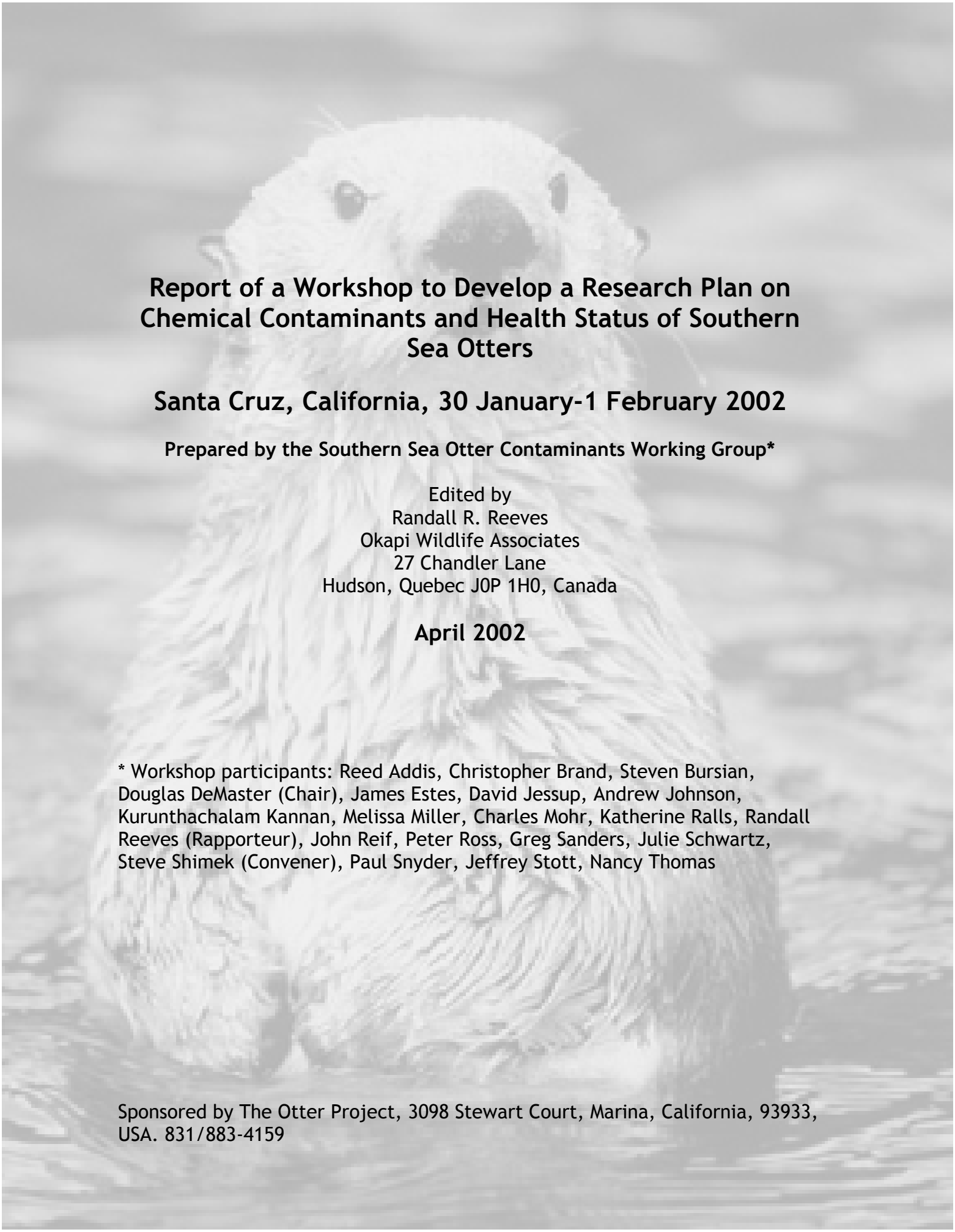

Report of a Workshop to Develop a Research Plan on Chemical Contaminants and Health Status of Southern Sea Otters

Santa Cruz, California, 30 January-1 February 2002

Prepared by the Southern Sea Otter Contaminants Working Group*

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Report of a Workshop to Develop a Research Plan on Chemical Contaminants and Health Status of Southern Sea Otters

Workshop Held, 30 January through 1 February 2002, Santa Cruz, California

1. Introduction

The southern sea otter, a geographically isolated subspecies, presently occupies a small portion of its historical range in California, encompassing some 250 miles of coastline from just south of Point Conception northward to approximately Half Moon Bay. There were probably at least 14,000 animals in California prior to commercial exploitation (DeMaster et al. 1996). Having been driven to near extinction by the early 20th century, the California population has recovered slowly (at a maximal rate of about 5%/yr since the late 1930s) and now numbers about 2,200 individuals (www.werc.usgs.gov/news/2001-06-12.html). A steady increase in abundance during the 1980s and early 1990s inspired hope that the population would continue to recover, but annual counts since 1995 have indicated that the trend has reversed and that the population is either stable or decreasing. There is grave concern about the following observations of this otter population: (a) it has never achieved rates of increase comparable to those of Alaskan populations (17-20%; Estes 1990); (b) it remains intrinsically vulnerable because of its low numbers and limited range; and (c) it now appears to be declining rather than recovering after decades of protection. Long-standing concern about the catastrophic consequences of a major oil spill remains, but other, perhaps more insidious, factors such as fishery bycatch, disease, malnutrition, and the effects of toxic contamination are now also considered to have potential relevance to the long-term security and recovery of this otter population (Southern Sea Otter Recovery Team 2000).

In view of these concerns, The Otter Project organized and convened a workshop in January 2002 to address one of the perceived threats to the southern sea otter population: toxic contamination. An international workshop on persistent contaminants and marine mammals sponsored by the Marine Mammal Commission in 1998 had stressed the need for "multidisciplinary studies to integrate physiological, behavioral, reproductive, clinical, pathologic, and toxicological data to evaluate the relationships of immune status, reproduction, and survival of individuals to population- and ecosystem-level trends" (O'Shea et al. 1999). This need clearly applies to the southern sea otter. Thus, the goal of the workshop was to develop an integrated, multidisciplinary research plan for investigating the relationship between contaminants and health status in southern sea otters. It was expected that the proposed research would have clear relevance to management, that it would

involve collaboration among scientists from various institutions, and that the results would provide a basis for concrete action to improve the sea otter population's chances of recovery. Participants were asked prior to the workshop to consider the following working hypothesis: "Chemical contaminants are affecting the health status of southern sea otters (specifically their susceptibility to infectious disease) and thereby contributing to an increase in mortality rate that in turn is limiting the population's ability to recover."

