



Movement, dive behavior, and survival of California sea lions (*Zalophus californianus*) posttreatment for domoic acid toxicosis

KATE THOMAS

JAMES T. HARVEY

Moss Landing Marine Laboratories,
Moss Landing, California 95039, U.S.A.
E-mail: kate_thomas@csumb.edu

TRACEY GOLDSTEIN

The Marine Mammal Center,
1065 Fort Cronkhite,
Sausalito, California 94965, U.S.A.

JEROME BARAKOS

Department of Radiology,
California Pacific Medical Center,
San Francisco, California 94115, U.S.A.

FRANCES GULLAND

The Marine Mammal Center,
1065 Fort Cronkhite,
Sausalito, California 94965, U.S.A.

ABSTRACT

Domoic acid (DA) is a neuroexcitatory toxin increasingly causing strandings and mortality of marine mammals. The hippocampus of mammalian brains, associated with learning, memory, and spatial navigation, is one of the predominant regions affected by DA exposure. California sea lions stranding from 2003 to 2006 as a result of DA toxicosis were classified as having acute ($n = 12$) or chronic neurologic ($n = 22$) clinical signs. Chronic neurologic cases were examined by magnetic resonance imaging to determine the extent of brain damage related to DA exposure. Brain damage included hippocampal and parahippocampal atrophy, temporal horn enlargement, and pathological T2 hyperintensity. Posttreatment, animals were fitted with satellite transmitters and their movement and dive behaviors compared with those of a control group. The only significant difference between acute and chronic animals was distance traveled per day. There were, however, significant differences between chronic neurologic cases and controls: chronic neurologic cases dove shallower for shorter durations, traveled further from shore, and spent less time hauled out and more time surface swimming than control animals. There was no relationship between severity of brain damage and behavioral patterns for chronic

neurologic cases. Sea lions with chronic neurologic changes had a poor prognosis for survival following release.

Key words: domoic acid, California sea lions, *Zalophus californianus*, magnetic resonance imaging (MRI), harmful algal blooms, hippocampal atrophy, *Pseudo-nitzschia*.

Domoic acid (DA) is a water-soluble neuroexcitatory toxin produced by the diatom *Pseudo-nitzschia* spp. Structurally, DA is related to kainic acid, an analogue of glutamate, which causes neuronal excitation (Teitelbaum *et al.* 1990, Lefebvre *et al.* 2001, Ananth *et al.* 2003a) and neuropathological changes predominantly affecting the hippocampus and limbic system. Several other regions of the brain including the thalamus, claustrum, secondary olfactory areas, and subfrontal cortex (Ananth *et al.* 2003b) also may be affected by DA exposure.

Since the first documented DA outbreak in 1987 (Teitelbaum *et al.* 1990), DA has been identified as the cause of mass stranding and mortality events of marine mammals and sea birds. In 1991 brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) stranded in Santa Cruz, California (Work *et al.* 1993), and in 1996 Beltran *et al.* (1997) documented 150 pelicans affected by DA in Cabo San Lucas, Mexico. The first documented marine mammal mortality event occurred along the central California coast in 1998 when more than 400 California sea lions (*Zalophus californianus*) died and 70 stranded from Santa Cruz south to San Luis Obispo (Lefebvre *et al.* 1999, Gulland 2000, Scholin *et al.* 2000). In 2000, 184 California sea lions (CSLs) stranded displaying clinical signs of DA toxicosis (Gulland *et al.* 2002) again along the California coast.

Since 1998 the number of CSLs stranding with DA toxicosis has increased (Goldstein *et al.* 2008). Many of the animals, in addition to the seizures described in 1998 and 2000, have chronic neurologic damage characterized by hippocampal atrophy (Silvagni *et al.* 2005, Goldstein *et al.* 2008). The hippocampal region of the mammalian brain is associated with learning and memory processes, spatial navigation, and spatial memories (O'Keefe and Nadel 1978, Aguirre *et al.* 1996, Clayton *et al.* 1999, Sprenger 1999, Ananth *et al.* 2003b, Broadbent *et al.* 2004). Clinical and experimental studies of humans and rodents with hippocampal lesions have documented signs of memory loss, difficulty relearning previously learned tasks, and an inability to form new memories adequately (Clayton *et al.* 1999, Scotville and Milner 2000). CSLs with DA toxicosis, therefore, also may have difficulty performing everyday tasks such as foraging and migrating.

