Domoic Acid Toxicity in California Sea Lions (*Zalophus californianus*) Stranded Along the Central California Coast, May-October 1998

Report to the National Marine Fisheries Service Working Group on Unusual Marine Mammal Mortality Events

Frances Gulland

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum NMFS-OPR-17
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In response to large numbers of California sea lions washing ashore dead and in obvious physical distress, individuals from the Marine Mammal Center, other stranding network participants, National Marine Fisheries Service, as well as numerous other State and Federal partners, were called into action to give aid to the animals and determine the cause of the event. In the course of this mortality event, many individuals with expertise in various disciplines contributed their efforts and insight into the initial response, sample collection, and final analyses. The results of their investigations are in this report. This report was prepared by the Marine Mammal Center under contract 40AAND801390.

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ABSTRACT

Between May 15 and June 19, 1998, 70 California sea lions (*Zalophus californianus*) and one northern fur seal (*Callorhinus ursinus*) stranded along the central California coast from San Luis Obispo to San Mateo County. Of these 70 animals, 54 were adult females, with 27 (50 %) pregnant; three were subadult females; two were juvenile females; one was a yearling female; six were subadult males and four were juvenile males (Appendix 1). All animals were in good nutritional condition and displayed similar clinical signs that were predominantly neurological. The animals had severe seizures that either became increasingly frequent, resulting in opisthotonus, then death, or became less frequent with the animals showing ataxia and decreased responsiveness to stimuli between seizures and eventually becoming clinically normal. Forty-eight of the 70 animals (69 %) died despite treatment.

Treatment consisted of supportive care with oral and subcutaneous fluids, antibiotic cover with penicillin, control of seizures using diazepam, lorazepam, and phenobarbitone, and reduction of cerebral edema using dexamethasone. Induction of parturition using dexamethasone and prostaglandin F2α was attempted in adult females with open cervices and dead intrauterine fetuses.

Hematological parameters in these stranded sea lions were within normal limits, as were serum biochemical profiles other than creatine kinase levels. The creatine kinase levels were elevated in most animals, presumably as a consequence of muscular damage during seizures. Levels of blood lead were normal in the eight affected animals tested, and brain cholinesterase levels were normal in the five affected animals tested.

Virus neutralization (VN) tests for phocine distemper virus (PDV) detected low levels of antibody in 10 of 34 animals (29 %). After two weeks, four of these 10 animals showed low positive, but rising, titers on VN. No signs of respiratory disease or novel neurologic signs were detected. After a further month, these four animals were seronegative to PDV. Three animals that had been in contact with seropositive animals remained seronegative. A retrospective survey of banked sera from 100 adult California sea lions that had stranded previously revealed that 20 % had low titers to PDV.

A variety of non-specific lesions were observed on gross *post mortem* examination. These included gastric ulceration and erosion with associated lymphadenopathy, bile stasis, pulmonary congestion and occasional subcutaneous hemorrhages. Sea lions that died within the first two days of stranding had diffusely pale myocardium with occasional focal areas of severe pallor. All pregnant females had necrotic placentae and dead fetuses.

Bacteria isolated from tissues at *post mortem* were typical of those isolated from stranded California sea lions in recent years. A novel calicivirus was isolated from myocardium of one animal (#3709).

The predominant histologic lesion in affected animals was neuronal necrosis, that was most severe in zones CA3 and CA4 of the hippocampi and the dentate gyri. There were also intramyelinic and neuropil edema and occasional foci of gliosis. In some animals that died within 48 hours of stranding, there was multifocal myocardial necrosis and inflammation.

Domoic acid, a biotoxin produced by a diatom, was detected in serum of 3 of 7 animals, urine 7 of 14 animals, and feces of 3 of 9 animals. Two of the positive fecal samples were examined by electron microscopy, and frustules of *Pseudo-nitzschia australis* were observed. No domoic acid was found in kidney, stomach washings, cerebrospinal fluid, or brain samples from affected animals. Analyses were carried out using a receptor binding assay (RBA) and High Performance Liquid Chromatography with Ultraviolet detection (HPLC/UV). HPLC / mass spectrometry (HPLC/MS and HPLC/MS/MS) carried out on a subset of samples from each tissue type provided independent confirmation of the chemical identity of the biotoxin.

A bloom of *Pseudo-nitzschia australis*

(*P.australis*) occurred in Monterey Bay during the latter half of May 1998, reaching its peak on or about May 22. The greatest concentration of *P. australis* recorded was ~200,000 cells per liter. Cells were concentrated near shore, possibly in response to nutrients of terrestrial origin brought to coastal waters by enhanced river outflow. Plankton samples were also analyzed for domoic acid using a receptor binding assay. The rise and fall of domoic acid in these samples corresponded to the rise and fall of *P. australis* observed in the plankton. Anchovies collected from the bay on May 22, 1998 had levels of domoic acid of 105.6 μg domoic acid/g tissue, whereas fish collected on June 10, 1998 had no detectable levels of domoic acid. Anchovies collected during the peak of the *P. australis* bloom that contained high amounts of domoic acid had *P. australis* frustules within their stomachs. In Monterey Bay, the bloom of *P. australis* was followed by a bloom of *P. pseudodelicatissima*. Anchovies collected from Monterey Bay reflected this change in species abundance in their stomach contents. No *P. australis* frustules or domoic acid were detected in stomach contents of these fish, and no domoic acid was detected in plankton during the *P. pseudodelicatissima* bloom.

Three of the 23 sea lions (#3815, #3822 and #3815) that survived treatment during the event in May and June 1998 were equipped with satellite and radio-transmitters prior to their release on November 15, 1998. Battery life of the satellite transmitters was three months. Satellite and re-sight data indicated that sea lions survived for at least 48, 64 and 94 days, respectively. Two sea lions traveled as far south as the Channel Islands, whereas the third sea lion remained in the vicinity of Año Nuevo Island. All three sea lions that were sightedduring the three-month telemetry period appeared healthy and displayed normal behavior.

During routine surveys around the Monterey Bay during the months of May and June 1998, a 3.5 fold increase in numbers of dead beach-cast birds and pinnipeds was detected compare to the same months of the previous year. The majority of the dead birds were Common Murres, Surf Scoters and Sooty Shearwaters. Large numbers of stranded sea lions were also detected along other central California beaches at the time. The extent to which these mortalities was due to domoic acid toxicity rather than as a consequence of starvation due to El Niño conditions is unclear, since most animals were not examined.

From July 2 to October 17, 1998, an additional 11 California sea lions stranded displaying similar clinical signs to those that stranded during late May and early June. Nine of these animals stranded between October 3 and October 17. Four animals survived and seven died. The dead animals had histologic lesions similar to those in the earlier cases. Domoic acid was detected in urine of two of these animals by the microplate assay. At the same time (early October), cells of *P. australis* were observed in Monterey Bay around the Santa Cruz and Capitola piers, but were at relatively low concentrations (<10,000 cells per liter). However, further offshore (outside of Monterey Bay), concentrations of *P. australis* were in excess of 100,000 cells per liter. It is likely that the source of domoic acid affecting sea lions during October was a bloom outside of Monterey Bay.

In summary, the combination of clinical signs, histopathological, toxicological, epidemiological and oceanographic changes led to the conclusion that domoic acid toxicity was the cause of this Marine Mammal Unusual Mortality Event. Domoic acid was first reported as a cause of toxicity in humans in 1987, when four people died and approximately one hundred were clinically ill following ingestion ofcontaminated mussels on Prince Edward Island, Canada. This event is the first documented occurrence of domoic acid toxicity in marine mammals.

INTRODUCTION

Within the California Marine Mammal Stranding Network, response to stranded marine mammals is shared between a number of organizations under the direction of the NMFS Southwest Regional Stranding Coordinator, Joe Cordaro. The Marine Mammal Center (TMMC) responds to calls on live animals from San Luis Obispo County in the south to Mendocino County in the north. Calls on dead marine mammals in the same range are responded to by a number of organizations, usually a different one in each county. The type of response and extent of sampling beyond the collection of level A data varies between agencies within the California Marine Mammal Stranding Network, due to different interests of volunteer members. In El Niño years, there is typically an increase in the number of pinnipeds, especially California sea lion (*Zalophus californianus*) pups and yearlings, stranding along the central California coast (Cordaro, 1997). In late 1997 and early 1998, strandings of emaciated animals increased as predicted in association with changes in sea surface temperature and decreasing food availability. The majority of these animals were emaciated, heavily parasitized California sea lions. In May and June 1998, 334 yearling sea lions were admitted to TMMC, compared to 92 in the same months of 1997. Strandings of adults are rare, and usually a consequence of human interactions or neoplasia (Gulland *et al.*, 1996). Thus, although TMMC admitted 334 yearling California sea lions in May and June 1998, the stranding of 70 adults and subadults in good nutritional condition showing severe neurologic signs was an unusual occurrence.

EPIDEMIOLOGY

Live stranded sea lions

From May 18 to June 19, 1998, 70 California sea lions (*Zalophus californianus*) and one northern fur seal (*Callorhinus ursinus*) stranded live along the central California coast between Oceano Dunes, San Luis Obispo County in the south and Half Moon Bay, San Mateo County, in the north. This unusual mortality event has been defined by these dates. A second event of 11 sea lions showing similar signs stranded between July 12 and October 17, with nine of them stranding in the two weeks from October 3, 1998. This second mortality event will be described in the discussion section of this document.

Criteria for inclusion in this unusual mortality event were as follows. All live animals included in this study displayed severe neurologic signs, were in good nutritional condition, and showed no other clinical signs of primary traumatic diseases or chronic infectious diseases. Dead animals were included if they showed good nutritional condition and severe neurologic signs prior to death or consistent pathologic findings of neurologic lesions like those described in the following pathology section. Evidence of tumors or "normal" parasitic diseases did not exclude animals in this study if the above criteria were met. Dead animals for which there was minimal diagnostic information were not included, therefore the 70 animals identified are likely to be an underestimate of the actual numbers of animals involved.

Figure 1 shows the spatial and temporal assessment of the strandings of the animals included in this event. The majority of the animals that stranded in the first week of the mortality event stranded along Oceano Dunes. In the second week of the mortality event, animals stranded in both Monterey Bay and Oceano Dunes, and on June 14 one animal stranded north of Monterey Bay, in San Mateo County.
All live animals admitted were transported to TMMC by volunteers following calls from thepublic about animals observed in distress. No surveys for affected animals were undertaken. The distribution of stranding sites was therefore affected by the intensity of public visitation to beaches along the coast. The area between the south end of Monterey Bay and Oceano Dunes, Big Sur, is inaccessible to the public due to lack of roads along the beach, so it is unclear whether or not clinically affected sea lions stranded along this stretch of coastline.

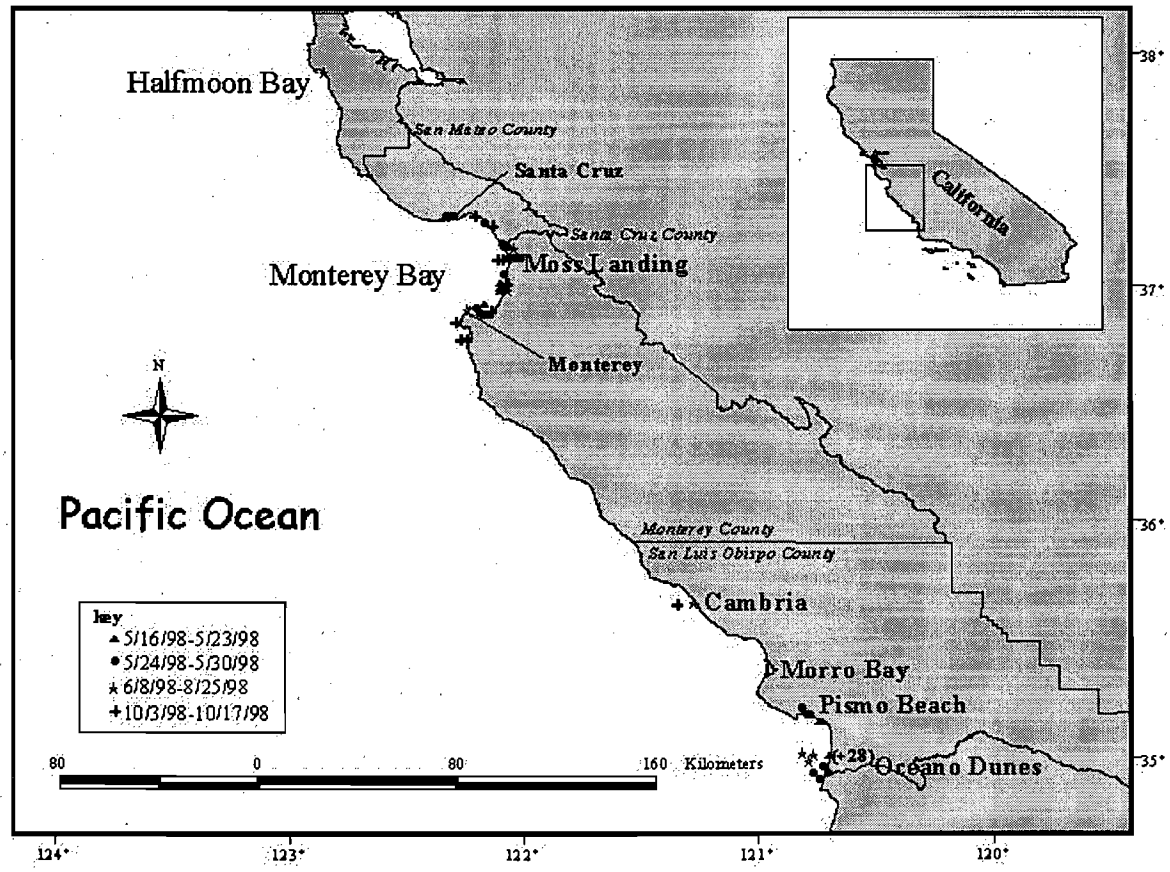
The majority of clinically affected animals were adult females, 50% of which were pregnant (Table 1). Although juvenile animalswere of both sexes, there were no adult male sea lions affected. This may be due to sex differences in dispersal along the California coast. The majority of the other 334 California sea lions that were admitted to TMMC in May and June 1998 were emaciated yearlings of both sexes.

Sea lions (other than yearlings) were aged by counting the number of dentine annuli in longitudinal sections of the upper left canine by Dr. Sue Chivers at the South West Fisheries Science Center, La Jolla California and Dr. R. DeLong, at the National Marine Mammal Laboratory (NMML) in Seattle, Washington. Ages of tooth-aged animals are listed in Appendix 1, and ranged from 3 to 13 years.

Table 1. Age structure of total affected and number of California sea lions which stranded or survived from May 16 to June 14, 1998.

Age Class	Females, affected	Females, survived	Males, affected	Males, survived
Adult	54	12		
Subadult	3	1	6	4
Juvenile	2	2	4	2
Weanling	1			

Figure 1. Stranding sites for sea lions stranding during the Unusual Mortality event in successive time periods in 1998. Numbers of animals stranding in each time period at each site are given within the circles.



