Sea Otter Population Biology at Big Sur and Monterey California: Investigating the Consequences of Resource Abundance and Anthropogenic Stressors for Sea Otter Recovery

Final Report to the California Coastal Conservancy and the U.S. Fish and Wildlife Service
(DRAFT)

October 15, 2013

Edited by:

M. Tim Tinker[1] (Principal Investigator)
University of California, Santa Cruz, CA

with contributions from:

Joe Tomoleoni[1], Ben Weitzman[1], Michelle Staedler[3], Dave Jessup[2], , Mike Murray[3], Melissa Miller[2], Tristan Burgess[4], Lizabeth Bowen[1], Keith Miles[1], , Nicole Thometz[5], Lily Tarjan[5], Emily Golson[6], Francesca Batac[2], Erin Dodd[2], Eva Berberich[2], Jessica Kunz[2], Gena Bentall[1], Jessica Fujii[3], Teri Nicholson[3], Seth Newsome[7], Ann Melli[4], Nicole LaRoche[5], Holly MacCormick[5], Andy Johnson[3], Laird Henkel[2], Chris Kreuder-Johnson[4], Pat Conrad[4]

1. U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA
2. Marine Wildlife Veterinary Care & Research Center, California Dept. Fish & Wildlife, Santa Cruz, CA
3. Sea Otter Research and Conservation Department, Monterey Bay Aquarium, Monterey, CA
4. Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA
5. Ecology and Evolutionary Biology Department, University of California, Santa Cruz, CA
6. Moss Landing Marine Lab, California State University of Monterey Bay, Moss Landing, CA
7. Department of Biology, University of New Mexico, Albuquerque, NM

Suggested Citation:
M. T. Tinker *et al.*, 2013. "Sea Otter Population Biology at Big Sur and Monterey California: Investigating the Consequences of Resource Abundance and Anthropogenic Stressors for Sea Otter Recovery". DRAFT Final Report to California Coastal Conservancy and U.S. Fish and Wildlife Service. University of California Santa Cruz, CA. *243* pages

Table of Contents

Chapter 1. Overview of Study Objectives, Hypotheses and Methods

M. Tim Tinker[1], Joe Tomoleoni[1], Dave Jessup[2] and Andy Johnson[3]

1. U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA
2. Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife
3. Sea Otter Research and Conservation Department, Monterey Bay Aquarium

Introduction

Sea otters (*Enhydra lutris nereis*) in California forage as an apex marine predator in nearshore habitat that occurs along a densely human-populated coastline. Sea otters are unusual among marine mammals in that they have an extremely high surface area to volume ratio, live outside of their thermal neutral zone, lack an insulating blubber layer utilized by most marine mammals, and consequently have one of the highest mass-specific metabolic demands of all marine mammals (Morrison et al. 1974, Costa and Kooyman 1982, Yeates et al. 2007). This trait, in conjunction with their distribution and the preponderance of filter feeding benthic invertebrates in their diet, makes southern sea otters especially susceptible to naturally occurring and human-induced stressors in their environment, and as such, they are effective sentinels of the health of California's coastal ocean (Jessup et al. 2007). Their utility as a sentinel (or indicator) of ecosystem health is further increased by their nearshore distribution, their extraordinary appeal to the general public (a fact that generates community support for monitoring efforts), and their tractability for observational study. In effect, sea otters can "tell us" how they encounter environmental stressors or acquire particular disease pathogens via detailed measurements of where they live and what they eat (Johnson et al. 2009). Sea otters also play a vital ecological role in coastal ecosystems as a keystone predator, and their relative abundance can have profound effects on both the productivity and biodiversity of kelp forest communities (Kenyon 1969, Estes et al. 1978, Estes et al. 2004).

Research over the past 15 years has provided a great deal of information on a wide range of specific cause(s) of mortality in southern sea otters, including starvation, predation, intra-specific aggression, intoxication, and a variety of infectious diseases (Kreuder et al. 2003); however, important questions remain about the ultimate drivers of these patterns, and about the nature of interactions among these causal factors. Studies of the diet, time-activity budgets, movements and behavioral patterns, demography (survival and reproduction), health metrics, and causes of death of tagged, free-ranging otters provide the core component of a research program aimed at elucidating the underlying drivers of population change. The overall objectives of this research program are to identify and prioritize conservation actions that will positively affect the recovery of the southern sea otter and improve ecosystem health. Comparisons of all these metrics across space and time, and between populations that differ with respect to one or more factors of interest (e.g. low vs. high density, or low vs. high exposure to pollutants), allow for a "quasi-experimental" approach to testing hypotheses about the relative impacts of various factors on population recovery. The results of one-such study, comparing sites with varying sea otter densities and per-capita prey abundance, highlighted the importance of prey resource availability in affecting sea otter behavior and health, and pointed towards density-dependent resource limitation as a key driver of population change in certain areas (Tinker et al. 2008, Tinker et al.

2012). At the same time, comparative studies of sea otter exposure to land-derived pathogens showed that there was considerable spatial variation in the rates of infection, and that these patterns were potentially related to point sources of pathogen pollution (Miller et al. 2002, Miller et al. 2007, Johnson et al. 2009, Miller et al. 2010a). More recently, it was found that the biotoxins produced by harmful algal blooms (HABs) can have significant acute and chronic effects on sea otter health (Kreuder et al. 2005, Miller et al. 2010b), and there is a possibility that land-based nutrient enrichment is contributing to increased frequency and severity of these HAB events.

Given the above findings, it is clear that elucidating the relative importance of - (in terms of driving population change) and relationships between - food resource abundance, environmental conditions, and human-contributed increases in pathogens and pollutants (including nutrient-driven HAB events) is critical in order to reach a full understanding of the underlying drivers of population change. For example, although previous research suggests that both food resource limitation and terrestrial input of pathogen pollution are contributing to elevated mortality, our ability to make specific recommendations for mitigation has been hampered by uncertainty about the relative importance of these factors, their interaction, and the specific pathways of exposure to disease causing pathogens. The current study was designed to investigate the causal links between the sluggish population performance of sea otters in central California and factors that could be driving variation in survival and reproduction, including food resource limitation and various types of terrestrial-based pollution (including pathogen pollution). Our overall goal was to identify and prioritize conservation actions that will positively affect the recovery of the Southern sea otter and improve ecosystem health. In order to achieve this goal we identified five specific objectives:

A) Describe the health status and basic ecology of sea otters at two study sites in central California, i) an area of relatively dense human population that is heavily impacted by anthropogenic influences and urban and agricultural effluents and ii) a more pristine area with minimal human impact.

B) Document patterns of mortality in the sea otter population, including spatial and temporal trends in the causes of death. Compare these patterns between high impact and low impact study sites.

C) Describe relationships between specific health threats and putative risk factors (such as habitat use, movement behavior, diet, foraging success, body condition, reproductive status), and contrast these between areas of high vs. low human impact. Conduct epidemiological analyses to pinpoint key risk factors for disease exposure.

D) Combine data from this study with similar data generated from previous studies, and conduct a comprehensive hazards analysis to identify the ultimate factors that are most important in affecting sea otter survival and thus limiting population growth.

E) Advise and assist the relevant management agencies in implementing appropriate conservation, regulatory, and policy development actions

We hypothesized that:

1) Sea otters living in areas adjacent to human population centers and areas heavily impacted by runoff or sewage are more likely to be exposed to pathogens and toxins of public health importance than those in more pristine areas.

2) Patterns of survival and causes of death will differ between heavily impacted and pristine environments, reflecting differences in pathogen and pollutant exposure.

3) Environmental risk factors will vary between sites, corresponding to the differing land-use patterns.

4) Sea otters from high-density populations (and/or areas that have been occupied longer) will exhibit lower rates of foraging success due to prey resource depletion, and these patterns will be reflected by i) greater percentage of time spent feeding, ii) more pronounced individual diet specialization, iii) poorer body condition, and iv) lower survival rates of both adults and pups.

Hypotheses 1-3 are premised upon the notion that the high levels of infectious disease and contaminants found in some sea otters are ultimately related to elevated land-to-sea transport of the causal agents, which is associated with urban and agricultural activities and landscape alteration. Hypothesis 4 is premised upon the notion that density-dependent resource depletion is a primary factor limiting population growth in central California. We note that none of these hypotheses are mutually exclusive, and our goal is to determine the relative degree to which each is supported or refuted by empirical data.

In order to test these hypotheses we examined the health, survival and basic ecology of sea otters at two locations along the central coast (Figure 1): the south end of Monterey Bay and Monterey Peninsula (MON) was selected as a site of high human impact, while the central portion of the Big Sur coast (BSR) was selected as a site of lower human impact (Figure 2). Within the current range of the southern sea otter, these sites represent the extreme ends of the spectrum of anthropogenic impact (e.g. urban, industrial and agricultural activities). Another advantage of these particular sites was that they contain generally similar sub-tidal benthic habitat and have supported high-density sea otter populations for many years, thereby limiting the influence of confounding effects. Additionally, field studies of sea otters have been conducted in the past at both sites, providing us with historical data sets for temporal contrasts. The study commenced in November 2008 and continued until February 2012. During the course of this study a total of 98 sea otters were captured, radio-tagged, and monitored closely in the field for up to 4 years, and another 37 were captured and sampled but not radio tagged. From these study animals we collected demographic, behavioral, dietary and life history data using state-of-the-art telemetric methods; measured individual exposure to pathogens and other stressors; and performed rigorous necropsies and cause-of-death analyses for all study animals that died during the course of the study. These data were combined with similar data available from previous research projects, and together subjected to extensive analyses designed to test the above-listed hypotheses and identify the ultimate factors most important in limiting population growth of southern sea otters in central California.

Methods

Live sea otter captures

All of the study animals were captured using standardized methods that utilize closed-circuit scuba techniques (Ames et al. 1983). Briefly, shore spotters with high-powered spotting scopes relayed information about potential target animals to dive crews, which operated out of small 17-20ft skiffs. Divers worked in pairs and each diver operated a trap with a capacity for one adult sea otter, 2 juveniles, or a mother/pup pair. Otters must be resting (preferably sleeping) for this method to be successful. Divers used closed-circuit oxygen rebreathers and electric propulsion vehicles to maneuver the traps underneath the floating sea otters (Figure 3) and engulf them within the trap's net bag, which was then closed by a purse line. The divers kept the animal and trap on the surface until the skiff arrived and the otters could be transferred to a sliding-lid capture box. Once secure within the capture box, the dive crew quickly transported the otter to veterinary facilities for anesthetic immobilization, health assessments, bio-sampling, surgical implantation with VHF radio transmitters and time-depth recorders (TDRs), tagging, and release to their initial capture site (Ames et al. 1983, Williams and Siniff 1983, Monson et al. 2001). The entire procedure, from capture to release, generally took from 2-3 hours.

In the Big Sur study region, 4 separate capture events occurred. The initial capture of 40 sea otters took place from November 5-11, 2008. A second capture event was conducted one year later, from November 2-5, 2009, and added an additional 18 sea otters to the study. From September 22-27, 2010, a third Big Sur capture event took place in which 11 new sea otters were added to the study while 5 previously captured sea otters were recaptured and resampled (TDRs were removed at this time). A fourth and final Big Sur capture event took place from November 8-10, 2011, at which time 2 previously captured animals were recaptured and resampled (TDRs removed), and 3 additional new otters were captured and flipper tagged but not implanted with instruments. A total of 72 individual sea otters were captured in Big Sur. Of those 72 otters, 45 were implanted with VHF radio transmitters and TDRs. The remaining 27 otters were determined not to be good candidates for surgery due to a variety of reasons (palpably pregnant females, young pups, or extremely poor health). In Monterey, a total of 63 individuals were captured during multiple 1- to 2-day capture events occurring opportunistically between 2007 and 2011. Of those 63 sea otters, 53 individuals received VHF radios and TDRs. The remaining 10 otters were determined not to be good candidates for surgery due to a variety of reasons (palpably pregnant females, young pups, or extremely poor health). Summary information for all 135 animals captured for this study is provided in Table 1.

Processing, Sampling, Health Assessments and Tagging

Upon arrival at the veterinary station (the Animal Health Lab at the Monterey Bay Aquarium, an onshore mobile veterinary facility, or an onboard ship veterinary lab), otters were initially allowed to rest and cool within the capture box that was floated adjacent to a tender vessel. Following this 10-20 minute soak time, the animals were moved to the veterinary laboratory for sedation, physical examination, collection of morphometric data, and acquisition of a set of biological samples (Figure 4A). The sea otters were chemically immobilized using standard doses of fentanyl citrate (0.22-0.33 mg/kg) and midazolam hydrochloride (0.07-0.11 mg/kg) administered intramuscularly (Monson et al. 2001). Once adequately sedated, typically after 6-10 minutes, the sea otter was removed from the capture box and

processed. During the anesthetic period, vital signs (heart rate, respiratory rate, end-tidal CO_2, blood pressure, blood oxygen saturation, and rectal body temperature) were monitored at 5 minute intervals. Anomalies were corrected as indicated. Following completion of the examinations, measurements, sample collections, and tagging procedures, the effects of the opiate, fentanyl, were reversed with an intramuscular dose of naltrexone hydrochloride (1.1-1.65 mg/kg).

For all captured study animals, a suite of standardized health parameters were recorded, including: weight, length, girth, fur condition and degree of grizzle, tooth-wear (and tooth-based age estimates: Figure 4B), and general body condition (see Chapter 2). A set of biological samples were collected from each animal, as summarized in Table 2, for use in analyses that are reported on in subsequent chapters of this report: for example, blood samples were collected for clinical health assessments (Chapter 2) and gene expression analyses (Chapter 3), vibrissae for use in stable isotope analyses (Chapter 6), etc. The animals were then prepared for surgical implantation of the VHF radio transmitters (80 x 22 x 50mm, ~160g, Advanced Telemetry Systems, Isanti, MN) and time depth recorders (TDRs, 67 x 17 x 17mm, ~27g, Wildlife Computers, Redmond, WA) using standardized surgical techniques (Figure 5; Williams and Siniff 1983). The VHF transmitter is used for telemetric monitoring and re-location of the otter post-release, and emits a radio signal at one second intervals on a unique frequency band that can be detected from up to 10km away. The TDR is a bio-logging instrument that records depth (via scaled conversion of pressure transducer readings) and internal body temperature at 2 second intervals for a 2-3 year period, and allows for detailed analysis of sea otter dive behavior and time-activity budgets (see Chapter 4). Each otter was also tagged with unique color/number coded polyethylene "Temple Tags" (livestock ear tags, Temple, TX) on their hind flippers (2 tags per otter), to allow for visual identification by field observers, and received a coded, passive transponder chip, implanted subcutaneously in the inner thigh, for later identification should the temple tags be lost.

During recapture events of previously tagged study animals, the otters were once again anesthetized and a second surgical procedure performed in order to retrieve the archival TDR for data collection. At that time all health parameters were re-assessed, tissue samples taken, and any missing flipper tags were replaced before release.

Live sea otter surveillance

Tracking and observation of the study animals occurred on a daily basis for the duration of the study, which commenced with the first day of captures in Big Sur on November 1, 2008, and continued without interruption until February 1, 2012. Field personnel were able to conduct shore-based surveys of both study sites, locating study animals using standard telemetric protocols (triangulation of radio signal and visual identification: Figure 6A) identical to the methods used in previous sea otter tracking studies (Siniff and Ralls 1991, Tinker et al. 2006). Initial locations were determined by driving and/or hiking throughout the study area while using a radio receiver and antenna to scan for telemetry signals. When a signal was obtained, trackers used a combination of binoculars and high powered (80x) spotting scopes (Questar Inc., New Hope, PA) to positively identify individuals via their unique flipper tag color combination (Figure 6B). Once individual animals were located, observers recorded a variety of bio-geographic, behavioral, and environmental data including: GPS position, survival, reproductive status and instantaneous behavior. Aerial flights were conducted periodically to locate missing study animals.

A total of 38,941 resights were recorded for tagged otters as part of this study (Table 1). To monitor time-activity budgets, detailed focal-animal observations, termed "activity budgets," on behavior, diet, distance-to-shore and fine-scale movements (habitat use) were collected for each study animal on a rotating schedule. These activity budgets were conducted during intense 6-hour sessions, but spanned the entire 24 hour period of one day, with an emphasis on the daylight hours when direct observational data could be collected.

Observational foraging data were collected from radio-tagged sea otters (Figure 6C) following well-established protocols (Ralls et al. 1995, Watt et al. 2000, Estes et al. 2003, Tinker et al. 2008). Field observations were collected 7 days per week throughout the study period, with teams of 1–2 observers making systematic searches of the study areas and sequentially targeting specific animals for foraging observations. Study animals were initially located by radio signal using standard telemetric techniques, and then visually monitored from shore using a 50-80X spotting scopes. Foraging bouts (defined as contiguous sequences of feeding dives made by the focal otter) typically lasted 1-4 hours, and data were recorded throughout the entire bout or for as many dives as possible. The information recorded during these bouts includes 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), outcome of each dive (i.e. whether or not prey was captured), species of prey captured, number and size of prey items, per-item handling time (number of seconds required to handle and consume each item), whether or not tools were used to handle the prey, and ambient conditions (including sea-state, wind, etc.). Prey size was recorded as the estimated diameter of the shell or maximum body dimension (excluding appendages), categorized into 5cm size-classes. For observations where prey could not be reliably identified to species, the items in question were assigned to the lowest possible taxonomic unit. Any items that could not be reliably categorized to any taxonomic level were listed as "un-identified prey" (although size class and # items would still be recorded for such items). Additional information recorded by observers included numbers of prey items that were stolen by or from the focal animal and, in the case of females with dependent pups, the number of items that were shared with the pups. All data were entered into GPS-enabled hand-held computers in the field and later transferred to a central relational database.

Dead sea otter surveillance

Field personnel made every effort possible to locate and collect any animals that died during the course of the study. A number of animals disappeared from the focal study areas, and in these cases aerial telemetry was used in an attempt to locate them. If a carcass was located by shore-based trackers or by plane, researchers were dispatched by foot, boat, or kayak to retrieve the carcass, which was placed on ice and transported immediately to the California Department of Fish and Wildlife's Marine Wildlife Veterinary Care and Research Center (CDFW-MWVCRC) for necropsy. Every sea otter carcass retrieved was subject to detailed necropsies by a veterinary pathologist and followed established protocols (see Chapter 10). In addition to determining the primary and contributing cause(s) of death, the pathologist supervised the collection of tissue samples for a variety of otter and ecosystem health studies.

Data Analyses

After completion of all field and lab-based activities described above, data were compiled in a relational Microsoft Access database (the "Wild Sea Otter Database", or WSOD) and prepared for statistical analyses and syntheses. The following suites of analyses were conducted and are summarized in subsequent chapters of this report:

- **Health Assessments** (Chapter 2): objective and subjective data from the physical examinations and blood diagnostic analyses were summarized for all study animals and compared among study sites, to determine whether there were substantial differences in gross health or clinical pathology.
- **Gene Expression Analysis** (Chapter 3): data on genetic biomarkers that measure expression of genes associated with immune responses to specific physiological assaults were summarized for all study animals and compared among study sites. This analysis was used to assess whether there was spatial (across sites) or temporal (across years) variation in environmental stressors encountered by study animals.
- **Movements and Home Range** (Chapter 4): data on weekly movements, annual dispersal distance, and annual home range use was evaluated and compared among study sites, to measure the degree of population spatial structure and determine habitat use patterns, and whether they varied as a function of physical differences between sites.
- **Dive Behavior and Time-Activity Budgets** (Chapter 5): archival TDR data were analyzed to compare dive depth, duration, and other characteristics of foraging bouts between animals from different age/sex classes and study sites. Time-activity budgets were estimated from each TDR record and compared, to determine whether there were site differences or effects of reproductive status in terms of percent time spent feeding (foraging effort).
- **Foraging Ecology** (Chapter 6): A comprehensive analysis of diet, feeding behavior and foraging success (rate of energy gain) was conducted using both observational data and analysis of stable isotope ratios from collected vibrissae. Comparisons were made among study sites and with other populations around the North Pacific, to infer the degree of prey resource abundance and how it varied among sites.
- **Body Condition** (Chapter 7): Data on age-specific body length and body mass were analyzed using growth functions, and residuals from these functions were used to compute indices of relative body condition for each study site (BSR, MON, plus data from 4 other past studies). Differences among sites were analyzed with respect to variation in foraging success.
- **Survival and Reproduction** (Chapter 8): Bayesian non-parametric proportional hazards models were fit to data on adult and pup survival at all study sites (BSR, MON, plus data from 4 other past studies), in order to estimate instantaneous hazard rates (and thereby annual survival rates) and identify factors that cause variation in these rates, such as sex, age, site effects, reproductive status and body condition.

- **Epidemiology** (Chapter 9): Serological analysis was used to determine whether study animals were infected with the protozoan parasite *Toxoplasma gondii*. Infection rates were then analyzed using logistic regression models to determine significant risk factors for disease exposure. Potential risk factors evaluated included sex, age, site effects, diet composition and movement behavior.
- **Causes of Mortality** (Chapter 10): Data from necropsies of all tagged animals that died during the study (and whose carcasses were recovered) were analyzed in order to compare broad disease and mortality patterns among sea otters that were tagged in the Monterey and Big Sur coastal regions.
- **Syntheses and Conclusions** (Chapter 11): The key results from the proceeding chapters are combined and integrated in order to evaluate the overall degree of support (or lack thereof) for the 4 main hypotheses. We discuss the implications of these results for our understanding of the factors that are most important in affecting sea otter health and survival, and thus limiting population growth in central California. We then discuss what this means with respect to future strategies of management and conservation.

References

Ames, J. A., R. A. Hardy, and F. E. Wendell. 1983. Tagging materials and methods for sea otters, Enhydra lutris. California Fish and Game **69**:243-252.

Costa, D. P. and G. L. Kooyman. 1982. Oxygen consumption, thermoregulation, and the effect of fur oiling and washing on the sea otter, Enhydra lutris. Can. J. Zool. **60**:2761-2767.

Estes, J. A., E. M. Danner, D. F. Doak, B. Konar, A. M. Springer, P. D. Steinberg, M. T. Tinker, and T. M. Williams. 2004. Complex trophic interactions in kelp forest ecosystems. Bulletin of Marine Science **74**:621-638.

Estes, J. A., M. L. Riedman, M. M. Staedler, M. T. Tinker, and B. E. Lyon. 2003. Individual variation in prey selection by sea otters: Patterns, causes and implications. Journal of Animal Ecology **72**:144-155.

Estes, J. A., N. S. Smith, and J. F. Palmisano. 1978. Sea otter predation and community organization in the western aleutian islands, Alaska. Ecology **59**:822-833.

Jessup, D. A., M. A. Miller, C. Kreuder-Johnson, P. A. Conrad, M. T. Tinker, J. Estes, and J. A. K. Mazet. 2007. Sea otters in a dirty ocean. Javma-Journal of the American Veterinary Medical Association **231**:1648-1652.

Johnson, C. K., M. T. Tinker, J. A. Estes, P. A. Conrad, M. Staedler, M. A. Miller, D. A. Jessup, and J. A. K. Mazet. 2009. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. Proceedings of the National Academy of Sciences of the United States of America **106**:2242-2247.

Kenyon, K. W. 1969. The sea otter in the eastern Pacific Ocean. North American Fauna **68**:1-352.

Kreuder, C., M. A. Miller, D. A. Jessup, L. J. Lowenstine, M. D. Harris, J. A. Ames, T. E. Carpenter, P. A. Conrad, and J. A. K. Mazet. 2003. Patterns of mortality in southern sea otters (Enhydra lutris nereis) from 1998-2001. Journal of Wildlife Diseases **39**:495-509.

Kreuder, C., M. A. Miller, L. J. Lowenstine, P. A. Conrad, T. E. Carpenter, D. A. Jessup, and J. A. K. Mazet. 2005. Evaluation of cardiac lesions and risk factors associated with myocarditis and dilated cardiomyopathy in southern sea otters (Enhydra lutris nereis). American Journal of Veterinary Research **66**:289-299.

Miller, M. A., B. A. Byrne, S. S. Jang, E. M. Dodd, E. Dorfmeier, M. D. Harris, J. Ames, D. Paradies, K. Worcester, D. A. Jessup, and W. A. Miller. 2010a. Enteric bacterial pathogen detection in southern sea otters (Enhydra lutris nereis) is associated with coastal urbanization and freshwater runoff. Veterinary Research (Les Ulis) **41**:Article No.: 01.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, and P. A. Conrad. 2002. Coastal freshwater runoff is a risk

factor for Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). International Journal for Parasitology **32**:997-1006.

Miller, M. A., M. E. Grigg, W. A. Miller, H. A. Dabritz, E. R. James, A. C. Melli, A. E. Packham, D. Jessup, and P. A. Conrad. 2007. Toxoplasma gondii and Sarcocystis neurona infections of pacific coastal sea otters in California, USA: evidence for land-sea transfer of biological pathogens. Journal of Eukaryotic Microbiology **54**:48S-49S.

Miller, M. A., R. M. Kudela, A. Mekebri, D. Crane, S. C. Oates, M. T. Tinker, M. Staedler, W. A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D. A. Jessup. 2010b. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS One **5**:Article No.: e12576.

Monson, D. H., C. McCormick, and B. E. Ballachey. 2001. Chemical anesthesia of northern sea otters (Enhydra lutris): Results of past field studies. Journal of Zoo & Wildlife Medicine **32**:181-189.

Morrison, P., M. Rosenmann, and J. A. Estes. 1974. Metabolism and thermoregulation in the sea otter. Physiol. Zool. **47**:218-229.

Ralls, K., B. B. Hatfield, and D. B. Siniff. 1995. Foraging patterns of California sea otters as indicated by telemetry. Canadian Journal of Zoology **73**:523-531.

Siniff, D. B. and K. Ralls. 1991. Reproduction, survival and tag loss in California sea otters. Marine Mammal Science **7**:211-229.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., D. F. Doak, J. A. Estes, B. B. Hatfield, M. M. Staedler, and L. Bodkin James. 2006. Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. Ecological Applications **16**:2293-2312.

Tinker, M. T., P. R. Guimarães, M. Novak, F. M. D. Marquitti, J. L. Bodkin, M. Staedler, G. Bentall, and J. A. Estes. 2012. Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecology Letters **15**:475-483.

Watt, J., D. B. Siniff, and J. A. Estes. 2000. Inter-decadal patterns of population and dietary change in sea otters at Amchitka Island, Alaska. Oecologia (Berlin) **124**:289-298.

Williams, T. and D. B. Siniff. 1983. Surgical implantation of radio telemetry devices in the sea otter. Journal of the American Veterinary Medical Association **11**:1290-1291.

Yeates, L. C., T. M. Williams, and T. L. Fink. 2007. Diving and foraging energetics of the smallest marine mammal, the sea otter (Enhydra lutris). Journal of Experimental Biology **210**:1960-1970.

Tables

Table 1. Summary information for sea otters captured, sampled, tagged and monitored as part of this study. Fields shown for each animal are the study site the otter was captured at, whether or not a VHF transmitter was applied, the sex and identification number, number of times the individual was captured and sampled, the date last captured, the capture site latitude and longitude, age class (juvenile, sub-adult, adult or old adult) and age estimate at time of capture (in years), and number of resights collected.

Study Site	VHF trans?	SEX	BRD_NO	# samples	Last Capture	Capture Latitude	Capture Longitude	Age class	Age est.	# resights
BSR	Yes	F	1067-08	2	23-Sep-10	N 35˚57.129	W 121˚29.767	a	10	639
BSR	Yes	F	1068-08	1	05-Nov-08	N 35˚54.601	W 121˚28.354	a	5	2
BSR	Yes	F	1069-08	1	05-Nov-08	N 35˚54.601	W 121˚25.654	a	8	163
BSR	Yes	F	1070-08	1	05-Nov-08	N 36˚00.660	W 121˚33.600	a	na	72
BSR	Yes	F	1073-08	1	06-Nov-08	N 36˚02.463	W 121˚35.206	a	4	450
BSR	Yes	F	1074-08	2	24-Sep-10	N 36˚07.829	W 121˚39.366	a	10	512
BSR	Yes	F	1075-08	1	06-Nov-08	N 36˚02.057	W 121˚35.015	s	3	608
BSR	Yes	F	1079-08	1	07-Nov-08	N 36˚02.489	W 121˚35.191	s	1.5	638
BSR	Yes	F	1081-08	1	07-Nov-08	N 36˚05.628	W 121˚37.424	a	10	196
BSR	Yes	F	1083-08	1	07-Nov-08	N 36˚09.218	W 121˚09.218	a	8	113
BSR	Yes	F	1084-08	1	07-Nov-08	N 36˚04.978	W 121˚37.094	a	6	152
BSR	Yes	F	1086-08	1	07-Nov-08	N 36˚06.091	W 121˚37.685	s	2	609
BSR	Yes	F	1088-08	1	10-Nov-08	N 36˚07.435	W 121˚38.688	a	4	622
BSR	Yes	F	1089-08	1	06-Nov-08	N 36˚02.057	W 121˚35.015	a	6	6
BSR	Yes	F	1090-08	1	03-Nov-08	N 36˚56.605	W 121˚29.074	o	12	56
BSR	Yes	F	1097-08	2	24-Sep-10	N 36˚11.291	W 121˚42.715	a	6	482
BSR	Yes	F	1098-08	1	11-Nov-08	N 36˚11.718	W 121˚43.431	s	2	279
BSR	Yes	F	1103-08	2	09-Nov-11	N 36˚02.744	W 121˚35.313	a	10	215
BSR	Yes	F	1105-08	1	11-Nov-08	N 36˚10.029	W 121˚41.213	a	4	2
BSR	Yes	F	1106-08	1	11-Nov-08	N 36˚10.041	W 121˚41.234	a	9	47
BSR	Yes	F	1135-09	2	09-Nov-11	N 36˚04.416	W 121˚36.752	a	5	1
BSR	Yes	F	1136-09	1	02-Nov-09	N 36˚ 2.170	W 121˚35.025	a	4	459
BSR	Yes	F	1139-09	1	03-Nov-09	na	na	na	4	27
BSR	Yes	F	1141-09	2	25-Sep-10	N 36˚06.629	W 121˚37.982	a	6	17
BSR	Yes	F	1142-09	2	24-Sep-10	N 36˚09.221	W 121˚40.154	a	4.5	323
BSR	Yes	F	1146-09	1	04-Nov-09	N 36˚04.503	W 121˚36.801	j	1	17
BSR	Yes	F	1148-09	1	05-Nov-09	N 36˚05.625	W 121˚37.558	a	5	76
BSR	Yes	F	1159-10	1	22-Sep-10	N 36˚00.365	W 121˚31.043	a	5	250
BSR	Yes	F	1161-10	1	22-Sep-10	N 36˚02.443	W 121˚35.182	a	4.5	295
BSR	Yes	F	1162-10	1	23-Sep-10	N 36˚05.007	W 121˚35.773	a	3.5	254
BSR	Yes	F	1166-10	1	25-Sep-10	N 36˚06.768	W 121˚38.293	a	4	294
BSR	Yes	F	1167-10	1	25-Sep-10	N 36˚06.754	W 121˚37.994	s	3	317
BSR	Yes	F	1168-10	1	27-Sep-10	N 36˚58.569	W 121˚29.502	a	6	246
BSR	Yes	F	1169-10	1	27-Sep-10	N 36˚54.887	W 121˚28.597	s	3	257

Table 1. Summary information for all study animals, continued (page 2 of 4)

Study Site	VHF trans?	SEX	BRD_NO	# samples	Last Capture	Capture Latitude	Capture Longitude	Age class	Age est.	# resights
BSR	Yes	M	1066-08	1	05-Nov-08	N 35˚57.129	W 121˚29.767	a	10	31
BSR	Yes	M	1091-08	1	03-Nov-08	N 36˚04.595	W 121˚36.662	a	9	7
BSR	Yes	M	1093-08	1	06-Nov-08	N 36˚02.463	W 121˚35.206	a	8	135
BSR	Yes	M	1094-08	1	03-Nov-09	N 36˚05.628	W 121˚37.424	a	8	325
BSR	Yes	M	1095-08	1	03-Nov-08	N 36˚00.660	W 121˚33.600	a	6	256
BSR	Yes	M	1096-08	1	03-Nov-08	N 36˚01.490	W 121˚34.693	a	9	584
BSR	Yes	M	1099-08	1	03-Nov-08	N 36˚06.091	W 121˚37.685	a	6	547
BSR	Yes	M	1137-09	1	03-Nov-09	N 36˚ 1.754	W 121˚34.834	a	8	367
BSR	Yes	M	1143-09	1	04-Nov-09	N 36˚06.764	W 121˚38.086	a	7	475
BSR	Yes	M	1147-09	1	04-Nov-09	N 36˚04.503	W 121˚36.807	s	2	410
BSR	Yes	M	1165-10	1	25-Sep-10	N 36˚06.750	W 121˚37.996	a	7	72
BSR	No	F	1071-08	1	05-Nov-08	N 36˚59.378	W 121˚30.035	a	4	8
BSR	No	F	1072-08	1	05-Nov-08	N 35˚59.378	W 121˚30.035	a	6	79
BSR	No	F	1076-08	1	06-Nov-08	N 36˚07.881	W 121˚39.104	a	9	8
BSR	No	F	1077-08	1	07-Nov-08	N 36˚07.881	W 121˚39.115	a	4	1
BSR	No	F	1078-08	1	06-Nov-08	N 36˚07.881	W 121˚39.115	o	11	13
BSR	No	F	1080-08	1	07-Nov-08	N 36˚02.489	W 121˚35.191	a	5	17
BSR	No	F	1082-08	1	03-Nov-08	N 36˚09.210	W 121˚40.262	a	6	9
BSR	No	F	1085-08	1	08-Nov-08	N 36˚08.711	W 121˚39.158	a	6	116
BSR	No	F	1087-08	1	08-Nov-08	na	na	na	na	624
BSR	No	F	1092-08	1	11-Nov-08	N 36˚04.275	W 121˚36.723	a	8	2
BSR	No	F	1104-08	1	11-Nov-08	N 36˚06.026	W 121˚37.609	a	6	108
BSR	No	F	1129-08	1	11-Nov-08	N 36˚10.029	W 121˚41.213	p	0.4	1
BSR	No	F	1138-09	1	03-Nov-09	na	na	a	4	75
BSR	No	F	1140-09	1	03-Nov-09	N 36˚06.629	W 121˚37.982	a	9	367
BSR	No	F	1144-09	1	04-Nov-09	N 36˚ 1.456	W 121˚34.786	a	4	46
BSR	No	F	1145-09	1	04-Nov-09	N 36˚02.428	W 121˚57.976	a	7	3
BSR	No	F	1149-09	1	05-Nov-09	N 36˚05.625	W 121˚37.558	p	na	520
BSR	No	F	1150-09	1	05-Nov-09	N 36˚06.613	W 121˚37.980	a	5	37
BSR	No	F	1151-09	1	05-Nov-09	N 36˚06.613	W 121˚37.981	a	9	86
BSR	No	F	1160-10	1	22-Sep-10	N 35˚59.494	W 121˚30.022	a	8	22
BSR	No	F	1163-10	1	24-Sep-10	N 36˚08.130	W 121˚39.294	p	0.38	3
BSR	No	F	1164-10	1	24-Sep-10	N 36˚09.221	W 121˚40.175	a	8	112
BSR	No	F	1177-11	1	08-Nov-11	N 36˚06.153	W 121˚37.543	o	12	7
BSR	No	F	1178-11	1	08-Nov-11	N 36˚06.153	W 121˚37.543	a	7	13
BSR	No	F	1179-11	1	10-Nov-11	N 36˚06. 75	W 121˚38.029	a	4	19
BSR	No	M	1100-08	1	11-Nov-08	na	na	na	na	4
BSR	No	M	1131-08	1	11-Nov-08	N 36˚11.273	W 121˚42.680	j	0.5	1

Table 1. Summary information for all study animals, continued (page 3 of 4)

Study Site	VHF trans?	SEX	BRD_NO	# samples	Last Capture	Capture Latitude	Capture Longitude	Age class	Age est.	# resights
MON	Yes	F	1000-05	5	11-Jan-12	N 36˚37.885	W 121˚55.191	o	11	877
MON	Yes	F	1011-06	2	28-Jul-10	N 36˚34.704	W 121˚58.641	a	9.5	248
MON	Yes	F	1032-07	5	15-Dec-10	N 36˚37.172	W 121˚53.903	a	9	594
MON	Yes	F	1033-07	2	15-Jul-08	N 36˚37.088	W 121˚53.779	a	6	220
MON	Yes	F	1034-07	1	24-Sep-07	N36˚54.787	W 121˚93.930	s	3	684
MON	Yes	F	1037-07	3	27-Oct-10	N 36˚37.767	W 121˚55.081	a	9	296
MON	Yes	F	1038-07	3	08-Sep-11	N 36˚34.704	W 121˚58.641	a	6	412
MON	Yes	F	1042-07	2	29-Jul-10	N 36˚34.704	W 121˚58.641	a	7	128
MON	Yes	F	1043-07	1	26-Sep-07	N 36˚34.072	W 121˚57.087	a	9.5	88
MON	Yes	F	1044-07	1	26-Sep-07	N 36˚34.072	W 121˚57.087	a	7	169
MON	Yes	F	1045-07	2	10-Sep-08	N 36˚34.517	W 121˚58.502	o	11	206
MON	Yes	F	1046-07	2	10-Jun-09	N 36˚34.549	W 121˚58.494	o	11	210
MON	Yes	F	1049-07	1	27-Sep-07	N 36˚36.635	W 121˚57.964	a	5.5	568
MON	Yes	F	1050-07	1	27-Sep-07	N 36˚34.556	W 121˚58.429	a	5.5	158
MON	Yes	F	1051-07	1	27-Sep-07	N 36˚34.556	W 121˚58.429	a	4	279
MON	Yes	F	1052-07	1	27-Sep-07	N 36˚34.556	W 121˚58.429	a	3.5	508
MON	Yes	F	1053-07	4	13-Feb-12	N 36˚36.400	W 121˚53.489	a	7.5	177
MON	Yes	F	1054-07	2	04-Sep-08	N 36˚36.773	W 121˚53.785	a	5	722
MON	Yes	F	1056-08	4	19-Apr-12	N 36˚36.754	W 121˚53.716	a	5	327
MON	Yes	F	1057-08	3	02-Sep-11	N 36˚37.339	W 121˚54.118	a	3.11	34
MON	Yes	F	1064-08	2	24-Aug-09	N 36˚38.527	W 121˚56.194	s	1.11	205
MON	Yes	F	1109-09	3	03-Feb-11	N 36˚37.450	W 121˚55.260	a	10	628
MON	Yes	F	1110-09	2	27-Oct-10	N 36˚36.742	W 121˚53.714	a	8	682
MON	Yes	F	1113-09	2	12-Jan-12	N 36˚34.704	W 121˚58.627	a	9	470
MON	Yes	F	1115-09	2	01-Sep-11	N36˚37.842	W -121˚55.114	a	9	629
MON	Yes	F	1117-09	2	28-Oct-10	N 36˚37.860	W 121˚54.931	a	4	499
MON	Yes	F	1119-09	1	09-Jun-09	N 36˚35.558	W 121˚57.973	s	2	434
MON	Yes	F	1122-09	3	01-Sep-11	N 36˚38.086	W 121˚55.260	a	8	583
MON	Yes	F	1123-09	2	02-Sep-11	N 36˚38.118	W 121˚55.322	a	6	576
MON	Yes	F	1124-09	1	16-Jun-09	N 36˚36.634	W 121˚57.243	s	3	563
MON	Yes	F	1125-09	2	11-Nov-12	N 36˚36.202	W 121˚52.883	a	8.5	531
MON	Yes	F	1126-09	3	29-Jul-10	N 36˚38.021	W 121˚54.651	a	10	510
MON	Yes	F	1127-09	3	07-Jun-10	N 36˚37.059	W 121˚53.873	a	7	525
MON	Yes	F	1128-09	2	28-Jul-10	N 36˚34.704	W 121˚58.641	a	6.5	492
MON	Yes	F	1134-09	3	03-Feb-11	N 36˚37.400	W 121˚53.500	a	5.5	603
MON	Yes	F	1153-10	2	19-Apr-12	N 36˚36.738	W 121˚53.727	s	5	345
MON	Yes	F	1155-10	1	01-Feb-10	N 36˚34.656	W 121˚58.719	a	6	62
MON	Yes	F	1157-10	2	02-Sep-11	N 36˚38.064	W 121˚55.265	a	8	310
MON	Yes	F	1158-10	2	12-Jan-12	N 36˚38.399	W 121˚56.065	a	10	297

MON	Yes	F	946-03	9	11-Feb-11	N 36˚36.981	W 121˚53.786	a	10	1557

Table 1. Summary information for all study animals, continued (page 4 of 4)

Study Site	VHF trans?	SEX	BRD_NO	# samples	Last Capture	Capture Latitude	Capture Longitude	Age class	Age est.	# resights
MON	Yes	M	1024-06	2	09-Jun-09	N 36˚33.873	W 121˚56.571	a	11	375
MON	Yes	M	1030-06	3	16-Dec-10	N 36˚37.967	W 121˚55.187	a	10	1008
MON	Yes	M	1065-08	2	22-Apr-09	N 36˚62.180	W 121˚90.255	a	7	4
MON	Yes	M	1108-09	2	09-Jun-10	N 36˚37.870	W 121˚55.203	a	10.5	275
MON	Yes	M	1114-09	4	02-Sep-11	N 36˚37.086	W 121˚54.054	a	9	738
MON	Yes	M	1116-09	1	28-May-09	N 36˚38.141	W 121˚56.697	a	4	33
MON	Yes	M	1118-09	1	28-May-09	N 36˚34.675	W 121˚58.626	a	5	35
MON	Yes	M	1156-10	2	11-Feb-11	N 36˚36.753	W 121˚53.715	a	7.5	526
MON	Yes	M	1170-10	1	16-Dec-10	N 36˚37.357	W 121˚54.063	a	4.5	241
MON	Yes	M	1172-11	1	03-Feb-11	N 36˚38.058	W 121˚55.146	a	7	27
MON	Yes	M	945-03	4	10-Jun-09	N 36˚38.282	W 121˚56.160	a	8	440
MON	Yes	M	985-04	5	01-Sep-11	N 36˚37.539	W 121˚54.755	a	11	547
MON	Yes	M	998-05	2	26-Feb-08	N 36˚37.526	W 121˚54.689	a	8	1225
MON	No	F	1028-06	3	14-Jul-08	N 36˚34.605	W 121˚58.745	s	4.5	750
MON	No	F	1111-09	2	08-Sep-11	N 36˚36.754	W 121˚53.710	a	8	186
MON	No	F	1112-09	1	24-Apr-09	N 36˚.576	W 121˚.979	a		17
MON	No	F	1120-09	1	09-Jun-09	N 36˚34.556	W 121˚58.497	a	8	72
MON	No	F	1130-08	1	11-Nov-08	na	na	a	na	1
MON	No	F	1132-09	1	17-Jun-09	N 36˚34.704	W 121˚58.641	a	6	47
MON	No	F	1133-09	1	08-Aug-09	N 36˚37.163	W 121˚53.982	a	8	66
MON	No	F	1154-10	1	01-Feb-10	N 36˚34.656	W 121˚58.719	a	5	4
MON	No	F	941-03	2	22-Apr-09	na	na	j	7	610
MON	No	M	1121-09	1	10-Jun-09	N 36˚34.549	W 121˚58.494	a	5	102

Table 2. Biological Samples collected from captured sea otters

Sample Type	Use
Blood	Hematology, clinical chemistry, infectious disease monitoring, biomarkers, contaminants
External swabs (integument, oral cavity, rectum, genital orifice	Infectious disease (bacterial, fungal, parasitic, viral), contaminants, genetics
Saliva	Hormonal assays
Feces	Diet assessment, infectious disease (bacterial, fungal, parasitic, viral), contaminants, biotoxin, hormonal assay
Adipose tissue	Fatty acids, contaminants
Liver biopsies	Histopathology, toxicology, contaminants, genetics, chemical analysis (i.e., Vit A analysis)
Skin plugs	Genetics
Vibrissae	Stable isotope
Tooth	Cementum aging
Fur	Hormonal assays, toxins, contaminants

Figures

Figure 1. Map of the central California coast, illustrating the current distribution of the southern sea otter and spatial variation in relative population density: sea otter habitat that is currently utilized by sea otters is shown as a colored band along the coast, with color coding corresponding to the number of sea otters per 500m of coast (as shown in the Legend). The locations of the two study sites for the current project are delineated with black squares.

Figure 2. Photographs of segments of the coastline within each of the two study sites. A) The Monterey study site along cannery row in Monterey, illustrating the high potential for human impacts on the nearshore marine ecosystem in this area. B) The Big Sur study site near Lopez Pt. in the center of the Big Sur study site, illustrating the minimal potential for human impacts on the nearshore marine ecosystem in this area.

Figure 3. Photographs illustrating the scuba-based methods used to capture sea otters. A) A pair of divers travelling underwater with the Wilson traps propelled ahead of them using electric propulsion scooters. B) Resting sea otters being captured within the Wilson traps.

Figure 4. Photographs illustrating the health assessments and biosampling of anesthetized sea otters. A) Veterinarians conduct careful physical exams and collect blood samples; B) A veterinarian examines the dental and oral health of a sea otter, recording all incidences of tooth wear, breakage, and the presence of oral lesions.

Figure 5. Photographs illustrating the surgical procedure used to implant telemetry tracking devices in study animals. A) A veterinary team performs abdominal surgery on an anaesthetized sea otter. B) The Time Depth Recorder (TDR) used to record diving behavior and internal temperature. C) The VHF transmitter used locate the study animals in the wild using standard wildlife telemetry techniques.

Figure 6. Photographs illustrating field monitoring of tagged study animals. A) A field observer uses a radio receiver/antenna to localize source of the VHF signal from a study animal, and a 50-80x telescope to visually monitor the study animal. B) A study animal about to dive shows its uniquely-colored flipper tags, used for visual identification. C) The view of a study animal from through a Questar spotting scope, demonstrating the potential for a trained observer to visually identify prey and record feeding behavior (in this case a mussel of size “1c” (3.5–5cm) is being handled and consumed).

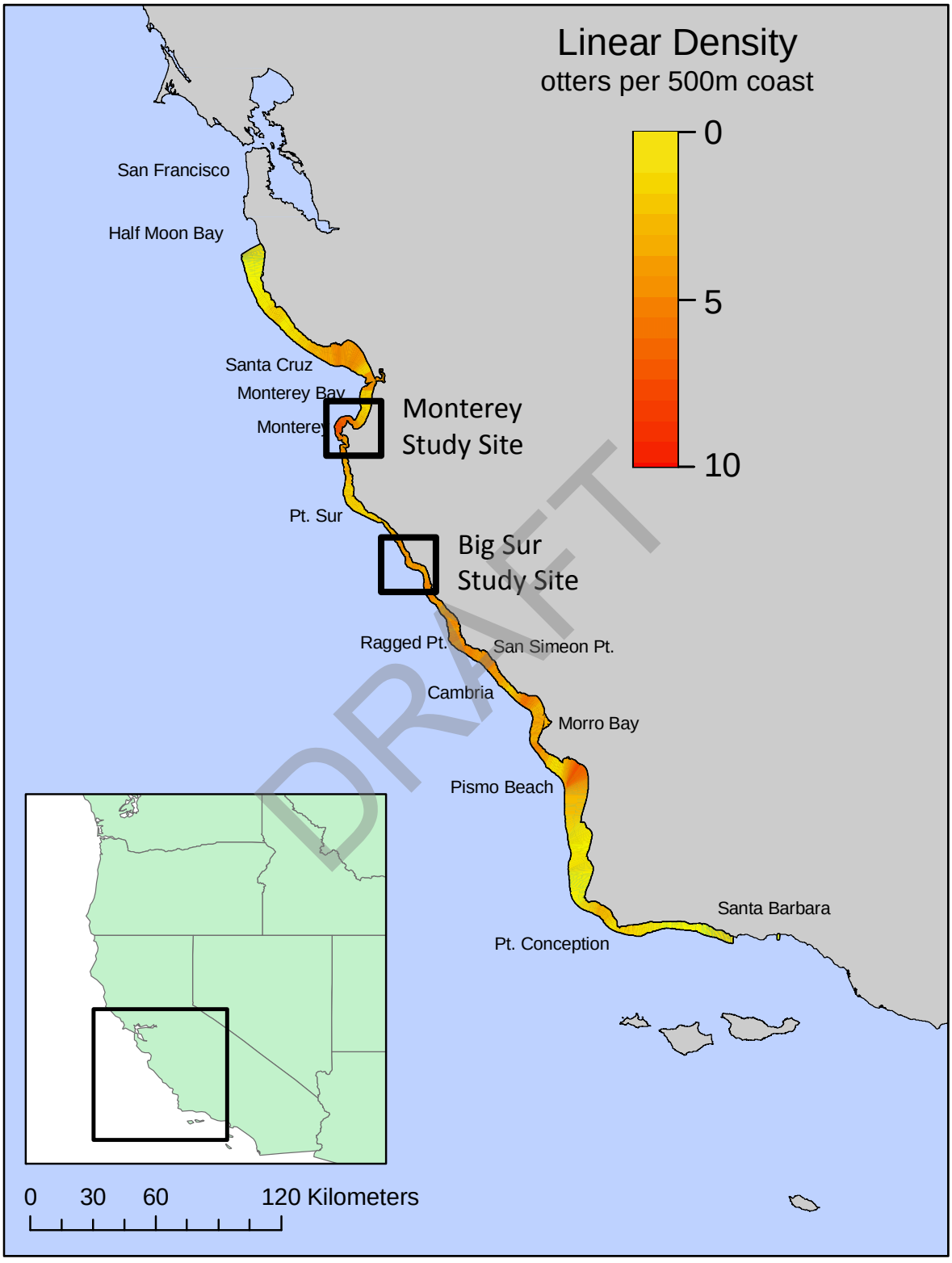

Linear Density
otters per 500m coast
0
5
10
San Francisco
Half Moon Bay
Santa Cruz
Monterey Bay
Monterey
Monterey
Study Site
Pt. Sur
Big Sur
Study Site
Ragged Pt.
San Simeon Pt.
Cambria
Morro Bay
Pismo Beach
Santa Barbara
Pt. Conception
DRAFT
0 30 60 120 Kilometers

Figure 1

Figure 2

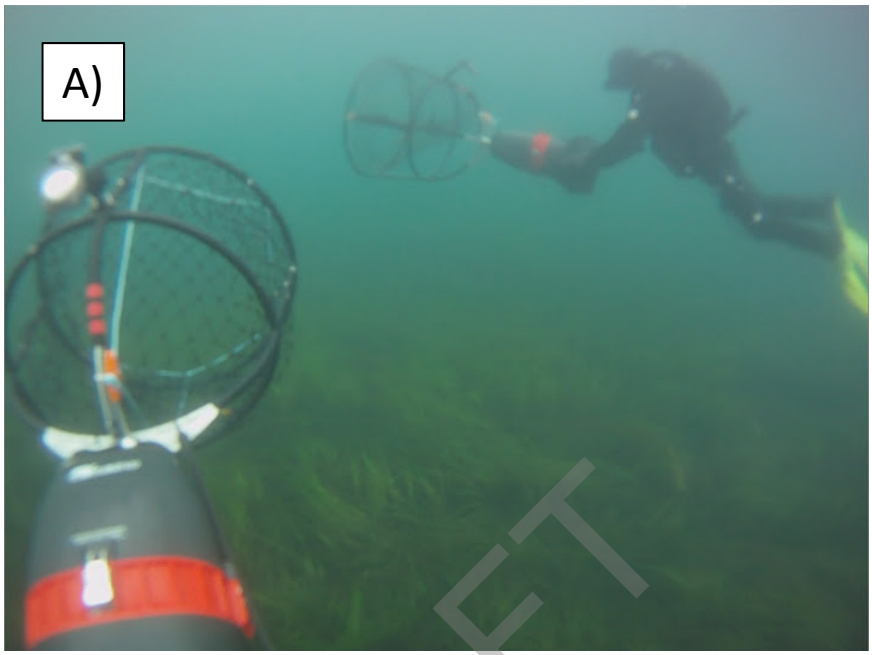


Figure 3

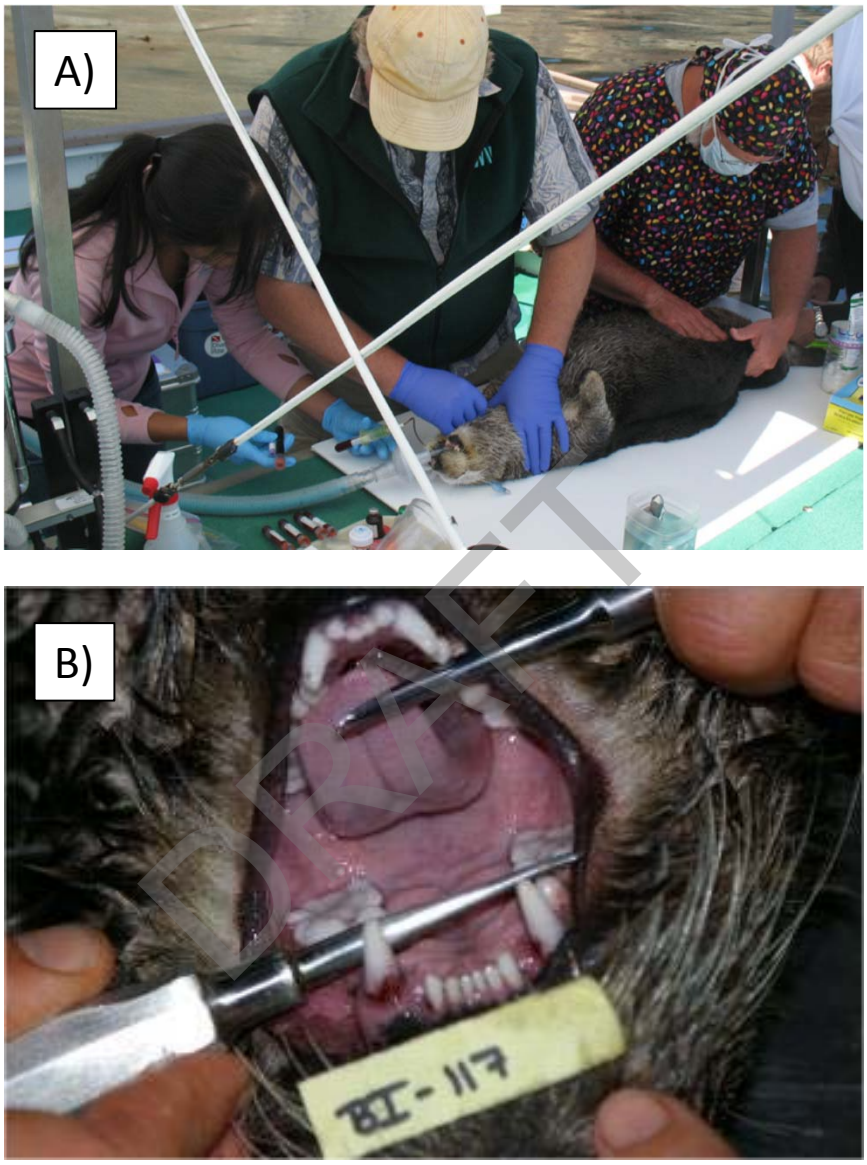
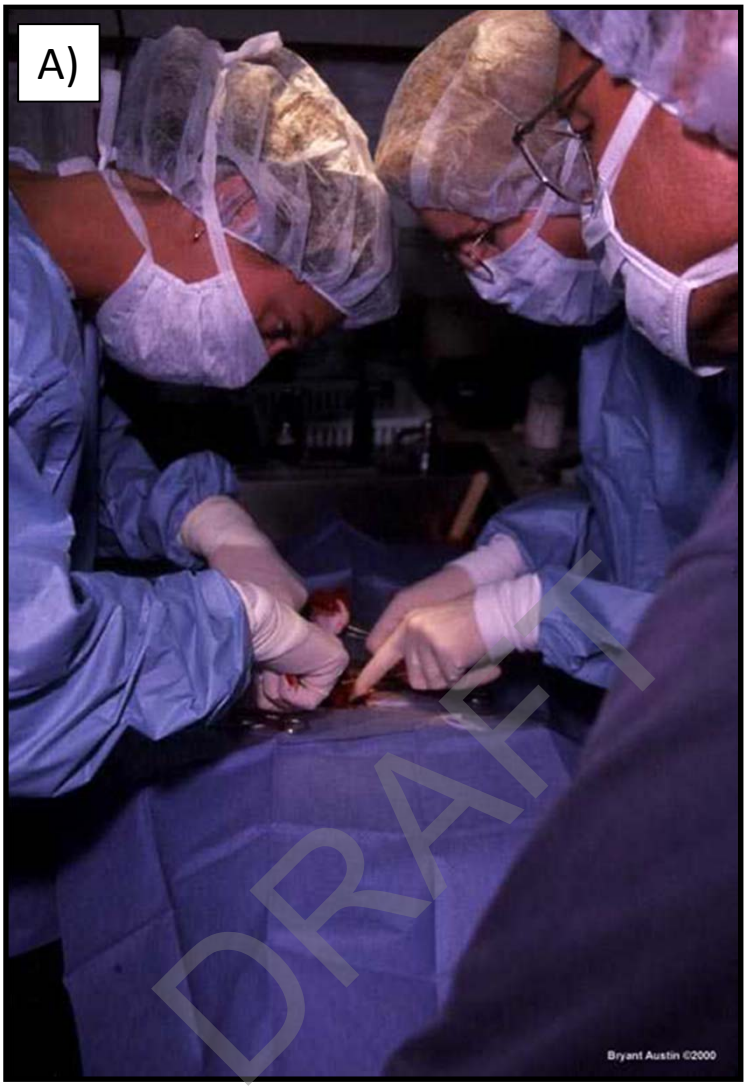


Figure 4



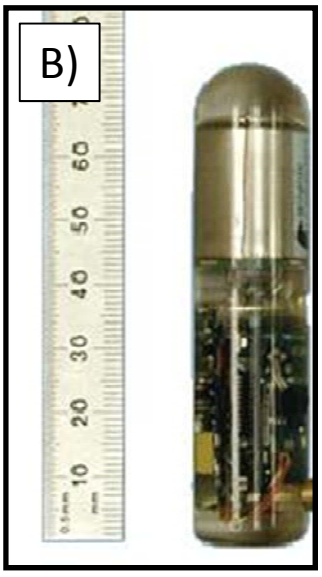
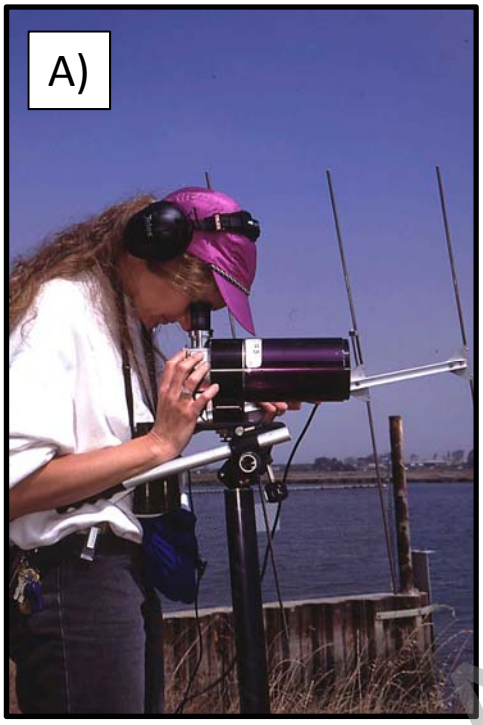


Figure 5



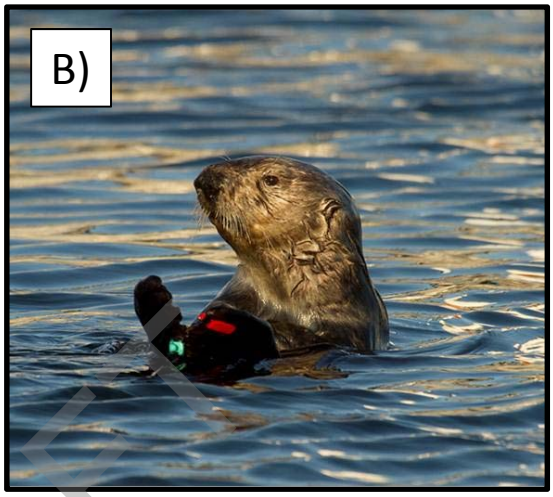


Figure 6

Chapter 2. Sea Otter Health Assessments at Big Sur and Monterey

Michael J. Murray[1] (DVM) and Tim Tinker[2]
[1]Director of Veterinary Services, Monterey Bay Aquarium, Monterey, CA
[2]U.S. Geological Survey Western Ecological Research Center, Santa Cruz, CA

Introduction

One of the most readily apparent questions to ask when comparing populations of sea otters in "pristine" habitat with those from an area with significant anthropogenic impact is "are the animals from the pristine habitat healthier?" We took advantage of the opportunity to conduct thorough examinations of sea otters from both geographic locations in order to answer this question. The determination of health status was made by integrating findings identified in complete physical examination of sedated sea otters with the results of complete blood count and serum chemistry panel analyses of blood samples submitted to a veterinary reference laboratory (IDEXX).

The nature of this study mandated making multiple changes in the clinical paradigm from the one traditionally practiced in domestic animal medicine. Much of the veterinarian's training and experience is found in the health management of individual patients. Assessments are made of numerous aspects of holistic picture, including clinical history, physical findings, and a myriad of laboratory and other ancillary tests of various body organs and systems, as well as specific infectious and non-infectious diseases. These are then accumulated and considered en masse, a health assessment is made, appropriate therapeutic measures are recommended, and a prognosis is given. When dealing with populations of free-ranging wildlife, the "patient" becomes the population, and the various "organs and body systems" are individual animals within the population. Health of this "patient" is not simply a statistical analysis of the incidence of health and/or disease within the population, but must also take into consideration a number of external factors, such as reproductive rates, resource availability, habitat use, and the effects of anthropogenic influences.

There are significant shortcomings in this approach to applying individual animal data to population level health assessment. Similarly structured epidemiological studies in human medicine are bolstered by exponentially larger sample sizes numbering in the thousands instead of the approximately 150 individual otters in this study. Obviously a larger "*n*" results in a more robust statistical interpretation of the data. Another deficiency common to nearly all wildlife health studies is the reliance on single samples, thereby losing the perspective that one gains by evaluating trends in observed and measured data over a longer time frame. Despite this limitation, a good faith effort has been made to interpret data as-is, without extrapolation towards potential future health status.

Nearly all spectra of infectious and non-infectious diseases have been recognized in either free-ranging or captive sea otters. The use of serologic testing to look for historic exposure or infection or antigen capture methods to evaluate the presence of pathogens or parts thereof in samples was considered, but a decision was made to not include such tests in the general health assessment (however, refer to Chapter 9 for a targeted epidemiological analysis of *Toxoplasma gondii* infections based on serology).

This decision reflected uncertainty about which pathogens should be evaluated in a comprehensive health screening, which tests are validated in sea otters, what constitutes a "positive" test, and whether a positive test was consistent with disease. On a more practical level, funding limitations prohibited broad-based serological screening for pathogen exposure. Instead, reliance was placed upon non-specific molecular genetic tests (gene expression analyses: see Chapter 3) to provide insight into the role of infectious disease in the health of sampled sea otters. A similar evaluation and decision was reached with respect to testing for the myriad of potential contaminants in sea otters.

Limitations notwithstanding, complete physical examinations from "nose to toes" were performed on all sea otters using traditional clinical methods. Complete blood counts and serum chemistry panels submitted to a veterinary reference laboratory were evaluated using published reference ranges, bearing in mind the limitations associated with the interpretation of each parameter. Data from each sea otter was interpreted independently and health scores assigned. It is the database of health scores that was utilized in the assessment of population health between Monterey and Big Sur populations.

Methods

Once captured using standard techniques, sea otters were transported to the appropriate veterinary laboratory, the Animal Health Lab at the Monterey Bay Aquarium, an onshore mobile veterinary facility, or an onboard ship veterinary lab. Prior to further handling, otters were allowed to rest within the capture box that was allowed to float adjacent to a tender vessel. This step served two major purposes. First, the movement of water in and out of the box through the capture box's ventilation holes facilitated thermoregulation, as otters held out of water during transit from capture site to lab site often developed some degree of hyperthermia. Secondly, this step allowed the animal to float inside of the box, a darkened, quiet environment, generally grooming and resting. This opportunity to "calm down" appears to reduce the effects of the catecholamine response from the initial capture. These endogenous hormones, epinephrine and norepinephrine, may have untoward effects on cardiac function during anesthesia and are known to artifacturally alter clinical pathology data.

Following this 10-20 minute soak time, the animals were moved to the veterinary laboratory for sedation, physical examination, collection of morphometric data, and acquisition of a set of biological specimens (see Chapter 1). The physical examination was conducted in a standardized fashion following traditional clinical methods starting at the nose and ending at the tip of the tail. Particular attention was paid to the oral cavity examination; a complete dental inventory and evaluation was performed and results recorded on dentition-specific data sheets. Other physical anomalies were recorded on standard data sheets by the attending clinician.

Blood samples were collected from the left or right external jugular vein into BD Vacutainer tubes using 19 or 21 gauge butterfly winged blood collection sets. Red/gray-topped serum separator tubes were used for serum chemistry panels and lavender-topped EDTA tubes for the complete blood counts. Blood smears were made directly from needles or syringes without anticoagulant effects. Serum was separated from the cellular components of the blood within 30 minutes of collection using a Clay Adams TRIAC centrifuge or its equivalent at a speed of 3500 RPM (1500 RCF) for 5 minutes. After the serum was decanted from the tube, an aliquot of 1.0 ml was refrigerated or frozen until time of transport to

the reference laboratory. The blood collected into the lavender-topped tube for the CBC was inverted multiple times to assure adequate mixing of the anticoagulant and then refrigerated until shipment. Blood smears were air dried placed in slide shipping containers and accompanied the serum and whole blood to the lab.

CBC and serum chemistry evaluations were done by Idexx Laboratories, a nation-wide veterinary reference laboratory used by many companion animal veterinary practices. Samples were taken by Idexx courier from a drop box to the reference lab for testing. A Sysmex XT-2000-I Automated Hematology Analyzer was used to perform the automated CBC. Serum chemistry parameters were analyzed on an Olympus Model AU 5400 Chemistry Immuno Analyzer.

Evaluation of individual sea otter health was made by retrospectively reviewing objective and subjective data from the following aspects of the examinations: physical exam, dental exam (incisors, canines, premolar/molars), packed cell volume (PCV), hemoglobin (Hgb), total white blood cell count (WBC), differential white cell count, serum chemistry panel, and subjective weight assessment. These specific parameters were selected not only because they reflect a paradigm for a typical health assessment of apparently healthy companion animals utilized in traditional clinical veterinary medicine, and it is consistent with the protocol utilized in the Pacific Nearshore Project funded by the U.S. Geological Survey, Ecosystem Mission and DOI on the Landscape Initiative to investigate biotic response to environmental variation in nearshore habitats of the northeast Pacific Ocean. The scoring matrix (Table 1) is a subjective, qualitative ranking of status when compared to "normal" with higher score reflecting increased deviation. A higher score is a "less normal" appearance.

Interpretation of clinical pathology data was done by integrating the clinical interpretation of the data by comparison to published reference ranges as well as the subjective, holistic evaluation of the entire suite of test results derived from the panels. It is important to note that the second evaluation, the complete clinical pathology data sets, was a comparison between the two populations, Big Sur and Monterey, not between either or both of these populations and the reference ranges. To do so is not only outside of the scope of this project, but is subject to valid criticism, as the published reference ranges often involve different subspecies of sea otter, varying sample handling methods, and different analysis equipment - all of these are potential sources for variability that were not addressed in this study.

Our overall goal was to answer two simple questions: 1) are there overall differences in sea otter health between study sites; and 2) if differences do exist, which health parameters are most important in distinguishing between sites? The multivariate nature of the data sets precluded a univariate statistical approach to analyses; instead, we used multivariate Discriminant Analysis (DA) to compare Big Sur and Monterey study animals with respect to health parameters. We conducted DA on both the matrix of subjective health scores and the matrix of clinical pathology results: for each dataset, the groups compared were sex/study site combinations (Big Sur females, Big Sur males, Monterey females, Monterey males). We used Wilks's lambda (λ_U) to test for differences among groups with respect to the discriminant functions computed from the underlying variables, and also assessed the percentage of individuals that could be correctly assigned to diet groups based on the discriminant classification

functions (we present both the raw classification matrix and the jackknife resampled classification matrix). We evaluated the "F-to-remove" statistic (F_{TR}) to determine which variables were most important for discriminating between groups: F_{TR} is a partial multivariate F statistic that tests the significance of the decrease in discrimination power should that variable be removed from the model. We report on between-group differences for those variables with F_{TR} values of >3.

Results

The health assessment dataset (Table 2) consists of a total of 144 sea otters, 69 from Big Sur and 75 from Monterey. At Big Sur the sample consisted of 58 females and 11 males, and at Monterey the sample consisted of 58 females and 17 males. Taken together, health profiles were significantly different between sexes and sites (λ_U =0.642, $F_{24,376}$=2.66, P=0.0001), although a discriminant function was only able to classify otters correctly by sex and study site 49% of the time (Jackknife classification accuracy = 45%). The variables most important in distinguishing between groups were physical weight (F_{TR} = 4.98), physical exam (F_{TR} = 9.27) and the blood differential counts (F_{TR} = 3.79). In terms of physical weight assessments, Big Sur females and males had higher scores and thus were in poorer condition than their Monterey counterparts (1.67 and 2.18 vs. 1.55 and 1.35, respectively) , although the difference was greater for males. In terms of overall physical exam, Big Sur females, males and Monterey females were all essentially equal (1.48, 1.54, 1.55 respectively), but Monterey males were in poorer condition (2.59). In terms of blood differential counts, Big Sur females showed more abnormalities than Monterey females (1.50 vs. 1.22), however Big Sur males showed fewer abnormalities than Monterey males (1.27 vs. 1.47).

The complete clinical pathology panel dataset (Table 3) represents 143 total samples comprised of 119 individuals, 97 of which were sampled once, 20 sampled twice, and 2 sampled three times. The difference in sample size between the health assessment data and that for clinical pathology is attributable to two factors, unacceptable logistical delay in getting samples delivered from collection site to laboratory (artifacturally spurious results) or submission to a reference lab other than IDEXX (inconsistent methodology). Considered altogether, there were significant differences in clinical pathology statistics between sexes and sites (λ_U =0.093, $F_{24,376}$=3.31, P<0.0001), and a discriminant function was only able to classify otters correctly by sex and study site 90% of the time (Jackknife classification accuracy = 63%). The variables most important in distinguishing between groups were serum creatinine (F_{TR} = 5.61), serum cholesterol (F_{TR} = 5.15), blood glucose (F_{TR} = 3.64), percent basophile (F_{TR} = 3.45) and absolute basophile counts (F_{TR} = 3.01). Serum creatinine was higher in Big Sur females and males as compared to Monterey females and males (0.39 and 0.56 vs. 0.38 and 0.44, respectively), as was serum cholesterol (198.0 and 157.8 vs. 156.8 and 132.5) and basophile metrics (0.07 and 0.31 vs. 0.03 and 0.17 for % basophile, 3.96 and 17.46 vs. 2.50 and 15.05 for basophile counts), while blood glucose in females was higher at Big Sur than at Monterey (137.75 117.54) but in males was lower at Big Sur (124.67 vs. 122.89).

Discussion

We did not see substantial variation in the health assessment scores of sea otters at the two study sites, and what differences we did see were insufficiently large to reliably distinguish between sites. At Big

Sur, otters scored slightly worse on assessments of physical weight (females and males), physical exams (males only) and blood differential counts (females only), although males at Monterey scored slightly worse than Big Sur on the latter metric. At both sites, individuals were in relatively poor body condition as compared to sea otters from other populations in California and in Alaska, British Columbia and Washington (see Chapter 7). The majority of the anomalies identified on physical examination were relatively minor in nature and not unexpected in populations that are at or near carrying capacity. Male sea otters tended to have a variety of peripheral limb injuries of varying degrees of seriousness likely associated with intra-specific trauma. Bite wounds, local and/or regional cellulitis and associated lymph node reaction, and occasional septic arthritis or osteomyelitis predominated.

Female sea otters had similar peripheral wounds, but they did not seem as common. There were, however, a significant number of injuries to the nose and muzzle of these otters. The injuries varied in nature from relatively minor and healing wounds to severe degloving injuries with loss of part or the entire nasal pad, maceration of tissue, and severe regional infection and inflammation. In some cases, the swelling associated with these wounds negatively impacted the otter's ability to nasal breath forcing the animal to open mouthed breathing. A number of females also demonstrated the systemic effects of long term negative calorie balance associated with pup rearing. These females were dramatically underweight, but also had physical and clinico-pathological changes associated with inanition, such as anemia, azotemia, and hypoproteinemia/hypoalbuminemia.

The incidence of dental disease seemed to be common in both populations. The evidence seemed to suggest that the severity of the anomalies present in the oral cavity increased with the animal's age; however, such a conclusion is significantly biased, as subjective age determination is determined in part by dental wear patterns. There may also be a relationship between dental disease and diet, as it is impossible to differentiate wear patterns, particularly in the pre-molars and molars, between the normal physiologic wear of tooth surfaces as they occlude (attrition) and the abnormal wear of tooth surfaces from contact with external objects (abrasion). The nature of this relationship, if it exists, remains to be described. Regardless, however, there is a demonstrable decline in the sea otter's health and overall condition once dental health deteriorates to a certain point. Available data cannot describe the nature of this effect; however, the potential increased incidence of apical tooth abscessation, regional osteomyelitis, bacteremia and secondary endocarditis, and simply oral cavity pain are not beyond the realm of possibility.

There were statistically significant differences in 5 of the 42 clinical pathology parameters either measured or calculated by the reference laboratory: creatinine, cholesterol, glucose, basophil %, and basophil absolute count. Absolute counts are calculated by multiplying the total WBC by the percentage of a particular cell type; it is the absolute count that is of clinical significance even though the differential percentage is reported. Interestingly, while there are statistically significant differences between populations for these parameters, none of the values reported, despite some being above or below published reference ranges, are considered clinically significant. This tends to imply that an explanation besides "disease" warrants consideration.

In this study, we found that serum creatinine levels were significantly higher in Big Sur sea otters, both male and female, than in Monterey sea otters. In clinical medicine, serum creatinine is considered to be an indicator test for renal disease, specifically glomerular filtration rate. In dogs and cats, however, it appears to be a relatively insensitive measure of kidney disease, as values tend not to elevate until approximately 75% of renal function is compromised.

There are aspects of creatinine metabolism which may be influenced by sea otter diets and/or body condition. Creatinine is a breakdown product of creatine phosphate found in muscle tissue. As a result, diets high in muscle tend to result in higher circulating creatinine. Since the compound tends to be produced endogenously at a relatively constant rate in muscle, animals with greater muscle mass tend to have higher levels. Conversely, decreased values are often seen in animals with muscle disease or systemic wasting. In this study, only one of the 41 sub-reference range results occurred in a male; the remainder were not surprisingly found in females.

Serum cholesterol values were also higher in Big Sur males and females than in Monterey otters. This parameter is rarely utilized clinically in domestic carnivores, despite its importance in health screening in primates. Dogs and cats are typically resistant to atherosclerosis due to the absence of an important transfer protein. As a result, this parameter is not typically considered clinically significant. Its presence on standardized serum chemistry profiles is likely a reflection of the manufacturers design emphasis on the human patient. There may be some degree of physiologic variation in serum cholesterol values based on the animal's diet, body condition, athleticism (exercise), and reproductive status.

Blood glucose values were statistically higher in Big Sur females and Monterey males than in their respective counterparts. These differences, while statistically relevant, did not appear to have any health-related relevance, as all values were within reference ranges. This parameter may be somewhat labile in free ranging sea otters. One might anticipate some degree of postprandial elevation in blood glucose, however, the degree to which this occurs is tied closely to the amount of carbohydrate in the meal. In this study, a postprandial effect may be significant, as many of the animals are captured after feeding bouts. Another cause for physiologic elevations in blood glucose is the transient hyperglycemia associated with catecholamine release, the "fight or flight response." Differences in behaviour between individuals, transport distance, and holding time may all influence the degree to which captured, free ranging sea otters manifest this adaptive phenomenon.

Basophile counts, both differential percentage and absolute, were also higher in Big Sur sea otters than in those from Monterey. As described for the other three statistically significant parameters, the overwhelming majority of basophil counts (all but 1Monterey male > reference range) were within reference range. Basophils are the most uncommon cell type found in peripheral blood. They develop and mature under the influence of interleukin-3 (IL-3). The function of the basophil is not well understood, however, it does appear to have a role in both immediate and delayed hypersensitivity, as well as increasing in the face of parasitism. In the case of sea otters, there may be a relationship between this cell's abundance and nasal mite (*Halarachne sp.*) infestation.

Evaluation of blood-based clinical pathology data collected one time from free ranging sea otters is inherently problematic. In the case of the sea otter, published reference ranges are based on samples collected at least a decade ago, analyzed using different methodology, and from a different subspecies (*Enhydra lutris kenyoni*). While the significance of this difference is difficult to quantify, its potential must be recognized. More importantly, however, the most effective use of clinico-pathologic data involves the evaluation of parameters over time in an effort to detect and interpret trends in the patient. Obviously, this is not possible, or is at least highly impractical, for free ranging wildlife. As a result, some of the information collected may represent clinically significant pathology in the earliest stages of disease. This shortcoming is unavoidable in wildlife medicine.

In summary, there was little evidence to support a biologically meaningful difference in health assessments, nor evidence of any major disease process impeding the health of individuals from either population. We recognize that this conclusion may to some degree reflect the lack of sensitivity associated with health evaluation based on physical examination and single, routine CBC/serum chemistry panels. Also, we recognize that even minor differences in disease exposure or health threats could translate into differences in survival or reproductive outcomes (see Chapter 8), and indeed we did find some very minor differences in health metrics that may have contributed to slightly lower survival for Big Sur animals. However, the results presented here suggest that there are no overt differences between the sites with respect to patterns of animal health or disease incidence. Samples collected and archived provide future opportunities to evaluate specific causes of sea otter pathology, either pathogen or contaminant related (e.g. refer to Chapter 9 for an analysis of protozoal infection rates), which may shed a different light on the data collected.

References

Arnemo JM, Caulkett. Stress in West G, Heard D, Caulkett N (eds) Zoo Animal and Wildlife Immobilization and Anesthesia.. Blackwell Publishing, Ames, IA. 2007: 103-110.

Bornez R, Linares MB, Vergara H, Haematological, hormonal and biochemical blood parameters in lamb: effect of age and blood sampling time. Livestock Science 121(1-2): 200-206, 2009.

Calvert CA and Thomason JD, Cardiovascular Infections in Greene CE (ed), Infectious Diseases of the Dog and Cat, 4th Ed. Elsevier Inc 2012: 912-936.

Evans EW. Proteins, Lipids, and Carbohydrates in Latimer KS (ed), Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th Ed. Wiley-Blackwell, Ames, IA. 2011: 173-209.

Glickman LT, Glickman NW, Moore GE, Goldstein GS, Lewis HB, Evaluation of the risk of endocarditis and other cardiovascular events on the basis of the severity of periodontal disease in dogs. J Am Vet Med Assoc, 234(4): 486-494. 2009.

Monson DH, McCormick C, Ballachey BF, Chemical anesthesia of northern sea otters (*Enhydra lutris*): results of past field studies. J Zoo Wildlife Med 32(2): 181-189, 2001.

Pohlman LM, Basophils, Mast Cells, and Their Disorders in Weiss DJ and Wardrop KJ (eds), Schalm's Veterinary Hematology, 6th Ed. Wiley-Blackwell, Ames, IA. 2010: 290-297.

Price HL, Ohnishi ST, Effects of anesthetics on the heart. Fed Proc 39(5): 1575-1579. 1980.

Rebar AH, Lipscomb TP, Harris RK, Ballachey BE, Clinical and clinical laboratory correlates in sea otters dying unexpectedly in rehabilitation centers following the *Exxon Valdez* oil spill. Vet Pathol 32: 346-350, 1995.

Tripathi NK, Gregory CR, Latimer KS. Urinary System in Latimer KS (ed), Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th Ed. Wiley-Blackwell, Ames, IA. 2011: 253-282.

Webb JL and Latimer KS. Leukocytes in Latimer KS (ed), Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th Ed. Wiley-Blackwell, Ames, IA. 2011: 45-82.

Williams TD, Rebar AH, Teclaw RF, Yoos PE. Influence of age, sex, capture technique, and restraint on hematologic measurements and serum chemistries of wild California sea otters. Vet Clin Path 21(4): 106-110, 1992.

Winer JN, et al., The Dental pathology of southern sea otters (*Enhydra lutris nereis*), J Comp Path, 149(2-3): 346-355. 2013.

Tables

Table 1. Scoring matrix for subjective health assessment

Parameter	Scoring Range	
	Low (normal)	High (abnormal)
Gross Condition		
Subjective Weight	1	5
Physical Exam	1	10
Dental Exam		
Incisor	1	5
Canine	1	5
Molar/Pre-molar	1	5
Blood Parameters		
PCV	1	5
Hgb	1	5
WBC	1	5
Differential Count	1	5
Serum Chemistry Panel	1	10

Table 2. (3 following pages) Health assessment scores for 144 sea otters captured at Monterey and Big Sur and screened with respect to 10 parameters. Refer to Table 1 for ranking scale of each parameter. Also shown for each individual is the otter identification number, date of capture, sex and study site.

Table 3. (12 pages, following Table 2) Clinical Pathology Results for 143 sea otters captured at Monterey and Big Sur. Blood samples were collected from each study animal at time of capture and sent to a diagnostic lab (IDEXX), which conducted tests and provided results on 42 standard blood parameters as shown. Also shown for each individual is the otter identification number, date of capture, sex and study site.

