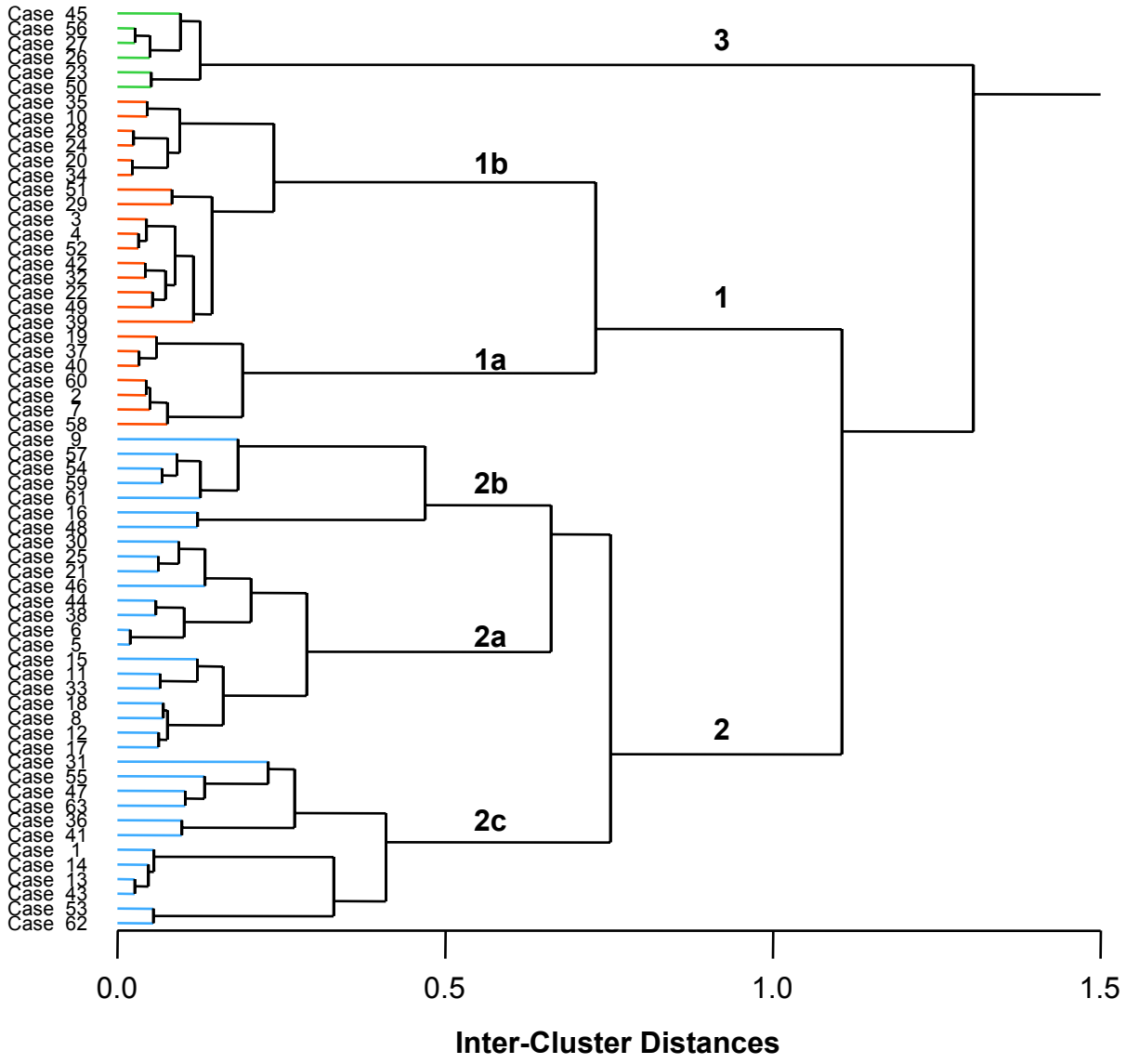

Figure 5.5. Graphical summaries of the results of a multivariate, hierarchical cluster analysis of diet composition for individual study animals in MBNMS. Part II. The dendrogram illustrates the divergent pattern of inter-node distances, such that animals within clusters are very similar, whereas animals in different clusters are highly dissimilar (each terminal node here represents a single study animal).

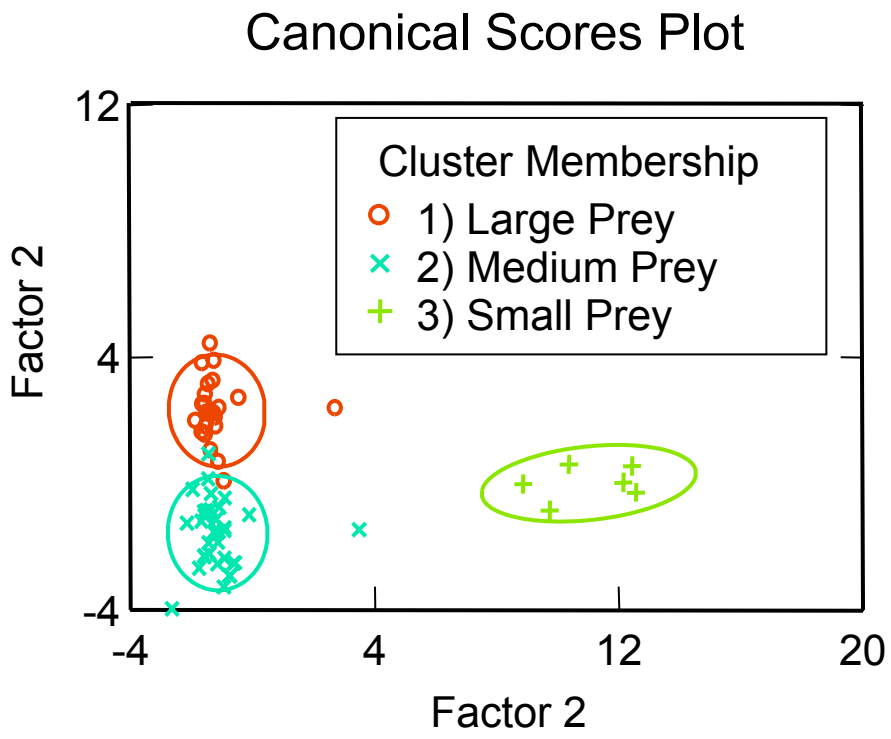

Figure 5.6. Canonical scores plot of the first two canonical factors from a multivariate discriminant analysis of dietary data from 63 radio-tagged sea otters in MBNMS. Canonical functions were derived from 13 separate predictor variables, corresponding to individual consumption rates of 13 prey functional groups. The *a-priori* classification variable was diet type, as determined from the results of a hierarchical cluster analysis (see Figure 3.4): type-1 animals consumed primarily large prey species, type-2 animals consumed a variety of medium-sized prey species, while type-3 animals consumed small prey species, primarily turban snails. See text (page 160) for further details.

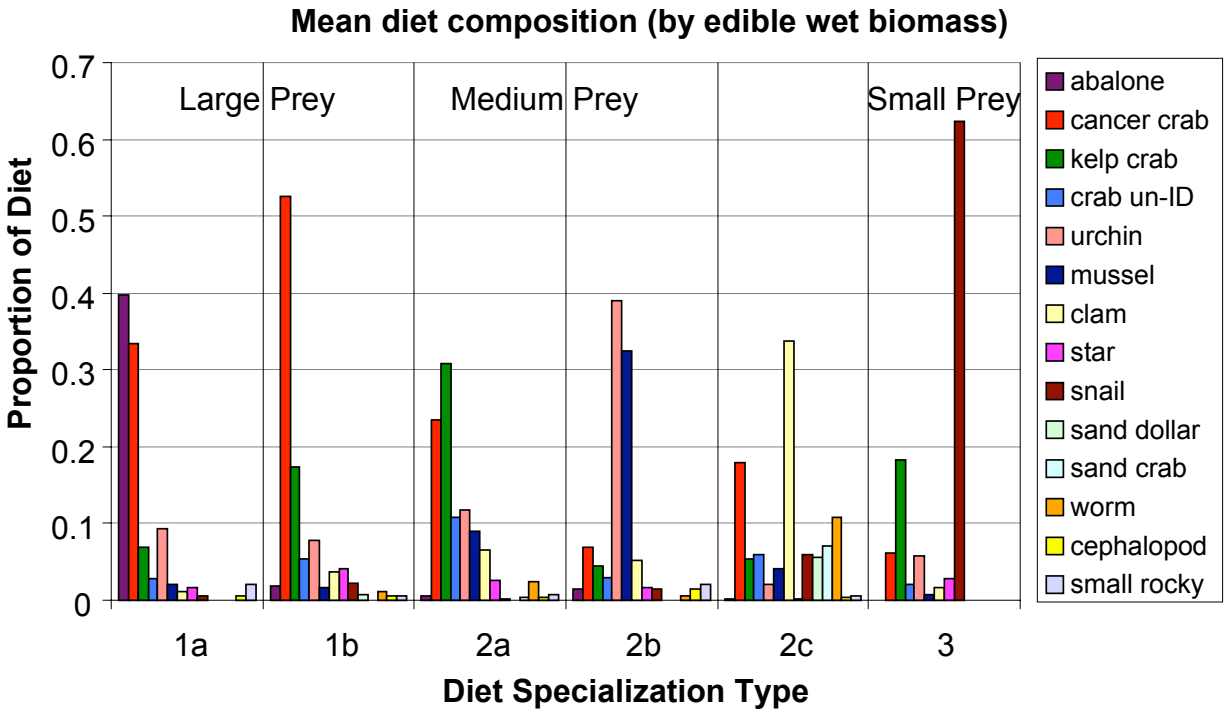

Figure 5.7. Average prey composition of sea otters assigned to 6 different diet specialization types. See text (page 160) for a full explanation of diet classification analysis. For each diet type, the relative importance of 14 prey functional groups is shown, expressed in terms of the proportional contribution to total wet edible biomass consumed.

also relatively invariant within MBNMS (area effect $p = 0.550$, sex effect $p = 0.943$); however, the between-bout variance in rate of energy gain differed between study areas and sexes: males had greater bout-to-bout variance in the rate of energy gain ($p<0.001$) and animals in the north (Monterey peninsula) showed greater variability than animals in the south (San Simeon; $p<0.001$). There was also a significant interaction effect ($p<0.001$), such that the difference between males and females was more exaggerated in the north than in the south. In addition to greater between-bout variance, there was also greater between-animal variation in the north: for example, the standard deviation in female rate of energy gain was 30% greater in Monterey than in San Simeon.

The average rate of wet mass intake by foraging otters was 10.42 g/minute overall, but this rate varied greatly depending on prey specialization (Table 5.5). For example, a female otter specializing on cancer crabs would have a typical intake of approximately 12 g of wet mass per minute of foraging time, while one specializing on turban snails and sea stars would have a typical intake of 7.8 g/minute. The individual otters showed an even greater variation in rates of wet mass intake, ranging from a high of over 23 to a low of 4 g per minute of foraging time (Table 5.2).

When we converted wet mass to energy, using our data on kilocalories per gram of wet mass for different prey species, we found that the variation in average intake rates for the different foraging specialist/diet types was somewhat less than for wet mass consumption, but still ranged from a little over 7 kcal per minute of foraging time for females of Type 2a (kelp canopy feeders) to more than 11 kcal per minute for males of Type 2c (sandy bottom feeders; Table 5.5). The population average was 8.84 kcal/minute; however individual otters showed a striking degree of variation in rates of energy intake, ranging from 4 to over 22 kcal per minute of foraging time (Table 5.2). The animals having the lowest rate of energy gain (Type 2a) also had the most diverse diet (Figure 5.8).

Although the average predicted foraging time required to meet energy needs was only a little over 10 hours, predictions for individual otters ranged from 4.91 to 20.48 hours (Table 5.2). Most of the 10 individuals with the shortest predicted required foraging time per day (4.91-7.27 hours) specialized on large prey, while all 10 individuals

Table 5.5. Foraging success for female and male sea otters according to diet type.

Sex	Diet type	Number of otters	Mean intake rate (g/min)	Standard deviation intake rate (g/min)	Mean Energy intake rate (kcal/min)	Std. Dev. Energy intake rate (kcal/min)
female	1a	7	12.10	3.81	10.43	3.45
female	1b	13	11.54	2.32	10.08	1.79
female	2a	11	8.02	2.27	7.31	2.07
female	2b	6	11.72	4.45	8.16	2.27
female	2c	10	9.20	3.71	7.86	2.62
female	3a	4	7.80	1.79	7.78	1.71
male	1b	3	10.94	2.33	10.33	2.10
male	2a	4	9.19	3.71	8.28	2.38
male	2b	1	23.55		10.63	
male	2c	2	14.41	11.32	11.37	9.79
male	3a	2	9.09	0.42	8.25	0.29

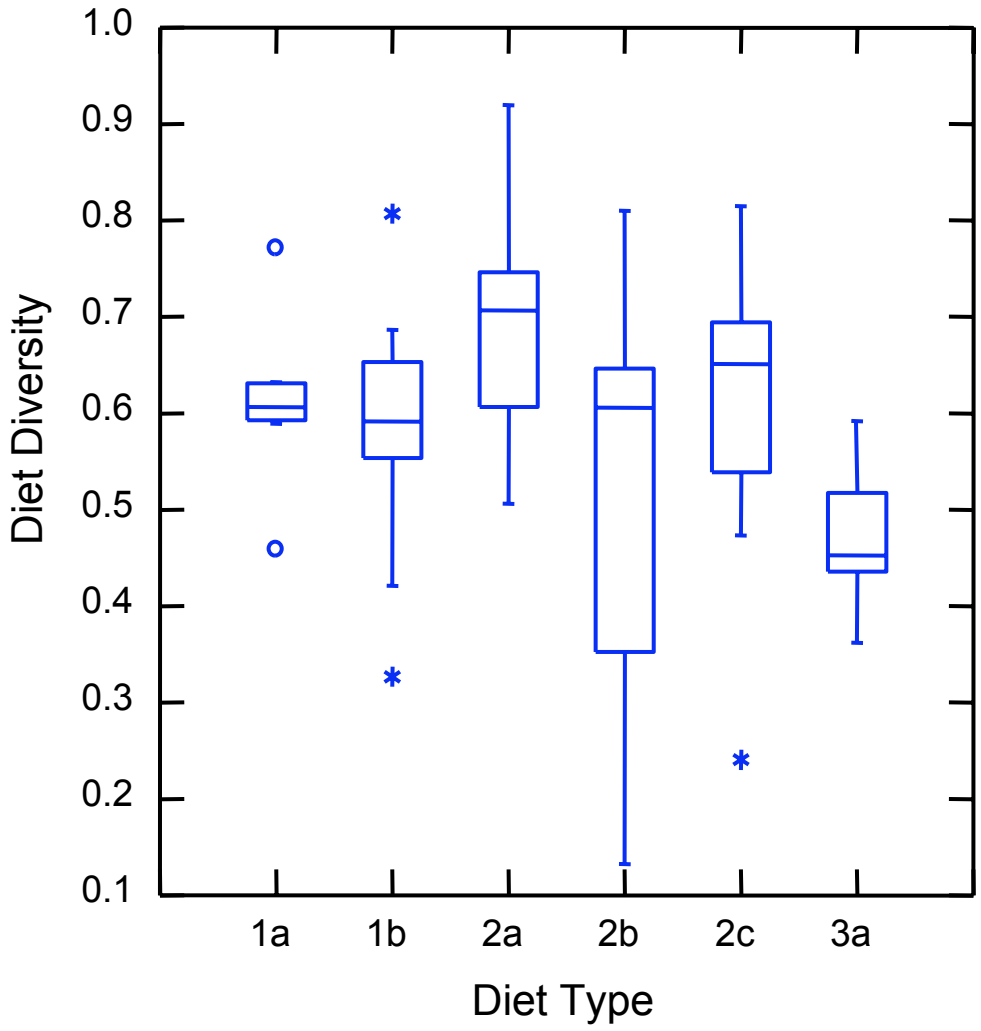

Figure 5.8. Box-plot showing the distributions of diet diversity measures for sea otters assigned to 6 different diet specialization types. Diet diversity was measured using the Shannon-Weiner index, calculated for each individual study animal on the basis of proportional contribution to the diet of 14 prey functional groups.

with the longest predicted foraging times (13.44 to 20.48 hours) specialized on medium-sized prey. The mean predicted activity budget (43% of the time spent feeding) was very close to the mean activity budget actually measured from these same animals (41%) using telemetric and archival TDR data sets (Tinker et al. 2006). The average daily food intake, given this activity budget, would represent approximately 30% of body mass, consistent with previously reported estimates (e.g. Costa 1982).

Nutrient composition of individual otter diets and otter diet types

By integration of data on prey consumption and prey composition, we were able to calculate the nutrient composition of the consumed diet for 63 individual otters. Of the nutrients evaluated, approximately half showed substantial variation between individuals: the median CV was 0.244, and for ten nutrients (fat, calcium, iron, copper, manganese, selenium, thiamin, riboflavin, vitamin A and vitamin E) the CV among individuals was equal to or greater than 0.25 (Table 5.6). For these 10 nutrients, variance component analysis indicated that approximately 2/3 of the variation among individuals (0.67 ± 0.167) was attributable to differences between the 6 diet types, and discriminant analysis indicated that 94% of individuals were correctly assigned to diet type solely on the basis of nutrient composition (Wilk's Lambda Approx. $F_{50,222} = 22.371$, $p<0.001$); accordingly, we restrict further discussion of nutritional variation to differences between diet types (Table 5.6).

Diet types differed in dry matter and energy on a fresh edible basis (ANOVA, Sidak-adjusted $p< 0.001$ for DM, $p = 0.006$ for energy), and on a dry matter basis for all major constituents tested (ANOVA, Sidak-adjusted $p < 0.002$ for 14 nutrients showing differences in ranking between diet types: see Table 5.6). Given the importance of energy to sea otters, it was noteworthy that the urchin and mussel diet (type 2b) had a higher energy content on a dry basis (4.2 kcal/g; Tukey's test, $p < 0.05$) than all diets other than the abalone and crab diet (type 1a), and that the snail diet (type 3a) was significantly lower in energy (2.5 kcal/g DM) than all other diet types (Tukey's tests, $p < 0.001$, all pairwise comparisons) (Table 5.5; Figure 5.9). This difference is attributable to two constituents: ash and fat. The ash content of the snail diet was much greater (39% DM) than all other diet types (18-25%; Tukey's tests, $p < 0.001$, all pairwise comparisons).