This report contains the workshop "proceedings" and is intended to supplement the pre-proposal document. The latter was developed immediately following the workshop for use by The Otter Project in its efforts to raise funds to support the recommended research. The main elements of this report are: (a) a description of the participants and workshop procedures; (b) summaries of invited presentations, including points that arose during question-and-answer sessions following the presentations; and (c) a section that identifies each component of the proposed research plan and provides a rationale, crude budget, and timescale for each of these components.

2. The Workshop

The workshop was held from 30 January to 1 February 2002 at the Chaminade conference center in Santa Cruz, California. Steve Shimek, Executive Director of The Otter Project, had organized the workshop and developed the list of invited participants in consultation with Otter Project board members and interested scientists. It was decided that the optimal number of participants should be in the range of 20-25, including scientists with expertise in sea otter ecology and life history, pathology, toxicology, epidemiology, immunogenetics, and population dynamics. Most of the individuals who attended the workshop are or have been directly involved in sea otter research sponsored by federal, state, or private agencies and institutions (see Appendix 1). The workshop was chaired by Doug DeMaster, and Randall Reeves served as rapporteur.

As indicated above, the purpose of the workshop was to explore possible links between contaminants and health status in the California sea otter population, and to develop the basis for an integrated, multidisciplinary research proposal to address critical uncertainties. In focusing on this topic, the workshop organizers intended to supplement, rather than compete with or detract from, ongoing studies of other known or potential mortality factors, including such things as nutritional stress, predation by sharks, and bycatch in fisheries. It was recognized that the southern sea otter's slow recovery at the best of times, and its recent stable or downward trend, were likely due to a combination of factors rather than a single cause. Therefore, it was acknowledged from the start that research should be pursued on a broad front and that multiple hypotheses should be considered in the search for ways to explain recent trends in the otter population.

The first day of the workshop was devoted to invited oral presentations and associated questions and discussions. The second day involved plenary discussion of the overall working hypothesis, followed by meetings of break-out groups to develop various elements of the research plan. Plenary discussions during the third and final day were aimed at producing an integrated research plan.

3. Topical Summaries

3.1 Biology, Status, and Trends in Southern Sea Otters

K. Ralls provided an overview based in part on her work in collaboration with J.A. Estes, B. Hatfield, and J. Ames. The sea otter is a near-shore species with a high metabolic rate. It feeds mainly on large invertebrates and tends to deplete its preferred prey. The sea otter's mating system is polygynous, and breeding males are territorial but otherwise wide-ranging. For example, 14 males captured and tagged near Point Conception in April 2001 were scattered all along the coast to as far north as Monterey Bay in September of the same year. Adult females tend to be more stationary than males. Breeding animals, including the territorial males, occur mainly in the center of the population's range. Carcass retrieval is not uniform along the coast; rather, most carcasses come from the periphery than from the center of the range.

Adult female sea otters are almost continuously under stress from either pregnancy or lactation. They produce a single pup each year, with six months of gestation followed by six months of pup dependency. Although pups are born year-round, there is usually a seasonal peak in the spring. Females that lose pups are normally inseminated again within a short time.

Standard shore counts of southern sea otters have been conducted annually since 1982. Although some effort at carcass retrieval has occurred along the California coast since 1968, routine and standardized necropsy protocols have been in place only since 1992, thanks primarily to the efforts of the California Department of Fish and Game in collaboration with the USGS National Wildlife Health Center. Currently, more than 3,000 animals are represented in the carcass database maintained by the Biological Resources Division of USGS.

A number of California sea otters were marked with flipper tags beginning in the 1970s, providing some preliminary insights about movements. In the mid-1980s, implanted radio transmitters were used to track otter movements and habitat use and to refine estimates of reproduction, survival, and tag loss (Ralls et al. 1989; Ralls and Siniff 1990; Siniff and Ralls 1991). Marking, tagging, and tracking stopped after the 1980s because of funding cutbacks premised upon: (a) the assumption that the otter population was steadily recovering, and (b)

the fact that there was a moratorium on oil development along the California coast.

The population trajectory since the late 1930s, when southern sea otters were "rediscovered" in the Big Sur area, suggests a steady increase at about 5%/yr, punctuated by two periods of abrupt decline, one from the late 1970s to early1980s and another in recent years. The 1976-84 downturn was clearly caused by mortality in the near-shore gillnet fishery. The cause(s) of the decline from 1994 onward is/are uncertain. Two other recent trends, neither of them associated with the trends in abundance are: (a) declining mass:length ratios; and (b) declining pup:independent ratios in the population counts. The first of these is most pronounced in females and applies particularly to the years since 1994. The mass:length change in females is closely correlated with the change in pup:independent ratios. Ralls interprets the latter to be due to increased pup mortality rather than decreased fecundity. The three estimates of fecundity from the 1980s were all higher than 0.90, making them comparable to those of other sea otter populations. Ongoing studies of marked animals will make it possible to distinguish the relative importance of fecundity and mortality in the current dynamics of the population.

There are two unusual patterns in the mortality records. A large proportion of carcasses are of prime-aged animals (3-10 years old), and mortality occurs throughout the year, with a peak in summer. In other sea otter populations, mortality is low for prime-aged individuals and deaths occur mainly in winter or early spring. Male sea otters in all populations die younger than females, on average.

Among the possible explanations for the recent decline in abundance are the following: (a) it is an artifact of survey procedures, (b) emigration of otters outside the survey area, (c) reduced fecundity, and (d) increased mortality. Various analyses have essentially ruled out the first two possibilities, and the third is considered unlikely on the basis of available evidence. Most indications point toward increased mortality as the main factor driving the decline.

Outstanding questions remain concerning the carcass data set, as follows:

(1) From 40 to 60% of carcasses are not recovered;
(2) It is possible to determine cause of death for only about 30% of recovered carcasses;
(3) The sample of carcasses for which cause of death is "known" may not be representative of the "unknown" component;
(4) The sample has an age bias, with an unrealistically small proportion of pups;
(5) There is a geographical bias in the sample because carcasses in the center of the population's range are more difficult to detect and salvage;

(6) Cause-of-death data are biased because of the fact that some causes are easier to detect than others; and
(7) It can be difficult to distinguish the ultimate and proximate causes of death.

For their analyses, Ralls et al. grouped the carcasses in the data set into four categories by cause of death – directly by human activity (bycatch, shooting, boat strikes, etc.), "natural" (starvation, disease), shark bites, and other. They also partitioned the data into four time periods – two when the population was increasing (1968-75, 1985-94) and two when it was decreasing (1976-84, 1995-99). There have been noticeable increases in the numbers of carcasses stranded during periods of population decrease, and vice-versa. An even clearer reciprocal relationship exists between the carcasses/population size ratio and the trend in overall abundance. The data suggest that mortality, rather than decreased fecundity, has been responsible for the downward trends in abundance. The incidence of shark attacks (expressed as shark deaths/population size) has increased significantly through time. Based on data from 1985 to 1999, there is no evidence that the incidence of fatal disease has changed significantly. The proportion of disease deaths has been consistently high (35-40%) for at least the last ten years.