Dead stranded marine mammals and mortality of other species

Data on stranded marine birds and dead marine mammals in the Monterey Bay were collected by the Monterey Bay National Marine Sanctuary BeachCOMBER program. The monitoring plan examines the sandy beaches within Monterey Bay and Carmel Bay, divided into 10 roughly equal-length segments for sampling. Data on beach-cast birds and mammals along the sandy beaches north of Año Nuevo Island to Bodega Head, Sonoma County, were collected by the Gulf of the Farallones National Marine Sanctuary (GFNMS) Beach Watch program. Pairs of volunteers survey these pre-defined segments on a monthly basis. Surveys are conducted during the first week of each month at low tide; therefore, encountered beach-cast animals represent those deposited during the previous month. For each encountered carcass, the following information is recorded: species, stage of decomposition, age, sex, evidence of scavenging, evidence for the cause of death, the presence of oil, and whether or not a photograph was obtained. A comments section is provided for documentation of any tags present on the carcass, length measurements, photograph roll and frame numbers, or any notes that would aid in post-identification of the encountered carcass. For seabirds, a toe is clipped each month in which the carcass is encountered, allowing determination of residence times and of the number of newly deposited birds. Prior to clipping a toe, the volunteer documents the number of toes previously removed. Beach-cast marine mammals are not marked.

The results of surveys in May and June 1997 and 1998 are shown in Table 2. They refer only to newly deposited birds, defined as birds with no previously clipped toes. Total beach-cast deposition of marine birds during May and June 1998 was greater at all beaches throughout the study area compared to the same period in 1997. In GFNMS, numbers of encountered carcasses were higher than the mean for these months over the previous three years (1994-1997). The causes of this elevated deposition are not clear. Many foraging guilds seem to have been affected, and overall species diversity was greater in 1998. In particular, overwintering seabirds were more frequently found beach-cast during the May and June 1998 surveys compared to the same period in 1997 (even though May 1997 was the first survey and therefore would be expected to have higher counts because it includes deposition for >1 month). Although no comparable data exist for April 1997, deposition rates of overwintering birds for April 1998 were also high. This indicates that resource limitations (perhaps caused by El Niño) during the winter months may have been a factor contributing to elevated seabird mortality from March through early June.

Table 2. Number of Dead Marine Birds and Mammals Reported by BeachCOMBER in Monterey Bay

	May 1997		June 1997		April 1998		May 1998		June 1998	
Marine Birds	**Total**	**%**	**Total**	**%**	**Total**	**%**	**Total**	**%**	**Total**	**%**
Common Murre	83	51.2	46	32.2	83	13.3	149	26.2	239	44.2
Sooty Shearwater	12	7.4	41	28.7	15	2.4	69	12.1	110	20.3
Unidentified gull	10	6.2	10	7.0	2	0.3	5	0.9	7	1.3
Western Grebe	9	5.6	11	7.7	46	7.4	12	2.1	1	0.2
Pacific Loon	9	5.6	5	3.5	45	7.2	46	8.1	29	5.4

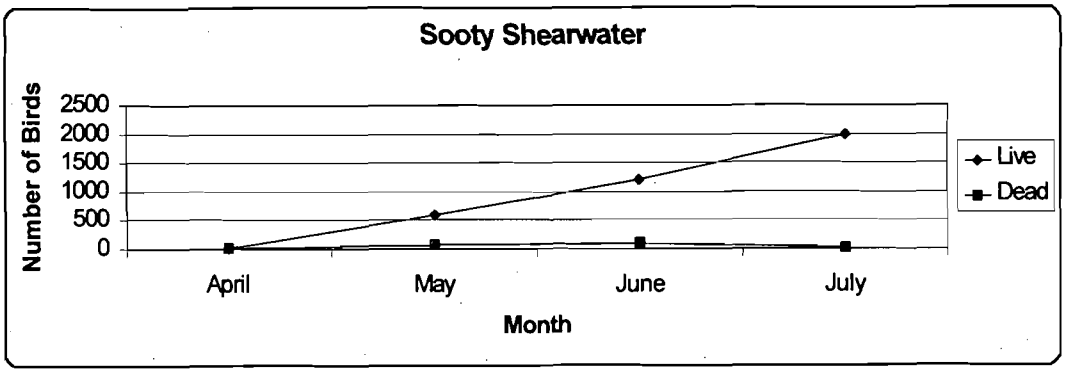
Marine Birds	Total	%	Total	%	Total	%	Total	%	Total	%
Western Gull	7	4.3	8	5.6	1	0.2			2	0.4
California Gull	7	4.3			1	0.2				
Brandt's Cormorant	3	1.9	3	2.1	30	4.8	13	2.3	7	1.3
Brown Pelican	2	1.2			7	1.1	1	0.2	1	0.2
Glaucous-winged Gull	2	1.2								
Heermann's Gull	2	1.2							3	0.6
Unidentified marine bird	2	1.2			1	0.2	2	0.4		
Pigeon Guillemot	1	0.6			1	0.2	18	3.2	12	2.2
Rhinoceros Auklet	1	0.6			40	6.4	16	2.8	7	1.3
Unidentified Alcid	1	0.6	1	0.7	6	1.0	3	0.5	1	0.2
Flesh-footed Shearwater	1	0.6								
Northern Fulmar	1	0.6	4	2.8	43	6.9	34	6.0	9	1.7
Unidentified Grebe	1	0.6	2	1.4	1	0.2	4	0.7	1	0.2
Arctic Loon	1	0.6								
Unidentified Loon	1	0.6	1	0.7					1	0.2
Cassin's Auklet			2	1.4	79	12.7	51	9.0	18	3.3
Double-crested Cormorant	1	0.6	1	0.7	3	0.5	1	0.2		
Brandt's/Double-breasted Cormorant	1	0.6								
unidentified Cormorant	1	0.6	1	0.7	4	0.6	2	0.4		
Surf Scoter		0.6			150	24.1	1	0.2	59	10.9
Mew Gull	1	0.6								

Marine Birds	Total	%	Total	%	Total	%	Total	%	Total	%
Red-breasted Merganser	1	0.6								
Horned Puffin			1	0.7						
Clark's/Western Grebe			1	0.7	7	1.1	12	2.1	2	0.4
California Gull			1	0.7			1	0.2	1	0.2
Common Loon					20	3.2	19	3.3	18	3.3
Unidentified Scoter					19	3.0	11	1.9	6	1.1
Herring Gull					2	0.3				
Red-throated Loon					3	0.5	1	0.2	1	0.2
Unidentified Shearwater			1	0.7	1	0.2	1	0.2	2	0.4
Pelagic Cormorant			1	0.7	4	0.6	4	0.7	1	0.2
Ancient Murrelet					1	0.2				
Clark's Grebe					1	0.2	1	0.2	1	0.2
Red-necked Grebe					1	0.2				
Bonaparte's Gull					1	0.2				
Black-legged Kittiwake					1	0.2	1	0.2		
Pomarine Jaeger					1	0.2				
Fork-tailed Storm-Petrel					1	0.2	1	0.2		
Unidentified Storm-Petrel					1	0.2	1	0.2		
Red Phalarope					1	0.2	1	0.2		
Black-footed Albatross			2	1.4						
Pink-footed Shearwater									1	0.2
Black Scoter							1	0.2		
Ashy Storm-Petrel							1	0.2		
Laysan Albatross									1	0.2

Marine Mammals	Total	%	Total	%	Total	%	Total	%	Total	%
California sea lion	19	55.9	31	59.6	16	59.3	12	36.4	83	59.7
Unidentified otariid	3	8.8	3	5.8	3	11.1	3	9.1	23	15.8
Northern elephant seal	1	2.9	0	0	0	0	2	6.1	9	6.5
Harbor seal	3	8.8	11	21.2	2	7.4	5	15.2.	12	8.6
Unidentified phocid	2	5.9	1	1.9	0	0	2	6.1	0	0
Unidentified pinniped	3	8.8	4	7.7	2	7.4	1	3.0	9	6.5
Pinniped total	31	91.2	50	96.2	23	85.2	25	75.8	136	93.8
Odontocete	1	2.9	1	1.9	1	3.7	5	15.2	4	2.9
Sea otter	2	5.9	1	1.9	3	11.1	3	9.1	5	3.6

There was a notable increase in beach-cast Common Murre (*Uria aalge*), Sooty Shearwater (*Puffinus griseus*), Surf Scoter (*Melanitta perspicillata*), Pacific Loon (*Gavia pacifica*), Common Loon (*Gavia immer*) and Cassin's Auklet (*Ptychoramphus aleuticus*) in May and June 1998 compared to the previous year. The causes for these differences are not known; however, several necropsied Surf Scotersexhibited large parasite loads (acanthocephalans). Cassin's Auklet, a small planktivore, may have been more susceptible than other species to the combination of resource limitation and severe winter storms caused by El Niño conditions. Increases in beach-cast marine birds have occurred in this area in previous El Niño events (Bodkin and Jameson 1991). However, it is possible that piscivorous Common Murre, Sooty Shearwater, Pacific Loon and Common Loon were impacted by domoic acid in sardines. By comparing live bird counts in Monterey Bay to beach-cast birdcounts, it is clear that the increased deposition was not simply an artifact of a greater number of birds in the Bay (Figure 2).

In contrast with seabirds, marine mammal carcasses could not reliably be marked at each survey, therefore, the results outlined in Table 2 and 3 include all carcasses found, regardless of residence time. Marine mammal deposition was relatively constant from March through early May of 1998, and the May 1998 count was nearly identical to the number encountered during May 1997. This pattern contrasts sharply with the results described above for seabirds. A large (4-fold) increase in the deposition of marine mammals occurred between the May 1998 and June 1998 samples, with the June 1998 sample being nearly three times higher than the June 1997 sample. The most frequently encountered species was the California sea lion(*Zalophus californianus*), comprising about 60% of all marine mammals in both years. The increase in marine mammal deposition between the months of June 1997 and June 1998 may have been caused by the unusual presence of female California sea lions in Monterey Bay during late spring/early summer. Previous studies have found a general migration northward from southern California breeding grounds during periods of resource limitation associated with El Niño (Trillmich and Ono 1991).



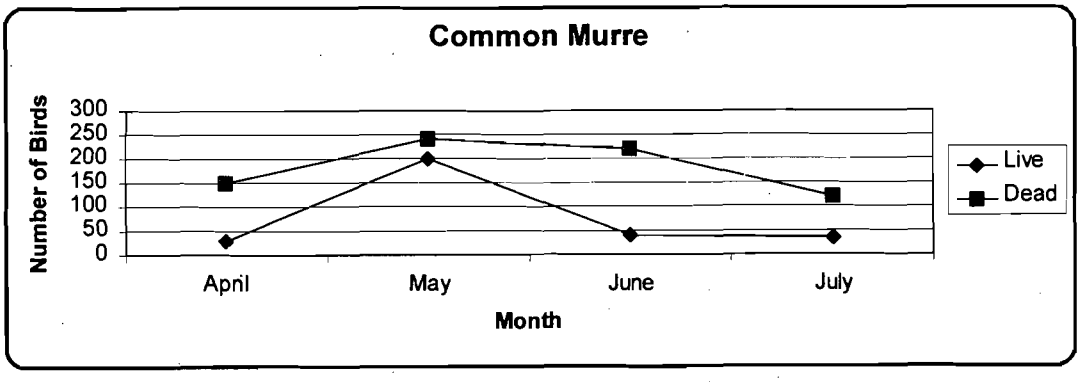


Figure 2. Counts of live and beachcast Common Murres and Sooty Shearwaters in Monterey Bay during 1998.

Table 3. Number of dead California sea lions reported to the California Marine Mammal Stranding Network in May and June 1998.

MAY 1998							
County	**Condition**	**Pup**	**Yearling**	**Subadult**	**Adult**	**Unknown**	**Total**
San Luis Obispo	Dead	0	0	0	2	6	8
	Alive	11	25	24	30	0	79
Monterey	Dead	1	3	2	1	2	9
	Alive	9	31	7	17	0	64
Santa Cruz	Dead	0	0	0	0	0	0
	Alive	0	13	4	8	0	25
San Mateo	Dead	3	6	1	3	2	15
	Alive	3	10	1	0	0	14
Total		27	88	39	61	10	214
JUNE 1998							
County	**Condition**	**Pup**	**Yearling**	**Subadult**	**Adult**	**Unknown**	**Total**
San Luis Obispo	Dead	0	0	0	2	13	15
	Alive	4	20	8	1	0	33
Monterey	Dead	12	8	12	11	3	47
	Alive	6	36	14		0	56
Santa Cruz	Dead	-	-	-	-	-	-
	Alive	0	31	4	2	0	37
San Mateo	Dead	3	24	5	4	4	40
	Alive	3	7	3	0	0	13
Total		28	126	46	20	20	241

CLINICAL SIGNS

The characteristic clinical signs in affected sea lions were ataxia, head weaving, muscle tremors, tetanic convulsions, rubbing and lethargy. Forty-eight (69 %) of the initial 70 affected California sea lions died or were euthanized. In animals that died, seizures became increasingly frequent, often progressing to *status epilepticus* before the animal died. If animals were repeatedly in *status epilepticus* for over an hour, and showed no improvement in clinical signs over subsequent days, or were comatose, they were euthanized (n=22). The seizures varied from a few minutes in duration, to over 30 minutes. The seizure duration was often affected by treatment, as seizuring sea lions were treated with diazepam, lorazepam and phenobarbitone symptomatically to alleviate tetany. Frequency of seizures varied from one to 30 in 24 hours. In animals that survived, the frequency of seizures gradually decreased over a one month period. Seizures were usually bilateral, but two animals repeatedly had unilateral seizures. Between seizures, sea lions showed varying degrees of lethargy and occasionally a characteristic rubbing behavior, in which the animals would rub the back of their necks and dorsum by rolling repeatedly on the pen floor. As assessment of the lethargy was subjective, a neurologic examination sheet (Appendix 4) was developed that could be used to give animals a numeric score. This could be evaluated daily by different personnel, and scores compared between days to determine an animal's clinical progress. A higher score would equate with a worse neurologic condition. In the first days after stranding, most animals had scores above 20. Apparently healthy animals prior to release had scores of between 7

and 10.

Other clinical signs observed included retching blood-tinged mucus, diarrhea, moist cough, and blepharospasm. Although animals retched mucus, no vomitus was observed, and on *post mortem* examination of animals that died, stomachs were empty.

Electrocardiograms were performed on three clinically affected animals both during seizures and between seizures. No abnormalities were detected.

Pathologic examination of animals that died or were euthanized during the event suggested that fetuses had died *in utero* sometimes days prior to the female's death. Ultrasonography was used to examine five animals that had distended abdomens. Of the five animals examined, all were diagnosed as pregnant, with no detectable fetal heart beat.

Magnetic resonance imagery (MRI) was used to examine the brain of one affected animal under anesthesia (# 3768) and one control animal. Although examination of histologic sections of the brain(#3768) after death revealed characteristic lesions of neuronal necrosis in the hippocampus, no lesions were detected by MRI when compared to the control animal.

CLINICAL PATHOLOGY

Blood analyses

Hematological results from affected sea lions at admission are shown in Appendix 2a. Hematocrits were elevated in many individuals, presumably as a consequence of dehydration. Three individuals (#4209, #4024 and #4220) had elevated leukocyte counts due to neutrophilia. These three animals stranded after the initial event, and all three had chronic abrasions.

The most consistent serum biochemical abnormality was an increase in creatine kinase ranging from 169 to 6,288 IU/L in animals tested (Appendix 2b). This elevation was believed to be a consequence of muscle damage during seizures and transport. Thirteen of 41 animals tested (32%) on admission had gamma-glutamyl transferase levels over 100 mg/dl. This elevation may have been due to bile stasis, as many animals on necropsy had distended gall bladders. Both hypo- (#3734) andhypernatremia (# 3901, #4217, #3719, #3815) were observed. Hyperphosphatemia was also observed in three animals (# 3734, #3806, and #3812). Two of the sea lions with hyperphosphatemia, # 3734 and # 3806, also had increased blood urea nitrogen, suggesting some of these biochemical changes could have resulted from impaired renal function. Both these animals were female and #3806 was euthanized; whereas #3734 died. Histologic examination of kidney tissue from # 3806 revealed interstitial nephritis, indicating that this clinical pathologic change was indeed a consequence of renal damage. However, no abnormalities were reported in kidney tissue from #3734.