Otter ID	Date	Site	Sex	Subj Wt	Physical Exam	Total Cond	Dental Exam			Total Dental	PCV	Hgb	WBC	Diff Count	Serum Chem	Total Bloods
							Incisors	Canines	Premolar/ Molars							
1170.10	12/6/10	MONT	M	3	1	**4**	1	1	1	**3**	1	1	1	2	1	**6**
945.03	6/10/09	MONT	M	1	2	**3**	2	3	3	**8**	1	1	2	1	1	**6**
985.04	6/8/10	MONT	M	2	3	**5**	3	3	4	**10**	1	1	1	1	1	**5**
998.05	2/26/08	MONT	M	1	6	**7**	1	1	2	**4**	1	1	1	2	1	**6**
1024.06	6/9/09	MONT	M	2	7	**9**	4	5	5	**14**	1	1	1	1	1	**5**
1030.06	4/23/09	MONT	M	1	3	**4**	2	2	2	**6**	1	1	1	2	1	**6**
1030.06	12/16/10	MONT	M	1	2	**3**	2	2	2	**6**	1	1	1	2	1	**6**
1065.08	4/22/09	MONT	M	1	3	**4**	2	2	2	**6**	1	1	1	2	1	**6**
1108.09	4/22/09	MONT	M	2	4	**6**	2	2	2	**6**	1	1	1	2	1	**6**
1108.09	6/9/10	MONT	M	1	1	**2**	2	2	4	**8**	1	1	1	1	2	**6**
1114.09	4/29/09	MONT	M	2	1	**3**	1	1	1	**3**	2	2	1	1	1	**7**
1114.09	12/15/10	MONT	M	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1116.09	5/28/09	MONT	M	1	3	**4**	1	1	1	**3**	1	1	1	2	1	**6**
1118.09	5/28/09	MONT	M	1	3	**4**	1	1	1	**3**	1	1	1	1	2	**6**
1121.09	6/10/09	MONT	M	1	2	**3**	2	1	1	**4**	1	1	1	1	1	**5**
1131.08	11/11/08	MONT	M	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1156.10	2/2/10	MONT	M	1	1	**2**	2	1	2	**5**	1	1	1	1	2	**6**
941.03	4/22/09	MONT	F	1	1	**2**	2	2	3	**7**	1	1	1	1	1	**5**
946.03	12/2/09	MONT	F	2	2	**4**	2	1	3	**6**	1	1	1	1	2	**6**
1000.05	6/17/09	MONT	F	1	1	**2**	2	1	1	**4**	1	1	1	1	1	**5**
1011.06	7/28/10	MONT	F	1	2	**3**	2	1	2	**5**	1	1	1	1	1	**5**
1016.06	4/29/09	MONT	F	2	2	**4**	3	2	2	**7**	1	1	1	2	1	**6**
1017.06	4/29/09	MONT	F	1	3	**4**	2	2	2	**6**	1	1	1	1	1	**5**
1028.06	2/6/06	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	2	**6**
1029.06	8/20/09	MONT	F	1	2	**3**	2	2	3	**7**	1	1	1	1	1	**5**
1032.07	9/23/09	MONT	F	1	1	**2**	2	1	2	**5**	1	1	1	1	1	**5**
1032.07	12/15/10	MONT	F	1	2	**3**	2	2	3	**7**	1	2	1	1	2	**7**
1037.07	4/23/09	MONT	F	3	3	**6**	2	2	2	**6**	1	1	1	1	1	**5**
1037.07	10/27/10	MONT	F	1	2	**3**	3	2	3	**8**	1	2	1	2	2	**8**
1038.07	7/29/10	MONT	F	1	1	**2**	1	2	1	**4**	1	1	1	1	1	**5**
1042.07	7/29/10	MONT	F	1	1	**2**	2	1	1	**4**	1	2	1	1	1	**6**
1046.07	6/10/09	MONT	F	2	4	**6**	3	3	4	**10**	1	1	1	2	2	**7**
1049.07	9/27/07	MONT	F	2	1	**3**	2	1	1	**4**	1	1	1	1	1	**5**
1050.07	9/27/07	MONT	F	2	1	**3**	1	1	1	**3**	1	1	1	1	1	**5**
1051.07	9/27/07	MONT	F	2	1	**3**	1	1	1	**3**	1	1	1	1	2	**6**
1052.07	9/27/07	MONT	F	2	1	**3**	1	1	1	**3**	1	1	1	1	2	**6**
1053.07	7/14/09	MONT	F	2	3	**5**	1	1	1	**3**	1	1	3	1	3	**9**
1053.07	6/7/10	MONT	F	2	1	**3**	2	1	1	**4**	1	1	1	2	1	**6**
1056.08	8/26/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1056.08	12/2/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1057.08	12/16/10	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1064.08	8/24/09	MONT	F	2	1	**3**	1	1	1	**3**	1	1	1	1	1	**5**
1109.09	4/22/09	MONT	F	3	3	**6**	2	2	3	**7**	1	1	3	2	1	**8**
1109.09	6/8/10	MONT	F	3	3	**6**	3	2	4	**9**	1	1	1	1	1	**5**
1110.09	4/23/09	MONT	F	1	2	**3**	2	2	2	**6**	1	1	1	1	1	**5**
1110.09	10/27/10	MONT	F	1	1	**2**	2	2	2	**6**	1	1	1	1	1	**5**
1111.09	4/23/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1113.09	4/29/09	MONT	F	1	1	**2**	2	2	1	**5**	1	1	1	1	2	**6**
1115.09	5/28/09	MONT	F	3	3	**6**	2	2	3	**7**	2	2	1	1	1	**7**
1117.09	5/28/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1117.09	10/28/10	MONT	F	3	3	**6**	1	1	1	**3**	1	1	3	1	2	**8**
1119.09	6/9/09	MONT	F	2	2	**4**	2	1	1	**4**	1	1	1	2	1	**6**
1120.09	6/9/09	MONT	F	1	2	**3**	3	3	3	**9**	1	1	1	1	2	**6**
1122.09	6/10/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1122.09	10/7/10	MONT	F	1	1	**2**	2	1	2	**5**	1	1	1	1	1	**5**
1123.09	6/10/09	MONT	F	2	2	**4**	2	1	3	**6**	1	1	1	1	1	**5**
1124.09	6/16/09	MONT	F	2	1	**3**	1	1	1	**3**	1	1	2	1	1	**6**
1125.09	6/16/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**

Otter ID	Date	Site	Sex	Subj Wt	Physical Exam	Total Cond	Dental Exam			Total Dental	PCV	Hgb	WBC	Diff Count	Serum Chem	Total Bloods
							Incisors	Canines	Premolar/ Molars							
1126.09	6/16/09	MONT	F	2	2	**4**	2	2	3	**7**	1	1	1	2	2	**7**
1126.09	7/29/10	MONT	F	2	2	**4**	2	2	3	**7**	1	1	1	1	1	**5**
1127.09	6/16/09	MONT	F	2	2	**4**	3	2	3	**8**	1	1	1	1	1	**5**
1127.09	6/7/10	MONT	F	2	1	**3**	3	2	3	**8**	1	1	1	2	1	**6**
1128.09	6/17/09	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1128.09	7/28/10	MONT	F	1	1	**2**	2	3	1	**6**	1	1	1	2	1	**6**
1129.08	11/11/08	MONT	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1132.09	6/17/09	MONT	F	1	1	**2**	3	3	3	**9**	1	1	1	1	2	**6**
1133.09	8/24/09	MONT	F	1	1	**2**	2	2	1	**5**	1	1	1	1	1	**5**
1134.09	8/24/09	MONT	F	1	1	**2**	2	2	2	**6**	1	1	1	1	2	**6**
1134.09	2/1/10	MONT	F	1	1	**2**	2	1	2	**5**	1	1	1	1	2	**6**
1153.10	2/1/10	MONT	F	1	1	**2**	2	1	2	**5**	2	1	1	1	2	**7**
1154.10	2/1/10	MONT	F	1	1	**2**	2	1	2	**5**	2	1	1	1	1	**6**
1155.10	2/1/10	MONT	F	1	1	**2**	1	1	1	**3**	2	1	1	1	1	**6**
1157.10	6/7/10	MONT	F	3	1	**4**	2	2	2	**6**	1	1	1	1	1	**5**
1158.10	7/28/10	MONT	F	3	3	**6**	2	2	3	**7**	1	1	1	2	1	**6**
1130-08	11/6/08	MONT	F	2	1	**3**	2	2	2	**6**	1	1	1	1	3	**7**
1091.08	11/6/08	BIGS	M	3	2	**5**	2	3	3	**8**	1	1	1	1	2	**6**
1093.08	11/6/08	BIGS	M	2	2	**4**	2	1	1	**4**	1	1	1	1	2	**6**
1094.08	11/7/08	BIGS	M	3	2	**5**	4	2	2	**8**	1	1	1	2	2	**7**
1094.08	11/3/09	BIGS	M	2	2	**4**	3	2	4	**9**	1	1	1	1	1	**5**
1095.08	11/5/08	BIGS	M	2	1	**3**	2	1	2	**5**	1	1	1	1	1	**5**
1096.08	11/7/08	BIGS	M	3	2	**5**	3	2	4	**9**	1	1	1	1	1	**5**
1099.08	11/7/08	BIGS	M	3	1	**4**	2	1	1	**4**	1	1	1	2	1	**6**
1137.09	11/3/09	BIGS	M	2	2	**4**	3	3	4	**10**	1	1	1	1	2	**6**
1143.09	11/4/09	BIGS	M	2	1	**3**	2	2	4	**8**	1	1	1	1	2	**6**
1147.09	11/4/09	BIGS	M	1	1	**2**	1	1	1	**3**	3	2	1	1	1	**8**
1165.10	9/25/10	BIGS	M	1	1	**2**	2	1	2	**5**	1	1	1	2	1	**6**
1067.08	9/23/10	BIGS	F	2	2	**4**	2	1	3	**6**	1	1	1	2	1	**6**
1067.08	11/5/08	BIGS	F	2	1	**3**	2	1	2	**5**	1	1	1	2	1	**6**
1068.08	11/5/08	BIGS	F	2	2	**4**	1	1	1	**3**	1	1	1	2	1	**6**
1069.08	11/5/08	BIGS	F	2	1	**3**	1	1	1	**3**	1	1	1	1	1	**5**
1070.08	11/5/08	BIGS	F	2	1	**3**	1	2	2	**5**	1	1	1	1	1	**5**
1071.08	11/5/08	BIGS	F	2	2	**4**	2	2	4	**8**	1	1	1	1	1	**5**
1072.08	11/5/08	BIGS	F	2	1	**3**	2	1	1	**4**	1	1	1	1	3	**7**
1073.08	11/6/08	BIGS	F	2	1	**3**	1	2	2	**5**	1	1	1	2	1	**6**
1074.08	11/6/08	BIGS	F	1	2	**3**	2	2	2	**6**	1	1	1	2	3	**8**
1074.08	9/24/10	BIGS	F	2	2	**4**	3	3	3	**9**	1	1	1	2	1	**6**
1075.08	11/6/08	BIGS	F	2	1	**3**	1	1	2	**4**	1	1	1	2	1	**6**
1076.08	11/6/08	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	2	2	**7**
1077.08	11/7/08	BIGS	F	2	1	**3**	2	2	2	**6**	1	1	1	2	1	**6**
1078.08	11/6/08	BIGS	F	1	3	**4**	3	3	3	**9**	1	1	1	2	1	**6**
1079.08	11/7/08	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1080.08	11/7/08	BIGS	F	1	1	**2**	2	1	1	**4**	1	1	1	1	1	**5**
1081.08	11/7/08	BIGS	F	2	2	**4**	3	3	4	**10**	1	1	1	1	3	**7**
1082.08	11/7/08	BIGS	F	1	2	**3**	2	2	2	**6**	1	1	1	2	3	**8**
1083.08	11/7/08	BIGS	F	2	2	**4**	3	2	3	**8**	1	1	1	2	1	**6**
1084.08	11/7/08	BIGS	F	1	1	**2**	1	2	1	**4**	1	1	1	2	1	**6**
1085.08	11/8/08	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1086.08	11/7/08	BIGS	F	2	3	**5**	1	1	1	**3**	1	1	1	2	1	**6**
1087.08	11/8/08	BIGS	F	2	7	**9**	1	1	1	**3**	1	1	4	1	4	**11**
1088.08	11/10/08	BIGS	F	2	1	**3**	2	2	1	**5**	1	1	1	1	1	**5**
1089.08	11/6/08	BIGS	F	2	1	**3**	2	2	2	**6**	1	1	1	2	1	**6**
1090.08	11/10/08	BIGS	F	2	4	**6**	2	2	4	**8**	1	1	1	2	2	**7**
1092.08	11/11/08	BIGS	F	3	4	**7**	2	2	2	**6**	4	4	1	2	2	**13**
1097.08	11/11/08	BIGS	F	2	1	**3**	1	2	1	**4**	1	1	1	1	1	**5**
1097.08	9/24/10	BIGS	F	2	1	**3**	3	2	2	**7**	1	1	1	2	2	**7**
1098.08	11/11/08	BIGS	F	3	1	**4**	2	1	1	**4**	1	1	1	1	2	**6**

Otter ID	Date	Site	Sex	Subj Wt	Physical Exam	Total Cond	Dental Exam			Total Dental	PCV	Hgb	WBC	Diff Count	Serum Chem	Total Bloods
							Incisors	Canines	Premolar/ Molars							
1103.08	11/11/08	BIGS	F	3	1	**4**	3	3	3	**9**	1	1	1	1	1	**5**
1104.08	11/11/08	BIGS	F	2	1	**3**	2	2	2	**6**	1	1	1	1	2	**6**
1105.08	11/11/08	BIGS	F	2	1	**3**	1	2	2	**5**	1	1	1	2	1	**6**
1106.08	11/11/08	BIGS	F	2	2	**4**	3	3	3	**9**	1	1	1	1	1	**5**
1135.09	11/2/09	BIGS	F	2	2	**4**	1	1	1	**3**	1	1	1	1	1	**5**
1136.09	11/2/09	BIGS	F	3	2	**5**	1	1	2	**4**	1	1	1	1	1	**5**
1138.09	11/3/09	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1139.09	11/3/09	BIGS	F	2	1	**3**	2	1	1	**4**	1	1	1	1	1	**5**
1140.09	11/3/09	BIGS	F	1	1	**2**	3	4	3	**10**	1	1	1	1	4	**8**
1141.09	11/3/09	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	1	3	**7**
1141.09	9/25/10	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	2	1	**6**
1142.09	9/24/10	BIGS	F	3	1	**4**	2	1	2	**5**	1	1	1	2	1	**6**
1144.09	11/4/09	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1145.09	11/4/09	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	2	3	**8**
1146.09	11/4/09	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1148.09	11/5/09	BIGS	F	2	1	**3**	2	1	1	**4**	1	1	1	2	1	**6**
1150.09	11/5/09	BIGS	F	1	1	**2**	2	2	1	**5**	1	1	1	1	2	**6**
1151.09	11/5/09	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	2	1	**6**
1159.10	9/22/10	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	2	1	**6**
1160.10	9/22/10	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	3	**7**
1161.10	9/22/10	BIGS	F	3	1	**4**	2	3	2	**7**	1	1	1	2	1	**6**
1162.10	9/23/10	BIGS	F	2	1	**3**	2	1	1	**4**	1	1	1	2	1	**6**
1163.10	9/24/10	BIGS	F	1	1	**2**	1	1	1	**3**	1	1	1	1	1	**5**
1164.10	9/24/10	BIGS	F	1	1	**2**	2	2	2	**6**	1	1	1	1	1	**5**
1166.10	9/25/10	BIGS	F	1	2	**3**	1	1	1	**3**	1	1	2	1	4	**9**
1167.10	9/25/10	BIGS	F	1	1	**2**	1	1	2	**4**	1	1	3	1	1	**7**
1168.10	9/27/10	BIGS	F	1	1	**2**	3	2	3	**8**	1	3	1	2	1	**8**
1169.10	9/27/10	BIGS	F	1	1	**2**	2	1	1	**4**	1	1	1	1	1	**5**

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
945.03	06/11/09	Mont	M	138	111	108	196	2.7	6.8	4.1	0.1	0.1	65	0.5	133	130	8.9	3.3	20	121	4.4	153	0.7	130
985.04	06/09/10	Mont	M	91	137	110	189	2.6	6.9	4.3	0	0	64	0.4	87	110	8.5	2.9	30	112	4.7	150	0.6	160
998.05	02/27/08	Mont	M	110	138	142	192	2.6	5.9	3.3	0.1	0.1	50	0.5	146	109	7.9	4.3	25	115	4	151	0.8	100
1024.06	06/10/09	Mont	M	66	188	188	147	2.5	6.7	4.2	0.1	0.1	50	0.4	126	148	8.5	5.9	24	116	4.9	150	0.6	125
1030.06	04/24/09	Mont	M	74	114	93	166	2.7	7	4.3	0.1	0.1	51	0.6	135	112	8.9	4.6	27	113	4.4	152	0.6	85
1030.06	12/17/10	Mont	M	105	166	145	169	2.6	7	4.4	0	0	71	0.4	151	146	8.2	3.7	26	113	4.1	149	0.6	177.5
1065.08	04/23/09	Mont	M	120	153	118	164	2.6	6.9	4.3	0	0	39	0.4	143	103	8.8	4.7	26	115	3.9	151	0.6	97.5
1108.09	04/23/09	Mont	M	98	176	158	293	2.4	6.8	4.4	0.1	0.1	51	0.5	128	102	8.4	4.4	27	111	4.5	147	0.5	102
1108.09	06/09/10	Mont	M	131	266	318	207	2.5	6.6	4.1	0	0	75	0.4	137	91	9	2.9	27	116	4.3	151	0.6	187.5
1114.09	05/01/09	Mont	M	98	180	173	1016	3	6.6	3.6	0.1	0.1	52	0.5	95	130	8.5	4.5	27	119	4.1	153	0.8	104
1114.09	04/28/10	Mont	M	90	103	133	253	2.7	6.5	3.8	0.1	0.1	64	0.4	103	112	7.8	4.3	27	119	4.5	154	0.7	160
1114.09	12/16/10	Mont	M	99	194	139	291	2.9	6.6	3.7	0.1	0.1	71	0.4	112	114	8.3	4.7	27	118	4.8	154	0.8	177.5
1116.09	05/29/09	Mont	M	116	231	250	341	2.9	6.3	3.4	0.2	0.1	53	0.5	162	101	8.4	2.9	21	118	3.6	153	0.9	106
1118.09	05/29/09	Mont	M	73	230	506	13014	2.7	6.3	3.6	0.1	0.1	43	0.4	147	181	8.4	4.4	27	117	4.2	155	0.8	107.5
1121.09	06/11/09	Mont	M	58	145	196	1695	2.8	6.9	4.1	0.2	0.1	53	0.5	135	93	8.7	3.5	23	120	4.1	157	0.7	106
1131.08	11/14/08	Mont	M	221	126	107	764	3.1	5.2	2.1	0.1	0.1	49	0.3	176	179	10.1	8.6	19	117	4.4	151	1.5	163.3
1156.10	02/03/10	Mont	M	100	87	98	294	2.7	7	4.3	0.2	0	73	0.5	126	102	8.3	3.6	22	117	4.1	153	0.6	146
1170.10	12/17/10	Mont	M	114	279	254	546	2.8	6.6	3.8	0.1	0	68	0.4	143	149	8.4	4.9	24	117	3.9	152	0.7	170

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
945.03	06/11/09	Mont	M	0	35	3.7	5.06	20	56.7	112	39.6	35.4	44	0	34	7	15	0	1628	0	1258	259	555	0
985.04	06/09/10	Mont	M	0	32	5.4	4.86	17.7	53.5	110	36.5	33.1	40	0	53	6	1	0	2160	0	2862	324	54	0
998.05	02/27/08	Mont	M	0	38	5.8	5.05	20.1	57.6	114	39.9	35	59	0	23	4	14	0	3422	0	1334	232	812	0
1024.06	06/10/09	Mont	M	0	31	6.5	5.17	19.7	56	108	38	35.1	47	0	40	4	9	0	3055	0	2600	260	585	0
1030.06	04/24/09	Mont	M	0	35	9.4	4.86	19.1	53.5	110	39.2	35.7	56	0	16	13	13	2	5264	0	1504	1222	1222	188
1030.06	12/17/10	Mont	M	0	36	10.1	4.64	17.5	54.2	117	37.7	32.3	43	0	31	2	24	0	4343	0	3131	202	2424	0
1065.08	04/23/09	Mont	M	0	39	8.3	5	20.7	54	108	41.4	38.3	72	0	11	7	10	0	5976	0	913	581	830	0
1108.09	04/23/09	Mont	M	0	33	7.7	4.88	20.2	54.1	111	41.5	37.4	64	0	16	6	14	0	4928	0	1232	462	1078	0
1108.09	06/09/10	Mont	M	0	35	7.4	4.99	18.8	57.2	115	37.8	32.9	68	0	24	8	0	0	5032	0	1776	592	0	0
1114.09	05/01/09	Mont	M	0	37	5.3	3.92	16.4	47.7	122	41.7	34.3	60	0	32	3	5	0	3180	0	1696	159	265	0
1114.09	04/28/10	Mont	M	0	34	7.9	4.64	18.4	54.9	118	39.7	33.6	66	0	3	3	1	0	5214	0	2370	237	79	0
1114.09	12/16/10	Mont	M	0	32	4.9	4.87	18.8	60.1	123	38.6	31.3	53	0	29	3	15	0	2597	0	1421	147	735	0
1116.09	05/29/09	Mont	M	0.1	43	8.3	5.26	21	55.5	106	39.9	37.8	35	0	49	3	12	1	2905	0	4067	249	996	83
1118.09	05/29/09	Mont	M	0	37	5	5.69	22.6	59.6	105	39.7	37.9	71	0	21	3	5	0	3550	0	1050	150	250	0
1121.09	06/11/09	Mont	M	0.1	38	8.4	4.84	19.2	53.9	112	39.8	35.7	75	0	18	7	0	0	6300	0	1512	588	0	0
1131.08	11/14/08	Mont	M	0	34	6.4	4.63	16.2	49.1	106	35.1	33.1	59	0	29	3	9	0	3376	0	1856	192	576	0
1156.10	02/03/10	Mont	M	0.2	37	4	4.17	19.5	47.8	115	46.7	40.8	68	0	21	3	8	0	2720	0	840	120	320	0
1170.10	12/17/10	Mont	M	0.1	39	6.6	4.96	17.6	55.2	111	35.4	31.8	42	0	37	6	15	0	2772	0	2442	396	990	0

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
941.03	04/24/09	Mont	F	84	68	65	204	2.7	6.8	4.1	0.2	0.1	38	0.4	128	147	8.8	4.5	28	116	3.8	155	0.7	95
946.03	12/03/09	Mont	F	130	104	74	152	2.9	7.1	4.2	0.1	0	82	0.4	151	132	8.5	3.9	25	111	3.9	146	0.7	205
1000.05	06/18/09	Mont	F	103	147	91	217	2.9	6.3	3.4	0.2	0.1	65	0.5	213	138	8.9	3.5	25	116	3.6	152	0.9	130
1011.06	07/29/10	Mont	F	109	163	96	153	2.9	6.6	3.7	0.2	0.1	68	0.3	188	106	8.2	3.7	21	126	4.2	158	0.8	226.7
1016.06	04/30/09	Mont	F	103	93	60	125	2.8	6.6	3.8	0.2	0.1	53	0.5	180	131	9	4.9	21	118	3.7	152	0.7	106
1017.06	04/30/09	Mont	F	82	108	81	287	3	7	4	0.6	0.2	51	0.3	128	107	8.9	3.8	22	117	3.6	153	0.8	170
1029.06	08/25/09	Mont	F	93	95	85	197	2.8	7	4.2	0.4	0.2	60	0.3	115	121	8.6	5	25	115	3.9	152	0.7	200
1032.07	09/23/09	Mont	F	79	73	72	201	3.1	6.5	3.4	0.1	0.1	66	0.4	120	125	8.8	5.5	25	116	4.6	152	0.9	165
1032.07	12/16/10	Mont	F	106	89	68	340	2.9	6.5	3.6	0.1	0.1	82	0.4	115	138	8.6	4.7	23	112	3.9	147	0.8	205
1037.07	04/24/09	Mont	F	90	118	122	687	2.9	6.7	3.8	0.2	0.1	67	0.5	239	129	9.1	5.1	29	117	3.8	160	0.8	134
1037.07	10/29/10	Mont	F	92	206	111	156	2.6	6.6	4	0.1	0.1	82	0.4	201	175	8.3	4.4	27	113	3.9	149	0.7	205
1038.07	07/30/10	Mont	F	107	116	90	224	2.8	6.3	3.5	0.1	0.1	66	0.2	187	113	8.5	5	20	118	4	154	0.8	330
1042.07	07/30/10	Mont	F	71	97	100	240	2.8	6.5	3.7	0.1	0.1	48	0.3	138	99	8.9	6.3	21	115	4.3	152	0.8	160
1042.09	11/05/09	Mont	F	79	198	160	191	2.8	6.9	4.1	0.1	0.1	74	0.3	172	119	9.1	2.3	26	116	3.7	153	0.7	246.7
1046.07	06/11/09	Mont	F	79	112	92	107	2.6	6.8	4.2	0.1	0.1	88	0.3	196	120	8.7	4	23	122	4.3	156	0.6	293.3
1053.07	07/15/09	Mont	F	91	148	196	684	2.7	7.3	4.6	0.1	0.1	55	0.4	128	134	9.3	6.1	30	111	4.5	151	0.6	137.5
1053.07	06/08/10	Mont	F	78	123	100	238	2.9	6.3	3.4	0.1	0.1	53	0.3	120	118	9.2	4.6	25	116	4.8	151	0.9	176.7
1056.08	08/25/09	Mont	F	144	156	144	358	2.8	6.4	3.6	0.2	0.1	60	0.4	109	136	8.8	4.6	25	118	4.4	151	0.8	150
1056.08	12/03/09	Mont	F	149	125	110	213	3	6.4	3.4	0.1	0.1	70	0.4	164	119	9.1	4.9	24	113	4	148	0.9	175
1057.08	12/17/10	Mont	F	91	128	108	298	2.8	6.2	3.4	0.1	0.1	49	0.4	173	89	9	4.5	24	114	3.7	152	0.8	122.5
1064.08	08/25/09	Mont	F	141	178	147	525	2.8	6.2	3.4	0.3	0.1	61	0.3	186	133	9	6.9	27	114	4.2	151	0.8	203.3
1109.09	04/23/09	Mont	F	52	128	141	635	2.1	6.9	4.8	0.1	0.1	52	0.5	124	116	8.7	4.6	26	115	4	1.5	0.4	104
1109.09	06/09/10	Mont	F	66	94	87	196	2.6	7.2	4.6	0.1	0.1	39	0.4	118	114	8.6	4	26	114	4	150	0.6	97.5
1110.09	04/24/09	Mont	F	99	64	95	150	2.7	7.2	4.5	0.1	0.1	51	0.4	149	122	9.1	5.2	27	114	4.1	153	0.6	127.5
1110.09	10/29/10	Mont	F	117	127	90	176	2.9	6.5	3.6	0.1	0.1	55	0.3	219	157	8.6	4.5	26	115	3.7	151	0.8	183.3
1111.09	04/24/09	Mont	F	102	155	159	1983	3	6.4	3.4	0.1	0.1	49	0.4	142	124	9.6	5.1	24	118	3.9	155	0.9	122.5
1113.09	04/30/09	Mont	F	110	132	97	647	2.8	6.4	3.6	0.1	0.1	76	0.4	169	132	8.8	6.3	22	118	3.7	153	0.8	190
1115.09	05/29/09	Mont	F	72	87	86	188	2.6	7.1	4.5	0.2	0.1	53	0.4	110	141	8.2	5.3	23	117	3.7	151	0.6	132.5
1117.09	05/29/09	Mont	F	126	209	120	361	2.8	6.2	3.4	0.2	0.2	42	0.3	165	120	9	7.2	23	116	3.5	152	0.8	140
1117.09	10/29/10	Mont	F	93	219	185	152	2.6	5.8	3.2	0.1	0.1	86	0.3	153	126	8.8	2.6	25	122	3.5	155	0.8	286.7
1119.09	06/10/09	Mont	F	95	180	144	477	2.8	6.3	3.5	0.2	0.1	49	0.4	162	113	8.7	5.5	23	117	4.2	151	0.8	122.5
1120.09	06/10/09	Mont	F	96	121	133	385	2.8	7.2	4.4	0.1	0.1	79	0.4	160	119	12.7	4.6	25	113	4.6	151	0.6	197.5
1122.09	06/11/09	Mont	F	98	86	75	276	2.7	6.6	3.9	0.1	0.1	43	0.4	150	120	9.1	6.8	21	120	3.8	154	0.7	107.5
1122.09	10/08/10	Mont	F	126	117	71	172	2.8	6.2	3.4	0.1	0	54	0.5	157	127	8.8	4.7	24	114	4	152	0.8	108
1123.09	06/12/09	Mont	F	136	119	130	613	3.1	6.8	3.7	0.3	0.1	41	0.4	190	95	8.6	4.1	20	115	4.1	153	0.8	102.5
1124.09	06/17/09	Mont	F	96	206	207	148	3	6.5	3.5	0.2	0.1	54	0.5	212	127	8.8	3.6	24	118	3.5	156	0.9	108
1125.09	06/17/09	Mont	F	109	113	120	320	2.8	6	3.2	0.1	0.1	69	0.4	127	131	9	4.1	27	116	4.1	154	0.9	172.5
1126.09	06/17/09	Mont	F	51	125	149	227	2.4	7.2	4.8	0.3	0.1	33	0.5	116	91	8.3	3.7	21	117	3.6	152	0.5	66

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
941.03	04/24/09	Mont	F	0.1	41	5.4	4.9	19.9	54	110	40.6	36.9	48	0	44	2	5	1	2592	0	2376	108	270	54
946.03	12/03/09	Mont	F	0.1	37	8.9	5.1	20.2	57.6	113	39.7	35.2	30	0	60	4	6	0	2670	0	5340	356	534	0
1000.05	06/18/09	Mont	F	0.1	42	5.2	4.81	19.6	52.3	109	40.8	37.6	39	0	49	2	10	0	2028	0	2548	104	520	0
1011.06	07/29/10	Mont	F	0.1	38	6.7	4.94	18.5	55.6	113	37.5	33.3	55	0	33	2	10	0	3685	0	2211	134	670	0
1016.06	04/30/09	Mont	F	0.1	41	6.3	5.22	19.4	54.3	104	37.1	35.7	43	0	38	1	18	0	2709	0	2394	63	1134	0
1017.06	04/30/09	Mont	F	0.4	43	7.3	4.9	19.1	54.3	111	39	35.2	41	0	57	0	2	0	2993	0	4161	0	146	0
1029.06	08/25/09	Mont	F	0.2	39	8	4.91	19.1	55.8	114	38.8	34.2	36	0	55	2	7	0	2880	0	4400	160	560	0
1032.07	09/23/09	Mont	F	0	33	7.8	4.52	18.8	50.9	113	41.6	36.9	40	0	46	8	6	0	3120	0	3588	624	468	0
1032.07	12/16/10	Mont	F	0	38	6.1	4.75	16.8	53.9	114	35.4	31.2	44	0	41	4	11	0	2684	0	2501	244	671	0
1037.07	04/24/09	Mont	F	0.1	42	9.9	5.34	21.1	59	111	39.5	35.7	78	0	13	6	3	0	7722	0	1287	594	297	0
1037.07	10/29/10	Mont	F	0	38	9.8	4.75	16.7	52.8	111	35.1	31.6	62	0	18	1	19	0	6076	0	1764	98	1862	0
1038.07	07/30/10	Mont	F	0	39	7.6	4.8	17.7	54.9	115	36.9	32.2	30	0	65	4	1	0	2280	0	4940	304	76	0
1042.07	07/30/10	Mont	F	0	35	7	4.29	16.7	52.9	123	38.9	31.6	66	0	22	7	5	0	4620	0	1540	490	350	0
1042.09	11/05/09	Mont	F	0	41	10.6	5.22	19.7	60.2	115	37.7	32.7	59	0	31	2	8	0	6254	0	3286	212	848	0
1046.07	06/11/09	Mont	F	0	36	5.7	4.86	19.8	56.1	116	40.7	35.2	50	0	34	3	13	0	2850	0	1938	171	741	0
1053.07	07/15/09	Mont	F	0	34	12.7	4.99	19.5	52.1	104	39	37.3	73	0	21	1	5	0	9271	0	2667	127	635	0
1053.07	06/08/10	Mont	F	0	31	7.7	4.95	18	52.1	105	36.4	34.5	41	0	46	2	11	0	3157	0	3542	154	847	0
1056.08	08/25/09	Mont	F	0.1	34	6.9	4.56	17.9	51.8	114	39.2	34.5	40	0	53	5	2	0	2760	0	3657	345	138	0
1056.08	12/03/09	Mont	F	0	37	6.8	4.98	20.6	58.9	118	41.4	35	42	0	50	3	5	0	2856	0	3400	204	340	0
1057.08	12/17/10	Mont	F	0	41	10	5.23	19	59.2	113	36.3	32	45	0	27	4	24	0	4500	0	2700	400	2400	0
1064.08	08/25/09	Mont	F	0.2	36	9.1	5.15	19.2	55.3	107	37.3	34.8	30	0	60	7	3	0	2730	0	5460	637	273	0
1109.09	04/23/09	Mont	F	0	38	11.8	4.53	18.3	50.2	111	40.3	36.4	84	0	11	4	1	0	9912	0	1298	472	118	0
1109.09	06/09/10	Mont	F	0	38	6	4.97	17.8	54.4	110	35.8	32.7	79	0	9	5	7	0	4740	0	540	300	420	0
1110.09	04/24/09	Mont	F	0	37	7.2	4.8	18.7	52.3	109	39	35.8	56	0	29	8	7	0	4032	0	2088	576	504	0
1110.09	10/29/10	Mont	F	0	41	6.9	5.4	19.1	60.9	113	35.5	31.4	39	0	51	0	10	0	2691	0	3519	0	690	0
1111.09	04/24/09	Mont	F	0	40	9.6	5.24	19.9	55.4	106	38.1	36	48	0	47	0	5	0	4608	0	4512	0	480	0
1113.09	04/30/09	Mont	F	0	41	7.4	4.38	17.5	49.3	113	40	35.6	52	0	38	3	7	0	3848	0	2812	222	518	0
1115.09	05/29/09	Mont	F	0.1	41	7.7	4.27	16.8	45.7	107	39.3	36.7	58	0	33	1	8	0	4466	0	2541	77	616	0
1117.09	05/29/09	Mont	F	0	43	9.2	5.16	19.9	53.1	103	38.7	37.5	54	0	34	2	10	0	4968	0	3128	184	920	0
1117.09	10/29/10	Mont	F	0	44	12.2	5	18.2	55.3	111	36.4	32.9	72	0	18	6	4	0	8784	0	2196	732	488	0
1119.09	06/10/09	Mont	F	0.1	36	7.1	5.39	20.2	58.7	109	37.5	34.5	47	0	35	2	16	0	3337	0	2485	142	1136	0
1120.09	06/10/09	Mont	F	0	33	7.7	5.12	19.9	57.7	113	39	34.5	64	0	24	3	9	0	4928	0	1848	231	693	0
1122.09	06/11/09	Mont	F	0	41	10.7	4.59	17.1	48.9	107	37.3	35	52	0	39	2	7	0	5564	0	4173	214	749	0
1122.09	10/08/10	Mont	F	0.1	38	7.1	5.17	17.7	56.6	110	34.3	31.3	51	0	36	4	9	0	3621	0	2556	284	639	0
1123.09	06/12/09	Mont	F	0.2	37	8.9	5.5	20.2	59.5	108	36.8	34	72	0	18	7	3	0	6408	0	1602	623	267	0
1124.09	06/17/09	Mont	F	0.1	45	11.3	4.7	19.2	53.1	113	40.8	36.2	63	0	25	8	4	0	7119	0	2825	904	452	0
1125.09	06/17/09	Mont	F	0	38	8	5.21	19.7	55.3	106	37.8	35.5	39	0	53	1	7	0	3120	0	4240	80	560	0
1126.09	06/17/09	Mont	F	0.2	42	6.7	5.22	20.1	55.3	106	38.4	36.3	62	0	23	3	12	0	4154	0	1541	201	804	0

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
1126.09	06/09/10	Mont	F	67	119	106	291	2.4	7.5	5.1	0.2	0.2	33	0.4	105	90	8.4	4.7	26	112	4.2	149	0.5	82.5
1126.09	07/30/10	Mont	F	99	106	84	157	2.7	6.9	4.2	0.2	0.1	40	0.4	112	90	8.5	4.5	21	116	4.1	151	0.6	100
1127.09	06/17/09	Mont	F	85	110	84	382	3	6.5	3.5	0.6	0.2	55	0.4	145	124	8.3	4.4	27	116	4	154	0.9	137.5
1127.09	06/08/10	Mont	F	87	170	159	215	2.7	6.3	3.6	0.2	0.1	60	0.3	179	131	8.8	4.3	26	115	3.6	151	0.8	200
1128.09	06/18/09	Mont	F	97	116	112	405	2.8	6.8	4	0.2	0.1	66	0.4	187	112	9	3.4	23	117	3.5	151	0.7	165
1128.09	07/29/10	Mont	F	69	90	84	183	2.7	7.1	4.4	0.2	0.1	45	0.3	146	110	8.7	3.3	26	116	3.4	149	0.6	150
1129.08	11/14/08	Mont	F	205	156	178	1164	0	5.5	2.5	0.2	0.1	48	0.3	173	143	9.9	6.2	21	120	4	154	1.2	160
1132.09	06/18/09	Mont	F	85	143	123	484	2.7	6.2	3.5	0.2	0.1	65	0.4	133	142	8.2	6.9	24	116	4.6	152	0.8	162.5
1133.09	08/25/09	Mont	F	73	129	117	273	2.7	6.9	4.2	0.1	0.1	75	0.4	104	106	9.1	5.3	25	113	4.5	150	0.6	187.5
1134.09	08/25/09	Mont	F	61	68	84	483	2.6	7.5	4.9	0.2	0.1	47	0.5	154	112	8.9	4.2	25	116	4	150	0.5	94
1134.09	02/02/10	Mont	F	78	113	126	422	2.7	7.6	4.9	0.1	0.1	54	0.3	113	119	8.7	5.4	22	120	4.7	155	0.6	180
1153.10	02/02/10	Mont	F	124	126	92	326	2.8	6.2	3.4	0.3	0.1	48	0.4	176	144	8.8	4.1	22	117	4	154	0.8	120
1154.10	02/02/10	Mont	F	86	190	124	222	2.5	7	4.5	0.1	0.1	66	0.3	224	186	8.2	3.9	20	117	4.1	150	0.6	220
1155.10	02/02/10	Mont	F	119	167	122	351	2.7	6.5	3.8	0.1	0.1	55	0.3	188	151	8.1	4.1	19	122	4.4	154	0.7	183.3
1157.10	06/08/10	Mont	F	68	111	97	193	2.7	6.9	4.2	0.1	0.1	49	0.4	142	116	8.6	4.4	31	111	4.3	150	0.6	122.5
1158.10	07/29/10	Mont	F	84	128	125	418	2.7	7.4	4.7	0.2	0.1	67	0.3	165	133	8.8	4.5	22	117	3.8	152	0.6	223.3
1130-08	11/10/08	Mont	F	129	276	237	598	3	7	4	0.2	0.1	108	0.5	202	144	8.3	6.1	24	115	4.1	155	0.8	216

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
1126.09	06/09/10	Mont	F	0	35	8.4	5.08	17.1	51.4	101	33.8	33.4	46	0	32	7	14	1	3864	0	2688	588	1176	84
1126.09	07/30/10	Mont	F	0.1	37	6.5	5.24	17.5	55.5	106	33.3	31.4	44	0	47	7	2	0	2860	0	3055	455	130	0
1127.09	06/17/09	Mont	F	0.4	39	5.4	4.97	19.2	53.1	107	38.6	36.1	42	0	52	0	6	0	2268	0	2808	0	324	0
1127.09	06/08/10	Mont	F	0.1	42	5.4	4.84	17.4	51.3	106	36	34	51	0	30	3	16	0	2754	0	1620	162	864	0
1128.09	06/18/09	Mont	F	0.1	43	7.9	4.72	19.7	52.6	111	41.6	37.4	51	0	34	1	14	0	4029	0	2686	79	1106	0
1128.09	07/29/10	Mont	F	0.1	44	9	4.37	17	50.9	116	38.9	33.4	54	0	35	1	10	0	4860	0	3150	90	900	0
1129.08	11/14/08	Mont	F	0.1	39	4.2	4.61	17	49.2	107	36.8	34.5	57	0	37	1	5	0	2394	0	1554	42	210	0
1132.09	06/18/09	Mont	F	0.1	33	6.1	4.97	18.2	50.7	102	36.6	35.8	58	0	31	8	3	0	3538	0	1891	488	183	0
1133.09	08/25/09	Mont	F	0	33	10.5	5.16	19.8	56.6	110	38.3	34.9	47	0	39	6	8	0	4935	0	4095	630	840	0
1134.09	08/25/09	Mont	F	0.1	38	8.4	5.23	19.3	55.8	107	36.9	34.6	58	0	38	2	2	0	4872	0	3192	168	168	0
1134.09	02/02/10	Mont	F	0	33	7.9	4.03	17.4	45.3	112	43	38.3	72	0	23	2	3	0	5688	0	1817	158	237	0
1153.10	02/02/10	Mont	F	0.2	39	7.8	4.04	19.8	48.4	120	49	40.9	40	0	53	2	5	0	3120	0	4134	156	390	0
1154.10	02/02/10	Mont	F	0	37	6.4	4.33	17.9	46.6	108	41.2	38.3	58	0	33	7	2	0	3712	0	2112	448	128	0
1155.10	02/02/10	Mont	F	0	35	7.3	4.28	19.1	50.4	118	44.6	37.9	48	0	42	6	4	0	3504	0	3066	438	292	0
1157.10	06/08/10	Mont	F	0	35	6.7	4.97	18.4	53.4	108	37	34.4	52	0	28	3	17	0	3484	0	1876	201	1139	0
1158.10	07/29/10	Mont	F	0.1	40	5.6	4.81	17.5	53.9	112	36.4	32.5	66	0	24	2	8	0	3696	0	1344	112	448	0
1130-08	11/10/08	Mont	F	0.1	38	4.9	4.86	19.2	57.2	118	39.4	33.6	51	0	32	8	9	0	2499	0	1568	392	441	0

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
1066.08	11/06/08	Big Sur	M	81	209	218	532	2.4	6	3.6	0.1	0.1	78	0.8	135	91	9.2	8	26	115	5.6	154	0.7	97.5
1091.08	11/10/08	Big Sur	M	89	234	210	504	2.5	6.4	3.9	0.1	0.1	78	0.5	157	135	8.2	6.3	27	120	4.7	154	0.6	156
1093.08	11/10/08	Big Sur	M	64	224	172	884	2.4	6.9	4.5	0.1	0.1	76	0.5	152	133	8.3	3.8	29	119	4.2	153	0.5	152
1094.08	11/10/08	Big Sur	M	65	168	180	743	2.2	5.9	3.7	0.1	0.1	76	0.5	149	90	8.1	3.5	28	120	4.6	154	0.6	152
1094.08	11/04/09	Big Sur	M	107	220	279	102	2.7	8	5.3	0.1	0.1	62	0.7	177	104	8.8	4.1	25	113	4.2	149	0.5	88.6
1095.08	11/06/08	Big Sur	M	64	159	214	2459	2.5	6.5	4	0.2	0.2	63	0.5	195	142	8.6	5.3	23	123	4.3	157	0.6	126
1096.08	11/10/08	Big Sur	M	92	186	172	1034	2.7	7	4.3	0.1	0.1	69	0.7	141	85	8.1	5.4	24	115	4.8	152	0.6	98.6
1096.08	11/05/09	Big Sur	M	126	281	234	309	2.5	7	4.5	0.1	0.1	65	0.6	177	115	8.2	4.8	28	116	4.6	153	0.6	108.3
1099.08	11/10/08	Big Sur	M	60	150	197	963	2.6	6.6	4	0.1	0.1	68	0.7	159	99	8.5	4.7	26	116	4.7	153	0.7	97.1
1137.09	11/04/09	Big Sur	M	107	224	228	246	2.5	6.6	4.1	0.1	0.1	76	0.5	133	143	8.3	3	27	116	4.1	153	0.6	152
1143.09	11/05/09	Big Sur	M	127	210	244	438	2.5	6.6	4.1	0.2	0.1	79	0.4	167	132	8.6	2	27	122	4.3	156	0.6	197.5
1147.09	11/06/09	Big Sur	M	119	178	153	744	2.8	5.9	3.1	0.1	0.1	62	0.4	174	120	9.2	4.9	21	123	4.1	156	0.9	155
1165.10	09/28/10	Big Sur	M	88	200	183	190	2.5	6.3	3.8	0.1	0.1	62	0.5	135	139	8.8	3.5	23	98	3.7	128	0.7	124

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
1066.08	11/06/08	Big Sur	M	0	28	7.4	5.06	18.8	56.3	111	37.1	33.4	61	0	29	0	9	1	4514	0	2146	0	666	74
1091.08	11/10/08	Big Sur	M	0	33	5.3	5.14	18.7	54.9	107	36.4	34.1	66	0	19	4	10	1	3498	0	1007	212	530	53
1093.08	11/10/08	Big Sur	M	0	36	5	5.17	20.3	58.9	114	39.3	34.4	60	0	26	4	10	0	3550	0	950	500	355	0
1094.08	11/10/08	Big Sur	M	0	33	7.5	4.45	18.3	54.6	123	41.1	33.5	58	0	15	2	25	0	4350	0	1125	150	1875	0
1094.08	11/04/09	Big Sur	M	0	35	6.6	5.02	18.9	61.4	122	37.6	30.8	64	0	23	6	7	0	4224	0	1518	396	462	0
1095.08	11/06/08	Big Sur	M	0	37	8.3	4.97	18.7	54.9	110	37.6	34.1	72	0	20	2	6	0	5976	0	1660	166	498	0
1096.08	11/10/08	Big Sur	M	0	32	4.7	5.25	19.9	58.9	112	37.8	33.7	66	0	19	2	13	0	3102	0	893	94	611	0
1096.08	11/05/09	Big Sur	M	0	33	5.5	5.58	21.2	62.7	112	38	33.8	56	0	33	2	8	1	3080	0	1815	110	440	55
1099.08	11/10/08	Big Sur	M	0	33	7.1	5.25	20.3	60.3	115	38.6	33.6	60	0	27	2	11	0	5734	0	2162	470	1034	0
1137.09	11/04/09	Big Sur	M	0	37	4.7	4.99	20.8	65.6	132	41.8	31.8	56	0	27	5	12	0	2632	0	1269	235	564	0
1143.09	11/05/09	Big Sur	M	0.1	36	4.5	4.99	19.8	59.7	120	39.7	33.2	57	0	35	1	6	1	2565	0	1575	45	270	45
1147.09	11/06/09	Big Sur	M	0	38	6.9	5.12	14.2	46.6	91	27.8	30.6	60	0	26	9	5	0	4140	0	1794	621	345	0
1165.10	09/28/10	Big Sur	M	0	35	7.3	5.68	19.8	62.7	110	34.9	31.6	50	0	28	3	19	0	3650	0	2044	219	1387	0

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
1067.08	09/24/10	Big Sur	F	120	170	105	227	2.8	6.8	4	0	0	63	0.3	168	126	9.4	3.5	25	111	4.3	146	0.7	210
1067.08	11/06/08	Big Sur	F	71	166	117	355	2.6	6	3.4	0.2	0.1	57	0.3	165	133	9.1	5.9	30	113	4.3	151	0.8	190
1068.08	11/06/08	Big Sur	F	106	152	151	1186	2.5	6.3	3.8	0.2	0.2	74	0.4	190	148	8.8	5.1	26	119	4	155	0.7	185
1069.08	11/06/08	Big Sur	F	112	296	237	1137	2.6	6.5	3.9	0.2	0.2	64	0.3	193	165	8.4	5.7	27	122	3.9	158	0.7	213.3
1070.08	11/06/08	Big Sur	F	99	147	248	2445	2.6	6.7	4.1	0.4	0.4	61	0.4	194	133	8.5	7.4	25	119	4	155	0.6	152.5
1071.08	11/06/08	Big Sur	F	86	269	288	1089	2.8	6.4	3.6	0.1	0.1	90	0.4	138	153	8.4	5.9	22	115	4.7	150	0.8	225
1072.08	11/06/08	Big Sur	F	93	178	205	526	2.5	6	3.5	0.3	0.3	109	0.5	173	176	8.8	4.4	23	120	4.6	155	0.7	218
1073.08	11/10/08	Big Sur	F	70	93	86	384	2.6	5.6	3	0.1	0.1	62	0.3	194	139	8	3	27	119	3.7	152	0.9	206.7
1074.08	11/10/08	Big Sur	F	113	273	313	397	2.8	6.6	3.8	0.1	0.1	101	0.5	222	129	8.5	4.6	28	114	4.4	152	0.7	202
1074.08	09/25/10	Big Sur	F	91	179	157	207	2.6	6.8	4.2	0	0	67	0.3	183	117	8	3.4	30	114	4	148	0.6	223.3
1075.08	11/10/08	Big Sur	F	140	159	143	1101	2.7	6.9	4.2	0.2	0.1	45	0.3	243	131	8.8	4.9	25	118	3.8	154	0.6	150
1076.08	11/10/08	Big Sur	F	112	105	104	209	2.4	6.3	3.9	0.1	0.1	84	0.3	200	92	8	4.3	26	119	4.2	155	0.6	280
1077.08	11/10/08	Big Sur	F	101	117	109	490	2.7	6.5	3.8	0.1	0.1	75	0.04	257	115	8.1	5.9	23	117	4.8	153	0.07	187.5
1078.08	11/10/08	Big Sur	F	75	80	99	583	2.4	6.4	4	0.1	0.1	68	0.5	306	169	8	4.3	29	118	4	154	0.6	136
1079.08	11/10/08	Big Sur	F	113	103	69	365	3	6.4	3.4	0.2	0.1	44	0.4	241	132	9.5	4.1	24	118	3.9	153	0.9	110
1080.08	11/10/08	Big Sur	F	110	97	107	670	2.7	6.6	3.9	0.2	0.1	49	0.5	217	129	8.5	5.8	27	117	4	153	0.7	98
1081.08	11/10/08	Big Sur	F	84	206	307	687	2.5	7.1	4.6	0.2	0.1	76	0.5	194	141	8.2	5.3	25	119	3.9	155	0.5	152
1082.08	11/10/08	Big Sur	F	119	211	154	797	2.6	5.9	3.3	0.1	0.1	100	0.5	174	186	7.9	4.9	23	119	4.3	152	0.8	200
1083.08	11/10/08	Big Sur	F	65	94	94	449	2.6	6.3	3.7	0.1	0.1	58	0.4	150	146	7.9	4.6	25	117	3.9	154	0.7	145
1084.08	11/10/08	Big Sur	F	96	121	113	661	2.9	6.8	3.9	0.2	0.1	64	0.5	214	118	8.5	4.8	26	116	4.3	152	0.7	128
1085.08	11/11/08	Big Sur	F	82	104	73	223	2.9	6.4	3.5	0.2	0.1	65	0.5	193	177	8.1	5.4	24	113	3.8	150	0.8	130
1086.08	11/10/08	Big Sur	F	83	149	137	564	2.8	7.1	4.3	0.2	0.1	58	0.4	221	160	8.3	5	23	120	4.2	153	0.7	145
1087.08	11/11/08	Big Sur	F	71	182	467	6137	2	6.5	4.5	0.2	0.1	153	1	155	85	4.8	10.2	13	112	4.4	145	0.4	153
1088.08	11/12/08	Big Sur	F	141	192	165	362	2.8	6.6	3.8	0.1	0.1	57	0.4	215	116	8.6	5.1	27	114	3.7	151	0.7	142.5
1089.08	11/10/08	Big Sur	F	71	138	130	492	2.7	7	4.3	0.1	0.1	72	0.5	196	80	8.2	4.8	24	121	3.9	155	0.6	144
1090.08	11/12/08	Big Sur	F	110	127	115	306	2.6	6.7	4.1	0.1	0.1	87	0.4	259	96	8.6	4.2	28	116	4.2	153	0.6	217.5
1092.08	11/14/08	Big Sur	F	80	107	134	131	1.9	6.8	4.9	0.1	0.1	77	0.3	413	136	8	5.5	26	116	4.2	152	0.4	256.7
1097.08	11/14/08	Big Sur	F	104	123	153	594	2.7	6.3	3.6	0.1	0.1	66	0.4	187	134	8.6	6.7	24	116	4.8	152	0.8	165
1097.08	09/28/10	Big Sur	F	95	115	103	205	2.7	6.2	3.5	0	0	83	0.3	186	132	8.4	5	21	119	3.9	152	0.8	276.7
1098.08	11/14/08	Big Sur	F	149	167	165	1064	2.8	6.4	3.6	0.2	0.1	82	0.3	223	132	9.8	4	24	115	4.2	150	0.8	273.3
1103.08	11/14/08	Big Sur	F	111	239	216	511	2.8	6.3	3.5	0.1	0.1	66	0.5	178	182	8.1	4.5	21	118	4.5	152	0.8	132
1104.08	11/14/08	Big Sur	F	90	102	150	484	2.7	6.5	3.8	0.1	0.1	82	0.4	160	152	8.3	7.3	24	118	4.5	156	0.7	205
1105.08	11/14/08	Big Sur	F	107	217	238	1633	2.6	6.2	3.6	0.2	0.1	62	0.3	158	152	8.5	2.6	16	123	4.2	152	0.7	206.7
1106.08	11/14/08	Big Sur	F	125	190	274	877	2.7	6.4	3.7	0.1	0.1	71	0.3	164	137	8	5.7	19	121	4.6	155	0.7	236.7
1135.09	11/04/09	Big Sur	F	56	172	135	279	2.5	7	4.5	0.1	0.1	67	0.4	196	117	8.4	3.7	28	113	3.7	150	0.6	167.5
1136.09	11/04/09	Big Sur	F	140	172	114	834	2.6	6.8	4.2	0.1	0.1	60	0.3	246	141	9.5	5	24	114	4.2	147	0.6	200
1138.09	11/04/09	Big Sur	F	84	150	102	271	2.5	6.2	3.7	0.1	0.1	61	0.4	205	143	8.9	3.3	25	113	4	149	0.7	152.5
1139.09	11/04/09	Big Sur	F	109	136	125	845	2.8	6.9	4.1	0.2	0.1	58	0.4	202	115	8.9	4.1	25	116	4.1	152	0.7	145

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
1067.08	09/24/10	Big Sur	F	0	34	9.9	5.17	19.7	58.1	113	38.2	33.9	44	0	36	2	18	0	4356	0	3564	198	1782	0
1067.08	11/06/08	Big Sur	F	0.1	35	9.7	5.45	19.7	58.2	107	36.1	33.9	64	2	26	3	5	0	6208	194	2522	291	485	0
1068.08	11/06/08	Big Sur	F	0	39	9.3	5.54	19.3	58	105	34.8	33.2	62	0	28	1	9	0	5766	0	2604	93	837	0
1069.08	11/06/08	Big Sur	F	0	41	11.1	4.35	18.1	53.5	123	41.5	33.7	61	0	28	7	4	0	6771	0	3108	777	444	0
1070.08	11/06/08	Big Sur	F	0	39	5.6	5.1	19.1	59.7	117	37.5	32	72	0	24	3	1	0	4032	0	1344	168	56	0
1071.08	11/06/08	Big Sur	F	0	32	5.8	5.11	19.6	58.9	115	38.4	33.3	53	0	45	2	0	0	3074	0	2610	116	0	0
1072.08	11/06/08	Big Sur	F	0	34	6.7	5.03	19.4	57.8	115	38.6	33.6	70	0	24	2	4	0	4690	0	1608	134	268	0
1073.08	11/10/08	Big Sur	F	0	41	4.7	5.36	19.6	58.2	109	36.6	33.7	37	0	38	3	21	1	2538	0	2021	141	0	0
1074.08	11/10/08	Big Sur	F	0	35	6.2	4.73	19.1	55.4	117	40.4	34.5	44	0	25	2	29	0	2728	0	1550	124	1798	0
1074.08	09/25/10	Big Sur	F	0	37	6.2	4.77	18.5	54.8	115	38.8	33.8	60	0	17	3	20	0	3720	0	1054	186	1240	0
1075.08	11/10/08	Big Sur	F	0.1	41	4.9	5.04	19.8	57.6	114	39.2	34.3	42	0	41	2	15	0	2058	0	2009	98	735	0
1076.08	11/10/08	Big Sur	F	0	37	8.9	4.98	19.4	57.5	116	38.9	33.7	19	0	39	3	39	0	1691	0	3471	267	3471	0
1077.08	11/10/08	Big Sur	F	0	32	9.3	5.16	19.9	58.1	112	38.5	34.2	40	0	20	4	35	1	3720	0	1860	372	3255	93
1078.08	11/10/08	Big Sur	F	0	39	6.7	4.94	19	55.5	112	38.4	34.2	41	0	27	1	30	1	2747	0	1809	67	2010	67
1079.08	11/10/08	Big Sur	F	0.1	39	4.3	5.13	19.3	59.1	115	37.7	32.7	49	0	36	4	11	0	2107	0	1548	172	473	0
1080.08	11/10/08	Big Sur	F	0.1	38	47	5.5	20.4	61.1	111	37.1	33.4	40	0	45	4	11	0	1880	0	2115	188	517	0
1081.08	11/10/08	Big Sur	F	0.1	40	5.6	4.33	18.3	54.4	126	42.3	33.7	72	0	15	3	10	0	4032	0	840	168	560	0
1082.08	11/10/08	Big Sur	F	0	35	6.1	4.74	18.2	54.9	116	38.4	33.1	47	0	30	5	18	0	2867	0	1830	305	1098	0
1083.08	11/10/08	Big Sur	F	0	39	7.1	4.81	18.7	57.1	119	38.9	32.8	45	0	34	4	17	0	3195	0	2414	284	1207	0
1084.08	11/10/08	Big Sur	F	0.1	35	7.3	5.36	20.7	60.3	113	38.6	34.3	38	0	40	3	19	0	2774	0	2920	219	1387	0
1085.08	11/11/08	Big Sur	F	0.1	39	6.3	4.78	18.5	56.1	118	38.7	32.9	49	0	40	2	9	0	3087	0	2520	126	567	0
1086.08	11/10/08	Big Sur	F	0.1	36	9.4	4.89	19.9	58.7	120	40.7	33.9	61	0	23	5	11	0	5734	0	2162	470	1034	0
1087.08	11/11/08	Big Sur	F	0.1	33	0.5	5.63	22.7	62.3	111	40.3	36.4	35	0	42	5	18	0	175	0	210	25	90	0
1088.08	11/12/08	Big Sur	F	0	41	8.1	4.46	19.2	53.7	120	43	35.7	33	0	59	0	8	0	2673	0	4779	0	648	0
1089.08	11/10/08	Big Sur	F	0	40	5.5	4.65	19.2	55.8	120	41.2	34.3	47	0	31	3	19	0	2585	0	1705	165	1045	0
1090.08	11/12/08	Big Sur	F	0	36	6.4	5.05	19.7	57.7	114	38.9	34.1	37	0	38	5	20	0	2368	0	2432	320	1280	0
1092.08	11/14/08	Big Sur	F	0	36	7.7	3.19	13.2	39.9	125	41.2	33	65	0	18	5	12	0	5005	0	1386	385	924	0
1097.08	11/14/08	Big Sur	F	0	32	6.4	4.67	19.2	54.3	116	41.1	35.3	52	0	39	1	8	0	3328	0	2496	64	512	0
1097.08	09/28/10	Big Sur	F	0	39	6.1	4.58	17.7	54.1	118	38.7	32.8	57	0	26	1	16	0	3477	0	1586	61	976	0
1098.08	11/14/08	Big Sur	F	0.1	36	8.1	5.13	19.6	56.9	111	38.1	34.4	41	0	49	1	9	0	3321	0	3969	81	729	0
1103.08	11/14/08	Big Sur	F	0	34	4.9	5	19.1	56.8	114	38.2	33.6	69	0	21	0	10	0	3381	0	1029	0	490	0
1104.08	11/14/08	Big Sur	F	0	35	8.7	4.71	19.4	55.9	119	41.1	34.6	54	0	36	1	9	0	4698	0	3132	87	783	0
1105.08	11/14/08	Big Sur	F	0.1	36	6.2	4.79	19	56	117	39.7	34	69	0	15	1	15	0	4278	0	930	62	930	0
1106.08	11/14/08	Big Sur	F	0	34	5.8	4.84	18.9	55.5	115	39	34	49	0	38	0	13	0	2842	0	2204	0	754	0
1135.09	11/04/09	Big Sur	F	0	41	7.1	5.56	20.8	66	119	37.5	31.6	71	0	16	6	7	0	5041	0	1136	426	497	0
1136.09	11/04/09	Big Sur	F	0	35	6.7	5.25	19.7	61.7	118	37.5	31.9	27	0	62	6	5	0	1809	0	4154	402	335	0
1138.09	11/04/09	Big Sur	F	0	37	7.4	5.2	19	60.5	116	36.6	31.4	29	0	62	1	8	0	2146	0	4588	74	592	0
1139.09	11/04/09	Big Sur	F	0.1	37	8.1	5.12	20.3	64.5	126	39.7	31.5	48	0	41	4	7	0	3888	0	3321	324	567	0

Otter ID (BRD #)	Date	Site	Sex	Alk Phos (U/L)	ALT (U/L)	AST (U/L)	CK (U/L)	Alb ((g/dL)	Tot Prot (g/dL)	Glob (g/dL)	Tot Bili (mg/dL)	Dir Bili (mg/dL)	BUN (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	Gluc (mg/dL)	Ca (mg/dL)	Phos (mg/dL)	TCO2 (mEq/L)	Chlor (mEq/L)	K (mEq/L)	Na (mEq/L)	A/G Ratio	B/C Ratio
1140.09	11/04/09	Big Sur	F	125	164	175	337	2.6	6.5	3.9	0.2	0.1	103	0.4	169	125	8.4	4.6	20	115	4.7	150	0.7	257.5
1141.09	11/04/09	Big Sur	F	122	158	109	380	2.9	7.7	4.8	0.1	0.1	70	0.5	230	134	8.4	2.2	22	115	3.8	149	0.6	140
1141.09	09/28/10	Big Sur	F	147	149	146	118	3	7.1	4.1	0.1	0.1	58	0.3	193	110	8.8	2.8	23	115	4	153	0.7	193.3
1142.09	09/25/10	Big Sur	F	73	147	108	199	2.9	6.7	3.8	0	0	57	0.3	171	122	9.5	5.2	28	113	4.2	149	0.8	190
1144.09	11/05/09	Big Sur	F	122	138	124	316	2.9	6.9	4	0.1	0.1	71	0.5	178	115	9.2	2.9	22	116	3.9	153	0.7	142
1145.09	11/06/09	Big Sur	F	106	155	108	302	2.8	7.5	4.7	0.1	0.1	66	0.6	238	136	8.7	3	24	112	3.7	150	0.6	110
1146.09	11/06/09	Big Sur	F	103	222	150	290	2.8	6	3.2	0.2	0.1	54	0.3	135	168	9.1	5	24	115	3.7	152	0.9	180
1148.09	11/06/09	Big Sur	F	88	146	97	248	2.7	6.9	4.2	0.1	0.1	68	0.6	188	173	8.8	3.3	22	117	3.9	153	0.6	113.3
1150.09	11/06/09	Big Sur	F	174	189	142	410	2.8	7.1	4.3	0.1	0.1	92	0.4	205	192	8.8	4.5	20	116	4.2	153	0.7	230
1151.09	11/06/09	Big Sur	F	115	117	146	1592	2.8	6.8	4	0.3	0.1	73	0.4	180	123	8.4	4.3	21	118	4.7	153	0.7	182.5
1159.10	09/23/10	Big Sur	F	98	119	99	297	2.9	6.6	3.7	0	0	64	0.4	186	157	9.2	5.6	19	114	4.3	151	0.8	160
1160.10	09/23/10	Big Sur	F	72	167	139	436	2.3	6.6	4.3	0	0	95	0.3	159	160	9.1	3.4	26	113	4	146	0.5	316.7
1161.10	09/23/10	Big Sur	F	93	156	117	406	2.9	6.6	3.7	0	0	63	0.4	156	193	9	6.5	23	115	4	150	0.8	157.5
1162.10	09/24/10	Big Sur	F	106	113	100	234	2.6	7.2	4.6	0.1	0.1	64	0.2	153	100	8.7	4	27	112	4	150	0.6	320
1163.10	09/25/10	Big Sur	F	268	122	101	449	3.3	6.1	2.8	0.1	0.1	40	0.2	192	140	10.1	8.5	27	115	4	151	1.2	200
1164.10	09/28/10	Big Sur	F	123	131	98	560	2.7	5.9	3.2	0.1	0.1	65	0.3	171	192	8.9	5.9	22	115	4.2	147	0.8	216.7
1166.10	09/28/10	Big Sur	F	119	1251	1841	144	2.8	6.5	3.7	0.2	0.2	63	0.4	279	119	8.7	3.6	21	116	3.6	151	0.8	157.5
1167.10	09/28/10	Big Sur	F	141	227	401	218	301	606	305	0.2	0.1	80	0.4	183	100	9.4	1.9	22	118	3.5	155	0.9	200
1168.10	09/29/10	Big Sur	F	52	122	113	220	2.4	6.9	4.5	0.1	0.1	65	0.4	146	128	8.4	4.4	24	114	4.2	148	0.5	162.5

Otter ID (BRD #)	Date	Site	Sex	Indir Bili (mg/dL)	Na/K Ratio	WBC (Thous/uL)	RBC (Million/uL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neut Seg (%)	Neut Band (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	Abs Neut (/uL)	Abs Band (/uL)	Abs Lymph (/uL)	Abs Mono (/uL)	Abs Eos (/uL)	Abs Baso (/uL)
1140.09	11/04/09	Big Sur	F	0.1	32	6.3	4.9	19.2	60.2	123	39.1	31.8	43	0	47	2	8	0	2709	0	2961	126	504	0
1141.09	11/04/09	Big Sur	F	0	39	6.6	5.41	20.2	65.1	120	37.3	31	58	0	33	1	7	1	3828	0	2178	66	462	66
1141.09	09/28/10	Big Sur	F	0	38	7.8	5.34	19.8	60.8	114	37.1	32.6	53	0	29	4	14	0	4134	0	2262	312	1092	0
1142.09	09/25/10	Big Sur	F	0	35	9	5.19	18.8	55.7	107	36.2	33.8	53	0	31	3	13	0	4770	0	2790	270	1170	0
1144.09	11/05/09	Big Sur	F	0	39	9.9	5.67	21.9	66.3	117	38.6	33	20	0	61	5	12	0	1980	0	6039	495	1188	0
1145.09	11/06/09	Big Sur	F	0	41	6.7	5.75	20.4	63.5	110	35.4	32	51	0	26	3	20	0	3417	0	1742	201	1340	0
1146.09	11/06/09	Big Sur	F	0.1	41	5.4	5.6	20	63	113	35.8	31.8	45	0	41	5	9	0	2430	0	2214	270	486	0
1148.09	11/06/09	Big Sur	F	0	39	8.2	5.13	20.3	63.5	124	39.5	31.9	56	0	22	4	18	0	4592	0	1804	328	1476	0
1150.09	11/06/09	Big Sur	F	0	36	7	4.99	20.2	63	126	40.4	32	42	0	39	8	11	0	2940	0	2730	560	770	0
1151.09	11/06/09	Big Sur	F	0.2	33	5.9	5.48	20.9	66.4	121	38.2	31.5	31	0	55	0	14	0	1829	0	3245	0	826	0
1159.10	09/23/10	Big Sur	F	0	35	7.8	5.66	20.6	63.6	112	36.4	32.4	30	0	48	3	19	0	2340	0	3744	234	1482	0
1160.10	09/23/10	Big Sur	F	0	37	7	4.89	17.4	52.1	106	35.5	33.4	58	0	29	3	10	0	4060	0	2030	210	700	0
1161.10	09/23/10	Big Sur	F	0	38	9	5.09	18.4	55.8	110	36.2	33	50	0	29	3	18	0	4500	0	2610	270	1620	0
1162.10	09/24/10	Big Sur	F	0	38	8.8	4.54	18.7	54.4	120	41.2	34.4	40	0	32	8	20	0	3520	0	2816	704	1760	0
1163.10	09/25/10	Big Sur	F	0	38	6.3	4.93	16.5	50.7	103	33.4	32.5	57	0	35	6	2	0	3591	0	2205	378	126	0
1164.10	09/28/10	Big Sur	F	0	35	5.4	4.68	17.8	53.8	115	37.9	33	61	0	24	3	12	0	3294	0	1296	162	648	0
1166.10	09/28/10	Big Sur	F	0	42	10.7	5.16	18.9	58.1	113	36.6	32.5	75	0	14	4	7	0	8025	0	1498	428	749	0
1167.10	09/28/10	Big Sur	F	0.1	44	13.1	4.83	18.9	56.6	117	39	33.3	68	0	28	1	3	0	8908	0	3668	131	393	0
1168.10	09/29/10	Big Sur	F	0	35	8.1	4.41	16.6	54.8	124	37.7	30.3	38	0	26	0	36	0	3078	0	2106	0	2916	0

Chapter 3. Gene Transcription: Immune and Detoxification Function in California Sea Otters

A.K. Miles[2], L. Bowen[1], M. Tim Tinker[1], and M. Murray[3]

[1]U. S. Geological Survey, Western Ecological Research Center, Santa Cruz Field Station, Santa Cruz, CA
[2]U. S. Geological Survey, Western Ecological Research Center, Sacramento, CA
[3]Monterey Bay Aquarium, Monterey, CA

Introduction

The California population of the southern sea otter (*Enhydra lutris nereis*) is classified as "depleted" under the federal Marine Mammal Protection Act and "threatened" under the federal Endangered Species Act. Historical abundance of southern sea otters was estimated at approximately 14,500, but now includes only about 2,800 animals in California, distributed between Half Moon Bay (San Mateo County) and Point Conception (Santa Barbara County). Since 1995, the recovery of the southern sea otter has seemingly stalled, leading to questions about population health and viability.

Suggested causes for declines in sea otter numbers are complex and have been attributed to a variety of ecological or human-influenced possibilities. Anthropogenic pressures such as oil spills are usually noted for their sudden dramatic impact on marine and coastal species. The acute effects of these disasters are evaluated by mortality estimations, clinical evaluation, and necropsy examinations (Lipscomb et al. 1993, Monson et al. 2000). The long-term or chronic effects of incidental or periodic events associated with anthropogenic insult to the environment are more difficult to assess. Studies often are restricted to demographic modeling, estimations of reproductive efficiency, or time-differential, age-specific survival rates, yet few biological markers are available that identify sub-lethal pathology attributable to direct anthropogenic influence.

Applying contemporary gene transcript analysis to identify genomic response to environmental stress or disease has the potential to transform studies of sea otter ecology and diagnostics (Burczynski et al. 2000; Bartosiewicz et al. 2001). Advanced technologies, based upon and developed from biomedical models of human physiology and disease, aid researchers with cutting-edge diagnostic tools for both domestic and wildlife veterinary applications (Burczynski et al. 2000; Bartosiewicz et al. 2001; Bowen et al. 2007, 2012; Miles et al. 2012; Sitt et al. 2008). Gene-based diagnostics of sea otters afford the opportunity for minimally invasive assessments of physiologic state in response to intrinsic and extrinsic factors, not only in individuals or populations but potentially at landscape scale (Acevedo-Whitehouse & Duffus 2009).

Gene transcript analysis affords the opportunity to detect the earliest observable signs of physiological perturbation, as gene transcripts are typically evident prior to clinical manifestations to environmental stressors (McLoughlin et al. 2006). Consequently, clinical application of quantitative gene transcript analysis technology will provide an invaluable addition to current approaches for monitoring potential ecosystem and individual health impairment (McLoughlin et al. 2006). The genes used in our analysis are fundamental to mediation of detoxification and immune function (Schwartz *et al*. 2004a,b), cellular

injury (Ghanem *et al*. 2006), signal transduction (Burchiel and Luster 2004), xenobiotic metabolism (Schwartz *et al*. 2004a,b), or tumorigenesis (Ramesh *et al.* 2004) (Table 1). Measurement of differential transcription of a selected suite of genes potentially can provide an early warning of compromised health and related environmental stressors in free-ranging animals. The suite of genes examined in this study identified potential effects on sea otters in Prince William Sound, Alaska, 20 years after the Exxon Valdez oil spill (Miles et al. 2012). We describe transcript profiles of these genes from populations of southern sea otters ranging from Monterey to Santa Barbara, California, with the particular goal of comparing two focal study sites (Big Sur and Monterey) and placing them within the context of variation from other sites throughout California.

Methods

Free-ranging target otters

A total of 180 sea otters were captured in California (43 Santa Barbara, 2012/2013; 57 San Luis Obispo, 2012; 51 Big Sur, 2008/2009/2010; 29 Monterey, 2009/2010). Sea otters were captured by scuba divers using Wilson traps (Wendell et al. 1996) and brought immediately to a shipboard station for processing. These sea otters (as well as reference captive sea otters) were anesthetized with fentanyl citrate and midazolam hydrochloride (Monson et al. 2001) prior to processing. Refer to Chapter 1 for more details on capture and sampling methodology.

Captive reference otters

Blood samples from 17 captive sea otters were obtained from the Monterey Bay Aquarium (Monterey, CA), Shedd Aquarium (Chicago, IL), Oregon Coast Aquarium (Newport, OR), and the Vancouver Aquarium (Vancouver, BC) in 2008, 2009, and 2010, and included both northern and southern subspecies (Bowen et al. 2012). These animals were identified as clinically normal by staff veterinarians at these aquaria during the time interval of blood collection.

Blood collection and RNA extraction

A 2.5 ml sample from each sea otter was drawn directly into a PAXgene™ blood RNA collection tube (PreAnalytiX, Switzerland) from either the jugular or popliteal veins and then frozen at - 20^{o}C until extraction of RNA (Bowen et al. 2012). Rapid RNA degradation and induced transcription of certain genes after blood draws has led to the development of methodologies for preserving the RNA transcription profile immediately after blood is drawn. The PAXgene™ tube contains a blend of RNA stabilizing reagents that protect RNA molecules from degradation by RNases and prevents further induction of gene transcription. Without this stabilization, copy numbers of individual mRNA species in whole blood can change more than 1000-fold during storage and transport. The RNA from blood in PAXgene™ tubes was isolated according to manufacturer's standard protocols, which included an on-column DNase treatment to remove contaminating gDNA (silica-based microspin technology), and the extracted RNA stored at -80^{o}C until analysis. All RNA was checked for quality on a nanodrop 2000 and achieved A260/A280 ratios of approximately 2.0 and A260/A230 ratios of less than 1.0.

cDNA creation

A standard cDNA synthesis was performed on 2 μg of RNA template from each animal. Reaction conditions included 4 units reverse transcriptase (Omniscript®, Qiagen, Valencia, CA), 1 μM random hexamers, 0.5 mM each dNTP, and 10 units RNase inhibitor, in RT buffer (Qiagen, Valencia, CA). Reactions were incubated for 60 minutes at 37°C, followed by an enzyme inactivation step of 5 minutes at 93°C, and then stored at –20°C until further analysis.

Real-time PCR

Real-time PCR systems for the individual, sea otter-specific reference or housekeeping gene (S9) and genes of interest were run in separate wells (Bowen et al. 2012, Miles et al. 2012; Table 1). Briefly, 1 μl of cDNA was added to a mix containing 12.5μl of QuantiTect SYBR Green® Master Mix [5mM Mg^{2+}] (Qiagen, Valencia, CA), 0.5μl each of forward and reverse sequence specific primers, 0.5μl of Uracil-N-Glycosylase (Invitrogen, Carlsbad, CA), and 10.0μl of RNase-free water; total reaction mixture was 25μl. The reaction mixture cDNA samples for each gene of interest and the S9 gene were loaded into 96 well plates in duplicate and sealed with optical sealing tape (Applied Biosystems, Foster City, CA). Reaction mixtures containing water, but no cDNA, were used as negative controls; thus approximately three - four individual sea otter samples were run per plate.

Amplifications were conducted on a 7300 Real-time Thermal Cycler (Applied Biosystems, Foster City, CA). Reaction conditions were as follows: 50°C for 2 minutes, 95°C for 15 minutes, 40 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 31 seconds, an extended elongation phase at 72°C for 10 minutes. Reaction specificity was monitored by melting curve analysis using a final data acquisition phase of 60 cycles of 65°C for 30 seconds and verified by direct sequencing of randomly selected amplicons (Bowen *et al.* 2007). Cycle threshold crossing values (C_T) for the genes of interest were normalized to the S9 housekeeping gene.

Statistical analysis

Analysis of qPCR data was conducted using normalized values, i.e., housekeeping gene threshold crossing (in qPCR, the point at which amplification is exponential) subtracted from the gene of interest threshold crossing for each animal (McLoughlin et al. 2006).

We used nonparametric statistical analyses because the cycle threshold (C_T) measure of gene transcription provided by qPCR may have a highly skewed, non-normal distribution (McLoughlin et al. 2006). We conducted multivariate, multi-dimensional scaling (MDS) analysis in conjunction with cluster analysis for statistical and graphical representation of individual sea otters clustered by similarity in transcription and not by pre-defined groups such as location (Primer E, v6, Plymouth, UK). Statistical comparisons of individuals by clusters were made using SIMPROF, which is a similarity profile permutation test for significance among *a priori*, unstructured clusters of samples. We used ANOSIM (Primer E) one-way nonparametric, multivariate analysis to test for differences in gene transcription among locations, i.e., sea otters captured at or near the coastlines of Monterey Bay, Big Sur, San Luis Obispo County, and Santa Barbara County and the captive sea otters. We used two-way ANOSIM to examine within locations by sex and between or among capture years. Capture years varied by location (e.g., sea otters were captured only in 2012 at the San Luis Obispo region), thus analysis of all locations

by years was not possible. Sea otters captured were biased towards adult animals (79 %), precluding any reliable characterization of juvenile gene transcription patterns, so age differences were not evaluated. Statistical significance was based on p values ≤ 0.05, and in the case of the ANOSIM tests, relative to the R Statistic value. We further classified clusters into groups based on the number of genes markedly (i.e., 3 or more C_T value) higher or lower than background values established for captive sea otters deemed clinically healthy (hereafter 'captives') (Bowen et al 2012). Hereafter, 'high or low' followed by a gene identifier is used only to report those cases where gene transcription C_T values were 'markedly' different.

Additionally, we used gene transcript profiling based on per gene and per otter response correlation, using normalized qPCR data obtained from each individual otter, which were subjected to hierarchical clustering with complete linkage disequilibrium (Genesis, Graz, Switzerland); this was used to generate a heatmap profile of gene expression and physiologic health status.

Results

Transcript Pattern Analysis

Transcription patterns were established for 180 sea otters from the central California coast. Sea otter gene transcription patterns differed among locations (ANOSIM, $p < 0.001$, Global $R = 0.21$, with 0 permuted statistics > Global R). Pairwise tests indicated sea otters from the San Luis Obispo area differed significantly ($p < 0.001$) from the background captive values as well as all other areas; these differences were mainly influenced by San Luis Obispo area sea otters that had markedly lower transcription of HDC, THRB, IL10, and MX-1 than those from the other locations (Table 2).

Sea otters from areas of Monterey Bay, Big Sur, or Santa Barbara did not differ from the captives ($p > 0.14$). However, transcription patterns of sea otters from Santa Barbara differed from those from Big Sur and Monterey ($p < 0.001$), which also differed significantly from one another ($p < 0.002$).

Santa Barbara area sea otters had markedly higher 5HTT and also low HDC and IL10 relative to sea otters from the other locations, and Monterey and Big Sur sea otters differed mainly in transcription of COX2 and CYT. The MDS analysis of gene transcription C_T values depicts separations of which the denser mass of clusters included the captives, with the remaining otters diffusely clustered into smaller groups or single animal outliers (Figure 1). The captives were the only defined group that clustered tightly. The 3-D MDS depiction indicated a better fit (i.e., Stress = 0.10 versus 0.14 for 2-D), and visual separation within the densest mass. SIMPROF analysis indicated highly significant ($p = 0.001 – 0.007$; 79 % of the clusters) or significant ($p = 0.01 – 0.05$) separations among 33 clusters that included outliers.

Forty-one (23 % of total) sea otters comprising 4 clusters had transcription patterns similar to captives; differences among these clusters comprised mean or outlier C_T values of targeted genes within 3 C_T of clinically normal captives. The majority (27) of these animals were from the area near Big Sur, and the remainder from areas at Monterey Bay (11) or north of Santa Barbara (3).

Qualifying the clusters into groups based on number of genes with markedly different C_T than the captives, 6 clusters comprising 19 (11 % of total) sea otters separated into 2 subgroups, one with both

elevated and low gene C_T values and the other with elevated C_T values relative to the captives. These were the only sea otters sampled that had transcription patterns that indicated reactive immune or defensive responses. A cluster including five sea otters from Santa Barbara had high 5HTT but low HDC and IL18, whereas one from Big Sur had high IL18 and low CYT and AHR. The remaining Monterey Bay area sea otter (ID 1117) in this group had the most concerning transcription pattern of any sea otter sampled in this study: high COX2, THR, IL10, DRB, MX1, and low HDC. The other subgroup had one sea otter with high IL18 and MX1, 7 Monterey Bay area sea otters with high CYT, and 1 Big Sur and 3 Santa Barbara sea otters with high MX1 and 5HTT.

All of the remaining clusters comprised average C_T values that were markedly lower than those established for captives. Most of the sea otters that comprised this low transcription set were from the southern half of the sea otter range in California, i.e., San Luis Obispo area (63%) and Santa Barbara (25%, mostly animals that were captured in 2013), with 6 % each from the northern sites of Big Sur and Monterey.

In the 4 – 6 gene groups, 18 sea otters had 6 genes, 13 otters had 5 genes, and 20 otters had 4 genes of markedly lower transcription than those patterns determined for captives. Within these groupings, which 6, 5, or 4 genes were markedly lower varied among the 51 (28 % of total) sea otters. Within the 6-gene group, HSP 70, IL10, MX1, HDC, and COX2 were most evident, and then THR, AHR, IL18, or CCR3 comprised the 6th gene. Transcription of CYT, 5HTT, and CaM were similar to C_T values of the captives. Within the 5-gene group low transcription of HSP70, IL18, and MX1 were prominent, followed by HDC, COX2, and IL10, and then CYT, CCR3, and 5HTT. In this group, only AHR and CaM were similar to the captives. Within the 4-gene group, low transcription of HDC, HSP70, IL10, and MX1 was evident for most (18 of 20) of these otters, whereas 2 otters had an altogether different set of 4 genes of low transcription. Transcriptions of THR, DRB, CCR3, 5HTT, and CaM were similar to those of the captives.

The next grouping of 69 sea otters (38 % of total) comprised 12 sea otters with 3 genes, 39 sea otters with 2 genes, or 18 otters with 1 gene markedly lower in transcription compare to captives. Sea otters from this set were more evenly spread across the range comprising 24 from San Luis Obispo, 19 from Santa Barbara, 16 from Big Sur, and 9 from Monterey Bay areas. Within the 3-gene group, most had low transcription of COX2, MX1, and either HDC or HSP70. In the 2-gene group, 72 % of the sea otters had low transcription of IL18 but the 2nd gene was HDC, COX2, HSP70, IL10, or MX1. Within the 1-gene group, 15 sea otters had low transcription of HDC and the remaining 3 low transcription of IL10.

Within site: sex by year transcript differences

Multivariate analysis of transcription patterns of sex by years indicated no difference at Monterey Bay between 2009 and 2010 ($p > 0.53$; Table 2). At Big Sur, transcription patterns among years differed (2008, 2009, 2010; $p < 0.04$) attributed to the difference between 2008 and 2010 ($p<0.001$), but did not differ by sex ($p > 0.38$). Although sexes were skewed toward more females captured, no marked or notable differences in transcription were apparent among the suite of genes. Transcription of HDC, CYT, AHR, and THRB decreased from 2008 to 2010 at Big Sur, while HSP70, IL18, and CCR3 increased but values were not markedly different from captive background levels.

Variation in transcription pattern at the Santa Barbara area were most evident where both sex ($p<0.04$) and years ($p<0.001$) differed (Table 2). Transcription of COX2 in female sea otters in 2012 was similar to captive levels but low in males in 2012 and in females in 2013. The genes THRB and 5HTT were markedly higher than captive values in both sexes in 2012, as well as CCR3 in females, but in 2013 these values were similar to background values. We found markedly (relative to background) low transcription of IL10 and MX-1 in 2013 than in 2012. Transcription of HDC in females captured in 2012 was similar to background but low to markedly lower by all other comparisons of sex or year.

Gene Specific Patterns

Cluster analyses did not reveal apparent transcription patterns in individual genes. Because of natural oil seeps near Santa Barbara, we examined elevated (relative to background captive levels) transcription of AHR and THRB among the sampled sea otters. Twenty-six sea otters had high ($\geq$ 2 C_T value difference) to markedly higher AHR transcription. These animals were from areas of Monterey Bay (7), Big Sur (8), and Santa Barbara (8), with a few (3) from San Luis Obispo. Similarly, 26 sea otters had elevated transcription of THRB, and in this instance, 17 of these animals were from Santa Barbara, 6 from Big Sur, and 3 from Monterey Bay. Also, we found that IL10 was elevated in 26 sea otters (10 Big Sur, 8 Santa Barbara, 7 Monterey Bay, 1 San Luis Obispo). Only 19 % (5) of these sea otters had elevated transcription of all three genes, and 1 additional animal had 2 of these genes with elevated transcription.

Heatmap Pattern Analysis

Hierarchical cluster analysis was conducted using individual sea otter transcription data (Figure 2). Profile responses were successful in identifying transcriptional differences between capture locations and years, and yielded varying sized clusters of sea otters consisting of up to 100% of otters from single populations and time points. The largest clusters comprised San Luis Obispo and Santa Barbara 2013 otters. Otters from Monterey Bay (2009), Big Sur (both years), Santa Barbara 2012, and captive also dominated clusters.