Table 5.6. Assessment of nutrient levels in otter diet types in MBNMS[1]

		Degree of Individual variability	Diet Type[2] 1a Abs & crabs		1b *Cancer* crabs		2a Kelp crabs+		2b Urchin/mussel		2c Clams/sandy		3a *Tegula* snails		Rec. for growth/reprod. Dog	Rec. for growth/reprod. Cat
Fat	**% DM**	**0.53**	5.13	Marg	5.22	Marg	5.28	Marg	9.65	Adeq*	**3.71**	**Low**	**3.77**	**Low**	8.50	9.00
Carbon	**% DM**	0.04	37.93		36.15		34.77		35.16		35.75		35.74		--	--
Nitrogen	**% DM**	0.09	9.22		8.253		7.65		7.18		8.34		8.36		--	--
Protein	**% DM**	0.09	57.61	High	51.52	High	47.69	High	44.67	High	51.71	High	52.22	High	22.5	22.5
Ash	**% DM**	0.24	17.79		24.5		24.83		20.32		25.29		39.21		--	--
Energy[3]	**kcal/g DM**	0.15	3.94	Adeq*	3.52	Marg	3.55	Marg	4.22	Adeq*	3.68	Marg	**2.50**	**Low***	4.36	4.36
Energy	**kcal/g WM**	0.11	0.90		0.837		0.81		0.83		0.76		0.89		--	--
Calcium	**% DM**	**0.50**	5.44	High	8.82	High	8.52	High	4.78	High	7.24	High	**19.28**	**Excess***	1.2	1.1
Phosphorus	**% DM**	0.18	0.80	Adeq	0.91	Adeq	0.87	Adeq	0.73	Adeq	0.78	Adeq	**0.49**	**Low***	1.00	0.80
Potassium	**% DM**	0.14	0.92	High	0.85	High	0.85	High	0.85	High	0.90	High	0.57	Adeq*	0.44	0.52
Magnesium	**% DM**	0.20	0.54	High	0.68	High	0.76	High	0.67	High	0.65	High	0.38	High	0.06	0.05
Iron	**mg/kg DM**	**0.89**	271	High	258	High	222	High	336	High	819	High	**1374**	**Excess***	88	80
Copper	**mg/kg DM**	**0.38**	29.6	High	42.2	High	25.9	High	13.5	Adeq*	30.5	High	45.1	High	12.4	8.8
Zinc	**mg/kg DM**	0.18	107	Adeq	104	Adeq	87	Marg*	90	Marg	112	Adeq	76	Marg*	100	75
Manganese	**mg/kg DM**	**0.71**	6.0	Marg	8.3	Adeq	8.8	Adeq	10.0	Adeq	25.1	High*	14.9	Adeq	7.2	7.2
Selenium	**mg/kg DM**	**0.26**	1.74	High	2.55	High	1.85	High	1.36	High	2.15	High	1.55	High	0.35	0.3
Thiamin	**mg/kg DM**	**0.33**	2.89	Marg*	**1.46**	**Low**	**1.37**	**Low**	**1.31**	**Low**	**1.34**	**Low**	**1.18**	**Low**	2.25	6.3
Riboflavin	**mg/kg DM**	**0.37**	77.8	High	108.7	High	95.3	High	51.3	High	57.8	High	36.4	High	5.3	4.0
Niacin	**mg/kg DM**	0.16	115	High	120	High	113	High	91	High	104	High	72	High	17	40
Vit. B6	**mg/kg DM**	0.19	2.57	Adeq	3.03	Adeq	2.74	Adeq	1.75	Marg*	2.29	Adeq	2.15	Marg	1.5	2.5
Vit A	**ug/kg DM**	**0.25**	1248	Marg	1243	Marg	1324	Marg	1513	Marg	1441	Marg	**642**	**Low***	1515	2000
Vit D3	**ug/kg DM**	0.31	17.9	Adeq	25.4	High	24.7	High	19.0	Adeq	18.0	Adeq	14.5	Adeq*	13.6	7.0
Vit E	**mg/kg DM**	**0.28**	132	High	140	High	116	High	78	Adeq*	86	Adeq*	75	Adeq*	30	38 - 120

1. Comparative data for 23 nutrients are shown, ten of which showed substantial levels of variability between individuals (coefficient of variation > 0.25; red highlighted font). Note that the majority of the individual variation for these nutrients (67%) was accounted for by differences between diet groups

2. Each nutrient for each diet is compared to the recommended levels for dogs and cats, and assessed as either low, marginal (Marg), adequate (Adeq), High or Excessive (Excess). If the mean for that diet is significantly different (Tukey's test, $p < 0.05$) from that of groups in the row bearing a different assessment category, it is marked with an asterisk. Nutrient levels of concern because they are low or excessive are so indicated in bold.

3. Energy recommendation converted to gross energy by adding 1.6 kcal per g protein; no additional digestibility correction applied.

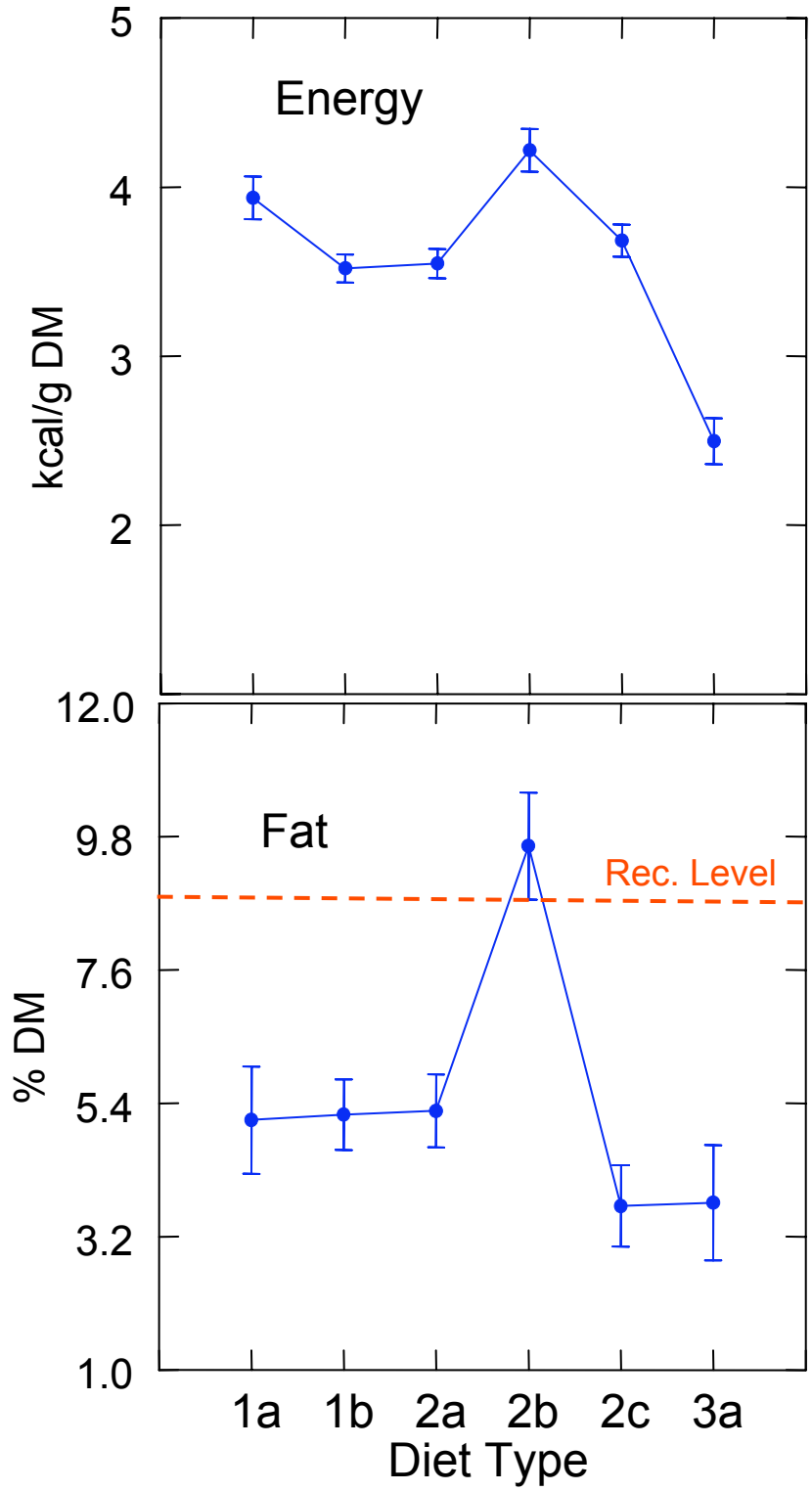

Figure 5.9. Variation among diet types in energy and fat. Means are least square means. Note diet 2b (urchins and mussels) is high in both fat and energy.

As discussed in Part 2, ash content is the primary determinant of the energy density on a dry basis in marine invertebrates. As a consequence of the inclusion of substantial quantities of high-fat urchins, the urchin and mussel diet (type 2b) was significantly higher in fat (9.7%) than all other diet types (2.7-5.3%; Tukey's tests, $p < 0.01$, all pairwise comparisons) (Figure 5.9). Inclusion of urchins in the diet increases fat and energy, whereas inclusion of snails increases ash and decreases energy.

Overall, most diets were ranked as "adequate" or "high" with regard to most nutrients, indicating the ability of sea otters to combine prey items in a manner that is nutritionally adequate. However there were some exceptions. As indicated in Part 3, most prey items were low in thiamin and vitamin A, and this is evident in the nutrient composition of the six diet types (Figure 5.10). Only diet 1a (abalone and crabs) was deemed marginal with respect to thiamin, all other diets were deemed low (Table 5.6). Most diets were considered marginal with respect to vitamin A, but diet 3a (turban snails) was low (Figure 5.10).

In comparing among the diet types, diets 1a, 1b, 2a, 2b and 2c are reasonably similar in nutritional quality, being adequate or high in nearly all nutrients except thiamin and vitamin A. Of these diet types, zinc was marginal in diets 2a (kelp crabs and generalist rocky) and 2b (urchins and mussels). By contrast, diet 3a (turban snails) was low in three nutrients (phosphorus, thiamin, vitamin A), marginal in two others (zinc and vitamin B_6), and excessive in two nutrients (calcium and iron) (Figure 5.10). This diet type was also lower in energy and higher in ash, as noted above. Based on these results, a diet specialization of turban snails may be nutritionally inadequate for sea otters.

DISCUSSION

Our analysis confirmed the major conclusions of Tinker (2004) regarding the diets of sea otters in the MBNMS and provided additional insights into the foraging ecology and diets of otters in this population. There is a high degree of dietary specialization and diets of individuals differ markedly from the average diet for the population. Nevertheless, definite dietary clusters can be discerned. Our improved data set provided increased resolution of the major foraging specialist/diet types in the population, allowing us to subdivide two of the original three dietary clusters identified

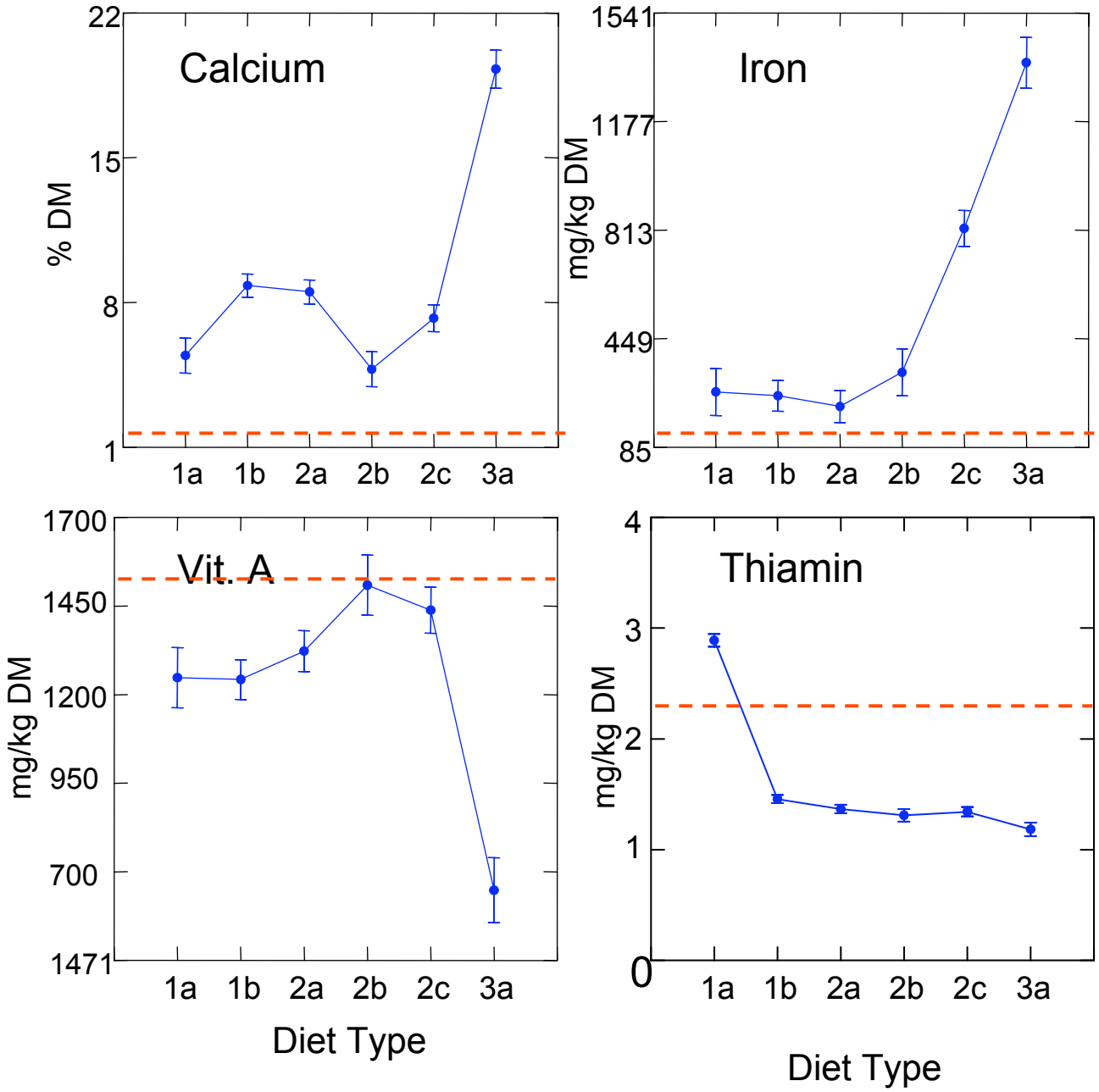

Figure 5.10. Diet type variation (least-square means) in four nutrients of concern: calcium, iron, vitamin A and thiamin. The red dashed line indicates approximate recommended level (see Table 5.6). Note that diet 3a (*Tegula* snail diet) is both very high in calcium and iron and very low in vitamin A and thiamin.

by Tinker (2004) and classify individuals into six rather than three diet types. Otters in these six dietary clusters differed in the types of prey consumed, in average rates of wet mass and energy consumption (Table 5.5) and in the proportions of nutrients ingested.

Although average rates of wet mass and energy consumption in MBNMS are low, as previously estimated by Tinker (2004), there is a striking degree of variation within the population. Females showed less variation in the rate of energy gain across feeding bouts than males, suggesting that the demands of pregnancy and pup-rearing favor more risk-averse foraging behavior in females. Greater variation between individuals in the northern part of MBNMS may reflect greater depletion of prey resources in this area, although otters recolonized both areas (Monterey Bay and Piedras Blancas) around 1960 in dispersing from the Big Sur coast (Riedman and Estes 1990).

Both the extreme dietary specialization and the low average rates of wet mass and energy consumption strongly suggest that the otter population in MBNMS is becoming increasingly food-limited. Furthermore, emaciation (adults) or starvation (pups) has been identified as a contributing cause of death in about 25% of fresh carcasses (Kreuder et al. 2003). However, the effects of food limitation are not equally distributed across the population because of dietary specialization and differences between the foraging abilities of individuals even within dietary types. While some individuals are doing well with respect to energy gain, others appear to be doing quite poorly, particularly some of the individuals specializing on medium-sized prey (Figure 5.9). Interestingly, individuals in dietary cluster 2a (the kelp canopy feeders) had both the most diverse diet and the lowest rate of energy gain, suggesting that a greater degree of dietary specialization is indeed associated with higher rate of energy intake, as predicted on the basis of theory (Estes et al 2003b).

Diet specialization is a workable strategy in response to resource limitation only if the specialized diets are adequate with regard to other nutrients as well as energy. By limiting the number of prey types, a predator is also more likely to restrict itself to a subset of available nutrients and may unintentionally select a nutritionally inadequate diet. The evolutionary consequences may be severe for such a choice, as nutritional deficiency may induce increased morbidity and mortality, increased susceptibility to the adverse effects of toxins and contaminants and reduction in reproductive output with loss

of fitness. If sea otters in MBNMS and vicinity are in the process of evolving greater dietary specialization, we may observe both nutritional successes and failures.

Evaluation of individual diet types suggest that this may be occurring. On the one hand, five of the six diet types appeared well-balanced with respect to most nutrients, but one, the snail based diet (diet type 3a), appeared replete with nutritional error: the diet appears to be too high in calcium and iron, too low in phosphorus, zinc, thiamin and vitamin A, and loaded with inorganic ash that has little if any nutritional value. On a dry matter basis, this is the diet with the lowest energy density, although we note that our analysis does not account for differences in energy expenditure, and so it is possible that animals with this diet specialization have lower energy costs (because of shallower dives or easier prey handling) which offsets the lower energy intake to some degree. Among the 63 otters in this study, only 6 had adopted the snail diet, and nutritional evaluation suggests that their prognosis is poor. Preliminary evidence suggests that otters feeding on snails may be more susceptible to certain infectious diseases (Tinker, pers. obs.), but further study is needed.

However, some nutritional questions remain with the other diet types as well. All diet types were deemed to be marginal or low in both thiamin and vitamin A (relative to terrestrial carnivore analogs). As indicated in Part 3, the low levels of these nutrients are troubling. On the one hand, thiamin and vitamin A are very labile compounds that are easily lost during handling, storage and analysis of seafood; for this reason aquaria and zoos almost always supplement with one or both nutrients when feeding marine mammals. Despite our careful efforts, we cannot rule out the possibility that losses occurred in this study, leading to underestimation of prey levels. However, our results are similar to prior data from renowned laboratories (Part 3), suggesting that analytic problems, if they exist, are widespread. If our results are accurate, one would expect widespread thiamin deficiencies in sea otters, which to our knowledge have not been reported (but see Part 3). Perhaps otters have evolved biochemical or physiologic mechanisms to survive on lower thiamin levels than terrestrial carnivores.

We find the ‘marginal’ to ‘low’ vitamin A levels in otter diets even more alarming, because vitamin A deficiency can be pernicious, undermining health in a wide variety of ways, and leaving animals susceptible to disease, persistent organic pollutants,

and reproductive difficulties. A 'low' diet is not necessarily deficient, but warrants closer evaluation: 1. analysis of more prey species and their seasonal variation; 2. assessment of vitamin A status of wild otters via tissue analysis; and 3. determination whether otters have the ability to utilize carotenoids as a source of vitamin A (Part 3).