Neurologic analyses

To rule out chemical pollutant exposure as a cause of the seizures, brain cholinesterase levels were assayed in five affected animals and control animals at California Veterinary Diagnostic Laboratory Systems (CVDLS) and were found to be normal (> 1.5 M/g/min) as compared to normal mammalian ranges. In addition, blood lead levels were examined in eight affected and control animals and were found to be below detectable limits.

Cerebrospinal fluid samples from four animals (#3837, #3794, # 3746, #3796) were spun and smears were examined after staining with Wright's stain. No inflammatory cells were observed.

Fecal analyses

Fecal samples from 18 animals were examined by Dr. R. DeLong at the National Marine Mammal Laboratory (NMML) for presence of prey species, but fish bones were only detected in samples from three sea lions. Vertebrae from anchovies (*Engraulis* spp.) were identified in 2 samples (#3801 and #3796) and probable sardine (*Sardinops sagax*) otoliths in one sample (#3760). These are common prey species of California sea lions in spring and summer (Antonelis *et al.*, 1984).

TREATMENT

Seizures were controlled symptomatically with diazepam (0.1 - 0.2 mg/kg intramuscularly (IM) or intravenously (IV) up to four times daily), or lorazepam (0.03 - 0.04 mg/kg IM up to twice a day). The latter was found to be more effective at controlling seizures. Long term control was achieved with phenobarbitone at 2 mg/kg IM initially, then orally once the sea lions recovered sufficiently to eat. Dexamethasone at 0.5 mg/kg IM was also given to reduce cerebral edema following seizures.

Supportive care was achieved with subcutaneous fluids (either Ringer's or 0.9% NaCl at 60 ml/kg subcutaneously) until the animals were eating consistently.

Parturition was induced in the five pregnant animals in which no fetal heart beat could be detected. Induction was performed in four animals by intramuscular injection of 500 g prostaglandin F2 (Estrumate), and in one animal by intravaginal administration of prostaglandin E (Misoprostol). The four given prostaglandin F2 gave birth to dead fetuses after 36 hours, while the one that received the drug intravaginally died within 12 hours. Post mortem examination revealed that this animal had a uterine rupture, an intra-abdominal fetus, and peritonitis of several days duration.

Two of the seizuring animals gave birth to live pups. One of the pups died within 48 hours of birth, the other did not nurse and the female showed no interest in it, so it was removed for hand-rearing. This pup did not survive.

GROSS *POST MORTEM* FINDINGS

All animals that died or were euthanized were in good nutritional condition with blubber layers over the sternum between 20 and 50 mm. The most common gross lesions observed that were considered features of the toxicity eventwere pallor of the myocardium (16/48), fibrinous plaques on the epicardium (5/48), and, more rarely, fibrinous fluid in the pericardial sac (2/48) (Table 4). These lesions were not observed in all cases, but were more frequent in sea lions that died within 48 hrs of stranding. Four animals with pallor of the myocardium had friable livers with a nutmeg pattern on the surface. Congestion of the meningeal blood vessels was observed in two cases, cerebral edema in one. Twenty three of the forty five females that died or were euthanized (51%) were peri-parturient. Placental necrosis was observed in five peri-parturient female sea lions that had not delivered a fetus prior to death. Uterine pathologies were observed in five sea lions. Two animals had uterine ruptures with intra-abdominal delivery of the fetuses, two had uterine torsions and one had a uterine prolapse that had become necrotic. Other gross lesions were those associated with parasitism - gastric ulceration and erosion and enlargement of the gastric lymph nodes (associated with *Anisakid* nematodes), cholecystitis (associated with *Zalophatrema hepaticum*) and pulmonary congestion (associated with *Parafilaroides* spp.) (Table 4). These lesions are common in animals normally stranded in this region. Enlarged tonsils were observed in 10 cases, and pus within the laryngeal pouches in two cases. Abscesses within the skeletal muscle and fascia were observed in three cases, one of which was gun-shot.

Five female sea lions had neoplasms. Two had leiomyomas of the uterus, and three had rough plaques of the vaginal mucosa that were found to be early epithelial carcinoma when examined histologically.

Table 4. Frequency of gross post mortem findings in 48 California sea lions.

Lesion	# animals
Pallor of the myocardium	16
Fibrinous fluid in the pericardial sac	2
Epicardial fibrinous plaques	5
Left atrial endocardial fibrosis	1
Friable liver, nutmeg pattern	4
Thickening of gall bladder wall	6
Atresia of common bile duct	1
Swelling of the kidneys	13
Placental necrosis	5
Uterine rupture	2
Uterine torsion	2
Prolapsed uterus	1
Uterine/vaginal neoplasia	5
Congestion of meningeal blood vessels	2
Cerebral edema	1
Keratitis/uveitis	5
Oral ulcers	6
Gastric erosions and ulcers	22
Enlarged gastric lymph nodes	5
Swollen gastric rugae	9
Blood tinged mucus in oropharynx	7
Pulmonary congestion	14
Pulmonary interstitial edema	6
Blood tinged froth in trachea and bronchi	3
Pus in laryngeal pouches	2
Abscess within muscle fascia	3 (gunshot 1)

MICROBIOLOGY

Bacteriology

A variety of bacteria were isolated from tissues of the sea lions after routine culture of swabs taken at post mortem examination (summarized in Table 5.). These bacteria species are not atypical from bacteria found in sea lions that strand along the California coast at other times (Thornton *et al.*, 1998). Uterine mucosa samples from 25 animals were submitted to National Veterinary Services Laboratory in Ames, Iowa, for Brucella isolation. All cultures were negative.

Virology

Swabs from pericardium, lung, liver and whole blood were submitted to Dr. Carol House, USDA, Plum Island, for viral isolation. A new calicivirus, designated San Miguel Sea Lion virus 18, was isolated from a pericardial swab from sea lion "Dean", #3709. This virus was identified using electron microscopy.

Virus neutralization (VN) tests for phocine distemper virus (PDV) detected low levels of antibody in sera from 10 of 34 animals (29 %). After two weeks, four of these 10 animals showed low positive, but rising, titers on VN. No signs of respiratory disease or novel neurologic signs were detected. After a further month, these four animals were seronegative to PDV. Three animals that they had been in contact with were also seronegative. A retrospective survey of banked sera from 100 adult California sea lions that had stranded previously revealed a seroprevalence of 20 % of low titers to PDV. The significance of these findings is under investigation by Drs. Carol House, USDA, Plum Island and Don King, University of California at Davis.

Table 5. Number of bacterial isolates from tissues of California sea lions at post mortem examination

Isolate	Tonsil n = 3	Laryngeal pouch n = 4	Lung n = 5	Liver n = 5	Brain n = 5	Uterus n = 3	Peri-cardium n = 2	Sub-lumbar lymph node	Gastric lymph node n = 2
Enterobacter cloacae	-	-	1	-	1	-	1	-	2
E. coli	3	4	2	2	3	1	2	-	1
Klebsiella pneumoniae	2	2	1	-	-	-	-	-	-
Aeromonas spp.	-	2	-	-	-	-	-	-	-
Proteus mirabilis	-	-	1	2	1	-	-	-	-
Edwardsiella tarda	-	-	-	2	-	-	-	-	-
Enterococcus spp.	-	-	-	2	-	1	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-
Corynebacterium spp.	-	2	-	-	-	-	-	-	-
Listeria ivanovii	-	-	1	-	-	-	-	-	1
Streptococcus viridans	1	1	-	-	-	1	-	-	-
Moraxella sp.	-	1	-	-	-	-	-	-	-
Fusobacterium sp.	-	-	-	-	-	-	-	1	
Clostridium perfringens	-	-	-	-	-	-	-	1	-
Plesiomonas shigelloides	-	-	1	1	1	-	-	-	-

HISTOPATHOLOGY

Tissues from 48 California sea lions, 4 California sea lion fetuses and one northern fur seal were examined microscopically with special emphasis on the central nervous system. Histopathology was performed at the University of California, Davis, Veterinary Medical Teaching Hospital (Dr. Linda Lowenstine and Dr. Paul Silvagni, pathology resident) (n =20 + 3 fetuses), the Armed Forces Institute of Pathology (under the direction of Dr. Tom Lipscomb) (n = 9 + one fetus), and at Colorado State University (Dr. Terry Spraker) (n = 19).

Brain

The most significant lesions were in the brain, and these varied with duration post stranding. Acute: All animals that died within the first 2 days (acute cases, n = 27) had vacuolation (presumed edema) in both the gray and white matter in many morphologic areas of the brain. There was always a striking laminar pattern of micro-vesicular vacuolation in the stratum radiatum of the anterior ventral hippocampus along with marked edema in the pyramidal cell layer of the dentate gyrus and the

hippocampus. In addition, 15 of these animals had severe acute neuronal necrosis involving most of the neurons of the dentate gyrus and many pyramidal cells in sectors CA3, CA4, CA1 and CA2 (in descending order of frequency). In another six animals there was minimal to mild necrosis in these same areas. All the hippocampal changes were segmental, involving almost exclusively the most ventral and anterior portions of "Ammond's horn". Sometimes the necrosis extended into the laminar cortex of theadjacent pyriform lobe and amygdala, or rostrally to involve the ventral rhinencephalon. In seven animals, no acute neuronal necrosis was observed in sections examined. In addition to the hippocampal and rhinencephalic lesions, which appeared to be contiguous, there was also necrosis of thalamic nuclei in two animals, and in the cuneate nucleus of the medulla in a further two animals.

Subacute

In animals that survived more than four days (short-term survivors, n = 21), edema and acute neuronal necrosis were much less apparent in the hippocampus, but there was obvious loss of neurons in dentate gyrus and hippocampal sectors as evidence of past necrosis. As survival time increased, reactive lesions such as capillary proliferation, mild diffuse gliosis, and mild perivascular lymphocyte accumulation became more apparent although ongoing acute necrosis of individual neurons could sometimes be appreciated. In some animals there was grossly apparent post necrotic hippocampal atrophy. Overt malacia (necrosis of large areas) with phagocytic glial cell ("gitter" cell) response was noted in the amygdala and pyriform lobes in nine animals and in midbrain (inferior colliculus/ lateral geniculate region) in one animal. The lesions in the amygdala and pyriform lobes were often grossly evident in fixed brain sections. In one animal that survived for six days, there was severe sub-acute asymmetrical edema causing a mass-like lesion and midline shift.

An additional finding in both groups of animals (rapid death and short-term survivors) was non-suppurative meningoencephalitis and choroiditis that varied from minimal to florid. In some of the short-term survivors, this was clearlya response to neuronal damage, but in others and in the acute death group, the lesion may have been preexisting. Non-suppurative encephalitis is a common finding in both California sea lions and southern sea otters stranding on the California coast. Chronic viral or protozoal infections are suspected, but not proven. One of the animals from this event did have protozoal tissue cysts in the brain and another one had possible endothelial tachyzooites, butinflammation did not appear to be directed toward them. Two additional animals had neuronal inclusions suggestive of a viral etiology, but electron microscopy, immunohistochemical stains and Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) were negative for morbillivirus (AFIP).

Cardiac

The heart was the second most frequently affected organ in terms of lesions that could not be attributed to known etiologic agents. Areas of myocardial pallor thought to represent necrosis and edema were grossly evident in several animals. Acute myofiber necrosis and edema were confirmed histologically in 21 of the animals that died early in the event. These varied from scattered areas of individual fiber necrosis to regionally extensive myocardial damage in two animals. In the short-term survivors, small areas of myocardial fibrosis, nuclear hyperplasia and hypertrophy, and mild non-suppurative myocarditis were noted. In one animal, small tachyzooite-like structures were associated with inflammation and in another animal *Sarcocystis*-like tissue cysts were seen. Rhabdomyolysis (muscle necrosis) was also seen in skeletal muscle of diaphragm and elsewhere in many of the animals and was thought to be secondary to the abnormal exertion of seizure activity.

Other

Other lesions identified histologically confirmed the gross diagnoses. These included cholecystitis and dochitis secondary to *Zalophatrema*, parasite-induced ulcers and stress-type acute gastric erosions and hemorrhages, pulmonary nematodiasis and verminous pneumonia. Additional common histologic lesions included: enteritis associated with trematodes or acanthocephlans, parasitic granulomas in liver and regional lymph nodes,lymphocytic interstitial nephritis, microfilaremia, vaginitis and cervicitis, cystitis, pharyngitis associated with acariasis, larval cestodiasis in colon, superficial colitis and acute enteritis.

Neoplasias

Interesting incidental findings were several neoplasms including: three cases of very early genital epithelial carcinoma; two uterine leiomyomas (one of which was external and pedunculated); one epithelium-predominant teratoma associated with the surface of the right kidney; one pharyngeal cleft cyst associated with the pineal gland; and one islet cell tumor of the pancreas. The prevalence of genital epithelial neoplasia in California sea lions in this group was 6.3% and the over-all prevalence of tumors (pharyngeal cyst excluded) was 14.6%. Another interesting case was a very old female with systemic amyloidosis.

Ocular lesions

The eyes from 12 animals (including one fetus) were examined. No lesions were noted in three animals and mild to moderate lesions noted in nine animals. The most common lesion (n = 7, including the fetus) was vacuolation (presumed edema) in the ganglion cell layer of the retina. Two animals had retinal hemorrhage, one animal had ophthalmitis, onehad bilateral corneal ulceration, one had degeneration of the outer molecular layer, and one had degeneration of the internal molecular layer. Four animals had more than one lesion. In none of the animals did the pattern of lesions mimic those described in rats with experimental domoic acid intoxication.

Reproductive lesions

Most of the females (51%) were either pregnant or recently post partum at the time of necropsy. Placentitis was apparent is five animals. Tissues from only four fetuses were examined histologically, due to the advanced autolysis of most of the fetuses recovered at gross examination. Of these four fetuses, one was severely autolyzed. One had a bacterial infection acquired *in utero* that had causedomphalophlebitis and pneumonia. The other two had large numbers of amniotic squames filling the bronchioles of the lungs. This is suggestive of fetal distress. In none of the fetuses were severe brain lesions noted (although domoic acid has been demonstrated to cross the placenta in rats).

In summary, the principal lesions that were unique to this unusual stranding event were in the brain and heart. The acute cerebral edema and neuronal necrosis were most severe in the dentate gyrus and pyramidal layers of the hippocampus in the anterior ventral region, with other areas of the brain less severely or consistently affected. Extent of lesions in these areas, especially pyriform lobe and amygdala, rhinencephalon, thallamic nuclei and lateral geniculate body and inferior colliculus of the midbrain may have been underestimated due to sectioning. In about 20% of the animals, only edema without necrosis was detected in sections of brain examined including hippocampus. Short term survivors had evidence of neuronal loss and gliosis (scarring) in the same regions as the acute cases with more evidence of extension into the pyriform lobe and amygdala in which overt malacia were often seen. The acute lesions in the hippocampus were compatible with exposure to an excitotoxin such as domoic acid as described in human cases and in experimental rats and macaques. Myocardial edema and necrosis were also features in animals that died rapidly during this event, and there was evidence of repair of

previous lesions in the short-term survivors. Myocardial necrosis has been reported in rats exposed to excitotoxins (kanic acid and N-methyl-D-aspartic acid), although it has not been previously described in domoic acid toxicosis (Rockhold *et al.*, 1989). Cardiac arrhythmia have been noted in humans exposed to domoic acid (Perl *et al.*, 1990).