Some of the animals in the "other" or "unknown" cause-of-death category presumably died from incidental capture in fishing gear. It is generally not possible to establish drowning as a cause of death for a stranded carcass. About 70% of the carcasses in the total data set (including reported but not necropsied and fresh-dead necropsied) are assigned to the "unknown" category, but there has been statistically significant variation in this percentage among the time periods. Numbers in the "unknown" category were 11.3% greater during the two periods of population decline. During the 1990s, there was a strong correlation between the number of otter carcasses recovered and finfish landings in the Morro Bay complex and the Monterey Bay area. The trap (pot) fishery for live fish has become extremely popular in near-shore waters of central California. Although there is no verifiable, direct evidence for entrapment by sea otters in these traps, there is likewise no reason to assume that they are not at risk from entrapment or entanglement in this fishery.

Following Ralls's presentation, several points were discussed. The declining pup/independent ratio suggests that either (a) fecundity has declined, or (b) there is a problem with pup survival to age 1. There is as yet no evidence of a fecundity problem with southern sea otters. Estes noted that in the California population about half of the pups do not survive to weaning age, whereas in rapidly growing sea otter populations pre-weaning pup mortality is very low. The relatively high observed mortality of prime-aged adults is a serious concern, as are the nature and variety of diseases documented in these animals. With regard to the live-fish trap fishery, Jessup pointed out that it

should be possible to modify the traps to prevent sea otters from being caught.

3.2 Diseases in Southern Sea Otters

Nancy Thomas summarized the results of necropsy studies of California sea otters based on gross necropsy, laboratory, and histopathology results (see Thomas and Cole 1996). The frequency of infectious diseases detected in dead southern sea otters has been about 40% since 1992, when the program of regular and consistent necropsy and sampling of salvaged carcasses began at the National Wildlife Health Center in Madison, WI, coordinated by the California Department of Fish and Game and the U.S. Fish and Wildlife Service. The 40% frequency applies to adults as well as the overall population, and it refers to what were judged to be fatal conditions.

Two lines of evidence have been cited as suggesting that sea otters in California have a generalized susceptibility to disease (Thomas and Cole 1996). One is the high frequency and large variety of diseases documented in carcasses. The other is that otter deaths have been attributed to diseases that are expected to cause only sporadic mortality in other species. Such conditions imply that immune function may be ineffective, whether related to genetic factors, contaminants, or habitat/nutrition (Thomas and Cole 1996). Emergent pathogens associated with the encroachment by humans, domestic animals, and invasive species represents another significant concern.

Two genera of parasitic acanthocephalans (thorny-headed worms) have been documented frequently in the intestines of southern sea otters. One genus of these worms (*Profillicolis* sp.) moves through the wall of the intestine and invades the abdominal cavity. The normal definitive hosts of these parasites are sea birds, and some crab species apparently function as intermediate hosts by ingesting eggs dispersed via bird droppings. Sea otters probably become exposed by eating infected crabs or other intermediate hosts. As aberrant hosts, however, the otters represent a dead end for these parasites.

Bacterial infections of numerous tissues and organ sites have been documented. Bacterial infections in otters often involve multiple organisms, and a variety of causative bacteria have been identified, e.g., *Streptococcus*, *Erysipelothrix*, *Vibrio parahemolyticus*, *E. coli*, and *Bordetella*. The origins of these infections are often not apparent. Affected otters may appear to have experienced sweeping infections and not to have "contained" them well, particularly those involving subcutaneous tissue or muscle.

Protozoan encephalitis is caused by two agents in sea otters. The final host for one of these, *Sarcocystis neurona*, is the Virginia opossum, a species introduced to California in the 1920s. The opossum sheds infectious oocysts in its feces. When ingested, the organisms in these oocysts can infect, proliferate, and encyst in the muscles of an intermediate host (e.g., sea otter, armadillo,

skunk, cat), but they may cause an inflammatory response during proliferation. When an opossum scavenges the infected muscle tissue, the parasite is able to complete its life cycle. The sea otter is not a good intermediate host because encephalitis becomes severe during the proliferation stage, and this probably represents another instance of aberrant parasitism.

The other agent is *Toxoplasma gondii*, a worldwide sporadid that sometimes afflicts humans. The final host of this parasite is the cat, which sheds infectious oocysts in feces. The parasite life cycle includes proliferation and encystation in tissue, similar to *S. neurona*. An infected intermediate host (a sea otter, for example) may become ill and, in the case of humans and domestic livestock, abort a fetus. Although exposure is more likely to lead to toxoplasmosis or encephalitis in very young or immunosuppressed individuals, cases do occur in subadult and adult sea otters. Not every otter exposed to *Toxoplasma* (as indicated by the presence of cysts and the results of serology) gets fatal disease. This differential vulnerability opens the possibility of studies to determine what factors influence resistance vs. susceptibility in otters exposed to *T. gondii*.

Coccidioides immitis, or San Joaquin Valley fever, is a soil-associated fungal disease that is usually contracted by inhalation of airborne anthrospores and can become widespread and systemic, invading the lungs, liver, spleen, and meninges of the brain in highly susceptible individuals. The fungus grows naturally and widely in certain arid environments, notably the San Joaquin Valley. It is another disease in which many individuals of a population may be exposed without contracting full-blown coccidioidomycosis (the fungus being confined in benign pulmonary nodules). Only in individuals that lack resistance does it become a disseminated disease.

A sea otter treated for paralysis at the Monterey Bay Aquarium was found to have damage in the white matter of its brain caused by nematode larvae identified as *Baylisascaris* sp., or raccoon roundworm. Again, the eggs of these parasites are shed in feces of the primary host. This pathogen's occurrence in sea otters (and in humans when it occurs) is clearly aberrant.

A general problem in evaluating disease risk in sea otters is determining whether increased incidence is due to: (a) higher rates of exposure to pathogens that have long been present in their environment; (b) exposure to novel pathogens to which the otters have little or no resistance; and/or (c) impaired immune competence in the otters (which could be caused by, e.g., poor nutrition, contaminants, or even loss of genetic variability such as in the MHC [see below]). Another possibility is that the relatively high incidence of disease is a longstanding feature of the southern sea otter's ecology. The question posed by Thomas and Cole in 1996 – "… are southern sea otters highly vulnerable to many diseases or are they undergoing high rates of exposure?" – remains unanswered.

Despite considerable effort searching for evidence of significant viral disease in California sea otters, little has been found to date (N. Thomas and M. Miller, pers. comm.). This lack of evidence is interesting but difficult to explain. Recent immuno-assays of southern sea otter serum revealed no evidence of exposure to morbillivirus (canine distemper virus, CDV, and phocine distemper virus, PDV; see Ham-Lammé et al. 1999; N. Thomas and D. Jessup, pers. comm.), suggesting that the population might be vulnerable to a catastrophic epizootic if they are susceptible to this virus.