In assessing the nutrient composition of sea otter prey, we noted that certain prey (particularly abalone) were very low in calcium and were unlikely to meet the calcium needs of growing otter pups or lactating otter females unless combined with high calcium prey (Part 4). In fact, otters feeding predominantly on abalone (diet type 1a) do just this, by predating on calcium-rich crabs as well, and hence this diet type is relatively high in calcium (5.4%). Some prey are also low in zinc, such that diets high in kelp crabs and sand-bottom prey (type 2a), urchins and mussels (type 2b) and turban snails (type 3a) were deemed marginal in zinc. Otter zinc status will depend to a large extent on their ability to absorb zinc from these prey species, but the bioavailability of these zinc sources are not known. Unfortunately, the high calcium loads of diet type 3a, and to a lesser extent of diet types 2a and 2b, may depress zinc absorption by analogy to digestive processes in other mammals (Part 4).

Our initial study design called for repeated sampling of prey species for determination of vitamin E levels, as we were concerned that bivalves and other low fat prey species might be low in vitamin E (Part 3). The two diet types in which bivalves predominate (type 2b, urchins and mussels, and type 2c, clams and sand-bottom prey) were significantly lower in vitamin E (78-86 mg/kg diet) than the three diets in which crabs feature heavily (types 1a, 1b, 2a; 116-140 mg/kg DM; Tukey's test, $p < 0.001$), but all of these diets were assessed as 'adequate' to 'high' in vitamin E. Although cats may need as much as 120 mg/kg DM vitamin E when fed diets rich in polyunsaturated and partly oxidized lipids (National Research Council 2006), it is unlikely that these conditions ever apply to sea otters at MBNMS. Should it prove that otters have elevated needs for vitamin E when faced with domoic acid or other toxin burdens, it may be important to investigate diet as a covariate in otter mortality events.

CONCLUSIONS AND RECOMMENDATIONS

We believe that the dataset we have generated on diet specialization by individual sea otters in MBNMS and vicinity, and its energetic and nutritional consequences, is unparalleled for a wild carnivore. Dietary specialization may allow individual sea otters to exploit food resources more successfully, while spreading the population-level top-down impacts across a broad array of prey taxa. From this perspective, diet specialization is an effective means of coping with deteriorating resource availability, if sufficient prey diversity is present to allow diverse feeding strategies to coexist.

On the other hand, diet specialization does not necessarily imply that optimal diets are always chosen. Our data suggest that one of the specializations exhibited by individual otters, a diet dominated by turban snails, may be nutritionally imbalanced, at least when compared to nutritional requirements of terrestrial analogs. Whether this diet will persist in the population, or disappear if these animals have reduced fitness, remains to be determined.

An important concept of this research is that other properties of food resources, besides abundance, patchiness and energy content, may limit populations.. An analogous situation exists in the threatened desert tortoise, in which high potassium levels in desert plants, coupled with modest water and nitrogen levels, have the consequence that most desert plants are of poor to moderate nutritional quality (Oftedal 2002). Tortoises compensate by becoming highly selective for a limited suite of species that are of high quality, but these are only found under optimal rainfall conditions (Oftedal et al. 2002). Due to lack of training and familiarity with nutritional sciences, conservation biologists and resource managers usually overlook the possibility that the quality of nutritional resources may limit animal populations. If, however, nutritional constraints limit the recovery of sea otters and other taxa, it is essential to demonstrate how this occurs so that appropriate conservation measures can be implemented.

Part 6. Comparison of diet and nutrition of otters in MBNMS to populations of otters at San Nicolas Island, California, and Glacier Bay, Alaska -- a comparative and collaborative approach

INTRODUCTION

Comparisons across sea otter populations in different locations have provided useful insights into many aspects of otter biology such as demography (Estes 1990; Estes et al. 1996) and population status (Bentall 2005; Laidre et al. 2006, Bodkin et al. 2007b). Recognizing the strength of this approach, we compared the diet, nutrition, and body condition of sea otters in MBNMS with those of otters at two locations where food resources are abundant and otter population size is increasing – San Nicolas Island, CA and Glacier Bay, AK – to gain a better understanding of the status of the MBNMS population in relation to its food resources.

The population at San Nicolas Island (SNI) is descended from sea otters translocated to the island from the MBNMS in the late 1980s. The SNI population currently numbers less than 50 individuals, is still quite small in relation to the available habitat, and is growing at approximately 9% per year (Bentall 2005). Bentall (2005) compared densities of three types of sea otter prey along the San Simeon region of the central coast with densities at SNI: urchins (red urchins, *Strongylocentrotus franciscanus,* and purple urchins*, S. purpuratus*), turban snails (red turban snails, *Lithopoma gibberosa,* and wavy turban snails, *Megastraea undosa*) and abalone (red abalone, *Haliotis rufescens*, and pink abalone, *H. corrugata*). Although prey densities at SNI may have declined somewhat since otters were introduced in the late 1980s (Bentall 2005), urchins and turban snails continue to be orders of magnitude higher than at San Simeon but there is little difference in abalone densities (Table 6.1). Abalone populations are reduced both at SNI (due to disease) and in MBNMS (due to otter predation and human harvesting) (Fanshawe et al. 2003). Bentall (2005) found that sea otters at SNI had less dietary specialization, higher mean rates of energy gain, better body condition, and shorter foraging times than those in the southern part of MBNMS.

Sea otters colonized the Glacier Bay area about 1995. The population in Glacier Bay was about about 2,400 animals, is increasing, and is thought to be below carrying

Table 6.1. Densities of some sea otter prey species at two locations in California.[1]

Species	Common name	San Simeon (2001-2004) n/m^2		San Nicolas (1998-1990) n/m^2		San Nicolas (2003-2004) n/m^2	
		Mean	Std Dev.	Mean	Std Dev.	Mean	Std Dev
Strongylocentrotus purpuratus	Purple urchin	0.0048	0.0131	28.24	25.98	11.46	13.10
Strongylocentrotus franciscanus	Red urchin	0.0004	0.0008	2.70	1.90	1.35	1.41
Lithopoma gibberosum	Red turban snail	0.0057	0.0094				
Megastraea undosa	Wavy turban snail			0.934	0.766	0.192	0.445
Haliotis rufescens	Red abalone	0.0006	0.0009	0.071	0.065	0	0
Haliotis corrugata	Pink abalone			0.048	0.075	0.0005	0.0015

1. Data from US Geological Survey semiannual subtidal surveys and PISCO subtidal monitoring program, as summarized by Bentall (2005)

capacity (Bodkin et al. 2007a). . Sea otters in and near Glacier Bay feed mainly on prey inhabiting soft-bottom substrates (e.g., clams) and are generally in good body condition (Bodkin et al. 2003, Bodkin et al. 2004) . Surveys of clam populations in Glacier Bay indicate high densities and biomass of clams (especially littleneck clams, *Protothaca staminea*, and butter clams, *Saxidomus gigantea*) in areas colonized or soon to be colonized by sea otters (Bodkin et al. 2003, 2007a), suggesting that these prey resources are still abundant within Glacier Bay.

Our study provided standardized data, including edible biomass and energy content in relation to size and nutrient content, for the principal sea otter prey species from these three locations. We were able to combine these data on sea otter prey with existing foraging data, which was collected in a similar manner at each location, to compare diets and rates of biomass and energy gain by sea otters at the three locations. We reasoned that if the sea otter population in MBNMS is impacted by reduced abundance of food resources, then we should find greater diet diversity at the population level, a higher degree of dietary specialization at the individual level, lower rates of edible biomass and energy intake, lower levels of key nutrients in their diet, and poorer body condition of individuals as compared to otters in the other two populations.

Additionally, we began collaborating with Dr. Seth Newsome, formerly at UC Santa Cruz but now at the Carnegie Institute in Washington, D. C., to determine if we can use stable isotope values in sea otter tissues (whiskers) and their prey species to study dietary specialization in sea otters. Because Dr. Newsome was able to carry out these analyses without charge, this new line of research is increasing the amount of scientific information obtained from the prey collection activities funded by MBNMS and MMC at no additional cost. This research also indicates the types of data needed for validation of fatty acids (Part 4) as an additional source of diet history information on individual otters.

METHODS

Foraging Observations

San Nicolas Island, CA - The foraging data from otters at San Nicolas Island were collected by a graduate student, Gena Bentall (assisted by field technicians from USGS, UCSC, and FWS), between 2003 and 2005, using the same methods that were

used in the MBNMS (described in methods section Part 5). Although 16 otters had been radio-tagged, we restricted our analysis to those 11 otters for which there were at least 300 known outcome foraging dives recorded: the resulting data set consisted of 170 foraging bouts comprising approximately 5,000 known-outcome foraging dives. An analysis of these data, including estimates of rates of energy gain, was provided by Bentall (2005). However, Bentall's analysis by necessity relied upon published estimates of edible biomass and energy content of the prey species consumed at San Nicolas Island, and the available information on these aspects of the prey species was incomplete and mostly from prey samples collected at locations other than San Nicolas Island. To rectify this, we collected and analyzed the prey species consumed by sea otters at San Nicolas Island in the same way that we had for prey species consumed in MBNMS -- see methods in Part 5. This resulted in a greatly improved database on the allometric relationships between size and edible biomass, as well as the energy content of the prey species consumed by sea otters at San Nicolas Island. We therefore reanalyzed the foraging data from San Nicolas Island, incorporating this new information and using the same methods as we used for otters in MBNMS (see methods in Part 5).

Glacier Bay, Alaska - The foraging data from Southeast Alaska were provided by James Bodkin of the US Geological Survey. They consisted of information on 629 feeding bouts (comprising 8,888 recorded feeding dives with known outcomes), collected in Glacier Bay from 1993 through 2005. Much of this information has been summarized in annual reports issued by James Bodkin and colleagues at the Alaska Science Center of the United States Geological Survey and the Glacier Bay National Park and Preserve of the United States National Park Service (Bodkin et al. 2001, 2002, and 2003). However, James Bodkin kindly provided the original data so that we could calculate diets in the same fashion as for MBNMS and San Nicolas. Observational data were collected in the same way as those collected for dives by otters in MBNMS and at San Nicolas Island, except that the otters were not individually identifiable. We also collected and analyzed the prey species consumed by sea otters in Glacier Bay in the same way that we did for prey species consumed in MBNMS and at San Nicolas Island (see methods section in Part 2 for development of allometric relationships between size and edible biomass and the energy content of prey species). We used these allometric and energetic data to

estimate rates of biomass and energy consumption for the Glacier Bay sea otter population by the same methods (Part 5) as we did for the MBNMS and San Nicolas Island populations. Even though otters in Glacier Bay were not individually identifiable, we were able to analyze the foraging data from Glacier Bay at the population level (treating individual feeding bouts as the statistical unit). Thus, our estimates of biomass, energy intake and required foraging time are directly comparable across all three populations.

Body size and condition

Because comparisons of body size and condition can provide a useful index of differences in ecological conditions, such as the relative availability of food resources, for sea otter populations in different locations (Laidre et al. 2006), we also compiled data on the mass and length of sea otters at various locations, including mainland California, San Nicolas Island, and near Glacier Bay, AK.

Nutrient composition of otter diets

Methods for estimating the content of various nutrients in sea otter prey species are given in earlier Parts. In order to calculate the composition of diets, it was necessary to undertake the four-step process described in Part 5:

1. We generated an average, representative value for each nutrient for each major prey type as recorded in the observational database.
2. All data were converted to a wet mass basis, to allow matching with the observational database.
3. We used the proportion of wet mass comprised by each prey type in each individual otter's diet to calculate the nutrient contribution of each diet item.
4. We summed these diet item contributions to get the nutrient composition of the individual otter diet on a wet mass basis, and then divided by dry mass to get the nutrient composition on a dry matter basis.

Step 1 entails a similar but somewhat different set of prey species at MBNMS (Part 5), San Nicolas Island, CA and Glacier Bay, Alaska. Therefore the species and data used to generate prey type averages differed somewhat as well.

San Nicolas Island: The macronutrient and macromineral data we used are primarily from San Nicolas Island (SNI) collections, but for trace mineral and vitamins much of the data were from samples from Monterey Bay National Marine Sanctuary and vicinity (MBNMS). The data used for each prey type are as follows:

- Abalone – We used average values for red abalone (*Haliotis rufescens*) and black abalone (*H. cracherodii*) from MBNMS (Part 5), as we were unable to find abalone at SNI while diving, although otters are observed to consume abalone at SNI.
- Cancer crabs – For macronutrients and macrominerals, we used average values for Pacific rock crabs (*Cancer antennarius*) and red rock (*C. productus*) crabs trapped near SNI. For trace minerals and vitamins we used values for *C. antennarius* and *C. magister* from MBNMS (see Part 5).
- Kelp crabs – We used macronutrient, macromineral and vitamin data from *Pugettia producta* from SNI but used trace mineral data for kelp crabs from MBNMS (Part 5).
- Crabs un-ID – We used average values for the two *Cancer* species and for *Pugettia,* as described above.
- Lobster – We used macronutrient and macromineral data for spiny lobster (*Panulirus interruptus*) collected at SNI. For vitamins we used values for kelp crabs from SNI. For trace minerals we used average values for Cancer crabs and kelp crabs from MBNMS (Part 5).
- Urchins – For macronutrients, macrominerals and vitamins we used data for red urchins (*Strongylocentrotus franciscanus*) from SNI; although we also had macronutrient and macromineral data for purple urchins (*S. purpuratus*) on SNI, this species is a minor part of the urchin diet on SNI (Bentall 2005). Trace mineral data were only available for purple urchins from MBNMS.
- Clams/bivalves – For macronutrients and macrominerals we used the average of rock scallops (*Crassadoma gigantea*) and California mussels (*Mytilus californianus*) from SNI. For vitamins and trace minerals we used average values

for littleneck clams (*Protothaca staminea*), California mussels and gaper clams (*Tresus nuttallii*) from MBNMS.

- Stars – For macronutrients and macrominerals we used the average of giant spined (*Pisaster giganteus*) and ochre stars (*P. ochraceus*) from SNI. For trace minerals and vitamins we used data for ochre stars from MBNMS.
- Snails – For macronutrients and macrominerals we used average values for three species from SNI: black turban snails (*Tegula funebralis*), Norris' top snails (*Norrisia norrisi*) and wavy turban snails (*Megastraea undosa*). For vitamins we used data for wavy turban snails from SNI. For trace minerals we used data for black turban snails from MBNMS.
- Cephalopods – For macronutrients and macrominerals we used average values for red octopus (*Octopus rubescens*) and market squid (*Loligo opalescens*) from MBNMS. For trace minerals and vitamins, we used average values for all other MBNMS taxa (Part 5).
- Small rocky – For macronutrients and macrominerals we used average values from a snail species (Kellet's whelk, *Kelletia kelletii*) and the owl limpet (*Lottia gigantea*) from SNI, taxa that were believed to represent some proportion of the rocky-bottom prey category. For trace minerals and vitamins we used average values for all other MBNMS taxa (Part 5).

Glacier Bay, Alaska: The macronutrient and macromineral data we used were primarily from collections made at Glacier Bay National Park, Alaska (GBNP), but for trace mineral and vitamins most of the data were from samples from MBNMS, with a few samples from San Nicolas Island (SNI). The data used for each prey type are as follows:

- Crabs – For macronutrients and macrominerals we used average values for Dungeness crabs (*Cancer magister*), tanner crabs (*Chionoecetes bairdi*) and red king crabs (*Paralithodes camtschaticus*) from GBNP. For trace minerals and vitamins we used values for *C. antennarius* and *C. magister* from MBNMS (see Part 5).

- Urchins – For macronutrients and macrominerals we used data for green urchins (*Strongylocentrotus droebachiensis*) from GBNP and red urchins (*Strongylocentrotus franciscanus*) from SNI; vitamin data were also from *S. franciscanus* from SNI. Trace mineral data were only available for purple urchins (*S. purpuratus*) from MBNMS.
- Clams – For macronutrients and macrominerals we used the average of Pacific littleneck clams (*Protothaca staminea*), Nuttall's cockle (*Clinocardium nuttallii*), bent-nosed macoma (*Macoma nasuta*) and butter clams (*Saxidomus gigantea*) from GBNP. For vitamins and trace minerals we used average values for littleneck clams (*Protothaca staminea*) and gaper clams (*Tresus nuttallii*) from MBNMS.
- Mussels – For macronutrients and macrominerals we used the average of horse mussels (*Modiolus modiolus*) and foolish mussels (*Mytilus trossulus*) from GBNP. For vitamins and trace minerals we used data from California mussels (*Mytilus californianus*) from MBNMS.
- Stars – For macronutrients and macrominerals we used the average of mottled sea stars (*Evasterias troschelii*) from GBNP and ochre stars (*Pisaster ochraceus*) from MBNMS. For trace minerals and vitamins, we used data for ochre stars from MBNMS.
- Snails – For macronutrients and macrominerals we used average values for hairy tritons (*Fusitriton oregonensis*) and ridged whelks (*Neptunea lyrata*). For vitamins we used data for black turban snails (*Tegula funebralis*) from MBNMS and wavy turban snails (*Megastraea undosa*) from SNI. For trace minerals we used data for black turban snails from MBNMS.
- Octopus – For macronutrients and macrominerals we used values for red octopus (*Octopus rubescens*) from MBNMS. For trace minerals and vitamins, we used data reported by USDA (2003) for *Octopus vulgaris*.
- Chiton – For macronutrients and macrominerals, we used values for gumboot chitons (*Cryptochiton stelleri*) from MBNMS. For trace minerals and vitamins we used average values for all other taxa, including mollusks.

- Worms – For macronutrients and macrominerals we used average values for Alaskan spoon worms (*Echiurus echiurus alaskanus*) and pile worms (*Nereis vexillosa*). For trace minerals and vitamins we used values for fat innkeeper worms (*Urechis caupo*) from MBNMS.
- Other (including barnacles) – For macronutrients and macrominerals we used average values for scallops (*Chlamys rubida*) and plate limpets (*Tectura scutum*) from GBNP as well as California sea cucumbers (*Parastichopus californicus*) from MBNMS. Although California sea cucumbers do not occur in Glacier Bay, we use this taxon as a proxy for *Cucumaria fallax*, a holothurian that occurs in Glacier Bay and is eaten by sea otters (Bodkin et al. 2003). For trace minerals and vitamins we used average values for all other taxa.