TOXICOLOGY

Sea lion serum, urine, kidney, fecal samples, and stomach washings were analyzed by the Northwest Fisheries Science Center (NWFSC) Marine Biotoxin group (Trainer), the National Ocean Service (NOS) Marine Biotoxins Program (Van Dolah, Powell, Doucette and Busman), and the University of California at Santa Cruz (UCSC) (Lefebvre) for domoic acid.

Four analytical techniques were used to detect the presence of domoic acid in either tissues or fluids. These methods were: receptor binding assay (Van Dolah et al, 1997), high performance liquid chromatography-with standard ultraviolet absorptance (HPLC-UV), high performance liquid chromatography with mass spectroscopy (HPLC-MS), and high performance liquid chromatography with tandem mass spectroscopy (HPLC-MS/MS). The limit of detection for domoic acid in serum using the receptor binding assay is approximately 50 ng/mL. The limit of detection of the HPLC-UV method is approximately 0.5 ug/ml with a spiked serum recovery of 98.7%. All samples were tested in NWFSC and NOS labs by receptor binding assay. All samples were analyzed by a receptor binding assay (Van Dolah *et al.*, 1997) which uses a cloned glutamate receptor and dilutions of a domoic acid standard of known concentration. A competitive displacement curve is generated and sample results are compared to controls. Glutamate dehydrogenase is incubated with all samples prior to analysis toeliminate endogenous glutamate activity. Samples with high levels of domoic acid on receptor binding assay were confirmed using high performance liquid chromatography with MS/MS at the NOS laboratory.

Serum

Serum from fourteen clinically affected animals were analyzed by the NWFSC lab, and serum from fourteen clinically affected animals (five of which were the same as that tested by NWFSC lab) and three negative controls were analyzed by the NOS laboratory using the same microplate receptor binding assay for domoic acid (Van Dolah *et al.*, 1997). At the NWFSC lab, domoic acid was detected in the serum of one (#3806) of 14 clinically affected sea lions (Table 6). At the NOS lab, two serum samples (CSL# 3734 and #3724) were positive with low levels of domoic acid (Table 6). Because high levels of glutamic acid may cause false positives in the receptor binding assay, the presence of domoic acid was further investigated at the NOS lab using the HPLC-UV method of Quilliam (1989), and by HPLC/MS. A total of 23 clinical samples and three control sera were analyzed by the HPLC-UV method. All were negative for domoic acid. The two serum samples positive by receptor assay (#3724 and #3734) also showed low levels of domoic acid by HPLC/MS.

Urine

At the NWFSC lab, urine samples from four animals were tested (two affected animals and two control animals). Domoic acid was detected in the urine of two affected sea lions (# 3807 and #3794) as compared to urine from two control animals using the receptor binding assay. One of these urine samples from the affected animals (#3807) also had weakly positive levels of domoic acid as detected by high performance liquid chromatography (HPLC) using standard UV absorbance at 242 nm. At the NOS lab, urine from eight clinically affected animals and three control animals were tested by both thereceptor binding assay and HPLC-UV. Four of these were positive by both methods (Table 6), and three additional samples were positive by receptor assay at levels below the detection limit

of the HPLC method. Urine from one animal (#3707) was also analyzed by HPLC/MS and confirmed positive for domoic acid.

Feces

Feces from seven clinically affected animals and two control samples were analyzed at the NOS lab by receptor assay and HPLC-UV. Fecal samples were extracted using a mussel extraction and SAX clean-up method of Quilliam *et al.,* (1995) before analysis by HPLC with spiked extraction recovery of 88.4 $\pm$ 8.4%, n = 3. Three samples were positive for domoic acid (Table7) and good quantitative agreement was seen between the receptor binding assay and HPLC analyses (Table 7).

Feces from eleven clinically affected sea lions were also analyzed at UCSC for the presence of domoic acid by HPLC using the extraction method of Quilliam *et al.*, (1989). Domoic acid was detected in samples from the same three animals as at the NOS lab (Table 7). In addition, fecal samples from seven control sea lions were analyzed. These animals were three captive sea lions from Long Marine Laboratory (LML # 1, 2, 3), three captive sea lions from MarineWorld in Vallejo, CA, and one sea lion from Maverick's Beach, CA (Clutch) that was euthanized at TMMC due to severe gunshot injury two months after the event (August 1998). Domoic acid was not detected in any of these samples.

Kidney, Liver, Muscle, Brain

Five kidney samples were analyzed at the NOS lab by HPLC-UV and were negative for domoic acid (Table 7). Samples were extracted and cleaned using the same procedure as described for feces, with a spiked kidney extraction recovery of 24.6%, n = 1. Liver, muscle, brain, intestinal mucosa and kidney samples from two animals (#3765 and # 3824)analyzed by HPLC at UCSC were also negative for domoic acid.

Stomach washings

A single stomach washing sample from a clinically affected animal (CSL 3708) analyzed by receptor assay at the NOS lab was negative for domoic acid.

Confirmation of domoic acid in fluids by mass spectrometry

Serum and urine samples with the highest concentrations of domoic acid, as determined by HPLC-UV, were subjected to HPLC/MS and HPLC-MS/MS analysis at the NOS lab for confirmation of the identity of the HPLC peak in question. For both methods, sample extracts were fractionated on a C18 column eluted with a gradient of 1-95% methanol in 0.1% TFA. A PE SCIEX API-III triple quadrupole mass spectrometer was used. The ionspray source was operated in positive ion mode utilizing nitrogen for the nebulization gas. The first quadrupole was used to pass only ions of nominal 312 m/z. The conditions in the second quadrupole were adjusted to allow a substantial amount of collisionally-induced dissociation. The third quadrupole was operated in multiple ion monitoring mode, where fragments ions of 161 and 266 m/z, as well as the residual parent ions at 312 m/z were allowed to pass to the mass spectrometer ion detector. The presence of domoic acid was confirmed in urine from # 3707 and #3758 and feces from # 3783 (Table 7).

Pseudo-nizchtia frustules

Ten of the eleven fecal samples analyzed at UCSC were also examined for the presence of diatom frustules (glass skeletons) using a compound microscope, (there was insufficient sample from CSL #3734 after HPLC analyses). Diatom frustules were only found in fecal samples that had tested positive for domoic acid (#3758 and #3783). Using scanning electron microscopy, frustules were identified as *Pseudo-nitzschia australis*.

Anchovies

Domoic acid was detected in anchovies (*Engraulis mordax*) collected from Monterey Bay and Morro Bay by HPLC-UV at the NOS

lab. Two samples of anchovies collected from Monterey Bay on May 22, 1998 had detectable levels of domoic acid, while fish collected on June 10, 1998 had no detectable levels (Table 8). Two samples of anchovies from Morro Bay on June 4 also had detectable levels of domoic acid (Table 8). Domoic acid was confirmed in two anchovy samples by HPLC/MS and one by HPLC/MS/MS (Table 8)

Summary of Toxicology

These results indicate that there is consistency between domoic acid assays performed at NOS, NWFSC and UCSC labs. The NOS and NWFSC labs run the same microplate receptor binding assay as a rapid screening method for domoic acid. Although highly effective as a screening method, positive results should be confirmed by analytical methods when testing novel tissue matrices. The HPLC-UV method is suitable only when tissue concentrations are sufficiently high, because the detection limit is poorer than that of the receptor assay. HPLC/MS or HPLC/MS/MS provides the most rigorous analytical confirmation, but are expensive and time-consuming to run. In the current study HPLC/MS/MS provided the necessary independent confirmation of the chemical identity of the toxin to unambiguously conclude the involvement of domoic acid in this event. The greater detection rate in feces and urine than serum (Table 9) suggests feces and urine should be routinely collected for detection of domoic acid in marine mammal die-offs. The low detection rate in serum is probably due to the rapid clearance of this water soluble toxin from blood. Clearance in rodents and primates following intravenous inoculation is under four hours (Truelove and Iverson 1994). Although stomach contents might be valuable for analysis,the stomachs of clinically affected sea lions in this event were empty, presumably due to vomiting and the time elapsed between ingestion and *post mortem* examination. Feces, thus, proved to be the most valuable sample fortoxicological analyses, as well as for determining diet.

Table 6. Presence of Domoic Acid in Serum from California Sea Lions

Animal ID	NWFSC Receptor assay	NOS Receptor assay	NWFSC HPLC/UV	NOS HPLC/UV	NOS HPLC/MS	NOS HPLC/MS/MS
CSL 3299 C		N/D		negative	N/D	
CSL 3451 C	Negative					
CSL 3489 C		negative		N/D	N/D	N/D
CSL 3510 C		negative		negative	N/D	N/D
CSL 3512 C		negative		negative	negative	N/D
CSL 3670	Negative					
CSL 3699	Negative	negative		negative	N/D	N/D
CSL 3700	Negative	negative		negative	N/D	N/D
CSL 3707		negative		negative	N/D	N/D
CSL 3708		N/D		negative	N/D	N/D
CSL 3719		negative		negative	N/D	N/D
CSL 3720	Negative					

CSL 3724		0.17 µg eq./mL		negative	positive	N/D
CSL 3727	Negative					
CSL 3731		negative		negative	N/D	N/D
CSL 3732		negative		negative	N/D	N/D
CSL 3733		negative		negative	N/D	N/D
CSL 3734		0.2 µg eq./mL		negative	positive	N/D
CSL 3735	Negative					
CSL 3744		N/D		negative	N/D	N/D
CSL 3744		N/D		negative	N/D	N/D
CSL 3746	Negative	N/D		negative	N/D	N/D
CSL 3747	Negative	negative		N/D	N/D	N/D
CSL 3748		N/D		negative	N/D	N/D
CSL 3749		negative		negative	N/D	N/D
CSL 3752		N/D		negative	N/D	N/D
CSL 3755		N/D		negative	N/D	N/D
CSL 3757		N/D		negative	N/D	N/D
CSL 3767						
CSL 3775	Negative					
CSL 3791	negative	N/D		negative	N/D	N/D
CSL 3793	negative					
CSL 3795	negative					
CSL 3796	negative					
CSL 3806	positive					
NFS 123		negative		negative	N/D	N/D

Table 7 Presence of Domoic Acid in Urine, Feces and Tissues from California Sea Lions

	NWFSC	**NOS**	**NWFSC**	**NOS**	**NOS**	**NOS**	**UC Santa Cruz**
Animal ID	**Receptor assay**	**Receptor assay**	**HPLC/ UV**	**HPLC/UV**	**HPLC/ MS**	**HPLC/MS/MS**	**HPLC/UV**
URINE		g eq./mL		g/mL			
CSL 3451 C	negative						
CSL 3707		3.72		14.05	positive	positive	
CSL 3726		0.12		2.68	N/D	N/D	
CSL 3741		0.03		negative	N/D	N/D	
CSL 3742		N/D		negative	N/D	N/D	
CSL 3749		0.72		2.38	N/D	positive	

CSL 3767 C	negative						
CSL 3794		3.72		14.05	positive	positive	
CSL 3800		0.12	positive	2.68	N/D	N/D	
CSL 3807		0.03		negative	N/D	N/D	
CSL 3841 C		N/D		negative	N/D	N/D	
CSL 3882 C		0.72		2.38	N/D	positive	
CSL 3883 C		N/D		negative	N/D	N/D	
NFS 123		0.12		negative	N/D	N/D	
FECES							
CSL 3734		1.31		5.23	N/D	N/D	2.79
CSL 3741		N/D		negative	N/D	N/D	negative
CSL 3747		N/D		negative	N/D	N/D	negative
CSL 3758		96.08		46.89	positive	N/D	45.26
CSL 3783		182.01		167.53	positive	positive	114.39
CSL 3806		N/D		negative	N/D	N/D	negative
CSL 3807		N/D		negative	N/D	N/D	negative
fc1a C		negative		negative	N/D	N/D	negative
fc4aC		negative		negative	positive	N/D	negative
KIDNEY							
CSL 3685		N/D		negative	N/D	N/D	negative
CSL3691		N/D		negative	N/D	N/D	negative
CSL3692		N/D		negative	N/D	N/D	negative
CSL3707		N/D		negative	N/D	N/D	negative
CSL3724		N/D		negative	N/D	N/D	negative

Table 8. Domoic acid levels in Anchovies from the Central California Coast

Sample site & Date	Receptor Assay NOS	HPLC/UV	HPLC/MS	HPLC/MS/MS
	g equiv/g	g/g		
5/22/98a Monterey Bay	71.30	100.35	N/D	Positive
5/22/98b Monterey Bay	69.67	123.04	Positive	N/D
6/4/98a Morro Bay	2.53	4.05	Positive	N/D
6/4/98b Morro Bay	0.27	0.82	N/D	N/D
6/10/98a Monterey Bay	N/D	Negative	N/D	N/D
6/10/98b Monterey Bay	N/D	Negative	N/D	N/D

Table 9. Samples positive for Domoid Acid by different laboratory analyses

	Receptor binding assay		HPLC/UV		HPLC/MS/MS	
	Positives	Number tested	Positives	Number tested	Positives	Number tested
Serum	3	26	1	21	2	2
Urine	11	14	9	15	2	2
Feces	0	0	3	13	1	1
Cerebrospinal fluid	0	3	0	1	0	0
Stomach washing	0	0	0	1	0	0
Gastric mucosa	0	0	0	1	0	0
Kidney	0	0	0	5	0	0

PLANKTON BLOOM

Within Monterey Bay, plankton samples are routinely collected several times per week from the Santa Cruz wharf and Monterey Coast Guard pier. Samples are also collected along several sites running due west of Moss Landing as part of the Monterey Bar Aquarium Research Institute (MBARI) upper water column time series project. Samples are subjected to DNA probe-based tests as a means of identifying and quantifying a variety of toxic and non-toxic *Pseudo-nitzschia* species as well as the toxigenic dinoflagellate *Alexandrium tamarense* (e.g., Scholin *et al.* 1996, 1997, submitted;). An example of the time series data collected from the Santa Cruz wharf is shown in Figure 3. During thespring bloom of diatoms, toxigenic *Pseudo-nitzschia* species were largely absent and no domoic acid was detected in plankton samples (Fig. 3, arrow #1). As the spring bloom progressed, *A. tamarense* was detected, and shellfish samples collected from the same area tested positive for paralytic shellfish poisons (Fig. 3, arrow #2). As the bloom of *Alexandrium* declined, the number of *Pseudo-nitzschia australis* increased sharply (arrow #3). The amount of domoic acid (= bars) in water samples rose in concert with increasing numbers of *P. australis* (arrow #4). As the bloom of *P. australis* reached its maximum, toxigenic cells were concentrated in a narrow band near shore. It is possible, although in no way proven, that the cells responded to nutrient enrichment of near shore waters as a result of enhanced river flows following late spring rains.

Anchovies collected from Monterey Bay during the height of the *P. australis* bloom contained high levels of domoic acid and high numbers of *P. australis* in their stomachs (Fig. 4). The *P. australis* bloom was replaced by a large *P. pseudodelicatissima* bloom (not shown on Fig. 3). Some *P. pungens* and *P. multiseries* were also present, but at relatively low levels. During this transition in species abundance, the concentration of domoic acid declined rapidly and precipitously in the upper water column (Fig. 3, arrow #5). Gut contents of anchovies collected at this time reflected this change in species abundance as well (Fig. 5), as they did not contain detectable levels of domoic acid. Reports of sickened sea lions fromMonterey Bay decreased in concert with the loss of *P. australis* from the upper water column and the rise in dominance of *P. pseudodelicatissima*. Notably, cultures of *P. australis* isolated from Monterey Bay produce domoic acid whereas isolates of *P. pseudodelicatissima* from the same area do not.