3.3 Contaminants and Health in Southern Sea Otters

The first reports of organochlorine levels in southern sea otters referred to ten animals collected in 1969 and 1970 (Shaw 1971). Of this sample, three had been shot, three had died of "natural causes," two had drowned, and two had been struck by boats. DDE, DDD, and DDT levels were measured in the livers of all ten animals, and total residues ($\sum$DDT) ranged from 0.032 to15 ppm (wet weight). Concentrations in fat (from which area of the body is not stated) ranged as high as 36, in kidney 12, brain 10, testis 14, and ovary 0.84 ppm.

Jarman et al. (1996; also see Bacon 1994; Estes et al. 1997; Bacon et al. 1999) used liver samples from adult male sea otters in relatively good condition collected between 1988 and 1992 to compare contaminant levels in three different sea otter populations. Importantly, the California and Aleutian Islands samples consisted of beach-cast carcasses, while the Southeast Alaska sample consisted of animals shot by hunters. Results must be interpreted with caution since the variable condition of stranded animals could have influenced contaminants results. Average total PCB levels ($\sum$PCB; sum of individual congeners) in California otters were intermediate between those in Aleutian and Southeast Alaskan otters (310 vs.170 vs. 8 μg/kg). The levels in Aleutian and California otters were significantly higher than those of Southeast Alaskan otters but not significantly different from each other. California otters, at 33 μg/kg, had the highest non-*ortho* PCB congener levels, but there was no significant difference among the three groups. The authors suspected a population-level effect of PCBs in California (and Aleutian; see Estes et al. 1997) sea otters. They considered pre-weaning pup mortality high enough to impair population growth (Riedman et al. 1994) and noted the correlation in mink between PCB exposure and reproductive problems and reduced kit survival. They also called attention to the fact that California and Aleutian otters had detectable levels of various other organochlorines "which could be acting synergistically to impair the health of these otter populations," once again citing experimental evidence from studies of mink.

Sea otters from California had significantly higher total DDT levels than the Aleutian and Southeast Alaska samples (Jarman et al. 1996). Estes et al. (1997) pointed out that the DDE/DDT ratio in sea otters from California was 100 times

higher than that of sea otters from the Aleutians, and they concluded that the DDE in California constituted residues from prior local use, whereas most of the organochlorine pesticide fractions in the Aleutians would have come from more recent use, probably in distant areas.

Mean polychlorinated terphenyl (PCT) levels in California sea otters were 1.3 times higher than those of Aleutian otters (46 vs. 35 μg/kg dry weight) although the difference was not statistically significant (Jarman et al. 1996). Sources of PCTs in California coastal waters could be local shipyards, atmospheric deposition, or both. Levels of chlordane, dioxins (PCDDs), and furans (PCDFs) were very low or undetectable in the otters from all three areas sampled.

Other published data on contaminant levels in southern sea otters include the following: (1) Kannan et al. (1998) reported hepatic concentrations of total butyltin compounds (∑BT) ranging from 40 to 9200 ng/g, with an overall mean of 1320 ± 2050 ng/g wet wt (n = 35) [male mean of 753 and female 1420, excluding the 9200 specimen as an outlier; fide Kannan, pers. comm.]. Total BT concentrations in kidney (n = 10) and brain (n = 5) tissue were, respectively, about 7- and 18-fold lower than in liver. (2) Nakata et al. (1998) reported hepatic concentrations of PCBs and DDTs in the ranges of 58-8700 and 280-5900 ng/g wet wt, respectively (n = 35). They found that concentrations of DDTs, PCBs, and chlordanes (CHLs) were greater in kidney than in liver, while HCH (hexachlorocyclohexane) concentrations were similar in both types of tissue. PCBs and DDTs were the predominant organochlorine contaminants found in otter livers, at concentrations as high as 8700 and 5900 ng/g wet wt, respectively. Like Estes et al. (1997), they found nothing to indicate recent inputs of organochlorine pesticides to California waters. (3) Kannan et al. (2001) found relatively low concentrations of perfluorooctane sulfonate (PFOS) in livers (<15 ng/g, wet weight).

Kurunthachalam Kannan provided an overview, co-prepared with Nancy Thomas, on contamination of dead southern sea otters with organotin compounds, organochlorines, and organofluorine surfactants. Apart from measuring tissue levels of various compounds, their studies have examined the relationships between contaminant levels and cause of death.

Tributyltin (TBT) was first used in 1961 and by the 1970s was widespread as an anti-fouling agent in marine paint formulations. Effects on non-target organisms such as oysters and gastropods were first recognized in 1974. In the1980s, it was shown that declines in European oyster populations due to "imposex" (e.g., females with male organs) were causally related to TBT exposure. Although the use of TBT in paints has been banned in the UK for certain classes of boats since 1987, and in the United States since 1988, these contaminants persist in sediments for several years and are still being released into the marine environment as paint is abladed from the hulls of large ships (see Kannan et al. 1998). The International Maritime Organization is seeking a

global ban on TBT use in marine paints by 2003, with the expectation that all ship hulls would be decontaminated by 2008.

Laboratory studies have shown that butyltins have immunosuppressive effects in fish and mammals. In sea otters, the liver is the site of greatest accumulation for these contaminants although it should be noted that BTs, unlike organochlorines, bind to proteins rather than lipids. Average TBT concentrations in liver tissues of dead southern sea otters were 376 ng/g, wet weight, in males, and 606 ng/g in females, with concentrations as high as 3020 ng/g recorded in one individual. TBT is cytotoxic at concentrations near 10,000 ng/g, suggesting that at concentrations above a few parts-per-million, liver tissue lesions would be expected to occur. In general, as pointed out by Kannan et al. (1998), females in their studied sample of 35 southern sea otter livers tended to have higher BT concentrations than males. There were large differences in $\sum$BT (dibutyltin, DBT, plus TBT plus monobutyltin, MBT) concentrations in otter carcasses salvaged from different parts of the coastline, with a strong peak in animals from Moss Landing and relatively high levels in those from Monterey Harbor and Morro Bay.

Of particular interest in the context of the workshop was Kannan et al.'s analysis of relationships between BT concentration and cause of death. In their sample of 34 animals (an outlier was eliminated from this analysis), 14 were judged to have died from infectious disease, 8 from trauma, 7 from unknown causes, and 5 from "miscellaneous problems" (including intestinal perforation, esophageal impaction, urinary obstruction, and neoplasia). BT concentrations in the disease group were significantly ($p < 0.05$) higher than those in the trauma and unknown classes. However, there was a wide range in BT concentrations within the disease group (40-5300 ng/g, wet weight), and at least some of the diseased animals may have had altered contaminant patterns as a consequence of fasting prior to death.