Discussion

Transcript Pattern Analysis

Transcript pattern analyzed using MDS, ANOSIM, gene specific patterns, and hierarchical cluster generated heatmap yielded corroborating results. A majority of sea otters, particularly those from San Luis Obispo, had patterns of inexplicably low gene transcription. The occurrence of low transcription has been observed in past analyses but the preponderance among California sea otters system-wide was noteworthy. We confirmed that the low transcription values were not a result of a laboratory artifact. We analyzed all samples in duplicate, and if any duplicate samples were > 1 C_T difference, they were re-analyzed. All samples were run with an internal reference standard (i.e., housekeeping gene, S9). If the internal reference was greater than 3 C_T values from the average internal reference value for control populations, the entire panel was re-run in duplicate; this was always a problem of insufficient RNA and those samples were omitted from analysis (i.e., < 1 % of the samples). Finally, California samples were

run with Alaska samples and re-run in different combinations to check for 'plate' or 'batch' effects, and no such effects were encountered.

Animals presenting abnormally low levels of select cytokine transcripts may be interpreted as indicative of abnormally low levels of leukocyte activity; such suppressed activity has been described for T helper lymphocytes derived from humans with chronic viral infections (Stott and McBain 2012). Alternatively, low transcription may be the result of unbalanced resource allocation. For example, immune defense exists to impede infections, but other ecological demands (e.g., nutrition, weather, predation) can supersede this, causing immune defenses to be compromised (Martin et al. 2011). It is optimal to increase the allocation to immune defense as reserves increase (Houston et al. 2007), however the costs associated with mounting an immune response create a delicate balance between a protective immune phenotype (transcription pattern in this case) and a potential misallocation of resources. Tradeoffs among components of the vertebrate immune system itself are common and occur when one portion of the immune system is up-regulated while another portion is down-regulated; activation of one branch of immune function may effectively disable an opposing branch (Pedersen and Babayan 2011). Failure to acknowledge or measure these trade-offs may lead to erroneous conclusions. What we may deem abnormally low transcription may simply be due to a lack of breadth in our transcript panel. Our panel was more specific to organic exposure and included other indicators such as microbial or inflammatory defenses but this panel was by no means an exhaustive evaluation of genes stimulated by potential environmental stressors in the areas examined.

Within site year by sex transcript differences

Within-site transcript level differences were most notable at Big Sur and Santa Barbara. In general there was minimal difference in transcript level between sexes but a distinct separation of transcript level among years for Big Sur sea otters. The Basin and Chalk wildfires in the Big Sur region of central California in 2008 occurred just prior to a scheduled study of sea otters in the adjacent nearshore marine environment. This timing provided an opportunity to assess the potential effects of wildfire on the immunological response of sea otters just downslope from where the fire burned. The Basin fire burned approximately 66,500 ha between 21 June and late July, while the Chalk Fire consumed an additional 6,240 ha between ignition on 27 September and containment by the end of October. For the two wildfires combined, 45 percent of the burned area lay within the drainage basin that discharges into the territory of the Big Sur sea otter population. The fires burned with mixed severity, with partial to complete consumption of the heavy vegetation within the perimeter.

There are two potential indirect impacts of these fires to the sea otter population that may manifest in a genetic analysis of toxicological effects. First, during and immediately after the wildfires, prevailing night-time wind patterns consistently carried ash and debris from the smoke column off-shore and onto the surface waters (WRCC 2012). This ash was observed intermixed in the surface water column by capture crews during the November 2008 sampling bout. Second, there are seven minor drainages that carry run-off into the adjacent nearshore environment from the burned area. From 1 – 3 November, the Big Sur Remote Automated Weather Station (RAWS) measured 2.6" of rain (WRCC 2012), the first appreciable rainfall in over six months. This storm event subsequently produced a run-off spike in the Big Sur River that exceeded 200 c.f.s., compared to the 10-15 c.f.s. discharge of the river in the summer

and early autumn months. Following this event, the Big Sur area experienced a slightly drier than normal winter, with 35" (91% of normal) precipitation for the water year ending in October 2009 (WRCC 2012). Gene responses were distinctly different between Big Sur temporal groups, identifying detoxification of PAHs (up-regulation of AHR, $p < 0.001$) and associated malignant transformation (up-regulation of HDC, $p = 0.05$) as the primary responses of sea otters to fire in 2008 compared to those captured in 2009 and 2010 (Bowen et al., in review). Down current effects of exposure to PAHs were evidenced in the 2008 sea otters by immune suppression (down-regulation of IL-10, $p < 0.0011$; down-regulation of Il-18, $p < 0.001$; down-regulation of DRB, $p = 0.01$). In general, gene transcription patterns in the 2008 sea otters were indicative of molecular reactions to organic exposure, malignant transformation, and decreased ability to respond to pathogens that may be consistent with short-term hydrocarbon exposure. Transcript patterns of Big Sur sea otters in 2009 and 2010 were indicative of a return towards baseline normal.

The separation of Santa Barbara sea otter transcript levels by year and sex was confounding. In general, 2012 Santa Barbara sea otters exhibited transcript patterns that may have been related to hydrocarbon exposure, i.e., elevated THRB and to some degree, AHR, a pattern unique to this area. This may reflect periodic exposure to the natural oil seeps that occur throughout Santa Barbara channel. Additionally, 5HTT was elevated in 2012, a marker of low stress phenotype. In contrast, low transcription in 2013 Santa Barbara sea otters was similar to that seen in otters from the San Luis Obispo area.

Overall, with the exception of low transcription in San Luis Obispo sea otters, any observed gene expression levels that differed from background values seemed more unique to individuals rather than influenced by the area in which sea otters resided – that is, these abnormal individuals occurred with approximately equal frequencies at all sites. Significant elevation of gene transcription was not apparent in any areas of the central California coast, including suspect areas such as the urbanized region encompassing Monterey Bay. We suggest that these patterns indicate immune responses reflective of those of healthy captive sea otters, and thus indicated that environmental conditions experienced by sea otters in central California generally fall within the "normal" range, with occasional pulses of challenging conditions occurring throughout the range associated with both natural events (e.g. wild fires, oil seeps) or human-caused stressors (e.g. organic pollutants or terrestrial pathogens). This conclusion is based on the observation that the majority of sea otters sampled were either similar in gene transcription to captive background levels or that had expression of only one to three genes markedly different from background. However, consistent patterns of low transcription at the SLO study site require further investigation.

Until now, it has only been feasible to study small numbers of genes, usually stemming from a candidate gene approach. However, recent advances in deep sequencing technology allow for the elucidation of an unprecedented breadth of gene pathways. Through the deep sequencing of transcriptomes, broad scale identification of gene transcription patterns can provide mechanistic understanding of protective immune phenotypes and immune proxies of health; causal links between molecular patterns and individual and population health become possible (Pedersen and Babayan 2011). We propose experimentation to use deep sequencing to identify those genes or suites of genes in the California sea

otters that may be drawing resources responsible for the low transcription profile in our current gene panel. Until such experimentation is realized, the cause cannot be substantiated.

References

Acevedo-Whitehouse K, Duffus LJ (2009) Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B,* **364**, 3429-3438.

Bartosiewicz M, Penn S, Buckpitt A (2001) Applications of gene arrays in environmental toxicology: fingerprints of gene regulation associated with cadmium chloride, benzo(a)pyrene, and trichloroethylene. *Environmental Health Perspectives,* **109**, 71–74.

Beineke A, Siebert U, Muller G, Baumgartner W (2007) Increased blood interleukin-10 mRNA levels in diseased free-ranging harbor porpoises (*Phocoena phocoena*). Vet Immunol Immunopathol 115: 100−106

Bommer UA, Thiele BJ (2004) The translationally controlled tumour protein (TCTP). Int J Biochem Cell Biol 36:379−385

Bowen L, Aldridge B, Miles AK, Stott JL (2006) Expressed MHC class II genes in sea otters (*Enhydra lutris*) from geographically disparate populations. Tissue Antigens 67: 402−408

Bowen L, Schwartz J, Aldridge B, Riva F, Miles AK, Mohr FC, Stott JL (2007) differential gene expression induced by exposure of captive mink to fuel oil: a model for the sea otter. EcoHealth 4: 298−309

Bowen L, Miles AK, Murray M, Haulena M and others (2012) Gene transcription in sea otters (*Enhydra lutris*); development of a diagnostic tool for sea otter and ecosystem health. Mol Ecol Res 12: 67−74

Bowen L, Miles AK, Kolden CA, Saarinen JA, Bodkin JL, Murray M, Tinker T. Effects of wildfire on sea otter (Enhydra lutris) immune function. In review.

Burczynski ME, McMillian M, Ciervo J, Li L, Parker JB, Dunn RT II, et al (2000) Toxicogenomics-based discrimination of toxic mechanism in HepG2 human hepatoma cells. *Toxicological Science,* **58**, 399–415.

Burchiel SW, Luster MI (2004) Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. *Clinical Immunology,* **98**, 2–10.

Chen Z, Wang H, Matsumura K, Qian P (2012) Expression of Calmodulin and Myosin Light Chain Kinase during Larval Settlement of the Barnacle *Balanus Amphitrite.* PLoS ONE 7: e31337

De Maio A (1999) Heat shock proteins: facts, thoughts, and dreams. Shock 11: 1−12

Dong L, Ma Q, Whitlock JP Jr (1997) Down-regulation of major histocompatibility complex Q1b gene expression by 2,3, 7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 272: 29614−29619

Ghanem MM, Batelli LA, Mercer RR, Scabilloni JF, Kashon ML, Ma JY, et al. (2006) Apoptosis and Bax expression are increased by coal dust in the polycyclic aromatic hydrocarbon-exposed lung. *Environmental Health Perspectives,* **114**, 1367–1373.

Goldsby RA, Kindt TJ, Osborne BA, Kuby J (2003) Immunology, 5th edn. WH Freeman and Company, New York, NY

Gurish MF, Humbles A, Tao H, Finkelstein S, Boyce JA, Gerard C, Friend DS, Austen KF (2002) CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with Trichinella spiralis. The Journal of Immunology 168:5730-5736

Harris SG, Padilla J, Koumas L, Ray D, Phipps RP (2002) Prostaglandins as modulators of immunity. Trends Immunol 23: 144−150

Houston, AI, McNamara, JM, Barta, Z & Klasing, KC, 2007, The effect of energy reserves and food availability on optimal immune defence, Proceedings of the Royal Society B: Biological Sciences, 274(1627):2835 – 2842 p.

Iwama GK, Mathilakath MV, Forsyth RB, Ackerman PA (1999) Heat shock proteins and physiological stress in fish. Am Zool 39: 901−909

Jenne DE, Tschopp J (1989) Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. Proc Natl Acad Sci USA 86: 7123−7127

Jennings KA, Loder MK, Sheward WJ (2006) Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. Journal of Neuroscience 30:8955-8964

Kibenge MJT, Munir K, Kibenge FSB (2005) Constitutive expression of Atlantic salmon Mx1 protein in CHSE-214 cells confers resistance to infectious salmon Anaemia virus. Virol J 2: 75

Kringel H, Iburg T, Dawson H, Aasted B, Roepstorff A (2006) A time course study of immunological responses in Trichuris suis infected pigs demonstrates induction of a local type 2 response associated with worm burden. International Journal for Parasitology 36:915-924

Krumm B, Meng X, Li Y, Xiang Y, Deng J (2008) Structural basis for antagonism of human interleukin 18 by pox-virus interleukin 18-binding protein. Proc Natl Acad Sci USA 105: 20711−20715

Li S, Xie L, Zhang C, Zhang Y, Gu M, et al. (2004) Cloning and expression of a pivotal calcium metabolism regulator: calmodulin involved in shell formation from pearl oyster (*Pinctada fucata*). Comp Biochem Physiol B Biochem Mol Biol 138: 235-243.

Lipscomb TP, Harris RK, Moeller RB, Pletcher JM, Haebler RJ, Ballachey BE (1993) Histopatholgic lesions in sea otters exposed to crude oil. Vet Pathol 30:1-11

Ma Q, Geng Y, Xu W, Wu Y and others (2010) The role of translationally controlled tumor protein in tumor growth and metastasis of colon adenocarcinoma cells. J Proteome Res 9: 40−49

Martin LB, Hopkins WA, Mydlarz LD, Rohr JR (2011) The effects of anthropogenic global changes on immune functions and disease resistance. Ann N Y Acad Sci 1195:129–148

McLoughlin K, Turteltaub K, Bankaitis-Davis D, Gerren R and others (2006) Limited dynamic range of immune response gene expression observed in healthy blood donors using RT-PCR. Mol Med 12: 185–195

Miles AK, Bowen L, Ballachey B, Bodkin JL, Murray M, Keister RA, Stott JL (2012) Variations of Transcript Profiles Between Sea Otters (*Enhydra lutris*) from Prince William Sound, Alaska, and Clinically Normal Reference Otters. Marine Ecology Progress Series 451: 201-212

Monson DH, Doak DF, Ballachey BE, Johnson A, Bodkin JL (2000) Long-term impact of the *Exxon Valdez* oil spill on sea otters, assessed through age-dependent mortality patterns. Proc Natl Acad Sci USA 97: 6562–6567

Monson DH, McCormick C, Ballachey B (2001) Chemical anesthesia of northern sea otters (*Enhydra lutris*): results of past field studies. Jnl Zoo Wildl Med 32:181-189

Oesch-Bartlomowicz B, Oesch F (2005) Phosphorylation of cytochromes P450: first discovery of a posttranslational modification of a drug-metabolizing enzyme. Biochem Biophys Res Commun 338: 446–449

Pedersen, AB, Babayan SA, 2011, Wild immunology, Molecular Ecology, 20(5):872-880

Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL(2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 453: 6–7

Raisuddin S, Kwok KWH, Leung KMY, Schlenk D, Lee J (2007) The copepod *Tigriopus*: a promising marine model organism for ecotoxicology and environmental genomics. Aquat Toxicol 83: 161–173

Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology,* **23**, 301–333.

Rigopoulou EI, Abbott WG, Haigh P, Naoumov NV (2005) Blocking of interleukin-10 receptor—a novel approach to stimulate T-helper cell type 1 responses to hepatitis Cvirus. Clin Immunol 117: 57–64

Schwartz JA, Aldridge BM, Lasley BL, Snyder PW, Stott JL, Mohr FC (2004a) Chronic fuel oil toxicity in American mink (Mustela vison): systemic and hematological effects of ingestion of a low-concentration of bunker C fuel oil. *Toxicology and Applied Pharmacology,* **200**, 146-158.

Schwartz JA, Aldridge BM, Stott JL, Mohr FC (2004b) Immunophenotypic and functional effects of bunker C fuel oil on the immune system of American mink (Mustela vison). *Vet Immunol Immunopathol* **101**, 179-90.

Sitt T, Bowen L, Blanchard MT, Smith BR, Gershwin LJ, Byrne BA, Stott JL (2008) Quantitation of leukocyte gene expression in cetaceans. *Developmental and Comparative Immunology,* **32**, 1253–1259.

Stott, J. L. and J. F. McBain. Fowler's Zoo and Wild Animal Medicine: Current Therapy. Ed: R Eric Miller & Murray Fowler, 7th Ed. , 2012, Longitudinal monitoring of immune System parameters of cetaceans and application to their health management, Chapter 63: 482-489 p.

Tabuchi M, Veldhoen N, Dangerfield N, Jeffries S, Helbing CC, Ross PS (2006) PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (*Phoca vitulina*). Environ Health Perspect 114: 1024−1031

Tanabe S, Iwata H, Tatsukawa R (1994) Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. Sci Total Environ 154: 163−177

Tsai MJ, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63: 451−486

Tsan MF, Gao B (2004) Cytokine function of heat shock proteins. Am J Physiol Cell Physiol 286: C739−C744

Tumpey TM, Szretter KJ, Van Hoeven N, Katz JM and others (2007) The *Mx*1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. J Virol 81: 10818−10821

Tuynder M, Fiucci G, Prieur S, Lespagnol A and others (2004) Translationally controlled tumor protein is a target of tumor reversion. Proc Natl Acad Sci USA 101: 15364−15369

Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453:106-109

Wegner KM, KalbeM, Rauch G, Kurtz J, Schaschl H, Reusch TBH (2006) Genetic variation in MHC class II expression and interactions with MHC sequence polymorphism in three-spined sticklebacks. Mol Ecol 15: 1153−1164

Wendell F, Pattison C, Harris M (1996) Sea Otter, *Enhydra lutris*, containment management: field studies and feasibility assessment. Calif Fish Game, Administrative Report 96-5. 10 p.

[WRCC] Western Regional Climate Center (2012). Data from West Wide Drought Tracker. Accessed December 23, 2012.

Zheng S, Song Y, Qiu X, Sun T, Ackland ML, Zhang W (2008) Annetocin and TCTP expressions in the earthworm *Eisenia fetida* exposed to PAHs in artificial soil. Ecotoxicol Environ Saf 71: 566−573

Tables

Table 1. Documented function of 13 genes identified in free-ranging sea otters sampled in Monterey (; 2009, 2010), Big Sur (2008, 2009), San Luis Obispo (2012), Santa Barbara (2012, 2013), and in clinically normal captive reference animals sampled in 2008, 2009, or 2010.

Gene	Gene function
HDC	The HDCMB21P gene codes for a translationally controlled tumor protein (TCTP) implicated in cell growth, cell cycle progression, malignant transformation, tumor progression, and in the protection of cells against various stress conditions and apoptosis (Bommer and Thiele, 2004, Tuynder et al. 2004, Ma et al. 2010). Up-regulation of HDC is indicative of the development or existence of cancer. Environmental triggers may be responsible for population-based, up-regulation of HDC. HDC transcription is known to increase with exposure to carcinogenic compounds such as polycyclic aromatic hydrocarbons (Bowen et al. 2007, Raisuddin et al. 2007, Zheng et al 2008).
COX2	Cyclooxygenase-2 catalyzes the production of prostaglandins that are responsible for promoting inflammation (Goldsby et al. 2003). Cox2 is responsible for the conversion of arachidonic acid to prostaglandin H2, a lipoprotein critical to the promotion of inflammation (Harris et al. 2002). Up-regulation of Cox2 is indicative of cellular or tissue damage and an associated inflammatory response.
CYT	The complement cytolysis inhibitor protects against cell death (Jenne and Tschopp 1989). Up-regulation of CYT is indicative of cell or tissue death.
AHR	The arylhydrocarbon receptor responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, polyhalogenated hydrocarbons, dibenzofurans, and dioxin (Oesch-Bartlomowicz et al. 2005). Depending upon the ligand, AHR signaling can modulate T-regulatory (T_{REG}) (immune-suppressive) or T-helper type 17 (T_H17) (pro-inflammatory) immunologic activity (Quintana et al. 2008, Veldhoen et al. 2008).
THR	The thyroid hormone receptor beta can be used as a mechanistically based means of characterizing the thyroid-toxic potential of complex contaminant mixtures (Tabuchi et al. 2006). Thus, increases in THR transcription may indicate exposure to organic compounds including PCBs and associated potential health effects such as developmental abnormalities and neurotoxicity (Tabuchi et al. 2006). Hormone-activated transcription factors bind DNA in the absence of hormone, usually leading to transcriptional repression (Tsai and O'Malley 1994).

Table 1, continued (page 2 of 3)

Gene	Gene function
HSP 70	The heat shock protein 70 is produced in response to thermal or other stress (Iwama et al. 1999, Tsan and Gao 2004). In addition to being expressed in response to a wide array of stressors (including hyperthermia, oxygen radicals, heavy metals, and ethanol) heat shock proteins act as molecular chaperones (De Maio et al. 1999). For example, heat shock proteins aid the transport of the AHR/toxin complex in the initiation of detoxification (Tanabe at al. 1994).
IL-18	Interleukin-18 is a pro-inflammatory cytokine (Goldsby et al. 2003). Plays an important role in inflammation and host defense against microbes (Krumm et al. 2008).
IL-10	Interleukin-10 is an anti-inflammatory cytokine (Goldsby et al. 2003). Levels of IL-10 have been correlated with relative health of free-ranging harbor porpoises, e.g., increased amounts of IL-10 correlated with chronic disease whereas the cytokine was relatively reduced in apparently fit animals experiencing acute disease (Beineke et al. 2007). Association of IL-10 transcription with chronic disease has also been documented in humans (Rigopoulou et al. 2005).
DRB	A component of the major histocompatibility complex, the DRB class II gene, is responsible for the binding and presentation of processed antigenn to T_H lymphocytes, thereby facilitating the initiation of an immune response (Goldsby et al. 2003, Bowen et al. 2006). Up-regulation of MHC genes has been positively correlated with parasite load (Wegner et al. 2006), whereas down-regulation of MHC has been associated with contaminant exposure (Dong et al. 1997).
Mx1	The Mx1 gene responds to viral infection (Tumpey et al. 2007). Vertebrates have an early strong innate immune response against viral infection, characterized by the induction and secretion of cytokines that mediate an antiviral state, leading to the up-regulation of the MX-1 gene (Kibenge et al. 2005).
CCR3	The chemokine receptor 3 binds at least seven different chemokines and is expressed on eosinophils, mast cells (MC), and a subset of Th cells (Th2) that generate cytokines implicated in mucosal immune responses (Gurish et al. 2002, Kringel et al. 2006). Up-regulation of CCR3 occurs in the presence of parasites (Gurish et al. 2002, Kringel et al. 2006).

Table 1, continued (page 3 of 3)

Gene	Gene function
5HTT	The serotonin transport gene codes for an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. This transport of serotonin by the SERT protein terminates the action of serotonin and recycles it in a sodium-dependent manner (Jennings et al. 2006, Squire et al. 2008). Increased transcription of 5HTT confers a low anxiety phenotype (Jennings et al. 2006).
CaM	Calmodulin (CaM) is a small acidic Ca^{2+}-binding protein, with a structure and function that is highly conserved in all eukaryotes. CaM activates various Ca^{2+}-dependent enzyme reactions, thereby modulating a wide range of cellular events, including metabolism control, muscle contraction, exocytosis of hormones and neurotransmitters, and cell division and differentiation (Chen et al. 2012). CaM has also been reported to be a pivotal calcium metabolism regulator in the shell formation (Li et al. 2004).

Table 2. Geometric mean, cycle threshold (Ct) of 13 genes identified in free-ranging sea otters sampled in Monterey (MONT; 2009, 2010), Big Sur (2008, 2009), San Luis Obispo (2012), Santa Barbara (2012, 2013), and in clinically normal captive reference animals sampled in 2008, 2009, or 2010.

		n	HDC	COX2	CYT	AHR	THRB	HSP70	IL18	IL10	DRB	MX1	CCR3	5HTT	CaM
CAPTIVES	ALL	17	5.90	6.78	2.41	11.01	13.30	9.62	-1.11	13.71	-0.78	10.99	4.59	10.97	-1.06
	M	7	6.92	7.81	2.77	11.00	13.94	9.35	-1.90	13.90	1.20	11.25	4.44	10.70	-1.43
	F	10	5.28	6.14	2.19	11.01	12.87	9.82	-1.10	13.57	-0.58	10.81	4.69	11.17	-0.97
MONT	ALL	30	6.62	5.72	1.92	10.07	13.59	10.99	1.33	13.77	0.70	12.31	4.83	11.56	0.29
BIG SUR	ALL	51	6.64	8.18	3.60	10.73	13.48	10.57	1.84	13.69	0.61	11.90	4.81	12.00	-0.21
	2008	27	5.93	8.54	3.09	10.01	12.93	10.87	2.35	14.16	0.43	11.13	5.38	12.05	-0.22
	2009	12	6.56	7.96	3.16	11.56	13.04	10.73	1.13	12.20	-0.72	12.91	4.78	11.74	-0.18
	2010	12	8.68	7.62	5.82	11.62	15.29	9.77	1.75	14.24	1.14	12.74	3.75	12.15	-0.21
SLO	ALL		9.23	8.75	2.34	11.04	16.65	12.82	1.53	18.44	0.29	14.95	5.38	11.98	0.34
	M	11	9.35	8.44	2.34	10.96	16.20	11.05	1.79	18.02	-0.47	14.58	5.73	11.23	-0.27
	F	45	9.19	8.83	2.41	11.06	16.76	13.29	1.44	18.55	0.31	15.04	5.29	12.17	0.37
SB	ALL	43	8.46	8.38	1.98	10.48	12.69	11.49	1.41	15.07	0.39	12.06	4.06	9.63	0.27
	M	22	8.64	8.88	1.91	10.74	12.18	11.76	1.30	14.56	0.35	12.18	4.35	9.52	0.29
	F	21	8.25	7.81	2.05	10.13	12.82	11.06	1.52	15.45	-0.44	11.79	3.70	9.60	0.25
	2012	20	8.70	7.89	2.49	10.01	10.73	10.32	1.16	12.59	0.36	10.54	3.39	8.16	0.24
	2013	23	8.27	8.84	1.62	10.92	14.67	12.61	1.66	17.61	0.42	13.56	4.75	11.11	-0.30

Figures

Figure 1. Multivariate, multi-dimensional scaling (MDS) analysis of gene transcription profiles of sea otters captured at 4 areas of the central California coast, 2008 – 2013, in comparison to captive healthy sea otters. Apparent is substantial clustering about the tightly ordered captive group, and numerous outliers in 3-D space.

Figure 2. Gene profiling: Transcription matrix of 13 target genes in sea otters captured in Monterey Bay (MB), Big Sur (BS), San Luis Obispo (SLO), Santa Barbara (SB), and captive reference (CAP) (Hierarchical clustering with complete linkage disequilibrium; Genesis Graz Switzerland). Green indicates higher relative transcription levels and red indicates lower relative transcription levels

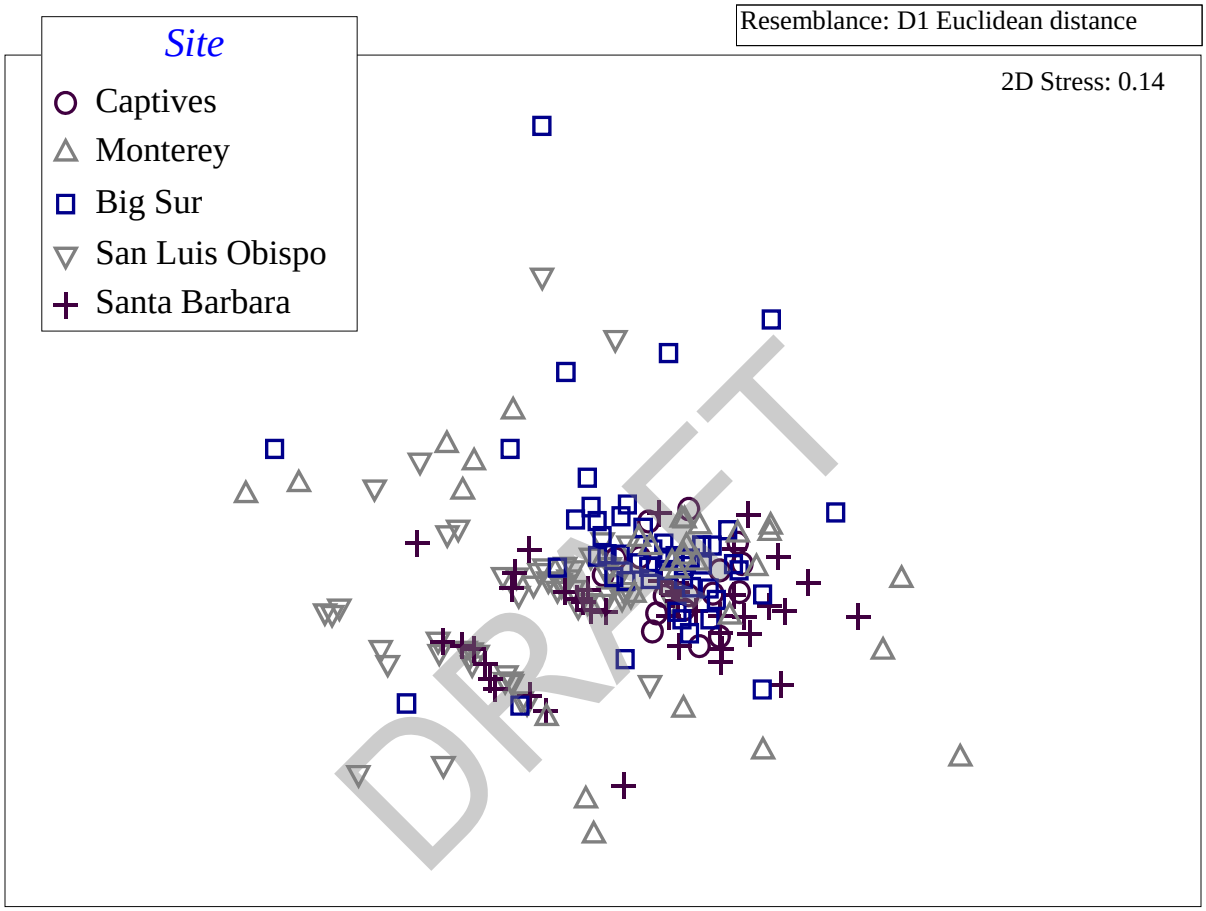

Site
Captives
Monterey
Big Sur
San Luis Obispo
Santa Barbara
Resemblance: D1 Euclidean distance
2D Stress: 0.14
DRAFT

Figure 1

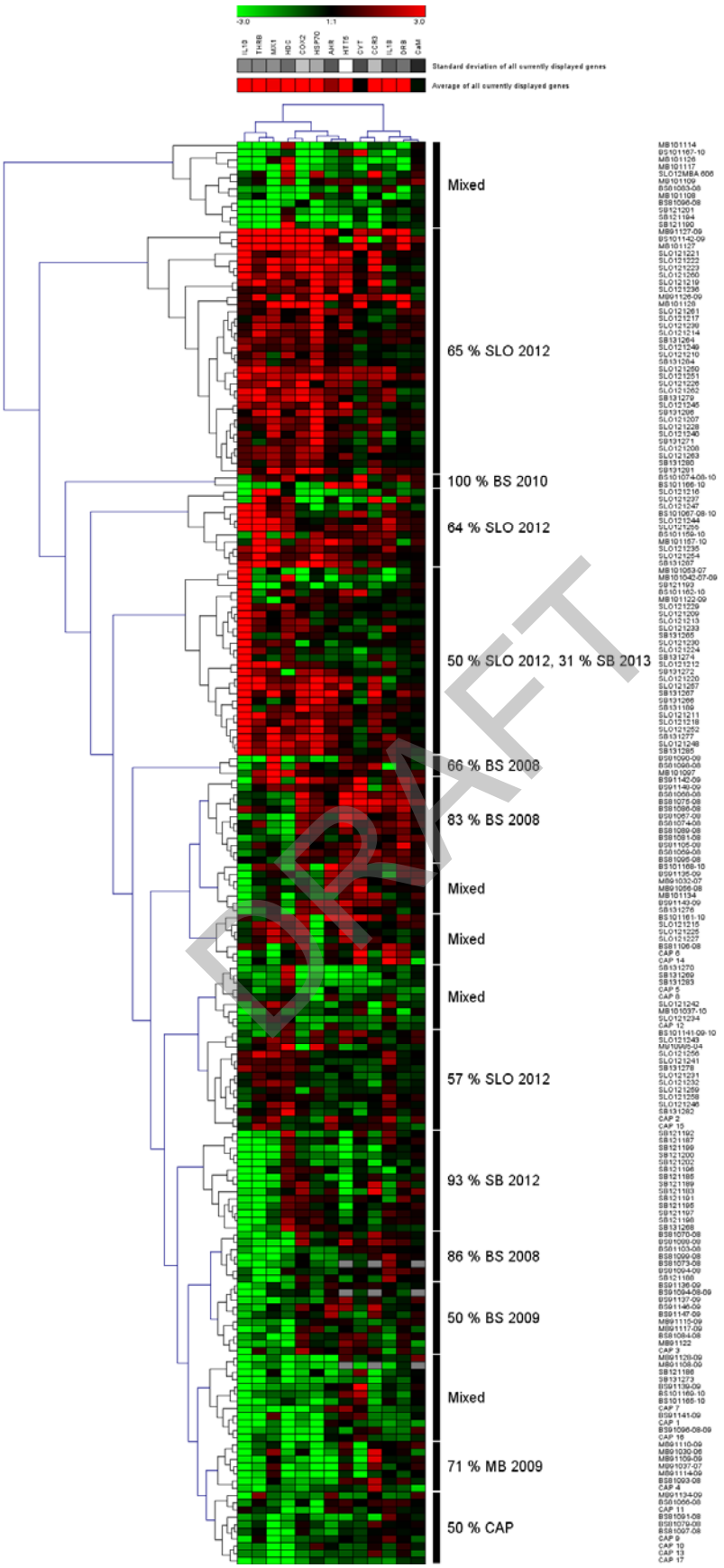


Figure 2

Chapter 4. Movement behavior and home range use of sea otters at Big Sur and Monterey

M. Tim Tinker[1], L. Maxine Tarjan[2], Emily Golson[3], Nicole LaRoche[2]

1. U.S Geological Survey, Western Ecological Research Center
2. Ecology and Evolutionary Biology Department, University of California Santa Cruz
3. Moss Landing Marine Lab, California State University of Monterey Bay

Introduction

Large mobile predators such as sea otters can exert strong community-level effects over large areas due to their capacity to move substantial distances, and at the same time this mobility can expose them to a broad range of spatially-dispersed health threats. As a result, properly understanding the community impacts of sea otter predation, as well as understanding the risks to population recovery associated with specific environmental or anthropogenic stressors, both require detailed information on movement behavior and home range use by individuals in the population. Data on movements and spatial use patterns also are important for characterizing population structure: that is, the spatial scale at which individuals within the population interact with each other, with their prey, and with their environment. Predator populations in which individuals are highly mobile are considered "unstructured", or well-mixed, such that individuals are likely to interact with any other individual, and will have similar exposures to various threats, irrespective of their geographic location at a given point in time. Conversely, predator populations in which individuals have small home ranges and restricted movements are "spatially structured", such that individuals only interact with other nearby individuals, and their risk of exposure to various threats is highly dependent on their geographic location. Understanding movement behavior and population structure is thus necessary to understanding the degree to which populations are limited by resource abundance or external threats at local rather than regional scales.

In studies of tagged or otherwise marked individuals, there are a number of accepted ways for measuring and describing movement behavior. At the most basic level, relocating tagged individuals at some regular time interval and measuring the net distance they have moved away from their location at the previous interval (their "net linear displacement", NLD), provides the fundamental data required for virtually all spatial models of population growth, disease dynamics, or demographic connectivity among habitat patches. For example, diffusion models and other so-called "Eulerian" spatial models can be parameterized from the NLD values of many individuals (from which estimates dispersal distance probability distributions) and used to estimate the rate at which populations will recover, invade, or otherwise spread into new habitat (Hastings 1996, Kot et al. 1996, Borger et al. 2008). More advanced models require not only the average NLD per unit time, but also information on the frequency distribution of this statistic, and how it varies between different classes of individuals within the population (Neubert et al. 1995, Shigesada et al. 1995). The magnitude and distribution of NLD values can also provide insight into the degree of population structure.

In contrast to population-level Eulerian models, "Lagrangian" models, such as random-walk models, focus on individual-level movement behavior over short, discrete time steps (Borger et al. 2008). Measurement of sequential step lengths and turning angles provides the data needed to parameterize correlated random walk models (CRW), which allow for a more mechanistic understanding of animal dispersal (Turchin 1998). For example, the degree to which observed distributions of NLD values conform to expected NLD distributions calculated from CRW models can provide insight into whether individual movements are "biased" (Kareiva and Shigesada 1983). Dispersal will be biased if individuals are avoiding certain habitat features, or are "attracted" to the center of their individual home range.

Another approach to describing the distribution of animals in space and time is the statistical analysis of individual home ranges. Seton (1909) recognized that animals restrict their movements to particular areas over time, and Burt (1943) termed this area a home range, defined as "that area traversed by an individual in its normal activities of food gathering, mating, and caring for the young." Geographically defining animal home ranges is necessary to answer critical questions in studies of habitat selection (Thomas and Taylor 2006, Borger et al. 2008), mating systems (Hedmark et al. 2007, Vanpé et al. 2009), and carrying capacity (Mitchell and Powell 2012), and in formulating expectations about the habitat and species with which an individual will interact.

According to an optimality approach to animal space-use, home ranges should be a product of animals maximizing benefits—resources contained within an area—while minimizing the costs of travel and resource acquisition (Mitchell and Powell 2012). Home ranges are therefore predicted to be finite in size because the cost of obtaining resources increases with travel distance. The benefits and costs of occupying a home range depend on resource availability and distribution. If resources are abundant, individuals can occupy a small range, but if resources are scarce or widely distributed, home ranges must be large to meet energetic demands and other requirements.

Sea otter habitats across California study sites differ in the availability of resources, such as kelp and invertebrate prey. Sea otter home ranges often encompass persistent patches of kelp canopy, which is preferred habitat for resting and feeding. As a keystone predator of kelp forest ecosystems, sea otters are capable of depleting preferred prey types upon establishment in an area (Estes et al. 1978). As the duration of sea otter occupancy differs across the California range, some sites are expected to be more food-limited than others. Previous work contrasted feeding by sea otters at San Nicholas Island, a site rich in invertebrate prey, and central California, an area with a longer-standing sea otter population that is resource-limited (Tinker et al. 2008a). The difference in prey availability was reflected in feeding rates and effort, with sea otters feeding at a rate two times higher at San Nicholas Island than in central CA, and spending half as much time dedicated to foraging. Differences in search and handling times of prey across California sites are expected to impact sea otter home range size.

The bathymetry of the habitat is also expected to influence home range shape and space-use in sea otters. Due to physiological limits in diving capabilities, the extent of the continental shelf has a large impact on the offshore availability of benthic prey. Among the study sites for this project, Monterey Bay has a more extensive continental shelf than Big Sur. Sea otters are therefore capable of using resources

farther offshore in Monterey. The shape of home ranges is likely influenced by these differences between habitats.

In addition to the probable effects of habitat type on home range characteristics, different resource requirements between the sexes is likely to influence animal space-use. Previous studies of the social structure of sea otters show that males range more widely than females and exhibit larger home ranges, except when they are engaged in defense of a small reproductive territory (Loughlin 1980, Ribic 1982, Jameson 1989). The objectives of this study are to examine the expected differences in home range size between males and females, and to determine if home range characteristics vary between the two main study sites. We hypothesize that: 1) home range size will vary depending on sex, with territorial males exhibiting the smallest home ranges, non-territorial males the largest, and females displaying intermediate home range sizes; 2) sea otters in resource-limited areas will have larger home ranges than individuals in resource-rich areas; and 3) home range area in Big Sur will be more dependent on the coastal extent of the home range than in Monterey. We will accomplish these objectives by quantifying home range characteristics using a new algorithm of home range estimation.

Methods

Movement behavior

Individual tagged otters at both study sites were re-sighted 3-7 days each week using radio telemetry, and their positions recorded into a GIS compliant database following standardized methods (see Chapter 1). Because the number of observations per week varied between individual otters, we selected (randomly) one location per week per otter as the fundamental unit of observation. The geographic coordinates for each data point (in units of decimal degrees and California 1983 Teale Albers projection) were also converted to a 1-dimensional coastal axis: specifically, linear interpolation was used to assign each re-sight location the value of the closest point along the "As The Otter Swims" (ATOS) line, defined by 500m intervals along the 10m isobath (Tinker et al. 2006). Note that we expressed the ATOS values in decimal units rather than rounding to the nearest integer ATOS value (e.g. a re-sight location that was 1/3 of the way between ATOS point 367 and 368 was assigned a value of 367.33).

The annual dispersal distance, or net linear displacement (NLD), for each otter was calculated as the absolute difference in decimal ATOS values between the otters location on an arbitrary re-sight date and its location at a 2nd arbitrary re-sight date between 350-380 days after the first date. For each otter we randomly selected (with replacement) 100 starting and ending points for this calculation, in order to obtain a distribution of representative annual NLD values for each otter. We used these data to fit probability density functions using maximum likelihood estimation techniques. Previous studies have utilized various probability distributions to describe sea otter dispersal distances, including exponential and leptokurtic distributions (Krkosek et al. 2007, Tinker et al. 2008b). In this case, visual examination of frequency histograms of individual NLD values revealed somewhat leptokurtic or "fat-tailed" distributions, characterized by many small values but a few very large values. Accordingly we selected the Weibull probability distribution to fit to empirical NLD observations: the Weibull density function is more flexible than the exponential distribution, and is described by a scale parameter (a) that specifies

the magnitude of the mean expected value, and a shape parameter (*b*) that determines the variance and skew:

$$f(x \mid a,b) = \frac{b}{a}\left(\frac{x}{a}\right)^{b-1} e^{-\left(x/a\right)^{b}} \quad (1)$$

Note that when *b* <1 the resulting distribution is more leptkurtic than the exponential and has higher variance, but when *b*=1 the Weibull converges to the exponential distribution (Figure 1). We fit Weibull distributions to NLD data for 6 groups of sea otters: males, adult females and sub-adult females from Big Sur, and males, adult females and sub-adult females from Monterey. We divided females into two age classes because previous analyses have shown that juvenile/sub-adult animals (individuals <4 years of age) may exhibit different movement behavior than adults (individuals ≥ 4 years of age), however we did not classify males by age class due to sample size limitations. For each group we calculated the mean and 95% confidence limits for the *a* and *b* parameters, and we evaluated whether NLD distributions differed between groups based on non-overlap of 95% confidence intervals. We then conducted variance component analysis in order to determine the degree to which variation in annual NLD values was explained by i) site differences, ii) age/sex differences, iii) among-individual differences, and iv) within-individual (year-to-year) differences. For this latter analysis we analyzed NLD data using a random-effects general linear model and restricted maximum likelihood estimation of variance components.

We next analyzed sea otter movements using a correlated random walk (CRW) model, to determine whether/how movements were biased. For this analysis we computed distributions of step-lengths and turning angles for each otter from contiguous sequences of weekly re-sight values. The step length for week *i* (l_i) was calculated as the absolute difference between decimal ATOS value at week *i* and the value at week *i*-1. The turning angle (θ_i) was assumed to be 0° if the otter had moved "up- coast" (decreasing ATOS values) from its location at week *i*-1, and 180° if the otter had moved "down-coast" (increasing ATOS values). The expected net linear displacement (NLD_E) for an otter over a specified period of time, *T*, was calculated as:

$$NLD_E(T) = \left[n(T)\cdot m_2 + 2m_1^2 \left(\frac{\Psi}{1-\Psi}\right)\left(n - \frac{1-\Psi^{(n(T)-1)/2}}{1-\Psi}\right)\right]^{0.5} \quad (2)$$

Where *n(T)* is the number of weekly intervals in period *T*, m_1 is the arithmetic mean of recorded step lengths, m_2 is the arithmetic mean of squared step lengths, and Ψ is the arithmetic mean of the cosine of recorded turning angles. We calculated NLD_E for values of *T* ranging from 4 weeks to 1 year, and used bootstrap re-sampling to account for measurement variance (1000 random samples of step lengths and turning angles were selected with replacement for each otter for each value of *T*). We next computed observed net linear displacement (NLD_O) for each otter, for each value of *T*, using the methods described above for calculating annual dispersal distance. Movement bias was then calculated

as NLD_O - NLD_E and plotted as a function of time for 4 groups of animals: i) adult females, ii) sub-adult females, iii) males whose re-sights were restricted to one area of the coast (and thus exhibited a unimodal spatial distribution), and iv) males whose re-sights were clustered in two or more widely separated areas of the coast (and thus exhibited a multimodal spatial distribution).

Home range analysis

Statistical methods to estimate individual home ranges utilize observed re-sighting locations to estimate the future probability of an individual occurring at any point in space, and to delineate a boundary encompassing some cumulative probability of occurrence. A notable limitation of existing methods is their inability to incorporate information about underlying environmental features that influence animal space-use. Due to this limitation, existing methods often perform well in encompassing areas that are used, but perform poorly at identifying unused areas. This limitation is particularly apparent for sea otters (Figure 2), whose space-use is restricted by the complex coastal boundary and water depth.

Recognizing the inadequacy of existing home range methods for describing sea otter home range behavior, we developed a new method of home range estimation (which we refer to henceforth as "Permissible home range estimation") that better reflects limiting habitat characteristics. To accomplish this, we transformed re-sight locations to reflect relevant landscape features: specifically, each re-sight location was assigned the value of the closest point along the one-dimensional ATOS line (the decimal ATOS value: see above), and the appropriate depth value was assigned via interpolation to the 10m high resolution multi-beam sonar bathymetric data collected by the California Department of Fish and Wildlife (CDFW Marine Region GIS Lab). Depth values were log-transformed to ensure normality and produce a variable that varied from $-\infty$ to ∞. Because home range boundaries could change over an animal's lifetime, two years of data were utilized for each home range estimate.

A bivariate kernel probability density function was fit to the decimal ATOS and *log*(depth) variables for each individual, and this function was then back-transformed to geographic space to define a permissible home range kernel density surface. The output of the algorithm includes a GIS layer of the kernel density grid (ESRI ArcMap Shapefile) as well as a polygon delineating the 90% kernel home range boundaries (Figure 3) and a vector of descriptive statistics, for use in comparing home range characteristics. Specifically, we computed the following home range statistics for each individual: i) total area (km^2); ii) the number of distinct centers of use (COU, defined as home range polygons separated by more than 2km); iii) the maximum distance between COU polygons; iv) the "coastline extent", or cumulative distance (km) of coastline contained *within* all home range polygons; and v) the "range span", or distance (km) between the northernmost and southernmost points of the home range.

Among-individual distributions of home range area, maximum distance between centers of use, coastline extent, and range span were highly right-skewed, and so were log-transformed for normality (Kolmogorov-Smirnov test, $p > 0.05$). Examination of the log-transformed distribution of coastline extent for males revealed a highly bimodal distribution with a clear break at value 2 (Figure 4), and this was confirmed by a significant Hartigans "dip" test for unimodality (Dip Stat = 0.104, P=0.0075; Hartigan JA and PM 1985). Accordingly we classified males into one of two categories: those with log-transformed coastline extent values of less than 2 were classified as residents (M1) and those with log-

transformed coastline extent values of greater than 2 were classified as transients (M2). Linear models were created and analyses of variance (ANOVA) were used to test for effects of site (Big Sur vs. Monterey) and sex (F vs. M1 vs. M2) on all home range characteristics, as well as the interactions between the main effects. Analyses of the maximum distance between centers of use were limited to individuals with home ranges having more than one center of use. Data on the number of centers of use were nonparametric, so the effects of sex and site were evaluated separately using Mann-Whitney U tests, and a χ^2 contingency test was used to evaluate the hypothesis that sex, site and number of centers of use were independent. Finally, we used a general linear model (GLM) to test for a functional relationship between home range area and coastline extent, assuming a log-linear relationship.

Results

The frequency distributions of observed values of sea otter annual dispersal (NLD) were well fit by Weibull probability density functions (Figure 5). Male otters at both sites exhibited more strongly leptokurtic distributions than females, with a long right tail to the distribution corresponding to "extreme" long-distance movements (Figure 5, A-B). In contrast, NLD distributions of females were less skewed, with almost all annual dispersal values between 0 and 20km (Figure 5, C-F). The sex-based difference in distribution skew was reflected by significantly lower "*b*" parameters for males relative to females (Figure 6), and thus a higher probability of making long-distance movements of over 20km (Table 1). Interestingly, sub-adult females at Big Sur had slightly lower mean NLD values than adult females, while at Monterey the sub-adult females exhibited slightly more skewed NLD distributions and greater mean NLD values (Figure 6). Overall, annual dispersal distances were similar between sites, but individuals at both sites were highly variable with respect to NLD, with among-individual and within-individual differences contributing more to variance than age and sex class differences (Figure 7).

The CRW model tended to over-estimate NLD values for both male and female sea otters, and the negative bias in observed NLD values (NLD_O - NLD_E) increased over time (Figure 8). Increasing negative bias in NLD_O compared to NLD_E is indicative of a centralizing tendency in sea otter movements, such that individuals are more likely to turn towards a "focal attractor" (e.g. a home range "center of use") the farther they are away from that point. Sub-adult females showed greater variation in movement bias than adult females, and males with multi-modal spatial distributions were also more variable (Figure 8), reflecting the occasional longer-distance movements of these animals (Figure 5).

Home range analysis

Permissible home range estimation outperformed other available methods—kernel density estimation (KDE, Silverman 1986; Worton 1989) and local convex hull analysis (LoCoH, Getz and Wilmers 2004)—for this species. Home range estimates were biologically accurate, excluding terrestrial areas and water beyond the depth range that is accessible to sea otters (compare Figures 2 and 3).

Average (± SD) home range characteristics (grouped by site and sex) are presented in Table 1. The number of distinct home range centers of use (COU) did not differ between sites (Figure 9a), either for females (χ^2=3.02, P=0.389) or males (χ^2=0.72, P=0.868). However, sex was a significant predictor of the number of COU (W = 2155, P < 0.01), with 80% or more of females having just one COU, while less than 60% of males at both sites had one COU. The number of COU also differed between resident males and

transient males (χ^2=36.68, P<0.0001), with more than 90% of resident males having one COU but 75% of transient males having 2 or more COU (Figure 9b).

Male sea otters had a greater maximum distance between COU than did females ($F_{1,78}$ = 18.29, P < 0.001), but there were no differences between sites ($F_{1,78}$ = 2.24, P=0.14) or interactions between sex and site ($F_{1,78}$ = 0.06, P =0.81). Similar patterns were found for other home range statistics (Figure 10): the total area (km^2) of home range polygons did not differ between sites ($F_{1,2}$ = 0.39, P=0.53), and there was no interaction between sex and site ($F_{2,136}$ = 0.89, P=0.45), but females had a larger home range than resident males and smaller home range than transient males ($F_{1,2}$ = 14.17, P<0.001); the coastline extent of home ranges did not differ between sites ($F_{1,2}$ = 0.30, P=0.58), and there was no interaction between sex and site ($F_{2,136}$ = 0.46, P=0.64), but the coastline extent of female home ranges was longer than that of resident males and shorter than that of transient males ($F_{1,2}$ = 26.83, P<0.001); and the total length of coastline spanned by otter home ranges did not differ between sites ($F_{1,2}$ = 0.57, P=0.45), and there was no interaction between sex and site ($F_{2,136}$ = 0.28, P=0.76), but female movements spanned a longer stretch of coastline than those of resident males and shorter stretch of coastline than transient males ($F_{1,2}$ = 25.51, P<0.001). Finally, coastline extent increased more rapidly as a function of home range area in Big Sur as compared to Monterey Bay: power functions fit to the data were highly significant and their slopes differed significantly (Figure 11; $F_{2,136}$ = 8.1408, P < 0.001).

Discussion

Sea otter movement behavior in the current studies was generally consistent with that observed in previous studies of sea otter movements (Ralls et al. 1996, Tinker et al. 2008b), in that female movements were more restricted than male movements, and sub-adult females were more likely to make occasional long-distance movements than adult females (at least in Monterey: Table 1). This analysis confirms the previously-reported observation that adult female sea otters exhibit strong site fidelity, rarely dispersing more than 20km from their current location within a 1-year period (Table 1), and thus are most at risk from (and good indicators of) local environmental or anthropogenic stressors. Interestingly, the best-fit Weibull distribution for adult females at Monterey had a smaller scale parameter *and* smaller shape parameter than the distribution for Big Sur females, meaning that the average dispersal distance of Monterey females was smaller than Big Sur females, yet there was a higher probability of Monterey females making a long distance movement of >20km. It is unclear why this difference exists: the longer average NLD of Big Sur females may simply reflect the fact that the narrow/steep slope of the continental shelf at Big Sur means that females have to cover more coastline to have access the same amount of prey resources (see home range analysis below), but the occasional long distance movements of Monterey females are more difficult to explain.

In contrast with females, the distribution of annual net linear displacement (NLD) values for males was strongly leptokurtic (Figure 5), and with a long right tail that was better described by a Weibull distribution than by the more traditional exponential distribution. Although the shape of male NLD distributions was identical at both sites (as measured by the shape parameter), the scale parameter was significantly greater at Big Sur, reflecting the longer average annual dispersal distances of males in this region (Figure 6). This difference may be explained by the fact that males at Big Sur had greater distances to travel to find abundant food resources than did Monterey males, given that Big Sur is

farthest from the north and south ends of the range where sea otter densities are lower and per-capita food abundance greater. However, although site and sex-based differences in movement behavior do exist, most of the variability in NLD was explained by individual variation, suggesting that variable life history and foraging strategies of sea otters probably contribute more to variation in annual movements than do environmental differences between Big Sur and Monterey. Understanding what factors explain this variation will be a productive area for further research.

For both females and males, the CRW analysis indicated a strong centralizing bias to sea otter movements (Figure 11). This bias indicates that sea otters tend to limit their movements to well-defined and relatively compact home ranges, within-which they move extensively but outside of which they are unlikely to move, particularly in the case of adult females. There is some reason to believe that this trait may be "hard wired" into sea otters, as female sea otters in Prince William Sound and SE Alaska have also been reported to exhibit restricted annual movements (Garshelis and Garshelis 1984, Bodkin et al. 2004). Regardless, an important consequence of this trait is that demographic processes in the population will be strongly spatially structured, since reproductive-age females are the demographically "relevant" component of the population. Of course the more mobile males do contribute to genetic connectivity, but because they do not directly contribute to intrinsic population growth (unless fertilization of estrous females were a limiting factor, which has never been reported) their movements will have no effect on demographic connectivity. This spatial structure of the southern sea otter population has enormous conservation implications: for example, it means that the factors that limit female survival and thus control population growth will tend to operate locally, not regionally. This is particularly likely to be the case for per-capita prey abundance, because the benthic invertebrates that sea otters feed on are sessile and their abundance varies enormously over short distances due to small-scale variation in recruitment and post-settlement processes (Gaines et al. 1985, Broitman et al. 2008). Together, these observations point towards a need to consider density-dependent processes and population status at scales of 10s of km, rather than at regional scales or at the scale of the entire population.

Home range estimation

Permissible home range estimation allowed for statistical descriptions of home range use that were consistent with sea otter biology. In particular, the resulting home range polygons did not include terrestrial areas and encompassed water depths that are accessible by diving sea otters (Figure 3). Accurate estimates of home range size and location have been previously unavailable for this species, so this methodological advance will be a major contribution to studies of sea otter ecology, with potential application to research on exposure to anthropogenic disturbance, encounter rates with pathogens, and access to resources. This method will also be applicable to ecological studies of other species whose home ranges are restricted by complex boundaries or across environmental gradients, such as water depth, primary productivity, or temperature. Increased accuracy in defining home ranges will allow researchers and resource managers to better understand habitat use requirements and ultimately improve conservation efforts for a variety of species.

Evidence based on body condition and foraging rates suggests that sea otters in Big Sur are slightly more resource-limited than sea otters in Monterey Bay (Chapters 6 and 7). However, despite this admittedly

small difference in apparent resource abundance we found no significant differences in home range areas between sites (although the average home range area was largest for males in Monterey), though there was a large amount of individual-level variability in this statistic. The similarity of home range area across sites is likely due to the relative similarity between sites in terms of habitat characteristics and population density. Although some difference in resource availability may exist between Monterey Bay and Big Sur, it may not be drastic enough to heavily influence sea otter home range use, and in fact results presented elsewhere in this report generally suggest that both sites are at or near carrying capacity. We suggest that an analysis including study sites that are significantly below carrying capacity, such as Santa Barbara Channel and San Nicolas Island, will be a fruitful next step.

Although absolute home range size did not differ between sites, there were subtle differences in space use of sea otters at the two sites that were likely related to differences in the distribution of resources and coastal bathymetry. In support of our hypothesis, we found that the coastline extent of home ranges increased with home range area much more rapidly in Big Sur than in Monterey Bay. This pattern reflects the fact that sea otters in Big Sur are only able to increase the area of their home range by extending it farther along the coastline, due to the narrow continental shelf at this site. In contrast, the wide continental shelf at Monterey Bay means that sea otters are able to access offshore resources, and thus can increase the area of their home range by extending it offshore.

These differences in habitat and home range shape have energetic implications for sea otters. The energy required to access all resources within a home range area depends on the shape of that area. A circular home range allows sea otters to access all of their resources by swimming a relatively short distance. An elliptical home range means that a sea otter needs to swim the full length of the ellipse to access all of the resources, thus expending more energy for travel. The greater energy requirement for long, narrow elliptical home ranges likely restricts the maximum home range area that can be maintained. Indeed, the maximum home range area in Big Sur (20.11 km^2) is under half the maximum home range area in Monterey Bay (45.22 km^2). Habitat bathymetry and the spatial distribution of resources therefore can have a profound effect on the maximum home range area that is attainable, and ultimately will affect the equilibrial population density in a given area.

Home range statistics were similar between females and males overall, however sex-based comparisons were complicated by the existence of two distinct strategies of home range behavior exhibited by males. At both sites, some of the males (“M1”, resident males) exhibited strong site fidelity, utilizing just one home range center that encompassed a small area and spanned a short stretch of coast. A second group of males (“M2”, transient males) were far more mobile, frequently moving between multiple home range centers distributed along the California coast, which together encompassed a larger area and spanned a long stretch of coast. These distinct home range strategies are apparently related to reproductive strategy: the M1 males in this study were all “territorial”, maintaining reproductive territories in kelp-dominated habitats where females tend to congregate for all or most of the year. In contrast, M2 males did not generally maintain breeding territories (or only did so for parts of the year), and instead moved regularly between male-dominated regions (generally soft-sediment, non-kelp habitats), with occasional opportunistic visits to female-dominated areas. These alternative reproductive tactics appear to be associated with dramatically different patterns of home range use. Interestingly,

female home range characteristics tended to fall midway between the home range metrics of the two male strategies.

Space-use patterns are important for population health because they can affect interactions between individuals and spatially-explicit stressors. While transient males tend to encounter many different individuals in the population, females and resident males are restricted to interactions with other local females. Moreover, the probability of encountering localized anthropogenic disturbances, pollution, or other features of the environment depends on the ranging capabilities of the individual: highly mobile transient males can potentially encounter a wider range of stressors as they move throughout central California, whereas resident males and females may be subject to greater intensity of local disturbances within their home range. Differences in home range use and movement patterns across sexes and mating tactics should therefore be considered when identifying threats to this species.

References

Bodkin, J. L., G. G. Esslinger, and D. H. Monson. 2004. Foraging depths of sea otters and implications to coastal marine communities. Marine Mammal Science **20**:305-321.

Borger, L., B. D. Dalziel, and J. M. Fryxell. 2008. Are there general mechanisms of animal home range behaviour? A review and prospects for future research. Ecol Lett **11**:637-650.

Broitman, B. R., C. A. Blanchette, B. A. Menge, J. Lubchenco, C. Krenz, M. Foley, P. T. Raimondi, D. Lohse, and S. D. Gaines. 2008. SPATIAL AND TEMPORAL PATTERNS OF INVERTEBRATE RECRUITMENT ALONG THE WEST COAST OF THE UNITED STATES. Ecological Monographs **78**:403-421.

Burt, W. H. 1943. Territoriality and home range concepts as applied to mammals. Journal of Mammalogy **24**:346-352.

Estes, J. A., N. S. Smith, and J. F. Palmisano. 1978. Sea otter predation and community organization in the western aleutian islands, Alaska. Ecology **59**:822-833.

Gaines, S., S. Brown, and J. Roughgarden. 1985. Spatial variation in larval concentrations as a cause of spatial variation in settlement for the barnacle, Balanus glandula. Oecologia **67**:267-272.

Garshelis, D. L. and J. A. Garshelis. 1984. Movements and management of sea otters [Enhydra lutris] in Alaska [USA]. Journal of Wildlife Management **48**:665-678.

Getz, W. M and C. C. Wilmers. 2004. A local nearest-neighbor convex-hull construction of home ranges and utilization distributions. Ecography **27**:489-505.

Hartigan JA and H. PM. 1985. The dip test of unimodality. Ann Statist **13:**70-84.

Hastings, A. 1996. Models of spatial spread: A synthesis. Biological Conservation **78**:143-148.

Hedmark, E., J. Persson, P. Segerstro, A. Landa, and H. Ellegren. 2007. Paternity and mating system in wolverines *Gulo gulo*. Wildlife Biology **13**:13-30.

Jameson, R. J. 1989. Movements, home range, and territories of male sea otters off central California. Marine Mammal Science **5**:159-172.

Kareiva, P. M. and N. Shigesada. 1983. Analyzing insect movement as a correlated random walk. Oecologia **56**:234-238.

Kot, M., M. A. Lewis, and P. Van Den Driessche. 1996. Dispersal data and the spread of invading organisms. Ecology (Washington D C) **77**:2027-2042.

Krkosek, M., J. S. Lauzon-Guay, and M. A. Lewis. 2007. Relating dispersal and range expansion of California sea otters. Theor Popul Biol **71**:401-407.

Loughlin, T. R. 1980. Home range and territoriality of sea otters near Monterey, California. . J. Wildl. Manage. **44**:576-582.

Mitchell, M. S. and R. A. Powell. 2012. Foraging optimally for home ranges. Journal of Mammalogy **93**:917-928.

Neubert, M. G., M. Kot, and M. A. Lewis. 1995. Dispersal and Pattern-Formation in a Discrete-Time Predator-Prey Model. Theoretical Population Biology **48**:7-43.

Ralls, K., T. C. Eagle, and D. B. Siniff. 1996. Movement and spatial use patterns of California sea otters. Canadian Journal of Zoology **74**:1841-1849.

Ribic, C. A. 1982. Autumn movement and home range of sea otters in California. The Journal of Wildlife Management **46**:795–801.

Seton, E. T. 1909. Life-histories of northern animals. An account of the mammals of Manitoba Scribner, New York.

Shigesada, N., K. Kawasaki, and Y. Takeda. 1995. Modeling Stratified Diffusion in Biological Invasions. American Naturalist **146**:229-251.

Silverman, B. W. 1986. Density Estimation for Statistics and Data Analysis. Chapman & Hall, London.

Thomas, D. L. and E. J. Taylor. 2006. Study designs and tests for comparing resource use and availability II. J. Wildl. Manage **70**:324-336.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008a. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., D. F. Doak, and J. A. Estes. 2008b. Using demography and movement behavior to predict range expansion of the southern sea otter. Ecological Applications **18**:1781-1794.

Tinker, M. T., D. F. Doak, J. A. Estes, B. B. Hatfield, M. M. Staedler, and L. Bodkin James. 2006. Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. Ecological Applications **16**:2293-2312.

Turchin, P. 1998. Quantitative analysis of movement: Measuring and modeling population redistribution in animals and plants. Sinauer Associates, Inc., Sunderland, Massachusetts.

Vanpé, C., N. Morellet, P. Kjellander, M. Goulard, O. Liberg, and A. J. M. Hewison. 2009. Access to mates in a territorial ungulate is determined by the size of a male's territory, but not by its habitat quality. The Journal of Animal Ecology **78**:42-51.

Worton, B. J. 1989. Kernel Methods for Estimating the Utilization of Distribution in Home-Range Studies. Ecology. **70**:164-168.

Tables

Table 1. Results of an analysis of annual net linear displacement (NLD) for sea otters at Big Sur and Monterey, grouped by sex and age-class. Scale (*a*) and shape (*b*) parameter estimates are shown for Weibull distributions fit to each data set (mean estimates and lower/upper 95% confidence limits are presented). Also shown are the mean movement distances for each group, and the cumulative probabilities of moving more than 20km from the starting location within a 1-year period, as calculated from the appropriate cumulative distribution functions.

Site	Sex/Age class	a (scale)	a (CI_{L95})	a (CI_{U95})	b (shape)	b (CI_{L95})	b (CI_{U95})	Mean move	Probability move>20km
Big Sur	Male, all ages	3.848	3.175	4.663	0.408	0.386	0.432	13.156	0.141
	Female, adult	4.141	3.902	4.395	0.756	0.731	0.782	4.883	0.037
	Female, sub-adult	3.706	3.357	4.090	0.737	0.696	0.780	4.384	0.031
Monterey	Male, all ages	1.591	1.424	1.779	0.398	0.386	0.411	5.935	0.065
	Female, adult	3.265	3.127	3.408	0.637	0.625	0.649	4.197	0.042
	Female, sub-adult	3.748	3.330	4.218	0.662	0.626	0.700	5.182	0.048

Table 2. Home range characteristics across study sites and sexes (average ± SD). Data are summarized for females (F), all males (M), resident males (M1), and transient males (M2) at both study sites.

Habitat	Sex	Sample size (n)	Area (km^2)	# of centers of use (COU)	Max distance between COU (km)	Coastline extent (km)	Range Span (km)
Big Sur	F	29	7.82 ± 3.84	1.21 ± 0.41	5.04 ± 3.78	14.72 ± 6.99	18.12 ± 10.44
	M	7	6.46 ± 3.66	1.57 ± 0.79	40.22 ± 40.97	10.54 ± 6.75	49.42 ± 65.67
	M1	3	2.74 ± 1.61	1.00 ± 0.01	n/a	3.94 ± 1.52	3.95 ± 1.52
	M2	4	9.25 ± 0.89	2.00 ± 0.82	40.22 ± 40.97	15.48 ± 3.67	83.53 ± 70.74
Monterey Bay	F	83	7.48 ± 7.89	1.23 ± 0.63	7.01 ± 12.90	12.34 ± 7.76	19.06 ± 27.68
	M	23	10.11 ± 12.60	1.83 ± 1.27	18.51 ± 17.21	10.81 ± 8.88	30.59 ± 39.70
	M1	11	2.07 ± 1.78	1.09 ± 0.30	n/a	3.53 ± 0.96	7.76 ± 14.75
	M2	12	17.49 ± 13.81	2.50 ± 1.45	18.51 ± 17.21	17.49 ± 7.43	51.52 ± 44.23

Figures

Figure 1. A) Plot of Weibull probability density functions used to model sea otter dispersal distance probabilities, illustrating the effect of variation in the shape parameter (*b*) on the degree of skew or leptokurtosis of the distribution. Note that when b=1 the Weibull is equal to the exponential probability density function. B) Same as above but plotted on log-transformed Y-axis, to illustrate how low values of *b* are associated with higher probabilities of very short or very long dispersal distances.

Figure 2. A) Home range estimate for sea otter 1317 (red) using kernel density estimation (KDE, h=475, 90% kernel) in geographic coordinate space (Monterey Peninsula, CA). Points represent the resights of sea otter 1317 (female). This method incorrectly defines terrestrial areas (grey) as part of the sea otter's home range. This method also over-estimates the depth usage of sea otters. Decreasing the smoothing parameter only serves to increase the number of satellite centers of use without significantly improving the problem of including terrestrial areas in the home range. B) Home range estimate for sea otter 1317 (red) using local convex hull analysis (LoCoH, 90% isopleth). Points represent the resights of sea otter 1317. Although LoCoH analysis is designed to recognize boundaries within a habitat, the coastline is too complex for accurate representation, and terrestrial habitat (grey) is still included in the home range estimate. In comparison to kernel density analysis (Figure 1), this method more accurately represents the offshore depth usage of sea otters.

Figure 3 GIS maps illustrating sea otter resights and the results of the "permissible home range estimation" for two sea otters at the Monterey study site. A) Daily resight locations (insert, yellow dots) for a female sea otter, and the associated 90% home range polygon (blue shaded area). B) Daily resight locations (yellow dots) for a male sea otter, and the associated kernel density surface, shaded from high probability of occurrence (purple) to low probability of occurrence (light blue). Note the bi-modal home range with two widely separated centers of use.

Figure 4. Frequency histogram of log-transformed values of "home range coastal extent" (the number of km of coastline contained within home range polygons) for male otters monitored in this study. The bimodal distribution is clearly evident, with an intermodal break-point at value 2: individuals to the left of this value were classified as M1 males, while individuals to the right of this point were classified as M2 males.

Figure 5. Frequency histograms of annual net linear displacement (NLD) values, and associated Weibull probability density functions (fit using Maximum Likelihood), for sea otters in the current study. A) data for male sea otters at Big Sur; B) data for male sea otters at Monterey; C) data for adult female sea otters at Big Sur; D) data for adult female sea otters at Monterey; E) data for sub-adult female sea otters at Big Sur; F) data for sub-adult female sea otters at Monterey.

Figure 6. Maximum Likelihood Estimates (MLE) for the two parameters of the Weibull probability functions fit to sea otter net linear displacement data (see Figure 5). Parameter *a* determines the scale (or mean expected value) while parameter *b* determines the shape of the distribution, with lower values creating more leptokurtic distributions (see Figure 1). Mean parameter estimates and 95% confidence intervals (error bars) are shown for males, adult females and sub-adult females at the Big Sur and Monterey study sites.

Figure 7. Results of a variance components analysis, showing the proportion of variation in annual net linear displacement values explained by 4 effects: between-site differences, age/sex class differences, among-individual differences, and within-individual variation.

Figure 8. The difference between observed net linear displacement (NLD_O) and expected net linear displacement (NLD_E, as calculated from correlated random walk models using methods described in the text) plotted as a function of time intervals over which NLD was estimated and measured, ranging from 4 weeks to 1 year. Grey lines show the NLD_O - NLD_E values for individual animals, while red lines show averages for all individuals in the study group. Data are plotted for 4 groups of animals, A) adult females, B) sub-adult females, C) males with unimodal home range distributions (resident males), and D) males with multimodal home range distributions (transient males).

Figure 9. A) Proportion of animals with different numbers of centers of use, compared between study sites and sexes (MON= Monterey Bay, BSR = Big Sur). B) Comparison of number of centers of use between females, M1 males and M2 males.

Figure 10. Boxplots of 4 Home range statistics compared between females, M1 males (residents) and M2 males (transients). In all cases the distributions reflect log-transformed values. The means of each home statistic differed significantly between groups.

Figure 11. Coastline extent (km) plotted as a function of home range area (km2) for otters in Monterey Bay and Big Sur. Power functions were fit to the data, and their slopes differed significantly ($Df = 1$, $F = 8.1408$, $P < 0.01$), indicating that coastline extent increased more rapidly with home range area for sea otters at Big Sur as compared to Monterey.

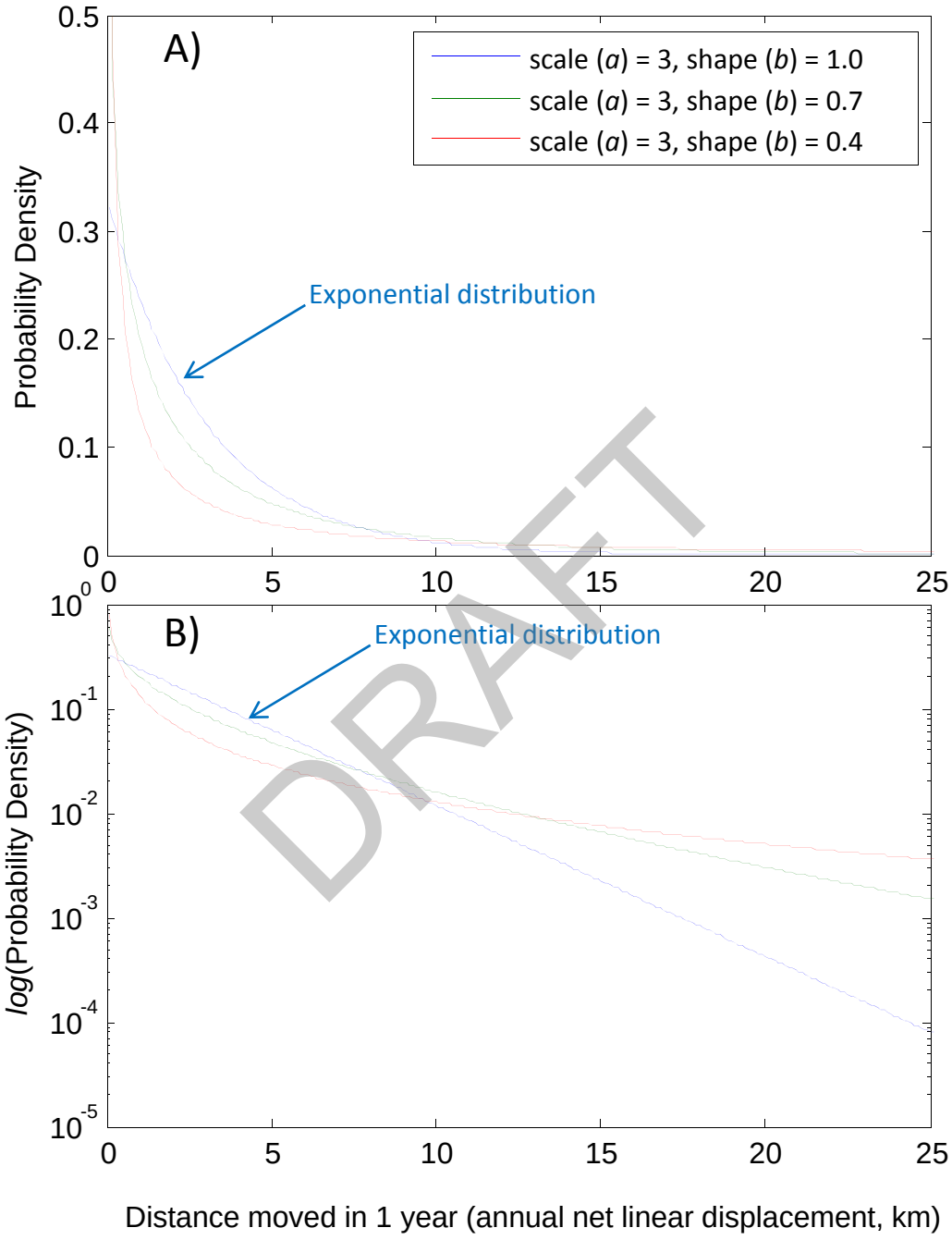


Figure 1

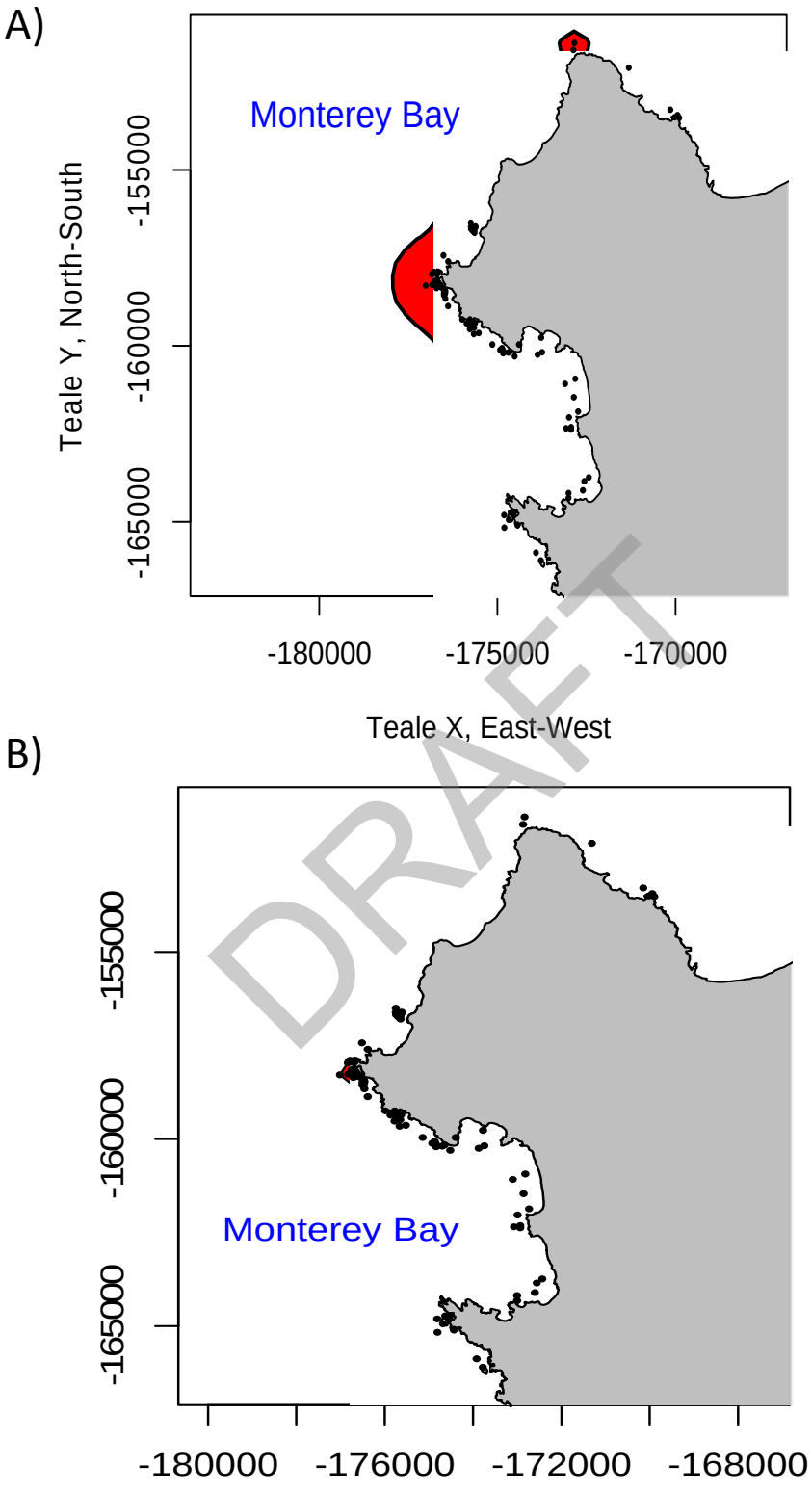
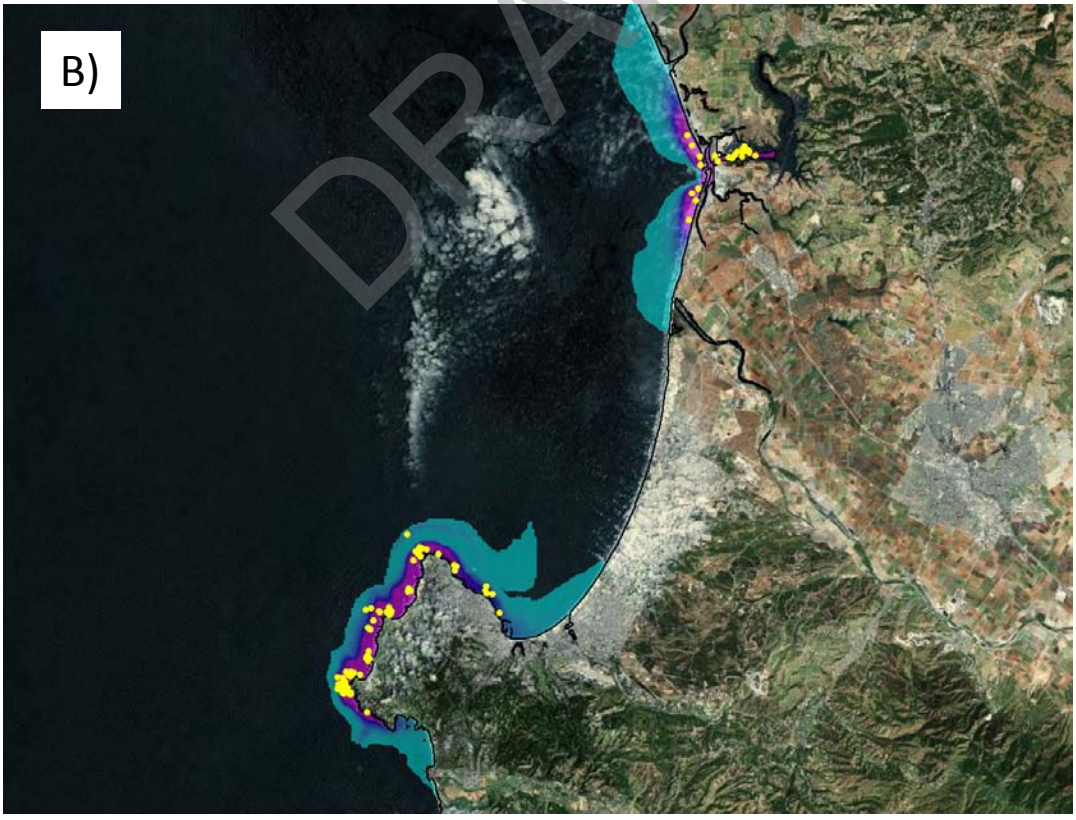


Figure 2



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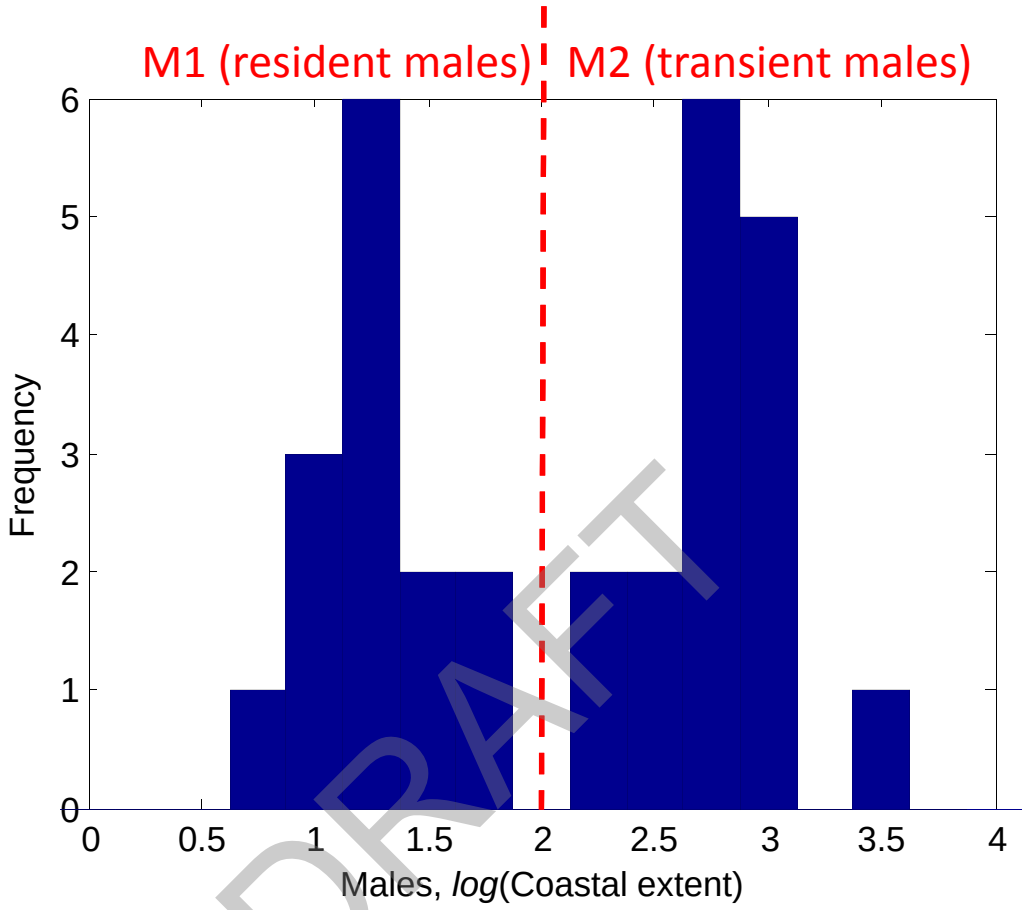