Evaluation of Nutrient Levels in Diets

Nutrient levels in San Nicolas Island and Glacier Bay were compared to levels observed at a population level in central California (MBNMS and vicinity). As both sex and individual identity were known for each of the 63 otters observed in central California and the 11 otters observed at San Nicolas Island, we opted to compare nutrient levels in SNI and MBNMS diets by two-way ANOVA, with location (MBNMS or SNI) and sex (male or female) as independent variables. For Glacier Bay statistical comparison was not possible as we did not know identities of individual otters and thus only had data at a population level. In both cases, the nutritional significance of observed differences from MBNMS were interpreted in relation to recommended nutrient levels discussed in Parts 2-5.

Stable isotope analyses of sea otters and their prey

We collected whiskers for stable isotope analyses from sea otters captured along the mainland coast of California and at San Nicolas Island, California, during other research projects. We were not able to obtain whiskers from Glacier Bay due to lack of appropriate permits. We subsampled prey collected for nutritional analyses for isotope analyses. Approximately 0.5 mg of powdered tissue samples was sealed into tin boats for isotopic analysis. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values were determined

using a Carlo-Erba elemental analyzer (NC 2500) interfaced with a Finnegan Delta Plus XL mass spectrometer (Carnegie Institute of Washington, Washington, DC).

Sea otter vibrissae were sampled from both wild caught and beach-cast animals collected within Monterey Bay, from Point Lobos (south) to the Santa Cruz Harbor (north). Vibrissae of wild captured animals were collected during periodic population assessments by the USGS and California Department of Fish and Game from 2000 to 2004 (n=9): details of the capture and handling of study animals are provided elsewhere (Tinker et al. 2006), and all activities were covered by federal, state and institutional permits issued to James Estes. Beach-cast animals were collected between 1998 and 2006 (n=22).

For $\delta^{13}C$ and $\delta^{15}N$ analysis, vibrissae were rinsed with a 2:1 chloroform/methanol mixture to remove surface contaminants. Cleaned vibrissae were sub-sampled into ~0.5 mg segments using nail clippers and sealed into tin boats for isotopic analysis. Carbon and nitrogen isotope values were determined using the mass spectrometer system described above. Depending on the length of each vibrissa the number of samples analyzed from each individual varied from 8 to 21 (mean = 16). As a control for the quality of keratin, we measured the carbon-to-nitrogen ([C]/[N]) ratios of each sample; atomic [C]/[N] ratios of all samples were 3.3-3.5, well within the range that characterizes unaltered keratin. Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}N = 1000*[(R_{sample}/R_{standard})-1]$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand or per mil (‰). Repeated measurements of a gelatin standard (n=100) yielded a standard deviation of ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

Figure 6.1 presents a schematic of our approach to studying dietary specialization in sea otters using isotopes: Figure 6.1A depicts predictions for a population of specialists in which the within individual component (WIC) of niche space, as measured in two-dimensional isotope space, is relatively small in comparison to the variance in isotope values across prey types (i.e., total niche width, TNW). Conversely, Figure 6.1B shows predictions for a population of generalists, in which the variability in individual consumer isotope values (WIC) is a larger proportion of the variance of prey isotope values (TNW).

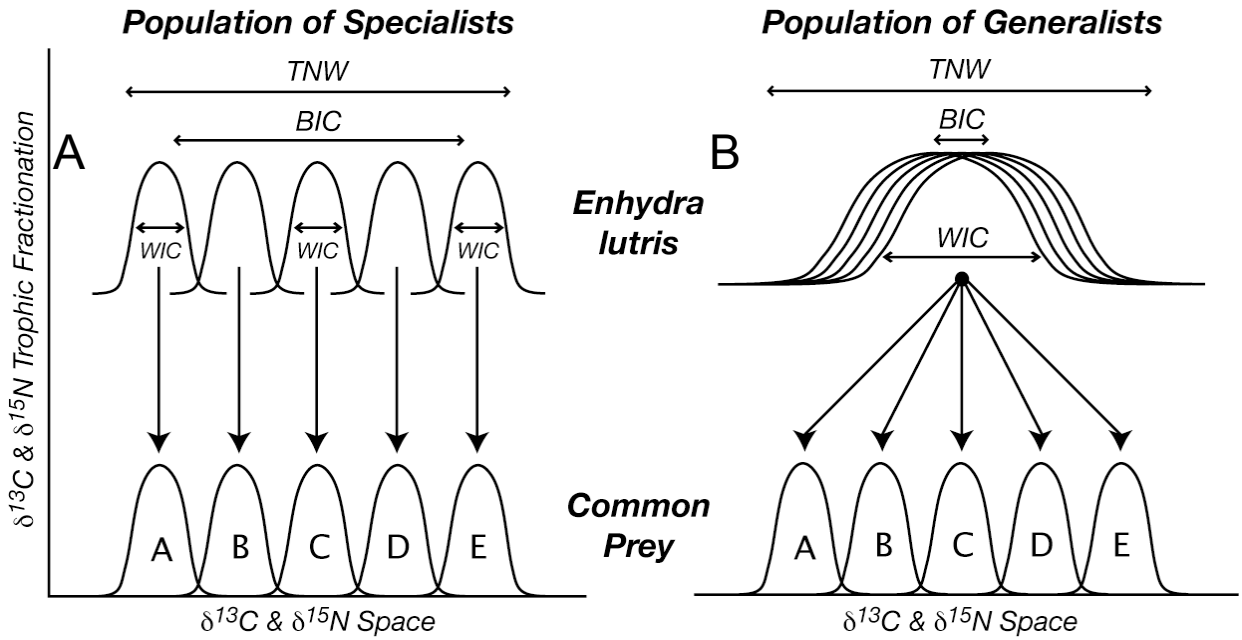

Figure 6.1. A schematic of our approach to studying dietary specialization in sea otters using isotopes, in which the x-axis represents the stable isotope "space" of prey and consumers in a particular population. Isotope "space" refers to the two-dimensional area in a δ13C or δ15N plot commonly used in isotope ecology. The y-axis represents specific trophic fractionations in isotope values, dependent on the isotope system utilized, between prey and consumer. Figure 4.1 A) depicts predictions for a population of specialists in which the within individual component (WIC) of niche space, as measured in two-dimensional isotope space, is relatively small in comparison to the variance in isotope values across prey types (i.e., TNW). Conversely, Figure 4.1 B) shows predictions for a population of generalists, in which the variability in individual consumer isotope values (WIC) is a larger proportion of the variance of prey isotope values (TNW).

Our approach builds on a recent theoretical study by Bearhop et al. (2004) outlining the potential use of isotope biochemistry to study individual specialization. Important to our study is a list of theoretical assumptions and factors that influence the isotope values of individuals and the prey they consume. First, individual prey types or groups of prey have distinct isotopic values (Figure 6.1). Second, isotope signatures at the base of the food web remain relatively constant over time. Seasonal variations in physical parameters such as temperature and nutrient availability can cause baseline changes in isotope values of primary producers, which cascade up food chains to primary and secondary consumers. Lastly, the analysis of tissues with relatively rapid turnover rates will likely provide the most accurate estimates of individual niche width. Tissues that grow continuously (i.e., feathers, whiskers, or hair) and can be serially sampled to produce a temporal record of dietary information are best suited for characterizing the within individual component WIC.

RESULTS

Otter diets at San Nicolas Island

Sea otter diets at San Nicolas Island differed from those in MBNMS in a number of important respects. First, in contrast to the varied prey base consumed in central California, the population-level diet at San Nicolas was relatively specialized and dominated by just a few prey types, the most significant being red urchins (Figure 6.2). Red urchins and purple urchins together represented approximately 75% of the diet on an edible wet mass basis, or 53% based on frequency of occurrence (Table 6.2). Aside from urchins, the prey types that comprised a significant part of the diet (>1% by mass) were kelp crabs, cancer crabs, gastropods (primarily wavy turban snails), spiny lobster and abalone. Note that bivalves were an insignificant part of the diet, despite being very important in other locations. The second obvious difference between San Nicolas and central California was the rate of biomass and energy intake while foraging. The mean rate of biomass consumption at San Nicolas (25 g/minute) was more than double that in MBNMS, a difference that was highly significant for both females and males ($P<0.001$, Figure 6.3), as was the difference in mean rate of energy gain ($P<0.001$, Figure 6.4). Accounting for the high rate of energy gain (18.6 kcal/minute), and using methods

Table 6.2. Population-level diet composition of sea otters at San Nicolas Island, California, categorized in terms of major ecological functional groups of prey species. [1]

Prey Type (functional group)	Number Recorded Occurences	Proportion of all occurences	Mean Intake (g/min)	Standard deviation	Proportion of total biomass
Urchin	1213	0.534	18.687	1.209	0.745
Kelp Crab	492	0.217	2.494	0.221	0.100
Cancer Crab	165	0.073	1.789	0.217	0.071
Snail	265	0.117	0.671	0.086	0.027
Lobster	29	0.013	0.637	0.145	0.025
Abalone	19	0.008	0.395	0.112	0.016
Clam	13	0.006	0.174	0.066	0.007
Crab, Un-ID	46	0.020	0.143	0.034	0.006
Cephalopod	16	0.007	0.067	0.015	0.003
All Other (rocky habitat)	7	0.003	0.010	0.003	0.000
Sea Star	2	0.001	0.001	0.000	0.000
Mussel	2	0.001	0.000	0.000	0.000
All Other (sandy habitat)	2	0.001	0.000	0.000	0.000
All Prey Types	**2271**		**25.07**	(± 2.109)	
Energy Intake (kcal/min)			**18.60**	(± 6.347)	

1. Based on foraging observations by Bentall (2005) and our biomass and energy data (Part 2).

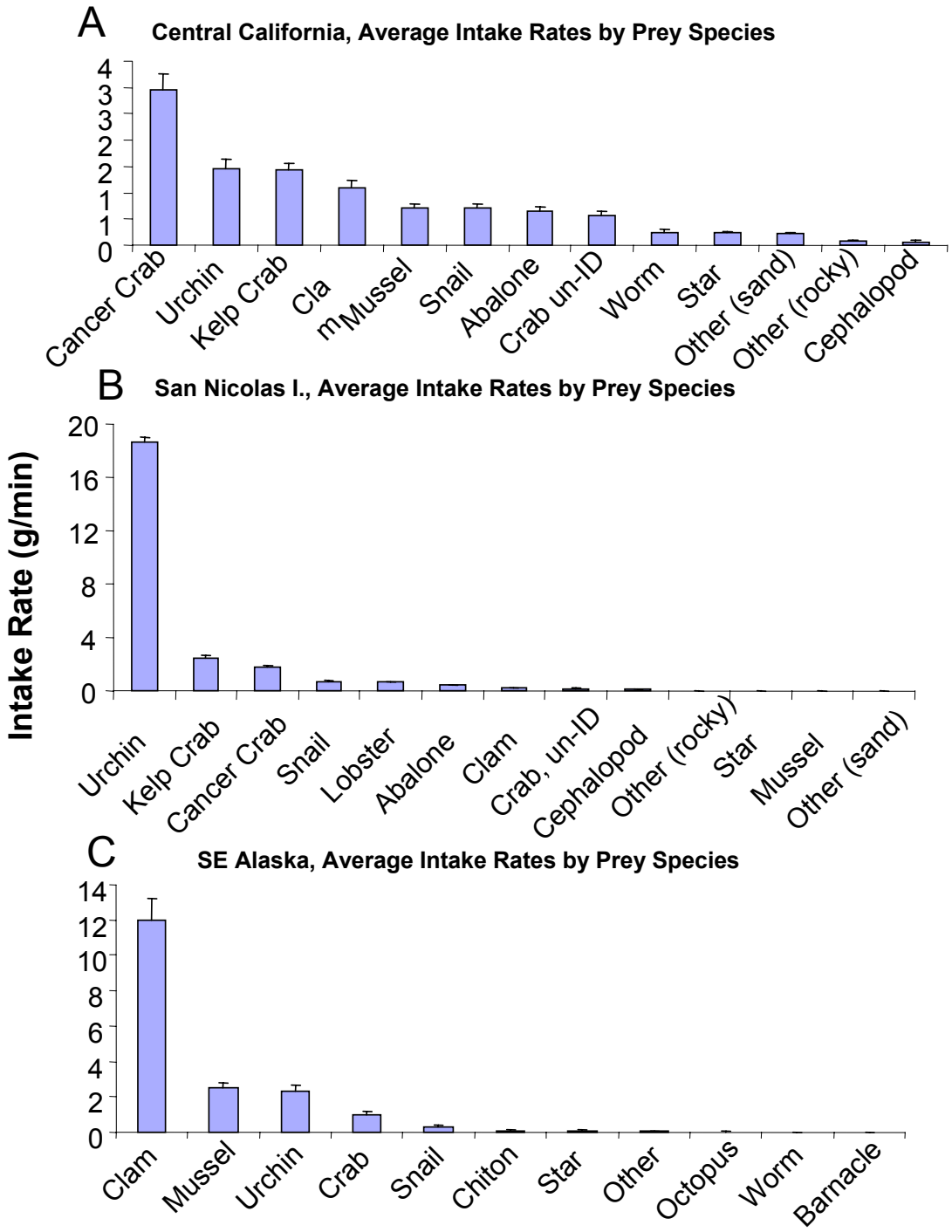

Figure 6.2. Frequency distributions of average prey consumption rates (corresponding to population-level diet composition on the basis of wet edible biomass) for sea otters in three different populations: A) central California, within the MBNMS, B) San Nicolas Island, in southern California, and C) Glacier Bay, in SE Alaska. Prey functional groups are sorted (from left to right) in terms of decreasing importance in the diet. The central CA population shows a relatively diverse diet (in terms of both richness and evenness of species consumed), the San Nicolas Island and Glacier Bay populations show less diverse diets, with just a few prey types dominating.

described in Part 5, we estimated that otters at San Nicolas would only need to feed for approximately 27% of their time in order to meet their expected metabolic costs, a value fairly similar to the 25% time feeding that was actually recorded using telemetric techniques and analysis of TDR archival data (Bentall 2005). The mean daily food intake, given this activity budget, would represent approximately 33% of body mass, at the high end reported for sea otters.

Otter diets in Glacier Bay Alaska

In contrast to otters in MBNMS or San Nicolas Island, the composition of the diet of the sea otter population in Glacier Bay, Alaska, based on edible biomass was nearly identical to that based on frequency of occurrence (Table 6.3). The Glacier Bay otters had a diet that was dominated by clams (65% wet mass) and also consumed large amounts of mussels (14%) and urchins (13%) (Figure 6.2). Only crab (5%) and snails (2%) comprised more than 1% of the remaining diet of Glacier Bay otters. Overall, the population level diet was less varied than the population-level diet in central California, while otters exhibited a higher mean rate of edible biomass intake (~18 g/min) (Figure 6.3) and energy intake (~12 kcal/min) (Figure 6.4). Using the energetic requirement algorithm described in Part 5, we predict that sea otters in Glacier Bay would need to spend an estimated 34% of their time feeding. Although data for individual otters are not available, it is thought that there is relatively little foraging/dietary specialization in Glacier Bay. Rather, dietary variation appears to reflect differences in the distribution of prey populations, with most individuals consuming a diet similar to that of other individuals foraging in the same area (Bodkin et al. 2003). This pattern again contrasts with the individual specialization seen in central California: it may result from some intrinsic property of the prey communities or feeding habitats (i.e. much available habitat in Glacier Bay is soft-sediment, in contrast to California), or it may be a reflection of the difference in population status (food and space are not thought to be limiting resources for otters in Glacier Bay, whereas they may be limiting in central California). Both population-level diet diversity and rates of energy intake at Glacier Bay are more similar to San Nicolas Island than to central California.

Table 6.3. Population-level diet composition of sea otters in Glacier Bay, SE Alaska, categorized in terms of major ecological functional groups of prey species. [1]

Prey Type (functional group)	Number Recorded Occurences	Proportion of all occurences	Mean Intake (g/min)	Standard deviation	Proportion of total biomass
Clam	4965	0.655	11.981	1.2809	0.653
Mussel	1032	0.136	2.542	0.2725	0.139
Urchin	1061	0.140	2.302	0.3450	0.126
Crab	241	0.032	0.941	0.2303	0.051
Snail	121	0.016	0.303	0.1159	0.017
Chiton	42	0.006	0.098	0.0391	0.005
Star	42	0.006	0.098	0.0421	0.005
Other	43	0.006	0.042	0.0173	0.002
Octopus	2	0.000	0.021	0.0190	0.001
Worm	21	0.003	0.009	0.0037	0.000
Barnacle	7	0.001	0.002	0.0014	0.000
All Prey Types	**7577**		**18.34**	(± 2.367)	
Energy Intake (kcal/min)			**11.75**		

1. Based on foraging observations by J Bodkin and colleagues at Glacier Bay National Park and our biomass and energy data (Part 2).

Table 6.4. Comparison of mean body mass and length for otters captured in one of 4 distinct populations: central California (at or near San Simeon), San Nicolas Island (southern California), SE Alaska (near Glacier Bay) and the Aleutian archipelago, Alaska.

	Sample size		Body mass (kg)		Body length (cm)		Mass: Length		Source
	Females	Males	Females	Males	Females	Males	Females	Males	
MBNMS, CA (PBLA)	27	8	19	26	116	125	0.161	0.208	Bentall 2005
Aleutians, AK 1960s-70s	1199	444	21	28	110	119	0.195	0.237	Laidre et al 2006
Aleutians, AK 1990s	142	56	24	32	123	132	0.196	0.245	Laidre et al 2006
near Glacier Bay, AK	28	12	24	37	123	137	0.197	0.272	J. Bodkin, unpublished data
San Nicolas Island, CA	9	10	24	35	123	134	0.199	0.264	Bentall 2005

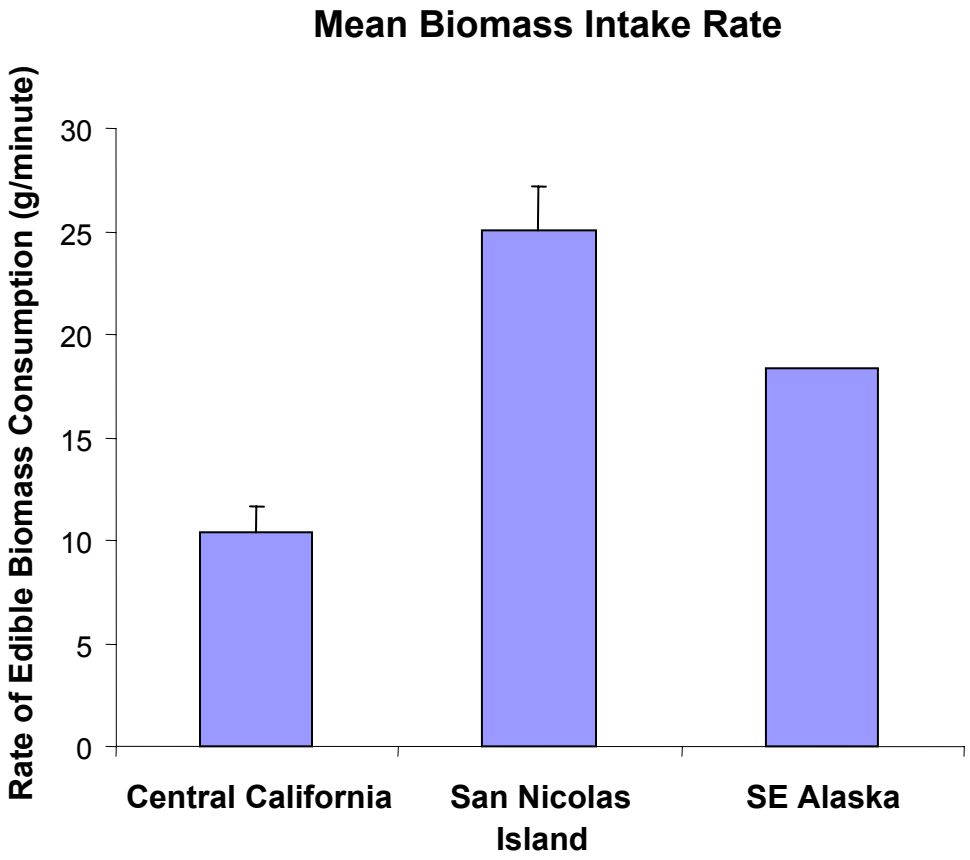

Figure 6.3. Comparison of mean prey intake rates while foraging (estimated grams of wet edible biomass consumed per minute) for sea otters in three different populations, central California, within the MBNMS, San Nicolas Island, in southern California, and Glacier Bay, in SE Alaska. Error bars indicate the variance between animals, measured as a single standard deviation; individual data were not available for SE Alaska, so no error bar is shown.