During the *P. pseudodelicatissima* bloom inside Monterey Bay, high concentrations of toxigenic *P. multiseries* were found south and especially north of that region. Thus, species composition of *Pseudo-nitzschia* populations found within Monterey Bay and contiguous waters of the central California coast can differ substantially.

Throughout the remainder of the year *P. australis* was not abundant in Monterey Bay.

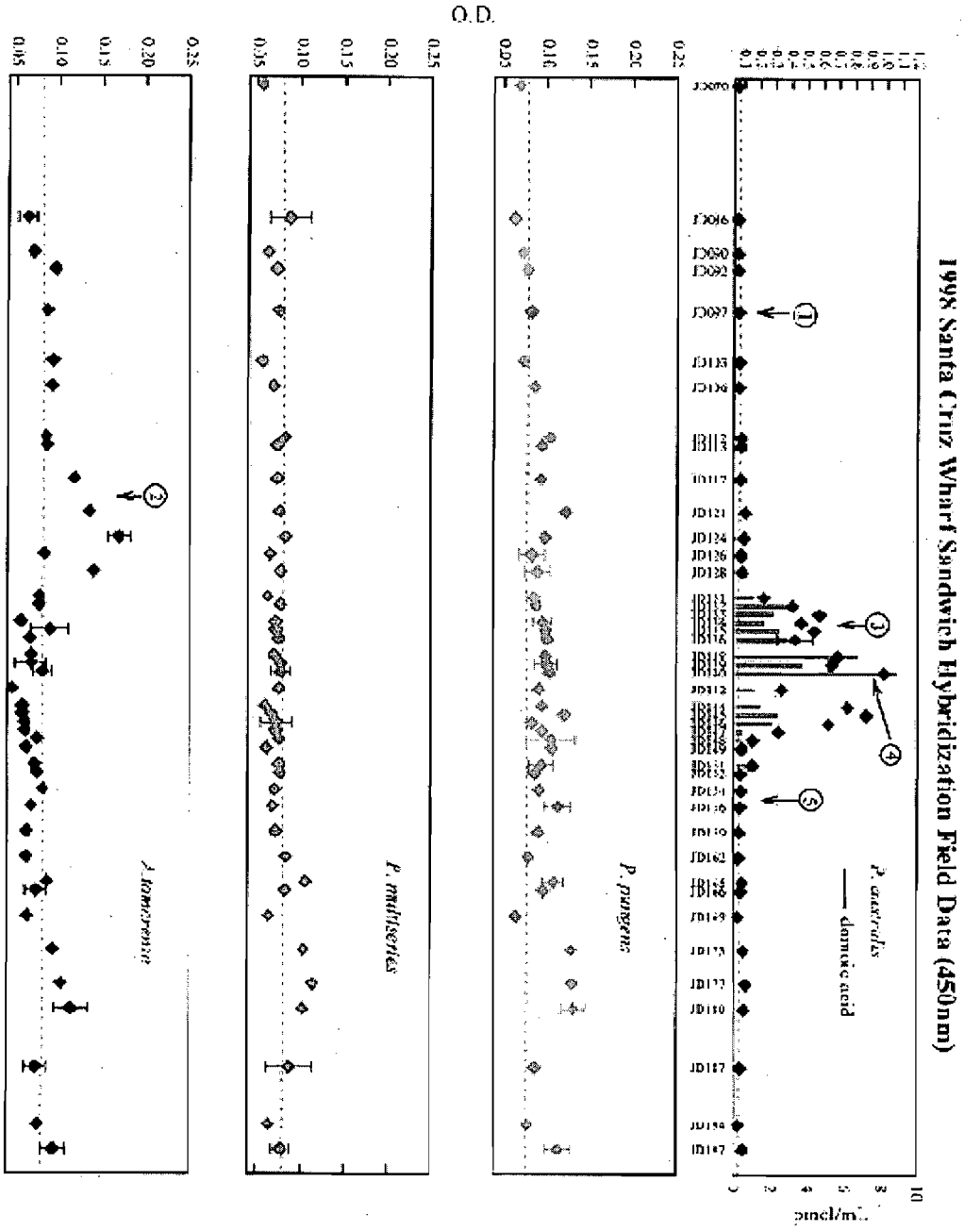


Figure 3. 1998 time series data collected at the Santa Cruz wharf station showing results of sandwich hybridization assays (optical density, O.D.) for *Pseudonizchtia australis, P. pungens, P. multiseries* and North American strains of *Alexandrium tamarense/catanella* plotted as a function of time (Julian day). Increasing O.D. indicates a rise in species abundance; the dashed line indicates lower limit of detection. Corresponding amounts of domoic acid associated with a particulate fraction of plankton are given as pmol toxin per mL water filtered (top panel, bars). Arrows refer to particular events described in the text.

Anchovy gut contents containing *Pseudo-nitzschia australis*, May 29, 1998.

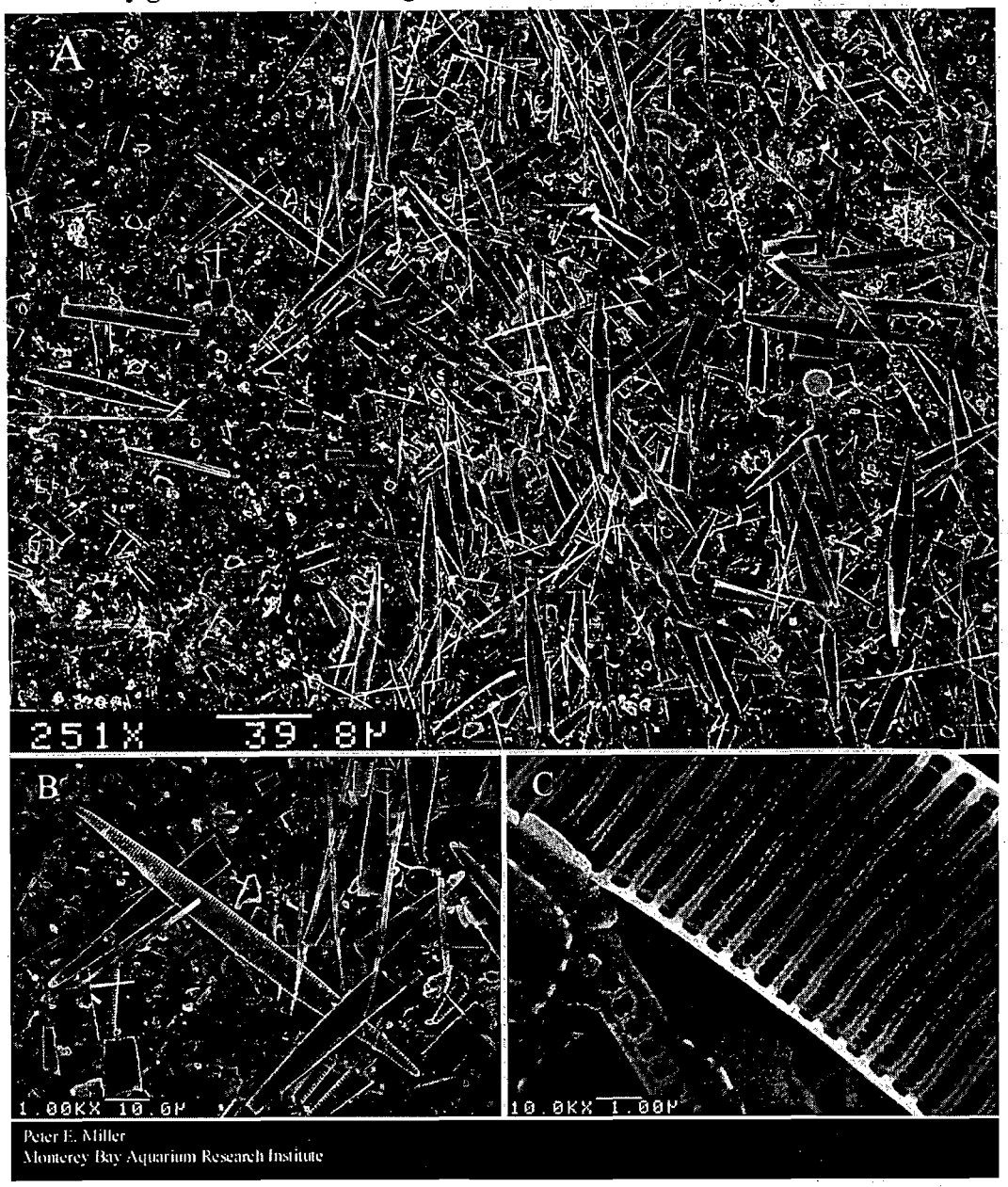


Figure 4.
Scanning electron micrographs of anchovy stomach contents at the height of the *P. australis* bloom. Anchovies caught in the Monterey Bay area had stomachs packed with *P. australis* frustules (A = overview, B and C = close-up showing frustules are *P. australis*).

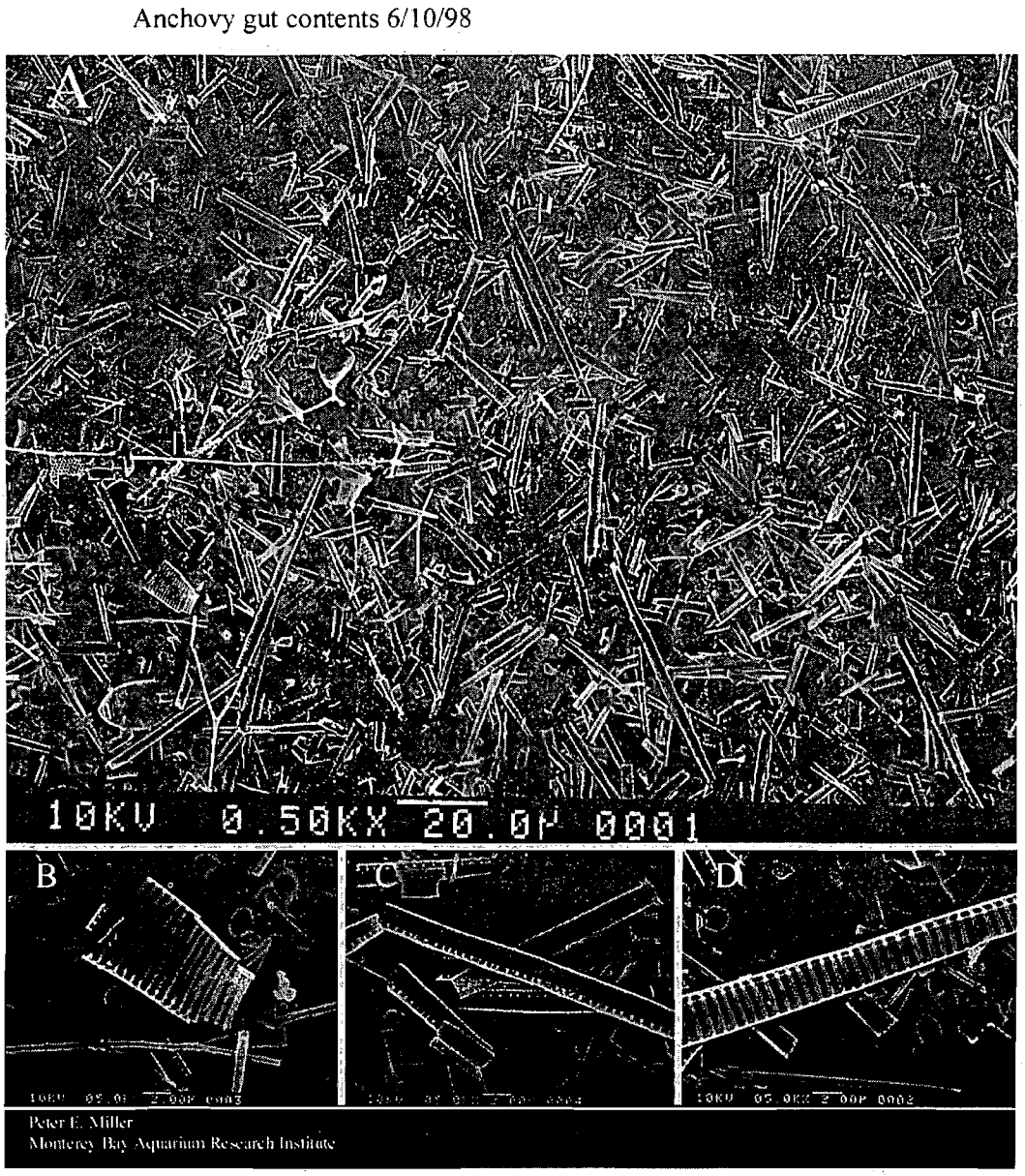


Figure 5.
Scanning electron micrographs of anchovy stomach contents after the *P. australis* bloom. Overview micrograph shows that anchovies caught in the Monterey Bay area had stomachs packed primarily with *P. delicatissima* (C), the dominant Pseudonizchtia in the water column at the time. Fragments of *P. australis* were also found (B), as well as frustules of *P. multiseries* (D), but the latter two species were not abundant.

DIAGNOSIS

The combination of clinical signs (scratching, seizuring, anchovies in fecal matter), histopathological (hippocampal necrosis), toxicological (domoic acid in serum, urine, feces, anchovies) and epidemiological findings (simultaneous mortality in sea lions and the presence of toxin-producing blooms) led to the diagnosis of domoic acid toxicity in the sea lions. This intoxication was due to blooms of Pseudonizchtia australis that were ingested by anchovies that in turn were eaten by California sea lions. Although a variety of incidental histopathologic and serologic changes were detected in the sea lions examined, they were not considered to be the cause of the mortality event. However, virus neutralization titers to phocine distemper virus, presence of protozoa in the brain and early neoplastic lesions warrant further investigation to determine their role in health of the California sea lion population.

POST RELEASE MONITORING

Three sea lions that survived the event were released with telemetry devices to monitor movements and survival after release. Adult females were selected, as animals of this sex and age are currently being tagged and monitored off San Miguel Island by Dr. Robert De Long and Sharon Melin from the National Marine Mammal Laboratory, and could be used as normal controls. The three animals selected had all shown typical clinical signs during the "event". One had seizures for only 48 hrs, one had repeated seizures for up to a week, and the third had seizures for up to one month post stranding. They were thus classed as mild, moderate and severe cases. None of the animals had been pregnant when stranded. As the tags are fixed to the animals using glue on the hair, the animals were not released until after the annual molt in September.