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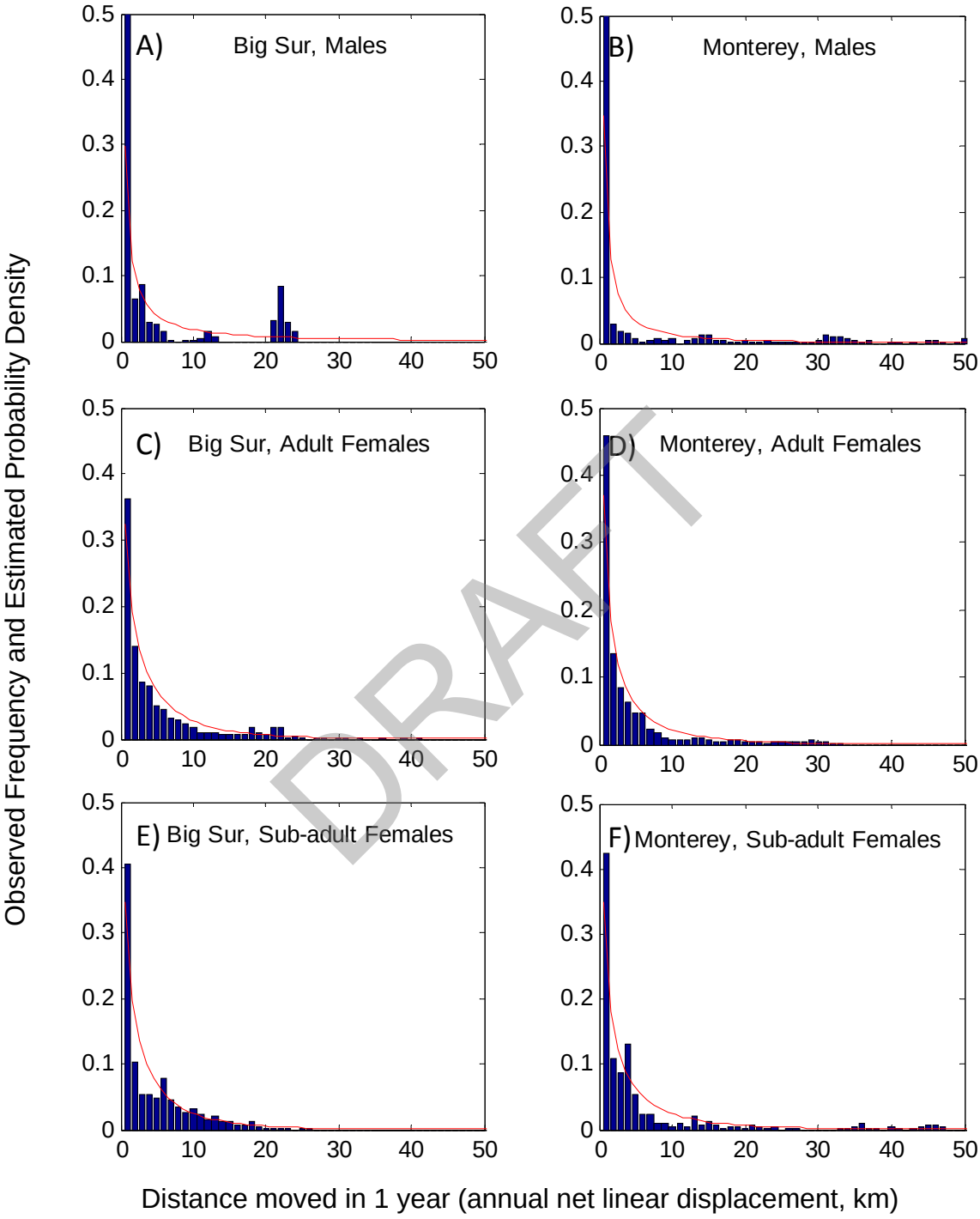
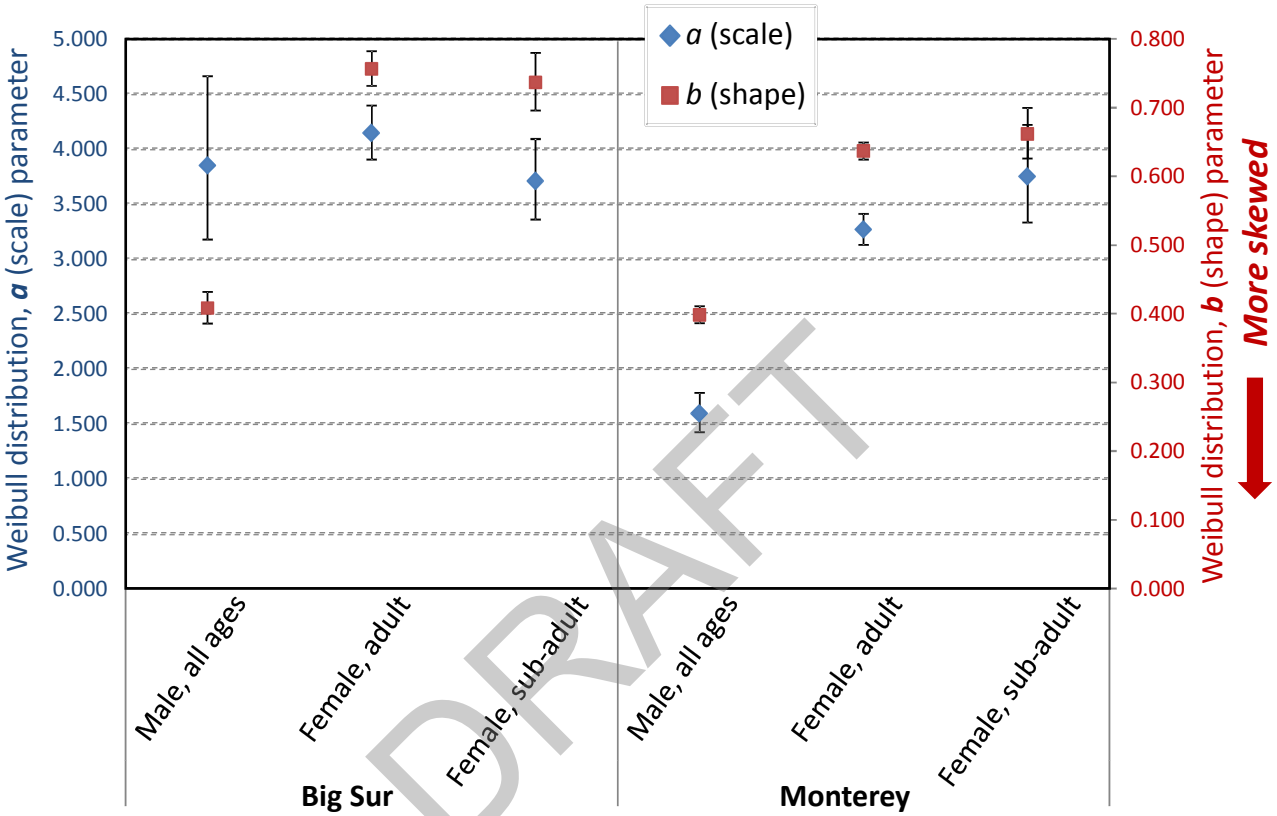


Figure 5



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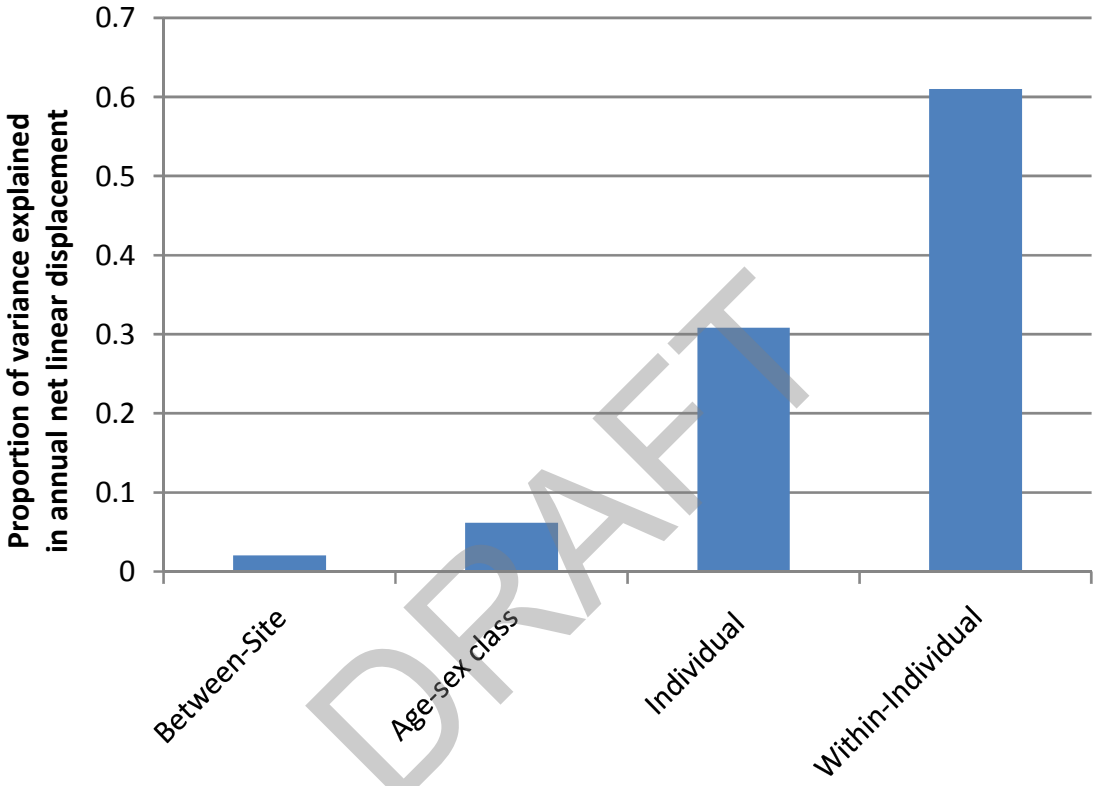

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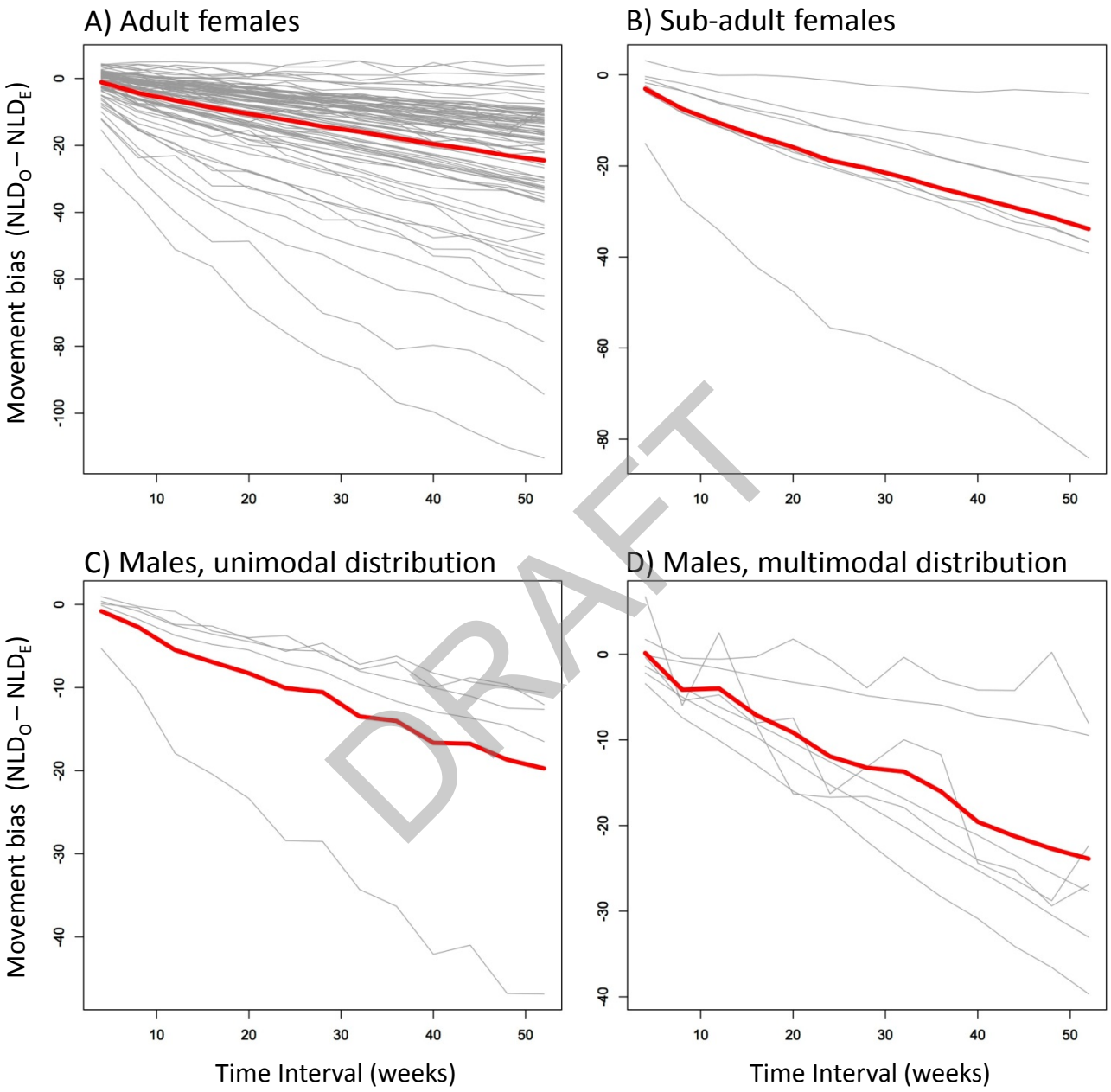
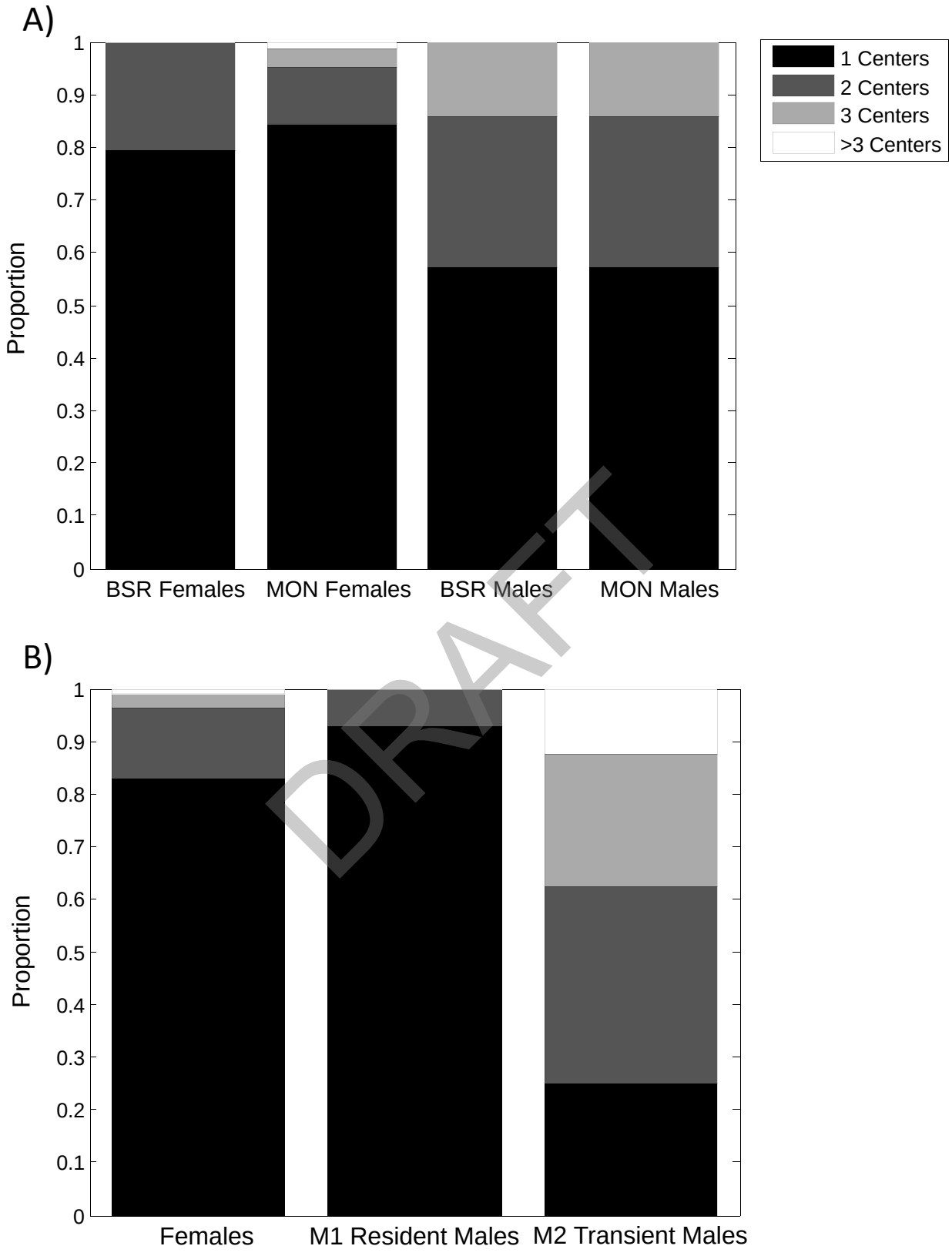


Figure 8



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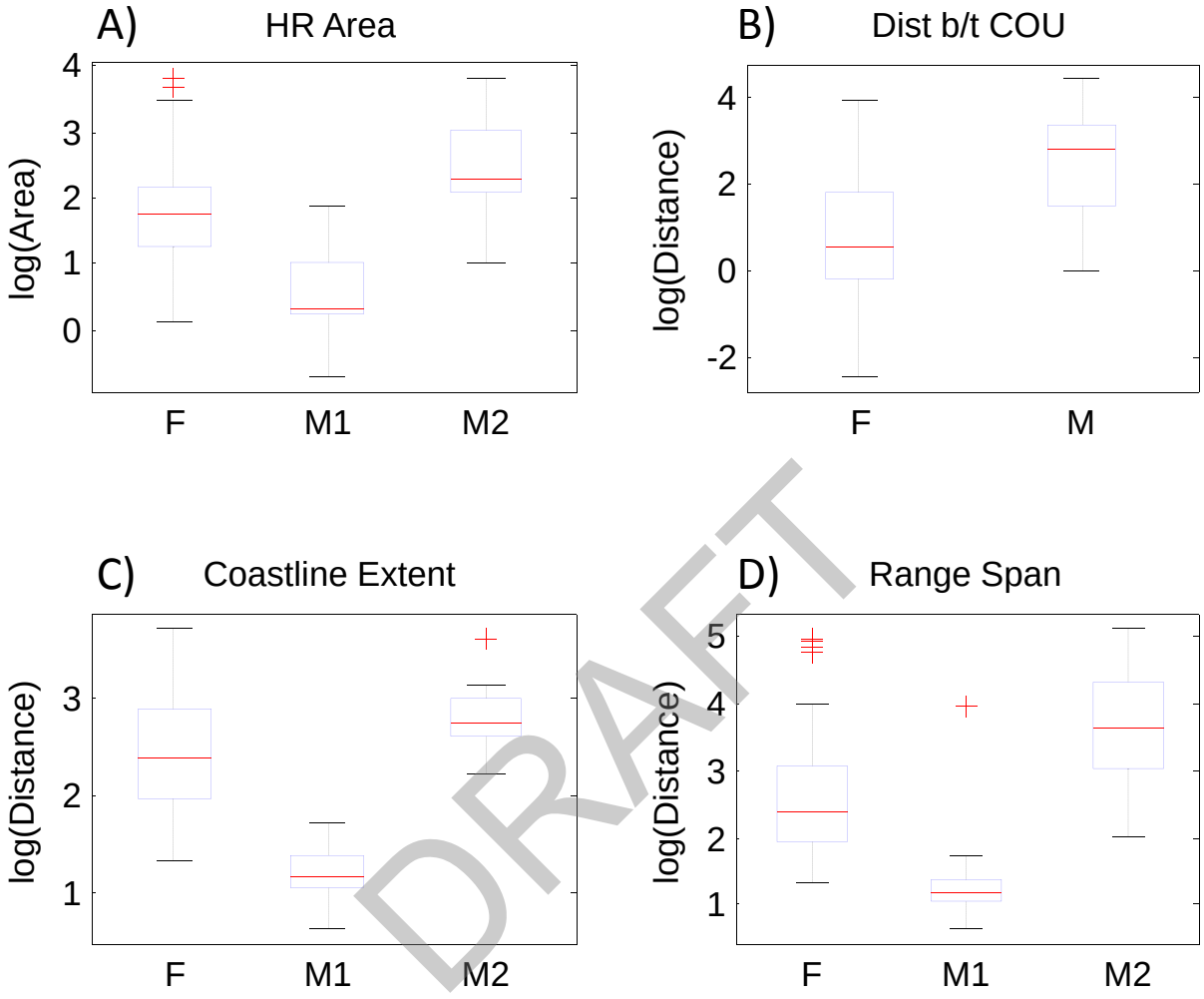


Figure 10

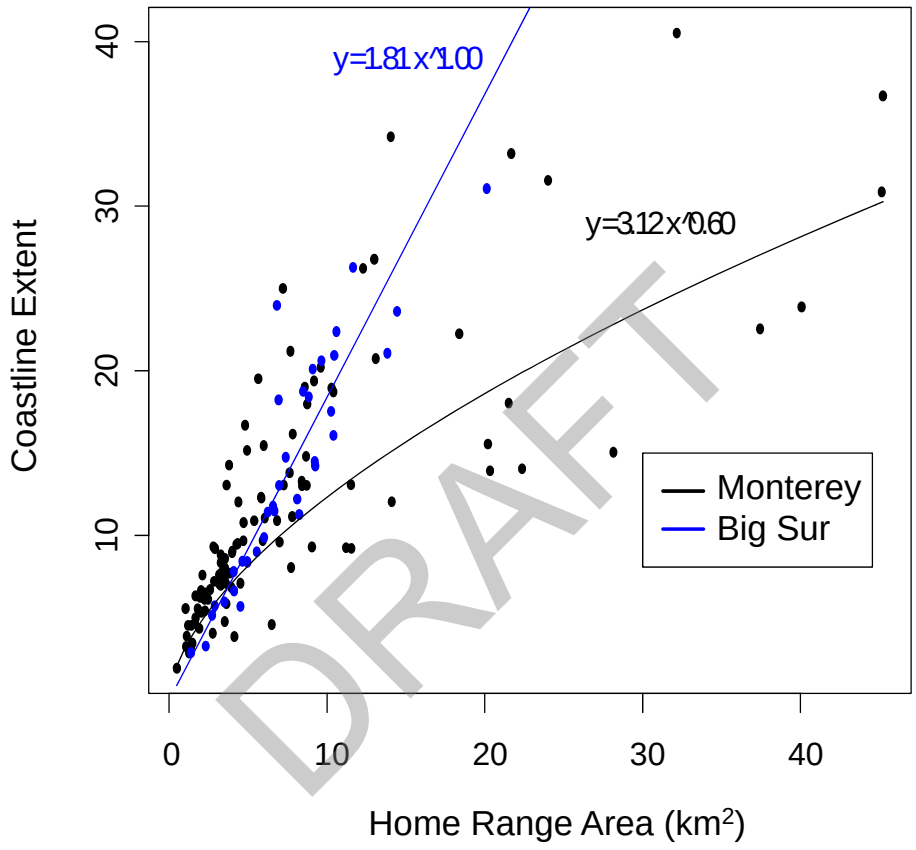


Figure 11

Chapter 5. Dive Behavior and Time-Activity Budgets

M. Tim Tinker[1], Nicole M. Thometz[2], Michelle M. Staedler[3], Teri Nicholson[3], Joe A. Tomoleoni[1]
1. U.S Geological Survey, Western Ecological Research Center
2. Ecology and Evolutionary Biology Department, University of California Santa Cruz
3. Sea Otter Research and Conservation Department, Monterey Bay Aquarium

Introduction

Sea otters have the highest mass-specific metabolic requirements of any marine mammal, and due to their lack of a blubber layer they have negligible energetic reserves (Kenyon 1969, Morrison et al. 1974, Yeates et al. 2007), thus foraging behavior and diving capacities are critical components of food acquisition and survival. As might be expected for an energy-limited apex predator, sea otter foraging activity and diet selection have been shown to be strongly dependent on population density and per-capita food abundance (Garshelis et al. 1986, Watt et al. 2000, Gelatt et al. 2002, Tinker et al. 2008), and density-dependent variation in foraging success is thought to be a primary determinant of equilibrium population size (Estes 1990, Bodkin et al. 2000). Although there is extensive published information on variation in foraging behavior and prey selection, much less is known about the details of diving behavior in sea otters. An investigation by Bodkin et al. (2004) of diving behavior of northern sea otters (*Enhydra lutris kenyoni*) in Port Althorp, Alaska, provided the first description of sea otter dive attributes, average dive depths, and differences in diving behavior between males and females. Foraging dives in Port Althorp typically occurred in depths of 2-30m; however, individuals occasionally made deeper dives of up to 100m depth. Females and males showed differences in foraging dive depths, with females diving <20 meter on 85% of their dives and males diving >45m on 50% of their dives (Bodkin et al. 2004). A summary of dive behavior in southern sea otters in central California showed similar patterns (Tinker et al. 2006). Sex-based differences in dive behavior are not surprising, given the larger size of males (Leith 1989, Burns 1999). Less is known about the effects of other factors that could influence the dive behavior of sea otters, including age, reproductive status, population density, prey distribution, benthic habitat quality and bathymetric characteristics of the local area.

Sea otters can adjust their diving behavior in response to reduced food abundance, making dives of greater depth or duration to find and retrieve prey. They can also increase the proportion of their daily activity budget spent feeding (thus reducing the time spent resting or in other activities). Comparisons of sea otter time-activity budgets have been made from Alaska to California, and have consistently shown that at higher population densities and lower per-capita food abundance, the percent time feeding can increase from 20% to up to 50% (Estes et al. 1982, Garshelis et al. 1986, Gelatt et al. 2002, Bodkin et al. 2007, Tinker et al. 2008). Different methodologies have been used to measure time-activity budgets, including scan sampling (Estes et al. 1986), radio telemetry (Loughlin 1980, Ribic 1982, Ralls and Siniff 1990), and time depth recorders or TDRs (Bodkin et al. 2007), but all have shown similar results. The relationship between activity budgets and resource abundance provides a useful tool for assessments of population status with respect to carrying capacity. However, it has also been pointed out that other factors contribute to variation to time-activity budgets, including sex and age-class

differences, and seasonal variation in prey abundance (Gelatt et al. 2002). Reproductive status in females may also affect foraging activity (Osterrieder and Davis 2011, Staedler 2011). These sources of variation can complicate inter-population contrasts, and so should be incorporated into analyses of activity budgets used to assess population status.

Another component of sea otter behavior that may vary in response to resource abundance is haul-out behavior. In a resource limited environment, hauling-out may enhance long-term individual reproductive success and survival by reducing thermal flux and thus overall energy requirements (Costa and Kooyman 1984, Yeates et al. 2007). The challenge has been to develop a reliable method of measuring sea otter haul-out behavior in the wild, which is not biased by daylight-dependent and line-of-sight observations (Harrold and Hardin 1986, Maldini et al. 2012). By applying logistic regression modeling to a predictable body-core-temperature feature of hauled-out sea otters, we developed a reliable and non-biased method to predict this behavior from continuous diving-depth and temperature data recovered from TDR records (Nicholson et al, manuscript in prep). We reasoned that investigating haul-out behavior of sea otters in Big Sur and Monterey might provide further insights into the behavioral responses of sea otters to limited prey resources at key life history stages, and the implications for sea otter reproductive success and survival.

We measured the dive behavior of sea otters in Monterey and Big Sur using archival time-depth recorders (TDRs), as part of the comparative study of sea otter ecology at these two sites. Our objectives were to 1) describe the diving behavior and basic dive attributes (e.g. mean and maximum dive depths and durations, bottom time, post dive intervals) of sea otters at two sites in central California; 2) identify sources of variation in diving attributes and behavior, including differences in location, age, sex, reproductive status and diet specialization; 3) using established methods, calculate time-activity budgets from TDR records in order to assess relative food resource abundance at the two study sites; 4) evaluate other potential sources of variation in activity budgets, including age, sex, reproductive status and diet specialization; and 5) examine factors that explain variation in haul-out behavior . The results of these analyses should shed light on the role of habitat differences and food resource abundance on affecting behavior and limiting sea otter populations in central California.

Methods

Dive Behavior

Archival data from TDRs were collected between September 2007 and December 2011. Archived data ranged from 66 days to over 1094 days, dependent on an individual otter's survival and battery life of the TDR, for a total of 377,496 hours of recorded data (Table 1). Data from 39 study animals from both Big Sur (2 males and 9 females) and Monterey (6 males and 22 females) were utilized for diving behavior analyses (see Chapter 1 for detailed methods of sea otter capture techniques, TDR implantation methods, and tracking methods). In addition to adult (n = 34) study animals, a small set of sub-adult (n = 5) animals, independent animals under 3 years of age, were also included in the analysis.

Because TDRs record diving behavior for an average of 1-2 years, many females in the study gave birth to (and raised until weaning) 1 or more pups during the period of TDR data logging. This allowed for an

examination of the effects of reproductive status on diving behavior. Specifically, females were categorized into one of four categories based on the presence/age of their pup: female with no pup (NP), female with very small pup (VP, pup ≤3 weeks old), female with small pup (SP, pup > 3 and ≤10 weeks old), and females with large pup (LP, pup > 10 weeks old). Data sets for each female were divided into the appropriate category based on their reproductive status (NP-LP) on each day of TDR data logging, and we treated each combination of individual/reproductive status as an independent record for statistical analyses. These records, together with data records from males (MA) and sub-adult females (SA), resulted in 6 demographic classes that were used for statistical comparisons.

Raw TDR data were downloaded from the TDR instruments and pre-processed using the software "Instrument Helper" (version 3.0; Wildlife Computers, Redmond WA) to correct depth readings for drift from the zero mark, and compile the depth readings (at 2-second intervals) into distinct dives and contiguous surface intervals. For each identified dive, a series of 6 descriptive parameters were calculated (all depths in meters and all times in seconds): maximum dive depth (*DEP*), duration of the sub-surface dive interval (*DT*), duration of time at spent at the bottom of the dive (*BT*, = 90% of maximum depth and thus not necessarily on the ocean floor), duration of the post-dive surface interval (*PDI*, the number seconds elapsed until the next dive), descent rate (*DRT*, vertical swim-speed from surface to bottom) and ascent rate (*ART*, vertical swim-speed from bottom to surface). These parameters were used to classify dives as feeding or non-feeding dives (i.e. dives conducted during grooming, travelling or social behavior), following previously published methods (Bodkin et al. 2004, Bodkin et al. 2007). Briefly, a logistic classification function was used to classify each recorded dive based on a combination of the above-described dive parameters:

$$\log\left(\frac{P}{1-P}\right) = \beta_1 + \beta_2\left(DT\right) + \beta_3\left(BT/DT\right) + \beta_4\left(ART\right) + \beta_5\left(DT\right)\left(ART\right) + \beta_6\left(BT/DT\right)\left(DRT\right) \quad (1)$$

where β_t is a vector of parameters fit by maximum likelihood, and all dives with P>0.5 were classified as feeding dives. Equation 1 was initially fit to a sub-sample of 5,000 "confirmed" dives (dives made by study animals which an observer was able to visually confirm as either 1=feeding or 2=non-feeding dive), and then validated by application to a second sub-sample of 1,000 confirmed dives, to ensure a classification accuracy of >99%. Best-fit values for β_t were:

[-5.115, 0.258, 0.487, -0.210, 0.063, 3.543]

We applied equation 1 to each TDR record to classify all dives, and then sub-divided the entire TDR record into contiguous "bouts" of similar activity states (inactive/resting = R, feeding = F, and "active other" = AO).

The net result of the above pre-processing steps was a sample of 86,441 feeding bouts comprising 3,857,794 foraging dives. Because individual dives within a feeding bout tended to be auto-correlated with respect to some dive parameters, we used feeding bouts as the basic statistical unit in all further analyses, to insure independence. For each distinct feeding bout we calculated a series of 12 statistics: number of dives per bout, bout duration, mean dive depth, mean dive duration (DT), mean post-dive interval (PDI), variance in post-dive interval (Variance PDI), mean bottom time (BT), the mean ratio of

bottom time to dive time (BT/DT), maximum dive depth, maximum dive duration, mean descent rate, and mean ascent rate.

Differences in dive attributes were first evaluated by location (fixed effect, two levels: Big Sur, Monterey) and by demographic class (fixed effect, six levels: NP, VP, SP, LP, M, SA), using a three-way PERMANOVA, in which the interaction between location and class was evaluated as a third factor. All dive attributes were normalized before analyses, Bray-Curtis similarity and 999 permutations were used. We then used a two-way PERMANOVA to test for differences between dive attributes by location and by class without including the interaction term and a one-way PERMANOVA to test for the influence of only the interaction term (location*class). Pair-wise comparisons for both the two-way PERMANOVA and one-way PERMANOVA were made *a posteriori.* When significant differences occurred, similarity percentages (SIMPER) analyses were used to identify the contribution of each factor to the observed differences. All PERMANOVA analyses and comparisons were made using PRIMER 6 & PERMANOVA+ software (Anderson et al. 2008).

In order to determine whether dive behavior varied as a function of prey selection and diet composition, a multiple Analysis of Variance (MANOVA) was used to test for differences in dive attributes between otters assigned to 5 different diet specializations (see Chapter 6 for details). We used Wilks lambda to evaluate the significance of the multi-variate model, and in the case of significance we used univariate F-tests to evaluate diet-based differences in individual dive parameters.

Haul out Behavior

Due to sample size limitations for males and sub-adult age classes, we limited our analysis of haul-out behavior to data from reproductive females from Big Sur (n = 6, 5 AD & 1 SA TDR days>324) and Monterey (n = 18 AD, TDR days >328). We reasoned that reproductive-age females presumably experience the greatest nutritional stress, especially during late-lactation when their foraging effort is greatest (Staedler 2011), and are also the demographic group if greatest interest in terms of understanding factors affecting population growth. To further improve statistical power for among-group comparisons at Monterey, we added data from another 15 adult females captured in Monterey prior to the current study (2006/2007).

To detect the occurrence of haul out behavior from TDR records, we used a logistic classification function similar in form to equation 1, that was based on measurable attributes of the temperature trace during prolonged (>3.5 hrs) non-diving (i.e. resting) intervals:

$$\log\left(\frac{P}{1-P}\right) = \alpha + \beta\left(\left(T_{st} - T_{\min}\right) + \left(T_{\max} - T_{\min}\right)\right) \quad (2)$$

where P is the probability that the otter is hauled out, T_{st} is the body temperature at the start of the resting period, T_{max} is the maximum body temperature occurring during the resting period, T_{min} is the minimum body temperature occurring during the resting period *prior* to the maximum temperature, and α and β are parameters fit by maximum likelihood. Equation 2 was fit to a sample of "training data" (temperature traces from known haul out and in-water resting periods), then validated by visual

confirmation of positive (hauled out) and negative (resting in water) predicted outcomes (T. Nicholson et al, manuscript in prep). After application of this model to data from the current study, we further validated model predictions with field observations of each study animal, and confirmed that the model performed at or above an 85% prediction success rate for each individual. To improve parsimony we rejected any haul-out bouts predicted when ambient air temperatures were >20^{o}C, as these conditions were most likely to lead to misclassification of at-sea resting bouts as haul-out bouts. We also rejected bouts characterized by short (<1 hr) but dramatic temperature anomalies or fluctuations: although a few of these intervals may have been incorrectly rejected, our goal was to identify and estimate the prevalence of prolonged and uninterrupted intervals ashore. For each day of each TDR record we then calculated the % time spent hauled out.

To evaluate factors important for predicting variation in haul out behavior, we used general linear models (GLM) to test for effects of study site (BSR vs. MON), average individual diving depth, season, and reproductive status. To account for seasonal effects we defined 2 seasons (winter and summer) based on relative differences in mean monthly air temperature measured by NOAA buoys off Monterey and Cape San Martin. For female reproductive status we used the same classifications identified above (NP, VP, SP and LP) but also added an "estrous" category for females that were visually confirmed to be engaged in mating activity (estrous generally occurs shortly after weaning a pup). We used maximum likelihood methods to fit a variety of models with different combinations of main effects, and used an information theoretic approach to select the best-supported model(s) with the smallest AIC values (Burnham and Anderson 2002).

Time-Activity Budgets

For each 24-hour period of each TDR record, we calculated a time-activity budget by summing the cumulative time spent in each of 3 activity states (see above for methods of classifying TDR records by activity state): F = feeding, AO = active-other, and R = inactive/resting (refer to Bodkin et al. 2007 for details). Figure 1 illustrates a typical 24-hour period from a TDR record, showing dive behavior and activity classifications. Time-activity budgets were then summarized for all otters, with each otter classified by location and sex, and for females we also calculated activity budgets for each of 4 reproductive states (NP, VP, SP and LP: see above for details of classification). We used two-way ANOVA to test for variation in percent time feeding due to sex and study site (MON vs. BSR), as well as interactions between these main effects. We then used single factor ANOVA to test for differences between study sites with respect to percent time feeding for females in different reproductive states (NP, VP, SP and LP). Pooling all female otters from both sites, we used mixed-effects ANOVA to test for differences in percent time feeding between reproductive states, with individual otters treated as a random effect. Finally, we used single factor ANOVA to test for differences in percent time feeding between otters assigned to 5 different diet specializations (see Chapter 6).

Results

Dive Behavior

Attributes of feeding dives and foraging bouts were generally similar at Big Sur and Monterey, with average dive depths of approximately 9m and average dive durations of approximately 1 minute at both

sites. The depth below which 99% of feeding dives occurred was 37m in Big Sur and 39m in Monterey, the deepest recorded dive (87m) occurred at Big Sur, while the longest recorded dive (472 seconds) occurred at Monterey. A complete summary of dive attributes at each site is provided in Table 2. Based on a complete model with interaction, dive attributes differed significantly by class (three-way PERMANOVA: $F_{5,73} = 3.95$ P = 0.001), but not by location ($F_{1,73} = 1.25$ P = 0.258) or by their interaction ($F_{5,73} = 0.50$ P = 0.947). Using a reduced model without interaction term, the influence of demographic class remained highly significant (two-way PERMANOVA: $F_{5,78} = 4.73$ P = 0.001) and the influence of location was marginally significant ($F_{1,78} = 2.58$ P = 0.055). Pair-wise tests were run for all demographic classes and nearly all were significantly different (P < 0.05) from one another (Table 3). Pairs that were marginally significant (P < 0.1) included MA and SA (t = 1.54 P = 0.059), VP and SP (t = 1.53 P = 0.074), and SP and SA (t = 1.67 P = 0.065). Groups that were not significantly different were NP and SA (t = 0.92 P = 0.48) and LP and SA (t = 1.24 P = 0.21).

The SIMPER analysis highlighted the dive attributes that contributed the most (cumulative contribution > 30%) to the differences found between each demographic class (Table 3). Maximum dive depth (Figure 2) and maximum dive duration (Figure 3) contributed most to the differences found between males (MA) and all female classes (NP-LP). Number of dives per bout also contributed greatly to the differences between MA and LP (males made fewer dives per bout). Variance in PDI, ascent rate, and maximum dive duration were the most important dive attributes between NP and VP (females without pups made longer dives and were less variable surface intervals than females with very small pups), while descent rate, maximum dive duration and ascent rate were the most important between NP and SP. Between NP and LP, the greatest contributing attributes to observed differences were number of dives per bout, bottom time and mean dive duration (females with large pups had more dives per bout and spent longer dives with more time at bottom). The most influential variables between VP and LP were variance PDI, number of dives per bout, and mean bout duration, but between VP and SA, differences were driven by maximum dive depth, maximum dive duration, and variance PDI (Table 3).

To test differences between individuals of the same reproductive state in different locations (Table 4), a one-way PERMANOVA was run with only the location*class interaction term. The interaction term was significant (one-way PERMANOVA: $F_{11, 73} = 2.5$, P = 0.001) and pair-wise comparisons were made. Only NP individuals differed significantly between locations (t = 1.76 P = 0.03), and all other pair-wise comparisons between individuals of the same class at each location were non-significant. The SIMPER analysis indicated that the dive attributes that contributed the most (cumulative contribution > 30%) to the difference between NP individuals in Big Sur and Monterey were mean bout duration, maximum dive duration, and number of dives per bout. In particular, females with no pup in Big Sur had longer maximum dives durations, longer foraging bouts and more dives per bout than those in Monterey.

Dive parameters differed significantly among otters having different diet specialization (λ_U=0.0008, $F_{12,22}$= 2,231, P <0.0001). In particular, diet specialists differed with respect to dive depth ($F_{1,33}$=5.94, P = 0.020), dive duration ($F_{1,33}$=6.65, P = 0.014), bottom time ($F_{1,33}$=6.59, P = 0.015), and ascent rate ($F_{1,33}$=4.75, P = 0.037). As shown in Figure 4, sea otters that specialized on *Cancer* crabs (type 1) and abalone (type 2) exhibited lower ascent rates and greater dive durations, depths and bottom times as compared to sea otters that specialized on mussels (type 3), turban snails (type 4) and clams (type 5).

Haul out Behavior

The model that best fit data on haul out frequency included effects of study site and mean diving depth. Females in Monterey hauled out more frequently than Big Sur females (4.1% ± 1.0 vs. 0.4% ± 0.07). None of the Big Sur females demonstrated haul out behavior at a biologically significant level. By contrast, Monterey females demonstrated great variability with respect to hauling out. Some (5) females rarely hauled out, others (8) hauled out frequently during portions of the year, and 2 hauled out throughout most or all of the year. These difference s may be partially explained by mean diving depth (Figure 5A): deeper divers (> 10m) hauled-out rarely if ever. We limited further analyses to sea otters from Monterey, in order to elucidate other effects more clearly after removing the effect of study site. The best-supported model included effects of reproductive status and season, with an additional random effect for individual otter (indicating that much of the variation was related to differences among individuals that were not explained by season or status). Haul-out behavior was strongly influenced by reproductive status, specifically the estrous period when females in our sample hauled-out most frequently (16.3% ± 1.2 SE, Figure 5B). Haul-out behavior did not vary significantly among the remaining categories of reproductive status. Season had a small effect: females tended to haul-out slightly more during winter than summer (9.4 % ± 6.6 SE vs. 6.5 ±% 0.9 SE). Reproductive status had the greatest relative effect on haul-out behavior (45.3%), followed by individual otter effects (26.2%), and seasonal differences (4.8%).

Time-Activity Budgets

Percent time foraging differed significantly between the two sites (Figure 6A) when all classes were pooled ($F_{1,35}$ = 9.08, P= 0.005), with sea otters at BSR spending more time feeding than otters at MON (45.49 ± 5.07 vs. 41.48 ± 4.68, respectively: see Table 5). Percent time in active-other behaviors also different between sites ($F_{1,35}$= 9.164, P = 0.005), with BSR otters spending less time in non-feeding activities than MON otters (5.76 ± 2.44 vs. 9.02 ± 2.62, respectively), but there were no differences in inactive/resting behavior ($F_{1,35}$= 1.667, P=0.205). The effect of sex on percent time feeding was not significant ($F_{1,35}$ = 1.577, P = 0.217), although the marginally significant interaction between sex and site ($F_{1,35}$ = 3.457, P = 0.071) reflected an especially high percent time spent feeding by BSR males (Table 5).

Limited sample sizes for sub-adult animals (BSR n=1, MON n=4) precluded testing for differences in behavior between sites and age classes. Pooling the data for both sites, we found no significant difference in time spent foraging between age classes (F=0.357. P= 0.555).

When we parsed data by reproductive status, we found that percent time feeding differed between study sites for females without pups ($F_{1,29}$ = 8.76, P = 0.007), such that females from BSR spent more time feeding than MON females (Table 6). However, there were no significant between-site differences in activity budgets for females with pups, irrespective of pup stage. Pooling data for both sites, a mixed-effects model indicated a strongly significant effect of female reproductive status on percent time feeding ($F_{3,50}$ = 127.4, P =<0.0001): specifically, the percent time feeding by females increased significantly (P<0.05) as pups grew from VP to SP, and from SP to LP stages (Table 7, Figure 6B).

We did not find any differences in activity budget between otters with different diet specializations ($F_{4,30}$= 0.766, P = 0.556).

Discussion

Sea otters in Big Sur and Monterey appear to exhibit similar diving behavior overall. This finding is not entirely surprising, considering that both locations have fairly similar benthic habitat types, and support high density sea otter populations where intra-specific competition for prey may be fairly high. Typical dive times were approximately 1 minute with average dive depths of 9 meters, although these parameters were highly variable (Table 2). Our data differ from data presented by Bodkin *et al.* (2004) in that we did not observe a clear bimodal foraging depth pattern. Sea otters in Port Althorp, AK, foraged mainly between 2 and 30 meters (84% of all dives), however many individuals, particularly males, showed a bimodal foraging depth distribution with a secondary modal feeding depth occurring at 30-60 meters (Bodkin *et al.* 2004). At Big Sur and Monterey, sea otters foraged mostly at depths of 3-15 meters and displayed progressively fewer foraging dives in deeper water, although the two Big Sur males exhibited a barely discernible secondary modal depth at 40-60m. Males dove to the greatest depths in our study (Figure 2), reaching maximum depths of 87 meters in Big Sur and 77 meters in Monterey. The difference in maximum dive depths between the two locations is likely a result of the differing bathymetry of each location (the coastal shelf drops off steeply at Big Sur, but is broad and shallow at Monterey), and not a result of any physiological differences in diving ability.

In contrast to the similarity in diving behavior across sites, we found that differences in age, sex, reproductive status and diet were associated with substantial differences in diving behavior. For example, sub-adults displayed a larger number of dives per bout (60.0±10.5) and longer mean bout durations (132.1±18.8) than their adult female counterparts (with the exception of those having large pups, as discussed below). In addition, sub-adults exhibited the second longest maximum dive durations (273.8±32.7) and spent the second longest amount of time foraging per day (47.6±2.5%). These results suggest that sub-adult sea otters in central California exhibit greater foraging effort than do adults (Table 2). Sub-adult animals are likely physiologically limited and behaviorally naive in comparison to adults (Burns 1999; Noren *et al.* 2002; Richmond, Burns, & Rea 2006) and these factors may ultimately require the increased foraging effort by sub-adult animals.

Adult females showed striking variation in diving behavior that corresponded with changes in their reproductive status. Females with very small pups drastically reduced their number of dives per bout, bout durations, maximum dive depths, and maximum dive durations in comparison to females with no pup (Table 3). The changes appear to be adaptive behavior adjustments for pup care, reflecting the high level of maternal attention a neonate sea otter pup needs to survive. Female sea otters with very small pups also showed greater post dive intervals and greater variance in these post dive intervals, likely due to the necessity of tending to their pup between dives. Reduced maximum dive depths for females with very small pups and small pups (Figure 2) is also probably an adaptive adjustment to improve pup survival outcomes, but implies a substantial reduction in potential foraging areas available to these females, with potential implications for foraging success.

As a pup matures from a highly dependent neonate into a large pup making diving and foraging attempts alongside its mother, females significantly alter their diving and foraging behavior yet again. The energetic toll of rearing young is high in sea otters (Thometz et al. 2014): in response to these high energetic demands, females with large pups show greater number of dives per bout and longer bout

durations, and also reduce the interval between dives (reflecting less time spent with the pup and more time diving for food). Females with large pups also greatly increase the overall amount of time they spend feeding (see below), but in spite of all these behavioral modifications females nearing the end of lactation display poor body condition due to depletion of body reserves over the 6-month pup dependency period, resulting in increased risk of mortality (see Chapters 7, 8 and 10, this report).

Another behavioral strategy that females may employ to mitigate costs of reproduction is haul out behavior, although opportunities for hauling out may vary seasonally and spatially. We found significant differences in hauling out between our two study sites: at Monterey many of the tagged females hauled out regularly, especially in response to estrous or mating, but females at Big Sur rarely hauled out. It may be that our sample size from Big Sur (n = 6) was too low to reliably estimate haul-out behavior at the population level; however, during field observation the behavior was only noted 3 times during the entire 3 year study period. One possible explanation for this difference is the differing geographic features and coastline topologies at the two sites: Monterey Peninsula is a winding and complex coastline with significant intertidal habitats that are protected from prevailing northwest winds and swell, providing ample haul out opportunities. By contrast, the Big Sur coastline is relatively linear and highly exposed, with minimal inter-tidal habitats, and so may simply provide fewer haul-out opportunities. Because hauling-out may act to reduce thermally-induced metabolic requirements during periods of nutritional stress, females from Monterey may thus experience a geographic advantage by having more haul-out opportunities, enabling individuals to reduce energy costs and potentially enhance their survival and long-term reproductive success (see Chapter 8). This advantage may be especially critical for females that have recently weaned a pup and are entering estrous in poor body condition with minimal energy reserves. Indeed, the incidence of mortality associated with "end-lactation syndrome" was slightly lower among Monterey females than among Big Sur females (see Chapter 10).

In addition to evaluating differences in haul out and dive behavior, the TDR data also allowed us to examine variation in time-activity budgets. The percent time spent feeding by sea otters at both sites was over 40%, quite high compared to growing populations where prey resources are abundant (Jolly 1997, Bentall 2005, Bodkin et al. 2007) but similar to populations thought to be at carrying capacity where prey resource abundance limits further growth (Estes et al. 1982, Garshelis et al. 1986, Gelatt et al. 2002, Tinker et al. 2008). We found that sea otters at the Big Sur study site spent slightly more time feeding than otters at Monterey (Figure 6A), suggesting that prey resources may be slightly more depleted at the Big Sur site. This difference was particularly apparent for Big Sur males, a fact that is consistent with the finding that males at Big Sur also showed much lower rate of energy gain while feeding (Chapter 6) and poorer body condition (Chapter 7) than males at Monterey. As expected based on previous analyses (Osterrieder and Davis 2011, Staedler 2011), female reproductive status was a major determinant of percent time feeding, which dropped to 24% for females with very small pups but increased to almost 50% for females with large pups (Figure 6B).

Understanding sea otter dive behavior is not as simple as describing differences between males and females: habitat type and bathymetry, population density, available resources, age, reproductive status, and diet specialization also play important roles in dictating diving behavior. For example, the

differences in dive attributes we found between sea otters that utilized different prey types (Figure 4) were consistent with earlier reports that diet specialization is reflected by variation in dive behavior (Tinker et al. 2007). Such behavioral differences are likely to reinforce diet specialization, as individuals become channeled into different behavioral strategies. Overall the dive behavior and time-activity budgets of sea otters at Big Sur and Monterey are indicative of populations that are strongly influenced by resource limitation, and exhibit patterns of variation that suggest adaptive responses to low prey availability coupled with high energy demands at critical life history stages. These patterns are likely to be reflected in survival and pup weaning success (see Chapter 8), and ultimately in population recovery.

References

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Bentall, G. B. 2005. Morphological and behavioral correlates of population status in the southern sea otter: a comparative study between central California and San Nicolas Island. Masters Thesis. University of California, Santa Cruz, CA.

Bodkin, J. L., A. M. Burdin, and D. A. Ryazanov. 2000. Age- and sex-specific mortality and population structure in sea otters. Marine Mammal Science **16**:201-219.

Bodkin, J. L., G. G. Esslinger, and D. H. Monson. 2004. Foraging depths of sea otters and implications to coastal marine communities. Marine Mammal Science **20**:305-321.

Bodkin, J. L., D. H. Monson, and G. G. Esslinger. 2007. Activity Budgets Derived From Time–Depth Recorders in a Diving Mammal. Journal of Wildlife Management **71**:2034-2044.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag, New York, NY.

Burns, J. M. 1999. The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. Canadian Journal of Zoology **77**:737-747.

Costa, D. P. and G. L. Kooyman. 1984. Contribution of specific dynamic action to heat balance and thermoregulation in the sea otter Enhydra lutris. Physiol. Zool. **57**:199-203.

Estes, J. A. 1990. Growth and equilibrium in sea otter populations. Journal of Animal Ecology **59**:385-402.

Estes, J. A., R. J. Jameson, and E. B. Rhode. 1982. Activity and prey selection in the sea otter - influence of population status on community structure. American Naturalist **120**:242-258.

Estes, J. A., K. E. Underwood, and M. J. Karmann. 1986. Activity-time budgets of sea otters in California [USA]. Journal of Wildlife Management **50**:626-636.

Garshelis, D. L., J. A. Garshelis, and A. T. Kimker. 1986. Sea otter time budgets and prey relationships in Alaska. Journal of Wildlife Management **50**:637-647.

Gelatt, T. S., D. B. Siniff, and J. A. Estes. 2002. Activity patterns and time budgets of the declining sea otter population at Amchitka Island, Alaska. Journal of Wildlife Management **66**:29-39.

Harrold, C. and D. Hardin. 1986. Prey Consumption on Land by the California Sea Otter Enhydra-Lutris. Marine Mammal Science **2**:309-313.

Jolly, J. M. 1997. Foraging ecology of the sea otter, *Enhydra lutris*, in a soft-sediment community. Masters dissertation. University of California, Santa Cruz, Santa Cruz, CA.

Kenyon, K. W. 1969. The sea otter in the eastern Pacific Ocean. North American Fauna **68**:1-352.

Leith, D. E. 1989. Adaptations To Deep Breath-Hold Diving Respiratory and Circulatory Mechanics. Undersea Biomedical Research **16**:345-354.

Loughlin, T. R. 1980. Radio telemetric determination of the 24-hour feeding activities of sea otters, Enhydra lutris. Pages 717-724 *in* C. J. Amlaner and D. W. MacDonald, editors. A handbook on biotelemetry and radio tracking. Permagon Press, Oxford, UK.

Maldini, D., R. Scoles, R. Eby, M. Cotter, and R. W. Rankin. 2012. Patterns of Sea Otter Haul-Out Behavior in a California Tidal Estuary in Relation to Environmental Variables. Northwestern Naturalist **93**:67-78.

Morrison, P., M. Rosenmann, and J. A. Estes. 1974. Metabolism and thermoregulation in the sea otter. Physiol. Zool. **47**:218-229.

Osterrieder, S. K. and R. W. Davis. 2011. Sea otter female and pup activity budgets, Prince William Sound, Alaska. Journal of the Marine Biological Association of the UK **91**:883-892.

Ralls, K. and D. B. Siniff. 1990. Time budgets and activity patterns in California sea otters. Journal of Wildlife Management **54**:251-259.

Ribic, C. A. 1982. Autumn activity of sea otters in California. J. Mammal. **63**:702-706.

Staedler, M. M. 2011. Individual variation in maternal care and provisioning in the southern sea otter (Enhydra lutris nereis): causes and consequences of diet specialization in a top predator. Masters Thesis. University of California, Santa Cruz.

Thometz, N. M., M. T. Tinker, M. M. Staedler, K. A. Mayer, and T. M. Williams. 2014. Energetic demands of immature sea otters from birth to weaning: implications for maternal costs, reproductive behavior and population-level trends. Journal of Experimental Biology **217**:2053-2061.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., D. P. Costa, A. Estes James, and N. Wieringa. 2007. Individual dietary specialization and dive behaviour in the California sea otter: using archival time-depth data to detect alternative foraging strategies. Deep Sea Research II **54**:330-342.

Tinker, M. T., J. A. Estes, K. Ralls, T. M. Williams, D. Jessup, and D. P. Costa. 2006. Population Dynamics and Biology of the California Sea Otter (*Enhydra lutris nereis*) at the Southern End of its Range. MMS OCS Study 2006-007. Coastal Research Center, Marine Science Institute, University of California, Santa Barbara, California. MMS Cooperative Agreement Number 14-35-0001-31063.

Watt, J., D. B. Siniff, and J. A. Estes. 2000. Inter-decadal patterns of population and dietary change in sea otters at Amchitka Island, Alaska. Oecologia (Berlin) **124**:289-298.

Yeates, L. C., T. M. Williams, and T. L. Fink. 2007. Diving and foraging energetics of the smallest marine mammal, the sea otter (Enhydra lutris). Journal of Experimental Biology **210**:1960-1970.

Tables

Table 1 Summary information on TDR deployments for study animals in the current study

Study Area	Otter ID	Sex	Age	Age Class	IMPLANT_DATE	TDR_STOP_DATE	TOTAL TDR DAYS	Pup Birth?	Pup Success?
BSR	5-093	F	8.0	A	30-Mar-09	13-Sep-09	167	Y	N
BSR	5-283	F	10.0	A	22-Mar-09	16-Jun-09	86	Y	N
BSR	6-043	F	9.0	A	12-Nov-08	9-Jun-09	209	Y	Y
BSR	6-209	F	6.0	A	30-Nov-08	5-Feb-10	432	Y	N
BSR	6-370	M	8.0	A	10-Nov-08	3-Nov-09	358	N/A	N/A
BSR	6-409	M	9.0	A	9-Nov-08	4-Nov-09	360	N/A	N/A
BSR	6-436	F	5.0	A	11-Nov-08	24-Sep-10	682	Y	Y
BSR	7-660	F	9.0	A	11-Nov-08	10-Nov-11	1094	Y	Y
BSR	6-067	F	4.0	A	3-Nov-09	9-Nov-11	736	N	N/A
BSR	6-553	F	2.5	SA	4-Nov-09	24-Sep-10	324	N	N/A
BSR	6-514	F	4.0	A	3-Nov-09	4-May-11	547	Y	N
MON	7-649	F	4.0	A	28-Sep-07	6-Oct-08	374	Y	Y
MON	7-609	F	4.0	A	26-Sep-07	19-Aug-08	328	N	N/A
MON	7-828	F	6.0	A	27-Sep-07	10-Sep-08	349	Y	N
MON	4-257	F	5	A	28-Sep-07	10-Sep-08	348	Y	N
MON	5-117	F	5.5	A	1-Feb-10	23-Jun-10	142	Y	Y
MON	6-765	F	8.0	A	7-Jun-10	16-Jun-11	374	Y	Y
MON	Jack	M	6.5	A	2-Feb-10	9-Jan-11	341	N/A	N/A
MON	ORWH	M	10.0	A	8-Jun-10	5-Feb-11	242	N/A	N/A
MON	5-217	F	6.0	A	2-Feb-10	12-Jun-10	130	N	N/A
MON	5-349	F	7.0	A	22-Apr-09	8-Jun-10	412	Y	Y
MON	7-722	F	5.0	A	30-Apr-09	24-Oct-10	542	Y	Y
MON	6-381	F	6.0	A	16-Jun-09	15-Aug-10	425	Y	Y
MON	1030-06	M	9.0	A	23-Apr-09	25-Aug-10	489	N/A	N/A
MON	1037-07	F	7.0	A	23-Apr-09	22-Jun-10	425	Y	Y
MON	7-633	F	5.5	A	17-Jun-09	28-Jul-10	406	N	N/A
MON	5-296	F	5.5	A	23-Apr-09	27-Oct-10	552	Y	Y
MON	6-485	M	6.0	A	30-Apr-09	27-Apr-10	362	N/A	N/A
MON	6-493	F	4.0	A	11-Jun-09	4-Sep-11	815	Y	Y
MON	7-747	M	10.0	A	22-Apr-09	8-Jun-10	412	N/A	N/A
MON	5-069	F	2.5	SA	28-May-09	28-Oct-10	518	N	N/A
MON	6-268	F	7.0	A	17-Jun-09	7-Jun-10	355	Y	Y
MON	Marigold	F	1.1	SA	24-Aug-09	29-Oct-09	66	N	N/A
MON	George	M	11.0	AA	9-Jun-09	31-Mar-10	295	N/A	N/A
MON	6-131	F	9.0	A	16-Jun-09	6-Jun-10	355	Y	N
MON	Lola	F	3.1	A	16-Dec-10	2-Sep-11	260	N	N/A
MON	Blanca	F	2.3	SA	3-Dec-09	26-Jun-11	570	Y	N
MON	1000-05	F	8.0	A	17-Jun-09	3-Oct-10	473	Y	Y
MON	1038-07	F	5.0	A	29-Jul-10	7-Aug-11	374	Y	Y

Table 2: Dive attributes of southern sea otters from two central California study sites, Big Sur (BSR) and Monterey (MON). Statistics shown represent arithmetic means for all individuals in each study site (± standard errors), with the exception of the last three columns which show the maximum recorded dive depth, the 99% percentile of dive depths (i.e. the depth below which 99% of all dives occurred) and the maximum recorded dive duration at each site.

Site	n	Dives Per Bout (#)	Bout Dur. (min)	Dive Depth (meters)	Dive Dur. (sec)	PDI (sec)	Variance PDI (sec)	BT/DT	Bottom Time (sec)	Max Depth per bout (meters)	Max Duration per bout (min)	Descent Rate	Ascent Rate	Max Depth record (meters)	Depth 99% Prcntle (meters)	Max Dive Dur (sec)
BSR	11	51.9(4.7)	134.0(8.3)	9.1(1.4)	66.7(5.1)	94.6(4.8)	91.5(4.8)	0.58(0.02)	41.0(3.7)	43.3(3.0)	217.5(14.5)	0.76(0.04)	0.84(0.07)	87	37	370
MON	28	42.0(2.7)	102.9(4.9)	9.2(0.8)	62.3(3.0)	99.4(2.8)	91.5(2.8)	0.61(0.01)	39.3(2.2)	43.9(1.8)	237.8(8.5)	0.75(0.02)	0.82(0.04)	77	39	472

Table 3: Dive attributes of southern sea otters in six classes. Data obtained from individuals in two different study sites (Big Sur and Monterey, CA) have been pooled for analysis. Values presented are means (±SE).

Class	n	Dives Per Bout (#)	Bout Duration (min)	Dive Depth (meters)	Dive Duration (sec)	PDI (sec)	Variance PDI (sec)	BT/DT	Bottom Time (sec)	Max Depth per bout (meters)	Max Duration per bout (min)	Descent Rate	Ascent Rate
NP	25	54.5(4.0)	129.3(7.2)	7.9(1.2)	61.3(4.4)	86.8(6.5)	82.2(4.2)	0.58(0.02)	37.2(3.2)	40.3(2.6)	258.9(12.5)	0.69(0.03)	0.69(0.06)
VP	17	24.9(5.0)	74.8(8.9)	9.7(1.5)	61.6(5.5)	122.6(8.1)	118.2(5.2)	0.60(0.02)	38.6(4.0)	26.7(3.2)	178.9(15.6)	0.79(0.04)	1.01(0.07)
SP	15	43.2(5.1)	114.4(9.2)	10.0(1.5)	67.7(5.6)	99.1(8.3)	93.9(5.3)	0.61(0.02)	43.5(4.1)	29.8(3.3)	177.4(16.0)	0.80(0.04)	0.94(0.07)
LP	15	61.1(5.1)	144.2(9.2)	9.1(1.5)	65.9(5.6)	76.2(8.3)	72.4(5.3)	0.61(0.02)	42.2(4.1)	39.4(3.3)	199.9(16.0)	0.73(0.04)	0.81(0.07)
MA	8	38.0(7.6)	115.9(13.7)	11.2(2.3)	71.0(8.4)	114.4(12.4)	97.1(7.9)	0.58(0.03)	44.5(6.1)	72.4(4.9)	277.0(23.9)	0.81(0.07)	0.86(0.11)
SA	5	60.0(10.5)	132.1(18.8)	7.2(3.1)	59.5(11.5)	83.1(17.0)	85.3(10.8)	0.58(0.04)	34.9(8.3)	52.9(6.7)	273.8(32.7)	0.70(0.09)	0.67(0.15)

Table 4: Dive attributes of southern sea otters in six classes determined for two different central California study sites. Values presented are means (±SE).

Site	Class	n	Dives Per Bout (#)	Bout Duration (min)	Dive Depth (meters)	Dive Duration (sec)	PDI (sec)	Variance PDI (sec)	BT/DT	Bottom Time (sec)	Max Depth (meters)	Max Duration (min)	Descent Rate	Ascent Rate
BSR	NP	8	65.1(6.6)	155.6(11.9)	8.7(2.0)	66.6(7.3)	77.6(10.8)	78.4(6.9)	0.57(0.02)	40.1(5.3)	36.8(4.3)	240.3(20.7)	0.72(0.06)	0.75(0.10)
BSR	VP	5	25.2(8.34)	81.2(15.0)	9.8(2.5)	64.9(9.2)	121.2(13.6)	121.1(8.7)	0.59(0.03)	40.4(6.7)	27.4(5.4)	180.4(26.2)	0.82(0.07)	1.04(0.12)
BSR	SP	5	49.5(8.4)	131.0(15.0)	8.8(2.5)	67.6(9.2)	92.2(13.6)	94.4(8.7)	0.60(0.03)	42.7(6.7)	30.4(5.4)	187.2(26.2)	0.78(0.07)	0.90(0.12)
BSR	LP	5	67.7(8.4)	159.4(15.0)	8.2(2.5)	64.3(9.2)	71.6(13.6)	72.2(8.7)	0.59(0.03)	39.7(6.7)	38.6(5.4)	189.2(26.2)	0.75(0.07	0.80(0.12)
BSR	MA	2	44.9(13.2)	143.7(23.7)	13.0(3.9)	79.2(14.6)	121.9(21.5)	96.3(13.7)	0.59(0.05)	50.2(10.5)	80.5(8.5)	270.0(41.4)	0.85(0.11)	0.92(0.19)
BSR	SA	1	59.3(18.7)	133.3(33.6)	6.4(5.5)	57.5(20.6)	83.3(30.4)	86.4(19.4)	0.56(0.07)	32.9(14.9)	46.0(12.1)	238.0(58.5)	0.66(0.16)	0.64(0.27)
MON	NP	17	44.0(4.5)	103.0(8.1)	7.1(1.3)	56.1(5.0)	96.1(7.4)	86.0(4.5)	0.59(0.02)	34.3(3.6)	43.9(2.9)	277.6(14.2)	0.66(0.04)	0.64(0.07)
MON	VP	12	24.6(5.4)	68.4(9.7)	9.7(1.6)	58.2(6.0)	123.9(8.8)	115.2(5.6)	0.61(0.02)	36.7(4.3)	26.0(3.5)	177.3(16.9)	0.76(0.05)	0.97(0.08)
MON	SP	10	37.0(5.9)	97.8(10.6)	11.2(1.7)	67.7(6.5)	105.9(9.6)	93.4(6.1)	0.63(0.02)	44.3(4.7)	29.2(3.8)	167.6(18.5)	0.82(0.05)	0.99(0.09)
MON	LP	10	54.6(5.9)	129.0(10.6)	9.9(1.7)	67.5(6.5)	80.8(9.6)	72.6(6.1)	0.64(0.02)	44.8(4.7)	40.2(3.8)	210.6(18.5)	0.72(0.05)	0.81(0.09)
MON	MA	8	31.2(7.6)	88.0(13.7)	9.5(2.3)	62.8(8.4)	106.9(12.4)	97.9(7.91)	0.57(0.03)	38.9(6.1)	64.3(4.9)	284.0(23.9)	0.78(0.07)	0.80(0.11)
MON	SA	4	60.6(9.4)	131.0(16.8)	8.0(2.8)	61.5(10.3)	83.0(15.2)	84.1(9.7)	0.59(0.03)	36.9(7.4)	59.8(6.0)	309.5(29.2)	0.74(0.08)	0.70(0.13)

Table 5 Comparison of mean percent time spent in 3 activity states (foraging, active-other, and inactive) for males (M), females (F) and sub-adult (SA) females at Big Sur (BSR) and Monterey (MON)

Behavior	BSR M (n=2)		BSR F (n=8)		BSR SA (n=1)		MON M (n=6)		MON F (n=12)		MON SA (n=4)	
	mean	SD±	mean	SD±	mean	SD±	mean	SD±	mean	SD±	mean	SD±
Foraging	50.83	0.75	44.14	5.12	44.70	.	40.47	4.36	41.76	3.98	47.54	4.29
Other	5.57	0.11	5.71	2.90	6.44	.	9.75	2.29	8.54	2.84	8.46	2.41
Non Active	45.39	0.69	50.13	4.30	48.86	.	49.78	5.97	43.99	4.42	44.00	2.54

Table 6 Summary of mean percent time spent in 3 activity states (foraging, active-other, and inactive) for female sea otters, grouped by study site (BSR vs MON), age class (adult = A, sub-adult = SA) and reproductive state (NP = no pup, VP = very small pup, SP = small pup, LP = Large pup). Values in **RED BOLD** indicate a significant difference between sites

Study site, age class and reproductive state	n	Foraging		Active-other		Inactive/Resting	
		mean	SD±	mean	SD±	mean	SD±
BSR, A, NP	8	**44.16**	4.50	**5.32**	1.96	50.52	4.50
BSR, A, VP	6	26.58	6.45	7.20	4.84	66.21	4.02
BSR, A, SP	4	39.95	8.65	8.42	6.40	51.63	2.27
BSR, A, LP	4	48.14	7.72	7.69	5.06	44.16	2.73
BSR, SA, NP	1	44.95	.	6.47	.	48.58	.
BSR, SA, VP	1	13.15	.	4.50	.	82.35	.
BSR, SA, SP	0	.	.	.	.	.	.
BSR, SA, LP	0	.	.	.	.	.	.
MON, A, NP	18	**39.06**	3.85	**9.09**	2.65	51.85	4.04
MON, A, VP	14	23.92	7.14	7.89	3.50	68.18	8.82
MON, A, SP	11	39.34	3.78	7.78	3.41	52.89	3.47
MON, A, LP	11	50.12	4.24	8.92	3.94	40.95	5.75
MON, SA, NP	4	47.89	4.39	8.49	.	43.62	.
MON, SA, VP	1	27.38	2.44	6.32	.	66.30	.
MON, SA, SP	1	45.46	2.46	6.58	.	47.96	.
MON, SA, LP	0	.	.	.	.	.	.

Table 7 Percent time and SD for foraging, active-other, and inactive (resting) behaviors for **all** females in the study, grouped by reproductive status of no pup (NP), very small pup (VP), small pup (SP) and large pup (LP)

Reproductive Status	Foraging	Active, Other	Non, Active
NP	41.07 ± 5.04	7.96 ± 2.82	50.34 ± 4.61
VP	24.32 ± 6.87	7.48 ± 3.63	68.20 ± 7.74
SP	39.87 ± 5.01	7.86 ± 3.89	52.26 ± 3.17
LP	48.14 ± 7.27	8.43 ± 3.55	43.43 ± 5.08

Figures

Figure 1. A TDR trace illustrating dive behavior over a 24-hour period for a typical sea otter. The left-hand vertical axis shows depth (m) of the otter at each point in time (black lines), while the horizontal axis shows time (hours), with dark blue axis shading representing nighttime hours and the light blue shading indicating daylight hours. The tidal height is indicated by the blue line (right-hand vertical axis). The activity state of the otter at each point in time is shown as a color-coded strip along the top horizontal axis. Note that foraging bouts (the five blocks of foraging activity, representing contiguous periods of feeding dives) are distributed throughout the 24 hour period, both day and night.

Figure 2: Mean and maximum dive depths for southern sea otters in six classes. Data from both Monterey and Big Sur locations pooled. Error bars display ±SE.

Figure 3: Mean and maximum dive durations for southern sea otters in six classes. Data from both Monterey and Big Sur locations pooled. Error bars display ±SE.

Figure 4: Box plots showing distributions of 4 dive attributes that varied as a function of individual diet specialization (diet groups 1 – 5, as defined in the legend). A) mean dive depth, B) mean dive duration, C) mean dive bottom time, and D) mean ascent rate. Data from both Monterey and Big Sur locations pooled.

Figure 5: Variation in the percent time spent hauled out by female sea otters in this study. A) Scatter plot showing variation in haul-out frequency as a function of mean dive depth; B) bar plot showing mean haul out frequency for females in different reproductive states.

Figure 6 Time-Activity budgets, as estimated from TDR data. A) Percent time spend in foraging (F), active other (AO) and non-active (R) behaviors is shown for sea otters from Big Sur (BSR) and Monterey (MON). B) Percent time feeding for otters from Big Sur (BSR) and Monterey (MON) in 4 different reproductive states: NP = no pup, VP = very small pup, SP = small pup, LP = Large pup.

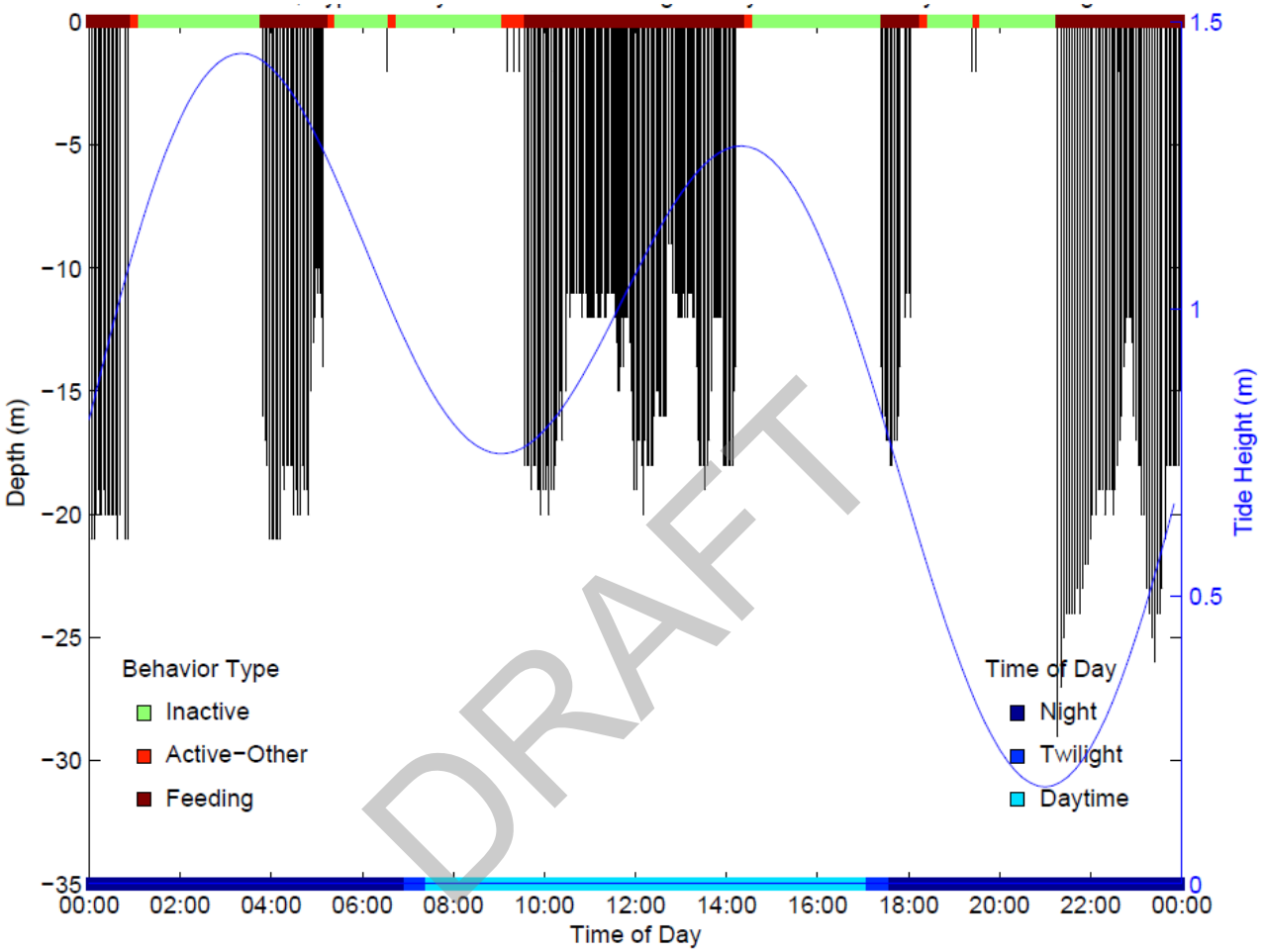


Figure 1

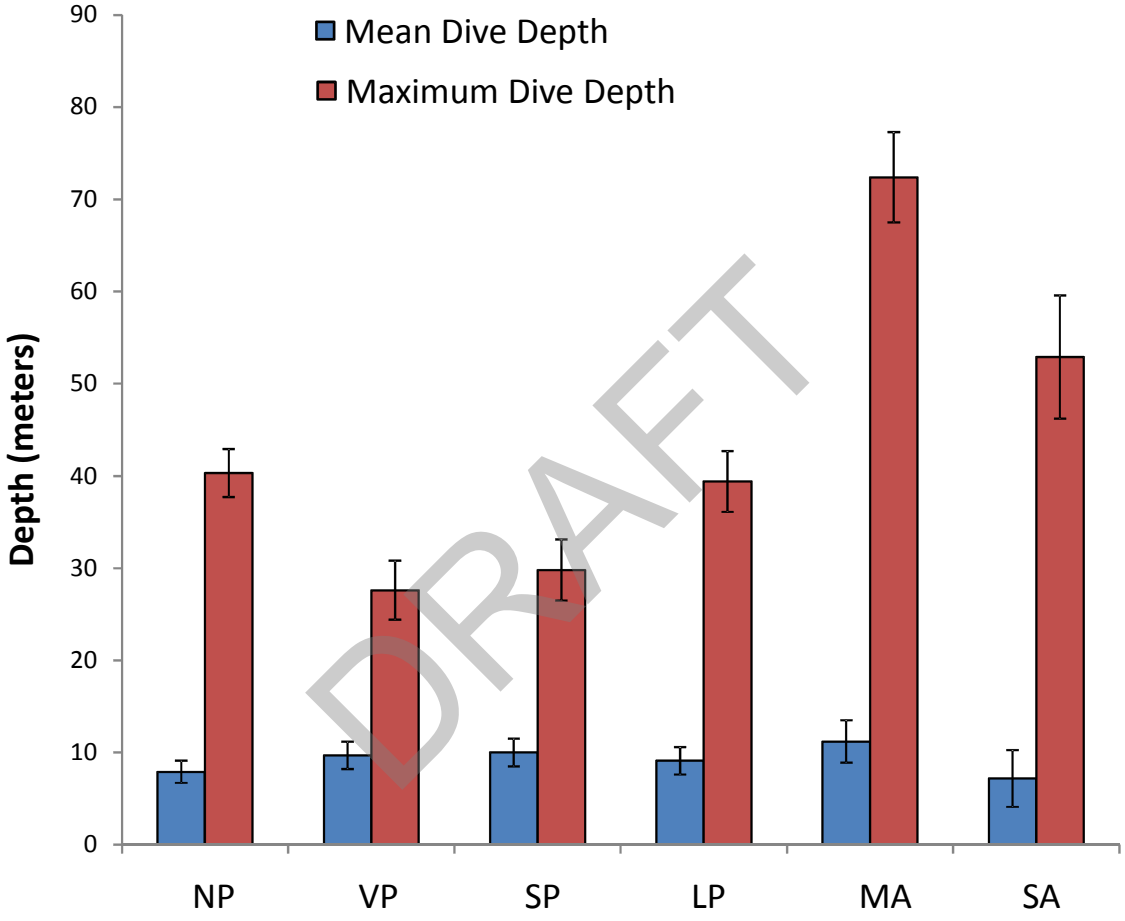


Figure 2

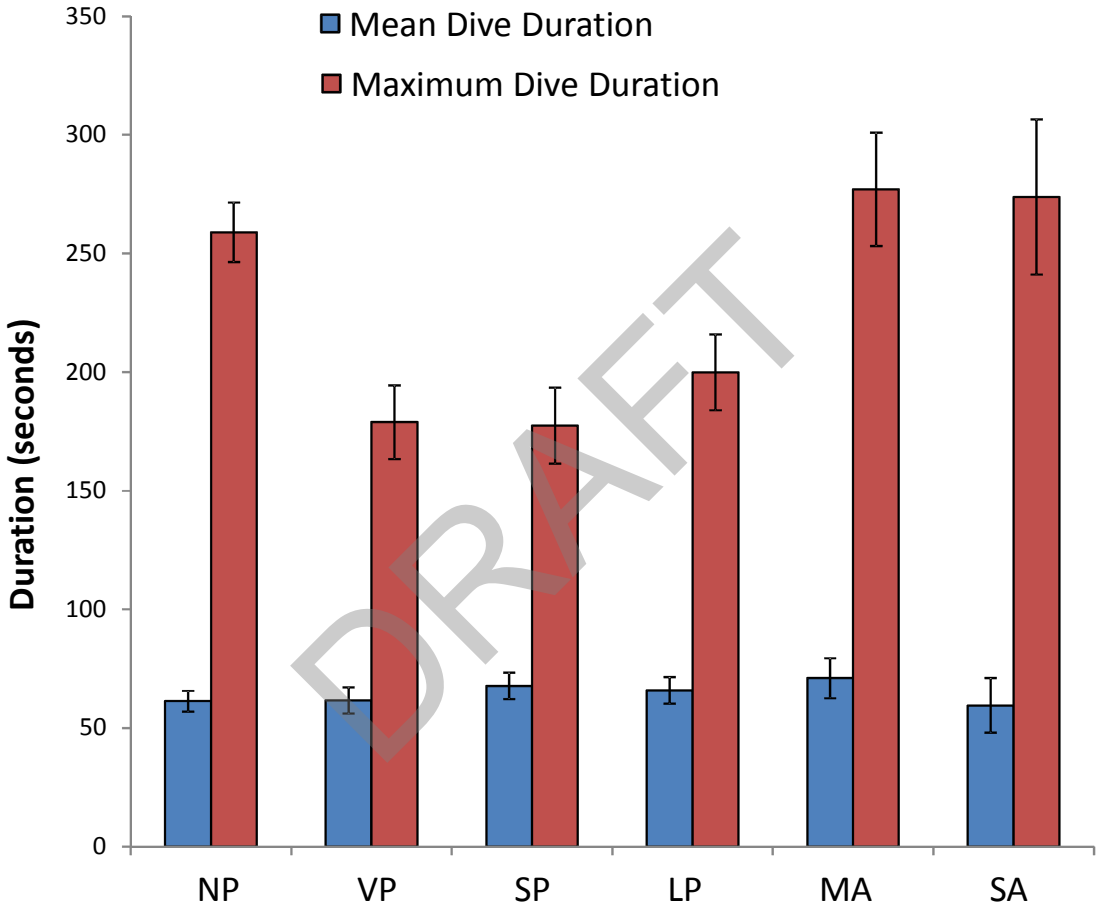


Figure 3

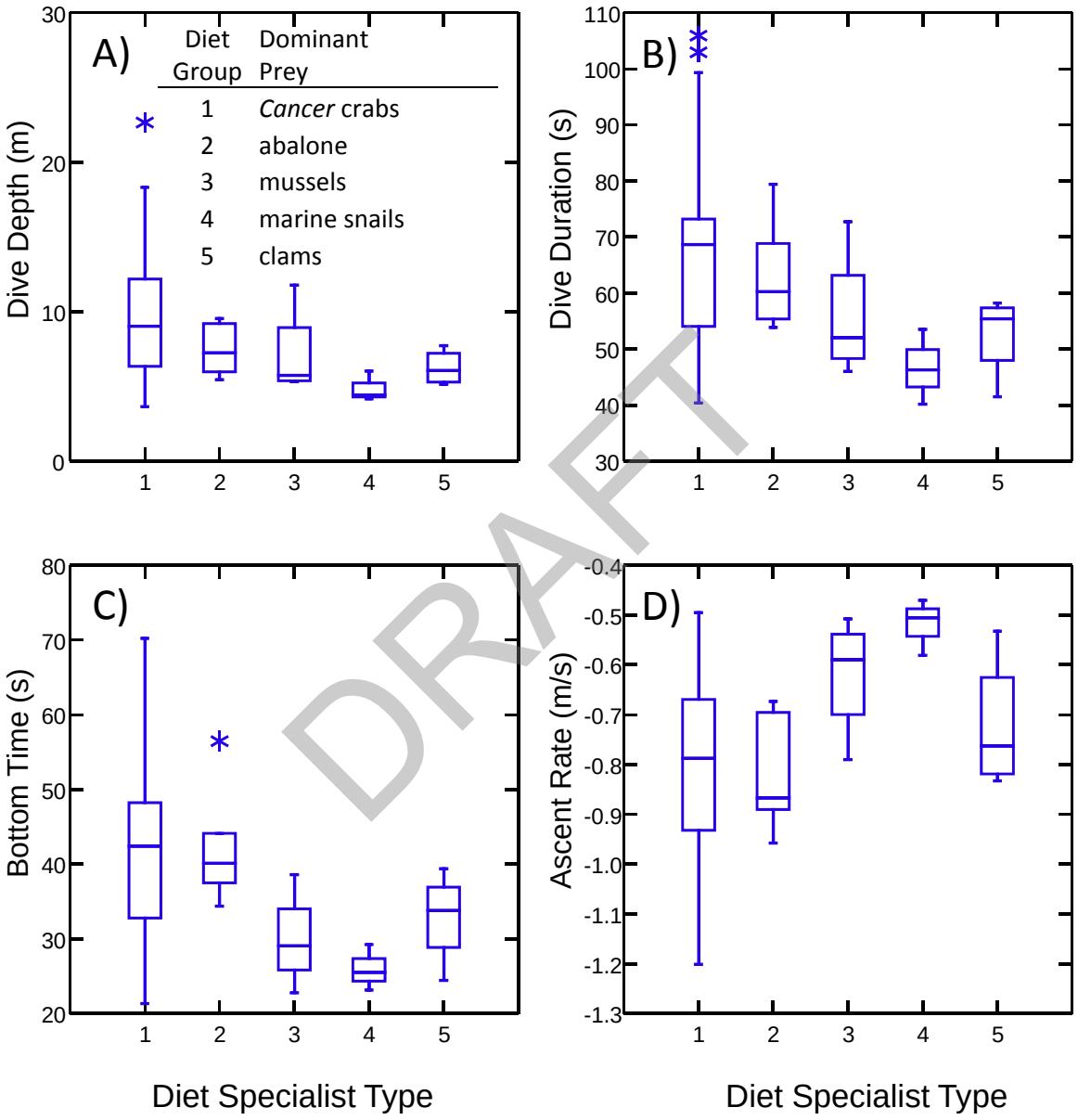
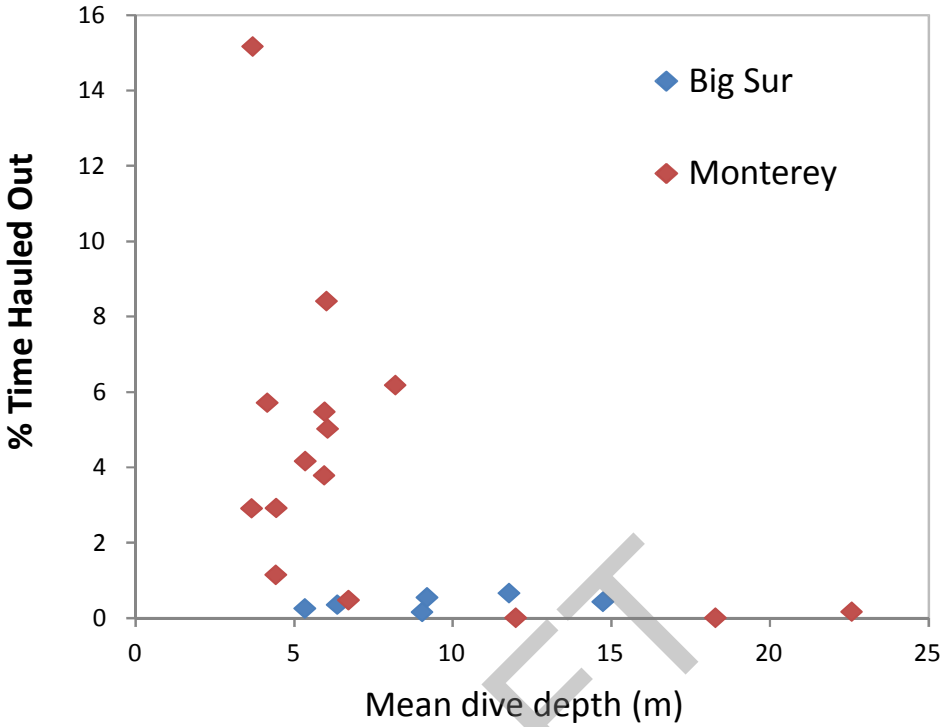


Figure 4

A)



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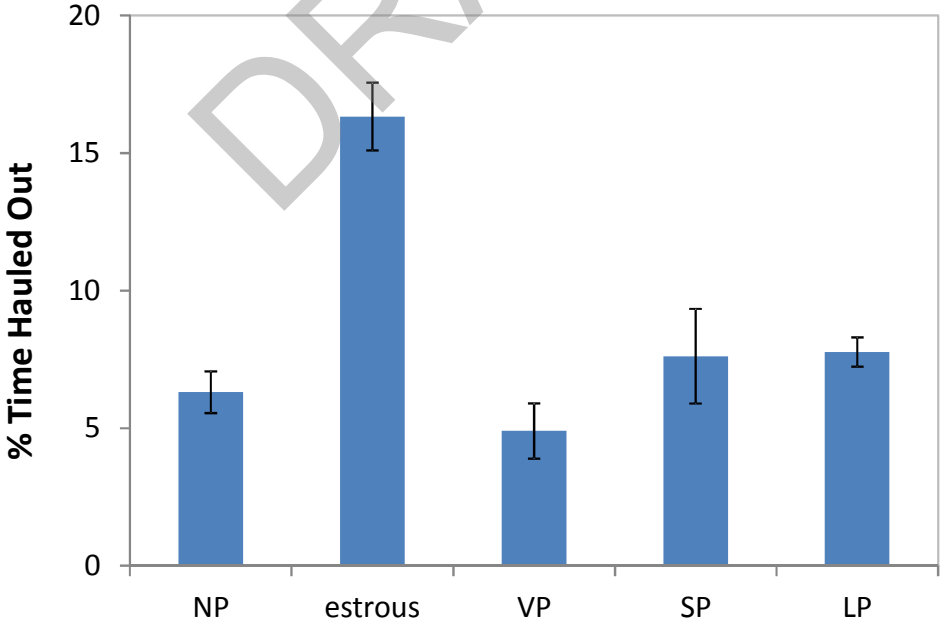


Figure 5

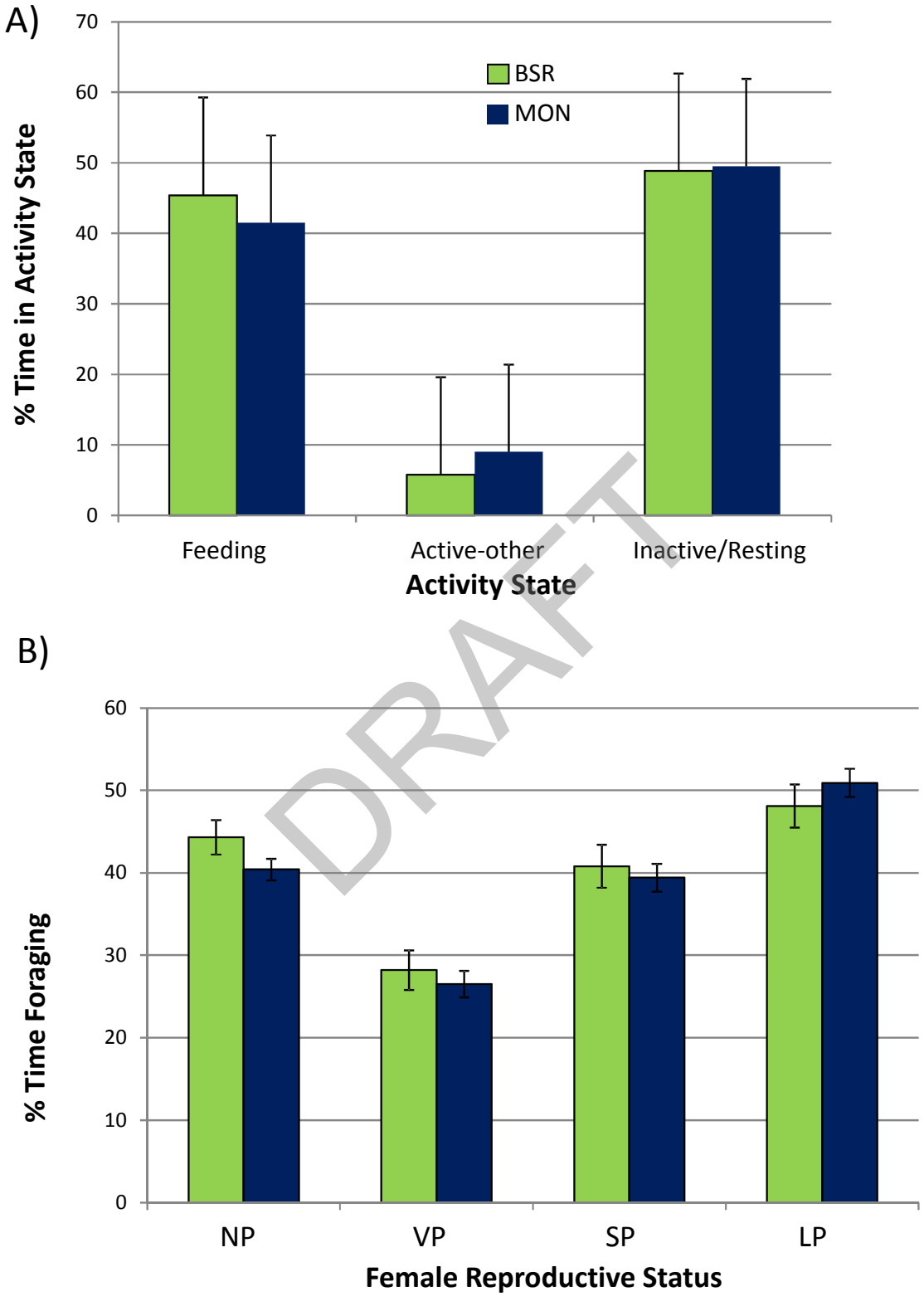


Figure 6

Chapter 6. Foraging ecology and tool use

M. Tim Tinker[1], Jessica Fujii[2], Seth Newsome[3], Gena Bentall[1], Michelle Staedler[2], Joe Tomoleoni[1]
1. U.S Geological Survey, Western Ecological Research Center
2. Sea Otter Research and Conservation Department, Monterey Bay Aquarium
3. Department of Biology, University of New Mexico, Albuquerque, NM

Introduction

The foraging ecology of sea otters has been well-studied in many previous studies (e.g. Calkins 1978, Estes et al. 1981, Kvitek et al. 1993, Doroff and Degange 1994, Ralls et al. 1995, Watt et al. 2000, Estes et al. 2003, Laidre and Jameson 2006, Tinker et al. 2007, Newsome et al. 2009), and information on sea otter diets and foraging behavior has been used both to infer the status of populations with respect to prey resource abundance (e.g. Garshelis et al. 1986, Dean et al. 2002) and to quantify sea otter impacts on prey populations (e.g. Hines and Pearse 1982, Ostfeld 1982, Kvitek and Oliver 1988, Estes and Duggins 1995). Studies of sea otter foraging ecology benefit from the unique properties of sea otter behavior: specifically, sea otters dive to the sea bottom to capture their prey but then return to the surface to handle and consume it while lying on their backs, usually within sight of shore, making it possible for a trained observer with a high powered telescope to directly record all aspects of diet composition and feeding activity from tagged individuals (see Chapter 1, Methods). The resulting data sets on individual diet and foraging behavior are unique among marine mammals and indeed represent one of the most comprehensive data sources on foraging ecology for any wild carnivore, and have allowed scientists to investigate how diet and predator-prey interactions contribute to individual health, fitness, population growth and even disease exposure (Johnson et al. 2009).