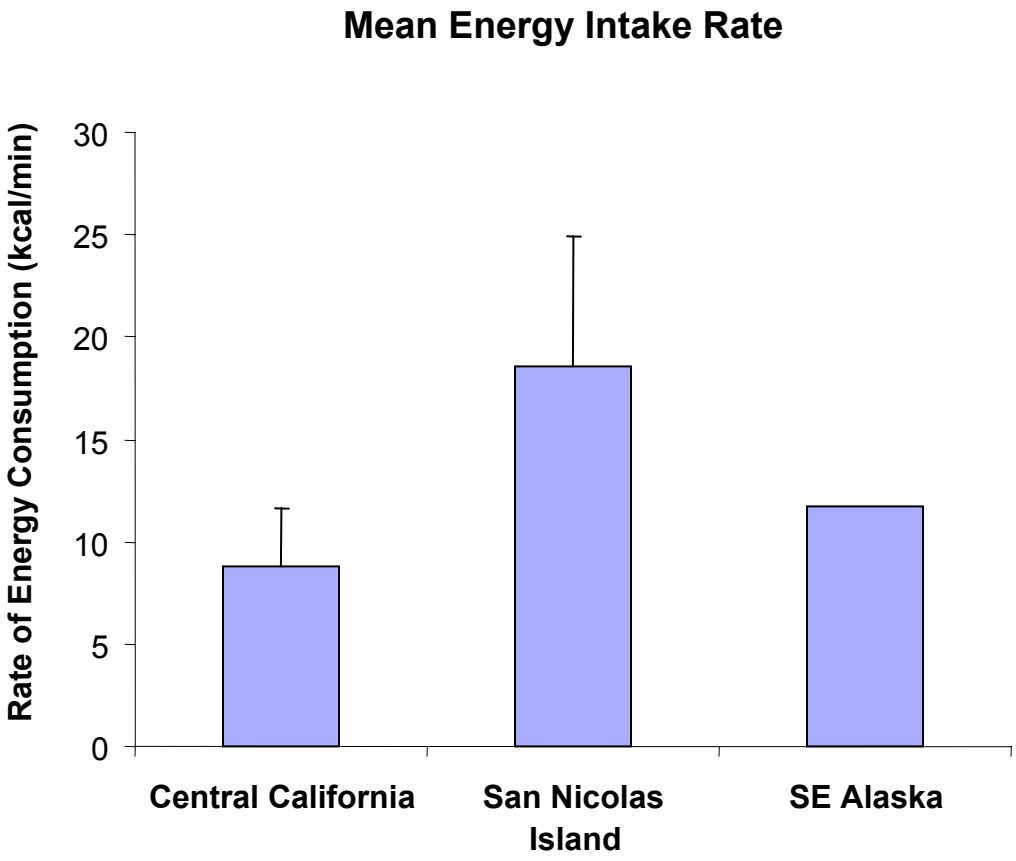

Figure 6.4. Comparison of mean energy intake rates while foraging (estimated calories consumed per minute) for sea otters in three different populations, central California, within the MBNMS, San Nicolas Island, in southern California, and Glacier Bay, in SE Alaska. Error bars indicate the variance between animals, measured as a single standard deviation: individual data were not available for Glacier Bay, so no error bar is shown.

Size and body condition

Table 6.4 compares mean body mass and length of otters captured in mainland California near the Piedras Blancas lighthouse with that of sea otters at San Nicolas, Glacier Bay, and the Aleutian archipelago. Because no data on the size of otters in Glacier Bay itself are available, Table 6.4 presents data from two recently colonized areas nearby: Port Althorp, about 40 km from Glacier Bay (colonized about 20 years ago) and Idaho Inlet about 25 km from Glacier Bay (colonized about 1995).

Body mass within sexes was remarkably similar in three areas with abundant food resources (Table 6.4). Females in all three locations weighed an average of about 24 kg, while males ranged from 32 to 35 kg. The similarity in size of animals from SNI and Alaska was surprising because *E. l. nerieis* was believed to be inherently smaller than the northern sub-species (Davis and Lidicker 1975; Reidman and Estes 1990; Wilson et al. 1991). However, sea otters of both sexes were actually lighter (21 and 28 kg respectively) in an Alaskan population near carrying capacity (the Aleutian Islands in the 1960's-70's) than they were at SNI. The otters from MBNMS were smaller than those in any of the other populations, with females weighing only 19 and males only 26 kg, respectively. The length data show a similar pattern, except that the mainland California animals were slightly longer than the Aleutian animals when the population was near carrying capacity. The mass:length ratio of otters in MBNMS was the lowest of any population yet measured.

Nutrient composition of diets

The average nutrient composition of the diets of sea otters at MBNMS, San Nicolas and Glacier Bay are presented in Table 6.5; the standard errors for the first two represent individual variation; individual identity was not known for Glacier Bay otters. Otter diets at San Nicolas Island were significantly higher in fat, potassium, magnesium, thiamin, vitamin B6 and vitamin E than otter diets at MBNMS (Table 6.5). Of these fat and thiamin may be of particular nutritional importance (Figure 6.5), given that the low levels in MBNMS diets, relative to recommended levels, were considered to be of concern (Parts 2-4). The diet consumed by otters in Glacier Bay had higher mean energy content (on a dry basis) than at MBNMS (Figure 6.5), associated with lower ash content

Table 6.5. Nutrient Composition of Sea Otter diets at MBNMS, San Nicolas Island and Glacier Bay, AK.[1]

		Central California			San Nicolas Island			Glacier Bay, AK	Central Cal. vs. San Nicolas MW-Rank Sum[2]
Constituent	**units**	**mean**	**sem**	**n**	**mean**	**sem**	**n**	**Pop. Value**	**p**
Dry Matter	% WM	23.54	0.58	63	14.67	0.60	11	11.97	0.001
Fat	% DM	5.29	0.35	63	10.10	0.70	11	3.41	0.001
Carbon	% DM	35.80	0.17	63	36.09	0.20	11	35.30	0.020
Nitrogen	% DM	8.13	0.09	63	6.50	0.31	11	8.52	0.001
Protein	% DM	50.63	0.58	63	40.61	1.95	11	47.62	0.001
Ash	% DM	24.92	0.77	63	25.13	0.33	11	20.81	0.001
Energy	cal/g DM	3584	67	63	3696	33	11	3753	ns (0.514)
Energy	kcal/g WM	0.825	0.012	63	0.541	0.020	11	0.449	0.001
Calcium	% DM	8.620	0.547	63	5.486	0.220	11	1.871	0.001
Phosphorus	% DM	0.802	0.018	63	0.700	0.023	11	0.909	0.001
Potassium	% DM	0.839	0.014	63	1.110	0.016	11	1.325	0.014
Magnesium	% DM	0.647	0.016	63	0.798	0.015	11	0.771	0.001
Iron	mg/kg DM	472.6	52.8	63	338.0	19.6	11	844.1	0.025
Copper	mg/kg DM	31.80	1.52	63	17.16	3.22	11	13.67	0.001
Zinc	mg/kg DM	97.22	2.17	63	67.75	5.02	11	96.52	0.001
Manganese	mg/kg DM	12.17	1.09	63	8.88	0.23	11	38.14	0.001
Selenium	mg/kg DM	1.989	0.066	63	1.045	0.172	11	1.761	0.001
Thiamin	mg/kg DM	1.53	0.06	63	7.50	0.56	11	2.89	0.001
Riboflavin	mg/kg DM	79.15	3.69	63	36.01	4.32	11	31.43	0.001
Niacin	mg/kg DM	107.16	2.21	63	106.09	2.11	11	145.64	0.002
Vit. B6	mg/kg DM	2.54	0.06	63	3.72	0.12	11	2.46	0.001
Vit. A	mg/kg DM	1273	39.6	63	1578	30.5	11	2591	ns (0.107)
Vit. D3	ug/kg DM	21.26	0.82	63	12.71	1.37	11	19.24	0.001
Vit. E	mg/kg DM	110.0	3.9	63	172.0	5.2	11	88.3	0.001

1. For Central CA (MBNMS) and San Nicolas, means and sem of individual diets; for Glacier Bay, only a population value is given.

2. Mann-Whitney Rank Sum test of difference between median values.

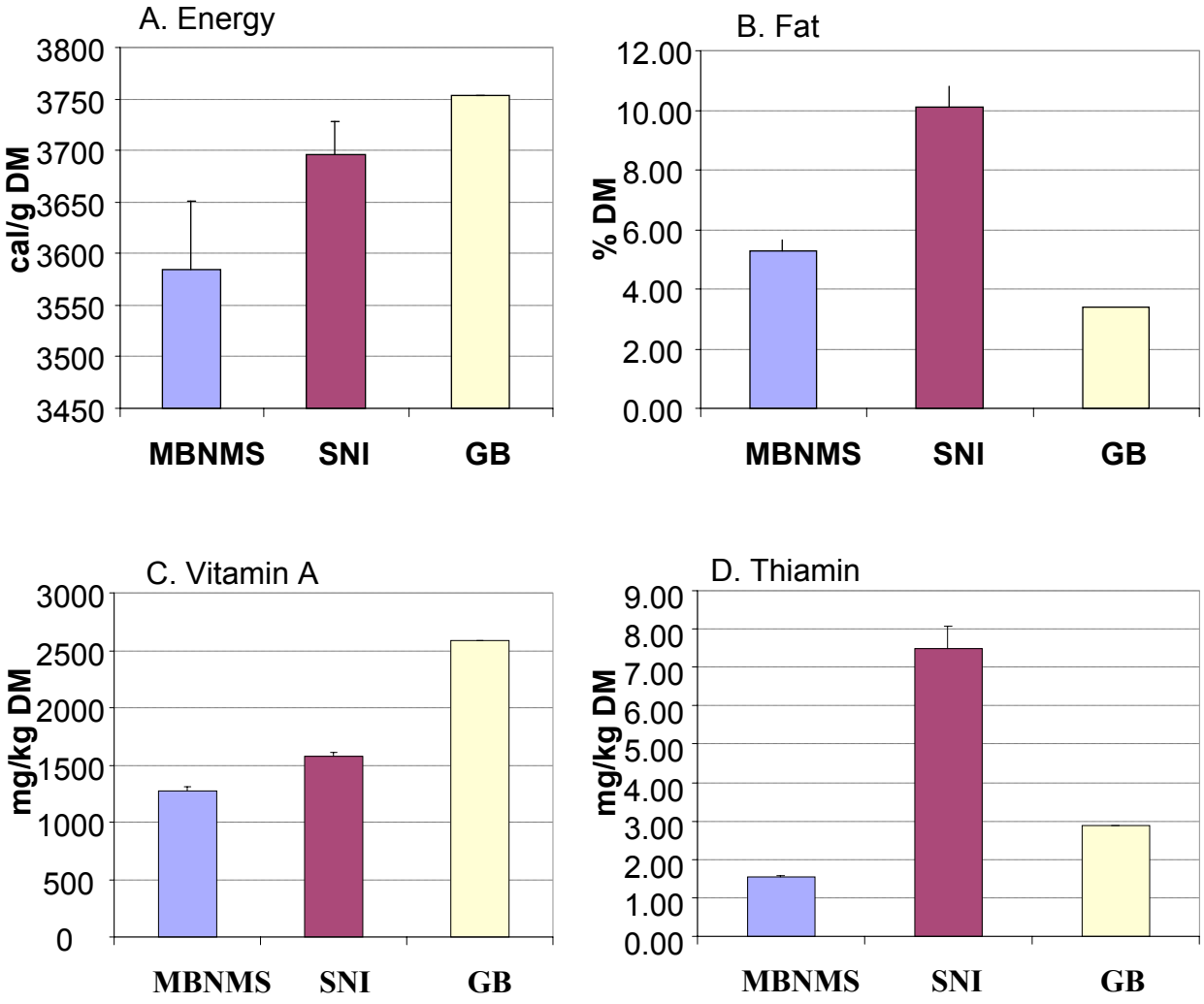

Figure 6.5. Comparison of the energy, fat, vitamin A and thiamin concentrations in population-level otter diets at MBNMS (blue), San Nicolas Island (red) and Glacier Bay, AK (yellow).

rather than higher fat content. Interestingly, this pattern was reversed when energy content was considered on the basis of wet edible biomass (Table 6.5), indicating that the low energy intake rate of sea otters at MBNMS relative to San Nicolas and Glacier Bay (Figure 6.4) is a function of reduced foraging success rather than low prey energy content. The Glacier Bay diet also appeared to be higher in thiamin and vitamin A than MBNMS (Figure 6.5), two nutrients that were considered marginal to low in MBNMS diets. Thus there is evidence that San Nicolas and Glacier Bay diets may be of higher nutritional quality than MBNMS diets.

The high calcium concentration in MBNMS diets was noted in Part 4 and 5 as a matter of concern, as high calcium (and high calcium:phosphorus ratios) can impair absorption of such minerals as phosphorus and zinc. The San Nicolas diet was significantly lower in calcium (5.5%) than the average for MBNMS (8.6%), and Glacier Bay diets were even lower still (1.9%). The high average calcium in MBNMS diets reflects, in part, the high calcium in diet types dominated by snails (diet type 3) and crabs (types 1b, 2a); the mineral levels of the San Nicolas diets resemble those of the urchin and mussel diet at MBNMS (Table 5.6). By contrast, the modest calcium level in the Glacier Bay diet, which is dominated by clams, has no parallel in otter diets in California, for even the MBNMS clam and sandy bottom diet (diet 2c) contains substantial amounts of high calcium prey such as sand crabs. Since a wide range of prey species from all three locations were analyzed for macrominerals (Part 4), this difference in dietary calcium levels among the three populations is robust.

The San Nicolas diet was significantly lower in dry matter, nitrogen, crude protein, phosphorus, iron, copper, zinc, manganese, selenium, riboflavin and vitamin D_3 than MBNMS diets, but the nutritional consequences of such differences are uncertain. On the one hand, protein, copper, selenium and riboflavin are high in MBNMS diets (Part 5), so that reductions are more likely to be beneficial than detrimental. The lower phosphorus and zinc in the San Nicolas diet might be of concern except that the lower calcium may compensate for this difference by allowing an increase in bioavailability. However, these vitamin and trace mineral comparisons are weakened by the fact that most of the analytic data were obtained from prey collected at MBNMS, and we cannot be certain that concentrations are the same at MBNMS, San Nicolas, and Glacier Bay

even for the same prey species, let alone related taxa. For nutrients of particular concern for sea otters, such as thiamin, vitamin A, zinc and w6 fatty acids (Part 4), more analytic data are needed for San Nicolas and Glacier Bay prey.

Stable isotopes

Our preliminary work on using isotopes to assess dietary specialization of sea otters is focusing on two main questions: 1) Is the large variation in sea otter carbon and nitrogen isotope values a function of dietary preferences?; and 2) If isotope variability is driven by diet, can we use carbon and nitrogen isotope values in sea otter whiskers as a proxy for individual dietary specialization? For example, could we assign individual sea otters to foraging specialist/diet types (as determined by visual observations of sea otter foraging behavior) by isotope analysis of whiskers collected from living individuals?

Figure 6.6 compares $\delta^{13}C$ or $\delta^{15}N$ values of sea otter whiskers and common sea otter prey species collected from multiple seasons and years at Monterey Bay/San Simeon (Figure 6.6A) and San Nicolas Island, California (Figure 6.6B). Colored ovals represent data from individual sea otters while points with error bars represent values for prey species or types. Because the degree of variation in carbon and nitrogen isotope values for a given prey species from different localities, seasons, and years within these areas was relatively small in comparison to differences among species, data were pooled to calculate the mean and standard deviations for individual prey species. Sea otter isotope values were plotted in "dietary space" by subtracting 2‰ from carbon and 3‰ from nitrogen isotope values to account for isotopic fractionation between prey and whisker keratin.