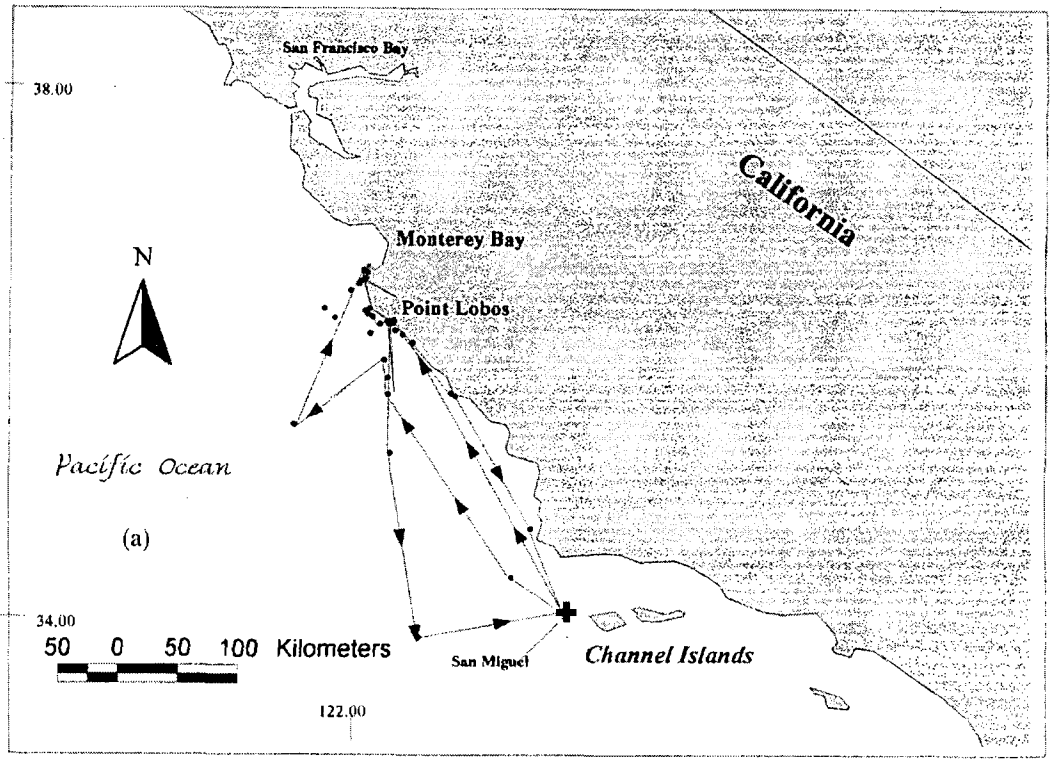
The three sea lions (CSL #3812, #3815 and #3822) were sedated with midazolam intramuscularly at 0.02 mg/kg (mixed with atropine at 0.02 mg/kg), then masked with isoflurane until relaxed but with intact gag reflex. Satellite-linked transmitters or platform transmitter terminals (ST-10 Argos PTT model; Telonics, Mesa, AZ) were glued to the dorsum between the scapulae using 5-minute epoxy. These tags were approximately 14 x 5 x 1 cm and weighed 0.2 kg. To conserve battery power and prolong the life of the PTTs, the duty cycle was set to transmit for 24 hrs every other day. Location data were collected by Service Argos. Radio transmitters (Advanced Telemetry Systems, Isanti, MN) were attached to the heads of each sea lion. These transmitters weighed 0.05 kg each and had 30 cm long whip antennae.

The sea lions were tagged on November 4 and released on November 6 at Weston Beach, Point Lobos State Reserve, Carmel, California (Fig. 6). This site was chosen as it was within the Monterey Bay, the area in which the sea lions stranded during the event, and is a protected area frequented by this species.

Two of these three sea lions were resighted. One animal was observed on December 10 1998 with a satellite transmitter and a VHF transmitter on Año Nuevo Island (Morris, pers. comm.), although its ID number was not determined. #3812 and #3815 were sighted on December 23 1998, 48 days after release. On this day, #3815 had a radio transmitter, but had lost the satellite transmitter. Satellite data indicate that the transmitter detached from the sea lion while on shore. Transmissions were received from the transmitter until February 7 1999. Sea lion #3812 was also sighted on January 8 1999 at Año Nuevo Island, with both transmitters attached.

Sea lion #3822 swam as far south as Point Benett, San Miguel Island (~325 km), where it remained for five days, before traveling north again to areas off the Big Sur Coast. Sea lion #3822 also made a second trip down to San Miguel Island, and returned north again (Figure 6a). A total of 170 positions was received for 64 days, until transmissions ceased on January 8 1999 off Point Lobos (36.519N, 121.954W). It is unclear whether signal cessation was a result of mortality, tag malfunction or tag loss. A total of 333 positions was received for sea lion #3812, which traveled as far north as the Farallon Islands, and as far south as the Channel Islands. Sea lion #3812 remained near the Channel Islands for one week, but returned to areas around Año Nuevo Island (Figure 6b). The last transmission from #3812 was 94 days post release on February 7 1999 off Pescadero Point, California (37.241N, 122.488W). Sea lion #3815 primarily remained within and around areas adjacent to Monterey Bay while the satellite tag was attached. Although the date of tag detachment from this animal is unclear, transmissions ceased on February 7, 1999. The failure of transmissions from two tags within a day of each other suggests that this was due to battery expiration rather than death of the sea lions.

At least two of the three satellite-tagged sea lions survived up to three months post release and data indicated they were using areas where wild sea lions could be found. Melin *et al.* (1993) found that female California sea lions from San Miguel Island, which were tagged with satellite-linked time depth recorders, foraged northwest of San Miguel Island during winter and spring months. Furthermore, tagged sea lions from San Miguel Island traveled as far as 460 km north along the mainland coast of California (Melin *et al.* 1993). Thus, movements of the three rehabilitated sea lions may have been typical.



DISCUSSION

This is the first report of domoic acid toxicity in marine mammals. However, as algal blooms producing domoic acid have been observed previously along the California coast (Walz *et al.*, 1994), and domoic acid toxicity was reported in brown pelicans off Monterey in 1991 (Work *et al.*, 1993), it is likely that this toxicosis has occurred previously in marine mammals. Clusters of stranded adult sea lions that showed similar neurologic signs to those in the animals in this 1998 event have been observed at other times along the California coast, although no cause was determined. In July 1978, 40 animals stranded in Ventura County displaying neurologic signs (Gilmartin *et al.*, 1979); in 1986, 11 sea lions showing opisthotonus and convulsions were admitted to TMMC (Vandenbroek *et al.*, 1987); in 1988, 38 sea lions and ten northern fur seals (*Callorhinus ursinus*) with similar signs were admitted to TMMC (Gage *et al.*, 1989) and in 1992 18 sea lions stranded in San Luis Obispo County displaying similar signs (Beckmen *et al.*, 1995). Similar histological changes in the hippocampus to those in sea lions from this 1998 event were observed by Dr. Linda Lowenstine in animals that died in the 1992 event. Two stomachs from seizuring sea lions during the 1992 seizure event were submitted for domoic acid analysis with negative results. However, the stomachs were devoid of contents. No suitable samples,i.e. serum, urine or feces, are available from earlier animals for retrospective analysis. As there was a bloom of *P. australis* in Monterey Bay in 1992 (Walz *et al.*, 1994), it is likely that domoic acid toxicity affected sea lions off California earlier than 1998. In addition, since this event there has been another similar occurrence in October 1998.

From July 12 to October 17, 1998 an additional eleven California sea lions stranded with similar clinical signs to the animals described in this report. Nine of the animals stranded from October 3 to October 17. Six animals were euthanized or died, and four animals recovered and were released. Four of six urine samples from sea lions in the October event analyzed for domoic acid by HPLC-UV were positive (Table 6), while one CSF sample was negative. Serum, urine, and cerebrospinal fluid (CSF) were tested by receptor binding assay only. Domoic acid was detected by RBA in three out of six urine samples from clinically affected animals. All serum and CSF samples tested were negative for domoic acid. During this time period, cells of *P. australis* did reach ~10,000 cells per liter at the Santa Cruz pier, however, much higher cell concentrations were observed outside of Monterey Bay, again highlighting the difference between water masses within and outside of the Bay. In this case, it is likely that the bloom of *P. australis* was fueled by upwelling and that cells and their associated toxin did not penetrate Monterey Bay to any great extent. This recent episode illustrates the quick response that stranding rates and pinnipeds may have to toxic blooms of *P. australis*.

The combination of oceanographic data, evidence of domoic acid in the prey eaten by the sea lions, clinical signs and histopathology are important in making a diagnosis of domoic acid toxicity, as detection of domoic acid in sea lion tissues is limited. The detection of domoic acid in only three serum samples by receptor binding assay is not surprising, as the plasma half-life is short in other species (21.6 minutes in rats, 114 minutes in monkeys; (Truelove and Iverson, 1994). Blood samples were not collected from sea lions until they arrived at TMMC after initial stranding, detection of the animal by the public and transport by road up to 200 miles. It is therefore likely that the domoic acid had been cleared from plasma by the time blood was collected in most cases. As domoic acid is water soluble and excreted in urine, it is also not surprising that it was detected in urine rather than plasma in some animals.

The even higher detection rate in feces may reflect poor absorption from the gastro-intestinal tract due to poor lipid solubility. After oral administration of domoic acid to rats, recovery from feces was complete (Iverson *et al.*, 1989).

In contrast to the toxicologic results, both clinical signs and histopathologic changes were characteristic of domoic acid toxicity as reported in other species, although a variety of incidental signs and lesions were detected. The seizures were dramatic, and presumably a consequence of domoic acid binding in the brain. Domoic acid is an excitatory neurotoxin that binds to neurones via high-affinity AMPA- and kainate-sensitive glutamate receptors to produce excitotoxic cell death (Iverson and Truelove, 1994; Larm *et al.*, 1997). In between seizures, the scratching behavior was reminiscent of the hallmark scratching syndrome documented in mice that distinguishes paralytic shellfish poisoning from domoic acid toxicity (Todd, 1993). Similar behavior was also described in pelicans intoxicated with domoic acid (Work *et al.*, 1993). The ability of animals to survive exposure, as demonstrated by the satellite-tracking data, is also consistent with the survival of exposed humans in the Canadian outbreak in people who ate contaminated mussels (Perl *et al.* 1990).

The significance of the virus neutralization titers to phocine distemper virus (PDV) in a few animals is unclear. However, the lack of accompanying histopathological changes consistent with morbillivirus infection, negative RT-PCR for morbillivirus on brain tissue, and the similar seroprevalence of PDV antibodies in banked sea lion sera suggest the presence of antibodies in sea lions dying during the event did not play a role in this event. In contrast, in the recent Mediterranean monk seal die-off, a controversy as to the cause of the die-off occurred, due to the isolation of a morbillivirus from tissue of affected animals, in addition to detection of saxitoxin (Osterhaus *et al.*, 1997; Costas and Lopez-Rodas, 1998).

Over the time frame of the "seizuring sea lion" event, large numbers of dead pinnipeds and sea birds were observed on the beaches around Monterey Bay. The causes of death of these animals were not determined. However, many were severely emaciated. Based on gross appearance and predictions of species likely to be affected by changes in food availability during El Niño events (Bodkin and Jameson 1991, Trillmich and Ono 1991), it is likely that many of these mortalities were a consequence of starvation due to food shortage. It is thus unclear how many sea lions in total were affected by domoic acid. The overall mortality due to domoic acid is likely to be more than 57 individuals, but is unknown. As the overall California sea lion population is estimated at 167,000 individuals, an event causing mortality in 0.00004 % does not constitute a threat to the population. However, the rapid and dramatic nature of the event suggests the potential for similar harmful algal blooms to impact endangered species, such as the Steller's sea lion or Hawaiian monk seal, before control measures can be implemented. In addition, the susceptibility of humans to domoic acid suggests that outbreaks of toxicity in marine mammals should be fully investigated and measures taken to prevent human morbidity and mortality.

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This investigation would not have been possible without the willing collaboration and enthusiasm of each of the organizations involved. Special thanks are due to Diane Paschall for assistance in putting this report together, the Monterey Bay Aquarium Research Institute for funding a workshop in the wake of the event to bring investigators together and facilitate exchange of data, Wanda Dietrich for editing and formatting this document, and the staff and volunteers of TMMC for their hard work caring for the sea lions during the event.

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List of Terms

dl	deciliter; one-tenth of a liter
g or gm	gram
HPLC	High Performance Liquid Chromotography
HPLC/UV	High Performance Liquid Chromotography with ultraviolet detection
HPLC/MS	High Performance Liquid Chromotography with mass spectrometry
HPLC/MS/MS	High Performance Liquid Chromotography with tandem mass spectrometry
IM	intramuscular
IV	intravascular
kg	kilogram; one thousand grams
mg	milligram; one-thousandth of a gram
ml	milliliter; one-thousandth of a liter
mm	millimeter; one-thousandth of a meter
M	mass
m/z	mass/charge
NOS	National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, South Carolina
NWFSC	Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington
RBA	Receptor binding assay
SAX	strong anion exchange
SSP	species
TFA	Trichloroacetic acid
TMMC	The Marine Mammal Center, Sausalito, California
ug	microgram; one-millionth of a gram
UCSC	University of California, Santa Cruz

Appendix 1A Dead Animals										
Acc #	Name	Admit date	Disposition	Disp Date	Sex	Age class	Age	Pregnant	Strand Location	Strand County
							Yrs			
CSL 3690	Jarkarta	5/18/98	Died	5/18/98	F	Adult	7	no	Monterey	Monterey
CSL 3691	Angelo	5/18/98	Died	5/18/98	F	Adult	9	no	Oceano Dunes	San Luis Obispo
CSL 3692	Jordan	5/16/98	Died	5/18/98	F	Adult	9	no	Oceano Dunes	San Luis Obispo
CSL 3699	Sallee	5/19/98	Euthanized	5/20/98	F	Adult	13	yes	Oceano Dunes	San Luis Obispo
CSL 3700	Alexandra	5/19/98	Euthanized	5/20/98	F	Adult	6	no	Oceano Dunes	San Luis Obispo
CSL 3707	Franky	5/20/98	Euthanized	5/21/98	M	Subadult	3.5	NA	Oceano Dunes	San Luis Obispo
CSL 3709	Dean	5/20/98	Died	5/20/98	F	Adult	11	no	Oceano Dunes	San Luis Obispo
CSL 3721	Toe	5/21/98	Died	6/3/98	F	Adult		yes	Oceano Dunes	San Luis Obispo
CSL 3724	Three	5/21/98	Died	5/22/98	F	Adult	10	yes	Oceano Dunes	San Luis Obispo
CSL 3725	Two	5/21/98	Died	5/21/98	F	Adult	8	yes	Oceano Dunes	San Luis Obispo
CSL 3726	Pedwar	5/21/98	Euthanized	6/2/98	F	Adult		yes	Oceano Dunes	San Luis Obispo
CSL 3729	Andromeda	5/22/98	Euthanized	6/1/98	F	Adult	11	no	Monterey	Monterey
CSL 3732	Neuf	5/22/98	Died	6/22/98	F	Adult		no	Oceano Dunes	San Luis Obispo
CSL 3734	Benny	5/22/98	Died	5/23/98	F	Subadult	4	no	Oceano Dunes	San Luis Obispo
CSL 3736	Eight	5/22/98	Died	5/22/98	M	Juvenile		NA	Oceano Dunes	San Luis Obispo
CSL 3741	Lumberjack	5/23/98	Died	5/29/98	F	Adult	7	yes	Marina	Monterey
CSL 3742	Chaos	5/23/98	Euthanized	5/31/98	F	Adult	10	no	Monterey	Monterey
CSL 3743	DOA	5/23/98	Died	5/23/98	F	Adult	4.5	yes	Marina	Monterey
CSL 3744	Reid	5/23/98	Died	5/23/98	F	Adult	6	no	Marina	Monterey
CSL 3745	Theiran	5/22/98	Died	6/12/98	M	Juvenile		no	Oceano Dunes	San Luis Obispo
CSL 3746	Finnegan	5/22/98	Euthanized	6/1/98	F	Adult	12	yes	Oceano Dunes	San Luis Obispo
CSL 3747	Fiona	5/22/98	Euthanized	6/2/98	F	Adult		yes	Oceano Dunes	San Luis Obispo
CSL 3748	Sixteen	5/22/98	Died	5/23/98	F	Adult		no	Oceano Dunes	San Luis Obispo
CSL 3749	Shamus	5/22/98	Died	5/24/98	F	Adult	11	yes	Oceano Dunes	San Luis Obispo
CSL 3757	Twenty six	5/23/98	Died	5/23/98	F	Adult		yes	Oceano Dunes	San Luis Obispo
CSL 3758	Twenty three	5/23/98	Died	5/23/98	F	Adult	8	no	Shell Beach	San Luis Obispo
CSL 3760	Oatmeal	5/24/98	Euthanized	6/2/98	F	Adult		Yes-fetus	Oceano Dunes	San Luis Obispo
CSL 3765	DOA	5/24/98	Died	5/24/98	F	Adult	4	yes	Monterey	Monterey
CSL 3768	Toddy	5/24/98	Died	11/25/98	M	Juvenile		NA	Sand City	Monterey
CSL 3770	Joule	5/24/98	Euthanized	6/4/98	F	Adult	7	Yes-fetus	Monterey	Monterey
CSL 3780	DOA	5/24/98	Died	5/24/98	F	Adult		yes	Watsonville	Santa Cruz
CSL 3783	Thirty two	5/23/98	Died	5/24/98	F	Adult	13	no	Oceano Dunes	San Luis Obispo
CSL 3791	Neville	5/24/98	Euthanized	6/2/98	F	Adult	12	no	Avila	San Luis Obispo
CSL 3793	Roller	5/25/98	Euthanized	6/4/98	F	Adult	8	Yes-stillbor	Watsonville	Santa Cruz