In the current study, we recorded and analyzed foraging behavior and diet of tagged sea otters at Big Sur and Monterey in order to achieve four main objectives: 1) determine whether sea otter diet composition was roughly similar at the two sites, or if there were differences in prey-use that could potentially contribute to variation in health or disease exposure risk; 2) measure the rate of energy intake of feeding sea otters at each site, in order to assess how the sites compared with respect to foraging success (and, by inference, with respect to resource abundance); 3) contrast foraging success of sea otters at Big Sur and Monterey with that of sea otters at other sites throughout California and around the north Pacific, including Washington, British Columbia, Alaska and Russia; 4) measure and compare the degree of diet diversity (at the population level) and diet specialization (at the individual level) between Big Sur and Monterey. These latter metrics have been found to provide insights into per-capita food abundance, with population-level diets becoming more diverse and individual-level diets more specialized when prey resource competition is high and per-capita food abundance is low (Tinker et al. 2008, Tinker et al. 2012).

We augmented the observational data with stable isotope analysis of vibrissae sampled from study animals, which provide further insight into diet composition and diversity at the population and individual level (Newsome et al. 2009). Briefly, the analysis of ratios of stable isotopes of carbon (δ13C) and nitrogen (δ15N) stored in inert tissues such as bone or vibrissae can be used to evaluate the diet of

predators, assuming that the stable isotope ratios of potential prey species are also known and are sufficiently distinct (Bearhop et al. 2004). The distribution of stable isotope ratio values on individual predators on the Cartesian axes of δ13C vs. δ15N provides a characterization of dietary niche of the predator population, the so-called "isotopic niche space" (Newsome et al. 2012). Because the vibrissae provide a longitudinal record of individual diets over approximately a 1 year period, it is possible to evaluate temporal variation in diet at the individual level (within-individual variation) and compare this to between-individual variation, thereby providing information about the degree of individual-level specialization (Newsome et al. 2009, Newsome et al. 2012).

Another aspect of sea otter foraging behavior that has often been noted but has not been well studied is tool use. The first well described record of sea otter tool use was made in California by Fisher (1939) with subsequent reports by Hall and Schaller (1964), Kenyon (1969), Houk and Giebel (1974) and Riedman and Estes (1990). Foraging sea otters will acquire a rock, empty shell, or other hard object from the ocean floor and use it to crack open their prey as either a hammer or an anvil. This behavior is an unusually conspicuous and well developed example of tool use for a non-primate mammal, and is thought to facilitate handling of well-armored invertebrate prey (Fisher 1939, Riedman and Estes 1990, Shumaker et al. 2011). We reasoned that an examination of tool use variation across populations, and the ecological drivers that maintain these behaviors, would potentially provide useful insights into the role of prey resource abundance in shaping behavior and limiting individual fitness.

Methods

We measured sea otter diet and foraging behavior following standardized field methods used in dozens of previous observational studies, as described in Chapter 1 of this report and documented in various publications (e.g. Tinker et al. 2008, Tinker et al. 2012). To obtain quantitative measures of the prevalence of various prey species in each individual's diet, field counts of prey capture frequency and prey shell diameter were converted to estimates of consumed biomass and caloric content using species-specific power functions for converting prey diameter to wet edible biomass and kcal per gram (Oftedal et al. 2007). These data were then analyzed using a Monte-Carlo procedure to estimate each individual's diet composition (in terms of the proportion of consumed biomass contributed by each prey taxa) and rate of energy gain, or kcal consumed per minute of feeding (Tinker et al. 2008). The Monte-Carlo procedure explicitly incorporates sampling uncertainty and adjusts for a number of recognized biases associated with direct observations of sea otter foraging (Tinker et al. 2012): a detailed description of the algorithm is provided in Appendix 1.

To account for inconsistencies in the taxonomic resolution of prey capture observations, all prey items were subsequently classified into 24 distinct functional groups of taxonomically and/or morphologically similar species (Table 1), henceforth referred to as "prey types". We limited all further analyses to those tagged sea otters for which a minimum of 10 foraging bouts and 300 feeding dives were recorded, resulting in a sample of 101 individuals (N = 36 individuals from Big Sur and N=65 individuals from Monterey).

To compare diet composition between sites, we graphically compared the relative contribution of each prey type to the population-level diet (the "average" diet of sea otters at each site). We then used

multivariate analysis of variance (MANOVA) to contrast the relative prevalence of key prey types in male and female diets at both sites, with sex and study site treated as fixed effects and individual otters as experimental units. For this analysis we limited analysis to those prey types comprising over 5% of diets overall. In cases where main effects were found to be significant (using Wilks's lambda, λ_U), we conducted post-hoc, pairwise comparisons of individual prey variables across treatment levels.

We analyzed the mean rate of energy gain for foraging otters at Big Sur and Monterey using Analysis of Variance (ANOVA), with sex and study site treated as fixed effects. We also evaluated how the rate of energy gain at Big Sur and Monterey compared to other sea otter populations for which similar analyses have been conducted (USGS, unpublished data). For this analysis we classified each sea otter population into one of three categories (based on their history, current abundance, annual growth rates, and other relevant published data for each site): 1) recently-established and rapidly growing populations, where food resource abundance is presumably not limiting to population growth; 2) long-established, stable or slowly increasing populations where resource abundance is thought to be limiting further growth; and 3) populations that have declined for reasons un-related to food abundance (e.g. disease or predation) and where per-capita food resources appear to be effectively unlimited. We graphically compared the mean rate of energy gain across sites in order to assess which of the above-listed categories Big Sur and Monterey populations were most similar to with respect to rate of energy gain.

We measured the dietary diversity (H_i) of each individual otter, *i*, using the Shannon Weiner index:

$$H_i = -\sum_j^J p_{ij} \log(p_{ij}) \quad (1)$$

where p_{ij} is the proportion of the diet of individual *i* that is made up of prey type *j*. We used ANOVA to compare H_i between study sites. The arithmetic mean value of H_i at each site was used to estimate the "within-individual component" of variation in dietary niche, or WIC. Diet diversity at the population level, or the total niche width (TNW), was calculated for each site as:

$$TNW = -\sum_j^J q_j \log(q_j) \quad (2)$$

where q_j is the proportion of the population-level that is made up of prey type *j*. To assess the degree to which individual otters exhibited specialized diets at each site, we calculated the WIC/TNW ratio at each site, values of which will be significantly less than 1 when there is individual diet specialization (Bolnick et al. 2002). We also calculated the degree to which individual diets overlapped with the population-level average diet, a metric referred to as the index of proportional similarity (*PS*):

$$PS = \frac{1}{N}\sum_i^N \left(1 - 0.5\sum_j^J \left| p_{ij} - q_j \right|\right) \quad (3)$$

where *N* is the total number of individuals in the sample and p_{ij} and q_j are as described above. As with WIC/TNW, values of *PS* significantly less than 1 indicate individual dietary specialization within the

population (Bolnick et al. 2002). To account for the differing sample sizes at Monterey vs. Big Sur, we used boot-strap resampling to estimate WIC/TNW and PSI for each site, with mean values, standard errors and 95% confidence limits calculated from 10000 randomly-drawn (with replacement) samples of 36 individuals for each site.

Assuming that significant levels of individual diet specialization were detected at each site, we used Hierarchical Cluster Analysis to classify otters into groups of animals having similar diet specializations, following previously-published methods (Tinker 2004, Tinker et al. 2007, Tinker et al. 2008). For this analysis we treated individual sea otters from both sites as experimental units, key prey types (those prey types comprising over 5% of diets overall) as variables, Euclidean distances were used as diet similarity measures, and clusters were identified using the Lance-Williams "flex beta" clustering algorithm with beta = -.25 (Lance and Williams 1967, Scheibler and Schneider 1985). We selected the optimal number of clusters based on profile plots of Root mean square standard deviation (RMSSTD) and the pseudo-F value (the optimal number of clusters is expected to produce a local minima of the RMSSTD and a maximal value of the pseudo-F value). We then used discriminant analysis (DA) to evaluate the effectiveness of the clustering classification: specifically, we used Wilks's lambda (λ_U) to test for differences among diet groups with respect to the discriminant functions computed from the underlying prey frequency variables, and we assessed the percentage of individuals that could be correctly assigned to diet groups based on the discriminant classification functions (we present both the raw classification matrix and the jackknife resampled classification matrix). We plotted a bar graph of the mean frequency of each prey type in the diets of individual otters belonging to each of the identified groups, in order to characterize each of these groupings (or "specialist types") based on the dominant prey type(s). We also examined whether there were significant differences between the two study sites in terms of the proportion of otters belonging to each diet group, constructing a contingency table and using Pearson chi square (χ^2) and Goodman-Kruskal's lambda ($\lambda_{G\text{-}K}$) to test for independence between study site and diet group.

Stable Isotope Analysis

Vibrissae from sea otters at Monterey and Big Sur were collected at time of capture (as described in Chapter 1) and sectioned to produce 20 evenly-spaced sub-samples per animal that reflected prey consumption at 20 periods over a 1-year period. In order to characterize the potential isotopic niche space represented by invertebrate prey species, field sampling of invertebrates were conducted during a series of diving and shore-based sampling trips in 2004, 2006, and 2008 at two California study sites: San Simeon/Cambria and Monterey Bay (Oftedal et al. 2007). In total, we analyzed 21 species of invertebrates that observational data show comprise >90% of prey consumed by sea otters in the respective study sites (Estes et al. 2003, Tinker et al. 2008, Tinker et al. 2012). Based on functional similarities, the 21 invertebrate species were condensed into 8 general prey types for the central California mainland coast (Table 4). Isotopic data for invertebrates collected from San Simeon/Cambria and Monterey Bay were combined for lack of significant differences in δ13C and δ15N of prey types from the two sites. Purple sea urchins (*S. purpuratus*) are more abundant in kelp forest communities on the central California mainland coast (Oftedal et al. 2007) and probably contribute more to sea otter diets than red sea urchins (*S. franciscanus*).

Prey specimens were rinsed of sediment and/or detritus, weighed, and measured using digital calipers. Inedible portions of prey (e.g., the spines and tests of sea urchins, carapace of large crabs and lobsters, snail and abalone shells) were removed prior to lyophilization. The dried edible portion was homogenized by grinding to a coarse powder in a Wiley mill. For reasons discussed elsewhere (Newsome et al. 2010), we did not lipid-extract any of the prey samples. Approximately 0.5 mg of the homogenized tissue sample was sealed into tin boats for isotopic analysis. Carbon (δ13C) and nitrogen (δ15N) isotope values were determined using a Carlo Erba elemental analyzer (NC 2500; Carlo Erba, Milan, Italy) interfaced with a Thermo-Finnigan Delta Plus XL mass spectrometer at the Carnegie Institution of Washington (Washington, DC). Isotopic results are expressed as δ values, δ13C or δ15N = 1000* [(R_{sample} - $R_{standard}$ / $R_{standard}$)-1], where R_{sample} and $R_{standard}$ are the 13C/12C or 15N/14N ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N2 for nitrogen. The units are expressed as parts per thousand, or per mil (‰). The within-run standard deviation of an acetanilide standard was ≤0.2‰ for both δ13C and δ15N values. We also measured the [C]/[N] ratios of each sample: mean (±SD) ratios for each prey type are presented in Table 1.

For δ13C and δ15N analysis, vibrissae were rinsed once with a 2:1 chloroform:methanol solution to remove surface contaminants. Cleaned vibrissae were then sub-sampled into ~0.5 mg segments using nail clippers. Dried vibrissae segments (~0.5mg) were sealed into tin capsules and δ13C and δ15N values were determined using the mass spectrometer system described above. As a control for the quality of keratin and bone collagen, we measured the [C]/[N] ratios of each sample. Atomic [C]/[N] ratios of all keratin and bone collagen samples were 3.3-3.5 and 3.2-3.4 respectively, well within the range that characterized unaltered protein (Ambrose 1990). To correct measured sea otter vibrissae isotope values for trophic discrimination ($\Delta_{diet\text{-}keratin}$), we used TDFs of 2.5‰ and 3.0‰ for δ13C and δ15N respectively (Newsome et al. 2010). Consumer TDFs are known to vary depending on the quality of prey consumed, growth rate, physiological condition, and/or excretion pathways (Vanderklift and Ponsard 2003, Caut et al. 2009). Our previous work on sea otters at San Nicolas Island, CA shows that TDFs can vary irrespective of diet (Newsome et al. 2010).

We compared isotopic niche diversity of sea otters at Big Sur and Monterey by graphical comparison of the dispersion of individual δ13C and δ15N values from each population. A convex hull polygon that encompassed all individuals was computed for each study site, and the area covered by each convex hull was calculated as a measure of population-level niche diversity. A second measure of total niche width in isotopic space (TNW_I) was calculated as the summed variance of δ13C and δ15N samples: note that TNW_I is distinct from but analogous to the TNW estimated from observational data on diets (see above). We used variance component analysis (using "Restricted Maximum Likelihood" REML methods) to estimate the proportion of total variation due to between-individual differences (BIC_I) and within-individual variation (WIC_I). The degree of individual niche-space specialization was then calculated as the WIC_I /TNW_I ratio, with lower values indicating more extreme individual specialization. A Bayesian stable isotope mixing model "mixSIR" (Moore and Semmens 2008) was used to estimate proportional contributions of 8 major prey categories (Table 4) to sea otter diets at Monterey and Big Sur. We then used the Bayesian posterior distributions of estimated prey contributions to compute two diet space

metrics: the index ε_I, which represents population-level diet specialization (values of ε_I can vary between 0 = ultra-generalist and 1 = ultra-specialist), and the index S_I, which represents individual-level diet specialization or individual-population diet similarity (values of S_I can vary between 0 = ultra-specialist and 1 = ultra-generalist). Note that S_I is distinct from but analogous to the *PS* index calculated from observational diet data (see above). A detailed description of the analytical methods used to calculate diet space metrics is presented elsewhere (Newsome et al. 2012).

Tool use Analysis

To examine tool use frequency variation between Monterey and Big Sur populations and among individuals, we calculated the mean percent of dives with observed tool use per forage bout at the population and individual level and compared with single factor ANOVA. We also developed a set of generalized linear mixed effects models (GLMMs) with binomial distribution and logit link functions, and used variance components analysis (calculated using Restricted Maximum Likelihood method) to examine the relative contributions of various factors to the likelihood that a tool would be used on a given dive. Factors evaluated for inclusion in the model included study site (Monterey vs. Big Sur), age class (adult or sub-adult), sex (male or female), foraging habitat (kelp forest, rocky intertidal, rocky sub-tidal open water, soft-sediment sub-tidal), type of prey captured, and an individual's diet specialization (see above and Table 3). Forage bouts were nested within individuals and treated as a random effect. For these analyses, prey types identified in Table 1 were further grouped into 9 categories, with all soft bodied prey items lumped together, as we did not expect sea otters to require tools for such prey. Dives where abalone were captured were excluded from these analyses due to the difficultly in identifying the use of tools associated with this prey item (Houk and Geibel 1974). Interactions between prey type and diet specialist type were included to explore the hypothesis that individuals would use tools differently for some prey items depending on their overall diet specialization. Wald's Test was used to identify significant terms with non-significant terms dropped in subsequent models. The best supported model was selected using corrected Akaike information criterion (AICc; Burnham &Anderson 2002). We further explored the interaction between prey type and diet specialization by comparing the probability of tool use by prey type for each diet specialization. Using Tukey's Honestly Significantly Different (HSD) analysis, we made pairwise comparisons for each diet type and prey type. All data manipulation and statistical analyses were completed using R.2.13.2 (R Development Core Team 2010).

Results

The diets of sea otters at both study sites were highly diverse, and generally included a similar suite of benthic invertebrate prey types (Figure 1). However, there were significant differences in diets between study sites (λ_U =0.820, $F_{8,91}$=2.49, P=0.0172) and between males and females (λ_U =0.814, $F_{8,91}$=2.60, P=0.0132). Sea otters at Big Sur consumed a higher proportion of abalone ($F_{1,98}$=7.19, P=0.0086), and a lower proportion of clams ($F_{1,98}$=6.97, P=0.0097) and *Cancer* crabs ($F_{1,98}$=5.84, P=0.0175) than did sea otters at Monterey (Figure 1). Males tended to consume more clams and worms than females ($F_{1,98}$=4.73, P=0.0321 for clams; $F_{1,98}$=8.62, P=0.0041 for worms) and fewer kelp crabs ($F_{1,98}$=6.45 , P=0.0127).

As well as site and sex-based differences in diet composition, there was also significant variation in the rate of energy gain while feeding. Sea otters at Big Sur had a lower rate of energy gain overall than did sea otters at Monterey ($F_{1,97}$=4.20, P=0.0431; Figure 2a); however, there was a significant site-by-sex interaction ($F_{1,97}$=5.74, P=0.0185) such that Big Sur males had a lower rate of energy gain than did Big Sur females, while Monterey males had a similar or higher rate of energy gain than did Monterey females (Figure 2b). Placed into a broader context, the rate of energy gain by sea otters at both sites was near the lower end of the range of values measured across 16 sea otter populations between California and Russia, and were most similar to values measured from long-established, stable or slowly increasing populations where resource abundance is thought to be limiting further growth (Figure 3).

Individual-level diet diversity of sea otters at Big Sur (H_i=1.09±0.096) was significantly lower ($F_{1,97}$=5.09, P=0.0264) than that of otters at Monterey (H_i=1.35±0.064), and female diet diversity tended to be higher than that of males ($F_{1,97}$=11.96, P=0.0008). While diet diversity was relatively low at the individual level, the population-level dietary niche was very broad due to individual diet specialization. Individual specialization occurs when individual diets comprise a small proportion of the population-level dietary niche and are thus dissimilar from the population average diet, as indicated by low values of WIC/TNW and *PS* indices. These indices were low at both sites, with values at Big Sur (WIC/TNW=0.56±0.035, *PS*=0.44±0.033, CI_{95}=0.38-0.51) somewhat lower though not statistically different from values at Monterey (WIC/TNW=0.62±0.026, *PS* =0.48±0.024, CI_{95}=0.43-0.53). Cluster analysis indicated that individual otters could be grouped into one of 5 different diet specialization types (Figure 4). An examination of these groups using discriminant analysis (DA) showed clear dietary differences (λ_U=0.0014, $F_{19,4,96}$=17.63, P<0.0001), and otters were correctly assigned to diet groups 97% of the time using a canonical discriminant function, with a jackknife classification accuracy of 90% (Table 2). The relative contribution of various prey types to the diets of otters in each specialist group is shown in Figure 5, and further details about each group are provided in Table 3. Otters that were classified into group 1 had the most diverse diets, with *Cancer* crabs being the most common prey, while otters from the remaining 4 groups tended to have more specialized diets that were dominated by just one or two prey types (Figure 5, Table 3). Contingency table analysis indicated significant interaction between study site and diet group (χ^2=15.05, *df*=4, P=0.005, $\lambda_{G\text{-}K}$=0.0972, P=0.0305): otters with type-2 diets (abalone specialists) were more common at Big Sur than Monterey, while otters with type-5 diets (clam specialists) more common at Monterey.

Stable Isotope Analysis

Invertebrate species common in the diet of southern sea otters spanned multiple habitats, trophic levels and ecologically defined functional groups, resulting in a broad range of values in both δ13C and δ15N (Table 4). Mean (±SD) δ13C and δ15N values for prey types ranged from -17.5‰ (±0.9) to -13.3‰ (±1.1) and 9.2‰ (±0.5) to 14.6‰ (±0.4) respectively. Variation in mean isotope values of individual prey types was relatively small in comparison to variation among prey types (Figure 6), and standard deviations for δ13C and δ15N were ≤1‰ for most prey types (Table 4). The combination of high isotopic variation among prey types but low variation within prey types created a large isotopic prey space that individual sea otters could potentially occupy.

The distribution of individual δ13C and δ15N values for sea otters at Big Sur and Monterey together occupied a large proportion of bivariate isotopic niche space (Figure 6), suggesting diverse diets at both study sites. While population-level niche diversity was almost identical at each site based on convex hull analysis (Figure 7A) and TNW_I (Figure 7B), there was very little overlap in the mean isotope values of individual sea otters from Big Sur and Monterey (Figure 6), suggesting significant dietary differences between sites. Most individuals from Big Sur had lower mean vibrissae $\delta^{15}N$ values in comparison to their counterparts from Monterey, and MixSIR analyses indicated that sea otters from Big Sur likely consumed a higher proportion of abalone and mussels, while sea otters from Monterey likely consumed a higher proportion of clams and *Cancer* crabs (Table 4). Sea otters at both study sites showed a high degree of individual niche specialization, although WIC_I /TNW_I ratios were slightly lower at Big Sur than they were at Monterey (Figure 7C) indicating slightly more extreme specialization at Big Sur. The distribution of diet space indices (incorporating individual differences and parameter uncertainty from the MixSIR posterior distributions) showed distinct patterns of variation at both sites (Figure 8). Population-level niche specialization (ε_I) was slightly greater at Big Sur than at Monterey (0.63 ± 0.18 vs. 0.45 ± 0.13, respectively). There was a bimodal distribution of individual-population diet similarity at both sites (S_I), although this pattern was more pronounced at Big Sur (Figure 8). This pattern indicates that some individuals at both sites had extremely specialized diets.

Tool use Analysis

The mean (± SE) frequency of tool use did not vary significantly between Big Sur (11.94 ±4.33% of dives) and Monterey (14.11± 2.61%; $\chi 2=116$, $p=0.663$). Otters from both sites exhibited a frequency of tool use ranging between 0% and >90% of their foraging dives (Figure 6). Much of the variation in tool use frequency was explained by an individual's diet specialization, with snail specialists being the only individuals who used tools in more 50% of their dives (Figure 7). Variance component analysis indicated that the prey type captured on a given feeding dive explained 33.43% of the variance in tool use probability, while the individuals' diet specialization explained a further 30.71% (after accounting for prey type), with the remaining variation explained by sex (3.73%), feeding habitat (0.16%), and unexplained between- and within-individual variation (31.97%). Study site and individual age each represented less than 0.0001% of the variance. The best-fit GLMM model (AICc =5091.7) included prey type and diet specialization as fixed effects, as well as their interaction, and forage bouts nested within individuals as a random effect (Table 5). Using Wald's Test, all included factors contributed significantly to the model fit (Table 6).

Post-hoc pairwise comparisons indicated that snails and other bivalves (including scallops and cockles) were the prey groups most likely to be consumed with the use of a tool, regardless of an individual's diet specialization (Figure 8). Snail specialists had the highest probability of tool use when consuming their core prey (Probability= 99.8%, $p<0.001$), but were also more likely to use tools across most other prey types than were otters from all other diet specialization groups. The probability of tool use while consuming mussels, Cancer crabs, kelp crabs, and other crabs was not significantly different from the probability of tool use while consuming soft bodied prey, irrespective of diet specialization (Figure 8).

Discussion

The diets of sea otters at Big Sur and Monterey (Figure 1, Table 1) included a broad range of benthic sub-tidal and intertidal invertebrates, consistent with previously published data on sea otter diets in central California (Estes et al. 1981, Hines and Pearse 1982, Ostfeld 1982, Ralls et al. 1995, Jolly 1997, Estes et al. 2003, Tinker et al. 2008, Tinker et al. 2012). There were two minor differences in diet composition between sites, one easily explained and the other more interesting. The greater prevalence of clams in the diets of Monterey sea otters is not surprising given the greater abundance of soft sediment benthic habitat around the Monterey peninsula and southern Monterey Bay, which supports abundant infaunal clam populations (Hallenbeck et al. 2012). The higher prevalence of abalone in the diets of Big Sur sea otters was more surprising, as sea otters have been present at high densities at Big Sur far longer than they have at Monterey , and given the well-described depleting effects of sea otter predation on abalone (Lowry and Pearse 1973, Hines and Pearse 1982), it might be expected that abalone depletion would be greater at Big Sur than Monterey. The fact that sea otters continue to rely heavily on abalone at Big Sur suggest that abalone populations in central California are able to sustain this level of predation, possibly because the positive indirect effects of sea otter predation (i.e. reduction of urchin competitors and increased kelp abundance) may equal or exceed the negative direct consumptive effects.

In addition to dietary differences between sites, there were also dietary differences between males and females, with males consuming more clams and worms and fewer kelp crabs. This pattern likely reflects a greater reliance on soft-sediment habitat by feeding by males. In central California, soft sediment habitats are thought to support less diverse, less energetically profitable prey communities than rocky, kelp-dominated habitats (Kvitek and Oliver 1988). Sub-adult and non-territorial males may be driven to using these sub-optimal foraging habitats due to intra-specific competition in kelp-dominated habitats (or due to active exclusion from those areas by territorial males), a pattern that is consistent with the lower rate of energy gain by males at the BSR study site. However this scenario is apparently inconsistent with the fact that males at Monterey actually had higher rates of energy gain than their female counterparts (Figure 2). This inconsistency may be explained in part by differences in the coastal bathymetry of the two study sites: in Monterey, males have ready access to the broad and shallow continental shelf of Monterey Bay, and the upwelling-driven productivity it supports; in contrast, Big Sur males have to travel great distances to access these same resources. Thus Big Sur males may be more geographically “blocked in” than males at Monterey. Another reason for this inconsistent pattern is that the Monterey sample contained (by chance) a higher ratio of territorial males to non-territorial males, and territorial males may have access to better foraging habitat.

Overall, the rate of energy gain for foraging sea otters was slightly lower at Big Sur than at Monterey (Figure 2), suggesting that food resource abundance may be more limiting for sea otters at Big Sur. However, when compared to a wide array of sea otter populations from around the north Pacific, foraging success for sea otters at both Big Sur and Monterey appeared to fall within the range of populations thought to be resource-limited (Figure 3), with otters at Big Sur just slightly more impacted. This pattern was entirely consistent with the patterns of individual diet specialization, a phenomenon which has been found to be related to the degree of resource competition (Tinker et al. 2008, Tinker et

al. 2012). Both Big Sur and Monterey sea otters exhibited substantial degrees of individual diet specialization, however Big Sur otters had slightly more specialized diets. Despite this difference, it was notable that the same types of diet specialization occurred at both sites, in largely similar frequencies (although there were more abalone specialists at Big Sur and fewer clam specialists; Figure 5). Together, these patterns suggest that a) Big Sur sea otters may be slightly more impacted by resource competition than Monterey sea otters, although both populations are at the lower end of the scale of resource abundance when compared to other populations; and b) diet specialization in sea otters, a behavioral response to resource limitation, tends to occur in a consistent fashion from site to site, perhaps driven by highly conserved patterns of prey profitability and the constraints of learning on prey handling skills (Tinker et al. 2009), and maintained by frequency dependence (Estes et al. 2003).

Stable Isotope Analysis

The patterns of niche variation inferred from stable isotope analysis were entirely consistent with, and supportive of, the patterns inferred from the observational data. At the population level, both study samples exhibited similarly diverse diets, occupying a large proportion of available niche space; however, these niche space distributions differed between the sites. The dietary differences inferred from isotope analysis closely matched the differences inferred from observational data, with abalone and mussels more prevalent at Big Sur and clams and *Cancer* crabs more prevalent at Monterey (compare Table 4 with Figure 1). This consistency between the two methods increases our confidence in both methods (since the data sets are entirely independent and thus unlikely to produce similar biases) and confirms previously published conclusions that Stable Isotopes provide a useful method of measuring diets in sea otters (Newsome et al. 2009, Newsome et al. 2010). The stable isotope analysis also resulted in estimates of individual diet specialization that were consistent with the observational data, indicating that sea otters at both sites exhibited substantial specialization, but this pattern was slightly more pronounced at Big Sur than at Monterey (Figure 7). The density plots of population-level dietary niche specialization (ε_I) vs. dietary similarity (S_I) illustrated that the degree of individual specialization within the population may be multi-modal, with some otters exhibiting extreme specialization and dissimilarity from the population average. These patterns have direct implications for sea otter health, as certain prey types are more likely to expose sea otters to disease-causing pathogens and environmental toxins (Johnson et al. 2009, Miller et al. 2010), and thus diet specialization can lead to greater variation in disease outcomes.

Tool use Analysis

We explored ecological and behavioral factors that may influence tool use variation among individuals. Tool use in sea otters has been repeatedly documented across populations but very little is known about individual variation. We found tool use frequency was largely dependent on the prevalence of difficult to access prey in an individual's diet. Neither Big Sur nor Monterey populations used tools at a high frequency, suggesting that this behavior is not universally required for successful foraging.

The most significant predictor of tool use was the type of prey being consumed. Average tool use frequency most likely did not vary between Monterey and Big Sur because their diets were relatively similar (Figure 1). The prey class most likely to be associated with tool use was snails (Figure 8). Although marine snails are relatively easy to capture, they have heavily calcified exoskeletons without

readily accessible meat. The most common snail species consumed were turban snails (*Chlorostoma spp*, formerly *Tegula spp*). These small (2-5cm) snails have thick, compact shells and small openings. Turban snails have a low per-capita energy return, and yet individual sea otters that specialized on snails are just as likely to reach their daily caloric demands as those in other specialist groups (Tinker 2004, Oftedal et al. 2007). Tool use is likely a key factor in making snails energetically profitable to sea otters.

The existence of individual diet specialization at Big Sur and Monterey provided an opportunity to explore the importance of prey type (environmental factors), feeding behaviors (learned behavioral factors), and their interaction in relation to tool use. Variation in tool use frequency among individuals in a population is most likely attributed to the individuals' diet and the necessity of learning how to use tools to consume their core prey items. Although the rate of tool use varied by prey type within each specialist group (Figure 7), snail specialists were more likely than other specialists to use tools on almost all other prey types (Figure 8).

Many individuals who specialized on prey items that were not associated with tool use (i.e. mussels and urchins) still used tools on occasion. The ability for individuals to "casually" use tools differs from sponging in bottlenose dolphins (Mann et al.) and larvae fishing by New Caledonian crows (Rutz et al.). Unlike with these other tool using species, tool use by sea otters may have a lower cost of learning. It is still unclear if the casual tool users are responding to a particularly difficult prey item, or if some other factor drivers their occasional tool use. If there is a very low cost associated with learning how to use tools, it is likely that the behavior is not more common because the benefit is also relatively low.

Age was excluded as a contributing factor to tool use occurrence in our models. We would expect to see individuals use tools more frequently as they became more proficient with age, as seen in chimpanzees and New Caledonian crows (Lonsdorf 2006, Holzhaider et al. 2010). In this case, a lack of any significant relationship is likely an artifact of our study samples. We would predict snail specialists to show the greatest age effect since tool use plays a large role in their foraging strategy. However, only 2 of our snail specialists were sub-adults at the time of capture. It is more likely that foraging observations from juveniles (just post-weaning) to sub adults would be necessary to see an age effect as this is the time period individuals must quickly learn foraging strategies to survive.

As the first quantitative study of sea otter tool use, we found that individuals vary in their frequency of tool use in response to their encounter rates with difficult to access prey. Like many tool using species, ecological factors (such as the physical characteristics of prey) play a critical role in explaining complex behaviors (Collins and McGrew 1987, Patterson and Mann 2011). Future studies would benefit from quantifying the cost and benefits of learning to use tools, as well as further exploration to how individuals learn to use tools.

References

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology **73**:1007-1012.

Bolnick, D. I., L. H. Yang, J. A. Fordyce, J. M. Davis, and R. Svanbäck. 2002. Measuring individual-level resource specialization. Ecology **83**:2936-2941.

Calkins, D. G. 1978. Feeding behavior and major prey species of the sea otter, Enhydra lutris, in Montague Strait, Prince William Sound, Alaska. Fish. Bull. **76**:125-131.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology **46**:443-453.

Collins, D. A. and W. C. McGrew. 1987. Termite fauna related to differences in tool-use between groups of chimpanzees (*Pan troglodytes*). Primates **28**:457-471.

Dean, T. A., J. L. Bodkin, A. K. Fukuyama, S. C. Jewett, D. H. Monson, C. E. O'Clair, and G. R. VanBlaricom. 2002. Food limitation and the recovery of sea otters following the 'Exxon Valdez' oil spill. Marine Ecology-Progress Series **241**:255-270.

Doroff, A. M. and A. R. Degange. 1994. Sea otter, Enhydra lutris, prey composition and foraging success in the northern Kodiak Archipelago. U S National Marine Fisheries Service Fishery Bulletin **92**:704-710.

Estes, J. A. and D. O. Duggins. 1995. Sea otters and kelp forests in Alaska: Generality and variation in a community ecological paradigm. Ecological Monographs **65**:75-100.

Estes, J. A., R. J. Jameson, and A. M. Johnson. 1981. Food selection and some foraging tactics of sea otters. Pages 606-641 *in* J. A. Chapman and D. Pursley, editors. Worldwide Furbearer Conference Proceedings, Volume 1. University of Maryland Press, Baltimore, Maryland.

Estes, J. A., M. L. Riedman, M. M. Staedler, M. T. Tinker, and B. E. Lyon. 2003. Individual variation in prey selection by sea otters: Patterns, causes and implications. Journal of Animal Ecology **72**:144-155.

Garshelis, D. L., J. A. Garshelis, and A. T. Kimker. 1986. Sea otter time budgets and prey relationships in Alaska. Journal of Wildlife Management **50**:637-647.

Hallenbeck, T. R., R. G. Kvitek, and J. Lindholm. 2012. Rippled scour depressions add ecologically significant heterogeneity to soft-bottom habitats on the continental shelf. Marine Ecology Progress Series **468**:119-133.

Hines, A. H. and J. S. Pearse. 1982. Abalones, shells, and sea otters: dynamics of prey populations in central California. Ecology **63**:1547-1560.

Holzhaider, J. C., G. R. Hunt, and R. D. Gray. 2010. The development of pandanus tool manufacture in wild New Caledonian crows. Behaviour **147**:553-586.

Houk, J. L. and J. J. Geibel. 1974. Observation of underwater tool use in sea otter, *Enhydra lutris linnaeus*. California Fish and Game **60**:207-208.

Johnson, C. K., M. T. Tinker, J. A. Estes, P. A. Conrad, M. Staedler, M. A. Miller, D. A. Jessup, and J. A. K. Mazet. 2009. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. Proceedings of the National Academy of Sciences of the United States of America **106**:2242-2247.

Jolly, J. M. 1997. Foraging ecology of the sea otter, *Enhydra lutris*, in a soft-sediment community. Masters dissertation. University of California, Santa Cruz, Santa Cruz, CA.

Kvitek, R. G., C. E. Bowlby, and M. Staedler. 1993. Diet and foraging behavior of sea otters in southeast Alaska. Marine Mammal Science **9**:168-181.

Kvitek, R. G. and J. S. Oliver. 1988. Sea Otter Foraging Habits and Effects on Prey Populations and Communities in Soft-Bottom Environments.*in* G. R. Vanblaricom and J. A. Estes, editors. The Community Ecology of Sea Otters. Springer Verlag Inc., New York.

Laidre, K. L. and R. J. Jameson. 2006. Foraging patterns and prey selection in an increasing and expanding sea otter population. Journal of Mammalogy **87**:799-807.

Lance, G. N. and W. T. Williams. 1967. A general theory of classification sorting strategies. I. Hierarchical system. Computer Journal **9**:373-380.

Lonsdorf, E. V. 2006. What is the role of mothers in the acquisition of termite-fishing behaviors in wild chimpanzees (Pan troglodytes schweinfurthii)? Animal Cognition **9**:36-46.

Lowry, L. F. and J. S. Pearse. 1973. Abalones and sea urchins in an area inhabited by sea otters. Marine Biology **23**:213-219.

Mann, J., B. L. Sargeant, J. J. Watson-Capps, Q. A. Gibson, M. R. Heithaus, R. C. Connor, and E. Patterson. 2008. Why Do Dolphins Carry Sponges? Plos One **3**:7.

Miller, M. A., R. M. Kudela, A. Mekebri, D. Crane, S. C. Oates, M. T. Tinker, M. Staedler, W. A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D. A. Jessup. 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS One **5**:Article No.: e12576.

Moore, J. W. and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters **11**:470-480.

Newsome, S. D., G. B. Bentall, M. T. Tinker, O. T. Oftedal, K. Ralls, J. A. Estes, and M. L. Fogel. 2010. Variation in δ13C and δ15N diet–vibrissae trophic discrimination factors in a wild population of California sea otters. Ecological Applications **20**:1744-1752.

Newsome, S. D., M. T. Tinker, D. H. Monson, O. T. Oftedal, K. Ralls, M. M. Staedler, M. L. Fogel, and J. A. Estes. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (Enhydra lutris nereis). Ecology **90**:961-974.

Newsome , S. D., J. D. Yeakel, P. V. Wheatley, and M. T. Tinker. 2012. Tools for quantifying isotopic niche space and dietary variation at the individual and population level. Journal of Mammalogy **93**:329–341.Oftedal, O. T., K. Ralls, M. T. Tinker, and A. Green. 2007. 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. . Monterey Bay National Marine Sanctuary, Monterey, CA.

Ostfeld, R. S. 1982. Foraging strategies and prey switching in the California sea otter. Oecologia **53**:170-178.

Patterson, E. M. and J. Mann. 2011. The Ecological Conditions That Favor Tool Use and Innovation in Wild Bottlenose Dolphins (Tursiops sp.). Plos One **6**.

Ralls, K., B. B. Hatfield, and D. B. Siniff. 1995. Foraging patterns of California sea otters as indicated by telemetry. Canadian Journal of Zoology **73**:523-531.

Rutz, C., L. A. Bluff, N. Reed, J. Troscianko, J. Newton, R. Inger, A. Kacelnik, and S. Bearhop. 2010. The Ecological Significance of Tool Use in New Caledonian Crows. Science **329**:1523-1526.

Scheibler, D. and W. Schneider. 1985. Monte Carlo Tests of the Accuracy of Cluster Analysis Algorithms: A Comparison of Hierarchical and Nonhierarchical Methods. Multivariate Behavioral Research **20**:283-304.

Tinker, M. T. 2004. Sources of variation in the foraging behavior and demography of the sea otter, *Enhydra lutris*. PhD Dissertation. University of California, Santa Cruz, CA.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., D. P. Costa, A. Estes James, and N. Wieringa. 2007. Individual dietary specialization and dive behaviour in the California sea otter: using archival time-depth data to detect alternative foraging strategies. Deep Sea Research II **54**:330-342.

Tinker, M. T., P. R. Guimarães, M. Novak, F. M. D. Marquitti, J. L. Bodkin, M. Staedler, G. Bentall, and J. A. Estes. 2012. Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecology Letters **15**:475-483.

Tinker, M. T., M. Mangel, and J. A. Estes. 2009. Learning to be different: acquired skills, social learning, frequency dependence, and environmental variation can cause behaviourally mediated foraging specializations. Evolutionary Ecology Research **11**:841-869.

Vanderklift, M. and S. Ponsard. 2003. Sources of variation in consumer-diet δ15N enrichment: a meta-analysis. Oecologia **136**:169-182.

Watt, J., D. B. Siniff, and J. A. Estes. 2000. Inter-decadal patterns of population and dietary change in sea otters at Amchitka Island, Alaska. Oecologia (Berlin) **124**:289-298.

Tables

Table 1. List of over 75 prey species (or higher taxa) consumed by sea otters over the course of the study. Because it was often difficult to distinguish taxonomically and/or morphologically similar species from a distance, all prey were grouped into 24 functional groups (referred to as "prey types" in the text).

Functional Group	Prey Common Name	Latin Name
urchin	red urchin	*Strongylocentrotus (Mesocentrotus) franciscanus*
	purple urchin	*Strongylocentrotus purpuratus*
Cancer crab	Pacific rock crab	*Cancer antennarius*
	dungeness crab	*Cancer (Metacarcinus) magister*
	red rock crab	*Cancer productus*
	Cancer crab, un-ID	*Cancer sp.*
kelp crab	northern kelp crab	*Pugettia producta*
	graceful kelp crab	*Pugettia gracilis*
sand crab	spiny mole crab	*Blepharipoda occidentalis*
	Pacific sand crab	*Emerita analoga*
other crab	decorator/masking crab	*Loxorhynchus crispatus*
	un-identified crab	
mussel	horse mussel	*Modiolus modiolus*
	California mussel	*Mytilus californianus*
	bay mussel	*Mytilus trossulus*
	mussel, un-ID	
clam	Nuttall's cockle	*Clinocardium nuttallii*
	giant rock scallop	*Crassadoma gigantea*
	sunset clam	*Gari californica*
	Macoma clam	*Macoma spp.*
	surf clam	*Mactromeris spp.*
	softshell clam	*Mya arenaria*
	geoduck clam	*Panopea generosa*
	scallop, un-ID	*Pectinidae spp. or Serripes spp.*
	rock jingle	*Pododesmus macroschisma*

Functional Group	Prey Common Name	Latin Name
	littleneck clam	*Prototheca (Leukoma) staminea*
(clam, con't)	Washington clam	*Saxidomus nuttalli*
	razor clam	*Siliqua patula*
	jackknife clam	*Tagelus californianus*
	tellin clam	*Tellina spp.*
	Pismo clam	*Tivela stultorum*
	gaper clam	*Tresus nuttallii*
	rough piddock	*Zirfaea pilsbryi*
	clam, un-ID	
marine snail	top snail	*Calliostoma spp.*
	red turban snail	*Lithopoma (Pomaulax) gibberosus*
	Nassa snail	*Nassarius fossatus*
	moon snail	*Polinices sp.*
	brown turban snail	*Tegula (Chlorostoma) brunnea*
	Monterey turban snail	*Tegula (Chlorostoma) montereyi*
	turban snail, un-ID	*Turbinidae*
	snail, un-ID	
abalone	black abalone	*Haliotis cracherodii*
	red abalone	*Haliotis rufescens*
	abalone, un-ID	
sea star	blood star	*Henricia sp.*
	brittle star	*Ophiuroidea*
	bat star	*Patiria miniata*
	ochre star	*Pisaster ochraceus*
	sunflower star	*Pycnopodia helianthoides*
	sea star, un-ID	
worm	pile worm	*Nereis sp.*
	polychaete, un-ID	*Polychaeta*
	peanut worm	*Sipunculus nudus*
	fat innkeeper worm	*Urechis caupo*

Functional Group	Prey Common Name	Latin Name
	worm, un-ID	
chiton	gumboot chiton	*Cryptochiton stelleri*
	lined chiton	*Tonicella sp.*
	Katy chiton	*Katharina tunicata*
	chiton, un-ID	*Polyplacophora sp.*
	mossy chiton	*Mopalia sp.*
	Stenoplax chiton	*Stenoplax fallax*
limpet	owl limpet	*Lottia gigantea*
tunicate	stalked tunicate	*Styela sp.*
cucumber	red sea cucumber	*Cucumaria sp.*
sponge	orange puffball sponge	*Tethya californiana*
small crustacean	acorn barnacle	*Balanus sp.*
	isopod	*Idotea sp.*
	gooseneck barnacle	*Pollicipes polymerus*
sand dollar	sand dollar	*Dendraster excentricus*
octopus	octopus	*Octopus sp.*
squid	market squid	*Loligo (Doryteuthis) opalescens*
lobster	spiny lobster	*Panulirus interruptus*
other kelp invert	coralline algae	*Corallina sp., Clathromorphum sp.*
	nudibranch	*Opisthobranchia*
fish	kelp greenling	*Hexagrammos decagrammus*
fish egg mass	greenling egg mass	*Hexagrammos sp.*

Table 2. Results of discriminant analysis of individual sea otter diets, used to evaluate the efficacy of diet-type groupings identified using hierarchical cluster analysis. The top matrix shows the percent of sea otters that were classified correctly into one of the 5 diet groups using the discriminant classification function computed from the relative prevalence of each of 24 prey types in each otter's diet. The bottom matrix shows the percent of sea otters that were classified correctly using a cross validation or 'jack-knife' classification, whereby all cases but one were used to develop a new discriminant classification function that was applied to the remaining case, and this process was repeated with each case left out in turn.

Classification Matrix (Cases in row categories classified into columns)

Group	1	2	3	4	5	%correct
1	11	1	0	0	0	92
2	0	48	1	0	1	96
3	0	0	18	0	0	100
4	0	0	0	8	0	100
5	0	0	0	0	13	100
Total	11	49	19	8	14	97

Jackknifed Classification Matrix

Group	1	2	3	4	5	%correct
1	11	1	0	0	0	92
2	1	44	1	2	2	88
3	0	3	15	0	0	83
4	0	0	0	8	0	100
5	0	0	0	0	13	100
Total	12	48	16	10	15	90

Table 3. Summary of the 5 diet groups identified using hierarchical cluster analysis. All sea otters at Big Sur and Monterey study sites were classified into one of the 5 groups based on their diet composition (see text for details). For each group, the table summarizes the predominant prey type(s) consumed by otters belonging to that group, their dietary diversity, and the number (*N*) of tagged otters from each group at each study site and overall.

Diet Group	Dominant Prey	Secondary Prey	Diversity	N, BSR	N-MBA	N, total
1	*Cancer* crabs	urchins, misc.	**1.49**	16	34	50
2	abalone		1.05	10	3	13
3	mussels	urchins	1.32	7	11	18
4	marine snails	kelp crabs	1.20	3	9	12
5	clams	*Cancer* crabs	1.29	0	8	8

Table 4. Stable Isotope characterization of 8 prey types used in the analysis of isotopic niche space. For each prey category the data fields shown are the most common species, number of samples used in stable isotope samples, mean δ13C value, standard deviation of δ13C values, mean δ15N value, standard deviation of δ15N values, the C/N ratio (± standard deviation), and the estimated and % contribution of each prey type (based on Bayesian mixture model analysis: see text) to the diets of sea otters at Monterey and Big Sur (± standard deviations).

Prey Type	***Species***	***n***	**$\delta^{13}C$**	**SD**	**$\delta^{15}N$**	**SD**	**[C]/[N]**	**est. % of diet MON / BSR**
Cancer Crabs	*Cancer antennarius, C. magister, C. productus*	*34*	-15.6	0.8	14.1	0.8	4.1 (0.3)	25.5 (1.2) / 20.2 (1.2)
Abalone	*Haliotis cracherodii, H. rufescens*	*22*	-15.5	0.9	9.5	0.9	3.8 (0.3)	11.0 (1.3) / 24.9 (2.0)
Purple Sea Urchins	*Strongylocentrotus purpuratus, S. franciscanus*	*16*	-17.0	1.1	9.4	0.4	4.7 (0.8)	10.5 (0.6) / 5.5 (0.4)
Clams	*Tresus nuttalli, Protothaca staminea, Saxidomus nuttalli, Macoma nasuta*	*56*	-15.5	1.0	11.4	0.7	4.1 (0.5)	9.7 (1.1) / 1.4 (0.2)
Northern Kelp Crabs	*Pugettia producta*	*27*	-13.3	1.1	11.6	0.8	4.8 (0.6)	8.7 (0.5) / 9.0 (0.6)
California Mussels	*Mytilus californianus*	*18*	-17.5	0.9	9.2	0.5	4.0 (0.4)	8.3 (0.4) / 19.9 (0.8)
*Tegula*Snails	*Tegula funebralis,T. pulligo, T. brunnea, T. montereyi*	*24*	-14.3	0.9	10.6	0.7	4.5 (0.4)	7.6 (0.5) / 2.5 (0.2)
Fat Innkeeper Worms	*Urechis caupo*	*16*	-15.6	0.7	11.7	0.6	4.3 (0.4)	4.7 (0.2) / 8.7 (0.6)

Table 5 Binary logistic generalized linear mixed effects models showing the relationship between the probabilities of occurrence of tool use with factors Population, Prey, Sex, Age, Habitat, and Diet Specialization. Forage bouts were random effect nested individuals.

Model	**Df**	**AIC_c**
Site + Diet Specialization+ Prey Type+ Age + Sex + Habitat	22	5111.8
Site + Diet Specialization* Prey Type+ Age + Sex + Habitat	46	5090.2
Diet Specialization*Prey Type	38	5091.7
5110, 5160		5098*

Table 6 Wald Test results for selected GLMM model.

Factor	**Wald's Test χ^2**	**d.f.**	**p-value**
Diet	61.0	4	1.8e-12
Prey	244.3	8	0.0
Diet*Prey	59.9	32	0.002

Figures

Figure 1. Histogram showing contribution of 20 prey types to the diets of sea otters at Big Sur and Monterey study sites. Refer to Table 1 for species included in each prey-type category.

Figure 2. Graphical comparisons of the estimated rate of energy gain while feeding (kcal/minute), corresponding to results of two-way ANOVA with interaction effect (see Text for details). Error bars represent 95% confidence intervals. A) Mean rates for all animals at Big Sur vs. Monterey. B) The interaction between sex and study site: males have a lower rate than females at Big Sur, but a higher rate than females at Monterey.

Figure 3. Graphical comparison of the mean rate of energy gain for sea otters in 16 sea otter populations from across their range in the north Pacific, based on field studies conducted using standardized methodologies. Populations are color-coded into 3 categories: Green = recently-established and rapidly growing populations, where food resource abundance is presumably not limiting to population growth; Orange = long-established, stable or slowly increasing populations where resource abundance is thought to be limiting further growth; and Purple = populations that have declined for reasons un-related to food abundance (e.g. disease or predation) and where per-capita food resources are abundant.

Figure 4. Results of a Hierarchical Cluster Analysis of sea otter diet composition. For this analysis, individual otters were considered cases and the relative frequency of the 11 most common prey types were the variables. A) the Dendrogram or "Cluster Tree", with branches "pruned" at Euclidean Distance of 0.1 to simplify presentation, such that each terminal node represents 3 – 10 individual otters. The terminal nodes of 5 distinct clusters are uniquely color-coded. B) Cluster validity plots used to select the optimal number of distinct clusters, based on a local minima of the Root mean square standard deviation (RMSSTD, on right) and a local maxima of the pseudo-F value (on left).

Figure 5. Histogram showing contribution of 11 key prey types to the diets of sea otters belonging to each of 5 diet-type groups (as identified by Cluster Analysis) at Big Sur and Monterey study sites. Refer to Table 1 for species included in each prey-type category.

Figure 6. $\delta^{13}C$ versus $\delta^{15}N$ biplots of California sea otter populations and their potential prey. Mean vibrissae and common prey (grey diamonds) $\delta^{13}C$ or $\delta^{15}N$ values for sea otter populations from Monterey Bay (blue circles) and Big Sur (red circles) on the central California mainland coast. We analyzed prey that represent >95% of consumed prey at the population level based on observational data (Table 4). Error bars associated with mean vibrissae isotope values denote standard error; ellipses associated with mean prey isotope values represent standard deviation. Refer to Table 4 for sample sizes and mean isotope values of prey types. Refer to *Methods* for an explanation of the trophic discrimination factors applied to each population.

Figure 7. Comparison of isotopic niche variation statistics at Big Sur and Monterey study sites: A) the total isotopic niche space occupied by sea otters at each site, as measured by convex hull area; B) summed variance of stable isotope values (TNW_I) at each site; C) individual niche-space specialization, calculated as the WIC_I /TNW_I ratio (lower values indicate more extreme individual specialization).

Figure 8. Density plots of population-level dietary niche specialization (ε_I) vs. individual-population dietary similarity (S_I) for sea otters at Monterey (top plot) and Big Sur (lower plot), based on stable isotope analysis. Density plots can be subdivided into quadrants: Individuals that are niche specialists with diets dissimilar to the population (Specialist/Dissimilar), niche specialists with diets similar to the population (Specialist/Similar), niche generalists with diets dissimilar to the population (Generalist/Dissimilar), and niche generalists with diets similar to the population (Generalist/Similar). Probability contour regions were set at 10% intervals; red represents high density, and light yellow represents low density.

Figure 9. A comparison of tool use frequency variation among individuals from Big Sur (top panel) and Monterey (bottom panel). At both locations, a small proportion of the sample population used tools at a very high frequency, while the majority of individuals used tools very infrequently.

Figure 10. Histograms of individual tool use frequency by diet specialization group (refer to text, Table 3 and Figure 5). A) Group 1 (*Cancer* crabs/urchins/misc.); B) group 3 (mussels/urchins); C) group 5 (clams/crabs/worms); and D) group 4 (snail specialists). Snail specialists were the only individuals who used tools on over 50% of their foraging dives.

Figure 11. Average frequency of tool use on different types of prey by otters belonging to 4 different diet specialization groups: checkered = group 3 (mussels/urchins); solid grey = group 5 (clams/crabs/worms); lined = group 1 (*Cancer* crabs/urchins/misc.); dotted = group 4 (snail specialists).

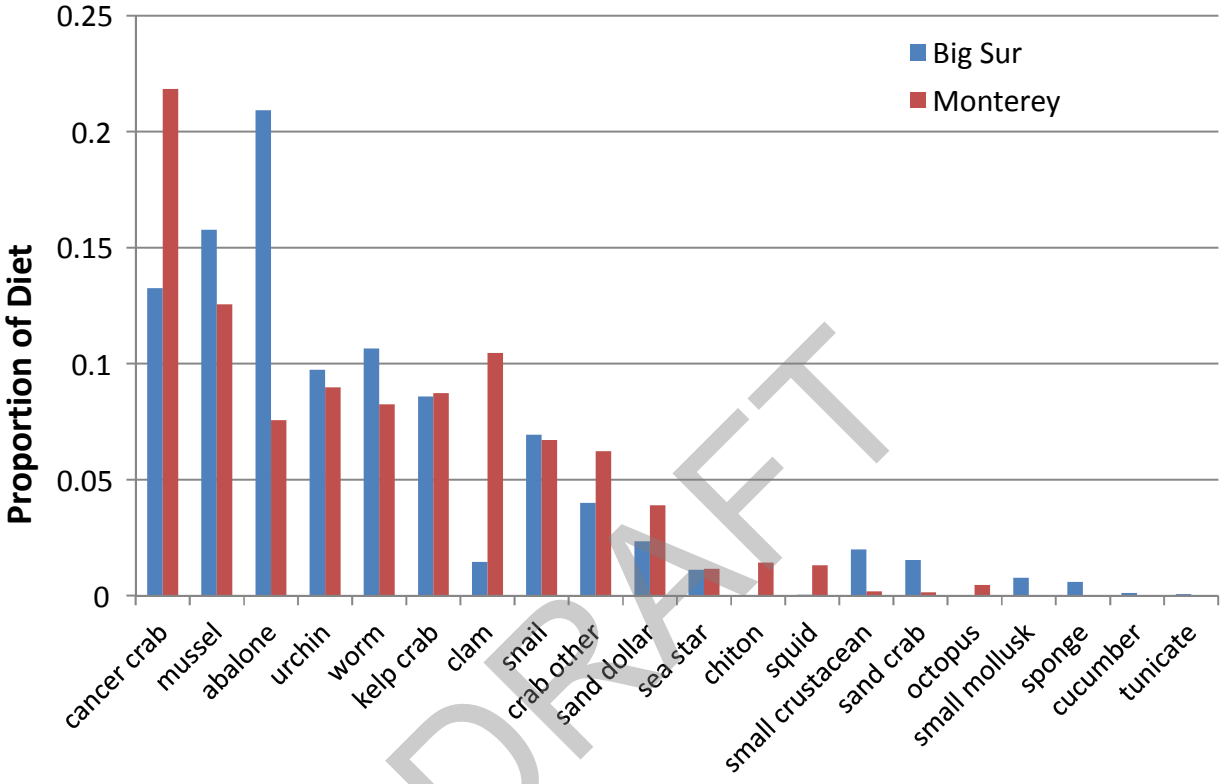


Figure 1

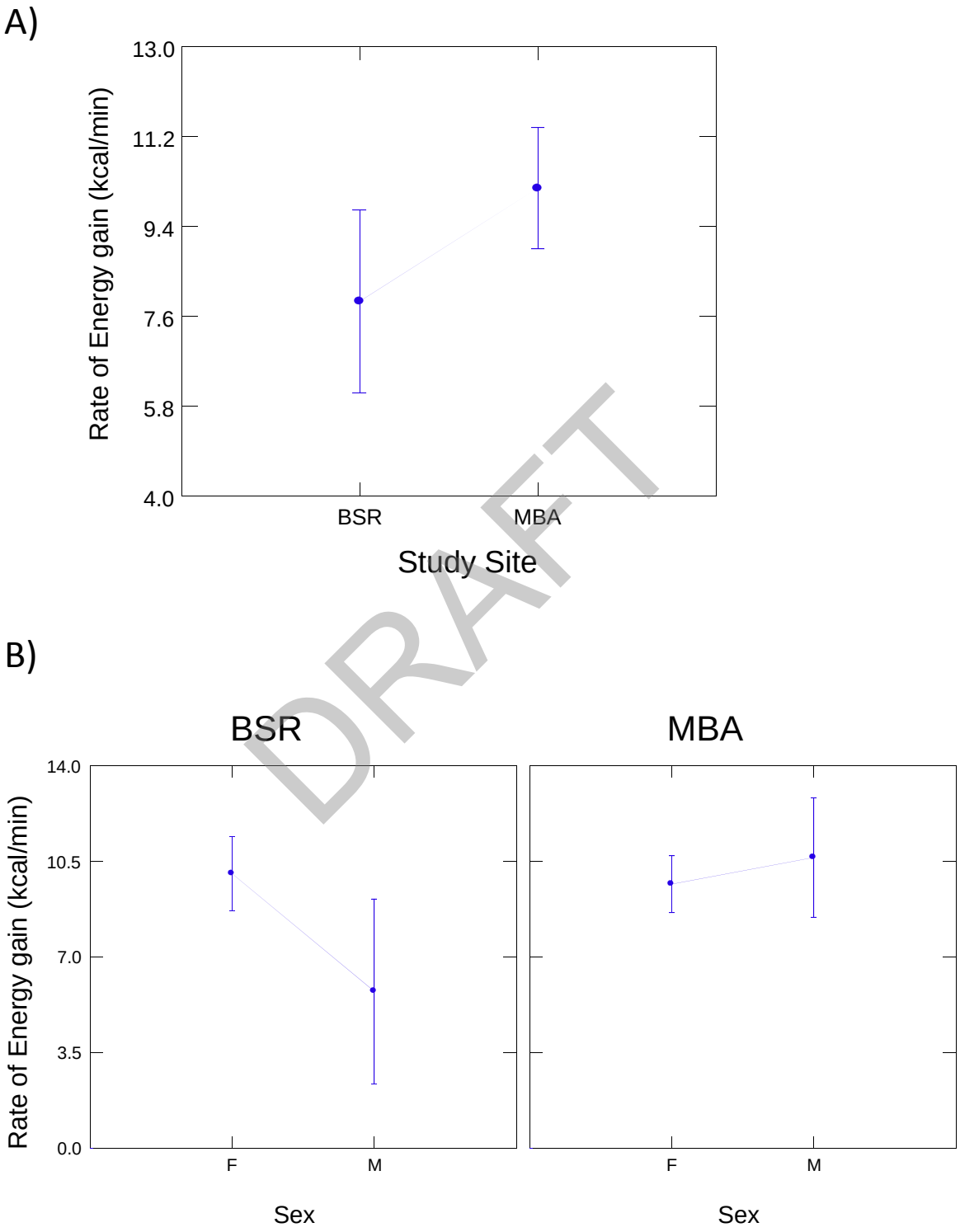


Figure 2

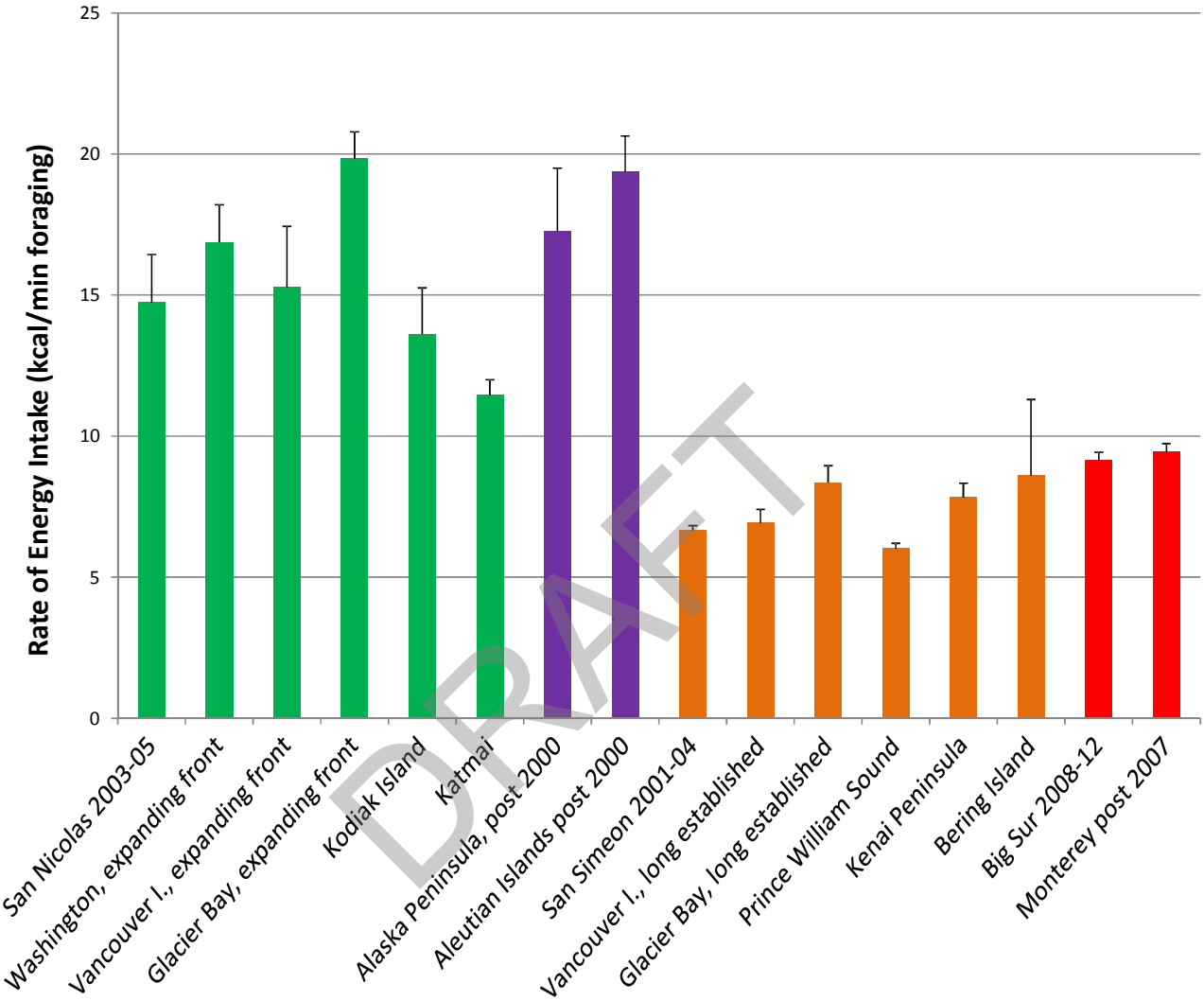
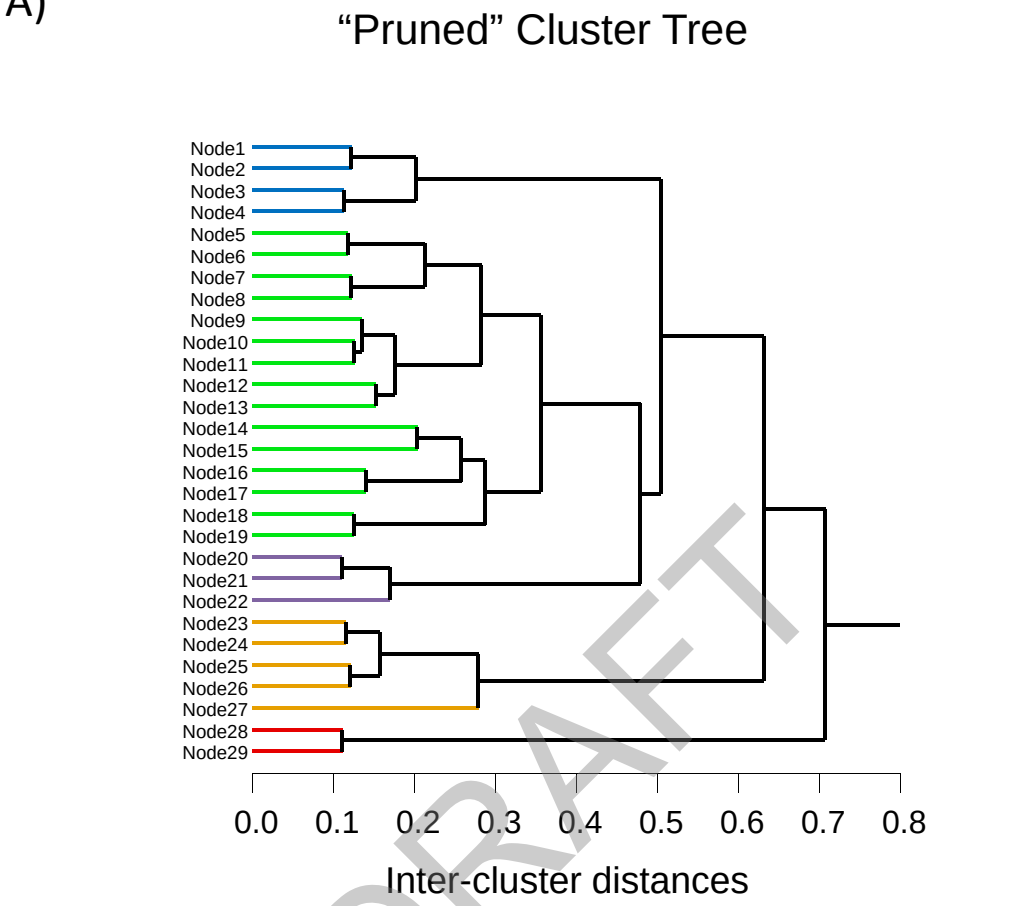


Figure 3

A)



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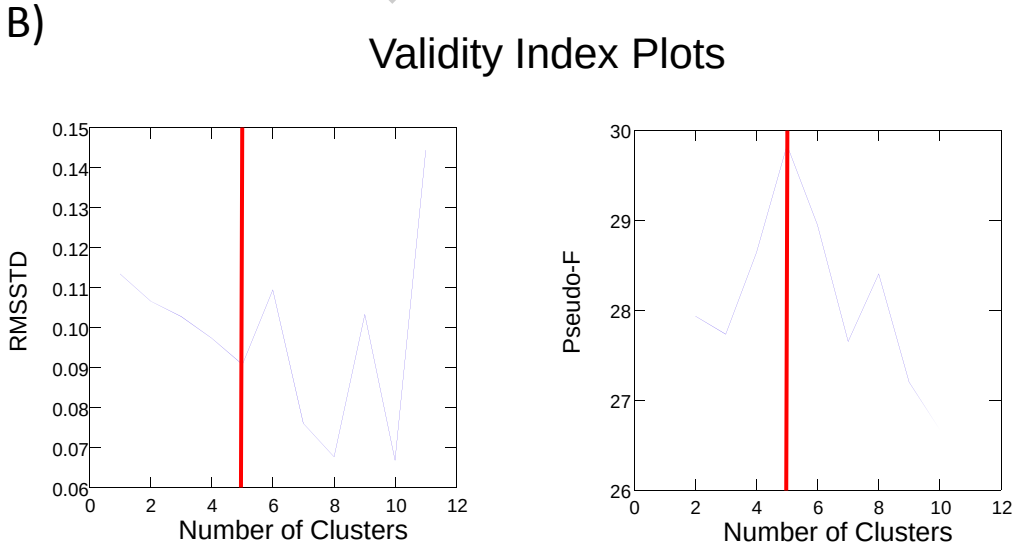


Figure 4

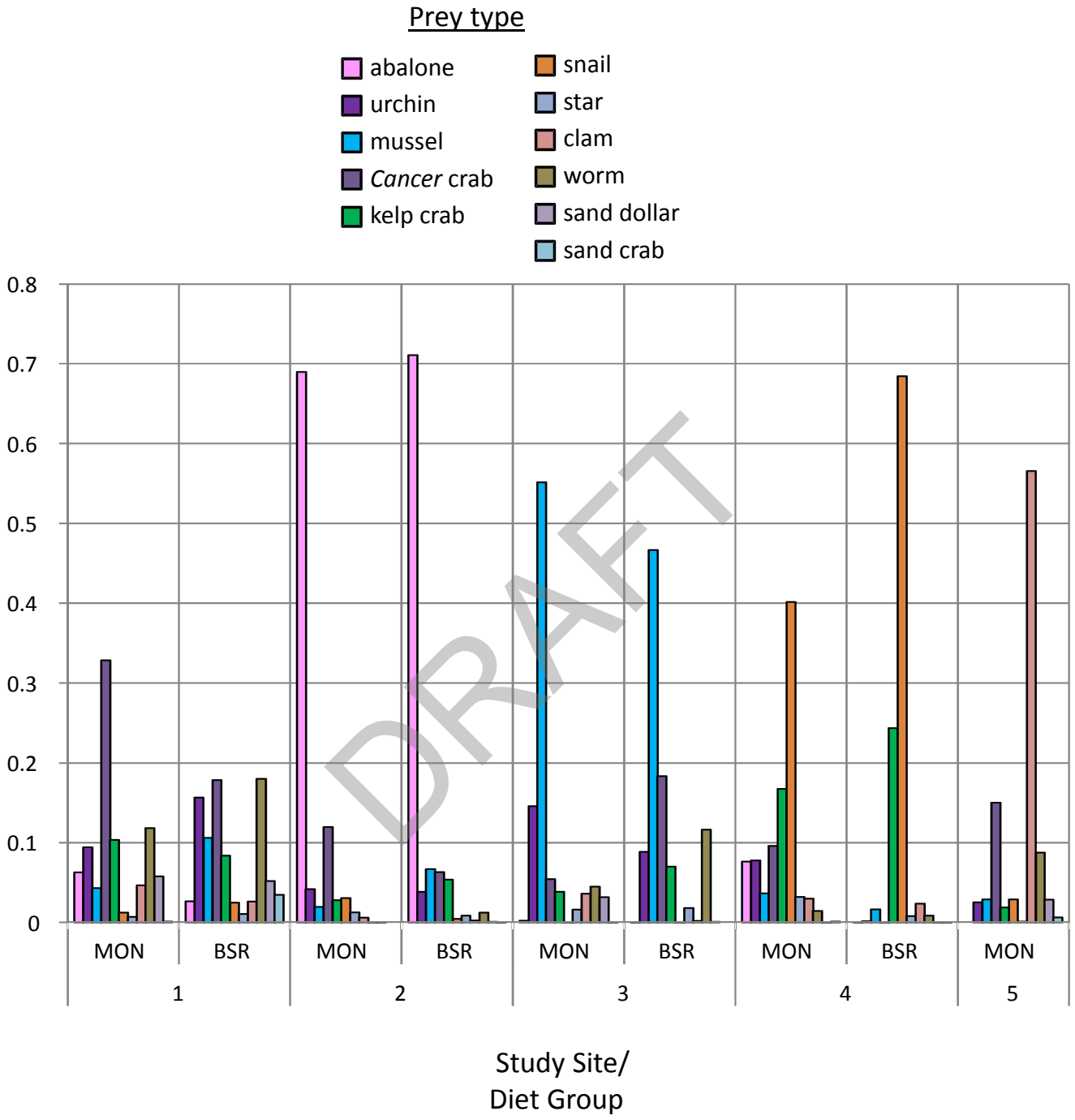


Figure 5

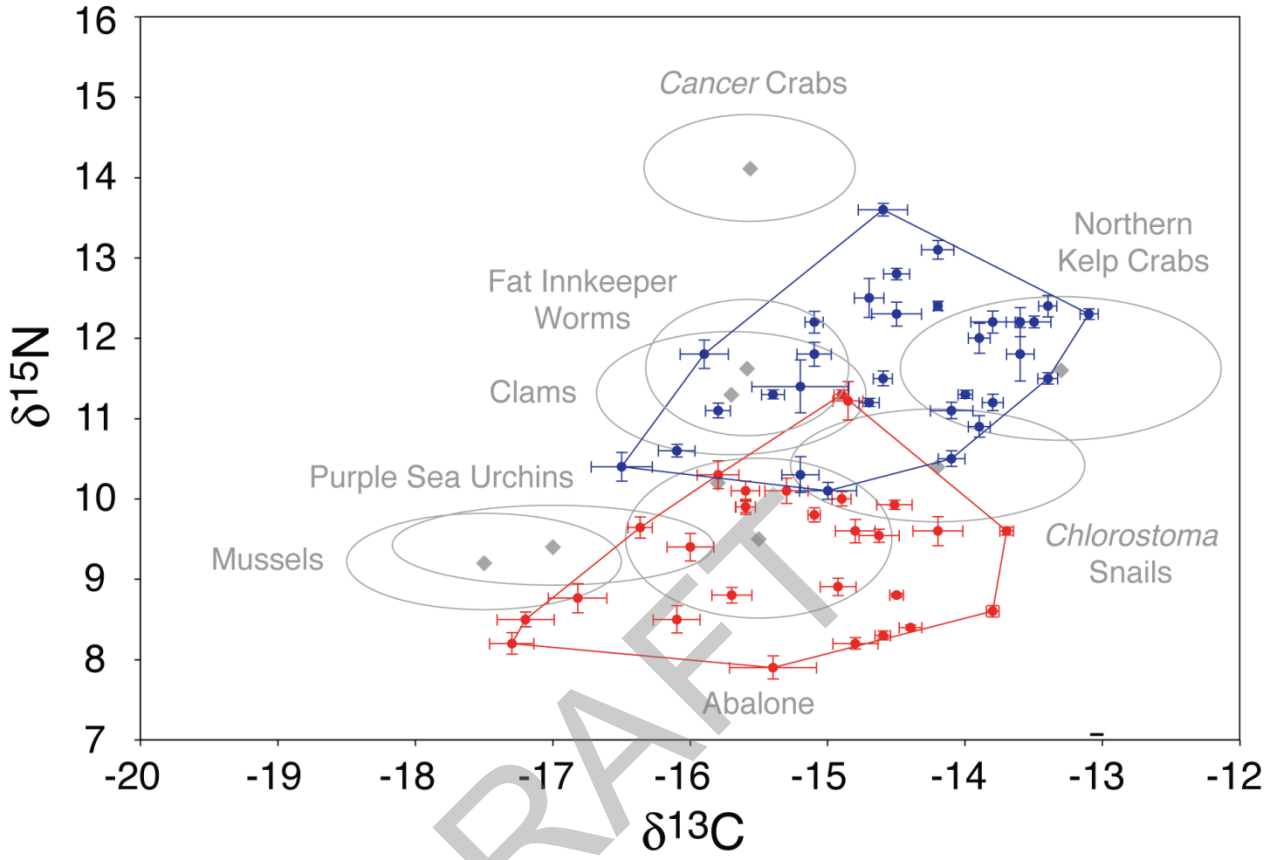
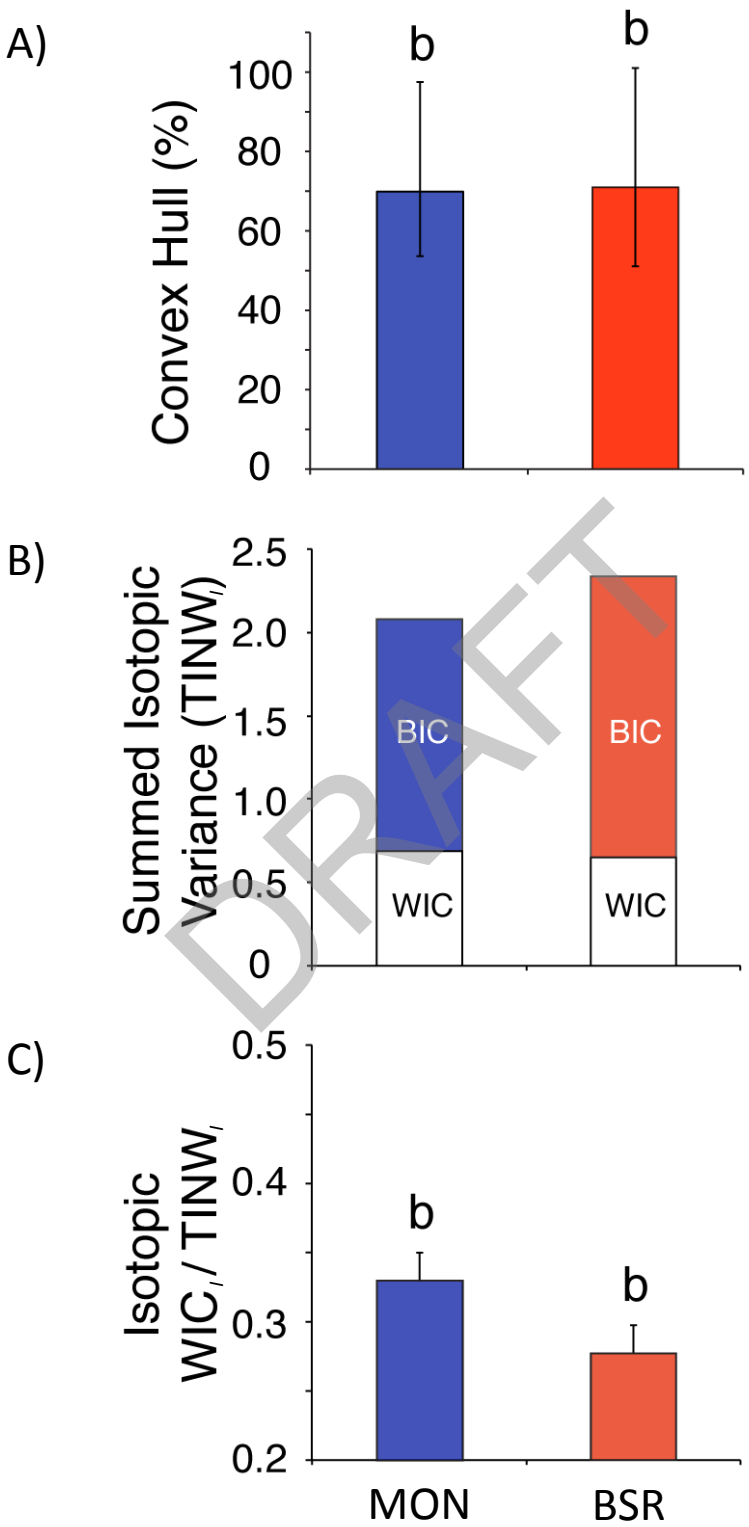