Kannan also presented a preliminary framework for assessing the risk posed by BTs to sea otters. He cited an experimental laboratory study that used dolphin and seal blood to determine a threshold blood concentration for decreased mitogen-induced lymphocyte proliferation (LOAEL, lowest observed adverse effect level) at 28 ng/mL for both TBT and DBT (Nakata et al. 2002). Since in marine mammals generally, the liver/blood concentration ratio for BT is 4:1, Kannan proposed 112 ng/mL as a reasonable immunotoxicity reference value for liver tissue (from all marine mammals, including sea otters). Using the reported range in BT liver concentrations for bottlenose dolphins (110-11,340 ng/g) as an equivalency standard (1-100), the BT "hazard quotients" (HQ) for dead southern sea otters sampled to date would range from 0.5 to 82 (very generally, HQs of less than 1 indicates less risk; 1, risk at the individual level; greater than 10, risk to the population level).

Kannan also summarized the data published by Nakata et al. (1998) on organochlorine pesticides and PCBs in dead southern sea otters. Geographical differences broadly similar to those found for BTs were found for these compounds, with a strong peak in hepatic PCB concentrations in Monterey Harbor (suggesting a nearby point source) and a somewhat lower peak in hepatic DDT concentrations in Moss Landing and Monterey Harbor. The ∑PCB concentrations in Monterey Harbor (ranging between 2100 and 8700 ng/g wet wt) were two or three times higher than the critical body residues estimated for reproductive effects in mink (Leonards et al. 1995).

Liver samples from 20 dead otters (compared with 34 samples for BTs, above) were used for comparisons of organochlorine concentration and cause of death (Nakata et al. 1998). For this sample, ∑DDT concentrations in liver were significantly higher in animals that died of infectious disease than in those that died of trauma or of "unknown" causes. Concentrations were also higher (but not significantly so) in the animals that died of "miscellaneous" causes, including neoplasia, emaciation, and esophageal impaction. A similar pattern was suggested for ∑PCB concentrations, but none of the differences was statistically significant. Concentrations varied widely among individuals. The authors acknowledged that their results were no more than suggestive and that larger samples were needed.

Kannan assessed the health risks of ∑PCB concentrations in southern sea otters in several ways. One used toxic equivalents (TEQs) based on exposure studies of seals, mink, and otters (standardized using toxic equivalency factors developed by the World Health Organization) (see Kannan et al. 2000), with sea otter TEQs ranging from 0.73 in Half Moon Bay (1 male) to 130 in Monterey Harbor (averaged for two females). A second approach used a threshold value for physiological effects (including immune suppression) of 8700 ng/g, lipid weight, in liver (from Kannan et al. 2000), resulting in HQs ranging from 0.46 in Halfmoon Bay to 26 in Monterey Harbor, with an overall mean of 4.2 (n=11). Finally, a third approach used mink data from Tillitt et al. (1996) to produce HQs for the sea otter sample from 0.012 in Halfmoon Bay to 2.2 in Monterey Harbor. All three approaches point to a potentially serious health risk to sea otters, and especially those that reside in Monterey Harbor. Kannan's estimates of HQs did not involve the application of safety factors either at the derivation of threshold concentrations or at the calculation of HQs. This means that the hazard quotients may be less conservative.

Kannan concluded his presentation with a list of further studies that are underway or contemplated, as follows:

1. Metabolic potential to organochlorines and food chain accumulation (work completed; manuscript in preparation).
2. Other contaminants – metals, PCDDs/DFs, PAHs, and fluorinated organics

3. Risk assessment from exposure to complex mixtures of contaminants: Additive? Synergistic? Antagonistic?
4. Geographical differences in contamination and health status: Californian vs. Alaskan vs. Russian sea otters and their prey items.
5. Analysis of environmental samples: sediment and food web.

During discussion following Kannan's presentation, it was suggested that the carcass sample used for disease/BT correlation analyses was perforce biased geographically toward harbor areas where input is likely to be high for both butyltins and pathogens, making it difficult to interpret the correlations found in the study. Portions of the California coast are likely receiving BT inputs continuously, e.g., from water flowing through PVC piping, ablation of paint on boat hulls, and small boatyards. Kannan indicated that the samples were collected from various locations along the central coast of California and that some of them came from relatively clean areas.

It was suggested that in developing a toxic-equivalency or hazard-quotient approach, it might be preferable to use direct measurements from whole blood rather than estimated blood toxicant levels obtained by extrapolation from liver measurements.

Estes made three observations potentially relevant to interpretation of Kannan's data:

1. There is a remarkable amount of individual variation in contaminant concentrations among sea otters, and this is similar to what is found in river otters and bottlenose dolphins.
2. Judging by what is known about demographic trends, it appears that the entire southern sea otter population is being affected throughout its range, thus making it difficult to pinpoint a particular problem area or areas.
3. Recent studies of California sea lions off California show dramatic declines over the past 30 years in $\sum$PCB and $\sum$DDT concentrations.

Kannan pointed out that because BTs bind to proteins rather than lipids, hair loss during the molt represents a way of getting rid of at least a portion of the BT body burden, at least for pinnipeds. This may not apply to sea otters, as they do not molt.

Ross emphasized the value of combining different lines of evidence, especially when extrapolations from other species or other chemical mixtures are involved. Also, he expressed concern about confounding factors such as age- or sex-related differences in diet, metabolism, and behavior, or the use of data from "stranded" animals that could have been sick and not eating for a period prior to death. Mobilization and metabolism of fat in such animals could increase contaminant concentrations in their body lipids. Kannan noted that

the latter should be less of a problem with BTs because they bind to proteins rather than lipids.

Sea otters, unlike marine mammals that store large amounts of energy in their blubber, tend to decline rapidly and die quickly because they cannot tolerate long periods of fasting. Kannan pointed out that variation in contaminant concentrations due to fat mobilization should be less of a factor in sea otters than in cetaceans because sea otters do not possess blubber. However, Estes pointed out that trauma-killed sea otters do have substantial lipid content in their bodies.

3.4 Immunotoxicology and its Application in Marine Mammals

Julie Schwartz presented an overview of her work in collaboration with Brian Aldridge, Jeff Stott, and Chuck Mohr at the University of California Davis. Their research focuses on the immune system as a key determinant of health status. This system is very sensitive to environmental factors, including biological and chemical agents. Assessments of immune function can be done with cells isolated from the blood of a live animal. Blood collection is relatively non-invasive and can be accomplished without injuring the animal. Traditional methods of measuring the activity of the immune system provide valuable but limited information. These methods include determining the composition (e.g., analysis of cell surface antigens [immunophenotype] by flow cytometry) and function (e.g., antibody and cytokine production, lymphocyte activation and proliferation) of immune cells.