Acc #	Name	Admit date	Disposition	Disp Date	Sex	Age class	Age Yrs	Pregnant	Strand Location	Strand County
CSL 3794	Muddy	5/25/98	Died	5/31/98	F	Adult	10	Yes-fetus	Moss Landing	Monterey
CSL 3796	Kristen	5/23/98	Died	6/1/98	F	Adult	7	Yes-fetus	Pismo Pier	San Luis Obispo
CSL 3800	Lady bug	5/26/98	Euthanized	5/27/98	F	Adult	6	Yes-fetus	Moss Landing	Monterey
CSL 3801	Whisper	5/25/98	Euthanized	5/31/98	F	Adult	9	no	Oceano Dunes	San Luis Obispo
CSL 3802	No Name	5/26/98	Euthanized	6/27/98	F	Adult		Yes-fetus	Monterey	Monterey
CSL 3803	Ouch	5/26/98	Euthanized	5/27/98	F	Adult	9	no	Pacific Grove	Monterey
CSL 3804	Kitty	5/26/98	Euthanized	6/3/98	F	Adult		no	Moss Landing	Monterey
CSL 3806	Flower	5/27/98	Euthanized	6/1/98	F	Adult	8	Yes-fetus	Monterey	Monterey
CSL 3807	Opal	5/27/98	Died	5/27/98	F	Adult	9	Yes-fetus	Aptos	Santa Cruz
CSL 3824	Sady	5/29/98	Euthanized	6/3/98	F	Adult	11	yes	Santa Cruz	Santa Cruz
CSL 3901	Palm	6/8/98	Euthanized	6/22/98	F	Adult		no	Pajaro Dunes	Santa Cruz
CSL 3931	Whitelight	6/14/98	Euthanized	6/14/98	M	Subadult		NA	Half Moon Bay	San Mateo
CSL 3950	Athena	6/16/98	Died	6/20/98	F	Adult		no	Pacific Grove	Monterey
CSL 3969	Digger	6/19/98	Died	6/19/98	F	Adult		NA	Oceano Dunes	San Luis Obispo

Appendix 1B Live Animals									
Acc #	**Name**	**Admit date**	**Dispositior**	**Disp Date**	**Sex**	**Age**	**Pregnant**	**Strand Location**	**Strand County**
CSL 3708	Sammy	5/20/98	Released	11/5/98	F	Adult	No	Oceano Dunes	San Luis Obispo
CSL 3717	Thelma	5/21/98	Released	6/25/98	F	Adult	No	Monterey	Monterey
CSL 3719	Tick	5/21/98	Released	7/18/98	F	Adult	Yes	Oceano Dunes	San Luis Obispo
CSL 3720	Tack	5/21/98	Released	7/18/98	F	Juvenile	No	Oceano Dunes	San Luis Obispo
CSL 3727	Solo	5/21/98	Released	6/14/98	M	Subadult	NA	Oceano Dunes	San Luis Obispo
CSL 3731	Ju-Ni	5/22/98	Released	8/5/98	F	Adult	Yes	Oceano Dunes	San Luis Obispo
CSL 3733	Pentobarb	5/21/98	Released	8/5/98	M	Subadult	NA	Oceano Dunes	San Luis Obispo
CSL 3735	Septimus	5/22/98	Released	8/5/98	M	Juvenile	NA	Pismo Beach	San Luis Obispo
CSL 3752	Pistachio	5/23/98	Released	6/25/98	F	Adult	Yes	Oceano Dunes	San Luis Obispo
CSL 3754	Pecan	5/23/98	Released	6/25/98	F	Subadult	No	Oceano Dunes	San Luis Obispo
CSL 3755	Macadamia	5/23/98	Released	7/22/98	F	Adult	No	Oceano Dunes	San Luis Obispo
CSL 3767	Newton	5/24/98	Released	8/5/98	F	Adult	No	Monterey	Monterey
CSL 3769	Pascal	5/24/98	Released	8/2/98	F	Adult	Yes	Watsonville	Monterey
CSL 3775	Skipper	5/24/98	Released	6/20/98	F	Juvenile	No	Oceano Dunes	San Luis Obispo
CSL 3776	Professor	5/23/98	Released	6/22/98	M	Subadult	NA	Oceano Dunes	San Luis Obispo
CSL 3777	Howell	5/24/98	Released	6/25/98	F	Adult	No	Los Osos	San Luis Obispo
CSL 3792	Tara	5/24/98	Released	6/14/98	M	Juvenile	NA	Pismo Beach	San Luis Obispo
CSL 3795	Mellow	5/25/98	Released	6/22/98	M	Subadult	NA	Watsonville	Santa Cruz
CSL 3812	Esperanza	5/28/98	Released	11/5/98	F	Adult	No	Santa Cruz	Santa Cruz
CSL 3815	Mahjong	5/28/98	Released	11/5/98	F	Adult	No	Santa Cruz	Santa Cruz
CSL 3822	Salace	5/29/98	Released	11/5/98	F	Adult	No	Moss Landing	Monterey
CSL 3835	Punchy	5/30/98	Released	7/22/98	F	Adult	Yes	Pismo Beach	San Luis Obispo

Appendix 2A, Admit hematology																					
Acc #	Name	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	segs	segs	bands	bands	lymph	lymph	monos	monos	eos	eos	baso	baso	plat
		x 10^3	x10^6	g/dl	%	fL	pg	g/dl	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	x 10^3
CSL 3708	Sammy	8.5	5.84	22.2	67.2	115.1	38	33	63	5.355	2	0.17	19	1.615	1	0.085	15	1.28	0	0	503
CSL 3717	Thelma	12.6	4.46	17.3	51.8	115.9	38.7	33.4	48	6.048	0	0	33	4.158	3	0.378	16	2.02	0	0	327
CSL 3719	Tick	14	3.56	15.5	39.3	110.4	43.5	39.4	63	8.82	0	0	23	3.22	7	0.98	7	0.98	0	0	392
CSL 3720	Tack	11.2	4.22	14.8	50.9	120.6	35.1	29.1	69	7.728	4	0.448	12	1.344	3	0.336	11	1.23	0	0	357
CSL 3727	Solo	11.8	4.5	17.5	53.3	118.4	38.9	32.8	70	8.26	1	0.118	16	1.888	13	1.534	0	0	0	0	246
CSL 3731	Ju-Ni	12.6	5.01	19.7	59.8	119.4	39.3	32.9	85	10.71	0	0	7	0.882	0	0	8	1.01	0	0	406
CSL 3735	Septimus	12.4	5.18	18.4	59.2	114.3	35.5	31.1	58	7.192	0	0	31	3.844	7	0.868	1	0.12	0	0	253
CSL 3752	Pistachio	12.5	4.84	18.3	52.9	109.3	37.8	34.6	81	10.13	0	0	12	1.5	2	0.25	5	0.63	0	0	319
CSL 3754	Pecan	13	5.7	21.1	60.6	106.3	37	34.8	61	7.93	0	0	19	2.47	7	0.91	13	1.69	0	0	199
CSL 3755	Macadamia	16.6	5.01	18.8	55.3	110.4	37.5	34	68	11.29	1	0.166	19	3.154	4	0.664	8	1.33	0	0	124
CSL 3767	Newton	13	4.67	17.7	58.9	126.1	37.9	30.1	64	8.32	0	0	25	3.25	10	1.3	1	0.13	0	0	258
CSL 3768	Skipper	9.4	4.68	16.3	54.3	116	34.8	30	52	4.888	0	0	21	1.974	11	1.034	13	1.22	2	0.19	125
CSL 3775	Toddy	18.3	4.24	15.9	48.6	114.6	37.5	32.7	84	15.73	2	0.366	12	2.196	1	0.183	0	0	0	0	214
CSL 3776	Professor	8.8	5.32	18.7	63.3	119	35.2	29.5	67	5.896	2	0.176	19	1.672	10	0.88	1	0.09	0	0	332
CSL 3795	Mellow	11.9	4.76	18.7	53.8	113	39.3	34.8	na	na	na	na	na	na	na	na	na	na	0	0	250
CSL 3812	Esparanza	10.4	5.18	20.2	68.1	131.5	39	29.7	76	7.904	3	0.312	13	1.352	5	0.52	3	0.31	0	0	206
CSL 3815	Mahjong	6.4	5.05	19.3	60.1	118.3	38	32.1	57	3.648	1	0.064	24	1.536	4	0.256	11	0.7	0	0	205
CSL 3822	Salace	10.1	4.68	18.1	58.4	124.8	38.7	31	72	7.272	4	0.404	15	1.515	3	0.303	4	0.4	1	0.06	430
CSL 3835	Punchy	10.2	4.73	17.7	50.5	106.8	37.4	35	51	5.202	1	0.102	27	2.754	4	0.408	14	1.43	0	0	359

Appendix 2B, Admit hematology																					
Acc #	Name	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	segs	segs	bands	bands	lymph	lymph	monos	monos	eos	eos	baso	baso	plat
		x 10^3	x10^6	g/dl	%	fL	pg	g/dl	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	x 10^3
CSL 3700	Alexandra	11.1	5.52	21.4	59.3	108.3	38.8	35.8	80	8.88	0	0	10	1.11	1	0.111	9	0.999	0	0	353
CSL 3707	Franky	11.6	6.16	23.3	65.3	106	37.8	35.7	13	1.51	0	0	13	1.508	2	0.232	16	1.856	0	0	291
CSL 3721	Toe	11.2	3.62	13	41.6	114.9	35.9	31.3	64	7.17	3	0.336	12	1.344	3	0.336	18	2.016	0	0	339
CSL 3726	Pedwar	12.7	5.77	20.8	64.6	112	36	32.2	73	9.27	2	0.254	13	1.651	2	0.254	10	1.27	0	0	253
CSL 3729	Andromeda	9.1	5.16	19.5	55.7	107.9	37.8	35	75	6.83	0	0	14	1.274	0	0	11	1.001	0	0	732
CSL 3732	Neuf	5.5	4.09	15.6	44.6	109	38.1	35	61	3.36	4	0.22	23	1.265	3	0.165	8	0.44	0	0	73
CSL 3741	Lumberjack	14.7	4.24	16.7	51.2	120.8	39.4	32.6	57	8.38	4	0.588	8	1.176	4	0.588	27	3.969	0	0	333
CSL 3742	Chaos	4	4.52	18.1	56.8	125.7	40	31.9	84	3.36	0	0	13	0.52	3	0.12	0	0	0	0	129
CSL 3745	Thieran	12.2	4.5	16.3	46.4	109.8	36.2	33	68	8.3	1	0.122	21	2.562	4	0.488	6	0	0	0	234
CSL 3746	Finnegan	6.7	5.22	18.5	55.5	106.3	35.4	33.3	55	3.69	1	0.067	12	0.804	3	0.201	29	1.943	0	0	298
CSL 3747	Fiona	11.5	4.31	17.9	50.1	116.2	41.5	35.7	74	8.51	1	0.115	17	1.955	7	0.805	1	0.115	0	0	400
CSL 3749	Shamus	8.2	4.37	16.7	49.6	113.5	38.2	33.7	69	5.66	4	0.328	9	0.738	6	0.492	12	0.984	0	0	420
CSL 3760	Oatmeal	16.2	4.31	16.9	48.4	112.3	39.2	34.9	73	11.8	2	0.324	10	1.62	1	0.162	14	2.268	0	0	329
CSL 3768	Toddy	9.4	4.68	16.3	54.3	116	34.8	30	52	4.89	0	0	21	1.974	11	1.034	13	1.222	2	0.188	125
CSL 3770	Joule	9.8	4.28	15.5	51.4	120.1	36.2	36.2	82	8.04	5	0.49	10	0.98	3	0.294	0	0	0	0	74
CSL 3791	Neville	8.7	4.11	16.4	49.5	120.4	39.9	33	64	5.57	4	0.348	18	1.566	8	0.696	9	0.783	0	0	212
CSL 3793	Roller	16.9	4.99	19.6	64.2	128.7	39.3	30.5	67	11.3	2	0.338	13	2.197	14	2.366	4	0.676	0	0	163
CSL 3794	Muddy	9.5	4.19	16.3	56.9	135.8	38.9	28.6	77	7.32	5	0.475	14	1.33	2	0.19	2	0.19	0	0	193
CSL 3796	Kristen	18	5.05	19.7	56.8	112.5	39	34.7	78	14	1	0.18	2	0.36	1	0.18	18	3.24	0	0	307
CSL 3802	No Name	8.7	4.35	16.4	44.7	102.8	37.7	36.7	77	6.7	1	0.087	14	1.218	3	0.261	5	0.435	0	0	189
CSL 3804	Kitty	10.1	5.32	21.8	61.7	116	41	35.5	84	8.48	0	0	4	0.404	7	0.707	5	0.505	0	0	76
CSL 3806	Flower	8.2	4.89	19.9	54.6	111.7	40.7	36.4	69	5.66	6	0.492	11	0.902	9	0.738	5	0.41	0	0	265
CSL 3824	Sady	10.2	5.1	18.8	59.7	117.1	36.9	31.8	82	8.36	3	0.306	10	1.02	3	0.306	2	0.204	0	0	126
CSL 3901	Palm	11.8	4.57	17.4	52.6	115.1	38.1	33.1	55	6.49	0	0	27	3.186	5	0.59	13	1.534	0	0	344
CSL 3950	Athena	8.3	4.96	18.8	55.2	111.3	37.9	34.1	88	7.3	0	0	5	0.415	4	0.332	1	0.083	0	0	357