Figure 6


A)
Convex Hull (%)
100
80
60
40
20
0
b
b
B)
Summed Isotopic Variance (TINW$_i$)
2.5
2.0
1.5
1.0
0.5
0
BIC
WIC
BIC
WIC
C)
Isotopic WIC$_i$ / TINW$_i$
0.5
0.4
0.3
0.2
b
b
MON
BSR

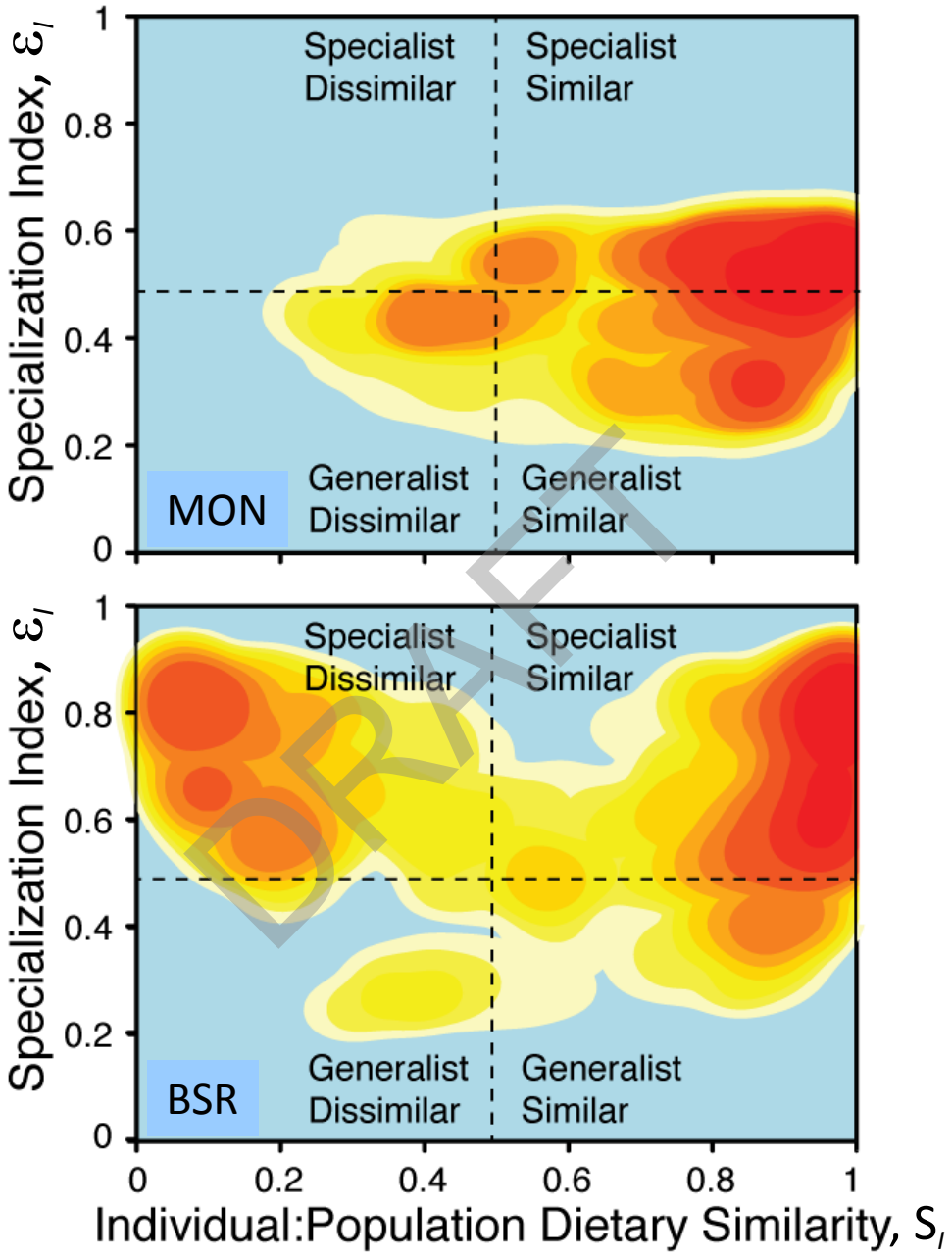
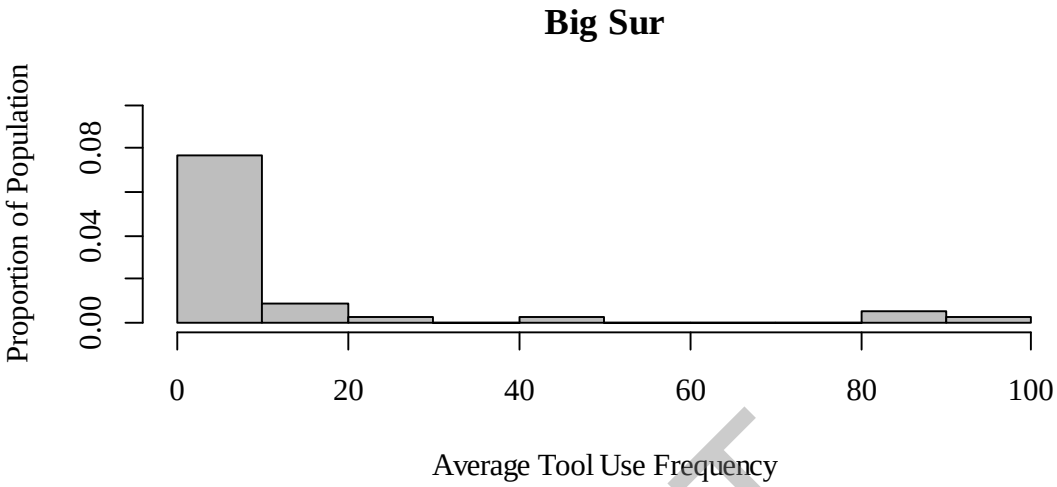


Figure 8



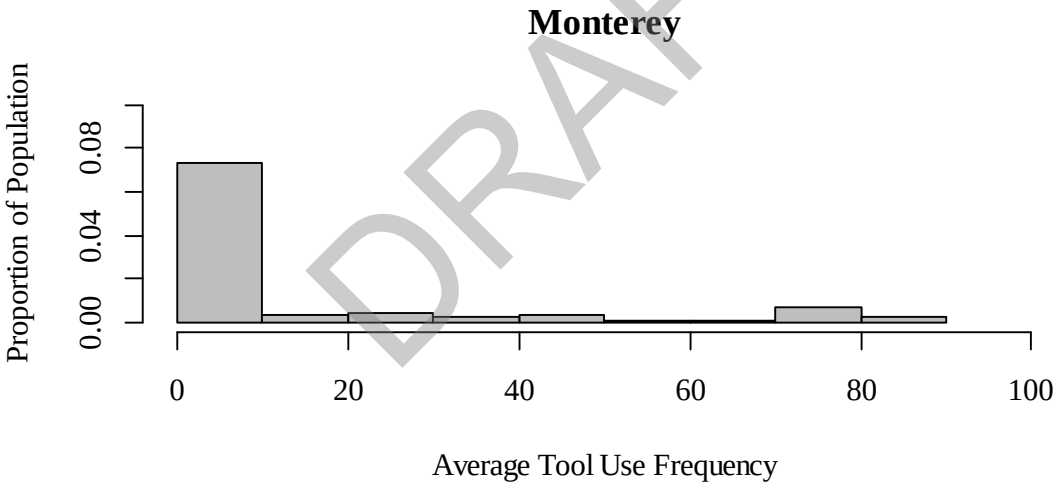


Figure 9

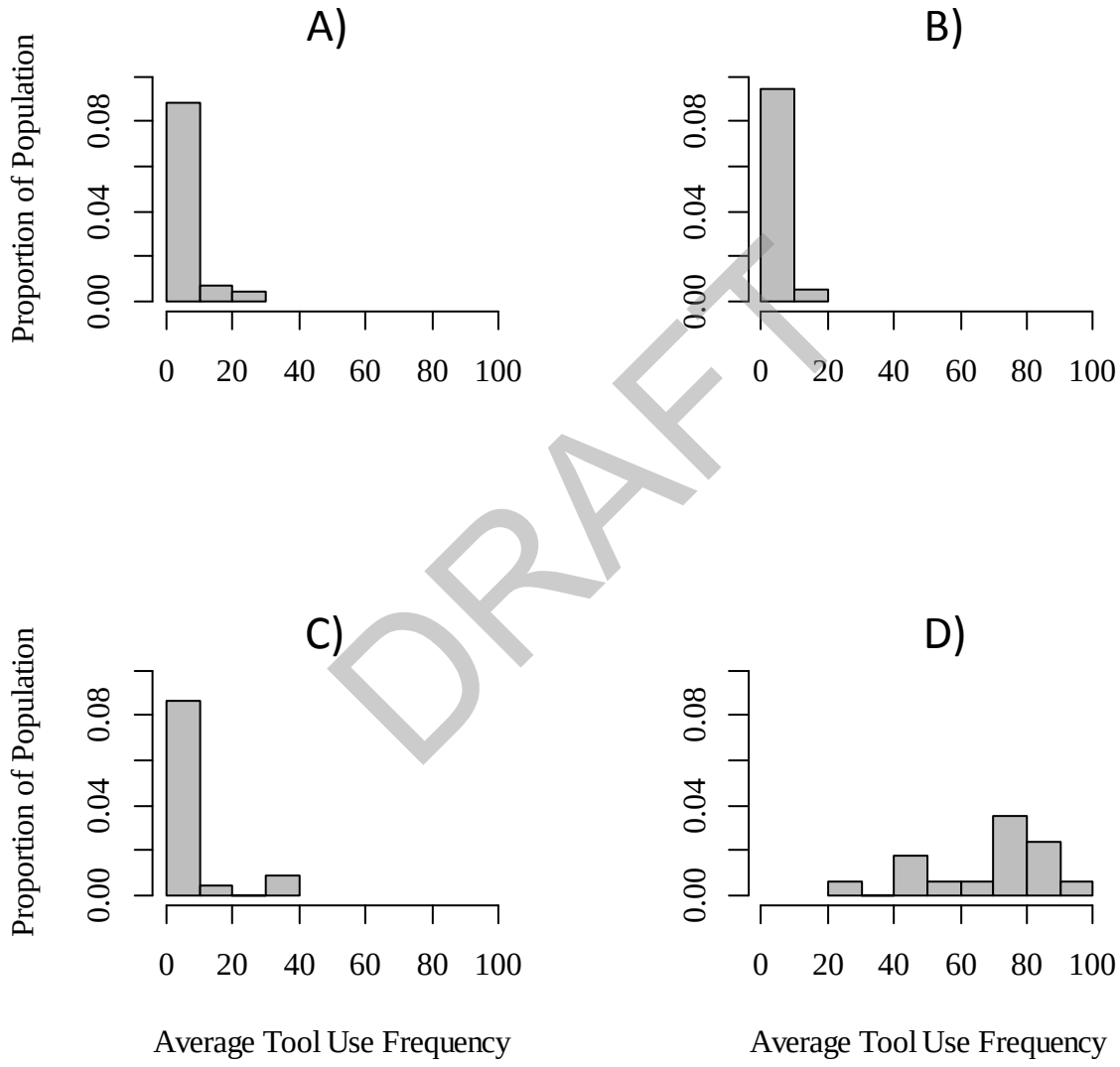


Figure 10

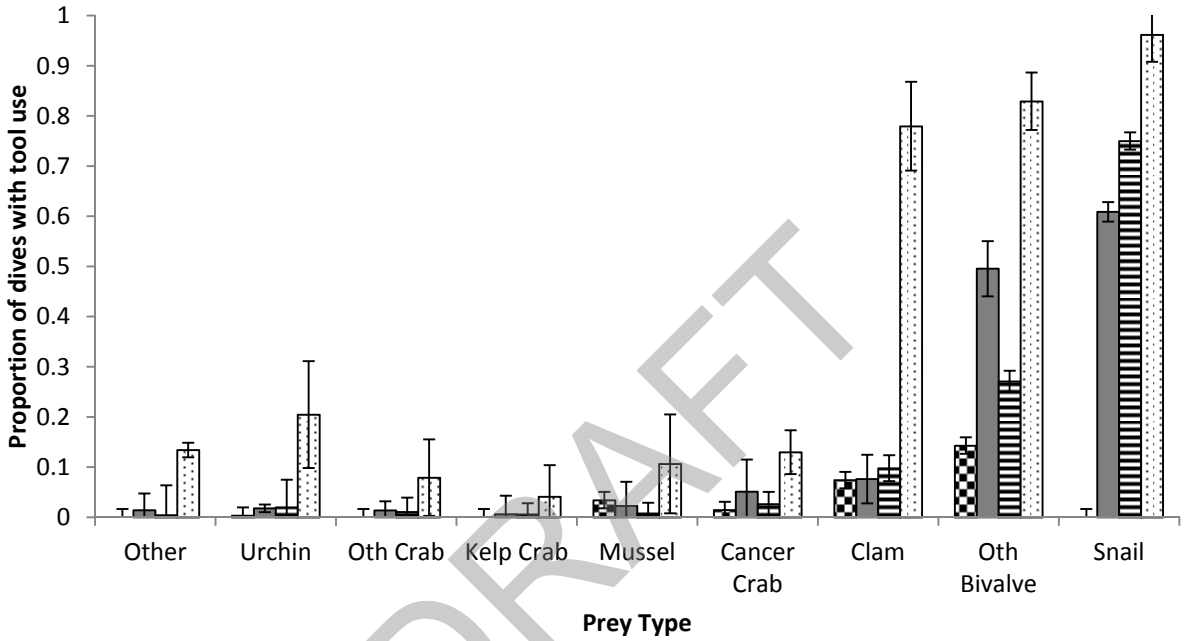


Figure 1 1

Chapter 7. Variation in body condition in California sea otters

M. Tim Tinker
U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA

Introduction

For populations of apex carnivores whose maximum population densities are ultimately limited by the abundance of their prey – that is, populations in which density-dependent changes in survival are associated with per-capita reductions in food resources – it is expected that there will be a cause-and-effect relationship between relative food abundance and one or more aspects of individual health and condition that are related to survival or fecundity. As a result, assessments of relative body condition of individuals within a population can provide insights into the status of that population with respect to carrying capacity. While simple in concept, identifying an appropriate metric with which to assess body condition can be challenging. Evaluating the body mass relative to body length is one of the most common methods of measuring relative body condition (Schulte-Hostedde et al. 2005), the most common approach being to estimate residuals from a regression of body mass (or log body mass) against some measure of structural size, such as body length. This type of index may be useful for assessing relative availability of food resources over the near-term, but it is subject to a number of statistical and biological limitations that can sometimes lead to spurious results (Green 2001). Another approach to assessing body condition, one that is less subject to short-term variation in conditions, is to evaluate age-specific length or mass, and/or the rate of body growth in juveniles (Fowler 1990). This can be done by measuring absolute body length or body mass after statistically controlling for age, which is accomplished by fitting some sort of asymptotic growth function (Stewart et al. 2005).

A number of different indices have been used in past analyses of sea otter body condition, including mass to length ratio, relative length at age and relative size at age (i.e. residuals from length or mass vs. age growth curves), and residuals from a log-linear function of body mass regressed against body length (Bodkin et al. 2002, Rotterman and Monnett 2002, Laidre et al. 2006, Tinker et al. 2008, Monson 2009). Different indices have different advantages and disadvantages: for example, mass:length ratio is very easy to compute but is confounded by age and length differences; relative length at age provides a good age-independent measure of structural size but does not provide insight into shorter term variation in muscle and fat stores associated with food abundance; residuals from a log-linear regression of body mass vs. body length can provide insight into short-term variation in body fat stores, but among-population comparisons are potentially confounded by differences in average structural size between sites. In general, the use of a number of different indices may be most useful, as different indices may reveal different components of body condition that vary at different scales. What is clear is that body condition in sea otters has been found to be related to resource abundance and population status in northern populations (Bodkin et al. 2002, Rotterman and Monnett 2002, Laidre et al. 2006, Monson 2009). Here we compare indices of body condition among different sub-populations of southern sea otter, in order to determine a) if there is variation in body condition among sites in California; b) if

variation in body condition is related to variation in foraging success, and c) if there is support for the hypothesis that sea otters at some locations are affected by reduced per-capita resource abundance.

Methods

We measured the body mass, total length and tail length of each otter captured at the BSR and MON study sites, as described in Chapter 1 (Introduction and Study Methods). We estimated the age of each animal at time of capture based on patterns of tooth wear and on degree of fur "grizzle" around the head and neck. These "field age estimates" were validated for a sub-sample of animals for which "tooth age estimates" (estimates based on cementum analysis of extracted pre-molars) were also available, and age estimates using the two techniques were found to be strongly correlated (R = 0.71), so we used field age estimates for all subsequent analyses (because tooth age estimates were not available for all study animals). We combined data from the current study with data collected previously at four other study sites: CAM = Cambria/San Simeon (2001-2004), PTC = Point Conception (2001-2004), SNI = San Nicolas Island (2003-2005), and MB1 = Monterey Bay Area (2000-2004: note that MON and MB1 samples were spatially similar but temporally distinct, and so were treated as independent study sites). Our total sample size for analysis of body condition was 440 sea otters, ranging in estimated age from 6 months to 18 years.

We compared sea otter body condition among sites by comparing relative body mass and total length after controlling for age effects (and thus the condition of each study animal was assessed relative to the "average" mass and length of all otters of that age). To accomplish this, we used maximum likelihood methods to fit sex-specific Von Bertalanffy growth models (Ratkowsky 1986) to the mass-vs-age and length-vs-age data for all otters from all 6 study sites:

$$L_{x,s} = c_{s,1} + a_{s,1}\left(1 - e^{-k_{s,1}(x-b_{s,1})}\right) \quad (1)$$

$$M_{x,s} = c_{s,2} + a_{s,2}\left(1 - e^{-k_{s,2}(x-b_{s,2})}\right) \quad (2)$$

Where x is the estimated age of each animal, M is total body mass, L is total body length, s is sex (1 = female, 2 = male), and c, a, k and b are the Von Bertalanffy growth function parameters. Note that L represents total length (nose to tail) rather than "length corrected for tail length" (L^c), as has been recommended elsewhere (Monson 2009), because we did not have tail length measurements for sea otters at 3 of the 6 study sites. To determine if variation in L provided a reliable estimate of variation in L^c, we plotted a regression of L vs. L^c for otters from BSR, MON and PTC, and determined that there was a strong linear relationship between these two measures (R^2 = 0.95), with no indication of site-specific differences in residual distributions (Figure 1). We thus concluded that total length (L) provided a reliable index for assessing variation in structural size, as long as among-site comparisons were limited to California. We used boot-strap re-sampling to account for sample-size differences among sites: specifically, for both males and females we randomly drew 20 otters (with replacement) from each study site, fit equations 1 and 2, then repeated this 1000 times and used the mean expected values to define the "average" length or mass at age for each sex.

We calculated two indices of relative condition for each animal as the residual values from the mean growth curves calculated from equations 1 and 2. Residuals from the length vs. age function represent an index of relative structural size after controlling for age: this structural size index (SSI) provides insights into long-term patterns of food abundance at a given site, as these long-term conditions determine the growth rates and asymptotic skeletal size of otters over the first 5-7 years of life. Residuals from the mass vs. age function provide a more comprehensive body condition index (BCI), reflecting both relative structural size as well as dynamic body stores (muscle and fat biomass) that can increase or decrease in response to short term variation in food abundance, animal health or reproductive status. These two indices are expected to be strongly correlated, since otters that have greater structural size at a given age clearly have the potential to reach larger body mass; however, short-term variation in conditions can lead to some variation in biomass for an otter of a given age and body length. Accordingly, we also computed the residuals from a linear regression of BCI vs. SSI, which represent a "dynamic condition index" (DCI), in order to assess short-term variation in conditions at each study site.

We graphically compared the SSI, BCI and DCI among sites using boxplots, and used Analysis of Variance (ANOVA) to test for statistically significant differences in all 3 indices among sites. We conducted separate analyses for males and females because data on movements and habitat use patterns suggested that males and females may experience local resource depletion in different ways and thus exhibit distinct patterns of variation in relative body condition. Finally, we used an inverse logit transformation of BCI to create a rescaled body condition index (BCI') for each sex at each study site that varied between 0 and 1:

$$BCI' = e^{BCI} \Big/ \left(1 + e^{BCI}\right) \tag{3}$$

We used bootstrap resampling (as described above) to account for sampling error and sample size differences among sites in calculating BCI'. To evaluate the role of prey resource abundance on body condition, we used linear regression analysis to test for a relationship between BCI' and the foraging success of study animals of each sex at each study site (specifically, the estimated rate of energy gain while feeding: see Chapter 6).

Results

Growth curves fit to the length-at-age data showed that sea otter structural size tends to reach asymptotic values at approximately 4-6 years of age (Figure 2), with males achieving a greater structural size (average asymptotic length = 127cm) than females (average asymptotic length = 118cm). Mass-at-age data also indicated an asymptote at around 4-6 for females (Figure 3A), but for males the asymptotic values were not reached until approximately 8 years of age (Figure 3B), suggesting that males may continue to increase in muscle mass for some years after reaching maximum structural size, perhaps increasing their chances at successfully defending a reproductive territory.

Structural size varied significantly among study sites for both males and females (Table 1). Female otters at BSR had lower SSI values than did female otters from all 5 other study sites, while females at MON had higher SSI values than females from BSR but lower SSI values than SNI (Figure 4A). Male otters at BSR had significantly lower SSI values than males from the SNI study site, but this was not the case for males at the MON study site (Figure 4B).

The overall index of body condition (BCI) also varied significantly among study sites for both males and females (Table 2), with patterns of variation generally matching those of the SSI. Female otters at BSR had lower BCI values than did female otters from the PTC and SNI study sites but did not differ statistically from females at MON, MB1 or CAM, while females from MON had lower BCI values than females from SNI but higher BCI values than females from CAM (Figure 5A). Male otters at BSR had lower BCI values than did male otters from MON, PTC and SNI study sites but did not differ statistically from females at MB1 or CAM, while males from MON had higher BCI values than males from BSR but did not differ from males at the other study sites (Figure 5B).

The Dynamic condition index (DCI) varied significantly among sites (Table 3); however, the patterns of variation were very different from the SSI and BCI indices. The values of DCI for females were higher at the CAM study site than all other sites, but there were no other statistically significant differences (Figure 6A). Males at BSR had higher DCI values than did males at MON and SNI, while males at MON had lower DCI values than males at BSR but did not differ significantly from males at any other study site (Figure 6B).

There was a strong positive relationship across sites between the mean scaled body condition index (BCI') and the estimated rate of energy gain while feeding ($R^2 = 0.748$, $F = 23.77$, $P = 0.001$), and this trend was consistent for both sexes (Figure 7).

Discussion

Sea otters in California show considerable variation in relative body condition, suggesting that there population status with respect to carrying capacity varies considerably from site to site. Differences in structural size (Figures 2, 4) indicate that sea otters at Big Sur and (to a lesser extent) Monterey in the early 2000's exhibit particularly low length-at-age, indicating slower growth rates among juveniles and lower asymptotic sizes of adults for both males and females. In contrast, otters at San Nicolas Island and Point Conception, the most recently colonized sites where population densities are lower than the other sites, exhibit significantly greater length at age than Big Sur animals. Otters from these two sites also show the greatest mass at age (BCI, figures 3 and 5), while otters from Big Sur and Cambria had the lowest mass at age. Interestingly, both females and males from the current sample at Monterey tended to have slightly better body condition than did otters from Monterey in the earlier sample, highlighting the fact that temporal variation in body condition does occur even at high density sites, potentially in response to annual variation in prey recruitment success, environmental stressors (e.g. disease prevalence), and other factors.

The dynamic condition index showed very different patterns, which were largely inconsistent with the other two indices. To some extent these patterns may reflect short term variation in dynamic body

reserves that are not necessarily consistent with longer-term patterns: for example, the fact that females at the CAM study site exhibited high DCI but low BCI and SSI could indicate a temporal trend towards higher prey abundance (such that animals that had experienced poor conditions during growth and development were experiencing more abundant food resources at time of sampling). However, such dynamic trends can be hard to interpret because sea otter fat and muscle mass can change so dramatically over short periods of time, and also reflect factors such as reproductive status or disease exposure (for example it is also possible that a higher proportion of females at CAM were in early term pregnancy).

Overall our results suggest that the BCI (relative mass at age) provides the most reliable index of body condition for comparing populations in California, as it captures age-specific differences in both structural size and dynamic body reserves. The strong relationship between BCI' and foraging success (Figure 7) shows that the per-capita abundance of key prey resources translates directly into differences in body condition, and these in turn can translate into differential survival and reproductive success (this report, chapter 8; Monson et al. 2000). Based on our comparison among sites, it would appear that both Big Sur and Monterey have relatively low food abundance relative to low density sites (e.g. PCI and SNI), but that at the present time this resource limitation appears to be most intense at the BSR study site.

References

Bodkin, J. L., B. E. Ballachey, T. A. Dean, A. K. Fukuyama, S. C. Jewett, L. McDonald, D. H. Monson, C. E. O'Clair, and G. R. VanBlaricom. 2002. Sea otter population status and the process of recovery from the 1989 'Exxon Valdez' oil spill. Marine Ecology-Progress Series **241**:237-253.

Fowler, C. W. 1990. DENSITY DEPENDENCE IN NORTHERN FUR SEALS (CALLORHINUS URSINUS). Marine Mammal Science **6**:171-195.

Green, A. J. 2001. MASS/LENGTH RESIDUALS: MEASURES OF BODY CONDITION OR GENERATORS OF SPURIOUS RESULTS? Ecology **82**:1473-1483.

Laidre, K. L., A. Estes James, M. T. Tinker, L. Bodkin James, D. H. Monson, and K. B. Schneider. 2006. Patterns of growth and body condition in sea otters from the Aleutian archipelago before and after the recent population decline. Journal of Animal Ecology **75**:978-989.

Monson, D. H. 2009. Sea otters (Enhydra lutris) and Steller sea lions (Eumetopias jubatus) in the North Pacific: Evaluating mortality patterns and assessing population status at multiple time scales. University of California, Santa Cruz.

Monson, D. H., J. A. Estes, J. L. Bodkin, and D. B. Siniff. 2000. Life history plasticity and population regulation in sea otters. Oikos **90**:457-468.

Ratkowsky, D. A. 1986. Statistical Properties of Alternative Parameterizations of the von Bertalanffy Growth Curve. Canadian Journal of Fisheries and Aquatic Sciences **43**:742-747.

Rotterman, L. M. and C. Monnett. 2002. Length-mass and total body length of adult female sea otters in Prince William Sound before and after the Exxon Valdez oil spill. Marine Mammal Science **18**:977-993.

Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. RESTITUTION OF MASS–SIZE RESIDUALS: VALIDATING BODY CONDITION INDICES. Ecology **86**:155-163.

Stewart, K., R. T. Bowyer, B. Dick, B. Johnson, and J. Kie. 2005. Density-dependent effects on physical condition and reproduction in North American elk: an experimental test. Oecologia **143**:85-93.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tables

Table 1. ANOVA results for among-site contrasts of SSI

Females

Source	SS	df	MS	F	Prob>F
Groups	1064.93	5	212.985	18.58	3.40629e-16
Error	3645.66	318	11.464		
Total	4710.59	323			

Males

Source	SS	df	MS	F	Prob>F
Groups	207.98	5	41.5954	2.48	0.0363
Error	1830	109	16.789		
Total	2037.98	114			

Table 2. ANOVA results for among-site contrasts of BCI

Females

Source	SS	df	MS	F	Prob>F
Groups	222.05	5	44.4103	8.35	1.95914e-07
Error	1691.11	318	5.318		
Total	1913.17	323			

Males

Source	SS	df	MS	F	Prob>F
Groups	289.79	5	57.9576	5.89	7.40264e-05
Error	1072.57	109	9.8401		
Total	1362.36	114			

Table 3. ANOVA results for among-site contrasts of DCI

Females

Source	SS	df	MS	F	Prob>F
Groups	141.62	5	28.3237	6.91	3.87694e-06
Error	1304.17	318	4.1012		
Total	1445.79	323			

Males

Source	SS	df	MS	F	Prob>F
Groups	130.789	5	26.1577	4.79	0.0005
Error	595.18	109	5.4604		
Total	725.969	114			

Figures

Figure 1. Plot of a linear regression (A) and residuals from the linear regression (B) of body length minus tail length vs. body length including tail, for sea otters at three different study sites (individual animals are shown color-coded by study site).

Figure 2. Scatter plots of body length vs. estimated age for sea otters at 6 different study sites in California. The best-fit Von Bertlanffy growth curves are plotted as blue lines. A) Length vs. age data for females. B) Length vs. age data for males.

Figure 3. Scatter plots of body mass vs. estimated age for sea otters at 6 different study sites in California. The best-fit Von Bertlanffy growth curves are plotted as blue lines. A) Mass vs. age data for females. B) Mass vs. age data for males.

Figure 4. Boxplots of the residuals from length vs. age growth curves (see Figure 2) plotted for sea otters at 6 different study sites in California. Values represent an index of structural size (SSI). A) SSI values for females. B) SSI values for males.

Figure 5. Boxplots of the residuals from mass vs. age growth curves (see Figure 3) plotted for sea otters at 6 different study sites in California. Values represent an index of overall body condition (BCI). A) BCI values for females. B) BCI values for males.

Figure 6. Boxplots of the residuals from a regression of BCI vs. SSI values (i.e. deviations from the expected mass at age vs. length at age relationship) for sea otters at 6 different study sites in California. Values represent an index of dynamic body condition (DCI). A) DCI values for females. B) DCI values for males.

Figure 7. Regression plot of the average logit-transformed Body Condition Index (BCI') vs. the average estimated rate of energy gain for male and female sea otters at 6 sites in California. Error bars represent 95% confidence intervals.

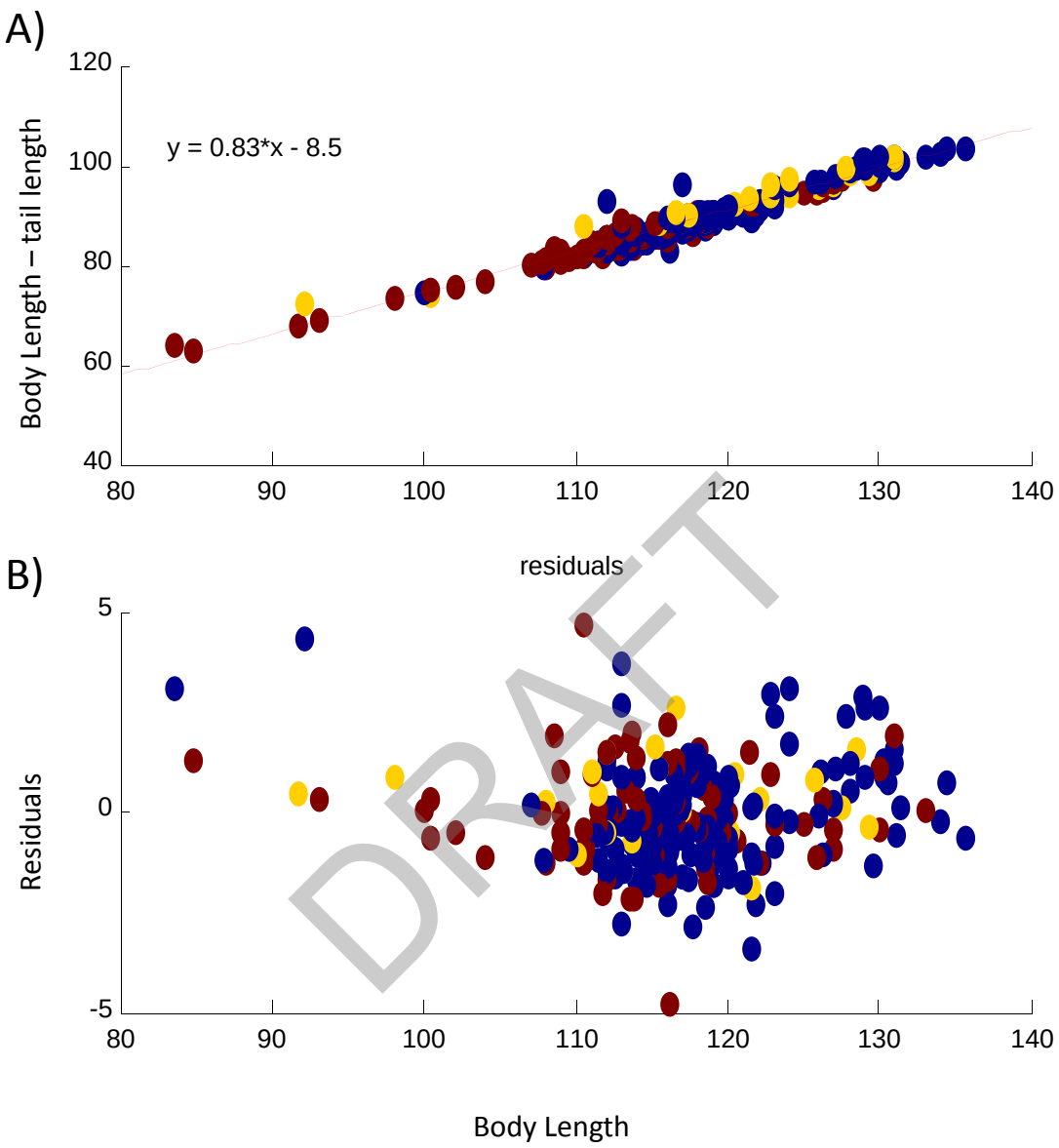
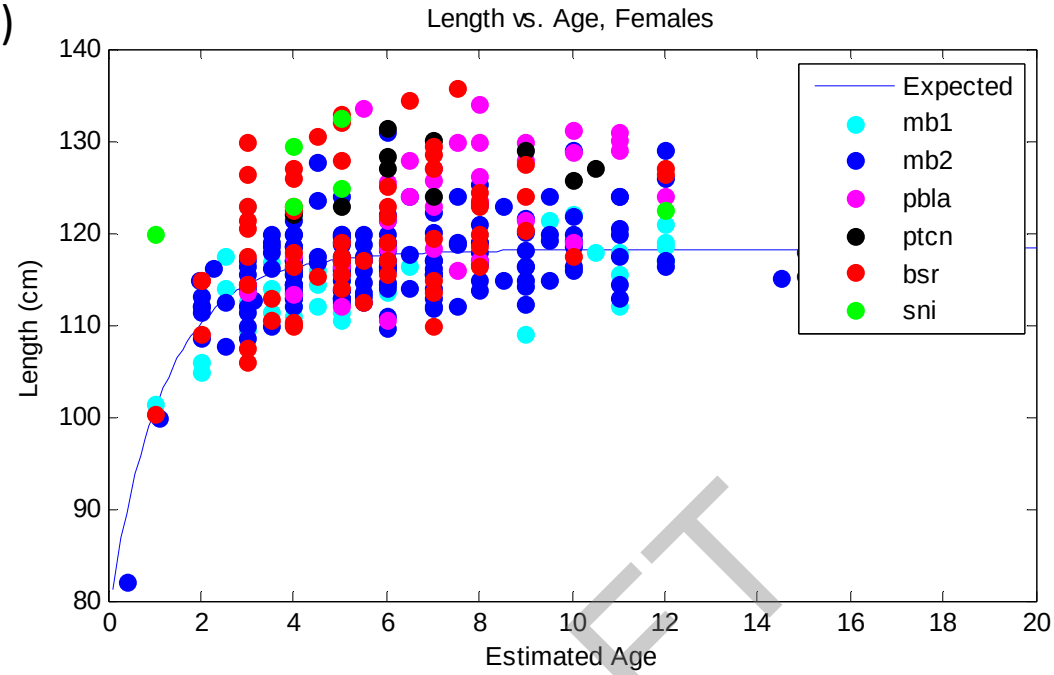


Figure 1

A)



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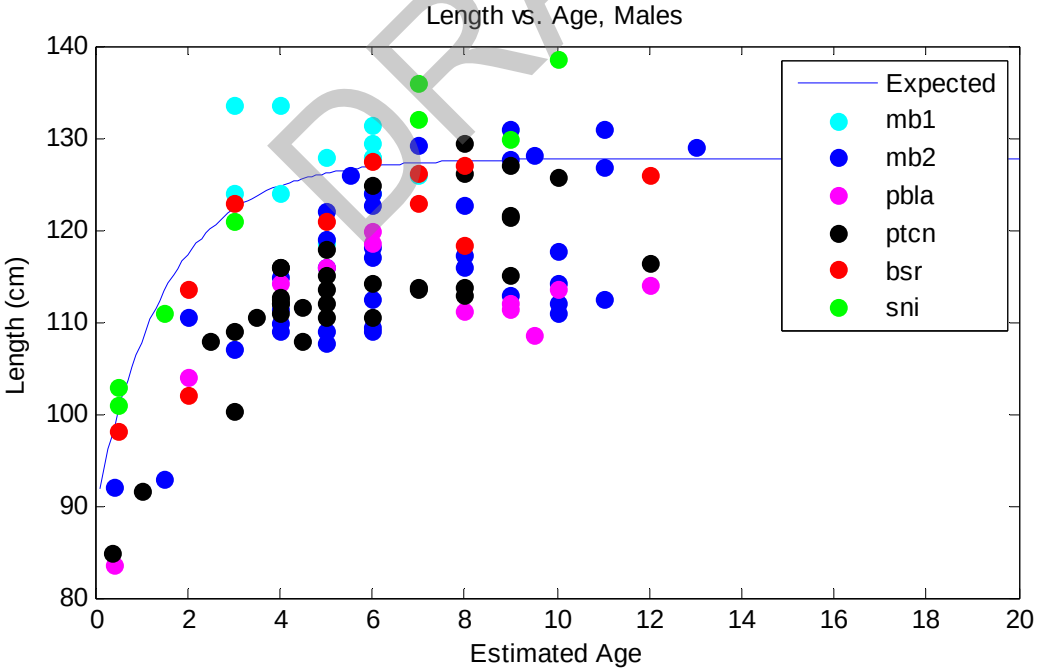
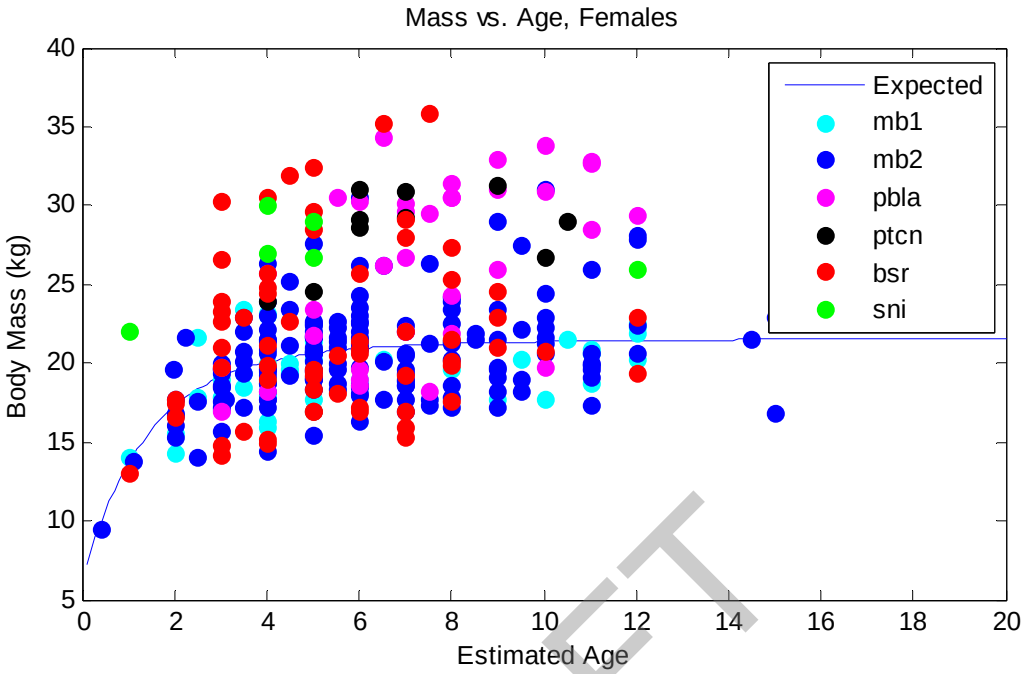


Figure 2

A)



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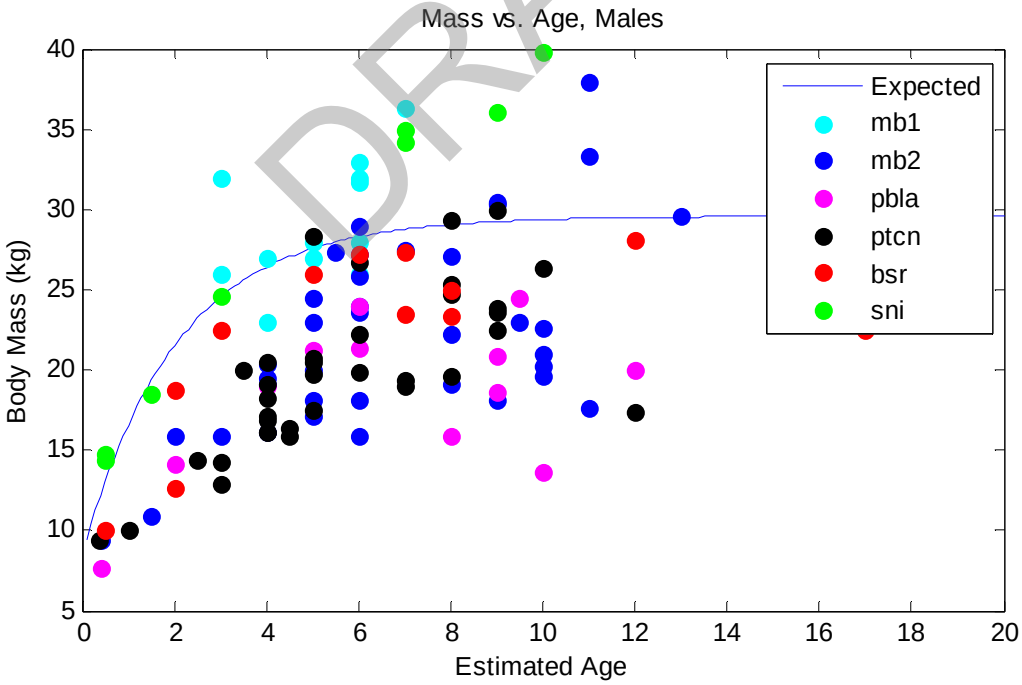
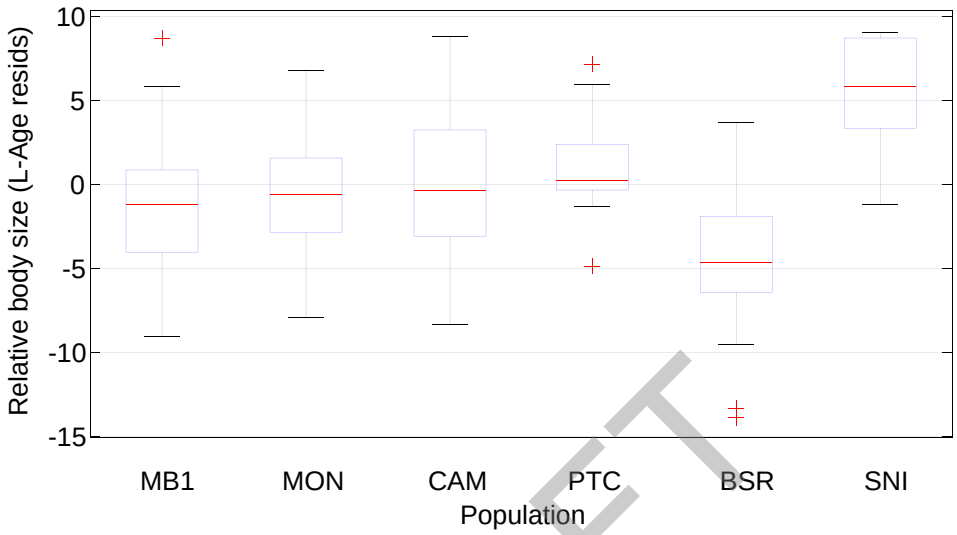


Figure 3

A)



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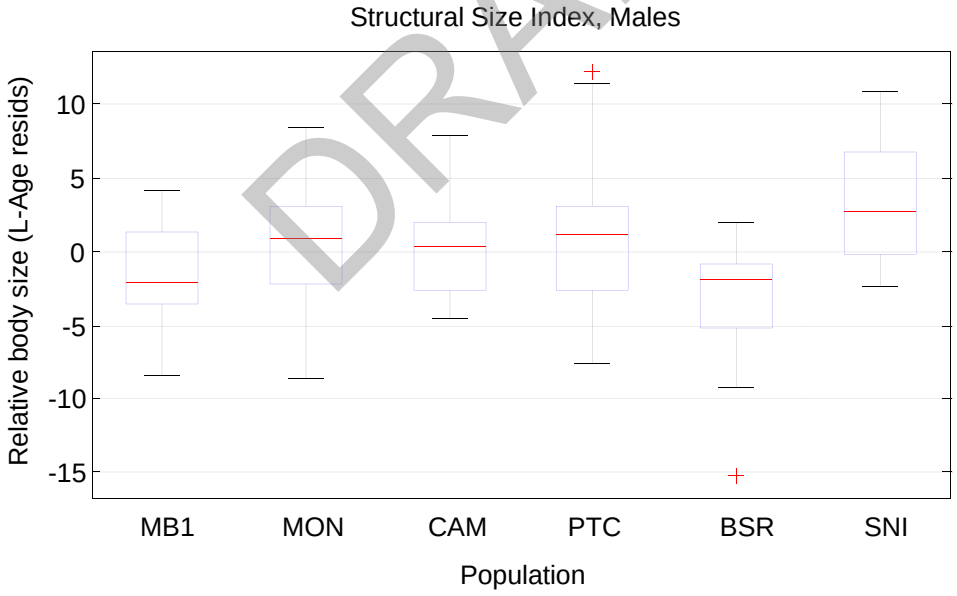
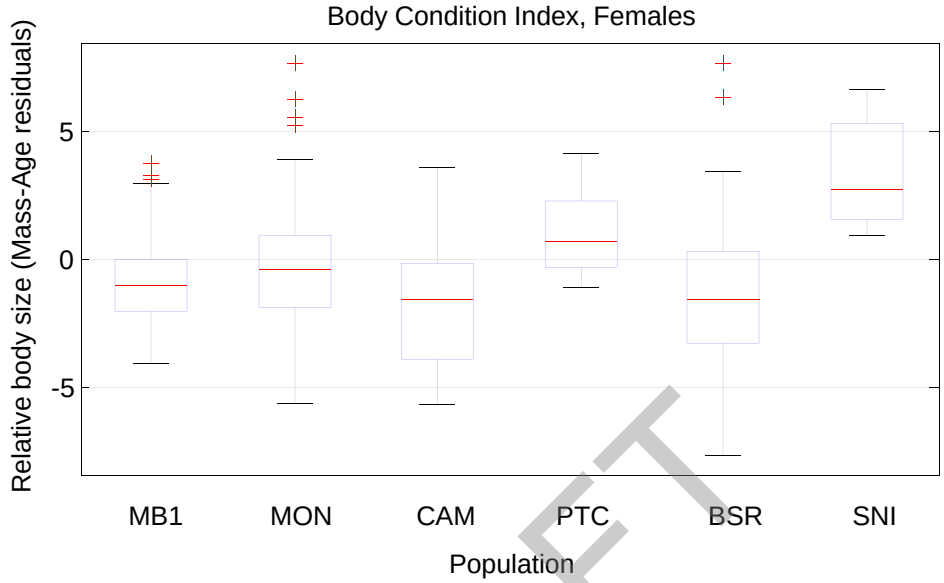


Figure 4

A)



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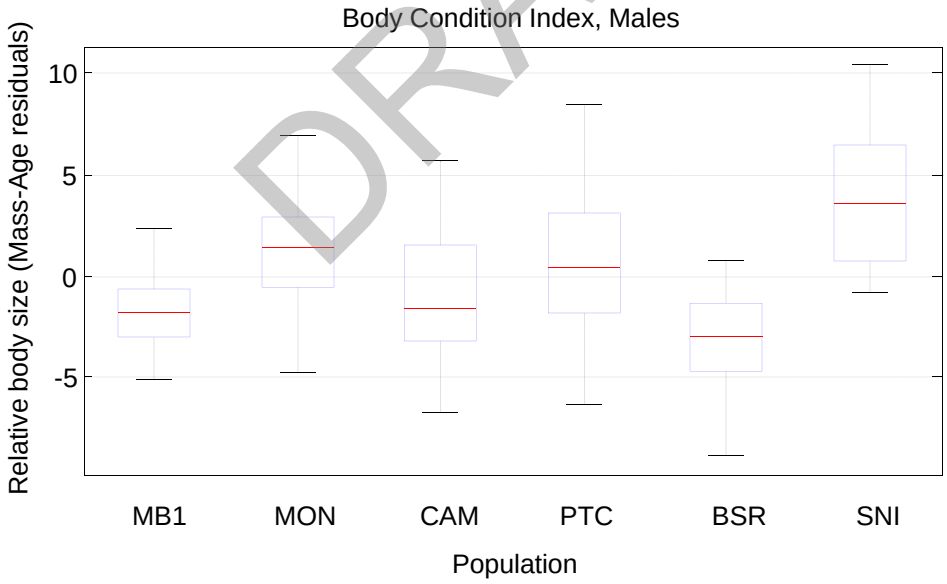
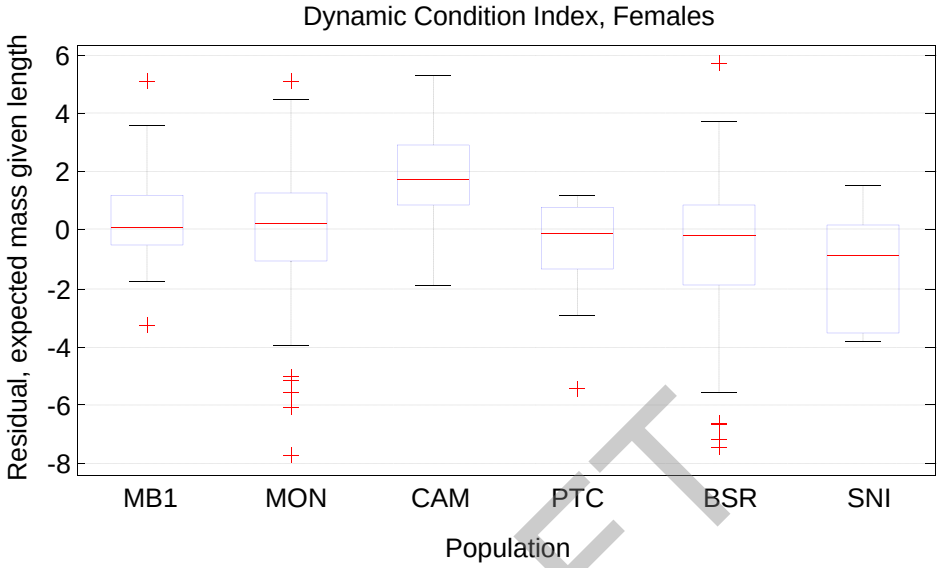


Figure 5

A)



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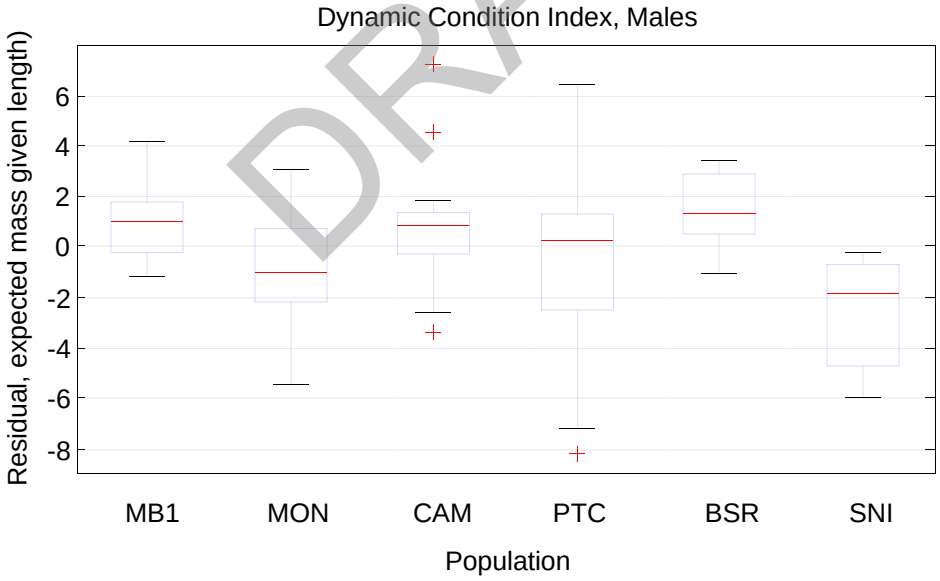


Figure 6

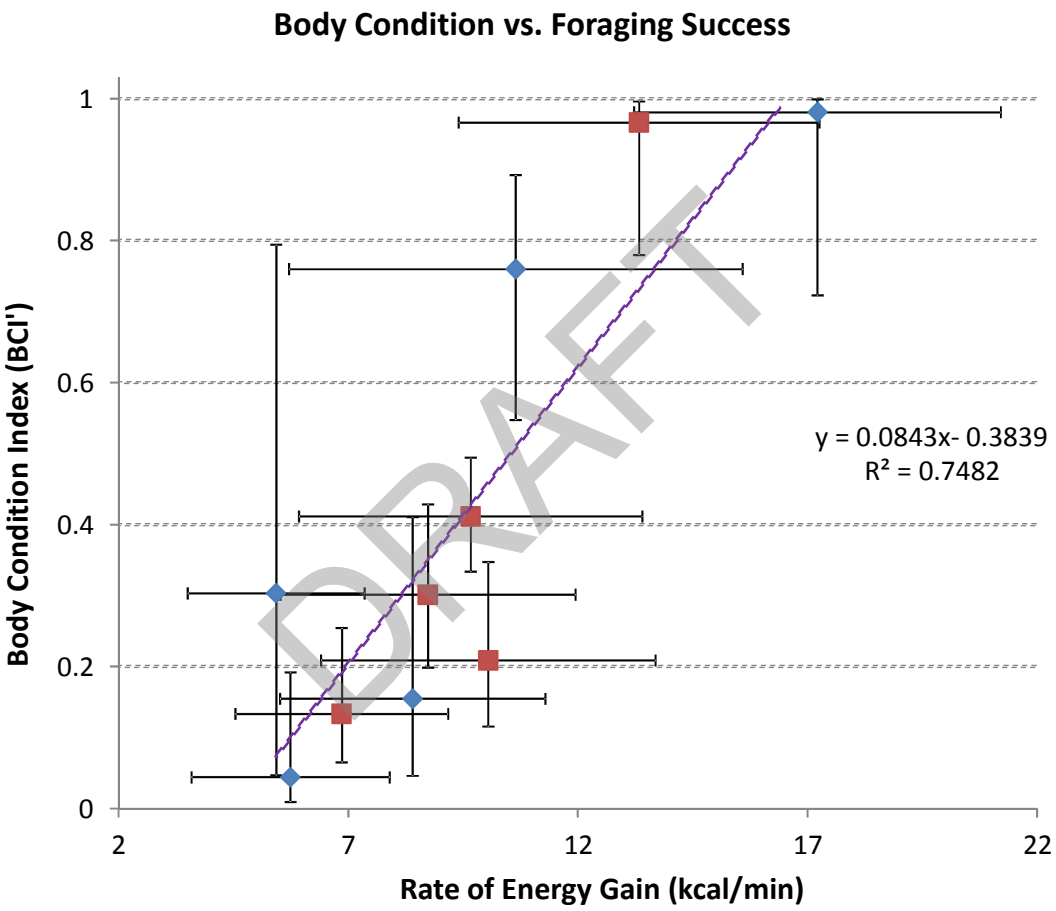

Body Condition vs. Foraging Success
Body Condition Index (BCI')
1
0.8
0.6
0.4
0.2
0
2
7
12
17
22
Rate of Energy Gain (kcal/min)
y = 0.0843x- 0.3839
R² = 0.7482
DRAFT

Figure 7

Chapter 8. Survival and Reproduction

M. Tim Tinker[1], Michelle Staedler[2], Gena Bentall[1], Joe Tomoleoni[1]
1. U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA
2. Sea Otter Research and Conservation Department, Monterey Bay Aquarium, Monterey, CA

Introduction

One of the central components of the comparative study of sea otter populations at Big Sur (BSR) and Monterey peninsula (MON) is the evaluation of survival rates at each site. Survival analysis is often at the heart of radio-tagging wildlife studies , providing biologists with an understanding of demographic drivers of population change, and insights into population viability and the degree to which various putative risk factors of concern to managers (such as anthropogenic or natural stressors) translate into demographically significant effects (Caughley 1966, Pollock et al. 1990, Beissinger and McCullough 2002). In the current study, we realized that a comparison of age and sex-specific survival could help to distinguish between alternate hypotheses about factors limiting sea otter population growth in central California. If anthropogenic stressors are a primary driver of population trends, we would expect that survival would be generally higher at the "pristine" site, BSR, and lower at our highly-impacted site, MON. In contrast, if population trends are more strongly driven by density-dependent processes associated with resource abundance, we might expect survival rates to be lower at BSR where food resources are apparently more limiting and body condition is poorer (see Chapters 6 and 7). Moreover, we might expect mortality risk for females to be highest the end-of-lactation period, when mothers are experiencing the greatest energetic demands associated with pup provisioning and lactation.

We evaluated survival rates of our radio-tagged sample of animals over the monitoring period, conducting comparisons between BSR and MON, as well as comparisons with similar data from previous monitoring studies (Tinker et al. 2006b). We also evaluated birth rates and pup survival rates (or weaning success rates) in these populations. Our analysis accounted for variation in survival caused by differences in age, sex, reproductive status and mean body condition. We employed an instantaneous hazards model for this analysis, treating survival as a continuous process observed at discreet intervals, with the likelihood of survival of an individual animal over a particular time interval given by its cumulative probability of avoiding mortality risks, or hazards, over that interval (Heisey and Patterson 2006). In essence, we can think of the instantaneous "hazards" at any point in time, *h(t)*, as an approximation of the conditional mortality probability over a short interval. Modeling instantaneous hazards as opposed to modeling survival directly has a number of biological and mathematical advantages, including the fact that instantaneous hazards are independent of time scale, and lead to simple multiplicative models (so called "proportional hazards models") where the relative levels of mortality risk associated with particular covariates can be estimated as hazard ratios (Heisey and Patterson 2006, Heisey et al. 2007, Halstead et al. 2012). We employed a non-parametric Kaplan-Meier approach (Sinha and Dey 1997) to estimate instantaneous proportional hazards from staggered-entry monitoring data, and then used these to estimate the contribution of various fixed and random effects to survival rates.

Methods

Survival

We used the telemetry-based re-sighting data from 245 radio-tagged sea otters at 6 study sites: BSR = Big Sur (current study), MON = Monterey Peninsula (current study), CAM = Cambria/San Simeon (2001-2004), PTC = Point Conception (2001-2004), SNI = San Nicolas Island (2003-2005), and MB1 = Monterey Bay Area (2000-2004: note that MON and MB1 samples were spatially similar but temporally distinct, and so were treated as independent study sites). These 6 sites varied both geographically and in terms of relative density, with SNI and PTC representing recently-established, low-density populations where prey resources were believed to be abundant during the period of study (Tinker et al. 2008a), while the remaining 4 sites represent high-density, long-established sea otter populations.

Our model incorporated both fixed effects of age, sex, reproductive status and mean body condition, as well as random site effects, also referred to as shared frailties (Banerjee et al. 2003, Halstead et al. 2012). Because independent hazards are multiplicative in nature (as with many time-dependent biological processes), it is more convenient to formulate hazard models in terms of *log*(hazards), which are additive and thus lend themselves to the fitting of linear models using maximum likelihood or Bayesian methods. Specifically, over a short time interval (t=r to t=r+1), the cumulative effects of all hazards for an individual of age i, sex j, at site k, having covariate values X_c, can be estimated by the "*log* unit cumulative hazard" (γ_r), which represents the additive effects of various *log*(hazards) within that time interval:

$$\gamma_{r,i,j,c,e,k} = \gamma_0 + \rho_r + \delta_{i,j} + \beta_1 X_c + \beta_2 Y_e + Z_k . \qquad 1$$

where γ_0 represents the baseline *log* hazard rate, ρ_r represents the effect of time-varying hazards (i.e. the hazard ratio associated with conditions specific to time interval r, relative to the baseline mean hazard rate), $\delta_{i,j}$ represents the effect of age-varying hazards (i.e. the hazard ratio associated with animals of age i and sex j relative to the baseline mean hazard rate), β represents a vector of parameter values each corresponding to the fixed-effects of a particular covariate (X_c and Y_e, described below), and Z_k represents any additional random effects (or shared frailties) associated with study site k that were not accounted for by any of the fixed-effects included in the model. The time-varying and age-varying hazards (ρ and δ respectively) were both treated as continuously-varying effects, estimated using non-parametric conditional auto-regressive (CAR) methods (Sinha and Dey 1997, Banerjee et al. 2003). Additional covariates evaluated in equation 1 included body condition and reproductive status. To evaluate the effect of body condition (X_c) on survival, each otter was assigned the appropriate mean sex- and site-specific body condition index (BCI, calculated as described in Chapter 7). Note that a negative value of β_1 would correspond to increased hazards for animals with lower than average BCI values, and reduced hazards for animals with higher than average BCI values. To evaluate the effect of reproductive status (Y_e) on survival, female otters were assigned a score of 0-1 at each time interval: 0 = female with no pup or a dependent pup of 1-5 months old, and 1 = "end-lactation period" status (females having dependent pups >5 months old or having weaned a pup within the last 30 days).

In addition to the full model shown in equation 1, we also evaluated simpler models with fewer effects, and used model DIC values to select the most parsimonious model (Spiegelhalter et al. 2002). Because equation 1 is expressed in terms of the log of the cumulative hazards, it is worth noting that the baseline instantaneous hazard rate is estimated by $exp(\gamma_0)$, while all other parameters represent the log of a hazard ratio, whereby a parameter value of 0 corresponds to a ratio of 1 (no significant effect), a value of <0 corresponds to a ratio of <1 or a reduction in hazard rates relative to baseline values, and a value of >0 corresponds to a ratio of >1 or an increase in hazard rates relative to baseline values.

While log hazards are convenient for statistical estimation, the metric we are really interested in is the conditional survival probability for a given otter over a specified time period. That is, given that an individual is alive at time *r*, what is the likelihood it will survive until time *s*? This probability can be calculated from instantaneous hazards as:

$$S(s \mid r) = exp\left[-\left\{ \int_{r}^{r+1} h(u)du + \int_{r+1}^{r+2} h(u)du + \ldots + \int_{s-1}^{s_N} h(u)du \right\} \right] \quad 2$$

where each of the integral terms on the right-hand side of equation 2 represents the "unit cumulative hazard" over a short time interval, and can be approximated by a point-estimate of the instantaneous hazard rate:

$$\int_{r}^{r+1} h(u)\,du \cong h(r) = \exp(\gamma_r) \quad 3$$

Thus the conditional survival can be calculated from a summation of log unit cumulative hazard values (γ_r, as calculated in equation 1) over the time period of interest:

$$S(s \mid r) = \exp\left[-\sum_{t=r+1}^{s} \exp(\gamma_t) \right] \quad 4$$

Bayesian MCMC fitting algorithms (implemented using the MATLAB and JAGS "Just Another Gibbs Sampler" programming environments) were used to fit equations 1 and 4 to interval-censored survival/mortality data from radio-tagged sea otters. Bayesian model fitting was conducted following standard procedures: all parameters were initiated with uninformative parameters, and 10,000 burn-in MCMC iterations were conducted to allow model convergence, before saving 10,000 iterations (thinned by a factor of 3) for evaluation of posterior distributions. We graphically evaluated the traces of 3 independently initiated MCMC chains and examined $\hat{r}$ values to ensure model convergence and stability (Brooks et al. 2011).

The Kaplan-Meier analysis of individual survival histories consists of identifying the left-censored and right-censored entry points for each monitoring record (the date first tagged and the date last observed), as well as the fate at the end of the monitoring period (died, still alive, or disappeared and

thus unknown fate). Our model was evaluated at time-step intervals of 1 week. Survival outcomes for each otter, over each observed time interval, were represented as random Bernoulli trials with probabilities determined by equation 4, and these comprise the binomial likelihoods maximized by the MCMC algorithm (Heisey et al. 2007).

We present results of the model fitting in terms of posterior distributions of log hazard ratios and the derived annual survival probabilities. The inclusion of terms in the final model was determined by model comparisons using DIC values (Spiegelhalter et al. 2002), and we evaluated the significance of model parameters by determining whether the 95% credible intervals (CI_{95}, which correspond loosely to the 95% confidence intervals for statistics computed using non-Bayesian methods) excluded 0, since a value of 0 corresponds to a hazard ratio of 1 (no significant effect). We tested for variation in survival among sites due to "random effects" (differences associated with environmental, ecological or anthropogenic influences) by evaluating the degree of overlap in the 95% credible intervals of Z_i. Because there could still be realized net differences in survival between sites, even in the absence of differences due to random effects (e.g. differences caused by differing distributions of covariate values between sites), we also calculated the 95% credible intervals for pairwise contrasts of age- and sex-specific survival rates among sites. To evaluate the overall effect of density-dependent variation in body condition on survival rates, we plotted the estimated site-specific adult survival rates as a function of site-specific body condition index values (with separate plots for males and females), and fit a non-linear power function of the form $y = p1 \cdot x^{p2}$. A positive relationship between body condition and survival rate would be represented by positive values of parameters *p1* and *p2* (with the 95% CI for each parameter excluding 0).

Reproduction

We used longitudinal records of observational data from radio-tagged individual females to estimate per-capita birth rates (number of pups born per year per female) and pup survival rates (also called weaning success rates). To estimate birth rates we used the algorithm of Eberhart and Schneider (1994):

$$\bar{b} = \frac{1}{K}\sum_{k=1}^{K} b_k \left(\frac{365}{N_k}\right) \qquad 5$$

where K is the total number of females monitored for at least 365 days, b_k is the number of observed births observed for female k, and N_k is the number of days female k was monitored. Because we determined that a significant number of pup births were missed when the pups died soon after birth, we restricted our analysis to those females having TDR implants, as the TDR records allowed us to reliably detect all birth events (Tinker et al. 2008b). We estimated overall mean birth rate and 95% Confidence Interval using boot-strap resampling, and also compared individual birth rates between BSR and MON using one-way ANOVA.

To estimate pup survival rates, we used Bayesian MCMC algorithms to fit an instantaneous proportional hazards model similar to the model used to analyze adult survival rates, but modified in order to estimate the instantaneous hazard rate over a 20-week pup dependency period (we assumed that pups

that survived to 20 weeks were successfully weaned: Siniff and Ralls 1991). This approach allowed us to measure variation in mortality risk over the course of the pup dependency period, and also to include risk covariates thought to affect pup survival probability (specifically, the mother's age, body condition, and site-specific random effects). In this model, pup age was substituted for time, and expressed in units of days since the pup's birth (t = 0 at the instant of birth). We estimated *log*(unit cumulative hazards) over the interval $t = d$ to $t = d + 1$ as:

$$\gamma_{d,a,c,k} = \theta_d + \varphi_a + \beta_3 X_c + Z_k \qquad 6$$

where θ_d represent baseline hazards assumed to vary with pup age, φ_a represents the effect of the mother's age (i.e. the hazard ratio associated with having a mother of a years of age, scaled relative to the baseline hazard rate for a 3-year old mother), β_3 is the parameter corresponding to the effect of the mother's body condition (X_c), and Z_k represents any additional random effects (or shared frailties) associated with study site k that were not accounted for by the fixed-effects included in the model. The mother's body condition was assessed based on her relative mass-at-age (BCI, calculated as described in Chapter 7), averaged across all capture events for that female. Note that a negative value of β would correspond to increased hazards for mothers with lower than average BCI values, and reduced hazards for mothers with higher than average BCI values. The age-varying hazards (θ and φ) were treated as continuously-varying effects, estimated using non-parametric conditional auto-regressive (CAR) methods (Sinha and Dey 1997, Banerjee et al. 2003).

The pup survival model was evaluated at time-step intervals of 1 day, and survival outcomes for each pup, over each time step, were represented as random Bernoulli trials with conditional probabilities given by:

$$S(s \mid d) = \exp\left[-\sum_{t=d+1}^{s} \exp\left(\gamma_t\right) \right] \qquad 7$$

Methods of Bayesian model fitting, evaluation and statistical assessment of parameter significance were identical to the procedures described above for analysis of independent otter survival. We used data from the BSR, MON, MB1, CAM and SNI study sites. We plotted the mean weaning success rate as a continuous function of mother's age and mother's body condition, and also compared average weaning success rates between Big Sur and Monterey.

Results

Survival

All model runs converged within 10,000 iterations, with well-conditioned posterior sample traces and $\hat{r}$ values less than 1.1. Evaluation of DIC values indicated a lack of support for inclusion of the continuous time-varying effect (ρ_r), but all other parameters were included in the best-supported model. A summary of parameter estimates for the *log*(hazards) model (equation 1) is provided in Table 1.

There was variation in hazards associated with age and sex differences (CAR parameters $\delta_{i,j}$), which resulted in "inverted-U" type survival curves for males and females that are typical for sea otters and other large mammals (Figure 1). The other covariates included in the model, body condition (relative mass-at-age) and reproductive status (for females) both had significant impacts on hazard rates (Table 1, Figure 2). The lower the mean body condition index, the greater the hazard rate, such that a reduction in body condition from "average" to "very poor" (e.g. CAM females) was associated with a 1.9x increase in hazard rate (CI_{95} 1.2-3.1), while an increase in body condition from average to very good (e.g. SNI females) was associated with a 2/3 reduction in hazard rate (CI_{95} 0.13-0.77). This effect was evident as a positive relationship between body condition and survival rates across all study sites for both females and males (Figure 3). For all study sites except SNI and PTC there was also a 3.6x increase in hazard rate (CI_{95} 1.9-6.3) for females when they were in "end-lactation" status.

After accounting for all fixed-effect covariates (age, sex, body condition and reproductive status), there was no statistically significant variation in hazard rates (based on overlap of 95% credible intervals) associated with random effects among study sites (Table 1), although MB1 tended towards slightly lower hazard rates while CAM tended towards slightly higher hazard rates (Figure 4). When the full model was evaluated at each site there were significant differences in survival between sites (Figures 5 and 6), but these were primarily driven by the differences in mean body condition and the proportion of end-lactation status females at each study site. Survival rates of adult females at Big Sur tended to be slightly lower than adult females at Monterey in the current study (significant at 90%, not significant at 95%; Figure 5B), and this pattern was even more evident for adult males (significant at 95%; Figure 6B). A summary of adult survival rate estimates for females and males is provided in Table 2, and Table 3 shows the mean age-specific survival rate estimates for females and males at each site.

Reproduction

Based on the number of pups born to tdr-implanted females (n=25) over the course of their monitoring period (1-3 years), the mean birth rate for adult females at both sites was 0.97 (95% Confidence Interval = 0.77 – 1.19). There was no significant difference in female birth rates between Big Sur and Monterey ($F = 0.97$, $df = 24$, $P = 0.335$).

Our analysis of pup mortality patterns was based on the observed survival outcomes for 413 pups born to study animals between 2001 and 2012 at 5 study sites. The probability of pup mortality varied as a function of pup age, as has been previously reported (Riedman et al. 1994, Monson et al. 2000, Tinker et al. 2006a), although the CAR model we used allowed for a more detailed examination of the nature of this variation (Figure 7). The instantaneous hazard rate was highest immediately after birth (when many new-born pups are abandoned), dropped sharply over the next week but then increased again between 3-6 weeks of age, and then dropped to low values for the period between 6 and 14 weeks of age. After approximately 14 weeks of age, the hazard rate again began to climb continuously up until the time of weaning (which generally occurs between 20-25 weeks of age).

The degree of mortality risk experienced by pups was affected by two factors, 1) the age of the pup's mother and 2) the relative body condition of the pup's mother. The pup hazard ratio dropped significantly for pups with mothers that were 8-years or older (Figure 8), while pups whose mothers

were in good body condition (e.g SNI females, mean BCI = 3.95) experienced a 30% reduction in hazards (CI_{95} = 2 - 49) relative to pups whose mothers were in average condition (Figure 9). As a result of these effects, average weaning success rates increased from 0.64 for 3-year-old mothers to 0.71 for 10-year-old mothers (Figure 9A), and likewise increased from 0.59 for mothers in poor condition (BCI = -0.3) to 0.73 for mothers in good condition (BCI = 0.3; Figure 9B). There were no significant differences in pup hazard rates associated with random effects among sites, however variation in mean female body condition among sites (Chapter 7) resulted in some differences in realized weaning success. The weaning success rate for females at Big Sur was slightly lower than for females at Monterey (mean difference = -.01), although this difference was only marginally significant (Figure 11). Age-specific weaning success rates for females at Big Sur and Monterey are provided in Table 4.

Discussion

The analysis of survival and reproduction rates of tagged study animals monitored in the current study and previously conducted telemetry studies in California revealed interesting patterns and provide key insights into the factors affecting population growth in central California. The Bayesian-based proportional hazards analysis produced estimates of age-specific survival that were consistent with previously published sea otter vital rates (Tinker et al. 2006b), but allowed for a greater flexibility in examining the contributions of various fixed and random effects to variation in survival. This was important as it allowed us to evaluate predictions associated with various hypotheses about factors limiting sea otter population growth in central California. In particular, if anthropogenic stressors were a strong driver of population trends in central California, we would expect higher survival at the "pristine" site (BSR) and lower survival at the highly-impacted site (MON); additionally, we would expect variation in hazard rates among sites that would correspond to the magnitude of environmental of anthropogenic stressors at these sites. The first prediction was not supported by the data: that is, survival rates were generally lower at BSR than at MBA (Figures 5 and 6). The second prediction was not strongly supported by the data either, although there was some suggestion of variation among sites (marginally significant) beyond that explained by body condition and reproductive status (Figure 4). Specifically, otters at the MB1 site experienced slightly lower hazard rates while otters at CAM experienced slightly higher hazard rates. This is interesting in light of earlier epidemiological analyses of study animals at these two sites, which indicated higher *Toxoplasma gondii* infection rates among sea otters at CAM than at MB1 (Johnson et al. 2009), and also elevated *Sarcocystus neurona* infection rates at CAM (Miller et al. 2010), suggesting the possibility that parasite exposure may have been contributing to differing survival rates at these sites in the early 2000's (Miller et al. 2002). However, no significant differences were found between BSR and MON with respect to site-specific hazard rates in the current study (beyond those associated with body condition and reproductive status, as discussed below), a pattern consistent with the similarity in parasite infection rates between these two areas (Chapter 9).

An alternative scenario to the one described above is that population trends are driven largely by density-dependent processes associated with resource abundance. Under this scenario, we would expect survival rates to be lower where food resources were more limiting and body condition (defined as relative body mass after controlling for sex and age differences) was poorer. This prediction was well supported by the data, with a significant positive relationship between survival rates and mean body

condition index across all sites, for both males and females (Figure 3). The inclusion of body condition as a covariate in the model explained much of the variation in survival rates (Figure 2A). Moreover, the elevated mortality risk associated with the end-lactation period for females in high density populations (Figure 2B) is also suggestive of the contribution of nutritional/energetic stress to elevated mortality and thus lower population growth. Females incur high energetic costs associated with pup rearing and provisioning (Thometz et al, unpublished manuscript) leading to increased foraging effort during this period (Chapter 5) and leaving them highly susceptible to any number of stressors they encounter when they come into estrous after weaning, such as parasite infections or aggression by males (Chapter 10). It is important to recognize that these results highlight important factors that affect survival probabilities, a key step in identifying the ultimate drivers of population growth, but they do not shed light on specific proximate mechanisms of mortality. Detailed analysis of cause-of-death in individual animals is required for this latter step, and the interaction between poor body condition associated with end-lactation period and exposure to environmental stressors is clearly evident in the necropsy results reported in Chapter 10.

Patterns of variation in pup survival were largely consistent with the results found for adult survival, in that female body condition was an important predictor of weaning success (Figure 10). The mother's age was also important, likely reflecting the fact that more experienced females were more likely to successfully navigate the challenges of pup rearing (Riedman et al. 1994, Monson et al. 2000, Tinker et al. 2006a). Variation in the magnitude of the hazard rate over the course of the pup dependency period (Figure 7) was consistent with expectations of life history theory (Monson et al. 2000). Specifically, females are expected to make a decision about whether to go through with pup rearing or to abandon the pup based on their own body condition at parturition: this corresponds to the high hazard rate in the weeks immediately after pup birth. Once they have committed to pup rearing the hazard rate is expected to drop, but as females near the end of the pup dependency the energetic load of pup provisioning may push some females beyond their metabolic and behavioral capacity to respond, resulting in a secondary increase in the hazard rates. These predicted patterns are well borne out by our results (Figure 7).

Combining all of our estimates of birth rates and survival rates of pups and independent otters, we calculated the expected population growth rates at Big Sur and Monterey using a projection matrix (following methods described in Tinker et al. 2006a), and compared these with the observed trends at each site from 2006-2010, based on census data from USGS and CDFW (www.werc.usgs.gov/seaottercount). At Big Sur, the growth rate predicted by a matrix model parameterized with vital rate estimates from our analysis of tagged otters was -3.0%, very similar to the observed trend of -2.5%. At Monterey, the growth rate predicted by a matrix model parameterized with vital rate estimates from our analysis of tagged otters was 4.5%, somewhat higher than the observed trend of 2.4% (though well within the range of parameter uncertainty). Overall our results successfully predict the direction and general magnitude of population trends at the two sites over the study period, suggesting that the measured rates of reproduction and survival of study animals were representative of the vital rates of other otters in the population.

Variation in sea otter survival rates across study sites in California points towards the role of density-dependent resource limitation in driving population trends in central California. Note that this conclusion does not preclude the importance of anthropogenic influences in contributing to overall mortality: for example, environmental stressors introduced by human activities may increase the net health impacts experienced by females during end-lactation. However, the spatial patterns in survival reported here suggest that such synergistic effects between anthropogenic stressors and resource-abundance are subtle, and not necessarily tied to point-source inputs of pollution. The latter point is emphasized by the fact that age-specific hazards for both adults and pups were highest at the "low impact" Big Sur site. Certain types of anthropogenic influences are indeed spatially diffuse: for example, terrestrial inputs of nitrogen-based fertilizers and other organic pollutants have been found to lead to increased frequency and intensity of toxic algal blooms, including those resulting in Domoic acid intoxication (Mos 2001), and these blooms occur across a broad geographic range and are not limited to areas immediately adjacent to the nutrient inputs (Lefebvre et al. 2002). Reducing anthropogenic inputs of nutrients may lower the cumulative health impacts to otters range-wide, and thus increase survival rates, at least to a point. Nonetheless, the strong effects of lowered body condition on hazard rates suggest that population abundance in areas of the highest sea otter densities (including both BSR and MON) is likely approaching the environmental carrying capacity, and thus continued recovery of the population will depend on range expansion and population growth in areas of lower density.

References

Banerjee, S., M. M. Wall, and B. P. Carlin. 2003. Frailty modeling for spatially correlated survival data, with application to infant mortality in Minnesota. Biostatistics **4**:123-142.

Beissinger, S. R. and D. R. McCullough. 2002. Population viability analysis. University of Chicago Press, Chicago, IL.

Brooks, S., A. Gelman, G. Jones, and X.-L. Meng. 2011. Handbook of Markov Chain Monte Carlo. Taylor & Francis US.

Caughley, G. 1966. Mortality patterns in mammals. Ecology **47**:906-918.

Eberhardt, L. L. and K. B. Schneider. 1994. Estimating sea otter reproductive rates. Marine Mammal Science **10**:31-37.

Halstead, B. J., G. D. Wylie, P. S. Coates, P. Valcarcel, and M. L. Casazza. 2012. Bayesian shared frailty models for regional inference about wildlife survival. Animal Conservation **15**:117-124.