New technologies are emerging in molecular diagnostics, and the fields of immuno- and toxicogenomics are expanding rapidly. The value of these novel technologies is that the up- or down-regulation of many genes, which provide the transcriptional messages important in mediating toxicological and immune reactions, can be assayed from a single sample of blood. This is a welcome development for researchers working on wildlife because they are often limited in the amount of blood that can be collected. Gene expression analysis can be used to identify which genes are activated by particular toxicants and to detect subtle changes in an animal's defense responses to the toxic insult. It is also possible to establish genetic signatures ("fingerprints") of particular toxicants.

The team at Davis is actively involved in use of these new molecular technologies in immune and toxicological assessments of captive and free-ranging animals. The fundamental goal of their work is to link contaminant exposure to health effects at the molecular level and to establish protocols for detecting exposure by reference to genetic markers. They view their work as providing discrete inputs to complex ecological models of such things as relationships between pollutants and pathogens.

Surrogate species, such as the mink, have been used to define and measure immunologic changes associated with exposure to environmentally relevant chemical contaminants, and to develop reagents and new techniques. Captive mink provide researchers with opportunities to conduct studies of controlled exposure to environmental stressors over long periods of time. Mink studies can include dose-response protocols and can model the pharmacokinetics of particular toxic chemicals. Samples taken from the mink in a controlled experimental setting are reliable, so the primary source of uncertainty is in how one extrapolates the results to another species, in this case the southern sea otter in the wild.

Mink have already been used to study the toxic effects of fuel oil. For example, changes have been observed in hematology (e.g., decreased red blood cells possibly leading to lowered oxygen carrying capacity, increased white blood cells signifying a proinflammatory state) and immune system function (e.g., increased lymphocyte proliferation, altered immune cell phenotype, and decreased mesenteric lymph node mass). Fuel oil also stimulates the induction of key metabolizing enzymes in the liver and increases the size of the adrenal glands), the later suggesting endocrine involvement. New technologies are being used with mink to assess gene expression in response to toxic chemicals, investigate mechanisms of effect, and identify relevant "biomarkers" of exposure.

Immunogenetics is another of the Davis laboratory's areas of expertise. Instead of using microsatellites, the conventional gene markers, Stott and associates are using the major histocompatibility complex (MHC) to track changes in the genetics of specific marine mammal populations. This gene complex is key to determining susceptibility to infectious disease. The advantage of using the MHC as a genetic marker rather than other markers is that it is sensitive to changes in population dynamics and to pathogen exposure.

Among the matters raised in discussion following the presentation were the cost and feasibility of developing a functional genomics approach to studying the role of contaminants and disease in limiting the recovery of southern sea otters. Participants also stressed the necessity of making the link between contaminant exposure (as indicated by observed gene induction) and pathology in sea otters.

3.5 Contaminants in Marine Mammal Food Webs

Peter Ross summarized his work on persistent organic pollutants (POPs) in marine mammal food webs. POPs include chemicals that are fat soluble and resistant to metabolic breakdown. POP exposure has been linked in studies of laboratory and captive animals to adverse health effects in fish-eating wildlife, including seals. Links to disease outbreaks in free-ranging populations have proven more difficult to establish (see Jepson et al. 1999 for an example of

good etiological study design). Nevertheless, certain relatively well-known persistent, bioaccumulative contaminants such as PCBs, PCDDs (dioxins), PCDFs (furans), organochlorine pesticides, and organotins have long been known to be immunotoxic, and weight-of-evidence arguments have been made to infer that they played a role in certain marine mammal die-offs (see Ross et al. 1996; Ross 2000, in press). It remains extremely difficult to establish direct mechanistic links between tissue contaminant levels and adverse effects in wildlife because free-ranging animals are exposed to complex mixtures of chemicals and their health is simultaneously affected by numerous other factors. However, a combination of approaches drawing upon experiments carried out with surrogate species, or other wildlife species, can often provide the basis for reasonable extrapolations. Such a "weight of evidence" approach is routinely used in human health research.

According to Ross, a central theme of contaminants research involving marine mammals should be to consider what these high-trophic-level animals can tell us about the state of the marine environment. Thus, in the case of southern sea otters, it is of interest to know as much as possible about the ecological pathways of the contaminants found in their tissues. This means understanding the nature of their food webs; the quality of the air, water, and sediments in their near-shore environment; and their levels of exposure to various types of contaminants, both old and new. POPs are distributed globally through atmospheric processes. Characterizing contaminants in a given food web helps to identify "local" and "global" sources, which is key to regulation and management.

Harbor seals have proven extremely useful as sentinels of POP contamination in coastal regions of the Northern Hemisphere. They are relatively abundant and easy to catch and handle. It is possible not only to obtain skin and blubber biopsies, but also to obtain liver biopsies from seals that are temporarily restrained. Harbor seals have been studied extensively by scientists in a variety of disciplines. They are omnivorous, with strong preferences for herring, hake, other local fish, and invertebrates. To a considerable extent, the harbor seal has become the "laboratory animal" for toxicologists who study marine mammals. Field and captive studies of harbor seals have implicated environmental contaminants in reproductive impairment, immunotoxicity, endocrine disruption, skeletal malformations, and disease outbreaks. The accumulated evidence suggests that current concentrations of persistent organic pollutants are sufficiently high to elicit significant population-level consequences for marine mammals inhabiting coastal areas in parts of the industrialized world.

The seals in Puget Sound have been heavily contaminated with PCBs, and historical chlorine-bleaching by pulp and paper mills has led to high dioxin and furan levels in seals in British Columbia's Strait of Georgia. Seals from the various parts of British Columbia and Washington can be readily distinguished

by principal components analysis on the basis of contaminant patterns. Killer whales in British Columbia and Washington have exceptionally high PCB levels. Male killer whales, in particular, have far higher concentrations than those reported for beluga whales in the St. Lawrence River, in eastern Canada. Interestingly, chinook salmon, one of the main prey species of inshore resident whales, acquire about 99% of their PCB burden while at sea in the North Pacific, well away from the natal rivers and estuaries from which they disperse as smolts. Studies of harbor seals and killer whales provide evidence that both local and international sources are contributing to contaminant burdens in marine mammals along the west coast of North America.

Given the difficulties of establishing cause-and-effect links between contaminant exposures and adverse health effects in wildlife, it is crucial to attempt to understand or eliminate "confounding factors" when interpreting both the *concentrations* of contaminants and the toxicological *effects* of these chemicals in studies of wild marine mammal populations. Consideration of confounding factors is needed in several phases of a study, including (a) study design, (b) sampling and field work, and (c) data assessment and interpretation. For example, vitamin A (retinol) is a useful biomarker of toxicity, but natural changes in vitamin A occur in harbor seal pups during the nursing period. Therefore, observed changes in vitamin A levels in tissue samples from harbor seals may be only partly due to toxic exposure. PCB levels in Pacific Northwest killer whales vary significantly by sex, population, and diet. Adult male "transients" (as opposed to "residents") are the most heavily contaminated class, likely because their diet consists mainly of marine mammals and, unlike females, they do not transfer any portion of their contaminant load to their offspring. Total PCB concentrations obscure differences in levels of various PCB congeners. For example, certain types of PCBs (III and IV) are metabolically eliminated by killer whales, while other types essentially accumulate in tissues until the animal dies. Pinnipeds get rid of about 25% of their body burden of organotins each year by molting. Cetaceans do not get this benefit, nor do sea otters.