Appendix 2C, Admit Blood Chemistries																								
Acc. #	Name	Na	K	Cl	Glu	BUN	Creat	Prot	Alb	Glob	Ur. Ac.	Ca	Phos	Tot Bil	Dir Bil	GGT	Alk P	AST	ALT	LDH	Iron	Chol	Trigl	CK
		meq/l	meq/l	meq/l	mg/dl	mg/dl	mg/dl	gm/dl	gm/dl	gm/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	u/l	u/l	u/l	u/l	mcg/dl	mg/dl	mg/dl	
CSL3699	Sallee	141	4	95	91	16	1.4	9.3	3	6.3	0.5	8.5	5.5	0.6	0.1	83	26	150	119	1599	46	246	75	na
CSL3700	Alexandra	142	4	96	88	16	1.4	9	3.1	5.9	0.5	8.7	4.7	0.4	0.1	62	45	204	116	2521	41	162	43	na
CSL 3708	Sammy	142	5.3	99	136	42	2.3	8.6	2.4	6.2	1.2	8.5	7.7	0.7	0.1	186	35	97	87	1567	22	253	82	na
CSL 3719	Tick	164	5.8	113	126	21	1.4	7.8	3.2	4.6	2.1	9	7.1	1.7	0.2	52	21	40	35	765	28	172	85	na
CSL 3720	Tack	143	5	100	103	22	1.4	9.8	3.5	6.3	3.1	9	7.6	2.4	0.1	87	12	56	39	1229	82	188	80	777
CSL 3721	Toe	143	5	10	103	22	1.4	9.8	3.5	6.3	3.1	9	7.6	2.4	0.1	87	12	56	39	1229	82	188	80	777
CSL 3724	Three	147	8.9	89	334	44	4.4	9.8	3.1	6.7	4.6	9.9	12	0.7	0.2	114	53	na	163	2664	193	190	90	na
CSL 3726	Pedwar	143	5.4	95	104	22	1.6	12.6	6.3	6.3	8.7	7.4	8.3	10	0.4	10	na	61	22	2958	na	247	284	na
CSL 3727	Solo	150	5.6	106	92	33	1.1	9	3.2	5.8	2	9	6.5	1.2	0.1	48	41	156	75	707	60	157	85	6288
CSL 3729	Andromeda	146	5.1	99	190	38	1.6	9.1	3.2	5.9	2.5	7.2	7.8	1.9	0.2	110	26	117	71	1747	79	216	97	1920
CSL 3731	Ju-Ni	157	5.3	105	113	16	1.6	11.2	5.6	5.6	6.9	8.4	7.8	7.7	1.5	19	na	125	41	2607	na	264	101	na
CSL 3732	Neuf	167	5	117	115	32	1.9	11.4	4.7	6.7	5	9.7	8.7	5.4	1.1	87	10	116	45	1561	165	212	186	na
CSL 3733	Pentobarb	151	4.8	105	121	14	1.3	8.3	33	6	2.6	8.6	7.8	1.8	0.2	51	20	149	67	1319	94	224	71	6367
CSL 3734	Benny	138	7.6	88	37	77	5.9	9.5	3.1	6.4	5.6	8.5	17	1.7	0.2	86	75	na	335	2761	92	162	111	na
CSL 3735	Septimus	149	4.8	111	70	62	0.9	8.7	3.2	5.5	3.8	8.6	6.5	2.4	0.2	76	45	81	82	952	220	186	215	750
CSL 3741	Lumberjack	148	5.2	100	77	21	1.7	7.4	2.7	4.7	1.2	8	9.5	0.5	0.2	72	37	83	65	1488	50	167	154	na
CSL 3745	Thieran	151	4.5	108	87	37	0.9	8.7	3	5.7	1.7	8.6	5.8	0.8	0.1	171	40	56	94	620	103	135	101	1033
CSL 3746	Finnegan	147	4.8	101	67	52	1.8	7.8	2.6	5.2	1.3	7.8	9.9	0.5	0.2	131	32	64	67	2250	66	206	115	na
CSL 3752	Pistachio	150	4.2	99	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
CSL 3754	Pecan	144	5.1	101	111	30	1.1	8.4	3	5.4	1.2	9.1	5.4	0.6	0.1	54	35	30	91	930	118	158	56	169
CSL 3795	Mellow	145	4.2	101	93	17	1.6	8.9	2.8	6.1	1.2	8.6	6.2	0.5	0.1	78	42	82	55	599	40	203	73	na
CSL 3801	Whisper	139	4.5	102	101	26	1.2	9.2	3.3	5.9	1.9	8.1	5.4	2.1	0.2	102	15	60	117	1606	135	181	57	311
CSL 3802	No Name	149	4.7	104	102	19	1.3	8.5	2.7	5.8	1.2	8.2	5.7	0.6	0.1	90	27	43	60	517	57	158	118	1311
CSL 3804	Kitty	151	4.7	100	167	37	1.5	8.3	2.8	5.5	1.5	8.4	6.9	1.3	0.2	137	26	70	80	885	101	213	50	623
CSL 3806	Flower	149	5.4	105	67	74	1.7	8.9	3	5.9	1.2	8.9	9	0.6	0.2	183	44	64	91	2075	43	184	100	912
CSL 3812	Esperanza	146	4.6	101	102	87	1.7	6.9	2.2	4.7	1.1	7.3	9.4	0.5	0.1	89	39	112	134	1762	132	107	46	na
CSL 3815	Mahjong	176	6.3	127	112	27	2.2	8.8	3.4	5.4	0.9	11	8.1	0.7	0.2	48	32	23	34	569	79	122	36	na
CSL 3822	Salace	159	5.4	111	106	19	0.6	8.99	3	6	na	9.8	7.8	0.78	na	na	31	90	83	na	na	112	na	na
CSL 3835	Punchy	158	6.1	118	115	17	1.4	6.8	2.4	4.4	1.1	8.9	7	0.9	0.2	52	27	81	51	1050	81	121	44	na
CSL 3901	Palm	167	6	112	90	66	3.1	10.2	3.3	6.9	1.2	11	12	0.5	0.2	105	34	56	30	760	96	288	62	na
CSL 3950	Athena	156	6.1	110	82	61	1.3	8.1	1.9	6.2	1.3	9.6	11	0.5	0.2	188	34	65	82	718	155	138	24	na

Appendix 3A Hematology, Pre-release																					
Acc #	Name	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	segs	segs	bands	bands	lymph	lymph	monos	monos	eos	eos	baso	baso	plat
		x 10^3	x10^6	g/dl	%	fL	pg	g/dl	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	%	Abs	x 10^3
CSL 3709	Sammy	17.5	3.62	13	40.9	113	35.9	31.8	36	6.3	1	0.175	36	6.3	4	0.7	8	1.4	0	0	338
CSL 3717	Thelma	9.4	4.23	17	46.8	110.6	39	35.3	40	3.76	3	0.282	32	3.008	2	0.188	23	2.162	0	0	395
CSL 3719	Tick	13.5	4.44	17	47.7	107.4	37.8	35.2	60	8.1	0	0	20	2.7	11	1.485	9	1.215	0	0	297
CSL 3720	Tack	12.6	4.3	16	46.4	107.9	37.2	34.5	46	5.796	0	0	35	4.41	5	0.63	8	1.008	1	0.126	292
CSL 3727	Solo	11.8	4.5	18	53.3	118.4	38.9	32.8	70	8.26	1	0.118	16	1.888	13	1.534	0	0	0	0	411
CSL 3731	Ju-Ni	19.2	4.19	16	44.8	106.9	37.9	35.5	77	14.78	1	0.192	13	2.496	2	0.384	7	1.344	0	0	287
CSL 3733	Pentobarb	12.8	4.19	16	46.2	110.3	38.9	35.3	48	6.144	1	0.128	44	5.632	3	0.384	1	0.128	0	0	225
CSL 3735	Septimus	12.4	4.4	18	49.1	11.6	40.6	36.7	64	7.936	1	0.124	25	3.1	8	0.992	2	0.248	0	0	352
CSL 3752	Pistachio	10.9	4.95	19	52.4	105.9	38.8	36.6	53	5.777	6	0.654	33	3.597	6	0.654	1	0.109	0	0	300
CSL 3754	Pecan	16.7	5.11	19	53.5	104.7	37.2	35.5	40	6.68	4	0.668	42	7.014	4	0.668	6	1.002	2	0.334	262
CSL 3755	Macadamia	17.8	4.69	17	50.6	107.9	36.2	33.6	72	12.82	2	0.356	17	3.026	5	0.89	4	0.712	0	0	369
CSL 3767	Newton	13	4.67	18	58.9	126.1	37.9	30.1	64	8.32	0	0	25	3.25	10	1.3	1	0.13	0	0	258
CSL 3769	Pascal	11.1	3.96	16	45.4	114.6	39.6	34.6	68	7.548	0	0	23	2.553	5	0.555	1	0.111	0	0	292
CSL 3775	Skipper	15.5	4.21	16	46.2	109.7	37.5	34.2	78	12.09	2	0.31	12	1.86	4	0.62	4	0.62	0	0	333
CSL 3776	Professor	10.7	4.72	18	52.3	110.8	38.6	34.8	59	6.313	1	0.107	25	2.675	4	0.428	11	1.177	0	0	325
CSL 3792	Howell	15.3	4.2	16	43.7	103	37.4	35.9	87	13.31	2	0.306	7	1.071	2	0.306	2	0.306	0	0	358
CSL 3795	Tara	13.9	4.36	16	45.4	104.1	36	34.6	72	10.01	1	0.139	10	1.39	4	0.556	13	1.807	0	0	530
CSL 3812	Mellow	16.1	3.65	15	40.8	111.8	41.6	37.3	85	13.69	3	0.483	5	0.805	7	1.127	0	0	0	0	421
CSL 3815	Esperanza	6.6	4.76	18	48	100.8	38.7	38.3	80	5.28	0	0	11	0.726	3	0.198	1	0.066	0	0	305
CSL 3822	Mahjong	7.6	4.21	17	44.2	105	39.2	37.3	51	3.876	0	0	39	2.964	3	0.228	5	0.38	5	0.33	532
CSL 3835	Salace	9.8	5.2	21	56.7	109	41	37.6	63	6.174	2	0.196	29	2.842	2	0.196	6	0.588	2	0.152	412

Appendix 3B, Blood Chemistries, Pre-release																							
Acc. #	Name	Na	K	Cl	Glu	BUN	Creat	Prot	Alb	Glob	Uri Ac	Ca	Phos	Tot Bili	Dir Bili	GGT	AP	AST	ALT	LDH	Iron	Chol	Trigl
		meq/l	meq/l	meq/l	mg/dl	mg/dl	mg/dl	gm/dl	gm/dl	gm/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	u/l	u/l	u/l	u/l	mcg/dl	mg/dl	mg/dl
CSL 3708	Sammy	144	4.3	101	3	39	1.8	10.9	2.3	8.6	1.4	9.8	8.4	0.3	0.1	209	46	91	72	1497	59	299	44
CSL 3717	Thelma	149	5.1	106	99	38	1.4	8.9	3.1	5.8	1.4	8.3	6.4	0.5	0.1	69	63	25	54	377	70	170	96
CSL 3719	Tick	149	4.9	102	86	33	1	9.3	3.1	6.2	2	9.1	7.4	0.9	0.1	50	42	52	64	852	146	230	140
CSL 3720	Tack	149	5.4	105	60	46	1.1	9.2	3.4	5.8	2.7	10	7.8	0.7	0.2	43	67	31	31	599	125	222	198
CSL 3727	Solo	150	5.6	106	92	33	1.1	9	3.2	5.8	2	9	6.5	1.2	0.1	48	41	156	75	707	60	157	85
CSL 3731	Ju-Ni	162	5.39	115	110	22.6	0.67	9.19	3.16	6.04	na	9.7	5.8	0.43	na	na	42	28	49	na	na	210	na
CSL 3733	Pentobarb	148	5.1	102	104	41	1.3	10.6	3.7	6.9	4.9	10	8.9	1.5	0.2	51	65	31	31	626	119	261	352
CSL 3735	Septimus	147	4.7	104	94	38	1.2	9.1	3.1	6	3.3	9.2	8.7	1.1	0.1	45	68	34	36	592	147	269	246
CSL 3752	Pistachio	143	5.3	97	99	15	1.7	8.8	3.2	5.6	1.2	9.1	6.3	0.7	0.2	51	23	23	23	617	43	196	35
CSL 3754	Pecan	156	5.2	108	82	48	1.2	9.8	3.3	6.5	2.7	10	9.3	0.9	0.1	na	32	73	47	57	584	164	209
CSL 3755	Macadamia	153	4.9	108	89	57	1	8.2	3	5.2	2.3	7.9	6.1	1	0.2	145	54	45	45	783	166	161	158
CSL 3767	Newton	146	4.7	104	55	36	1.1	9.7	2.8	6.9	2.2	8.6	5.4	0.5	0.1	59	41	39	43	692	115	148	118
CSL 3769	Pascal	151	5.3	112	73	46	1	8.9	2.9	6	3.1	9	7.1	1.7	0.2	83	33	38	38	915	124	182	196
CSL 3775	Skipper	147	4.7	111	86	61	1	8.9	2.9	6	2.3	9.2	5.5	1.2	0.2	92	30	33	50	392	115	141	179
CSL 3776	Professor	154	4.8	107	47	50	1	9.5	3.4	6.1	3.3	9	9.1	1.3	0.2	44	82	75	26	2689	138	178	166
CSL 3792	Howell	150	5.5	107	90	39	1.1	9.3	3.1	6.2	2	8.2	6.6	1.5	0.1	48	19	116	41	1198	101	188	77
CSL 3795	Tara	146	4.5	103	113	53	1.1	8.5	3	5.5	1.5	9.5	6.3	0.5	0.2	95	57	58	50	417	124	200	97
CSL 3812	Mellow	155	5.2	108	115	48	1.3	8.9	3.3	5.6	1.2	9.7	6.7	0.3	0.1	68	50	29	47	423	49	183	94
CSL 3815	Esperanza	149	3.7	105	119	15	1.7	8.4	2.4	6	0.6	8.2	4.5	0.4	0.1	43	37	14	16	440	53	148	20
CSL 3822	Mahjong	153	4.3	107	76	19	1.8	9.2	2.7	6.5	1.1	8.7	6.9	0.3	0.1	38	54	50	26	1972	82	123	na
CSL 3835	Salace	156	4.89	114	172	22.9	0.9	11.2	4.14	7.06	na	9.4	8.4	na	na	na	85	na	10	na	na	130	na

APPENDIX 4: Neurologic examination of the California sea lion

Acc. No: **Name:** **Date:** **Time:** **Observer:**

Posture
1. Sitting up on fore flippers
2. Recumbency: ventral, sternal, lateral R L, dorsal;
3. Body twisted to R L
4. Opisthotonus

Responsiveness
To audible or visual approach:
1. Charges person
2. Moves away at speed
3. Moves away hesitantly
4. Attempts to move but can't
5. Lifts head
6. Moves eyes to observe
7. No response
8. Seizuring

***To touching lumbar area*:**
1. Does not allow due to aggressive behavior
2. Moves away rapidly
3. Moves away slowly
4. Lifts head
5. Attempts to move but unable
6. Unresponsive

Locomotion
Ground:
1. Moves rapidly around pen using all 4 flippers
2. Moves reluctantly around pen with all 4 flippers
3. Moves around pen but does not use hind-flippers
4. Ataxic
5. Scoots on ventrum without using fore-flippers
6. Attempts to get up on fore flippers but falls over
7. Unable to use fore and hind-flippers on same side (hemiplegia)
8. Circling
9. Immobile

Pool:
1. Swims normally, leaps over side of pool easily
2. Swims normally, gets out down ramp
3. Floats without using flippers
4. Cannot enter pool