Heisey, D. M. and B. R. Patterson. 2006. A Review of Methods to Estimate Cause-Specific Mortality in Presence of Competing Risks. Journal of Wildlife Management **70**:1544-1555.

Heisey, D. M., T. L. Shaffer, and G. C. White. 2007. The abcs of nest survival : Theory and application from a biostatistical perspective. Studies in avian biology **34**:13-33.

Johnson, C. K., M. T. Tinker, J. A. Estes, P. A. Conrad, M. Staedler, M. A. Miller, D. A. Jessup, and J. A. K. Mazet. 2009. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. Proceedings of the National Academy of Sciences of the United States of America **106**:2242-2247.

Lefebvre, K. A., S. Bargu, T. Kieckhefer, and M. W. Silver. 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon **40**:971-977.

Miller, M. A., P. A. Conrad, M. Harris, B. Hatfield, G. Langlois, D. A. Jessup, S. L. Magargal, A. E. Packham, S. Toy-Choutka, A. C. Melli, M. A. Murray, F. M. Gulland, and M. E. Grigg. 2010. A protozoal-associated epizootic impacting marine wildlife: Mass-mortality of southern sea otters (Enhydra lutris nereis) due to Sarcocystis neurona infection. Veterinary Parasitology **172**:183-194.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, and P. A. Conrad. 2002. Coastal freshwater runoff is a risk factor for Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). International Journal for Parasitology **32**:997-1006.

Monson, D. H., J. A. Estes, J. L. Bodkin, and D. B. Siniff. 2000. Life history plasticity and population regulation in sea otters. Oikos **90**:457-468.

Mos, L. 2001. Domoic acid: a fascinating marine toxin. Environmental Toxicology and Pharmacology **9**:79-85.

Pollock, K. H., J. D. Nichols, C. Brownie, and J. E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs **107**:1-98.

Riedman, M. L., J. A. Estes, M. M. Staedler, A. A. Giles, and D. R. Carlson. 1994. Breeding patterns and reproductive success of California sea otters. Journal of Wildlife Management **58**:391-399.

Sinha, D. and D. K. Dey. 1997. Semiparametric Bayesian Analysis of Survival Data. Journal of the American Statistical Association **92**:1195-1212.

Siniff, D. B. and K. Ralls. 1991. Reproduction, survival and tag loss in California sea otters. Marine Mammal Science **7**:211-229.

Spiegelhalter, D. J., N. G. Best, B. P. Carlin, and A. Van Der Linde. 2002. Bayesian measures of model complexity and fit. Journal of the Royal Statistical Society: Series B (Statistical Methodology) **64**:583-639.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008a. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., J. Bodkin, M. M. Staedler, G. Esslinger, and D. H. Monson. 2008b. Using TDR records to detect life history events in sea otters. Third International Biologging Science Symposium Monterey, CA.

Tinker, M. T., D. F. Doak, J. A. Estes, B. B. Hatfield, M. M. Staedler, and L. Bodkin James. 2006a. Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. Ecological Applications **16**:2293-2312.

Tinker, M. T., D. F. Doak, J. A. Estes, B. B. Hatfield, M. M. Staedler, and J. L. Bodkin. 2006b. Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. Ecological Applications **16**:2293-2312.

Tables

Table 1 Parameter estimates for *log*(hazard) function from Bayesian survival analysis (see text, equation 1). Note that exp(γ_0) represents *log*(baseline instantaneous hazards), while all other parameters represent *log*(hazard ratios)

Parameter	Description	mean	std. dev	CI_95_L	CI_95_H
γ_0	baseline *log* hazards (= 5-yr old female)	-3.238	1.982	-7.251	0.209
$\beta(1)$	body condition effect	-0.327	0.133	-0.600	-0.078
$\beta(2)$	reproductive status effect (end lactation)	1.268	0.299	0.661	1.837
Z(1)	random site effect, Big Sur, 08-12	-0.006	0.321	-0.702	0.655
Z(2)	random site effect, Monterey, 08-12	-0.113	0.294	-0.751	0.439
Z(3)	random site effect, Monterey 01-04	-0.443	0.346	-1.195	0.048
Z(4)	random site effect, Cambria, 01-04	0.324	0.323	-0.205	1.030
Z(5)	random site effect, Pt. Conception 01-04	0.145	0.366	-0.500	0.981
Z(6)	random site effect, San Nicolas I. 03-05	0.070	0.490	-0.862	1.156
$\delta(1,1)$	age-specific hazards, females age 1	-0.187	2.139	-4.077	4.056
$\delta(2,1)$	age-specific hazards, females age 2	-1.259	2.019	-4.823	2.753
$\delta(3,1)$	age-specific hazards, females age 3	-2.055	1.988	-5.525	1.845
$\delta(4,1)$	age-specific hazards, females age 4	-2.468	1.976	-5.921	1.393
$\delta(5,1)$	age-specific hazards, females age 5	-2.492	1.962	-5.913	1.369
$\delta(6,1)$	age-specific hazards, females age 6	-2.248	1.954	-5.667	1.650
$\delta(7,1)$	age-specific hazards, females age 7	-1.755	1.948	-5.142	2.151
$\delta(8,1)$	age-specific hazards, females age 8	-1.537	1.942	-4.909	2.386
$\delta(9,1)$	age-specific hazards, females age 9	-1.286	1.940	-4.650	2.617
$\delta(10,1)$	age-specific hazards, females age 10	-0.960	1.936	-4.293	2.945
$\delta(11,1)$	age-specific hazards, females age 11	-0.931	1.937	-4.286	2.941
$\delta(12,1)$	age-specific hazards, females age 12	-0.698	1.939	-4.051	3.188
$\delta(13,1)$	age-specific hazards, females age 13	-0.486	1.939	-3.856	3.372
$\delta(14,1)$	age-specific hazards, females age 14	-0.247	1.949	-3.655	3.592
$\delta(15,1)$	age-specific hazards, females age 15	-0.029	1.975	-3.598	3.675
$\delta(16,1)$	age-specific hazards, females age 16	0.119	2.055	-3.703	3.869
$\delta(17,1)$	age-specific hazards, females age 17	0.048	2.225	-4.347	3.991
$\delta(18,1)$	age-specific hazards, females age 18	-0.097	2.570	-5.475	4.390

Table 1 (continued, page 2 of 2)

Parameter	Description	mean	std. dev	CI_95_L	CI_95_H
δ(1,2)	age-specific hazards, males age 1	0.084	2.049	-3.545	4.148
δ(2,2)	age-specific hazards, males age 2	-0.748	2.003	-4.319	3.178
δ(3,2)	age-specific hazards, males age 3	-1.265	2.039	-4.979	2.678
δ(4,2)	age-specific hazards, males age 4	-1.614	2.081	-5.455	2.267
δ(5,2)	age-specific hazards, males age 5	-1.561	2.031	-5.275	2.264
δ(6,2)	age-specific hazards, males age 6	-1.206	1.962	-4.726	2.720
δ(7,2)	age-specific hazards, males age 7	-0.900	1.936	-4.322	3.027
δ(8,2)	age-specific hazards, males age 8	-1.019	1.941	-4.427	2.959
δ(9,2)	age-specific hazards, males age 9	-1.243	1.958	-4.672	2.759
δ(10,2)	age-specific hazards, males age 10	-1.458	1.978	-4.925	2.500
δ(11,2)	age-specific hazards, males age 11	-1.554	1.989	-5.057	2.365
δ(12,2)	age-specific hazards, males age 12	-1.439	1.981	-4.943	2.529
δ(13,2)	age-specific hazards, males age 13	-1.211	1.975	-4.733	2.805
δ(14,2)	age-specific hazards, males age 14	-1.113	1.989	-4.675	2.947
δ(15,2)	age-specific hazards, males age 15	-1.176	2.037	-4.849	3.009
δ(16,2)	age-specific hazards, males age 16	-1.426	2.169	-5.394	2.955
δ(17,2)	age-specific hazards, males age 17	-1.743	2.473	-6.687	3.044
δ(18,2)	age-specific hazards, males age 18	-2.059	3.044	-8.699	3.466

Table 2 Adult (5-yr-old) annual survival rate estimates for females (top) and males (bottom) at 6 study sites.

Sex	Study Site	mean	std. dev	CI_95_L	CI_95_H
Females	Big Sur, 08-12	0.925	0.028	0.863	0.969
	Monterey, 08-12	0.950	0.018	0.909	0.980
	Monterey 01-04	0.957	0.019	0.913	0.984
	Cambria, 01-04	0.878	0.044	0.781	0.948
	Pt. Conception 01-04	n/a	n/a	n/a	n/a
	San Nicolas I. 03-05	0.983	0.012	0.951	0.997
Males	Big Sur, 08-12	0.736	0.130	0.450	0.935
	Monterey, 08-12	0.932	0.036	0.849	0.984
	Monterey 01-04	0.877	0.069	0.714	0.974
	Cambria, 01-04	0.818	0.081	0.639	0.950
	Pt. Conception 01-04	0.900	0.048	0.793	0.974
	San Nicolas I. 03-05	0.962	0.029	0.886	0.995

Table 3 Annual age-specific survival rate estimates for females (top) and males (bottom) at 6 study sites

		Study Area					
Sex	Age	Big Sur, 08-12	Monterey, 08-12	Monterey, 01-04	Cambria, 01-04	Pt. Concept., 01-04	San Nic., 03-05
Female	1	0.682	0.779	0.810	0.636	n/a	0.929
	2	0.793	0.859	0.880	0.680	n/a	0.956
	3	0.894	0.929	0.940	0.830	n/a	0.979
	4	0.928	0.953	0.960	0.884	n/a	0.986
	5	0.930	0.954	0.961	0.886	n/a	0.986
	6	0.911	0.941	0.950	0.857	n/a	0.982
	7	0.859	0.906	0.920	0.777	n/a	0.971
	8	0.828	0.884	0.901	0.731	n/a	0.964
	9	0.784	0.853	0.875	0.668	n/a	0.954
	10	0.715	0.803	0.831	0.572	n/a	0.937
	11	0.707	0.798	0.827	0.563	n/a	0.936
	12	0.646	0.752	0.786	0.484	n/a	0.919
	13	0.583	0.703	0.743	0.408	n/a	0.901
	14	0.504	0.639	0.686	0.320	n/a	0.877
	15	0.426	0.573	0.625	0.243	n/a	0.849
	16	0.372	0.524	0.580	0.194	n/a	0.827
	17	0.398	0.548	0.602	0.217	n/a	0.838
	18	0.451	0.594	0.645	0.267	n/a	0.858
Male	1	0.635	0.749	0.627	0.628	0.645	0.870
	2	0.639	0.872	0.779	0.667	0.812	0.936
	3	0.697	0.922	0.862	0.786	0.883	0.961
	4	0.775	0.944	0.900	0.843	0.916	0.973
	5	0.764	0.941	0.895	0.836	0.912	0.971
	6	0.681	0.917	0.854	0.774	0.877	0.959
	7	0.594	0.889	0.807	0.706	0.837	0.945
	8	0.630	0.901	0.827	0.734	0.853	0.951
	9	0.691	0.920	0.859	0.781	0.881	0.960
	10	0.742	0.935	0.885	0.820	0.903	0.968
	11	0.763	0.941	0.895	0.835	0.911	0.971
	12	0.738	0.934	0.882	0.816	0.901	0.967
	13	0.683	0.918	0.855	0.775	0.877	0.959
	14	0.656	0.909	0.841	0.755	0.866	0.955
	15	0.674	0.915	0.850	0.768	0.873	0.958
	16	0.735	0.933	0.881	0.814	0.900	0.967
	17	0.799	0.951	0.912	0.861	0.926	0.976
	18	0.849	0.964	0.935	0.897	0.946	0.982

Table 4 Weaning success rates for females at Big Sur and Monterey study sites

Female Age	Big Sur, 08-12	Mont., 08-12
1	0	0
2	0	0
3	0.6243	0.636
4	0.615	0.627
5	0.620	0.632
6	0.652	0.663
7	0.668	0.679
8	0.648	0.660
9	0.640	0.652
10	0.684	0.694
11	0.730	0.740
12	0.758	0.767
13	0.781	0.789
14	0.798	0.805
15	0.804	0.810

Figures

Figure 1. Average age-specific annual survival rates for females (top panel) and males (bottom panel) across all study sites, with mean estimated values indicated by the solid red line and 95% credible intervals (CI_{95}) indicated by the grey shaded areas.

Figure 2. Bayesian posterior distribution**s** for model estimates of β_1, the effect of body condition (top panel), and β_2, the effect of reproductive status (bottom panel). In both cases the parameters represent the log of the hazard ratios, and so a value of "0" corresponds to a ratio of 1 (no change in hazard rates associated with the effect), while values significantly greater or less than 0 represent a change in hazard rate associated with the effect.

Figure 3. Relationship between southern sea otter body condition (BCI', relative mass at age) and survival rates for females (top panel) and males (bottom panel), as estimated from data collected at 6 study sites throughout California. Fitted power functions are shown with 95% confidence intervals: there are significant, positive relationships between survival and body condition across study sites for both females and males.

Figure 4. Bayesian posterior distribution**s** for model estimates of random differences in survival at 6 study sites throughout California. The random effect parameters represent the log of the hazard ratios for otters at each site after controlling for differences due to fixed effects included in the model. A value of "0" corresponds to a ratio of 1 (no change in hazard rates relative to the overall mean value), while values significantly greater or less than 0 represent increases or decreases in hazards associated with that site.

Figure 5. Bayesian posterior distribution**s** for model estimates of adult (5-year old) female survival at 4 study sites in California (top panel), and posterior distribution for estimated difference in survival rates between adult females at Big Sur and Monterey (bottom panel: a negative value indicates lower survival at Big Sur relative to Monterey).

Figure 6. Bayesian posterior distribution**s** for model estimates of adult (5-year old) male survival at 4 study sites in California (top panel), and posterior distribution for estimated difference in survival rates between adult males at Big Sur and Monterey (bottom panel: a negative value indicates lower survival at Big Sur relative to Monterey).

Figure 7. Log hazard rates for pups as a function of pup age (measured in weeks since birth). Note that lower log hazard rates indicate higher survival probability. Mean estimated values indicated by the solid red line and 95% credible intervals (CI_{95}) indicated by the grey shaded areas.

Figure 8. Log hazard ratio for pups as a function of mothers age (years), with a value of 0 indicating a hazard ratio of 1 relative to a 3-year-old female (the baseline value). Note that negative log hazard ratios indicate higher survival probability relative to a 3-year-old. Mean estimated values indicated by the solid red line and 95% credible intervals (CI_{95}) indicated by the grey shaded areas.

Figure 9. Bayesian posterior distribution for model estimates of β_3, the effect of mother's body condition on pup survival. The parameter represents the log of the hazard ratios, and so a value of "0" corresponds to a ratio of 1 (no change in hazard rates relative to a female in "average" condition), while a value significantly less than 0 represents a reduction in hazard rate associated with mothers in higher than average condition (or an increase in hazards for mothers in poor condition).

Figure 10. Weaning success rates (pup survival) plotted as a function of mothers age (top panel) and mothers body condition (BCI, relative mass at age). Mean estimated values indicated by the solid red line and 95% credible intervals (CI_{95}) indicated by the grey shaded areas.

Figure 11. Bayesian posterior distribution**s** for model estimates of weaning success for an adult (5-year old) female at the Big Sur and Monterey study sites (top panel), and posterior distribution for estimated difference in weaning success rates between Big Sur and Monterey (bottom panel: a negative value indicates lower pup survival at Big Sur relative to Monterey).

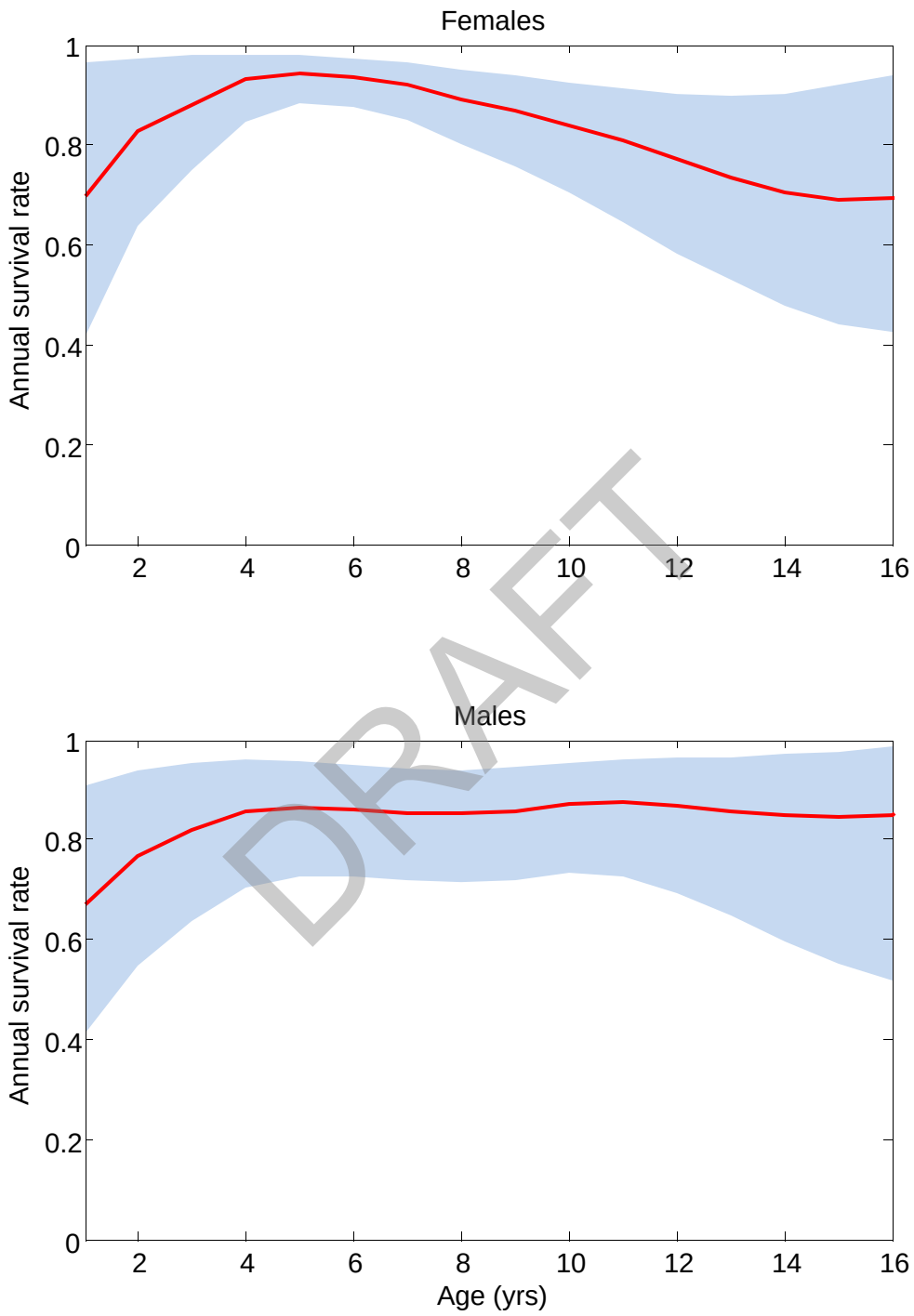
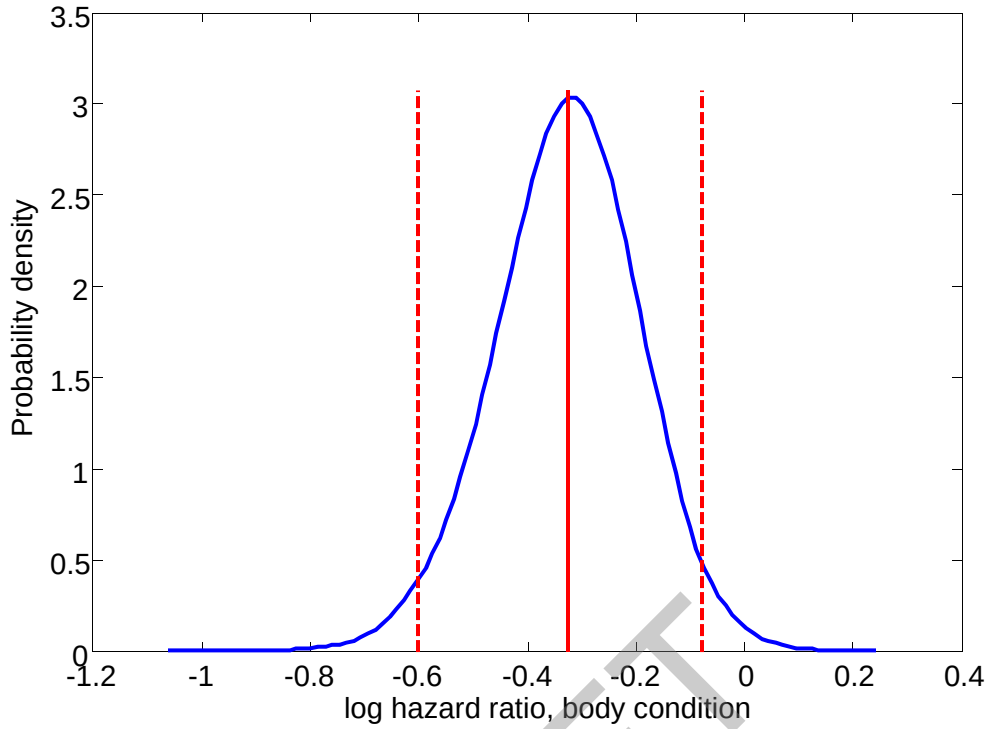
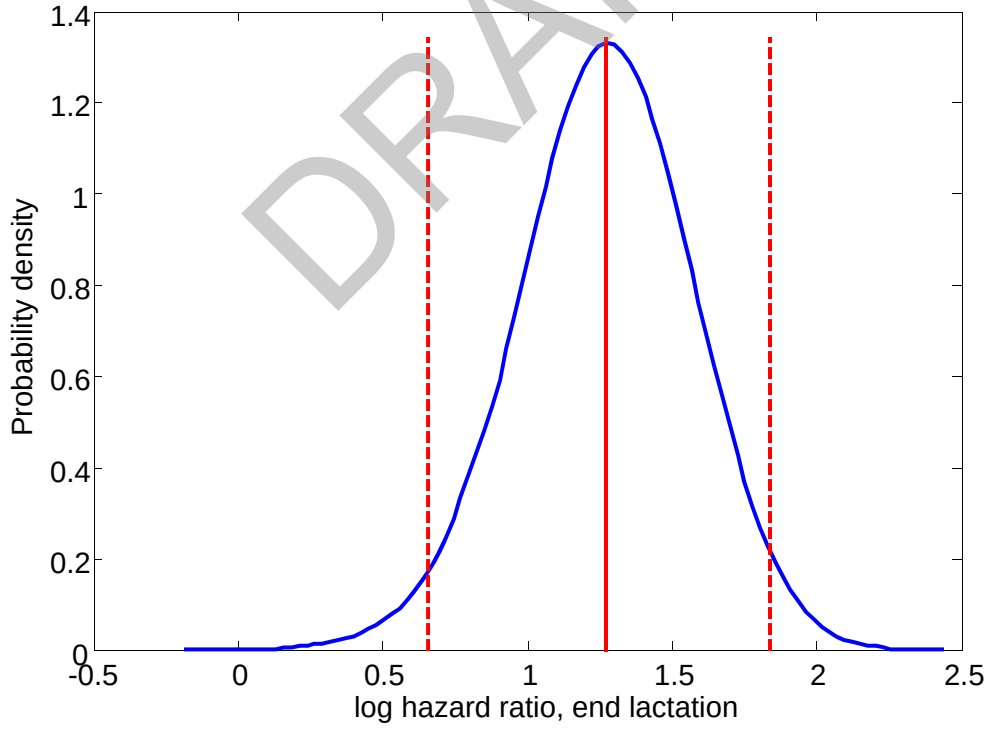


Figure 1





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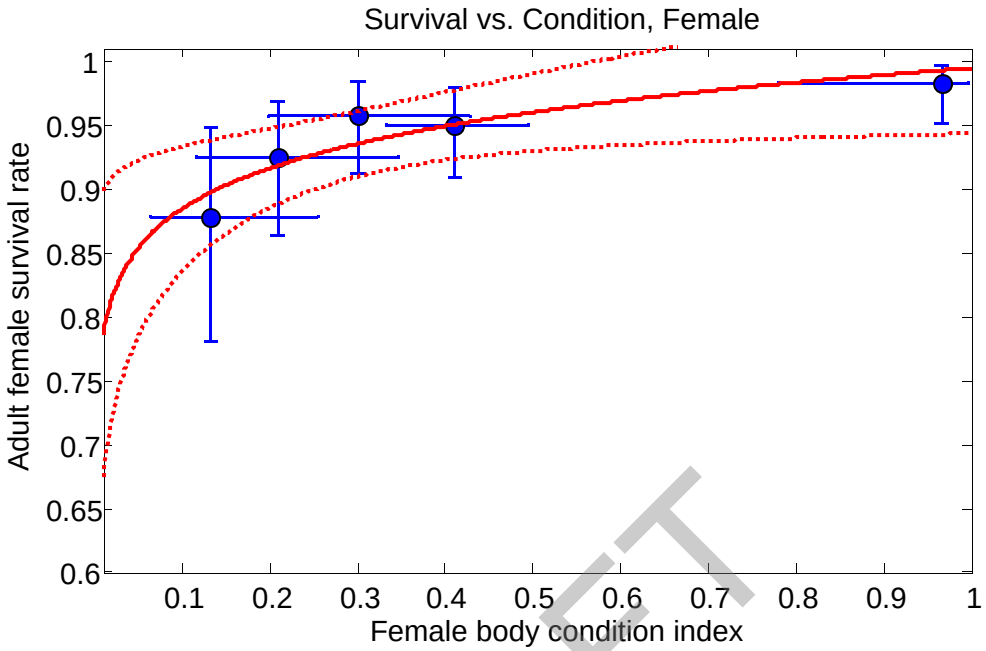

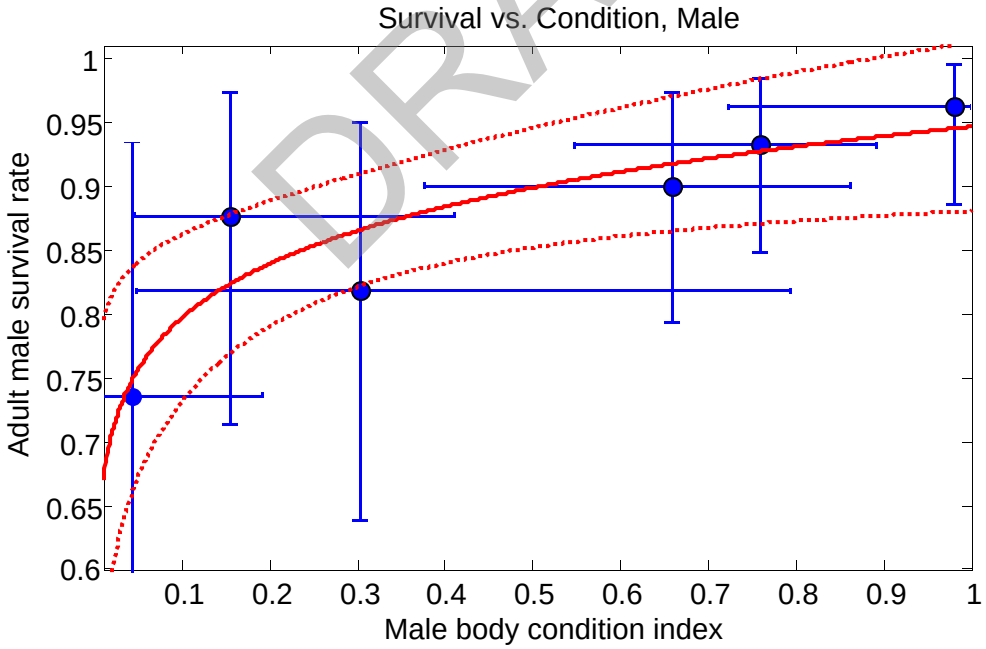

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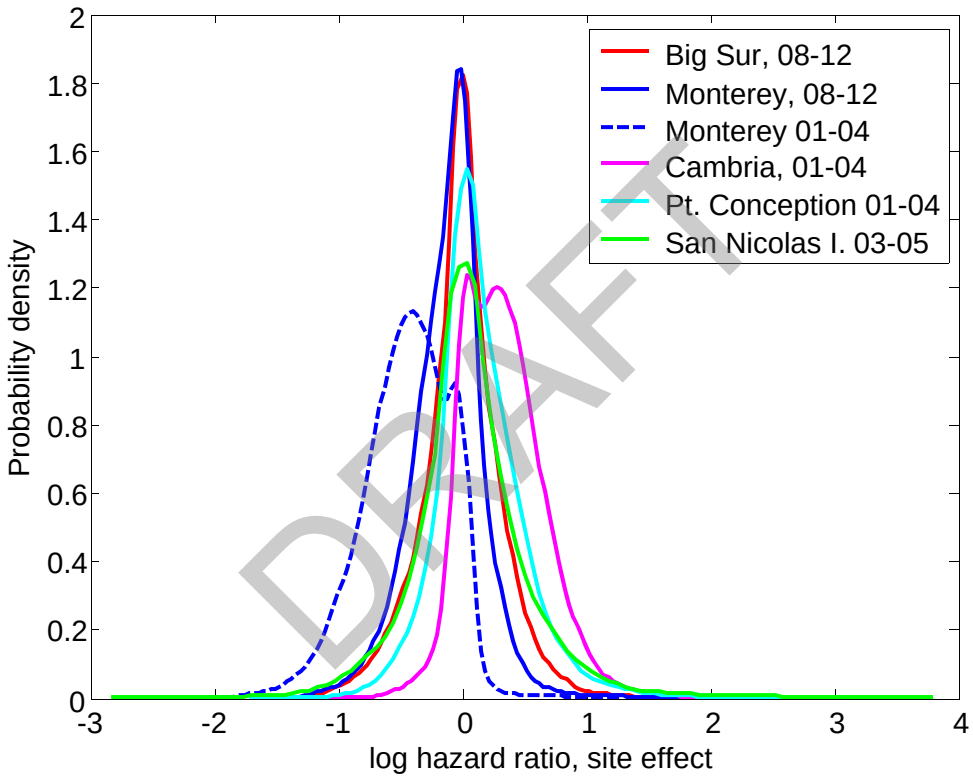

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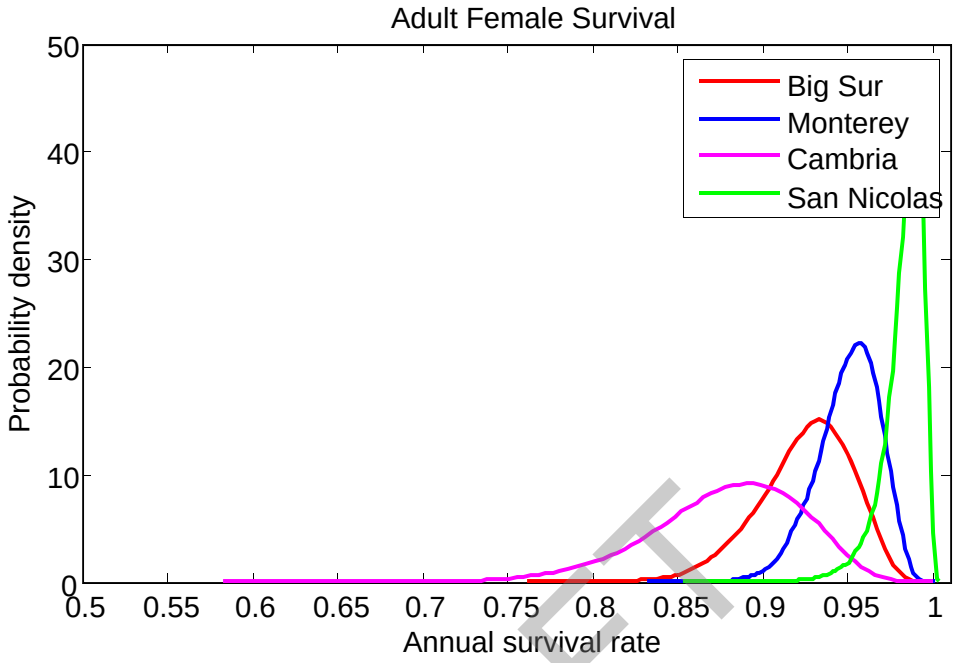

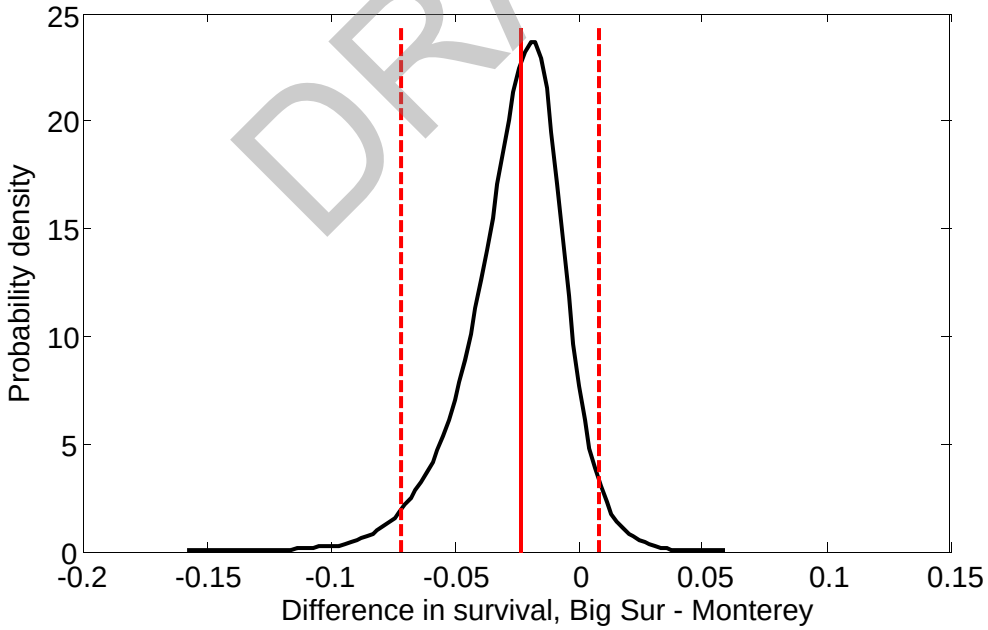

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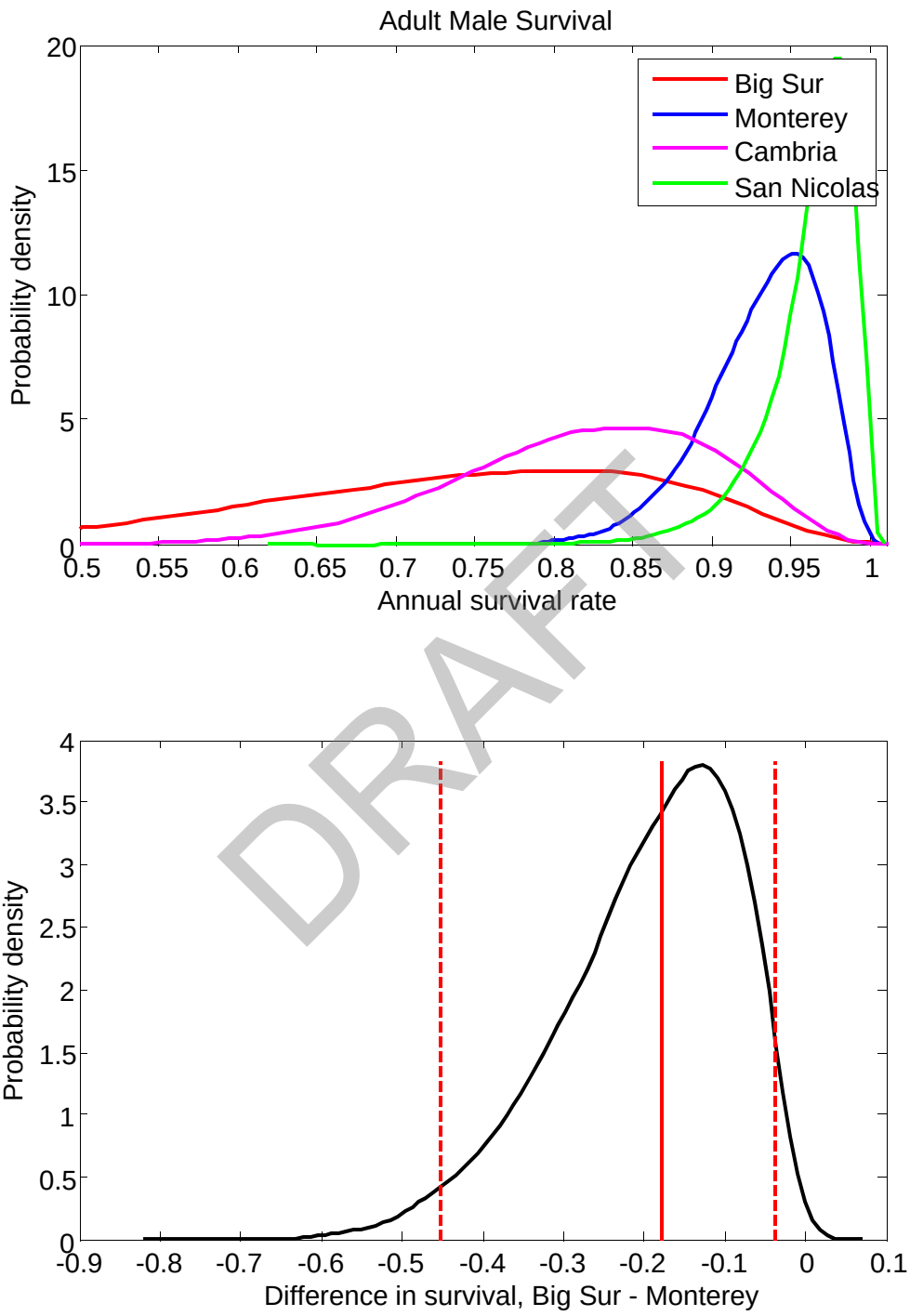

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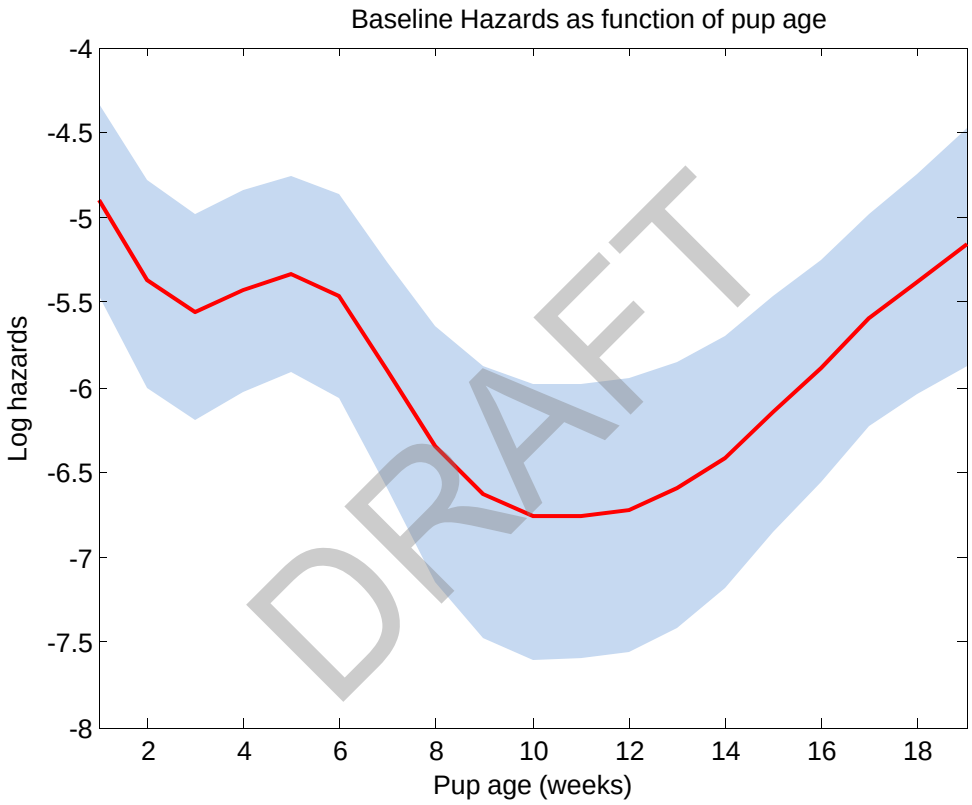

Figure 7

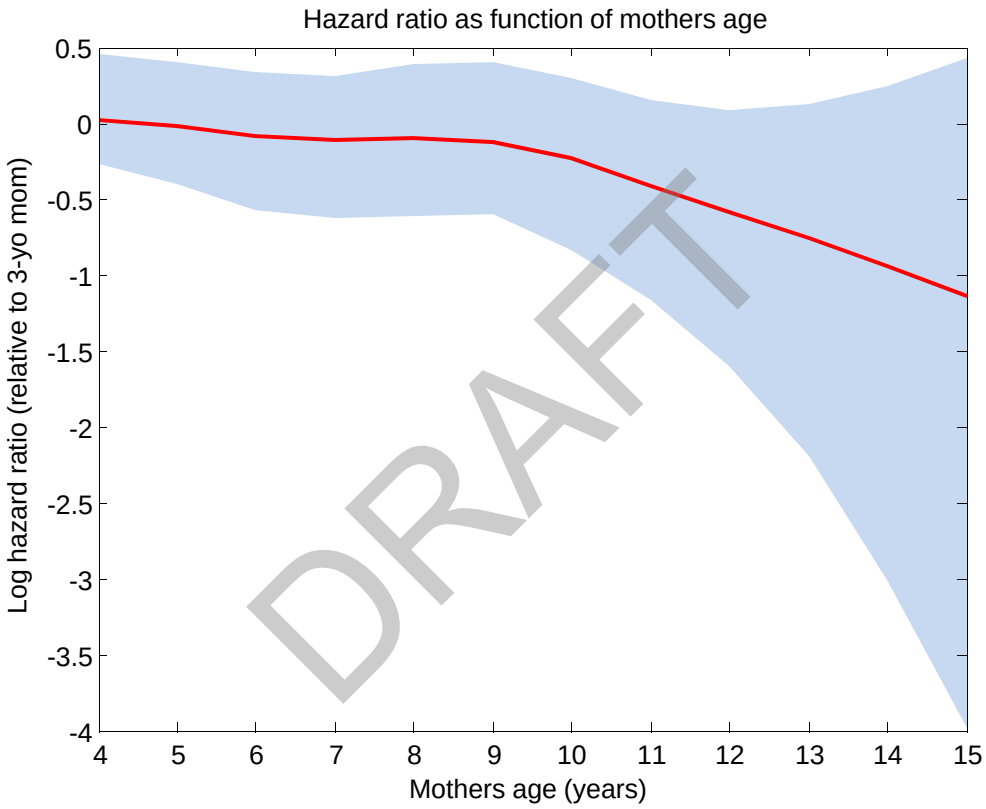

Figure 8

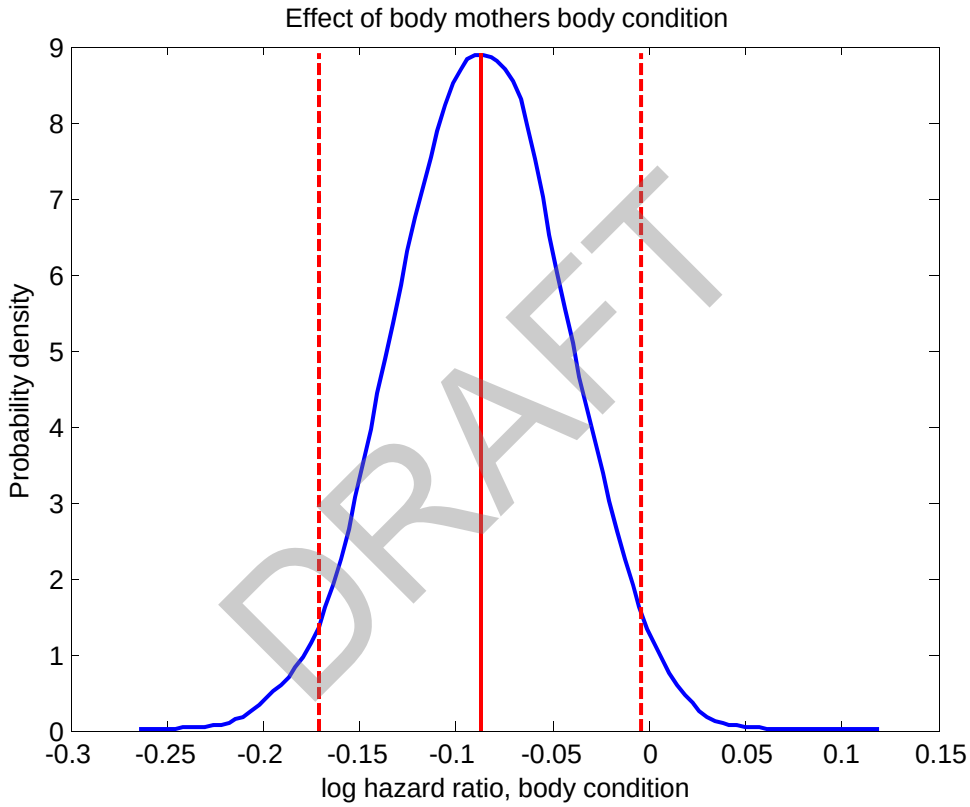

Figure 9

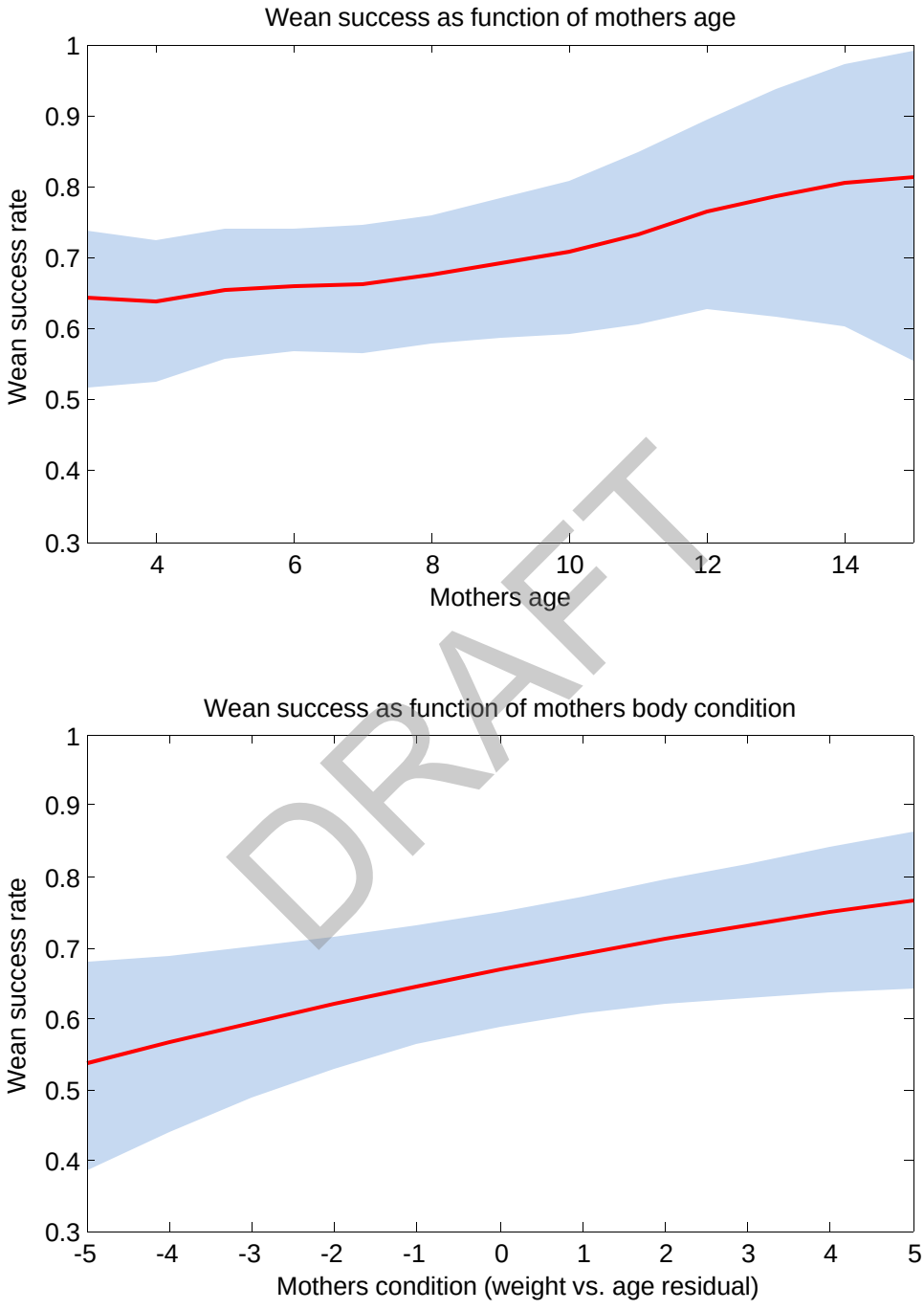

Figure 10

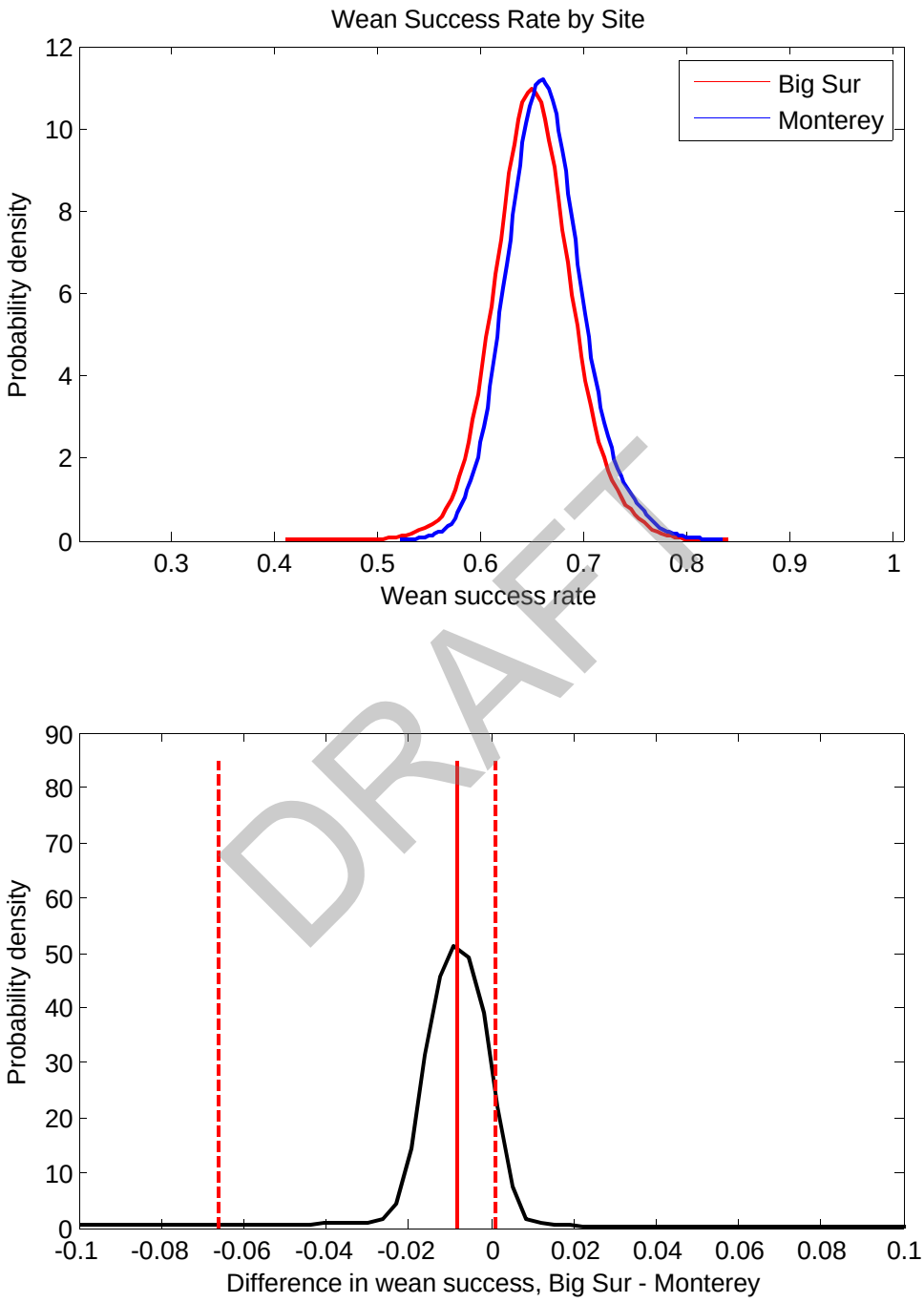

Figure 11

Chapter 9. Epidemiological Analysis of Protozoal Infections in Sea Otters at Big Sur and Monterey

Tristan Burgess[1], M. Tim Tinker[2], Christine Johnson[1], Holly MacCormick[3], Ann Melli[1] and Pat Conrad[1]

1. Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA
2. U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA
3. Ecology and Evolutionary Biology Department, University of California, Santa Cruz, CA

Introduction

Protozoal disease, including disease caused by the parasite *Toxoplasma gondii* (Miller et al. 2004), is an important cause of mortality in southern sea otters (Kreuder et al. 2003). Along with the closely-related parasite *Sarcocystus neurona* (henceforth *Sn*), *T. gondii* (henceforth *Tg*) is considered an important component of the land-to-sea transfer of infectious parasites that affect sea otter health (Miller et al. 2007, Conrad et al. 2009). The basis for assuming that *Tg* and *Sn* originate from land is the well-documented fact that the definitive hosts for *Sn* parasites are opossums (Miller et al. 2010), while the definitive hosts for *Tg* parasites are members of the cat family, including domestic cats, bobcats and mountain lions (Miller et al. 2002a). Understanding the factors that cause variation in the infection rates of sea otters with *Tg* is important because 1) protozoal disease is a major cause of mortality in sea otters, and information on the mechanisms of infection can lead to improved management policy and conservation actions; 2) to some extent we can use *Tg* infections as a model and proxy for exposure to other terrestrial pathogens and pollutants, in order to understand the role of land-to-sea pollution on sea otter health (Conrad et al. 2005, Jessup et al. 2007); and 3) Sea otters represent a useful sentinel species on the basis of their high trophic level, spatial philopatry and tractability for study, and thus information gleaned from epidemiological analysis of sea otter infections can be applied more broadly to nearshore marine ecosystems.

Past research has shown that a number of environmental, behavioral and demographic variables can lead to variation in infection risk for *Tg*. Coastal freshwater run-off is one factor that has been shown to increase risk of exposure to *Tg* (Miller et al. 2002a). Other reported risk factors include sea otter age, sex, mobility and prey choice (Johnson et al. 2009). The role of diet as a risk factor for infection is an interesting feature of the sea otter study system, and reflects two key facts: a) many disease-causing parasites that affect sea otters are acquired through prey consumption and not by contact with conspecifics, and b) sea otters in central California exhibit very high degrees of individual specialization in diet, such that individuals living in the same location at the same time utilize different subsets of the available prey species (Estes et al. 2003, Tinker et al. 2008, Tinker et al. 2012). During the course of the current study, extensive data on sea otter habitat use, movements and foraging ecology were collected, allowing us to test their potential contribution to variation in *Tg* infections.

Using results from serological analysis of blood samples collected from all study animals, we measured and compared patterns of *Tg* infection in sea otters from Big Sur and Monterey. We used standard epidemiological techniques to test for factors that could increase infection risk, including geographic

location and proximity to urban and agricultural centers, age, sex, home range size, mobility and diet composition. Our goal was to test one of the primary hypotheses of the Monterey/Big Sur comparison study:

> 1) Sea otters living in areas adjacent to human population centers and areas heavily impacted by runoff or sewage are more likely to be exposed to pathogens and toxins of public health importance than those in more pristine areas.

Methods

Study Population

Samples from all 135 study animals (Chapter 1, Table 1) were included in serological analysis, along with samples from an additional 25 sea otters captured and sampled in Monterey between 2006 and 2008, for a total of 159 individuals. Data on diet composition and home range use were assembled for all of these animals for which adequate observational data existed.

Serum collection and analysis

Whole blood was collected from otters captured as described in Chapter 1. Blood samples were allowed to clot and centrifuged at 1500 x *g* for 10 minutes and stored at -70°C until testing. Serum was drawn off and tested using an indirect fluorescent antibody test (IFAT). This test has been standardized by serial dilution and a cutoff of $\geq$ 1:320 has been determined to be optimal detection of *Toxoplasma gondii* infection in southern sea otters of known infection status (Miller et al. 2002b).

Risk Factors

Basic risk factors that were evaluated for association with *T. gondii* infection status included study site (Big Sur vs. Monterey, assumed to be a proxy for proximity to human influences), age, length, weight and sex. In addition to these variables, we assessed potential dietary risk factors including diet type (individuals were assigned to 1 of 5 dietary groupings using hierarchical cluster analysis of species-specific prey consumption rates: see Chapter 6), diet diversity (Shannon index) and snail consumption (as a percentage of biomass consumed). Home range area (km^2), the number of centers of use (see Chapter 4) and the total linear span of the home range (km) were assessed as indices of individual mobility.

Univariate Analysis

Chi-square tests, fisher's exact tests (when expected cell counts were less than 5) and t-tests (for continuous variables) were used to assess the association of *Tg* antibody status and potential risk factors. P-values < 0.05 were considered significant and odds ratios and 95% confidence intervals were calculated for categorical variables. Analyses were conducted using R 2.15 (R core development team).

Logistic Regression Analysis

Logistic regression models were used to examine effects of individual animal variables (age, length, weight, sex), diet type and the movement/habitat use metrics on *Tg* infection status. Variables with P<0.25 in the univariate analysis were initially used to build a multivariate model in a modified forward stepwise fashion. The addition of other excluded variables was assessed after the initial model selection

stage by adding these to the model and checking for improved model fit. Models were selected on the basis of reduced AIC and likelihood ratio tests. A first order site-sex interaction term was added specifically to test the hypothesis that disease risk might vary between sites for females but not for males because the greater site fidelity of females (see Chapter 4) makes them more reliable indicators of local environmental influences. Collinearity of predictors was assessed using linear regression. Collinear predictors were assessed by autoregression and residuals of the autoregression model were used to assess whether residual predictive value remained. Odds ratios and 95% confidence intervals were calculated to measure the strength of the association between each risk factor and *Tg* serological status. Analyses were conducted using R 2.15 (R core development team).

Results

On the basis of univariate analysis (Table 2) body length (P=0.001) and body weight (P=0.005) were significantly associated with *Tg* infection status, but these two variables were strongly correlated ($P<2.2x10^{-16}$) and both were associated with age ($P=1.32x10^{-11}$; $P=7.34x10^{-9}$). Sequential autoregression of length (Wald P<0.001) and weight (P=0.009) against age found the residuals were significant predictors of infection status while simultaneously accounting for age, but neither approach yielded a superior model (based on AIC) to that including length alone, so only length was used in further models.

Diet class was a significant predictor of disease risk, but not all classes differed significantly. The best fitting model compared diet classes 2 and 4 to a reference category containing classes 1, 3 and 5. Class 2 (abalone and cancer crab) did not differ significantly from reference but model fit (based on AIC) was improved by the retention of this category. Class 4 (primarily marine snails and some kelp crab) was associated with a significant increase (P=0.024) in infection risk. Infection risk did not differ significantly between sites in the univariate analysis (P=0.327), but when stratified by sex, female otters exhibited significantly greater infection risk at Big Sur compared with Monterey Bay (P=0.012). Infection risk did not differ by site among males (P=0.271). For this reason an interaction term of site*sex was included in the final multivariate model.

The multivariate model (Table 3) shows the relative contributions of the various risk factors while adjusting for differences between sample populations. The final model identified increasing length (P=0.022) and membership of the snail specialist dietary grouping (P=0.013) as significant risk factors. Additionally, the site by sex interaction was significant (P=0.02) in this model, and the inclusion of this variable improved model fit based on AIC. This term indicates that association of site with observed *Tg* antibody prevalence differed significantly between sexes. After adjusting for differences in all significant variables identified, the odds ratio (95% CI) for *Tg* seropositivity for females at Big Sur, compared with those at Monterey was 22.18 (1.64-299.15), whereas the same ratio for males was 1.46 (0.07-29.52).

Discussion

Our analyses confirmed earlier studies (Miller et al. 2002a, Johnson et al. 2009) in showing that there is substantial variation in *Tg* infection rates in sea otters in central California, and that much of this variation can be explained by differences in risks associated with location, age, sex and diet. Some of the risk factors we found were entirely consistent with earlier work, while other patterns were more surprising.

The finding of increasing infection rates with age was not entirely surprising because individuals presumably have a constant infection risk when consuming prey (assuming that some small proportion of prey are transport hosts for the parasite), but once infected they will remain infected, so the cumulative likelihood of exposure to the parasite should increase with age. However, if infection with the parasite carries some mortality risk, we might expect a "survivor bias" to emerge over time that would act to reduce the realized infection rates of older animals. A survivor bias occurs when infected individuals are more likely to drop out of the population due to disease, leaving a higher proportion of un-infected adults. The combination of these two opposing trends might be expected to lead to a non-linear, declining (or asymptotic) age effect. This may explain the finding that body length was a better predictor of infection risk than was age estimate. Body length is an increasing, asymptotic function of age (see Chapter 7), and may in some cases be a superior predictor of age than estimation based on tooth wear, especially in younger animals. Length might also be a better predictor of *Tg* infection than age because of its asymptotic nature, if infection rates are also an asymptotic function of age.

As with a previous analysis of *Tg* infection risks (Johnson et al. 2009), individual diet specialization was found to be a significant predictor of infection likelihood. In our analyses, three of 5 diet specialist types showed no difference in infection risks, but two of the diet types were associated with increased risk. Otters that specialized on marine snails were 42x more likely to be infected with *Tg* than were otters specializing on other prey types, consistent with previously published findings (Johnson et al. 2009). Abalone specialists also had a tendency towards greater infection risk than did other diet types, in contrast with the findings of Johnson et al (2009); however, this latter effect was not statistically significant (Table 4). Our findings add further support to the conclusions of Johnson et al (2009) about the association between marine snail consumption and the risk of infection with *Tg*. This is especially significant considering that the study animals used in the current analysis are entirely independent from the sample of animals used in the earlier analysis, and represent an entirely different area of the coast (Big Sur). The close agreement in findings between the two studies suggests that the processes underlying the snail-*Tg* association are relatively consistent over time and space. Ongoing research supported by the National Science Foundation (P. Conrad *et al*) is aimed at elucidating these mechanisms.

Sea otter mobility was not a significant predictor of infection risk, at least as measured by the three variables we used in the analyses (total home range area, the number of distinct centers of use, and the total linear span of the home range). We had expected that otters that utilized a larger portion of the coastline might have greater likelihood of passing through an area where environmental loading of *Tg* oocysts was higher, and thus have a higher risk of infection, a pattern reported by Johnson et al (2009) for *Sn* infection risk. Our failure to find such a pattern in the current study could reflect a lack of statistical power, or could indicate that individuals that integrated exposure risks over a larger area (i.e. more mobile otters with longer home range areas) experienced less risk than less mobile individuals that happened to have a home range within a high risk area. In other words, *Tg* infection risk varies locally, and spending lots of time within a high risk area represents a far greater risk than spending a bit of time in many areas. A final possible explanation for our results is that any increased risk associated with greater mobility was better explained by other factors: for example, males (that are generally more

mobile) had a 3.6x higher risk of infection than females (that are less mobile); however, this general sex effect was complicated by the unusually high infection risk of females at Big Sur.

The most unexpected finding from our analyses was the difference in *Tg* infection risk between study sites: females from the Big Sur study site had a 39x greater risk of infection than did females in Monterey (after controlling for other effects such as body length and diet type). There was also a suggestion of higher infection risk for Big Sur males as compared to Monterey males, but this effect was not statistically significant. The fact that the site effect was more pronounced for females is itself not surprising, as females exhibit far greater site fidelity (see Chapter 4) and thus are more reliable indicators of local infection risks than males, whose greater mobility tends to integrate risks over a broader area. What was surprising was the nature of the site effect: females at Big Sur had home ranges that were far from any human population centers or areas impacted by agricultural runoff, storm drains, or other point sources of land-sea pollution, and yet had far higher infection rates than Monterey females whose home ranges encompassed all of these anthropogenic stressors. While perplexing at first, closer consideration of the causes of *Tg* infection suggest a number of possible explanations for this pattern. Sea otters are thought to become infected with *Tg* after consuming prey that contain oocysts (the infective stage of the parasite) that have been shed by a felid, transported from the land to the sea, and incorporated into the marine food web. All steps in this chain are likely to differ between the Monterey and Big Sur study sites. Monterey has a higher density of domestic and feral house cats, while Big Sur has fewer house cats but a high density of bobcats and mountain lions that also carry the *Tg* parasite (L. VanWormer, unpublished manuscript; Miller et al. 2008). Hydrological processes differ greatly between the two sites as well: the Monterey area has many storm drains that drain urban areas with impervious surfaces, as well as a number of major rivers (the Carmel and Salinas rivers) that drain large agriculturally-dominated watersheds; conversely, Big Sur has many small, unpopulated watersheds with very steep terrain, such that particles deposited on land can be transported quickly to the marine environment during rain events. Finally, the steep bathymetry and rocky sub-tidal habitat at Big Sur means that kelp beds that are very close to shore (CDFW Marine Region, GIS Unit; Hallenbeck et al. 2012), most notably at watershed pour points, and so sea otters feeding and resting in these kelp beds are in close proximity to putative inputs of *Tg* oocysts; in contrast, the shallow bathymetry of the Monterey study site means that kelp beds are farther from shore and there are no kelp beds close to major watershed pour points. Any or all of these physical/environmental differences may contribute to the differing risk of infection at Big Sur and Monterey: for example, it is clear that wild felids at Big Sur must be a significant source of infective oocysts, but the hydrological and bathymetric characteristics of this area may facilitate transfer of oocysts from land to the marine food web, and thus magnify infection risk.

In conclusion, our results were not supportive of our main hypothesis: we found that sea otters at Monterey that were adjacent to human population centers and areas heavily impacted by runoff or sewage were ***not*** more likely to be exposed to pathogens than otters at the more pristine Big Sur site, at least in the case of *Toxoplasma gondii*. In fact our results showed exactly the opposite pattern, and thus point towards other risk factors such as individual age, prey use patterns, and (potentially) the particular physical/environmental characteristics of terrestrial watersheds and coastal landscapes. Our results also

highlight the importance of wild felids as contributors of *Tg* to the marine environment (Miller et al. 2008). Overall these results suggest a need for a more nuanced understanding of disease processes in sea otter populations. Such an understanding will almost certainly include the role of land-sea pollution, but this factor must be placed in the context of specific mechanisms of exposure/infection that incorporate both environmental characteristics of the habitat and ecological interactions of the parasites and hosts.

References

Conrad, P. A., M. A. Miller, C. Kreuder, E. R. James, J. Mazet, H. Dabritz, D. A. Jessup, F. Gulland, and M. E. Grigg. 2005. Transmission of Toxoplasma: Clues from the study of sea otters as sentinels of Toxoplasma gondii flow into the marine environment. International Journal for Parasitology **35**:1155-1168.

Conrad, P. A., E. VanWormer, K. Shapiro, M. Miller, C. Kreuder-Johnson, T. Tinker, M. Grigg, J. Largier, T. Carpenter, and J. K. Mazet. 2009. Tracking Toxoplasma Gondii from Land to Sea. American Journal of Tropical Medicine and Hygiene **81**:198.

Estes, J. A., M. L. Riedman, M. M. Staedler, M. T. Tinker, and B. E. Lyon. 2003. Individual variation in prey selection by sea otters: Patterns, causes and implications. Journal of Animal Ecology **72**:144-155.

Hallenbeck, T. R., R. G. Kvitek, and J. Lindholm. 2012. Rippled scour depressions add ecologically significant heterogeneity to soft-bottom habitats on the continental shelf. Marine Ecology Progress Series **468**:119-133.

Jessup, D. A., M. A. Miller, C. Kreuder-Johnson, P. A. Conrad, M. T. Tinker, J. Estes, and J. A. K. Mazet. 2007. Sea otters in a dirty ocean. Javma-Journal of the American Veterinary Medical Association **231**:1648-1652.

Johnson, C. K., M. T. Tinker, J. A. Estes, P. A. Conrad, M. Staedler, M. A. Miller, D. A. Jessup, and J. A. K. Mazet. 2009. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. Proceedings of the National Academy of Sciences of the United States of America **106**:2242-2247.

Kreuder, C., M. A. Miller, D. A. Jessup, L. J. Lowenstine, M. D. Harris, J. A. Ames, T. E. Carpenter, P. A. Conrad, and J. A. K. Mazet. 2003. Patterns of mortality in southern sea otters (Enhydra lutris nereis) from 1998-2001. Journal of Wildlife Diseases **39**:495-509.

Miller, M. A., P. A. Conrad, M. Harris, B. Hatfield, G. Langlois, D. A. Jessup, S. L. Magargal, A. E. Packham, S. Toy-Choutka, A. C. Melli, M. A. Murray, F. M. Gulland, and M. E. Grigg. 2010. A protozoal-associated epizootic impacting marine wildlife: Mass-mortality of southern sea otters (Enhydra lutris nereis) due to Sarcocystis neurona infection. Veterinary Parasitology **172**:183-194.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, and P. A. Conrad. 2002a. Coastal freshwater runoff is a risk factor for Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). International Journal for Parasitology **32**:997-1006.

Miller, M. A., I. A. Gardner, A. Packham, J. K. Mazet, K. D. Hanni, D. Jessup, J. Estes, R. Jameson, E. Dodd, B. C. Barr, L. J. Lowenstine, F. M. Gulland, and P. A. Conrad. 2002b. Evaluation of an indirect fluorescent antibody test (IFAT) for demonstration of antibodies to Toxoplasma gondii in the sea otter (Enhydra lutris). Journal of Parasitology **[print] 88**:594-599.

Miller, M. A., M. E. Grigg, C. Kreuder, E. R. James, A. C. Melli, P. R. Crosbie, D. A. Jessup, J. C. Boothroyd, D. Brownstein, and P. A. Conrad. 2004. An unusual genotype of Toxoplasma gondii is common in California sea otters (Enhydra lutris nereis) and is a cause of mortality. International Journal for Parasitology **34**:275-284.

Miller, M. A., M. E. Grigg, W. A. Miller, H. A. Dabritz, E. R. James, A. C. Melli, A. E. Packham, D. Jessup, and P. A. Conrad. 2007. Toxoplasma gondii and Sarcocystis neurona infections of pacific coastal sea otters in California, USA: evidence for land-sea transfer of biological pathogens. Journal of Eukaryotic Microbiology **54**:48S-49S.

Miller, M. A., W. A. Miller, P. A. Conrad, E. R. James, A. C. Melli, C. M. Leutenegger, H. A. Dabritz, A. E. Packham, D. Paradies, M. Harris, J. Ames, D. A. Jessup, K. Worcester, and M. E. Grigg. 2008. Type X Toxoplasma gondii in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. International Journal for Parasitology **38**:1319-1328.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., P. R. Guimarães, M. Novak, F. M. D. Marquitti, J. L. Bodkin, M. Staedler, G. Bentall, and J. A. Estes. 2012. Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecology Letters **15**:475-483.

Tables

Table 1: Univariate analysis of putative categorical risk factors for *Toxoplasma gondii* antibody status in n=159 southern sea otters (*Enhydra lutris*) sampled 2007-2010 at Monterey and Big Sur. *P*-values are chi-square *p*-values for site and Fisher's exact test for sex and diet specialization.

Risk Factor	Group	Seroprevalence	Odds Ratio	95% CI	*P*-Value
Sex	Female	0.12 (n=130)	1	-	
	Male	0.32 (n=28)	3.63	1.39-9.47	0.0162
Site	Monterey	0.13 (n=94)	1	-	
	Big Sur	0.18 (n=65)	1.55	0.65-3.7	0.4467
Diet	Type 1	0.06 (n=48)	1.00	-	0.1065
	Type 2	0.25 (n=12)	5.00	0.87-28.86	
	Type 3	0.06 (n=16)	1.00	0.1-10.35	
	Type 4	0.25 (n=12)	5.00	0.87-28.86	
	Type 5	0.29 (n=7)	6.00	0.8-44.95	
	Unknown	19 (n=64)	3.46	0.92-13.04	

Table 2: Associations of continuous variables with *Toxoplasma gondii* antibody status in n=159 southern sea otters (*Enhydra lutris*) sampled 2007-2010 at Monterey and Big Sur. Welch two-sample *t*-test.

Risk Factor	*t*	df	*P*-Value
Age	-2.775	35.67	0.0087
Length	-3.56	29.83	0.0013
Weight	-3.086	28.04	0.0045

Table 3: Binary logistic regression models showing the predicting *Toxoplasma gondii* antibody status on the basis of body length, site, sex, diet type (note that for this analysis, the 5 diet specialization types were collapsed to just 3: type 2, type 4, and type 1/3/5), % snails in the diet, home range area (hrarea), number of distinct home range centers of use, and the "range span" (length of coastline encompassed by all movements).

Model	Df	AIC
length+site+snail	84	50.351
length+diet+site	83	46.221
length+diet+site+snail+cou	81	45.584
length+diet+site+snail+diversity	81	45.492
length+diet+site+snail+hrarea	81	45.485
length+diet+site+snail+span	81	45.401
length+diet+snail+sex+sex:site	80	44.936

Table 4: Multivariable logistic regression model of risk factors for *Toxoplasma gondii* serum antibody status. Note that the final model does not contain snail consumption as a proportion of biomass, since (although the variable slightly improved model fit) it was not a significant predictor of antibody status, and inclusion of this variable precluded useful estimation of the other variables due to inadequate power.

Risk Factor		**Adjusted odds ratio**	**95% CI**	***P*-Value**
Length		1.53	1.04-2.23	0.0291
Diet	All other diet types	1.00	-	
	Abalone specialists	5.18	0.29-93.07	0.2642
	Snail specialists	42.26	1.98-900.87	0.0165
Sex	Female	1.00	-	
	Male	0.86	0.01-125.12	0.9527
Sex:Site	Female:Big Sur vs Monterey	38.72	1.22-1226.93	0.0381
	Male:Big Sur vs Monterey	4.33	0.18-103.88	0.366

Chapter 10. Preliminary Findings from Necropsy of Tagged Sea Otters from the Monterey-Big Sur Study

M. A. Miller[1], E. Dodd[1], E. Berberich[1], F. Batac[1], Laird Henkel[1], J. Kunz[1] and Tim Tinker[2]
[1]California Department of Fish & Wildlife, Marine Wildlife Veterinary Care & Research Center
[2]United States Geological Survey, Western Ecological Research Center

Background

All VHF transmitter-implanted otters from the Monterey and Big Sur coastal regions that stranded, died, and where the carcass was recovered, plus any VHF transmitter-implanted animals that were euthanized during the course of the study (July 2008 through March 2012), were submitted to the Marine Wildlife Veterinary Care and Research Center (MWVCRC) for postmortem examination. For animals that were recovered in an advanced state of postmortem decomposition, necropsy typically consisted of gross examination with photographs of any potential lesions. In some cases, tissues were collected for microscopic examination, and postmortem radiographs were performed to rule out gunshot as a cause of death, or to confirm and characterize bone lesions. For animals that were received in fresh or moderate postmortem condition, the examination process was more extensive, consisting of gross necropsy, microscopic examination of all major tissues, bacterial and/or fungal culture and biochemical analysis of feces, urine, liver or other samples for the presence of marine and freshwater biotoxins as funding permitted. Subsamples of many tissues, urine, serum, bile, pericardial fluid and cerebrospinal fluid were also cryoarchived at -80°C to facilitate future diagnostic testing. Postmortem radiographs were performed for some cases, as directed by case history and presentation. Tissues examined on the microscope included: multiple lymph nodes, spleen, liver, pancreas, kidney, adrenal gland, bladder, reproductive tract, ovary, testis, epididymis, heart, aorta, peripheral nerves and ganglia, multiple skeletal muscles, diaphragm, tongue, tonsil, soft palate, esophagus, thyroid and parathyroid glands, thymus, omentum, pituitary gland, trigeminal nerve and ganglion, cerebrum, cerebellum and brainstem. Microscopic examination of hematoxylin and eosin-stained tissues was completed by veterinary pathologists, and final reports were prepared that took into consideration information from gross and microscopic examination, bacterial culture, biochemical analysis and any available antemortem tests.

Summary of lesions, test results and diagnoses

In addition to detailed necropsy reports, summary tables were prepared to compare broader disease and mortality patterns among sea otters that were tagged in the Monterey and Big Sur coastal regions. Table 1 summarizes the stranding location, stranding date and overall demographical information for 17 tagged sea otters from the Monterey and Big Sur sample populations that have been submitted for necropsy as of March, 2012. Nearly all were female (82%), and 94% were adults or aged adults. Slightly over half (53%) were examined in fresh postmortem condition, 18% were moderately decomposed and 29% were severely decomposed

or mummified. Equal proportions of necropsied otters were examined from Monterey Bay (53%, n = 9) and the Big Sur coast (47%, n = 8). All but one animal was recovered from the same portion of the sea otter range where they had originally been tagged (eg Monterey or Big Sur).

For 16 otters with known nutritional condition (minus one mummified animal), 31% died with fair to abundant adipose stores, while 69% had scant or no fat at the time of necropsy. Roughly equal proportions of sea otters with fair to abundant adipose stores were recovered from the Monterey Bay (3) and Big Sur (2) region, and one of the three Monterey Bay animals had originally been captured in Big Sur. Sixteen animals had intact gastrointestinal tracts: Of these, 73% had scant or no food in the gastrointestinal tract, and the remainder contained moderate or abundant digesta. Fragments of crab carapace, especially large (cancer) crabs were apparent in digesta at necropsy, along with mussel shell fragments and a variety of invertebrate parts (Table 1). Three of the 17otters were alive when recovered; one otter died post-stranding and the other two were humanely euthanized after evaluation by veterinarians due to a poor clinical prognosis. Of 10 adult female sea otters that were fresh enough for accurate determination, 9 had moderate or severe nose wounds at the time of necropsy. Of 9 adult females that were fresh enough for determination, 4 (44%) were actively lactating at the time of death, and 3 additional females had prominent mammary glands, suggestive of recent pregnancy. The estimated number of completed pregnancies per adult female sea otter, as determined by counts of the number of corpora albicans visible on both ovaries (not including follicles or corpora lutea indicative of current, pre-implantation cycling or pregnancy), averaged 2.59 (range 0-5). Both adult male sea otters were actively producing sperm at the time of death, although a low number of spermatozoa were observed in the testicle and epididymis of sea otter 5738-10 (an aged adult) on histology.

Congenital malformations

Numerous physical conditions noted during necropsy of tagged Southern sea otters could represent either normal variation or congenital malformations. Because the Southern sea otter population has experienced a significant bottleneck over the past century, it is important to monitor these conditions. All physical abnormalities that were observed during the current study appeared to be mild and incidental. These conditions were found in the heart, kidneys and reproductive tract (Table 1), and included cardiac valve hemal cysts (n = 2), para-ovarian cysts (n = 3) and renal cortical cysts (n = 1). Most defects appeared to be clinically silent, and are fairly common in Southern sea otters (M. Miller unpub. data). However, one adult female otter exhibited unilateral ovarian agenesis, a condition that could affect fertility (Please see attached photo).

Antemortem and postmortem bacterial culture

Table 2 summarizes the results of aerobic and anaerobic bacterial culture. Bacterial data were available for 12 of the 17 tagged sea otters. All bacterial isolation and identification was performed at the UC Davis Veterinary Medical Teaching Hospital. In Table 2, samples with a black box surrounding the sample date were obtained during live capture, while all other isolates

were obtained at necropsy. Swabs of potential lesions were submitted for aerobic +/- anaerobic bacterial culture using non-selective media. Fecal samples were submitted for assessment via a "Fecal Pathogen Protocol" that was developed during a prior study of Southern sea otter enteric bacterial flora (Miller et al. 2010). Opportunistic bacterial pathogens detected in live otter feces included *Streptococcus infantarius* ss *coli* (n = 1), *Vibrio alginolyticus* (n = 2) and *Vibrio parahemolyticus* (n = 2). Only once were the same bacterial pathogens detected in feces of live otters and feces or tissues of the same animal postmortem.

Localized or systemic bacterial infection was a common primary or contributing cause of death for stranded otters (Table 5). Underlying causes of these infections included shark bite, mating wounds, anthropogenic trauma, and possibly, dental disease or enterocolitis. The spectrum of bacteria isolated from the tagged otters is similar to isolates obtained from random-source stranded sea otters (Kreuder et al 2003). The bacterial isolates encompass a range of weak opportunistic pathogens (e.g., non-hemolytic *E. coli*), moderate pathogens (e.g., *Streptococcus phocae*), and primary pathogens (e.g., *Erysipelothrix rhusiopathiae*). Mixed infections of aerobic and anaerobic bacteria were common in bite wounds, abscesses, and associated lymph nodes.

Antemortem and postmortem tests for biotoxins

Table 3 summarizes the results of antemortem and postmortem tests for the presence of freshwater or marine biotoxins, including domoic acid (DA), microcystin (MC), saxitoxin (SX), okadaic acid (OA), nodularin (NO) and anatoxin-a (AN) in samples from tagged sea otters. Two assays were used for biotoxin detection: 1.) Enzyme-Linked Immunosorbent Assay (ELISA); a relatively inexpensive test that indirectly measures toxin activity and 2.) Liquid chromatography/mass spectrometry (LC/MS); a sensitive, specific and expensive assay that measures biotoxin content. Both ELISA and LC/MS assays were used for DA detection; only ELISA was used for SX detection; and only LC/MS was used to detect all other toxins. Of 14 necropsied otters with samples available for testing for DA, 8/14 (57%) tested positive for DA above established minimum detection limits (MDL) in one or more samples, and 2 animals (14%) exhibited urine DA concentrations >200 PPB, suggestive of acute DA intoxication. Several of the DA-negative animals only had serum available for testing. Because of the short systemic half-life of DA and the relative insensitivity of serum as a screening sample, it is possible that additional otters would have tested DA-positive if urine or gastrointestinal content were available for analysis.

Two of the 6 LC/MS-tested sea otters were confirmed positive for the freshwater cyanotoxin MC, and this potent toxin may have been a contributing factor in the animals' deaths. Liver histopathology was suggestive of mild/early microcystin exposure in one case (Please see figures below), and mild, idiopathic icterus and septicemia were noted for the second animal. A third sea otter that was not tested due to financial constraints exhibited a soft, friable, liver grossly (Please see figures below). Microcystin testing should be performed for additional animals as funding permits, based on available samples. Because conventional diagnostic tests only detect free

(unbound) microcystins, and because these toxins bind covalently (e.g. strongly) to tissue receptors, a high potential exists for false-negative tests.

Sea otter GI content or feces tested low-positive for SX for 5 otters. The significance of this finding is not well understood, but merits investigation. Like DA, SX is widely distributed throughout the California coastal ecosystem. Thus, characterizing association with illness or death can be difficult when found at low concentrations. Tests for OA and assays for the cyanotoxins AN and NO were negative in all cases.

Significant findings from gross necropsy, histopathology and diagnostic testing

Table 4 provides a summary of findings from gross necropsy, histopathology and diagnostic testing for each tagged otter, and includes subjective weighting of the relative severity of each finding. These findings are further distilled into a primary and contributing cause(s) of death in Table 5. As reported in prior studies (Kreuder et al 2003), it is common for Southern sea otters to exhibit multiple concurrent or inter-related disease processes at the time of death, as Table 4 illustrates. With the exception of markedly autolyzed animals where detailed lesion characterization was not possible, nearly all of the tagged otters exhibited >1 concurrent disease process that was at least moderately severe. As shown in Table 4, discovery of 3 or more independent pathological processes impacting otter survival at necropsy is common, and many have strong connections with anthropogenic pollution.