In assessing the effects of POPs on the health of free-ranging wildlife populations, many other factors must be considered. For example, in the case of killer whales, these would include: stress from increased vessel traffic; limited genetic variation; quality and quantity of prey (e.g., large variation in salmon stocks); individual age, sex, and reproductive status; and pathogen exposure. Any of these factors could be additive, synergistic, or antagonistic with respect to a given animal's burden of immunotoxic contaminants.

Marine mammals can be useful "sentinels" of ecological risk, particularly when data on their contaminant levels through time are coupled with data on contaminants in their prey and in sediment core profiles from the region. For example, the modeled PCB history of northern "resident" killer whales in British Columbia shows an increasing trend that peaked in the 1970s, followed

by a steady decrease until the late 1990s. Total PCB concentrations in harbor seals from Puget Sound declined dramatically from the early 1970s to mid-1980s but have changed little since then. While these trends are encouraging, it is nevertheless important to recognize that current PCB levels in these organisms may be high enough to have toxic effects. Moreover, although many of the older POPs have been largely regulated, many newer replacement POPs with similar properties to PCBs and dioxins are being used, are not regulated, and are increasing in the environment.

During discussion following Ross's presentation, questions were raised concerning the sea otter's diet and how it differs from those of piscivorous marine mammals like harbor seals and killer whales. Although southern sea otters rarely or never eat fish, they are nonetheless exposed to persistent chemicals that bioaccumulate in the coastal food chain. It was also re-emphasized that sea otters to not have a blubber layer. They do, however, maintain body lipid reserves. Like other mammals, sea otters are probably unable to eliminate POPs readily, making them vulnerable to similar patterns of health effects. This assumes that vertebrate species have a common physiology and that similar mechanisms of toxicity associated with POPs are to be expected across taxa.

3.6 Sea Otter Ecology

Jim Estes provided comments based on his long experience with sea otters in both Alaska and California. In his view, there is a danger that the reductive focus on contaminants could prove misleading and distracting. He and several other participants expressed concern that disproportionate attention given to contaminants could divert public attention away from equally or more important issues such as disease exposure and fishery bycatch. He emphasized that contaminants and disease are not synonymous. Although there is compelling evidence that disease is a serious problem for southern sea otters, the evidence that contaminants are contributing to this population's failure to recover is thin by comparison. Moreover, it seems likely that the southern sea otter's demographic problems are chronic and date back to at least the early 20^{th} century.

Most of the relevant research on southern sea otters to date has been based on studies of carcasses, but that focus will never be adequate. Much more work needs to be done on live and superficially healthy animals. Further, studies of southern sea otters in isolation is likely to prove inadequate, and it is important to employ a comparative approach and take advantage of the extensive data on other sea otter populations, some of which have shown much faster and more sustained growth than this population.

Estes pointed out that the California mussel can serve as a useful index of contamination of nearshore ecosystems from central Baja California (Mexico)

northward to at least Kodiak Island (Alaska). A broad-scale mussel sampling effort could be extremely revealing in terms of what it could tell us about sea otter exposure to contaminants. Many contaminants (e.g., PCBs and butyltins) appear to be very aggregated spatially, thus the need for large samples collected over a wide spatial scale. Workshop participants were confident that data from Mussel Watch could be used to accomplish at least some of what Estes was proposing, and it was suggested that sediment data from USGS sedimentologists might also contribute to identifying "hotspots" of exposure to various contaminants along the California coast.

Estes also summarized the main elements of an ongoing study coordinated by the USGS Western Ecological Research Center (WERC), as follows:

- Live-capture, tag, sample, and instrument 150-200 animals over 3 years, beginning in February 2001
- Captures to be equally distributed at 3 sites: Monterey Bay, Piedras Blancas, and Point Conception
- Site selection was intended to be roughly representative of the spatial range of the population, and methodology is broadly similar to that of Ralls and Siniff during the 1980s – thus there is potential for meaningful comparisons
- Animals to be flipper-tagged, instrumented with VHF radio tags, PIT tags, and time-depth recorders
- Intensive monitoring of movements and activities (almost daily observations)
- Tooth extraction for age determination at capture, blood collection, body measurements
- Liver biopsies are *not* included in the sampling protocol and are not covered by the existing permit
- Recapture at 1 year after initial capture to recover instruments and resample
- Selection for relatively healthy non-pups; females to be released without instrumenting if palpably pregnant
- Major objective is to get a window on mortality of prime-aged animals – healthy animals have about a 15% chance of dying in a given year, so it is expected that about 60-70 of the sampled otters will die at some time during the study period

Estes stressed that it would not be possible to increase the power of the study by expanding the number of capture sites, largely because of manpower requirements and logistics. Any increase in power would need to come from increasing the number of animals sampled and/or from gathering additional types of information from the animals that are handled. He noted that at least a few animals would be caught incidentally or by mistake during the live-capture operations, and such animals would represent opportunities to obtain

some additional samples. The recapture success rate is expected to be in the range of 50-75%. Judging by experience in previous studies, it should be possible to recapture nearly all of the animals captured initially in Monterey Bay.

Estes also pointed out that many blood and other tissue samples (e.g., liver biopsies) are available from sea otters in Alaska and Washington, and that some of these have been or are being screened by Wally Jarman in Utah. A complete, up-to-date inventory of what is available and what is being done would be useful for planning further collections and analyses.

A striking feature of sea otters is the great variability in food habits between individuals. This variability has not yet been examined to the extent that it could be, using fatty acid and stable isotope analyses.

Estes' ongoing study of live sea otters was viewed by workshop participants as an opportunity for collaboration (see below). During discussion, both Estes and Jessup urged that a staged, stepwise approach be taken, and that more analyses be carried out with tissues in-hand to evaluate the plausibility of a contaminant problem in southern sea otters, before committing major resources to contaminants research.

3.7 General Discussion

During a general discussion following the presentations, a number of points were made that are recorded here because of their relevance as background to the proposal.