Isotope analysis of common prey items showed three patterns important to our approach in assessing dietary specialization in sea otters. First, there is a relatively broad range in isotope values of common sea otter prey items in both MBNMS and SNI, with $\delta^{13}C$ and $\delta^{15}N$ values varying by ~5-6‰. These results confirm that sea otters in these areas have a potentially large total niche-width, spanning several trophic levels and functional groups (filter feeders, grazers, benthic predators). Second, variation in isotope values of individual prey is relatively small in comparison to observed variation between prey species. The mean values for prey items presented in Figure 6.6 are pooled averages

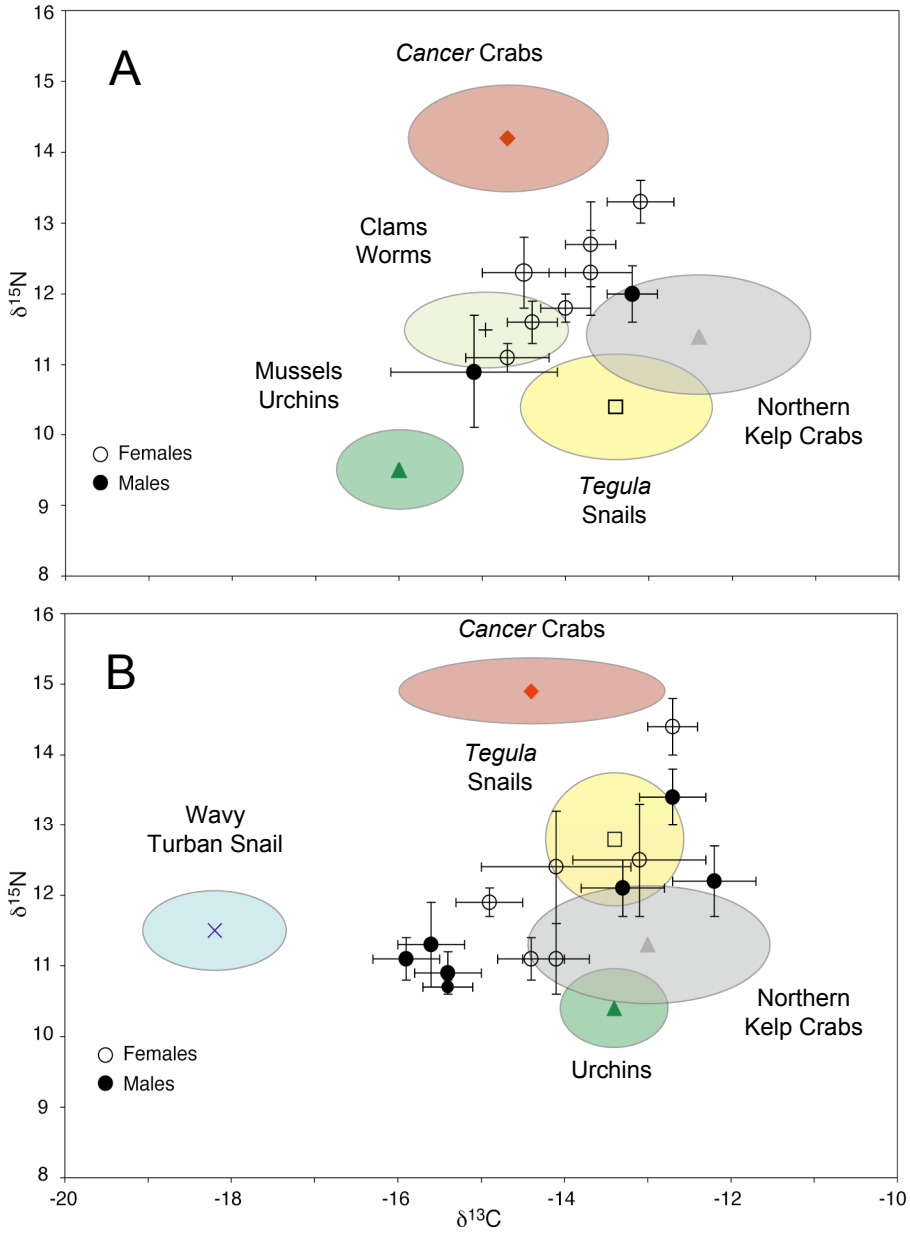

Figure 6.6. Mean $\delta^{13}C$ or $\delta^{15}N$ values of common *Enhydra* prey items and mean keratin $\delta^{13}C$ or $\delta^{15}N$ values of individual sea otters along the central and southern California coast (A) and San Nicolas Island (B). Colored ovals (prey items) or error bars (individual otters) represent one standard deviation.

of individuals (n=8-12) collected over all four seasons in multiple years. This is especially important since isotope values of primary producers in many marine ecosystems are known to vary on seasonal timescales, which would add an additional layer of complexity in the interpretation of isotope values of primary and secondary consumers. Thus, the segregation in carbon and nitrogen isotope space of common sea otter prey collected over multiple seasons and years strengthens our ability to characterize long-term (multi-seasonal to decadal) dietary patterns. Lastly, our pilot data show that it is important to analyze (when possible) common prey available to populations in different regions because isotope values of similar prey types can significantly change due to local oceanographic or ecological conditions. For example, sea urchins and *Tegula* snails in Monterey Bay (Figure 6.6A) have lower carbon and nitrogen isotope values than the same prey types at San Nicolas Island in the Channel Islands (Figure 6.6B).

In respect to sea otter isotope values (closed and open circles, Figure 6.6), a comparison of inter- versus intra-individual isotope values present a few intriguing patterns. First, similar to isotope results for common prey, variability in $\delta^{13}C$ values is slightly larger than variation in $\delta^{15}N$ at the individual and population level. Second, intra-individual variation in both $\delta^{13}C$ and $\delta^{15}N$ values is relatively small in comparison to variation among individuals. Statistical tests confirm that individual isotope values are significantly different from one another with only a few exceptions (MANOVA, F-Test, $P<0.05$).

DISCUSSION

The southern sea otter (*Enhydra lutris nereis*) population at MBNMS is considered threatened under the Endangered Species Act because of the modest numbers of animals occupying a limited range where they are deemed vulnerable to oil spills (U.S. Fish and Wildlife Service 2003). The slow recovery of this population -- the growth rate has never exceeded 5% -- is of concern, especially in view of the fact that all other remnant sea otter populations in Washington and Alaska have increased at much higher rates (Estes 1990, Bodkin et al. 1999). One possible explanation of the slow recovery, and the relative high rates of mortality, is that the MBNMS population is food limited.

Survey data suggest that at least several preferred prey types are occur in lower densitites in MBNMS compared to San Nicolas Island (Table 6.1). Two species of sea urchins and turban snails are at reduced densities compared to San Nicolas Island (Bentall 2005) and abalones are also greatly reduced compared to historical levels (Fanshawe et al. 2003). We were unable to compare prey densities in MBNMS with those in Glacier Bay due to lack of comparable data on prey occurring in soft-bottom substrates. However, clams are abundant in much of Glacier Bay, although areas that have been occupied by otters show reduced densities and biomass of clams (Bodkin et al. 2003, 2007). Reduction of the size and density of such prey species as red urchins, abalone and clams has been correlated to sea otter occupation of a given area (Kvitek et al. 1988; Riedman and Estes 1990; Estes and Duggins 1995; Jolly 1997; Fanshawe et al. 2003; Lairdre and Jameson 2006), and sea otters occupied the southern MBNMS prior to declines of these prey species (Ebert 1967; Ebert 1968; Wild and Ames 1974). The remoteness of San Nicolas Island, being the outermost of the Channel Islands and one with access restricted by the U.S. Navy, also limits the impact of human activities on invertebrate fauna.

Comparisons of multiple dietary measures across sea otter populations were consistent with the hypothesis that sea otter diets in MBNMS are impacted by reduced per-capita abundance of prey resources:

First, the otter population in MBNMS exhibits a very diverse diet relative to other populations at San Nicolas and Glacier Bay (e.g. Table 2.1). This is similar to the situation in coastal Washington where an otter population that has occupied the outer coast for several decades now consumes a greater variety of prey, with a predominance of bivalves, as compared to otters in the newly occupied Strait of Juan de Fuca, where red urchins comprise about 60% of the foraging observations (Laidre and Jameson 2006). The discrepancy in Washington may be even greater on a biomass basis if the situation is similar to San Nicolas wherered urchins account for a greater proportion of biomass than of observed foraging events due to their large size (Table 6.2).

Second, individual otters in MBNMS show dietary specialization as compared to those at San Nicolas (data on individual otters were not available from Glacier Bay), a pattern which has been theoretically proposed to be a response to food limitation (Glasser

1982; Schindler et al. 1997). Bentall (2005) found that sea otters at San Nicolas Island showed little individual-level specialization, with most individual diets having almost complete overlap with the population-level diet. Because sea otters at San Nicolas are descended from sea otters in central California (the founders of the population having been translocated from MBNMS in the late 1980s) and there is broad overlap in the potential invertebrate prey species at San Nicolas and MBNMS, this difference in degree of individual specialization is likely a reflection of differing levels of food availability.

Third, average rates of edible biomass and energy consumption by sea otters in MBNMS are much lower than those at San Nicolas and Glacier Bay. Consequently, estimates of foraging time per day required to meet energetic requirements are much higher for MBNMS otters than for those at other locations. These estimates of required foraging time agree well with the observed percentage of time the MBNMS and San Nicolas otters spend foraging: 41% and 25%, respectively.

Lastly, a number of key nutritional indicators – fat, thiamin, vitamin A and calcium – suggest that the MBNMS diet is on average of lower quality than sea otter diets at San Nicolas and/or Glacier Bay. Limited food resources would also seem to be the most parsimonious explanation for the poor body condition of sea otters at MBNMS, relative to other areas (Table 6.4). The smaller *absolute* size of otters in central California was not entirely surprising, as *E. l. nerieis* has been previously described as somewhat smaller than the two northern sub-species (Davis and Lidicker 1975; Reidman and Estes 1990; Wilson et al. 1991). Indeed, what was more surprising was that animals from SNI were comparable in both mass and length to animals from Glacier Bay and from the Aleutian Islands in the mid 1990's, at a time when the population was declining and both food abundance and body condition were increasing (Estes et al. 1998, 2004; Laidre et al. 2006). While admittedly based on a rather small sample size from San Nicolas Island, these data suggest that the morphological differences between the sub-species may not be as great as previously thought, and with abundant food resources the California sea otter can grow to a similar size and weight as northern sea otters. In any case, the extremely low mass to length ratio of otters from MBNMS (lower even than that recorded for otters in a population thought to be at carrying-capacity, in the Aleutian Islands in the 1960's-70's) is most consistent with a scenario of resource-limitation.

CONCLUSIONS AND RECOMMENDATIONS

In sum, multiple comparisons with other sea otter populations suggest that sea otters in MBNMS are food-limited: the broad range of prey species eaten by the population, the extreme dietary specialization shown by individuals, the low rates of edible biomass and energy consumption, the increased foraging time required to meet energetic needs, small body size, poor body condition and lower dietary quality. Ideally our conclusions would be supported by temporal and spatial contrasts of sub-tidal abundance of all key prey species; unfortunately, however, such data are, for the most part, non-existent. Nonetheless, we believe that the consistency in all metrics that we have measured (as summarized above) provides a fairly compelling weight of evidence argument for resource limitation.

Our initial explorations of stable isotope variation in MBNMS sea otters and their prey suggest that isotopic analysis offers great promise as a tool for comparing nutritional status and foraging ecology across and within sea otter populations. The use of stable isotope analyses may enable us to evaluate dietary specialization in sea otter populations in the absence of foraging data, thus extending comparisons across space and time. Stable isotopes could also be combined with fatty acid analyses (Part 4) as part of a multi-pronged effort to elucidate dietary histories of individual sea otters. Such information would be very valuable in attempts to link diet types of individual otters, as well as population-level changes in food choice, to such parameters as disease, morbidity, mortality, reproductive performance and body condition. Isotopic comparison of extant populations with those from historic and/or archaeological contexts may be able to shed light on long-term trends in diet that could underlie stagnation in population growth or periodic declines in sea otter populations in California. We recommend that such work be given a very high priority.

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APPENDICES

Appendix I. Collected sea otter prey species, by collection location.

Prey Species Collected			Collection Locations			
			CENTRAL CALIFORNIA		ALASKA	CHANNEL ISLANDS
#	Scientific Name	Common Name	North	South	Glacier Bay National Park	San Nicolas Island
1	*Acanthina punctulata*	spotted unicorn	Monterey Bay	San Simeon		Cosign Cove
2	*Acanthina spirata*	angular unicorn		Estero Bay		
3	*Acmaea mitra*	white-cap limpet		Rancho Marino		
4	*Asterias vulgaris*	northern sun star			N. Marble Is.	
5	*Blepharipoda occidentalis*	spiny mole crab	Elkhorn Slough	Morro Bay		
6	*Calliostoma annulatum*	purple-ringed top snail	Stillwater Cove	Point Estero; San Simeon		
7	*Calliostoma canaliculatum*	channeled top snail	Stillwater Cove; Monterey Bay	Point Estero; San Simeon		
8	*Calliostoma ligatum*	blue top snail	Pebble Beach; Stillwater Cove	Estero Bay; San Simeon		
9	*Cancer antennarius*	Pacific rock crab	Half Moon Bay; Monterey Bay	Estero Bay		Offshore
10	*Cancer anthonyi*	yellow rock crab		Estero Bay		
11	*Cancer gracilis*	slender crab		Morro Bay		
12	*Cancer magister*	Dungeness crab	Monterey Bay; Half Moon Bay	Cambria Rock; Estero Bay	GBNP - subtidal	
13	*Cancer productus*	Red rock crab	Macabee Beach	Rancho Marino		Offshore
14	*Ceratostoma foliatum*	Leafy hornmouth		Point Estero; San Simeon		
15	*Chionoecetes bairdi*	tanner crab			Beardslee Is.; Sundew Cove	

16	*Chlamys* sp.	scallop			N. Marble Is.	
17	*Clinocardium nuttallii*	basket cockle	Elkhorn Slough	Morro Bay	Geike; Sundew Cove	
18	*Collisella ochracea*	yellow limpet		Rancho Marino		
19	*Collisella pelta*	shield limpet		Rancho Marino		
20	*Colus halli*	Hall's colus			GBNP - subtidal	
21	*Crassadoma gigantea*	rock scallop		Cambria		SE Shore
22	*Cryptochiton stelleri*	gumboot chiton	Stillwater Cove	Point Estero; San Simeon		
23	*Ctenodiscus crispatus*	mud sea star			GBNP - subtidal	
24	*Cypraea spadicea*	smooth brown cowry				W. end
25	*Dendraster excentricus*	sand dollar		Morro Bay		
26	*Dermasterias imbricata*	leather star	Stillwater Cove	Point Estero	N. Marble Is.	
27	*Diodora aspera*	rough keyhole limpet		Cambria Rock		
28	*Echiurus echiurus alaskanus*	Alaskan spoonworm			Geike; RAN's 24 & 120	
29	*Emerita analoga*	Pacific sand crab	Elkhorn Slough	Estero Bay		
30	*Euspira lewisii*	Lewis's moon snail		Morro Bay		
31	*Evasterias troschelii*	mottled sea star			RAN 24; Willoughby Island	
32	*Fissurella volcano*	volcano limpet		Rancho Marino		
33	*Fusitriton oregonensis*	hairy triton			GBNP - subtidal	W. end
34	*Haliotis cracherodii*	black abalone	Pebble Beach	Rancho Marino		
35	*Haliotis rufescens*	Red abalone	Monterey Bay	Point Estero		
36	*Hapalogaster cavicauda*	furry crab	Macabee Beach			

37	*Hemigrapsus nudus*	Purple shore crab	Pebble Beach	Rancho Marino; San Simeon		Cosign Cove
38	*Hemigrapsus oregonensis*	Yellow shore crab	Elkhorn Slough	Morro Bay		
39	*Henricia leviuscula*	blood star	Macabee Beach; Stillwater Cove			
40	*Hiatella arctica*	arctic hiatella			Geike; Sundew Cove; RAN 86	
41	*Hyas lyratus*	Pacific lyre crab			GBNP - subtidal	
42	*Katharina tunicata*	black katy			Willoughby Island	
43	*Kelletia kelletii*	Kellet's whelk	Monterey Bay			N Shore; SE Shore; West End
44	*Lepidopa californica*	California mole crab		Estero Bay		
45	*Leptasterias polaris*	knobby six-rayed star			N. Marble Is.	
46	*Leptasterias pusilla*	small slender sea star	Pebble Beach	Rancho Marino; San Simeon		
47	*Lithopoma gibberosa*	red turban snail	Pebble Beach	Point Estero		
48						
49	*Loligo opalescens*	market squid	Monterey Bay			
50	*Lottia gigantea*	owl limpet	Pebble Beach	Rancho Marino		Thousand Springs
51	*Loxorhynchus crispatus*	moss crab	Macabee Beach	San Simeon		
52	*Loxorhynchus grandis*	sheep crab		Point Estero		
53	*Macoma balthica*	Baltic macoma			Geike; RAN's 120 & 179	
54	*Macoma nasuta*	Bent-nosed clam	Elkhorn Slough	Morro Bay	Geike; Sundew Cove; RAN 86	
55	*Macoma secta*	white sand clam	Elkhorn Slough	Morro Bay		

56	*Macoma* spp.	(various macoma clams)			Geike; Sundew Cove; RAN 86	
57	*Mediaster aequalis*	red sea star	Stillwater Cove			
58	*Megastraea undosa*	wavy turban snail				N. Shore, SE Shore, W. end
59	*Megathura crenulata*	giant keyhole limpet	Macabee Beach; Stillwater Cove	Point Buchon		SE Shore
60	*Mimulus foliatus*	foliate kelp crab	Macabee Beach			
61	*Mitra idae*	Ida's miter		Point Estero; San Simeon		W. end
62	*Mopalia lignosa*	woody chiton	Pebble Beach			
63	*Mopalia muscosa*	mossy chiton		Estero Bay		
64	*Mya truncata*	truncated softshell-clam			Geike; Sundew Cove; RAN 179	
65	*Mytilus californianus*	California mussel	Pebble Beach	Estero Bay; Rancho Marino		Thousand Springs; Cosign Cove
66	*Mytilus edulis*	bay mussel		Morro Bay		
67	*Modiolus modiolus*	horse mussel			GBNP	
68	*Mytilus trossulus*	foolish mussel			Geike; RAN'S 86 & 120	
69	*Neotrypaea californiensis*	bay ghost shrimp	Elkhorn Slough	Morro Bay		
70	*Neptunea lyrata*	ridged whelk			Sundew Cove	
71	*Nereis vexillosa*	pile worm			RAN 120	
72	*Norrisia norrisi*	Norris' top snail				N shore, SE shore, W end
73	*Nucella lima*	File dogwinkle			RAN'S 24 & 120	
74	*Ocenebra circumtexta*	circled rock snail		Rancho Marino; San Simeon		
75	*Octopus rubescens*	red octopus	Monterey Bay			

76	*Ophiura sarsi*	notched brittle star			N. Marble Is.	
77	*Opisthopus transversus*	mottled pea crab	Elkhorn Slough; Macabee Beach			
78	*Orthasterias koehleri*	rainbow star	Stillwater Cove			
79	*Orthopagurus minimus*	toothshell hermit		San Simeon		
80	*Pachygrapsus crassipes*	striped shore crab	Pebble Beach	Rancho Marino; San Simeon		Cosign Cove
81	*Pagurus granosimanus*	grainyhand hermit	Pebble Beach	Rancho Marino		
82	*Pagurus hemphilli*	maroon hermit	Pebble Beach; Stillwater Cove	San Simeon		
83	*Pagurus hirsutiusculus*	hairy hermit	Stillwater Cove	Rancho Marino; San Simeon		
84	*Pagurus ochotensis*	Alaskan hermit crab			GBNP - subtidal	
85	*Pagurus samuelis*	blueband hermit	Pebble Beach			
86	*Panulirus interrruptus*	Spiny lobster				N shore
87	*Panopea generosa*	geoduck clam		Morro Bay		
88	*Paralithodes camtschaticus*	red king crab			GBNP, subtidal, Sundew Cove	
89	*Parastichopus californicus*	California sea cucumber		Point Buchon	N. Marble Is.	N Shore; SE Shore
90	*Paraxanthias taylori*	lumpy crab	Macabee Beach			Thousand Springs
91	*Patiria miniata*	bat star	Stillwater Cove	S. of Estero Bay		N Shore
92	*Pisaster brevispinus*	short spined sea star		Point Estero		
93	*Pisaster giganteus*	giant spined star	Monterey Bay	Point Estero		N shore, SE shore; West End
94	*Pisaster ochraceus*	ochre star	Monterey Bay; Pebble Beach	Estero Bay; Rancho Marino		Thousand Springs; Cosign Cove