In addition to the two otters mentioned above with possible acute neurotoxic DA exposure, sea otters with chronic and/or recurrent DA exposure may develop progressive heart muscle damage and cardiac failure (cardiomyopathy syndrome). Three of 14 tagged sea otters with sufficient detail for accurate determination exhibited gross and/or microscopic lesions of mild to severe cardiomyopathy syndrome. Other suspected causes of cardiac muscle damage in sea otters include infection by protozoal parasites and viruses, and exposure to other environmental biotoxins in addition to DA.

In the current study, livers from two of the 6 otters tested were biochemically positive for the freshwater cyanotoxin MC, and a third, untested otter had a visibly friable liver at necropsy, possibly indicative of MC intoxication. Both microcystin and DA have the ability to cause acute, subacute and chronic effects in exposed animals and humans. The complexity of interactions between these HAB toxins and other important causes of sea otter disease are poorly understood, but may be important; strong toxin-pathogen interactions, with possible facilitation of infectious disease by HAB toxins is suspected. At present, approximately 40 Southern sea otters are suspected to have died with MC as a primary or contributing cause of death since 1999 (M. Miller, unpublished data).

Also common among examined animals was localized and/or systemic bacterial infection, spanning a range of opportunistic and primary pathogens. Some of the bacteria inhabit marine or

estuarine environments, while others may be derived from fecally-contaminated river outflows. Many or all may be concentrated in the tissues of filter-feeding invertebrates that are consumed by sea otters and humans, such as mussels and clams. For 11 sea otters with sufficient detail for accurate determination, 91% exhibited localized and/or systemic bacterial infections at necropsy, and these infections were considered severe for 64% of examined animals.

The low proportion of sea otters with significant infections by acanthocephalan parasites and protozoa in this study partly reflects sample bias: Both groups of pathogens tend to cause clinically severe disease in immature animals. However, nearly all of the otters in the current study (16/17) were adults or aged adults. This low observed prevalence may also reflect dietary preferences, cyclic or long-term trends in parasite prevalence, and other factors that could not be measured. Some otters exhibited lesions that could be consistent with less well characterized pathogens, such as *Bartonella* spp.. These cases can be submitted for confirmatory testing when sufficient funds are identified.

Trauma-associated death was observed for 8 sea otters in the current study. Of 13 animals that were fresh enough for accurate determination, trauma of any cause was found in 8 cases, including confirmed or suspected shark bite (n = 4), confirmed or suspected boat strike (n = 2) and infection secondary to recent surgery (n= 2). Fight-associated trauma was also found in a single sea otter that died with confirmed shark bite.

Emaciation was a consistent finding at necropsy, which is not surprising, given the presence of chronic disease in the majority of stranded animals. Of 16 animals where nutritional condition could be accurately assessed at necropsy, 31% were in excellent or good nutritional condition at the time of death, and 69% were thin or emaciated. Nearly all of the thin or emaciated sea otters exhibited severe, concurrent, subacute or chronic disease processes at necropsy (Table 4). The potential for emaciation to be associated with prey resource limitation in certain portions of the range is discussed in detail in other chapters of this report.

The current study also provides important insight on influences of sea otter biology, the reproductive cycle, intraspecific interactions and nutritional stress on the health of the Southern sea otter population. One important finding that resulted from this Coastal Conservancy-supported research is an improved understanding of the importance of end-lactation syndrome (ELS) as an obstacle to Southern sea otter population recovery. ELS is a disease syndrome that effects only reproductively-active female sea otters. Factors associated with sea otter death due to ELS include the stress of pregnancy and pup care, nutritional stress, concurrent disease and aggression by male sea otters during mating. For 12 adult female sea otters that were sufficiently fresh for accurate determination, caloric stress due to pregnancy and postpartum pup care was a possible contributing factor for death of 58% of adult females, and ELS was a primary cause of death for just under 17%. This result is especially concerning because survival of reproductive-age female sea otters is critical to achieve population recovery.

Table 5 summarizes the primary and contributing cause(s) of death for each otter. These determinations were made after carefully considering all available data for each case, including the clinical history, as well as results from gross necropsy, histopathology and available diagnostic tests. The primary and top two contributing cause(s) of death were determined for 15 of the 17 tagged sea otters. Advanced autolysis prohibited identification of antemortem lesions for the remaining 2 animals. The most prominent primary causes of death were presumptive or confirmed shark bite (n = 4), bacterial septicemia (n = 3), mating trauma (n = 2) and end-lactation syndrome (ELS) (n = 2). All 4 otters with ELS and/or mating trauma as the primary cause of death originated from the Big Sur coastline, while shark bite cases were divided between Monterey (n=2) and Big Sur (n = 2). Both suspected boat strike cases were recovered from Monterey Bay, including Moss Landing Harbor, a known high-risk area for boat strikes due to close proximity of large numbers of sea otters and heavy boat traffic.

The most common secondary, or contributing causes of death included intoxication by harmful algal bloom (HAB) toxins (n = 6, including 4 possible cases of DA intoxication and 2 possible cases of MC intoxication, based on limited testing). Also noted were 4 additional cases of bacterial infection, plus ELS and emaciation (2 cases each).

When pooled across the primary and top 2 contributing causes of death, the most significant lesions across 15 tagged, necropsied otters with sufficient tissue detail for lesion identification included bacterial infection (n = 9), possible or suspected biotoxin intoxication (n = 6), ELS (n= 4), shark bite (n = 4), emaciation (n=4), mating trauma (n=2), poor dentition (n=2) and presumed boat strike (n=2). When divided by location where each sea otter was tagged, resided and was recovered (e.g. Monterey Bay or Big Sur), cases where bacterial infection was either a primary or contributing cause of death were divided between Monterey Bay (n = 4) and Big Sur (n= 5). A similar pattern was observed for otters with DA intoxication as a primary or contributing cause of death. In contrast, 2 of the 4 ELS cases, and both mating trauma cases were recovered along the Big Sur coast.

This Coastal Conservancy-supported research has helped focus attention on ELS as an important target for improving Southern sea otter population recovery. Knowledge gained through the current study should help catalyze scientific study of ELS. An improved understanding of possible links between poor population recovery, nutritional stress and ELS in the central part of the sea otter range helped lead to abolishment of the “no otter zone”, allowing expansion of the sea otter population into historical range in Southern California and, hopefully, achieving population recovery over the coming years.

Because the sample size is small in this preliminary review of findings from tagged, necropsied sea otters, inferring population-level trends should be performed with caution. Re-assessment of disease patterns will be completed in the future when a larger sample of tagged sea otters has been examined by veterinary pathologists. In addition, comparing data from detailed postmortem examinations with information gleaned from ongoing field monitoring efforts, antemortem tests

and foraging data will be critical. Factors that limit the precision of lesion interpretation include postmortem scavenging and autolysis; obstacles that are commonly encountered during prospective observational studies of wide-ranging, tagged, wild marine animals like sea otters. An additional obstacle is limited ability to test all animals for all potential pathogens and toxins, due to cost constraints and sample availability. In spite of these unavoidable obstacles, support by the Coastal Conservancy has enabled scientists to complete a landmark study. The Monterey Bay/ Big Sur study is the first of its kind to employ a “life history” approach to examining in detail the intersection between sea otter biology, their environment and the ecology of disease. By synergizing effort of sea otter biologists, veterinarians and pathologists, experts in statistical modeling, toxicology and other scientific disciplines, we have learned a great deal about the complex interplay between sea otters and their environment. As monitoring of tagged otters and postmortem examinations continue over the next few years, additional information will be obtained that will continue to guide sea otter conservation efforts.

References

Kreuder, C., Miller, M.A., Jessup, D.A., Lowenstine, L.J., Harris, M.D., Ames, J.A., Carpenter, T.E., Conrad, P.A., and Mazet, J.A. (2003), Patterns of mortality in southern sea otters (*Enhydra lutris nereis*) from 1998-2001. Journal of Wildlife Diseases, 39(3): 495–509.

Miller, M.A., Byrne, B.A., Jang, S.S., Dodd, E.M., Dorfmeier, E., Harris, M.D., Ames, J., Paradies, D., Worcester, K., Jessup, D.A., and Miller, W.A. (2010). Enteric bacterial pathogen detection in southern sea otters (*Enhydra lutris nereis*) is associated with coastal urbanization and freshwater runoff. Vet. Res. 41 (1) 01.

Tables

(Next 6 pages)

Table 1: Stranding location, date and demographical information for tagged, necropsied sea otters from Monterey and Big Sur

Table 2: Aerobic and anaerobic bacterial culture findings for tagged, necropsied sea otters from Monterey and Big Sur

Table 3: Findings from antemortem and postmortem tests for domoic acid and cyanotoxins for tagged, necropsied sea otters from Monterey and Big Sur

Table 4: Findings from gross necropsy, histopathology and diagnostic testing for tagged, necropsied sea otters from Monterey and Big Sur, including subjective weighting of relative lesion severity

Table 5: Overview of lesions, implant entrapment frequency and primary and contributing cause(s) of death for tagged, necropsied sea otters from Monterey and Big Sur

BRD	1090	1106	1069	1074	1139	1089	1093	1094	1033	1064	1051	1155	1042	1037	1016	1109	1024
Area	BSR	BSR	BSR	BSR	BSR	BSR	BSR	BSR	MON	MON	MON	MON	MON	MON	MON	MON	MON
Sex	Female	Female	Female	Female	Female	Female	Male	Male	Female	Female	Female	Female	Female	Female	Female	Female	Male
Sea Otter#	5423-08	5582-09	5594-09	6120-11	6207-11	6278-11	5416-08	5650-09	5545-09	5651-09	5728-10	5818-10	6104-11	6130-11	6234-11	6400-12	5738-10
DateFound	11/25/2008	8/24/2009	9/14/2009	5/31/2011	9/16/2011	11/8/2011	11/8/2008	11/23/2009	7/7/2009	11/19/2009	3/22/2010	6/12/2010	5/12/2011	6/18/2011	10/5/2011	3/11/2012	3/29/2010
Live Strand	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	Yes
Euthanized	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	Yes
Date Euthanized or Died	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	11/8/2008	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	10/5/2011	Unknown	3/29/2010
Necropsy Date	11/27/2008	8/27/2009	9/16/2009	6/1/2011	9/20/2011	11/9/2011	11/10/2008	11/24/2009	Unknown	11/24/2009	3/25/2010	6/14/2010	5/17/2011	6/21/2011	10/6/2011	3/13/2012	3/30/2010
Age	Aged Adult	Adult	Adult	Aged Adult	Adult	Adult	Adult	Adult	Adult	Subadult	Adult	Adult	Adult	Aged Adult	Adult	Adult	Aged Adult
Carcass Condition	Mod Decomp	Adv Decomp	Fresh	Fresh	Fresh	Fresh	Fresh	Adv Decomp	Mumm/Frag	Adv Decomp	Mod Decomp	Fresh	Adv Decomp	Mod Decomp	Fresh	Fresh	Fresh
ATOS	625	552	637	574	563	592	601	346	321	387	237	406	422	385	368	380	321
Nose Wound Size	Medium	Decomposed	Large	Large	Large	Large	None	Small	Decomposed	Decomposed	Small	Medium	Decomposed	Large	Large	Large	Medium
Nose Wound Freshness	White	Decomposed	Pink	Pink	Pink	Pink	None	White	Decomposed	Decomposed	White	Red	Decomposed	Pink	Red	White	Red
Teeth Condition	Poor	Unknown	Good	Poor	Good	Fair	Good	Fair	Good	Unknown	Good	Good	Fair	Fair	Fair	Fair	Poor
Nutritional Condition	Emaciated	Emaciated	Emaciated	Emaciated	Emaciated	Emaciated	Fair to Good	Excellent	Unknown	Emaciated	Excellent	Emaciated	Emaciated	Poor	Emaciated	Fair	Poor
Subcutaneous Fat	Fair	None	None	None	None	None	Fair	Abundant	Unknown	Scant	Abundant	None	None	Scant	Scant	Fair	Scant
Tarry Digesta	Not Obvious	Obvious	Obvious	Obvious	Obvious	Obvious	Not Obvious	Not Obvious	Unknown	Obvious	Unknown	Obvious	Obvious	Not Obvious	Slight	Not Obvious	Slight
Food Gut	None	None	None	None	Little	Medium	None	Little	Unknown	Medium	Unknown	None	Medium	Full	None	None	Little
Prey					cancer crab in intestines	Mostly urchins, crab, Tegula operculum		Inkeeper, Crab		crabs and algae	GI - sea star, snail, cancer crab	sea water and melena	mussel, crab	cancer crab, octopus, kelp crab			Clams, other un-ID soft tissue.
Acanthocephalans	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unknown	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Parasites	C	C	C	C	C	C>P	C	C=P	Unknown	C	C	C>P		C	P>C	P>C	C
Intestinal	Mild	Mild	Mild	Mild	Moderate	Moderate	Mild	Mild	Unknown	Mild	Moderate	Moderate	None	Moderate	Moderate	Mild	Mild
Abdominal	None	None	None	None	None	None	None	None	Unknown	None	Unknown	None	None	None	Moderate	None	None
Lactating	Not noted	Y (Scant)	Y (Lots, & prom. mammary glands)	No, but mammary tissue prominent	Yes (Milk collected)	N	N/A	N/A	Unknown	N/A	No, but prominent mammary glands	No	Y (Scant)	No, but mammary tissue prominent	Yes (Scant milk, small, flat, atrophied mammary glands)	N?	N/A
Estimated # of pregnancies	5	2	3-3.5	1	2	3	N/A	N/A	Unknown	N/A	Unk	2.5	1+ (adv. Decomp.)	5	3.5+	5	N/A

Parasites: C=Corynosoma, P=Profilicollis

BRD	Area	Sex	Sea Otter#	Necropsy Date	Collection Date	Culture Site	Arcanobacterium phocae, Archanobacterium spp.	Campylobacter sp	Clostridium difficile	Clostridium perfringens	E. Coli	E. coli 0157	Photobacterium damselae	Salmonella sp.	Streptococcus bovis/equinus	Streptococcus phocae	Vibrio alginolyticus	Vibrio parahemolyticus	Vibrio sp.	Miscellaneous
1090	BSR	Female	5423-08	11/27/2008	11/10/2008	Feces		-	-	-		-		-	-				-	
"	"	"	"	"	11/27/2008	Feces		-	-	-		-			-				-	Salmonella saintpaul
"	"	"	"	"	11/27/2008	Incision							+							Proteus sp, Shewanella putrefaciens
"	"	"	"	"	11/27/2008	Liver														
"	"	"	"	"	11/27/2008	Lymph Node - Inguinal							+							
"	"	"	"	"	11/27/2008	Muscle					+									
"	"	"	"	"	11/27/2008	Spleen														
"	"	"	"	"	11/27/2008	Transmitter							+							
1106	BSR	Female	5582-09	8/27/2009	11/11/2008	Feces		-	-	-		-		-	-					Vibrio cholerae
1069	BSR	Female	5594-09	9/16/2009	11/5/2008	Feces		-	-	-		-		-	-				-	
"	"	"	"	"	9/16/2009	Abscess											+			Bacilli
"	"	"	"	"	9/16/2009	Lymph Node - Retropharyngeal	+									+	+			Nonenteric Bacteria
"	"	"	"	"	9/16/2009	Wound	+									+	+			Shewanella putrefaciens
1074	BSR	Female	6120-11	6/1/2011	11/6/2008	Feces		-	-	-		-		-	-				-	
"	"	"	"	"	9/24/2010	Feces		-	-	-		-		-	-		+			
"	"	"	"	"	6/1/2011	Heart Blood					+									NFG 3
"	"	"	"	"	6/1/2011	Liver														
1139	BSR	Female	6207-11	9/20/2011	11/3/2009	Feces		-	-	+		-		-	-			+		
"	"	"	"	"	9/20/2011	Abscess, Right cheek	+													Fusobacterium necrophorum, Pasteurella multocida ss multocida, Streptococcus Beta-Hemolytic
"	"	"	"	"	9/20/2011	Lymph Node - Retropharyngeal														Bacteroids sp /Prevotella sp
"	"	"	"	"	9/20/2011	Pleural Fluid	+													Peptostreptococcus anaerobius
"	"	"	"	"	9/20/2011	Transmitter Surface					+						+			Staphylococcus pseudintermedius, Streptococcus Beta-Hemolytic
1089	BSR	Female	6278-11	11/9/2011	11/6/2008	Feces		-	-	-		-		-	-			+		
"	"	"	"	"	11/9/2011	Lymph Node - Inguinal														
"	"	"	"	"	11/9/2011	Mammary Tissue										+	+			Actinomyces-like
"	"	"	"	"	11/9/2011	Stifle, Left - joint										+				
"	"	"	"	"	11/9/2011	TDR pocket														
1093	BSR	Male	5416-08	11/10/2008	11/6/2008	Feces			-	-		-		-	-		+			
"	"	"	"	"	11/10/2008	Abdominal Fluid											+			
"	"	"	"	"	11/10/2008	Abscess											+			
"	"	"	"	"	11/10/2008	Feces		-	-	-		-		-	-		+			
"	"	"	"	"	11/10/2008	Heart Valve											+			
"	"	"	"	"	11/10/2008	Lymph Node - Inguinal											+			
"	"	"	"	"	11/10/2008	Lymph Node - Sublumbar											+			
"	"	"	"	"	11/10/2008	Spleen														
"	"	"	"	"	11/10/2008	TDR pocket											+			
"	"	"	"	"	11/10/2008	Transmitter Surface														
1094	BSR	Male	5650-09	11/24/2009	11/7/2008	Feces		-	-	-		-		-	-				-	

BRD	Area	Sex	Sea Otter#	Necropsy Date	Collection Date	Culture Site	Arcanobacterium phocae, Archanobacterium spp.	Campylobacter sp	Clostridium difficile	Clostridium perfringens	E. Coli	E. coli 0157	Photobacterium damselae	Salmonella sp	Streptococcus bovis/equinus	Streptococcus phocae	Vibrio alginolyticus	Vibrio parahemolytic	Vibrio sp	Miscellaneous
1051	MON	Female	5728-10	3/25/2010	N/A	No cultures at necropsy														
1155	MON	Female	5818-10	6/14/2010	6/14/2010	Lymph Node - Axillary														Erysipelothrix rhusiopathiae
"	"	"	"	"	6/14/2010	Lymph Node - Inguinal														Erysipelothrix rhusiopathiae
"	"	"	"	"	6/14/2010	Spleen														Erysipelothrix rhusiopathiae
"	"	"	"	"	6/14/2010	Transmitter Surface														Erysipelothrix rhusiopathiae
1042	MON	Female	6104-11	5/17/2011	9/26/2007	Feces		-				-		-					-	
"	"	"	"	"	N/A	No cultures at necropsy														
1033	MON	Female	5545-09		N/A	No cultures at necropsy														
1064	MON	Female	5651-09	11/24/2009	N/A	No cultures at necropsy														
1037	MON	Female	6130-11	6/21/2011	6/21/2011	Lymph Node - Retropharyngeal														
"	"	"	"	"	6/21/2011	Pus	+										+			Corynebacterium sp, Klebsiella ornithium, Proteus vulgaris
1016	MON	Female	6234-11	10/6/2011	4/29/2009	Feces		-	-	-		-		-					-	Streptococcus infantarius ss coli
"	"	"	"	"	10/6/2011	Lymph Node - Inguinal										+				
"	"	"	"	"	10/6/2011	Lymph Node - Sublumbar					+					+	+	+		
"	"	"	"	"	10/6/2011	Transmitter Surface														
"	"	"	"	"	10/6/2011	Wound, right hind limb										+	+	+		Fusobacterium necrophorum
1109	MON	Female	6400-12	3/13/2012	3/13/2012	Lymph Node - Mesenteric							+							
"	"	"	"	"	3/13/2012	Transmitter Surface														
1024	MON	Male	5738-10	3/30/2010	3/30/2010	Feces		-	+	+		-		-		+			-	
"	"	"	"	"	3/30/2010	Lymph Node - Axillary										+				
"	"	"	"	"	3/30/2010	Peritoneal Fluid				+			+							
"	"	"	"	"	3/30/2010	Trachea				+						+				Serratia-Like

+ = growth

- = no growth

BRD	Area	Sex	Sea Otter#	Necropsy Date	Sample Date	Tissue Type	Saxitoxin	Domoic Acid	Microcystin	Nodularin	Anatoxin-A	Okadaic Acid
1106	BSR	Female	5582-09	8/27/2009	11/11/2008	Serum		-				
"	"	"	"	"	11/5/2008	Feces	+/-	-				
"	"	"	"	"	11/5/2008	Serum	-	-				
"	"	"	"	"	9/16/2009	Milk	-	+				
"	"	"	"	"	9/16/2009	Serum	-	-				
"	"	"	"	"	9/16/2009	Urine	-	+				
1074	BSR	Female	6120-11	6/1/2011	11/6/2008	Serum	-	-				
"	"	"	"	"	9/24/2010	Serum	-	-				
"	"	"	"	"	6/1/2011	Liver		-	-	-	-	-
1139	BSR	Female	6207-11	9/20/2011	9/20/2011	Liver		+	-	-	-	-
"	"	"	"	"	9/20/2011	Urine		+++	-	-	-	-
1089	BSR	Female	6278-11	11/9/2011	11/6/2008	Serum	-	+				
"	"	"	"	"	11/9/2011	Liver		+	-	-	-	-
"	"	"	"	"	11/9/2011	Urine		+++	-	-	-	-
1093	BSR	Male	5416-08	11/10/2008	11/6/2008	Feces	+	+				
"	"	"	"	"	11/6/2008	Serum	-	-				
"	"	"	"	"	11/6/2008	Serum		-	-	-	-	-
"	"	"	"	"	11/10/2008	Liver		-	+			-
"	"	"	"	"	11/10/2008	Feces	-	-				
"	"	"	"	"	11/10/2008	Urine	-	-				
1094	BSR	Male	5650-09	11/24/2009	11/7/2008	Serum	-	-				
1051	MON	Female	5728-10	3/25/2010	3/25/2010	Stomach Contents	+/-	+				
1155	MON	Female	5818-10	6/14/2010	2/1/2010	Serum	+	+				
"	"	"	"	"	6/14/2010	Serum	-	-				
"	"	"	"	"	6/14/2010	Urine	-	+				
1042	MON	Female	6104-11	5/17/2011	7/29/2010	Serum	-	-				
1037	MON	Female	6130-11	6/21/2011	4/23/2009	Serum	-	-				
"	"	"	"	"	6/21/2011	Colon Contents		+	-	-	-	-
"	"	"	"	"	6/21/2011	Liver		-	-	-	-	-
"	"	"	"	"	6/21/2011	Serum		-	-	-	-	-
"	"	"	"	"	6/21/2011	Stomach Contents		-	-	-	-	-
1016	MON	Female	6234-11	10/6/2011	4/29/2009	Serum	-	-				
1109	MON	Female	6400-12	3/13/2012	4/22/2009	Serum	-	-				
"	"	"	"	"	6/8/2010	Serum	-	-				
1024	MON	Male	5738-10	3/30/2010	3/30/2010	Feces	+	+				
"	"	"	"	"	3/30/2010	Serum	-	-				
"	"	"	"	"	3/30/2010	Stomach Contents	+/-	+				
"	"	"	"	"	3/30/2010	Urine	-	+				
"	"	"	"	"	3/30/2010	Liver			+/-			

- = not detected

+ = <100 ppb, ++= 100-200 ppb, +++=>200ppb

-/+ = detected below MDL

BRD	Area	Sex	Sea Otter#	Carcass Condition	Dental Disease	Bacterial Infection	Abscess	Sepsis	Acanth. perit.	Protozoal Encephalitis	Domoic acid	Microcystin	Cardiomyopathy	Nose wound	End-lactation syndrome	Lactating?	Emaciation	Fight trauma	Shark bite	Other trauma	Gastric ulcers & melena
1090	BSR	Female	5423-08	Fr	2	3	3	3	0	0	0	Unk	0	1	0	0	0	0	0	3 (recent surgery)	0
1106	BSR	Female	5582-09	Adv	Unk	Unk	Unk	Unk	0	0	Unk	Unk	0	Unk	3 (pres. based on findings and Hx)	Y (Scant)	3	Unk	Unk	Unk	2
1069	BSR	Female	5594-09	Fr	0	3	3	3	0	0	0 to 1	Unk	0	3	3	Y (Lots, & prom. mammary glands)	3	0	0	0	3
1074	BSR	Female	6120-11	Fr	3	0	0	0	0	0	0	0	0	3	0	No, but mammary tissue prominent	3	0	0	0	3
1139	BSR	Female	6207-11	Fr	0	3	3	3	0	0	3	0	0	3	1 (Died of bacterial & resp. dz.)	Yes (Milk collected)	3	0	0	0	Y
1089	BSR	Female	6278-11	Fr	1	3	3	3	1 (Resolving)	0	3	0	2	2	0	N	Y	0	3 (Pres.)	0	3
1093	BSR	Male	5416-08	Fr	0	3	3	3	0	0	0 to 1	2 to 3 (contrib.)	0	0	N/A	N/A	1	0	0	3 (recent surgery)	Y
1094	BSR	Male	5650-09	Adv	1	Unk	Unk	Unk	0	Unk	Unk	Unk	Unk	2	N/A	N/A	0	2	3 (Conf.)	0	0
1033	MON	Female	5545-09	Mum/ Frag	0	Unk	Unk	Unk	Unk	0	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
1064	MON	Female	5651-09	Adv	Unk	Unk	Unk	Unk	0	0	Unk	Unk	Unk	Unk	N/A	N/A	2	Unk	Unk	Unk	2 to 3
1051	MON	Female	5728-10	Mod	0	Unk	Unk	Unk	0	0	2	Unk	0? (Not noted)	0	0	No, but prominent mammary glands	0	0	3	0	Unk
1155	MON	Female	5818-10	Fr	0	3	0	3	0	0	1 to 2	Unk	0	2	1 (Poss. as contrib. factor)	No	3	0	0	0	3
1042	MON	Female	6104-11	Adv	1	Unk	Unk	Unk	0	0	0	Unk	Unk	Unk	1 (Possible, as contrib. factor)	Y (Scant)	3	Unk	0	0	3
1037	MON	Female	6130-11	Mod	1 to 2	1	1	0	0	0	3	0	3	2	1 (contrib.)	No, but mammary tissue prominent	2	N	0	0	1
1016	MON	Female	6234-11	Fr	1	2	2 (Localized)	0	2	0	Unk	Unk	0	2	1 (coming out of ELS)	N (Small, flat, atrophied mammary glands)	0	0	0	3 (Boat strike)	0
1109	MON	Female	6400-12	Fr	1	1 to 2	1 to 2 (Perit., seg. enteritis)	1 to 2 (Poss chronic *Bartonella*)	2	0	Unk	Unk	0*	1 to 2	N/A (old, not cycling?)	N?	0	0	3 (Pres.)	0	Y
1024	MON	Male	5738-10	Fr	3	3	1 to 2 (teeth)	3	0	0	1 to 2	Unk	1 to 2	1 (D/T abnormal locomot.)	N/A	N/A	2	0 *	0 *	3? (*Prior boat strike, pres.)	2

0=none, 1=mild, 2=moderate, 3=Severe

Fr = Fresh dead, Mod. = Moderately decomposed, Adv. = Advanced decomposition, Mum/Frag. = Mummified/ fragmented, Unk = Unknown,

BRD	Area	Sex	Sea Otter#	Overview of lesions	Primary Cause of Death	Contributing #1	Contributing #2
1090	BSR	Female	5423-08	Surgery-assoc. infection & sepsis, rhabdomyolysis, dental Dz,	Rhabdomyolysis/Necrosis	Septicemia - Bacterial	Dental Abscess/Tooth Wear
1106	BSR	Female	5582-09	ELS pres., Gastric ulcers	End Lactation Syndrome	Ulcers - Gastric	TDR Entrapment
1069	BSR	Female	5594-09	ELS, Infected nose/ face wounds, septicemia, GI ulcers, septal emphysema, matted coat	End Lactation Syndrome	Bacterial - Abscess	Septicemia - Bacterial
1074	BSR	Female	6120-11	Mating trauma, dental disease, emaciation, GI ulcers & melena, terminal drowning, adrenal thrombus	Nose Wound	Dental Abscess/Tooth Wear	Emaciation/Starvation
1139	BSR	Female	6207-11	Mating trauma with secondary infection, pyothorax, septicemia, septal emphysema, pneumothorax, Strong DA +, ELS, prior (healed) trauma to dorsal skull	Nose Wound / Bacterial - Abscess	Septicemia - Bacterial	Domoic Acid Intoxication - Presumptive
1089	BSR	Female	6278-11	Prior trauma of Unk origin, secondary infection, DCM, strong DA+, nose wound, emaciat., acanth perit.	Shark Attack - Presumptive	Bacterial - Abscess	Domoic Acid Intoxication - Presumptive
1093	BSR	Male	5416-08	Surgery-assoc. infection & sepsis, MC intox., rhabdomyolysis, wet coat	Septicemia - Bacterial	Microcystin Intoxication - Presumptive	Rhabdomyolysis/Necrosis
1094	BSR	Male	5650-09	Autolysis, confirmed shark bite, intraspecific fight wounds, scavenging	Shark Attack - Confirmed	Trauma-Fight trauma	None
1033	MON	Female	5545-09	Unknown	Unknown	Unknown	Unknown
1064	MON	Female	5651-09	Autolysis, emaciation	Unknown	Unknown	Emaciation/Starvation
1051	MON	Female	5728-10	Shark bite, DA	Shark Attack - Presumptive	Domoic Acid Intoxication - Presumptive	None
1155	MON	Female	5818-10	Erysipelothrix sepsis, wet saturated coat, emaciation, healing nose wound, gastric ulcers & melena, dehydration, terminal drowning	Septicemia - Bacterial	Emaciation/Starvation	Pelage - Matted
1042	MON	Female	6104-11	Prior jejunal perf., secondary ometnal adhesion, tertiary partial colon obstruction by adhesed omentum. Poss ELS as contributing factor	Intestinal Perforation	Impaction - Colon	Emaciation/Starvation
1037	MON	Female	6130-11	Poor coat, DA suspect, Cardiomyopathy, thin, infected nose wound, moderate tooth wear	Cardiomyopathy	Domoic Acid Intoxication - Presumptive	End Lactation Syndrome
1016	MON	Female	6234-11	Boat strike with bone fractures, secondary bacterial cellulitis and regional lymphadenitis, recovering ELS, acanth. Perit., melena, dehydration	Boat Strike	Bacterial - Abscess	End Lactation Syndrome
1109	MON	Female	6400-12	Shark bite, shock, AP, Segmental enteritis, *cardiac valve lesions may be supportive of chronic bartonellosis	Shark Attack - Presumptive	Hypovolemia/Shock, Presumptive	Acanthocephalan Peritonitis
1024	MON	Male	5738-10	Severe sepsis, prior blunt trauma with old jaw fracture, chest wound, liver wound (Poss boat strike), bad teeth, emaciation, fight wounds, gastric ulcer & melena, nasal mites, minimal icterus	Septicemia - Bacterial	Boat Strike - Presumptive	Dental Abscess/Tooth Wear

Figures

Figures 1 – 17: Photos taken during necropsy of tagged sea otters, illustrating specific pathological conditions or results (next 13 pages).

Figure 1. Intestinal content, providing insight on this animal's "last meal": Fragments of sea urchin exoskeleton and spines

Figure 2. A *Tegula* operculum from a sea otter that had been eating snails

Figure 3. Intestine of an otter with a colon impaction: The content is tightly packed and contains fragments of cancer crab carapace and mussel shells

Figure 4. Sea otter with a mild nose wound

Figure 5. Sea otter with a severe nose wound

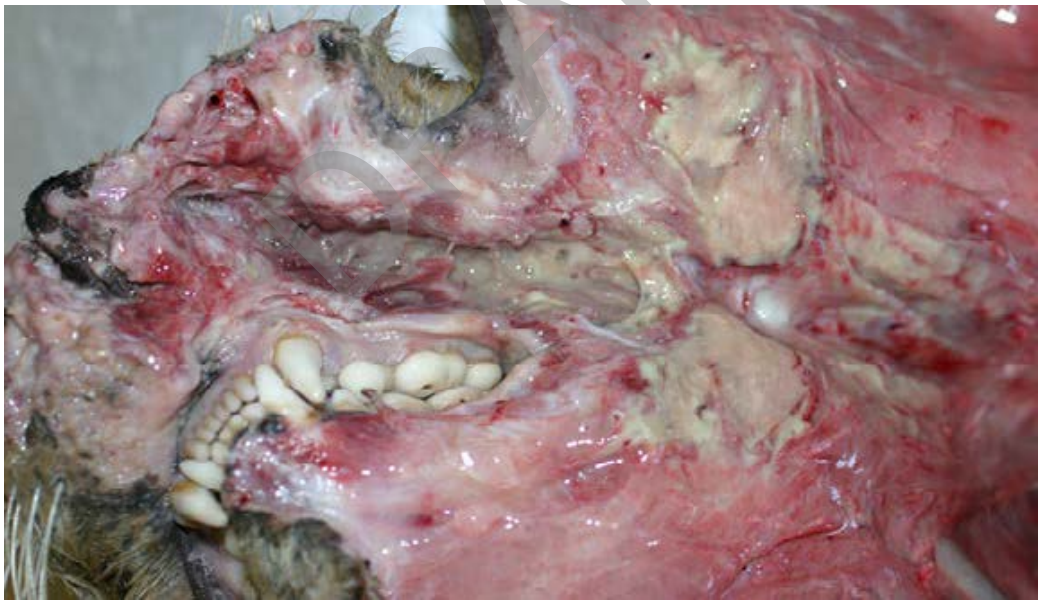

Figure 1. Sometimes otters develop bacterial infections after mating-related trauma. These infections often travel away from the original bite location along tissue planes

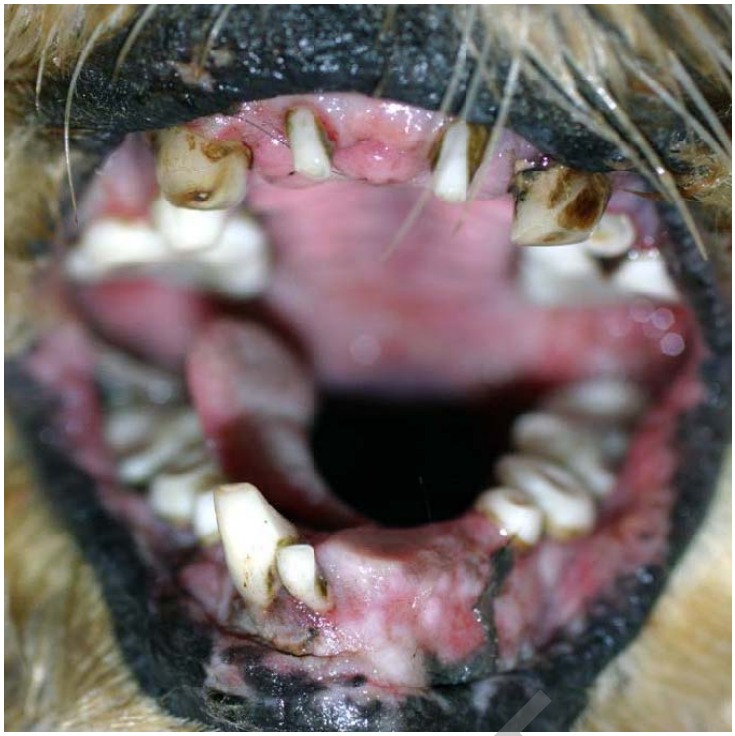

Figure 7. An old sea otter, with severely worn and missing teeth. This otter also had a partially healed mandible fracture -Note increased thickening of the mandible at the site of the fracture (bottom center).

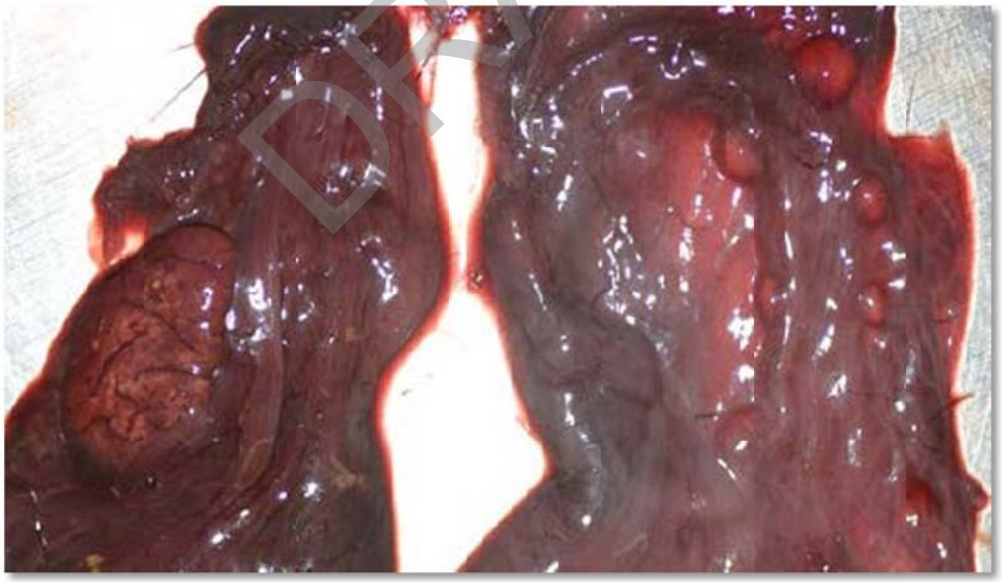

Figure 8. Normal ovary left, missing ovary, right. Note that a normal, wavy, undulating fallopian tube is still visible, just to the right of the thin flap of tissue where the ovary should be (right center).

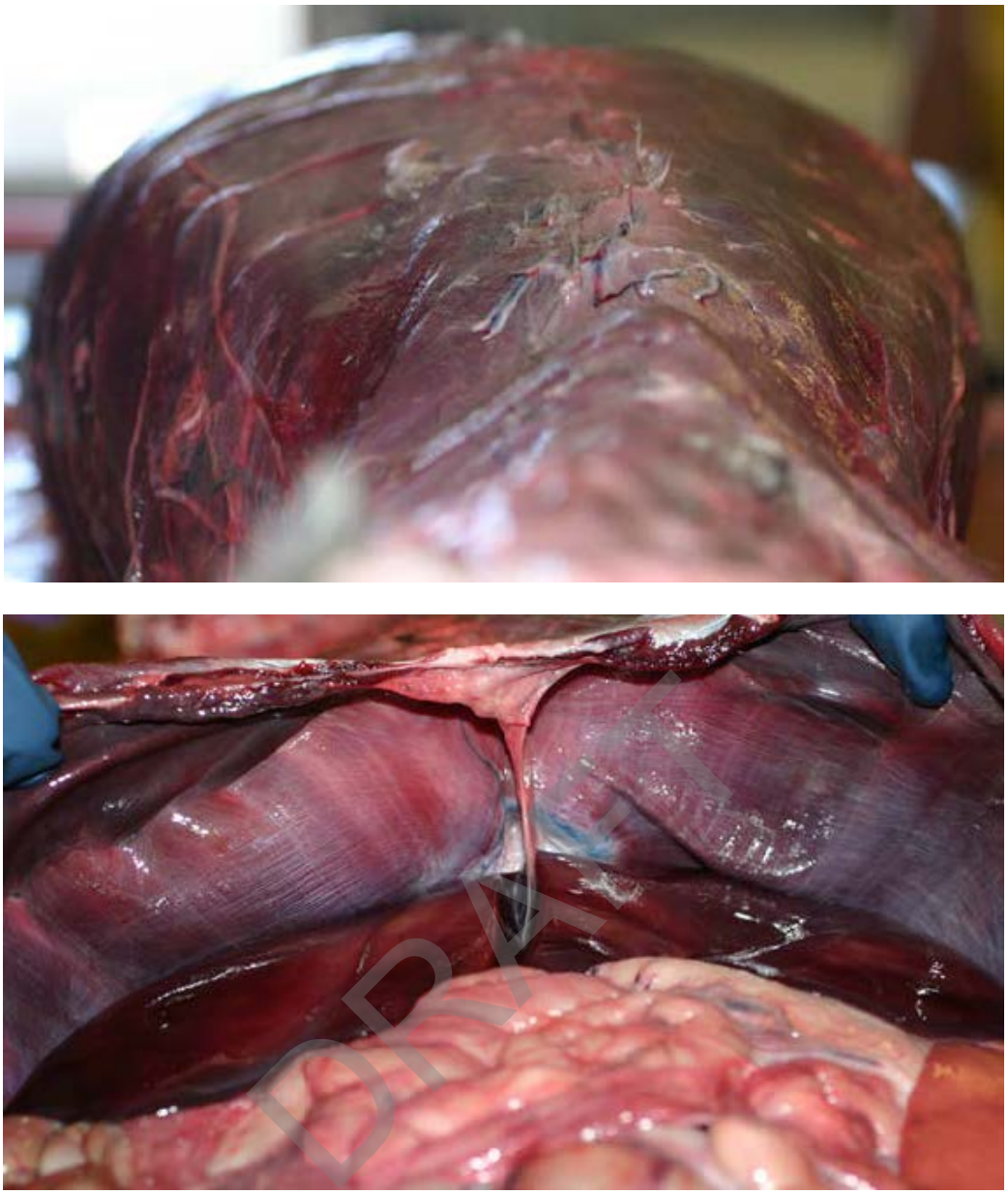

Figure 9. Sea otter with a chest full of pressurized air or gas (pneumothorax), characterized by an abnormal, “barrel-shaped” chest (upper photo) and a diaphragm that is pushed back toward the abdomen (bottom). This pneumothorax was associated with a bacterial infection. Pneumothorax is rapidly fatal because it impairs breathing.

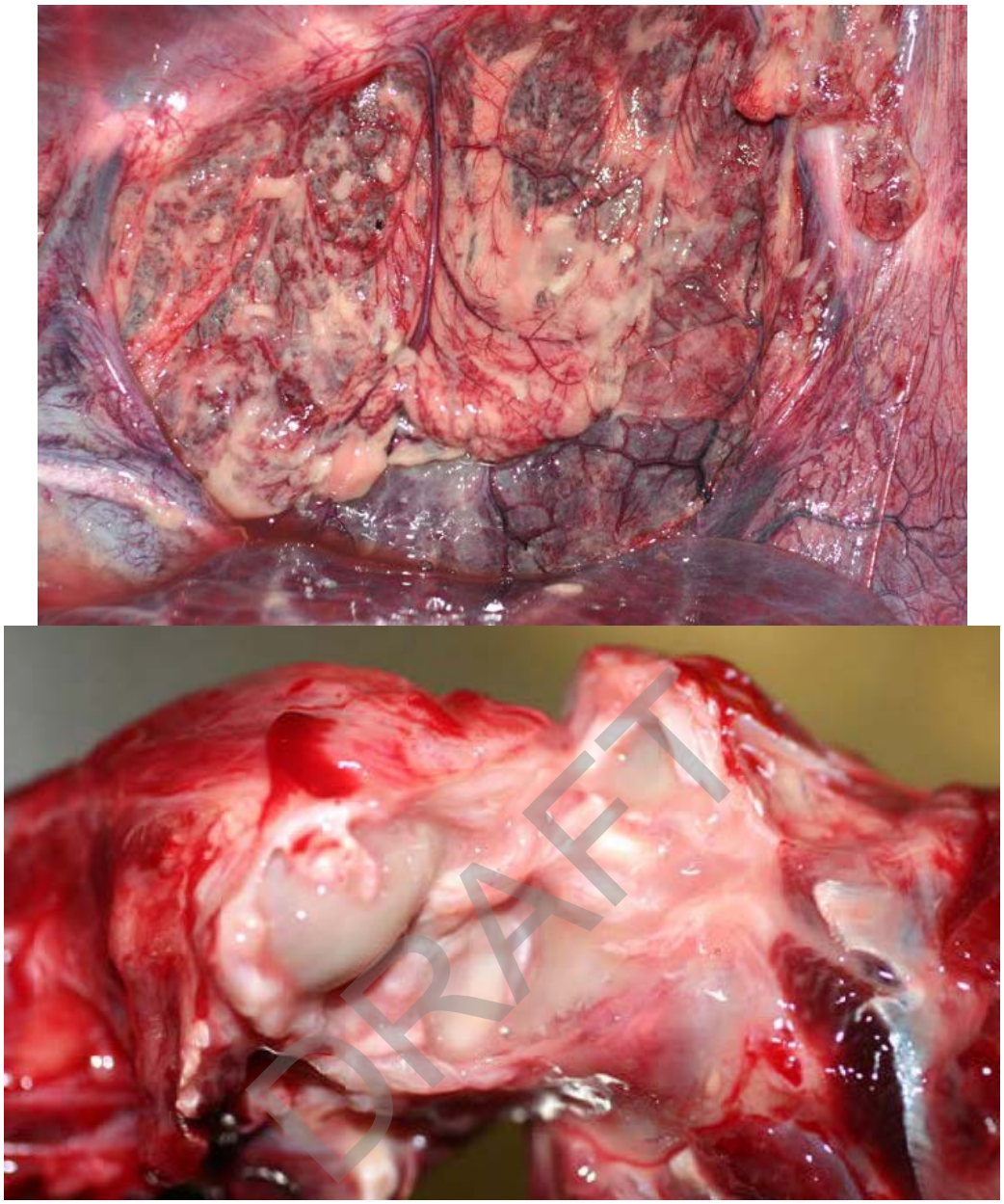

Figure 10. Same otter as in figure 9, showing a severe bacterial infection of the chest cavity (top) and a knee joint (bottom).

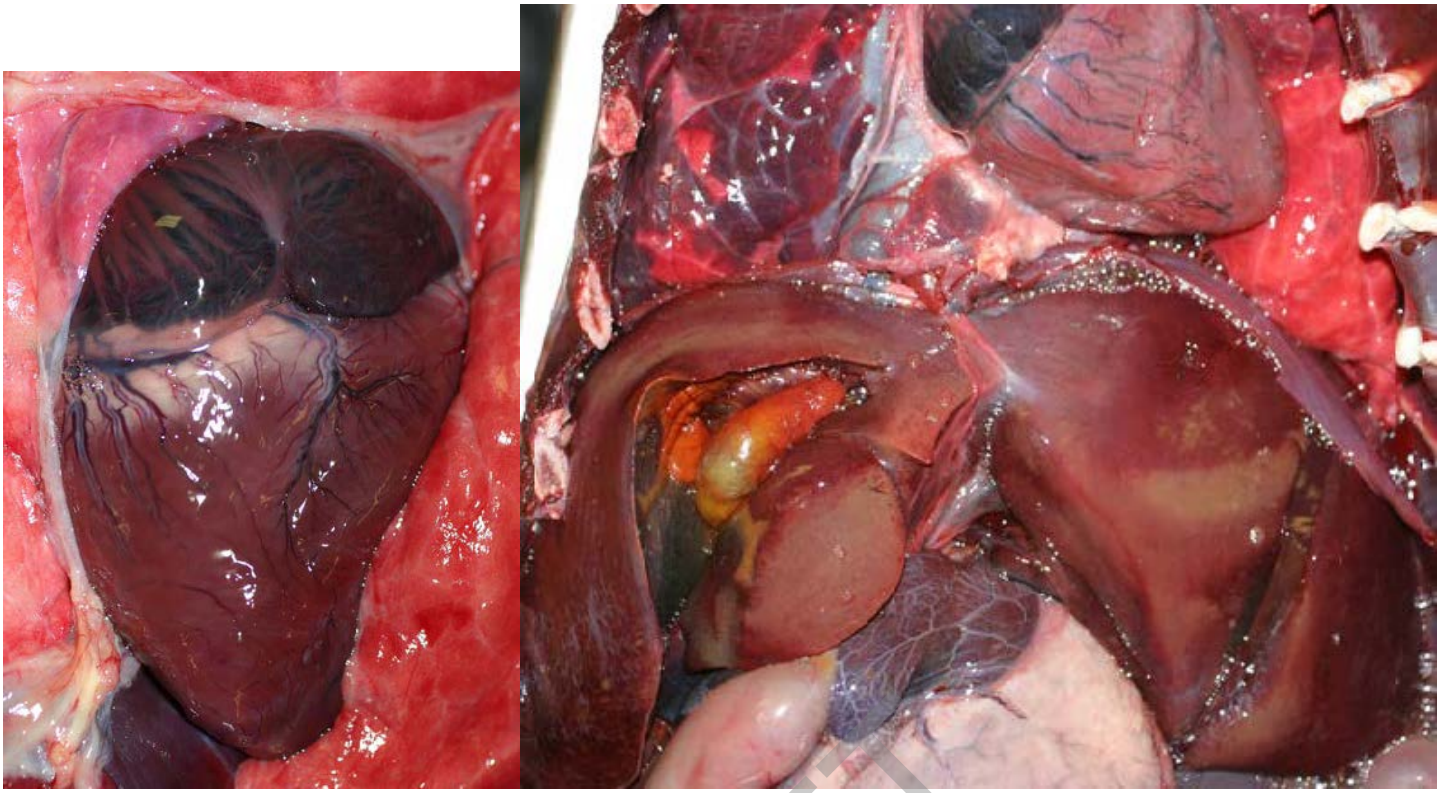

Figure 11. A normal sea otter heart (left), compared with the heart of an otter with cardiomyopathy syndrome (upper right center).

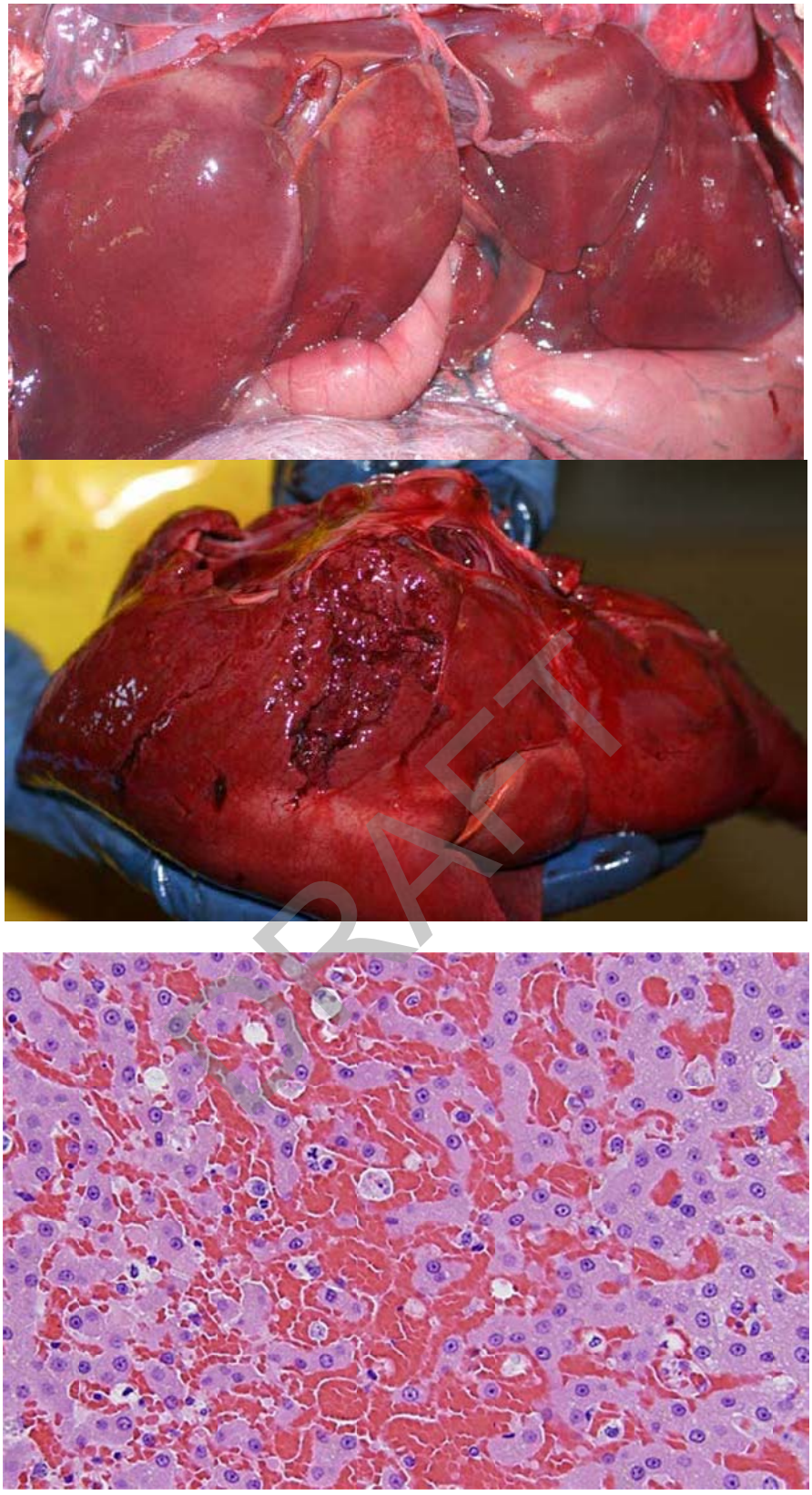

Figure 12. Lesions suggestive of microcystin intoxication: Pale, swollen or mottled liver (top), increased friability (fragility) of liver tissue (center) and microscopic evidence of liver cell death (bottom).

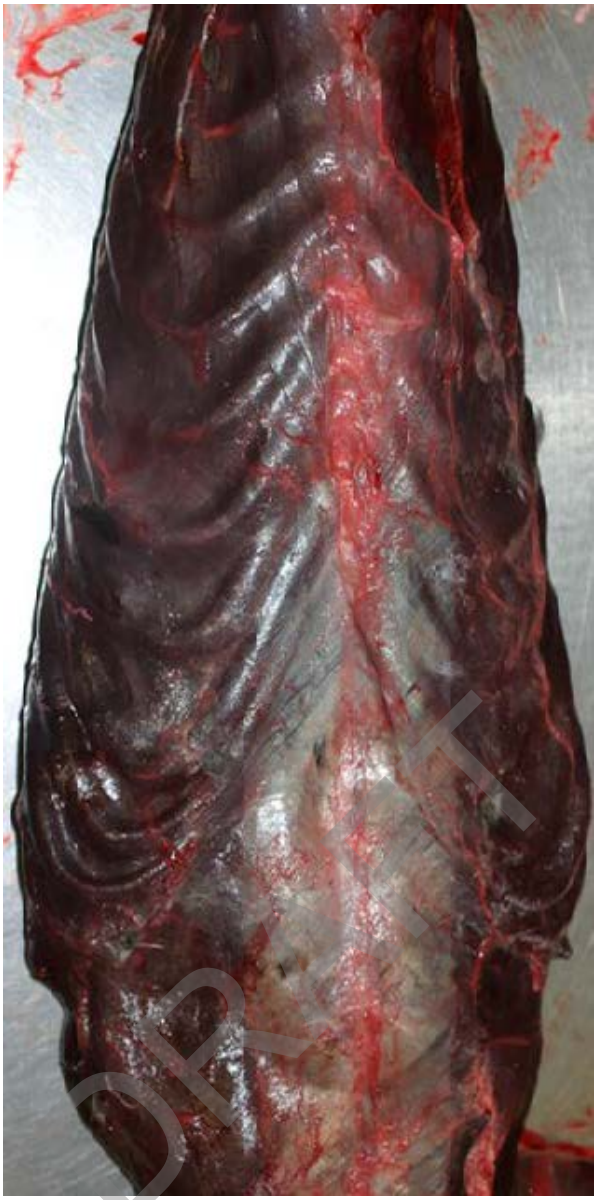

Figure 13. A severely emaciated sea otter, typical of adult females dying with end-lactation syndrome (ELS).

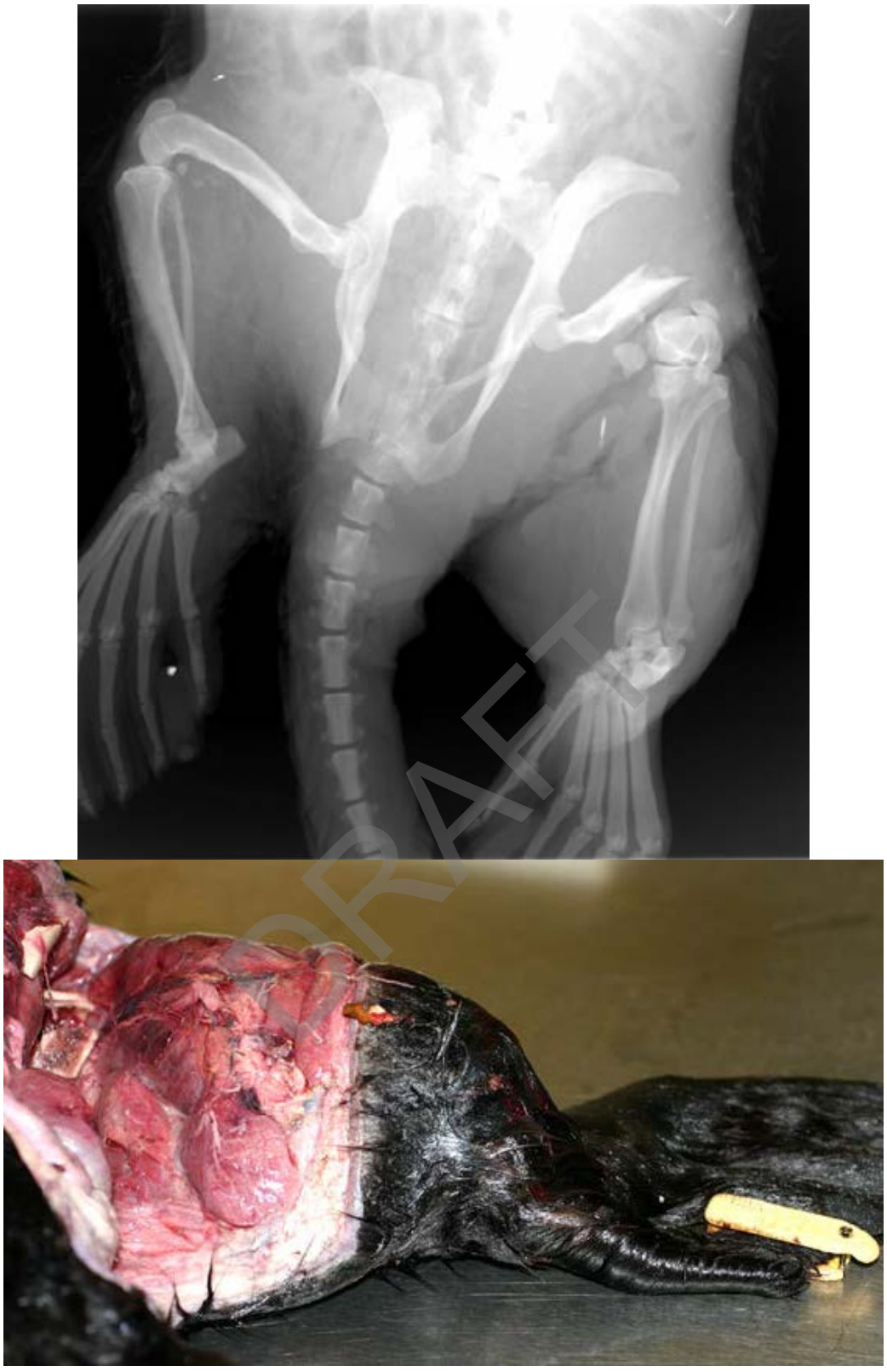

Figure 14. A sea otter with a deep laceration and femur fracture due to boat propeller strike. Radiograph (top) and gross photo (bottom).

Figure 15. Lesions suggestive of severe blood loss due to trauma or other causes: Pale gums (upper left), small heart with collapsed chambers (upper right) and small, pale liver (bottom center).

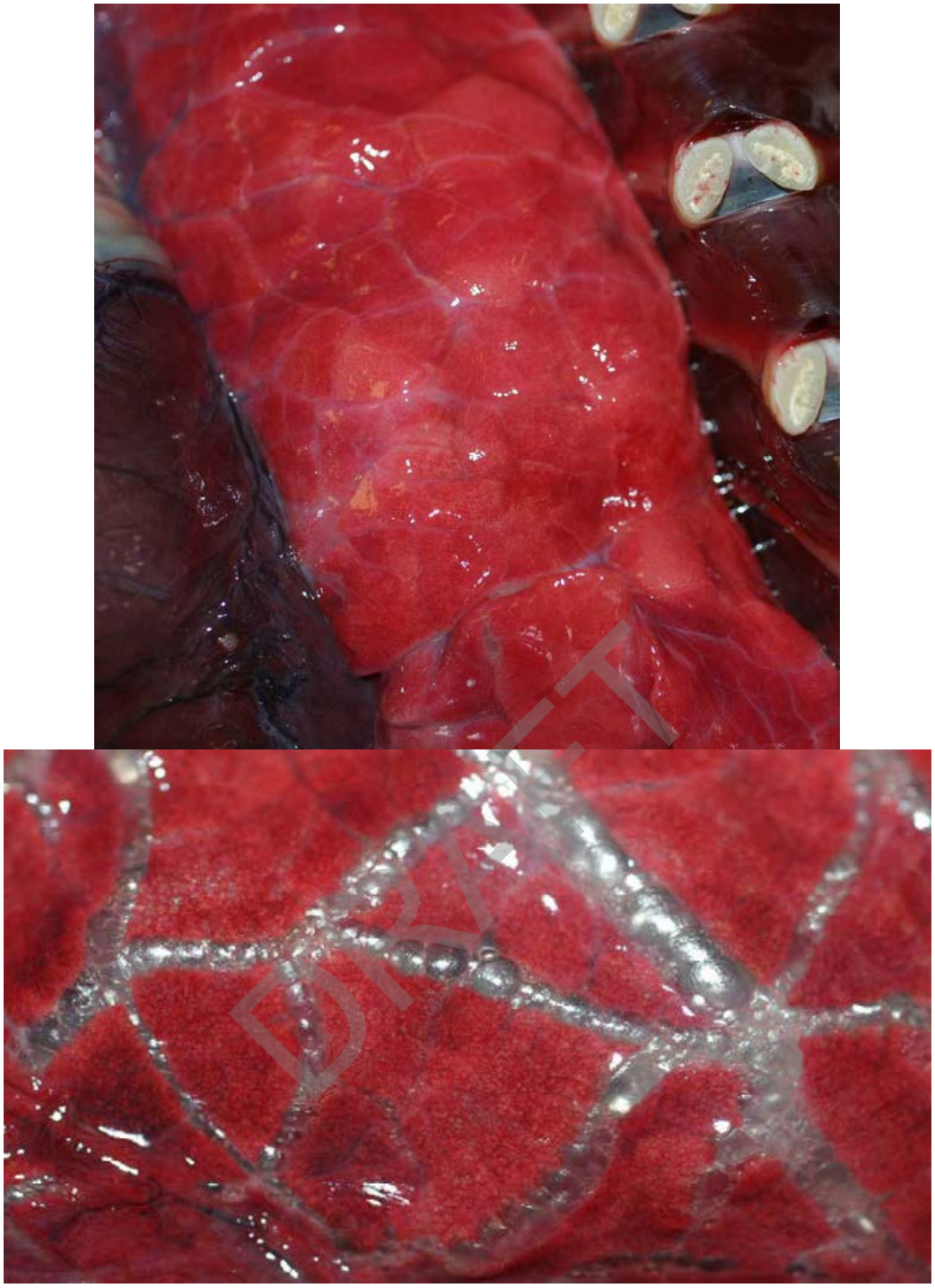

Figure 16. Normal lung tissue (top), compared with an otter with severe pulmonary emphysema (bottom). This lesion is non-specific-It is typically associated with an animal that has been breathing hard just prior to death.

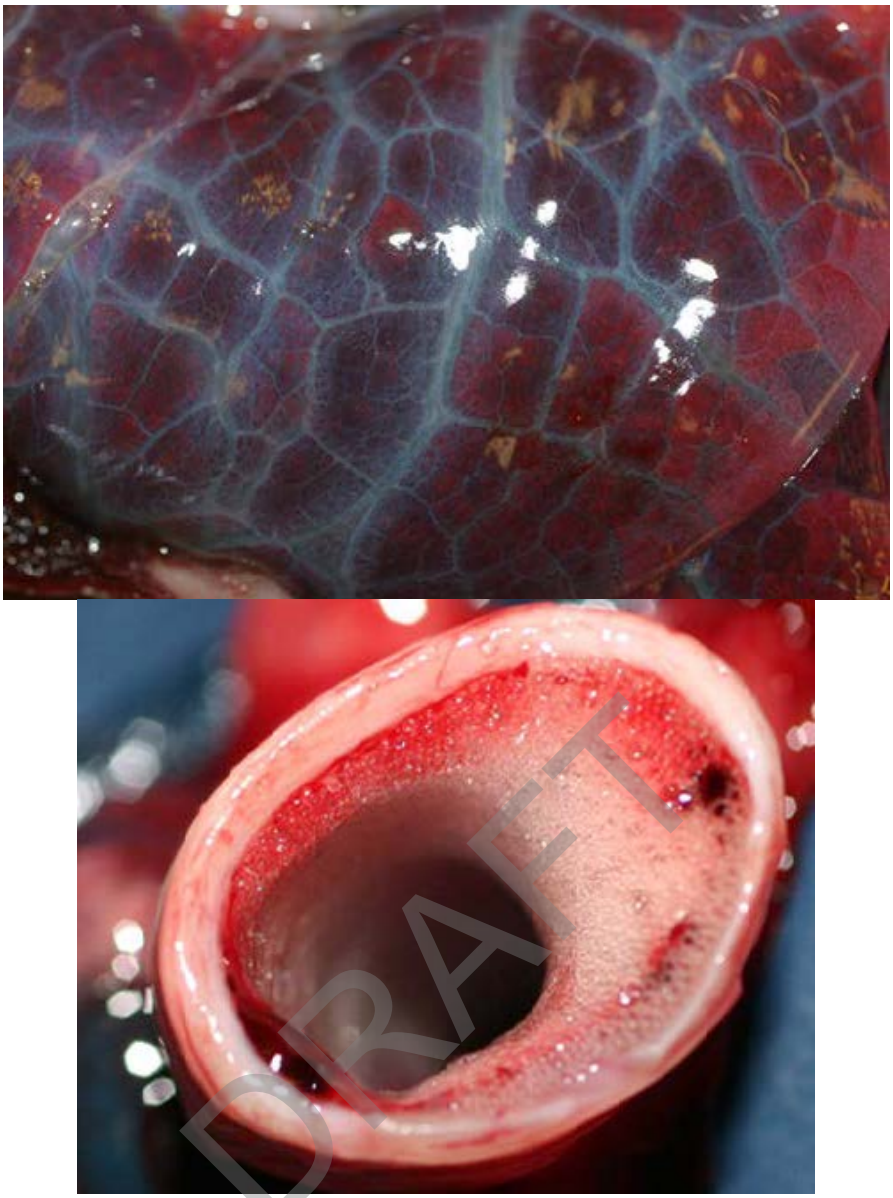

Figure 17. Pulmonary edema, characterized by expansion of the spaces between pulmonary lobules by clear or blue-tinged fluid (top), and build-up of pink or white froth in the trachea and bronchi (bottom). Depending on the circumstances, this lesion may indicate primary drowning, terminal drowning (eg aspiration of seawater near the time of death in an animal that is dying of other causes), heart failure, or other health problems.

CHAPTER 11: Synthesis and Conclusions

M. Tim Tinker
U.S Geological Survey, Western Ecological Research Center, Santa Cruz, CA

General Conclusions

The various modules of the Big Sur-Monterey population study reported on in the preceding chapters represent the culmination of one of the most expansive studies of sea otter biology ever conducted. The breadth of topics covered and the diversity of results is somewhat overwhelming, and distilling all this information down to a few simple conclusions is no easy task. All of the analyses presented in this report were conducted with the aim of testing one or more of the primary hypotheses, often using multiple lines of inquiry. Considered together, the various lines of investigation encompassed by this study were generally consistent with respect to their degree of support (or lack of support) for each of the 4 primary hypotheses, as described below.

1) Sea otters living in areas adjacent to human population centers and areas heavily impacted by runoff or sewage (e.g. Monterey) are more likely to be exposed to pathogens and toxins of public health importance than those in more pristine areas (e.g. Big Sur). This hypothesis was ***not supported*** by the results of our study. An epidemiological analysis of *Toxoplasma gondii* infections (Chapter 9) indicated that sea otters in the highly impacted site were significantly *less* likely to be exposed than were otters from the pristine area. Necropsies of study animals that died during the study revealed that the frequency of the domoic acid exposure (a bio toxin produced from diatom blooms) as a contributing cause of death was approximately equal between the two study sites (occurring in 50% of recovered carcasses at Big Sur and 44% of recovered carcasses at Monterey: Chapter 10). Gene expression analysis revealed no significant differences between sites in terms of gene up-regulation patterns indicative of physiological responses to pathogens or toxins, with the exception of elevated response to organic contaminants in sea otters from Big Sur in 2008 (possibly due to effects of Big Sur wild fires that year: Chapter 3). We did not conduct lab tests of blood contaminant levels, due to funding constraints (although blood samples have been archived to permit such analyses in the future), so we cannot rule out the possibility that there may have been differences in exposure to specific contaminants that were consistent with this hypothesis; however, physical exams and blood diagnostic tests showed no evidence of health effects that would suggest such a pattern, and in fact the minor differences in health parameters that were found indicated more abnormalities in sea otters from Big Sur, the pristine site (Chapter 2).

2) Patterns of survival and causes of death will differ between heavily impacted and pristine environments, reflecting differences in pathogen and pollutant exposure. This hypothesis was ***not supported*** by our results. The comprehensive analysis of sea otter survival and weaning success showed that sea otters from the pristine site (Big Sur) had lower age-specific survival rates than did sea otters from the heavily impacted (Monterey) study site, and female weaning success rates showed a similar pattern (Chapter 8). These differences were explained almost entirely by the differences in resource abundance and body condition of animals at the two sites: after controlling for the effect of age-specific

body mass, there were no significant differences in survival rates between the two sites. In terms of causes of death of study animals (Chapter 10), necropsies indicated that the same suite of causal factors were evident at both sites in roughly equal proportions, with the exception of boat strikes which only occurred at the Monterey site (2 out of 9 cases).

3) Environmental risk factors will vary between sites, corresponding to the differing land-use patterns. This hypothesis was for the most part ***not supported*** by our results, with some important caveats. As discussed above for hypothesis 1, the health assessments (Chapter 2) and gene expression analyses (Chapter 3) suggested no consistent differences in environmental risk factors, with the exception of up-regulation of certain genes in sea otters captured at Big Sur in 2008 suggestive of increased exposure to organic contaminants. Epidemiological analysis (Chapter 9) indicated that exposure to the protozoal parasite *Toxoplasma gondii* was significantly greater at Big Sur than at Monterey. Thus our results do indicate variation in environmental stressors across sites, and over time, however the differences we found were not clearly attributable to differences in human population densities or land-use patterns.

4) Sea otters from high-density populations (and/or areas that have been occupied longer) will exhibit lower rates of foraging success due to prey resource depletion, and these patterns will be reflected by i) greater percentage of time spent feeding, ii) more pronounced individual diet specialization, iii) poorer body condition, and iv) lower survival rates of both adults and pups. This hypothesis was ***well supported*** by data collected in the current study and in previous similar studies. Sea otters at Big Sur and Monterey study sites (both of which have supported high density populations for many years) had relatively low rates of energy gain while feeding as compared to low density, growing populations in California, Washington, British Columbia, Alaska and Russia (Chapter 6). Big Sur sea otters experienced slightly lower energy intake rates than did otter in Monterey, and also spent slightly more time feeding (Chapter 5) and exhibited slightly greater levels of diet specialization (Chapter 6), although these latter metrics were high at both sites as compared to low density populations. A comparison of body condition and survival rates across 6 sites in California (Chapters 7 and 8, respectively) showed that lower foraging success in high-density sea otter populations was reflected by poorer body condition and reduced survival and pup weaning success rates, with strongly significant correlations between all of these parameters.

Based on the above hypothesis tests, it is clear that not all of our a priori predictions were supported by empirical data sets, requiring a re-evaluation of some of our assumptions about factors driving trends in sea otter abundance in central California. Fortunately, the enormous scope and inter-disciplinary nature of this project, and the extensive sample sizes available from both the current study and from previous similar studies conducted over the past 15 years (e.g. Miller et al. 2002, Bentall 2005, Tinker et al. 2006, Johnson et al. 2009, Staedler 2011), provide us with an excellent opportunity to "update" our understanding of southern sea otter population biology. Four general conclusions about the sea otter populations of central California, and the factors driving trends in abundance, have emerged from this work: 1) density-dependent population regulation driven by per-capita resource abundance is the most significant factor currently limiting population growth in the center part of the range (approximately Monterey peninsula to Estero Bay); 2) spatial and temporal variation in environmental and anthropogenic stressors also have significant effects on sea otter health, but patterns of variation are complex and not

simply a function of proximity to human populations; 3) exposure to environmental stressors (either natural or anthropogenic in origin) does not act independently of resource limitation; and 4) sea otter populations are structured at small spatial scales, and the processes that regulate population abundance (including density-dependent resource abundance) also occur locally. The significance of each of these points is discussed in the following sections.

Density-Dependent Population Regulation

Density-dependent resource limitation appears to be the dominant factor driving variation in reproductive success and survival of sea otters in central California. Sea otters experienced high survival/reproductive success in low density populations near the edge of the range where prey resources were abundant (e.g. San Nicolas Island, Santa Barbara Channel), and low survival/reproductive success in high density populations near the center of range (Big Sur, Monterey and San Simeon) where per-capita resource abundance was low. The hypothesis that per-capita resource abundance was depleted in the latter areas was supported by multiple, independent lines of evidence including low forage success, high percent time feeding, and poor body condition. The differences in estimated survivorship schedules and reproductive success between sea otters in Monterey and Big Sur and sea otters in San Nicolas Island were explained almost entirely by differences in body condition (Chapter 8), and the resulting estimates correctly predicted observed population growth rates at these areas. Thus sea otters in Big Sur appear to be at a carrying capacity determined by resource abundance: however, the phrase "resource limitation" sometimes causes confusion for those unfamiliar with the language and concepts of population ecology, so it is worth correcting a few common misunderstandings about this concept:

A) "Resource limitation" does not mean that there is a paucity of invertebrates or lower productivity in a given area: indeed, high density sea otter populations tend to occur in areas of high productivity (as measured by invertebrate recruitment and growth rates) such as Monterey peninsula. Resource limitation implies that there is decreased *per-capita* availability of preferred, high energy prey (e.g. large red urchins), leading to increased reliance on lower quality prey by many individuals, with consequences for one or more population vital rates.

B) Resource limitation does not mean that all sea otters will be equally affected by low food abundance. To the contrary, the emergence of individual prey specialization in resource-limited populations (Tinker et al. 2008a) means that different individuals will be differently affected by competition for specific prey types, leading to increased variation in foraging success and body condition. This variation means that many individuals will in very poor condition but some individuals will be in very good condition.

C) Resource limitation does not imply that starvation will be a common cause of death; it does imply that *average* demographic rates will vary as a function of per-capita resource abundance, until at some point birth rates and death rates are equal and the population reaches equilibrium abundance. The proximate causes of death for animals in a resource-limited population can be highly variable, including infectious disease, intra-specific aggression, intoxication, and many other pathological conditions. "Starvation" *per se* may occur rarely, or not at all.

Variation in Environmental Stressors

Environmental stressors affecting sea otter health appear to vary both temporally and spatially within the sea otters range, and results presented throughout this report suggest that this variation is more complex than can be explained by a simple "dirty vs. pristine" axis, or simply by proximity to human activities. For example, *Toxoplasma gondii* infection risk was highest for female sea otters in the pristine Big Sur site, and much lower for females in the highly impacted Monterey site (Chapter 9). We found evidence for variation in environmental health threats associated with both natural stressors (fires, oil seeps, HAB toxins: Chapter 3) and human caused stressors (anthropogenic pollution associated with bacterial infections: Chapter 10). Exposure to some of these stressors may reflect variation in natural or anthropogenic sources, as well as geographic characteristics of watersheds and marine habitats, but can also be mediated by ecological interactions between sea otters and their prey. For example, in the case of *T. gondii* infections, sea otters that specialized on consuming marine turban snails had a risk of infection 42x higher than otters that specialized on other prey types, irrespective of their location. And while protozoal infections varied significantly among sites, other stressors appeared to be more ubiquitous throughout the sea otter's range: for example, domoic acid intoxication was found as a contributing cause of death in otters at both sites, reflecting the fact that the diatom blooms that produce these toxins are widespread and not associated with point source pollution (Lefebvre et al. 2002).

Synergistic Interactions between Resource Limitation and Environmental Stressors

Just as importantly, the impact of environmental stressors on health and survival appears to be related to individual physiological condition and nutritional status. End Lactation Syndrome (ELS) was found to be a major cause of mortality for females in this study. Females that died of ELS were often exposed to other stressors or factors that contributed to their negative outcome (e.g. domoic acid poisoning, bacterial infections), but also were characterized by extremely poor body condition at the end of a pup dependency period. In the high density populations of central California, entering the end lactation period was associated with a 3.6x increase in mortality risk for females (Chapter 8), but this was not the case for females in the low density population at San Nicolas, where females had much higher foraging success (Chapter 6), spent less time feeding (Chapter 5), and were in much better body condition (Chapter 7). These findings suggest that resource limitation and exposure to environmental stressors are likely synergistic, because females are less capable of coping with exogenous stressors when they are in poor condition, particularly at the end of lactation when body reserves have been exhausted.

Population Structure

Analysis of home range behavior and individual movements of tagged sea otters in this study (and supported by previous analyses) reveals that the southern sea otter population is structured demographically at relatively small spatial scales. Put another way, there is very limited mixing of the demographically relevant components of the population (adult and sub-adult females) between habitat areas only 50km apart. Many of the factors affecting sea otter survival and driving population trends can also vary at small spatial scales: for example, the relative abundance and productivity of invertebrate prey populations, or point sources of pollution. The result of these two facts is that population regulation is, for the most part, a local rather than a regional process. Because of this fact, the question "what factor is

limiting the sea otter population in California" is actually somewhat nonsensical, and should be replaced by the question "what factors are currently most important in limiting population growth in area X?"

Management Implications and Recommendations

The results and conclusions presented in this report and summarized in the above paragraphs have a few important implications for future management and conservation strategies. First, managers need to recognize that simple, universal explanations for population trends are unlikely to be fruitful, due to the fact that population regulation occurs locally rather than regionally, and that environmental stressors and density-dependent processes also vary extensively throughout the range (and over time). Conservation research and management actions should instead be tailored to specific geographic areas and population threats. One example of this approach is the recently initiated (and Coastal Conservancy-supported) project in Elkhorn Slough, designed to inform habitat improvement and restoration actions to support sea otters within this particular estuary. Emerging threats that affect specific geographic areas at present include shark-bite mortality in the Estero Bay – Pismo Beach area (USGS/CDFW, unpublished data) and microcystin intoxication in the Monterey Bay area (Miller et al. 2010). It is worth noting that some stressors may occur more broadly (such as Domoic acid intoxication: Kreuder et al. 2005) and these factors may benefit from coordinated conservation efforts at regional scales.

Second, it is increasingly clear that much of the center part of the sea otter's range appears to be at (or near) carrying capacity, and this means that it is unrealistic to expect that management actions will substantially increase densities in these areas. Understanding the causes of mortality in these areas is still valuable, as lessons learned are often broadly applicable; however, the focus of conservation actions should be aimed at improving the potential for population growth in areas that are still well below carrying capacity (i.e. areas nearer the range peripheries). Moreover, the recovery of southern sea otters to the optimum sustainable population abundance (OSP) identified for California (USFWS 2012) will require range expansion to the north and south of the current distribution: efforts to facilitate or accelerate the rate of range expansion may have a greater impact on the overall rate of recovery than any other conservation action.

Third, prioritization of conservation and management actions should be based on rigorous demographic sensitivity analyses, if the goal is to maximize the potential for recovery. Such analyses (often referred to as "Population viability Analyses", or PVA models) can be used to assess the magnitude of expected benefits associated with particular actions. For example, actions to reduce mortality in an area dominated by juvenile males will have negligible effects on population recovery, but a small reduction in mortality in a female-dominated area near the range periphery could have enormous effects. The estimates of vital rate parameters and dispersal distance statistics presented in this report (Chapters 8 and 4, respectively) provide the basic ingredients needed to develop such PVA models using well established techniques (Monson et al. 2000, Tinker et al. 2006, USFWS 2010). It is becoming increasingly clear that these models must appropriately factor in population structure, movement behavior and range expansion (e.g. Tinker et al. 2008b) in order to provide realistic results.

Finally, the Big Sur-Monterey population study provides a model for how collaborative, multidisciplinary research can make progress in answering challenging conservation questions involving highly complex

ecological interactions. The recovery of southern sea otters from their near-extinction in the north Pacific fur trade in many ways represents a great conservation success story; however, the population still only occurs in a portion of its former range, remains threatened, and is still well below the optimal sustainable population level (OSP) identified by US Fish and Wildlife Service (USFWS 2012). Questions about the factors affecting the slow process of population recovery have been difficult to resolve because they require untangling myriad, complicated interactions between sea otters and their ecosystems. Success in the "untangling process" requires expertise in physiology, behavior, animal health, molecular ecology (e.g. stable isotope and gene expression methods), veterinary pathology, parasitology, epidemiology, and quantitative population biology. Clearly there is no single research lab or agency that has expertise in even half of those disciplines; however, all of these skills are represented by the collaboration of scientists and experts that contributed to this study (a group loosely referred to as the "Sea otter research alliance"). The result of this collaboration has been substantial progress in our understanding sea otter ecology, and this progress has enabled us provide concrete recommendations to managers that reflect the best available science. We believe that ongoing work by this group, bolstered by new collaborations with experts from other fields, will continue to provide tangible results to benefit conservation of sea otters and their ecosystems.

References

Bentall, G. B. 2005. Morphological and behavioral correlates of population status in the southern sea otter: a comparative study between central California and San Nicolas Island. Masters Thesis. University of California, Santa Cruz, CA.

Johnson, C. K., M. T. Tinker, J. A. Estes, P. A. Conrad, M. Staedler, M. A. Miller, D. A. Jessup, and J. A. K. Mazet. 2009. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. Proceedings of the National Academy of Sciences of the United States of America **106**:2242-2247.

Kreuder, C., M. A. Miller, L. J. Lowenstine, P. A. Conrad, T. E. Carpenter, D. A. Jessup, and J. A. K. Mazet. 2005. Evaluation of cardiac lesions and risk factors associated with myocarditis and dilated cardiomyopathy in southern sea otters (Enhydra lutris nereis). American Journal of Veterinary Research **66**:289-299.

Lefebvre, K. A., S. Bargu, T. Kieckhefer, and M. W. Silver. 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon **40**:971-977.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, and P. A. Conrad. 2002. Coastal freshwater runoff is a risk factor for Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). International Journal for Parasitology **32**:997-1006.

Miller, M. A., R. M. Kudela, A. Mekebri, D. Crane, S. C. Oates, M. T. Tinker, M. Staedler, W. A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D. A. Jessup. 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS One **5**:Article No.: e12576.

Monson, D. H., D. F. Doak, B. E. Ballachey, A. Johnson, and J. L. Bodkin. 2000. Long-term impacts of the Exxon Valdez oil spill on sea otters, assessed through age-dependent mortality patterns. Proceedings of the National Academy of Sciences of the United States of America **97**:6562-6567.

Staedler, M. M. 2011. Individual variation in maternal care and provisioning in the southern sea otter (Enhydra lutris nereis): causes and consequences of diet specialization in a top predator. Masters Thesis. University of California, Santa Cruz.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008a. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America **105**:560-565.

Tinker, M. T., D. F. Doak, and J. A. Estes. 2008b. Using demography and movement behavior to predict range expansion of the southern sea otter. Ecological Applications **18**:1781-1794.

Tinker, M. T., D. F. Doak, J. A. Estes, B. B. Hatfield, M. M. Staedler, and L. Bodkin James. 2006. Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. Ecological Applications **16**:2293-2312.

USFWS. 2010. Southwest Alaska Distinct Population Segment of the Northern sea otter (*Enhydra lutris kenyoni*) - Draft Recovery Plan. . U.S. Fish and Wildlife Service, Region 7, Alaska, Anchorage, AK.

USFWS. 2012. Final Supplemental Environmental Impact Statement on the Translocation of Southern Sea Otters. Ventura, CA.