3.7.1 Factors possibly affecting population recovery

The question of sea otter mortality in pot fisheries for live fish and lobsters along the central California coast needs to be addressed urgently. Although there is reason to believe that some mortality of sea otters occurs in these fisheries, no direct observations have been reported. Estes stressed that even with substantial observer effort, there is danger of a false negative conclusion. In other words, given the very large numbers of trap-days in the fishery, otter captures would be rare events and could easily be under-estimated, or even missed entirely, by an observer program with insufficient coverage. Participants were uncertain whether NMFS had plans to implement an observer program in these fisheries specifically to assess sea otter bycatch.

Ralls noted that Eastern North Pacific Southern Oscillation (ENSO, commonly known as El Niño) events had been shown to cause high pup mortality in southern sea otters, but she believed that such events could not account for a basic shift in health status of the population. She urged that more attention be paid to the role of nutrition, and that condition indices for both live and dead

sea otters should be improved. In response, Jessup pointed out that blood values are not very reliable indices of condition because they tend to provide only a snapshot of the animal's immediate "condition" rather than its overall nutritional status.

3.7.2 Mortality patterns

The higher-than-expected mortality of prime-aged animals, particularly females, was seen as a likely driving force in the population's recent decline. Similar age distributions of beach-cast sea otter carcasses have been observed in only two other instances – one consequent to the Exxon Valdez incident in Prince William Sound, Alaska, and the other the current sea otter crash in the Aleutians (see Estes et al. 1998). DeMaster pointed out that in sea otter population dynamics there is an almost 1:1 relationship between adult female survival and the overall population trend. Indeed, Estes suggested that both the short-term population downturns (1976-84 and 1994-present), and the longer-term "sluggishness" of southern sea otter population's recovery (maximal rate of 5%/yr), appeared to be due to the high mortality of prime-aged animals. All sea otter populations that he has studied appear to be regulated by mortality rather than reproduction. There is little evidence of density-dependent changes in reproductive rates in sea otters; reductions in population growth rate generally do not occur until the population gets very near carrying capacity.

Estes added that the southern sea otter population clearly differs from other studied sea otter populations in that it fails to exhibit the typical pattern of a peak in mortality during late winter and early spring. He believes that the anomalous mortality patterns of southern sea otters, with respect to both age distribution and seasonality, hold the key to explaining the population's failure to recover.

3.7.3 Dynamics between immune suppression and disease

Problems with the immune system could be caused by at least three factors, alone or in combination – contaminants, loss of genetic variability in the MHC, or exposure to a virus. It is possible that, at least in some instances, susceptibility to disease could reflect little more than novel host/pathogen interactions. One cannot rule out the possibility that the immune systems of southern sea otters are fine, and that the incidence and severity of at least some of the diseases observed in this population are the result of new exposures to pathogens. Many factors other than immune suppression can determine whether an animal becomes ill – genetics; exposure to viruses, bacteria, and other pathogens; contaminants; nutrition; age and reproductive status; and environmental conditions.

Immune suppression does not automatically lead to illness but does put an animal at risk for developing a life threatening disease. A major challenge is to link immune system stress (or dysfunction) with sickness, debilitation, or death. It is important to investigate the history of an animal's, or a population's, exposure to infectious agents. This means that screening of blood and other tissues from live as well as dead otters should include the search for antigenic challenge and successful immunologic response. As Brand repeatedly emphasized during workshop discussions, selection of controls should particularly include the identification of animals that have been exposed to a disease agent but not succumbed as a result. According to Jessup, lymphocyte function assays have been conducted on some live sea otters, but sample size and sampling conditions have not been optimal. These assays must be carried out quickly on samples obtained immediately upon capture (i.e., with the animal having undergone minimal capture stress). Results are relative, making comparisons among sampling days difficult.

3.7.4 Surrogate species

It is virtually impossible to contemplate controlled studies with sea otters that would provide definite answers to questions about potential links between contaminants and disease. However, surrogate species often provide feasible ways of establishing the mechanism or mode of action that links exposure to effects. Laboratory conditions are required for studies that depend upon the ability to control contaminant dosages and the age, sex, health, and other features of the experimental animals.

Although it may not always be feasible, or even necessary, to have a mechanistic understanding of cause-and-effect linkages, it is certainly desirable. For example, captive studies of ducks (surrogates) provided an explanation of how pesticides were affecting reproduction in raptors and brown pelicans, and this knowledge proved critical in convincing Congress to take decisive action to restrict pesticide use (see Risebrough 1999).

Mohr explained that surrogate studies were extremely useful for validating biomarkers. In addition, Ross pointed out that laboratory studies that allowed dose variation of contaminants also offered opportunities to challenge animals with controlled exposures to disease.

3.7.5 Other factors that affect the immune system

Many kinds of stress can affect an organism's immune system either temporarily or permanently. Pregnancy and lactation are natural stressors that compromise immune function because they are so energetically demanding. As indicated above, adult female sea otters almost never get a rest from the demands of reproduction, and this might increase their susceptibility, particularly if their plane of nutrition is for some reason sub-optimal. The

added metabolic costs of carrying a substantial contaminant burden could increase the normal stress of pregnancy and lactation.

3.7.6 Comparative approaches

Reijnders (1999) has emphasized the value of sampling animals over a gradient of contamination, i.e., animals from both relatively clean and relatively polluted areas. The "clean" animals can provide reference values for assessing the contaminant profiles of the studied population. In the case of southern sea otters, Monterey Bay and Moss Landing are highly contaminated compared with other parts of the population's range (see Kannan's overview, above). It was noted that rehabilitated sea otters often come from "clean" areas, but they are usually released into the Moss Landing area where exposure to both contaminants and disease is relatively high. Using stranded animals as sources of reference values is contraindicated.

A similar strategy of sampling across a range of areas with differing levels of pollution (in terms of both contaminants and disease) would apply to studies of sea otter prey. It was noted in this regard that Mussel Watch involves regular sampling of 22-30 sites in California, spanning the entire range of the southern sea otter.

4. Proposal

All participants agreed on the need for a broad-based, multifactorial approach, recognizing that multiple threats were likely implicated in the mortality-driven decline (or abnormally slow recovery) of southern sea otters. Therefore, the study proposed here, with its focus on contaminants, must not be seen as competing with or precluding other avenues of research or monitoring. In fact, the completed and ongoing work by the CDF&G and USGS is vital, as is expanded effort to document interactions between southern sea otters and fisheries.

The workshop broke into three subgroups to develop proposals for inclusion in the overall research package. These subgroups were organized around the following three themes: (1) How to complement and supplement the current capture-release program (outlined in 3.6, above); (2) Effects of contaminants on the sea otter immune system; and (3) Causes of slow recovery/decline of the southern sea otter population. Each group was encouraged to produce two or three discrete tasks for consideration as components of the overall research program. These suggested tasks were presented and discussed during the third day of the workshop. The following tasks comprise the workshop's agreed package. It should be noted that there is overlap (redundancy) between the descriptions of tasks given here and those given in the preproposal document. The complete preproposal text of each task is included here, along with