95	*Protothaca staminea*	Pacific littleneck clam	Elkhorn Slough		Geike; Sundew Cove, Ran 86	
96	*Pseudopythina compressa*	compressed montacutia			Geike	
97	*Pugettia producta*	northern kelp crab	Monterey Bay	Estero Bay; Rancho Marino		SE Shore
98	*Pugettia richii*	cryptic kelp crab	Macabee Beach; Stillwater Cove	San Simeon		
99	*Pycnopodia helianthoides*	sunflower sea star	Stillwater Cove	Point Estero		SE Shore
100	*Saxidomus gigantea*	Washington butterclam			GBNP, Geike	
101	*Saxidomus nuttalli*	California butterclam	Elkhorn Slough			
102	*Scyra acutifrons*	sharpnose crab		Point Buchon		
103	*Siliqua patula*	Pacific razor clam		Estero Bay		
104	*Siphonosoma ingens*	sipunculid worms	Elkhorn Slough	Morro Bay		
105	*Solen sicarius*	sickle razor clam		Morro Bay		
106	*Stenoplax fallax*	Fallax chiton		Cambria		
107	*Stenoplax heathiana*	Heath's chiton	Macabee Beach			
108	*Strongylocentrotus droebachiensis*	green urchin			Sundew Cove; RAN 86	
109	*Strongylocentrotus franciscanus*	red urchin	Macabee Beach	San Simeon		N Shore; SE Shore; Thousand Springs
110	*Strongylocentrotus pallidus*	white urchin			Willoughby Island	
111	*Strongylocentrotus purpuratus*	purple urchin	Macabee Beach; Pebble Beach; Stillwater Cove	Estero Bay; Rancho Marino; San Simeon		N Shore, Thousand Springs
112	*Styela montereyensis*	stalked tunicate		Point Buchon		
113	*Stylasterias forreri*	fish-eating star	Stillwater Cove			
114	*Tectura scutum*	plate limpet			Willoughby Island	

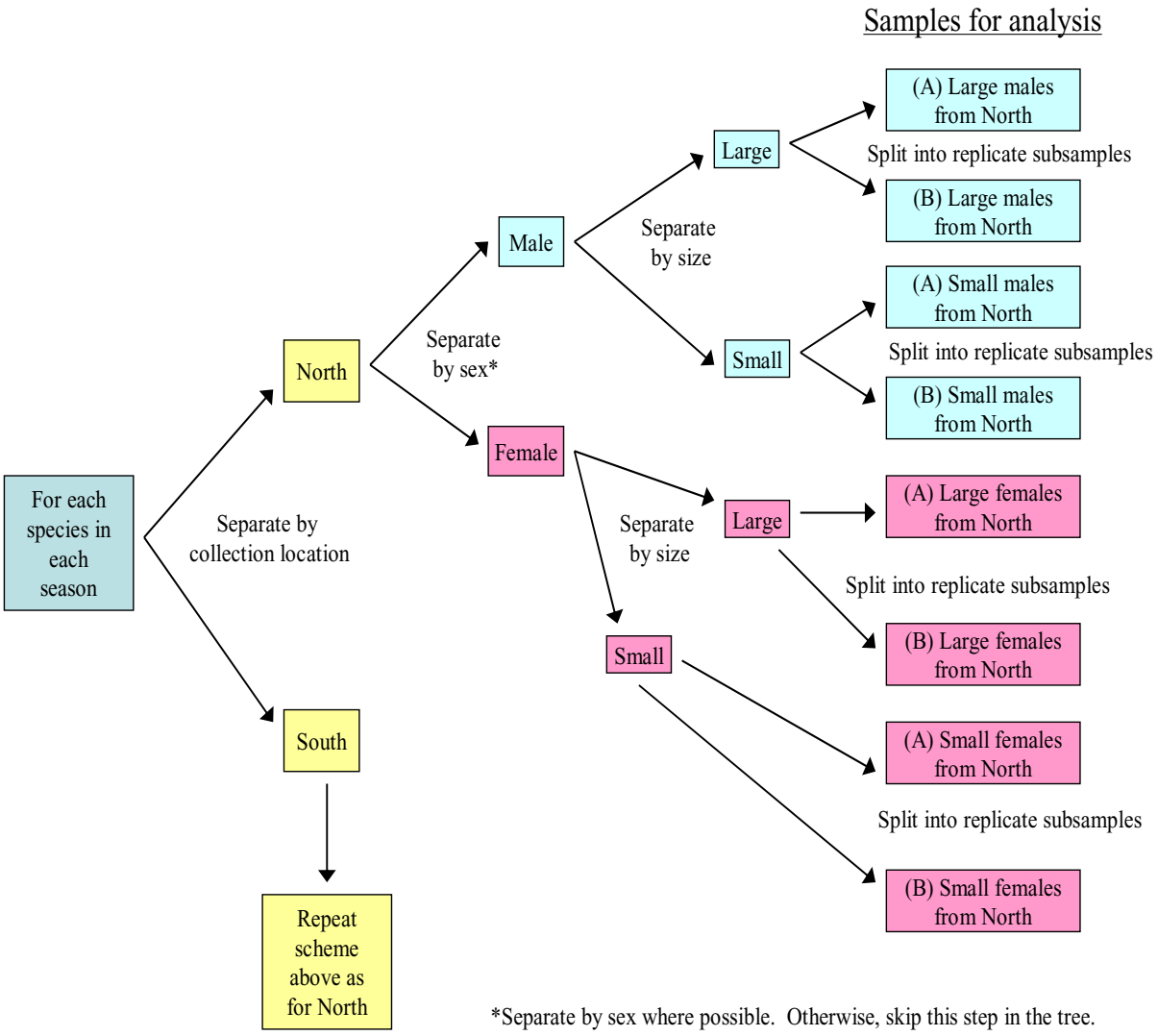
115	*Tegula brunnea*	brown turban snail	Monterey Bay; Pebble Beach; Stillwater Cove	Estero Bay; Rancho Marino		
116	*Tegula eiseni*	banded turban snail				Thousand Springs
117	*Tegula funebralis*	black turban snail	Monterey Bay; Pebble Beach; Stillwater Cove	Estero Bay; Rancho Marino		Cosign Cove
118	*Tegula montereyi*	Monterey turban snail	Monterey Bay; Pebble Beach; Stillwater Cove	Estero Bay; Rancho Marino; San Simeon		
119	*Tegula pulligo*	dusky turban snail	Monterey Bay; Pebble Beach; Stillwater Cove	Estero Bay; Rancho Marino; San Simeon		
120	*Tegula regina*	Queen turban snail				West end
121	*Tivela stultorum*	Pismo clam	Elkhorn Slough	Estero Bay		
122	*Tresus nuttallii*	Pacific gaper	Elkhorn Slough	Morro Bay		
123	*Upogebia pugettensis*	blue mud shrimp		Morro Bay		
124	*Urechis caupo*	fat innkeeper worm	Elkhorn Slough	Morro Bay		

Appendix II. Example of a Processing Protocol for Crabs.

The following protocol was developed and used by laboratory technicians for the processing of crab species and to determine sex. Note that photos and graphics were downloaded from public domain websites for within-lab use and should not be replicated without contacting the source site.

OP.CR. Processing Protocols (by species)
June-July 2004

Separating individuals into lab samples (for all species):
Not all samples that were bagged together in the field will be kept together in the lab. Samples may be separated according to the following scheme:



*Separate by sex where possible. Otherwise, skip this step in the tree.

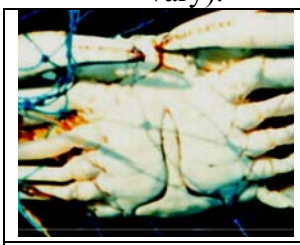
Ultimately, it is desirable to have at least several "replicates" for each location, sex (where possible), and size class (i.e., North Females, Small), and at least two "duplicates" of each "replicate" (i.e., North Females Small A and N F Sm B). Division of samples by the tree shown above will help to determine any difference in nutritional content between sexes, size classes and geographical locations. If any females of the species are gravid (and if there were enough individuals collected in the field), lab samples should be organized so that gravid and non-gravid females are analyzed separately. For larger or more important prey species, individuals may be analyzed individually rather than combined into samples.

1. *Pugettia richii*
   Cryptic Kelp Crab
   - Edible parts to a sea otter (on average, per Tim Tinker): legs crunched/eaten whole, all soft body tissue removed from carapace and eaten: for crabs <4cm, whole organism consumed.
   - Protocol:

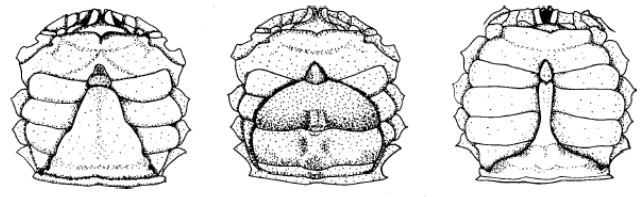
     1. First, gather all the individuals from one field sample together on a tray and bring the tray to the sink area. Using a wooden-handled metal probe, carefully scrape away all ice from the crabs (make sure not to scrape away body parts as well). When as much ice has been removed as possible, use the "Artificial sea water" (see instructions for mixing below) to rinse each individual, removing all remaining ice and frost. Place the individuals on a paper-towel-lined tray to dry, and bring to the processing area (repeat for each field sample – if each field sample is done separately, it is much easier to keep samples and individuals straight).
     2. Maximum carapace width and frozen mass should be measured for each individual, or up to a maximum of 50 individuals, and recorded on the Prey Species Data Sheet. Use a digital caliper to measure the max. carapace width, which on kelp crabs appears to be from outside edge to outside edge of the largest anterolateral teeth. Record dimension and frozen mass data on the species' Prey Species Data Sheet.
     3. If max. carapace width is <4cm, no further processing is required. If >4cm, the dorsal carapace should be removed. This is most easily done using a scalpel (disposable or stainless steel) to cut between the dorsal carapace and the legs, from the (caudal) point where the dorsal carapace meets the uropod, all the way around the body to the (cranial) point where the eyes are located. Once this cut is complete, the dorsal portion of the carapace may be gently pulled off, leaving legs and all soft parts attached to the abdominal sections.
     4. The assigned **Lab ID**; the ID(s) of the **field sample**(s) from which individuals from each sample originated; the total number of individuals in the sample (**n=**); the **sex** (M/F), **size class** (sm/lg), and **geographical location** (N/S) of the individuals in each sample; the **date** the sample was processed; the **gross frozen mass**; and the **gross edible mass** (mass of >4cm crabs, after carapace removed) should be recorded on the Master Otter Prey Sample Spreadsheet, in hard copy and on the computer.

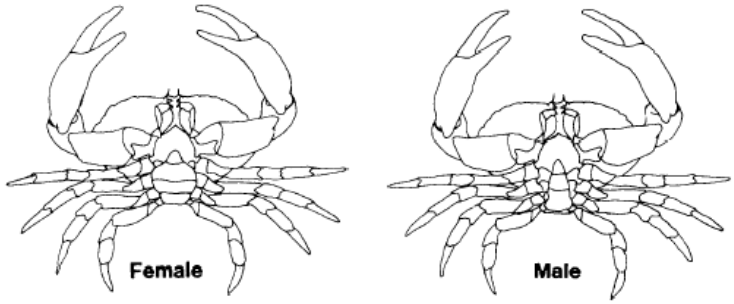
5. Samples should be stored in pre-tared weighboats labeled with the sample's lab ID and species abbreviation, and placed in the stand-up freezer on aluminum-foil covered cookie trays.

- To mix artificial sea water: Mix 1 scoop of Aquarium Salt Mix to 1 gallon (1 gal. = 3785 mL) of distilled water, and mix thoroughly using a magnetic stirring bar and magnetic stirrer. Measure the salinity in ppm using a hydrometer, and record the salinity level in the project's green lab book. If the salinity does not fall between 0.020 and 0.023 ppm, add saline mix or distilled water to bring the salinity within that range.
- To sex *P. richii:* Sex may be determined by looking closely at the shape of each individual's abdomen. Males (A) have a tall, narrow, T-shaped abdomen. Immature females (B) have a wide and triangular abdomen, and mature females (C) have a wider, more broadly rounded abdomen. Gravid females (D) are more easily identified because of the masses of eggs carried between the abdominal shield and the body (color of eggs will vary).

			
(C) Male	(B) Immature Female	(C) Mature Female	(D) Gravid Female
* note: images are of blue crabs, but abdomen shape is similar in all crab species that may be sexed in this manner			

Other Examples:





- Inedible matter: the detached dorsal carapace should be set aside, in a Whirl-Pak or Ziploc bag labeled "Inedible parts for" followed by the sample's lab ID, your initials, and the date. This inedible matter should then be returned to the labeled boxes in the walk-in freezer.

2. *Pugettia producta*
   Kelp crab
   - Edible parts to a sea otter (on average, per Tim Tinker): legs crunched/eaten whole, all soft body tissue removed from carapace and eaten: for crabs <4cm, whole organism consumed
   - Protocol:
     1. First, gather all the individuals from one field sample together on a tray, and bring the tray to the sink area. Using a wooden-handled metal probe, carefully scrape away any ice that may have accumulated during freezing, making sure not to scrape away body parts as well. When as much ice has been removed as possible, use the Artificial Sea Water (see instructions for mixing, above) to rinse each individual, removing all remaining ice and frost. Then place the individuals on a paper-towel-lined tray to dry off, and bring to the processing area.
     2. The maximum carapace width (on kelp crabs, the max. carapace width is measured from outside edge to outside edge of the anterolateral teeth) and the frozen mass should be measured for up to a maximum of 50 individuals, and recorded on the Prey Species Data Sheet. Maximum carapace width should be measured using a digital caliper. Record this data on a Prey Species Data Sheet, in hard copy and on the computer.
     3. If max. carapace width is <4cm, no further processing is required. If >4cm, the dorsal carapace should be removed using a scalpel (disposable or stainless steel) to cut between the dorsal carapace and the legs, from the (caudal) point where the dorsal carapace meets the uropod, around the body the (cranial) point where the eyes are located. Once this cut is complete, the dorsal portion of the carapace may be gently pulled off, leaving legs and all soft parts attached to the abdominal sections.
     4. The assigned Lab ID; the ID of the field sample from which individuals from each sample originated; the total number of individuals in the sample (n=); the sex (M/F), size class (sm/lg), and geographical location (N/S) of the individuals in each sample; the date the sample was processed; the gross frozen mass; and the gross edible mass (mass of >4cm crabs, after carapace removed) should be recorded on the Master Otter Prey Sample Spreadsheet, in hard copy and on the computer.
     5. Samples should be stored in pre-tared weighboats labeled with the sample's lab ID and species abbreviation, and placed in the stand-up freezer on aluminum-foil covered cookie trays.
   - To sex *P. producta*: use the same guidelines as for *P. richii*.
   - Inedible matter: hard parts that were removed from an individual should be set aside, in a Whirl-Pak or Ziploc bag labeled (in black Sharpie): "Inedible parts for," the sample's lab ID, your initials, and the date. The inedible matter should then be returned to the walk-in freezer.

3. *Emerita analoga*
   Sand crab
   - Edible parts to a sea otter (per Tim Tinker): whole organism consumed
   - Protocol:
     1. Use a wooden-handled metal probe to scrape away any ice that may have accumulated during freezing. When ice has been removed, use Artificial Sea Water to rinse away any remaining ice and frost. Place the individuals on a paper-towel-lined tray to dry off, and bring to the processing area.
     2. There is no dissection for the sand crab; measure the maximum carapace dimension (length from the anterolateral teeth to where the posterior end of the carapace), the total body length (from the tip of the anterolateral teeth to the end of the body – do not spread out the uropod segments, but measure to the natural end point), and frozen mass for a maximum of 50 individuals, and record this data on a Prey Species Data Sheet, in hard copy and on the computer.
     3. Field samples will then be separated into lab samples according to the sample separation tree above. Lab ID; the ID of the field sample from which individuals from each sample originated; the total number of individuals in the sample; the sex (M/F), size class (sm/lg), and geographical location (N/S) of the individuals in each sample; the date the sample was processed; and the gross frozen mass should be recorded on the Master Otter Prey Sample Spreadsheet, in hard copy and on computer.
     4. Samples should be stored in pre-tared and labeled weighboats on aluminum-foil-covered cookie sheets in the stand-up freezer.
   - To sex *E. analoga*: Females have 3 pairs of modified pleopods attached to the abdomen segment posterior to the 3 pairs of walking legs. These modified pleopods are not covered by chitin, and are slim and translucent in appearance (they are used to hold eggs when gravid). Males have 1 pr. modified pleopods posterior to the walking legs; these are *not* attached to the abdomen, and have a bulbous gland near their base (testes), which may be blue-tipped.

Male

Female (pleopods circled)

Gravid female

   - Inedible matter: no inedible matter for *E. analoga*.

4. *Lepidoda californica*
    - Edible parts to a sea otter (on average, per Tim Tinker): whole organism consumed.
    - Processing:
      1. Scrape away any ice that has accumulated on samples using a wooden-handled metal probe, and rinse each individual with Artificial Sea Water to remove any remaining ice or frost. Place the individuals on a paper-towel-lined tray to dry off and bring to the processing area.
      2. There is no dissection for the *L. californica*; processing consists of measuring the maximum carapace width and frozen mass for a maximum of 50 individuals, and recording this data on the Prey Species Data Sheet, in hard copy and on computer.
      3. Field samples will then be separated into lab samples according to the sample separation tree. Lab ID; the ID of the field sample from which individuals from each sample originated; the total number of individuals in the sample; the sex (M/F), size class (sm/lg), and geographical location (N/S) of the individuals in each sample; date the sample was processed; and the gross frozen mass, should all be recorded on the Master Otter Prey Sample Spreadsheet, in hard copy and on computer.
      4. Samples should be stored in pre-tared and labeled weighboats, then placed on aluminum-foil-covered cookie sheets in the stand-up freezer.
    - To sex *L. californica*: unknown.
    - Inedible matter: no inedible matter for *L. californica*.

5. *Blepharipoda occidentalis*
   Mole crab
    - Edible parts to a sea otter (on average, per Tim Tinker): for crabs <4cm, whole organism consumed; for crabs >4cm, carapace removed
    - Protocol:
      1. Carefully scrape away any ice that has accumulated on samples using a wooden-handled metal probe, and rinse each individual with Artificial Sea Water to remove any remaining ice or frost. Place individuals on a paper-towel-lined tray to dry off, and bring to the processing area.
      2. Using the digital caliper, measure the maximum carapace length and the total body length (from the tip of the anterolateral teeth to the segments of the uropod visible on dorsal surface), as well as the frozen mass, for a maximum of 50 individuals. Record this data on the species' Prey Species Data Sheet, in hard copy and on computer.
      3. If the maximum carapace length is >4cm, the carapace and hard parts of the abdominal shield should be removed. This is best done using a disposable or stainless steel scalpel to cut between the dorsal carapace and the legs, from either side of the uropod (caudal) around to the eyes (cranial). Once this cut is made, the dorsal carapace may then be gently pulled off, starting at the caudal and pulling towards the cranial end.

4. Field samples should be separated into lab samples according to the sample separation tree. Lab ID; the ID of the field sample from which the sample's individuals originated; the total number of individuals in the sample(n=); the sex (M/F), size class (sm/lg), and geographical location (N/S) of the individuals in each sample; the date the sample was processed; and the gross frozen mass and gross edible mass, should all be recorded on the Master Otter Prey Sample Spreadsheet (in hard copy and on computer).
5. Samples should be stored in pre-tared and labeled weighboats on aluminum-foil-covered cookie trays in the stand-up freezer.

- To sex *Blepharipoda occidentalis*: Females have 3 pairs of modified pleopods attached to the abdomen segment posterior to the 3 pairs of walking legs. These modified pleopods are not covered by chitin, and are slim and translucent in appearance (they are used to hold eggs when gravid). Males have 1 pr. modified pleopods posterior to the walking legs; these are *not* attached to the abdomen, and have a bulbous gland near their base (testes), which may be blue-tipped.
- Inedible matter: parts that were removed from an individual (e.g., dorsal carapace) should be set aside, in a Whirl-Pak or Ziploc bag labeled (in black Sharpie): "Inedible parts for," the sample's lab ID, your initials, and the date. The inedible matter should then be returned to the walk-in freezer.

6. *Cancer magister*
Dungeness crab
   - Edible parts to a sea otter (per Tim Tinker): claws cracked open and meat removed, other legs crunched/eaten whole, all soft body tissue removed from carapace and eaten: for crabs <4cm, whole organism consumed.
   - Protocol:
   1. Scrape ice off of frozen samples using a wooden-handled metal probe, and rinse each individual with Artificial Sea Water to remove any remaining ice or frost. Place individuals on a paper-towel-lined tray to dry and bring to the processing area. Use the digital caliper to measure the maximum carapace width (on Dungeness crabs appears to be from outside edge to outside edge of the lowest anterolateral teeth on the dorsal carapace) and a balance to measure the frozen mass for a maximum of 50 individuals.
   2. It is likely that all specimens will be >4cm, in which case the carapace and hard parts of the abdominal shield must be removed. This may be accomplished using a sturdy, pointed knife; a scalpel; a set of pliers; and a wooden-handled metal probe. Remove legs and claws beforehand.
   3. First, slide the knife tip underneath the hard sections of the abdominal shield. Use the knife as a lever to crack these sections, and then pull them away from the body (these usually crack off in small pieces, and the process goes rather slowly). Then, use the knife to cut along the edge where the dorsal carapace meets the section of carapace that slopes

inwards to meet the abdominal sections of the body. Press the knife tip hard into the carapace to puncture it, and make sure the sharp edge of the knife is facing away from you and along the edge you wish to cut. Wiggle the knife slightly to crack the carapace. Push the knife forward along the edge, and continue wiggling it slowly to crack the carapace and make forward progress. Cut all the way around the body in this manner, from each side of the uropod on the caudal surface, around to the eyes on the cranial surface.

4. When the cut is made, insert a probe or scalpel under the posterior edge of the carapace to ensure the carapace is completely separated from the soft matter underneath. Then, slowly and gently pull the carapace off from caudal end to cranial end, scraping off any soft matter that adheres. Crack off the side sections of the carapace.
5. Break the claws off at joints with hands, then use the knife or pliers to crack the long edges. Sometimes the probe may be inserted and the meat inside easily scraped out; otherwise, crack an edge and then scrape out meat with a probe or scalpel.
6. If edible matter will fit into a large weighboat, this is best; if not, the large Tupperware-like plastic containers in the supply room may be used (metal containers may leave trace amounts of minerals on samples, and should not be used). When labeling, write directly on the containers, as lab tape will come off in the lyophilizer. Any container used must be shorter than the long end of a credit card to fit on lyophilizer shelves.
7. Field samples should be separated into lab samples according to the sample separation tree. Lab ID; the ID of the field sample from which the sample's individuals originated; the total number of individuals in the sample(n=); the sex (M/F), size class (sm/lg), and geographical location (N/S) of the individuals in each sample; the date the sample was processed; and the gross frozen mass and gross edible mass, should all be recorded on the Master Otter Prey Sample Spreadsheet.
8. Samples should be stored in pre-tared and labeled weighboats on aluminum-foil-covered cookie trays in the stand-up freezer.

7. *Cancer antennarius*
   No common name
   - <u>Edible parts to a sea otter (on average, per Tim Tinker):</u> claws cracked open and meat removed, other legs crunched/eaten whole, all soft body tissue removed from carapace and eaten: for crabs <4cm, whole organism consumed.
   - <u>Protocol:</u>

     1. Scrape ice from frozen samples using a wooden-handled metal probe, and rinse each individual with Artificial Sea Water to remove any remaining ice or frost. Place individuals on a paper-towel-lined tray to dry and bring to the processing area. It may be useful to allow a few extra minutes here, to allow further thawing (this will make removing the soft parts easier). Use the digital caliper to measure the maximum carapace

width (on Dungeness crabs, this appears to be from outside edge to outside edge of the lowest anterolateral teeth on the dorsal carapace) and a balance to measure the frozen mass for a maximum of 50 individuals.

2. It is likely that all specimens will be >4cm, in which case the dorsal carapace and hard parts of the abdominal shield must be removed. This may be accomplished using a sturdy, pointed knife; a scalpel; a set of pliers; and a wooden-handled metal probe. Remove legs and claws beforehand.

3. First, slide the knife tip underneath the hard sections of the abdominal shield. Use the knife as a lever to crack these sections, and then pull them away from the body (these usually crack off in small pieces, and the process goes rather slowly). Then use the knife to cut from the posterior point where dorsal carapace meets the abdomen, around to the transverse line. There will be a fairly obvious *line* in the carapace that slopes upward from the dorsal carapace-abdomen juncture, to the edge of the dorsal carapace; attempt to cut along that line. Press the knife tip hard into the carapace to puncture it. Make sure the sharp edge of the knife is facing away from you, and wiggle the knife slightly to crack the carapace and move the knife forward.

4. When this cut is made, set the knife down and use the pliers to crack the rest of the carapace edge. Use the pliers to break away the anterolateral teeth, so that there is a gap between the dorsal carapace and the lower carapace from the transverse line to the eyes. Insert a probe or scalpel under the posterior edge of the carapace to ensure carapace is completely separated from the soft matter underneath, then pull the carapace off from caudal to cranial end, scraping off any soft matter that adheres.

5. Break the claws off at joints with hands, then use the knife or pliers to crack the long edges. Sometimes the probe may be inserted and the meat inside easily scraped out; otherwise, crack an edge and then scrape out meat with a probe or scalpel.

6. If edible matter will fit into a large weighboat, this is best; if not, the large Tupperware-like plastic containers in the supply room may be used (metal containers may leave trace amounts of minerals on samples, and should not be used). When labeling, write directly on the containers, as lab tape will come off in the lyophilizer. Any container used must be shorter than the long end of a credit card to fit on lyophilizer shelves.

7. Large and important prey items like cancer crabs may be sampled as individuals – do not recombine for lab samples unless there are enough individuals. Field samples should be separated into lab samples according to the sample separation tree. Lab ID; the ID of the field sample from which the sample's individuals originated; sex (M/F), size class (sm/lg), and geographical location (N/S) of individuals in each sample; the date the sample was processed; and the gross frozen mass and gross edible mass, should all be recorded on the Master Otter Prey Sample Spreadsheet.

8. Samples should be stored in pre-tared and labeled weighboats on aluminum-foil-covered cookie trays in the stand-up freezer.

Appendix III. Example of a Processing Protocol for Stars.

The following protocol was developed and used by laboratory technicians for the processing of star species. Note that photos and graphics were downloaded from public domain websites or general text books for within-lab use and should not be replicated without contacting the source.

OP.ST. Processing Protocols

1. *Pisaster ochraceus*
   Ochre star

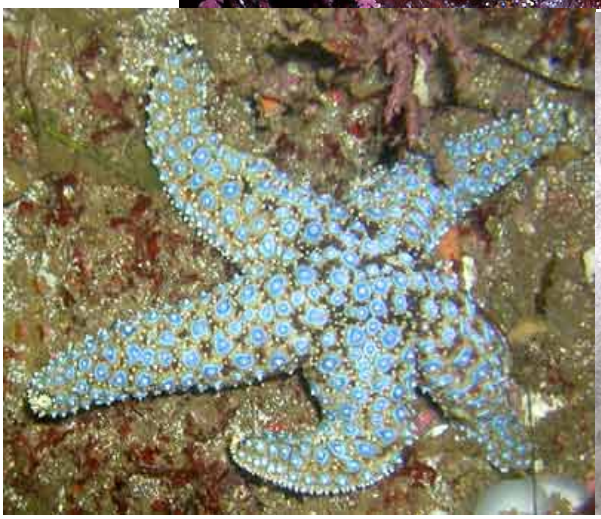
 [1]

possible color morphs

observed color morphs

This is the most common species of sea star the Sea Otter Nutrition Project has encountered and collected in California (as of Sept 2004), found mainly in tidepool and piling areas. The purple and orange examples shown above are the most commonly observed color morphs in the project's study area, though there are known to be many "transitional" morphs between the two (John Pearse, personal communication 9/2004). It is not possible to sex *P. ochraceus* externally unless the spawning material is directly observed; when in reproductive season, males exude a whiteish/cream fluid (sperm) and females a fluid that is more tan/buff in color (ova). Outside of reproductive season, arms must be severed, gonads removed, and a sample placed on a slide and viewed under a microscope to distinguish between the sexes. (Some bright salmon-pink egg masses have been observed in the lab, but this is not consistent and depends on gonad ripeness.)

2. *Pisaster giganteus*
   Giant spined star



This is the second-most common species of sea star SONP has found, especially diving and among rocks and pilings under the Coast Guard Pier in Monterey, California. Also may not be sexed externally unless spawning is observed; also requires use of microscope to characterize gonad material as sperm or ova. Costa (1976) cites *P. giganteus* as the main sea star eaten by sea otters in California.

General Anatomy

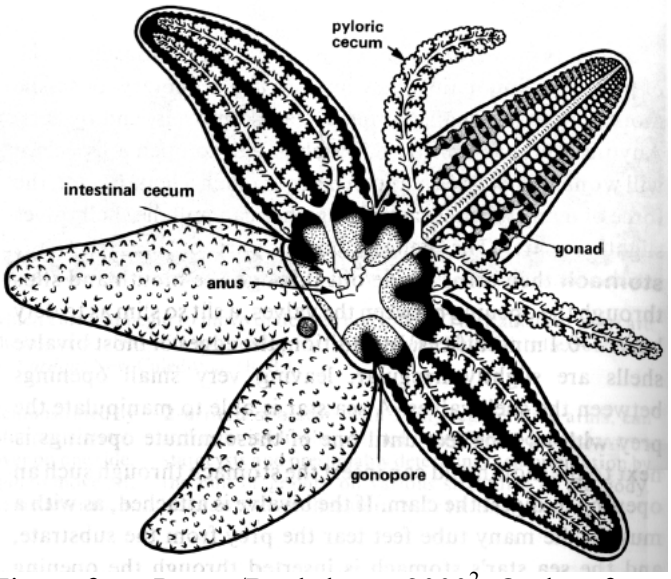


Figure from Pearse/Buchsbaum 2000[2]: Oral surface of Asteroid sea star. Two pyloric ceca line each arm, with the gonads lying underneath.

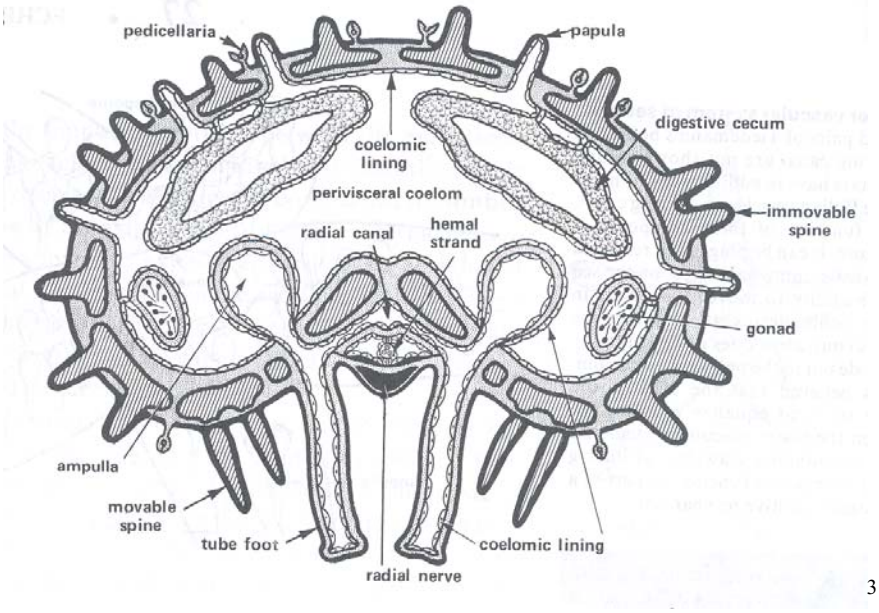


Figure from Moore 2001[3]: Internal cross-section of arm, showing position of pyloric (here labeled "digestive") ceca and gonads.

- Edible parts to a sea otter: One to two arms severed from the main body of each star; gonads and pyloric cecum sucked out from arm and body cavities; star then tossed back into water. Popular food for pups during maternal foraging dives, although not necessarily a preferred food.
- Protocol:

  1. Remove one sample from freezer at a time. Mass entire frozen sample (Ziploc included) and note size of Ziploc (Q=quart, G=gallon) on the Prey Species Data Sheet (when entering this data onto the computer, calculate the sample's total mass by subtracting the Q/G Average Ziploc mass from the mass of the entire field sample). Pour A.S. into sample's Ziploc bag (guideline: volume of water added should be approximately equal to 1/3 the volume of the sample) and let thaw. If bag is leaky (sea star spines often appear to damage Ziplocs), remove sample from bag and place in Pyrex tray with shallow layer of A.S. Stars bagged together are often stuck to one another and must be thawed out for a time before separation; also, edible material may not be removed until star is almost completely thawed. A metal spatula or a wooden-handled dissection probe can be used to carefully scrape ice away and separate stars. *Thawing suggestions: when samples were thawed on 9/28/04, large stars took 2-3.5 hours to thaw enough so that processing was possible. After about 4-4.5 hours, however, internal material was "goopy" and more difficult to remove. To prevent this from occurring, remove multiple stars at ½ hour intervals from the freezer, so that each star spends roughly equal time (2.5-3.5 hours) thawing prior to processing, and so that no star is left thawing for additional time.*
  2. Lay separated individuals on a clean metal tray. Use a digital caliper to measure the length of the longest or straightest arm on each star, and mass each whole individual star. Record these dimensions on the Prey Species Data Sheet for all individuals (as it is unlikely that 30-50 individual stars of each species will be collected, it will be necessary to record all dimensions measured on all stars).
  3. After measuring and thawing all individuals, separate the field samples into lab samples based on size and N/S origin. For large stars, a sample of 2-3 individuals should provide enough mass; for small stars, 3-5 may be required. Record the assigned **Lab ID**; the ID(s) of the **field sample**(s) from which individuals in each lab sample originated; the total number of individuals in the sample (**n=**); the **geographical location** (N/S) and **size class** (sm/lg) of the individuals; the **date** of the sample's processing; and the **gross whole mass** of each lab sample (i.e., mass of all individuals in the sample prior to processing) on the Master Otter Prey Sample Spreadsheet, in hard copy and on the computer.
  4. Use a sharp knife or lab scissors to sever one arm (ray) from the star's main body, cutting as close to the central disk as possible. Visible at the

base of the ray where it joins the central disk are 2 gonopores (see diagram); to collect a maximum of edible material as easily as possible, it is recommended that the cut to sever an arm be made below these gonopores. Pull the arm away from the body and use blunt/round-ended forceps to reach inside the arm and central disk cavities to remove all pyloric cecum material (usually a brown- or gray-green) and gonad material (buff/pale orange-colored; gelatinous in texture).

5. Mass pyloric cecum and gonad material separately, then combine in weighboat. Repeat the dissection process to obtain gonad and p.c. material from all 5 arms; mass this as "gross total edible mass" on the Prey Species Data Sheet. Mass emptied arms and central disk as "gross inedible mass," place in a labeled Whirl-Pak bag, and return to freezer.
6. Samples should be stored in pre-tared weighboats labeled with the sample's lab ID and species abbreviation, and placed in the stand-up freezer on aluminum-foil covered cookie trays.
7. Time guidelines: each star (small or large) takes from 20-40 minutes to process (i.e. massing whole organism, measurement, severing of all 5 arms, removal and massing of p.c. and gonad from each arm, etc.).

Additional Information

- To mix artificial sea water: Mix 1 scoop of Aquarium Salt Mix to 1 gallon (1 gal. = 3785 mL) of distilled water, and mix thoroughly using a magnetic stirring bar and magnetic stirrer. Measure the salinity in ppm using a hydrometer, and record the salinity level in the project's green lab book. If the salinity does not fall between 0.020 and 0.023 ppm, add saline mix or distilled water to bring the salinity within that range.
- Seasonal spawning: see literature in Sea Otter library
- Sex ratio: uncertain

Additional species SONP has encountered

3. *Pisaster brevispinus*
   Short-spined sea star

 [1]

4. *Patiria miniata*
   Bat star

[1] http://www.enature.com/search/show_search_byShape.asp?curGroupID=8&shapeID=1072
[2] Vicki Pearse, John Pearse, Mildred Buchsman, and Ralph Buchsman. Living Invertebrates. © 2000 Blackwell Scientific Publications, Boston, MA.
[3] Moore, Janet. An Introduction to the Invertebrates. © 2001 Cambridge University Press, Cambridge, UK