CSLs are a sexually dimorphic otariid that inhabit the California current, and breed on the Channel Islands during late spring and early summer (Reidman 1990). During nonbreeding seasons, adult males disperse north as far as Washington State, whereas most females remain in the Southern California Bight (Reidman 1990, Weise 2006). Lactating females and juveniles tagged on San Nicholas Island and adult males tagged in Monterey, California, traveled less than 65 km from shore in search of prey (Kuhn 2006, Weise 2006, Orr¹). Adult male sea lions, however, traveled 450 km from shore in search of prey during a 2005 warm water anomaly (Weise 2006). Kuhn (2006) reported maximum dive depth for foraging lactating females was 482 m, whereas Weise (2006) reported adult males dove to maximum

¹Unpublished data provided by Tony Orr, School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat Street, Seattle, WA 98105.

depths of 475 m. Juveniles, however, were reported diving to shallower depths (167 m; Orr¹).

Kuhn (2006) also determined lactating females spent 67% of their time at sea, with 42% of that time diving, and 33% of their time ashore (*i.e.*, hauled out). Similarly, Weise (2006) found adult male sea lions spent 49% of their time hauled out and 51% of their time at sea. At sea, 62% of their time was spent swimming at the surface, whereas 37% of time was spent diving (Weise 2006).

The purpose of this study was to determine if CSLs could be successfully rehabilitated following DA exposure, and whether acquired neurological damage influenced movement and dive behaviors. CSLs in rehabilitation suffering from chronic effects of previous DA toxicosis were evaluated neurologically using magnetic resonance imaging (MRI). Satellite telemetry was then used to evaluate their movements and dive behavior postrelease, and parameters were compared with healthy wild animals as well as the severity of brain damage. Results from this work will allow informed decisions regarding humane treatment of CSLs suffering from chronic neurologic effects of DA exposure.

METHODS

Diagnosis and Treatment

From 2003 to 2006, stranded CSLs with clinical signs of DA toxicosis (unresponsiveness, head weaving, scratching, flipper biting, ataxia, and seizures) were treated at The Marine Mammal Center (TMMC) in Sausalito, California. As described by Greig *et al.* (2005), sex and age classes were determined by genital morphology, body length, weight, and stage of sagittal crest development. On admission, animals suspected of suffering from DA toxicosis were classified as acute or chronic neurologic cases as defined by Goldstein *et al.* (2008). Animals classified as acute cases were defined as those that stranded in the vicinity of a toxic bloom of *Pseudo-nitzschia* spp. and with a minimum of five other animals admitted to TMMC with neurological signs within a 48-h period (Goldstein *et al.* 2008). Animals were considered chronic neurologic cases if one of the following criteria was fulfilled: (1) the animal stranded with neurological signs when no known *Pseudo-nitzschia* spp. blooms were occurring, (2) the animal was previously diagnosed with acute DA toxicity and restranded exhibiting neurological signs, or (3) the animal exhibited intermittent seizures at least 2 weeks apart or 2 weeks after admission to the TMMC.

Affected CSLs with signs of DA toxicosis were given subcutaneous fluids until they could eat solid foods and then were fed thawed herring (*Clupea pallasii*). Treatment for DA symptoms included controlling seizures with diazepam, lorazepam, or phenobarbitone and use of dexamethasone to reduce cerebral edema (Gulland 2000). Animals classified as having chronic neurologic signs received radiographs to rule out trauma or gunshot, cerebrospinal fluid analysis to rule out meningitis, and serology to rule out toxoplasmosis as causes of neurological signs. MRI was then used to assess brain morphology.

Magnetic Resonance Imaging Interpretation

CSLs with chronic neurological impairment were sedated in preparation for MRI using a combination of medetomidine (0.07 mg/kg) and tiletamine-zolazepam (1 mg/kg; Haulena and Gulland 2001). Imaging procedures followed were described by Goldstein *et al.* (2008). Images were interpreted by a neuroradiologist experienced in epilepsy imaging without knowledge of the clinical or pathology data. To determine

the effect of intraobserver variation on the imaging interpretations, all images were reviewed again by the same neuroradiologist 3 months later and interpreted in a blinded fashion. Imaging allowed for a visual assessment of parenchymal brain damage, including atrophy of the hippocampus and parahippocampus, enlargement of temporal horns, and detection of pathological T2 hyperintensity. Hippocampal atrophy, parahippocampal atrophy, and temporal horn enlargement were classified as unilateral or bilateral damage with severity classified as mild, moderate, or severe. Bilateral damage was classified as symmetrical (severity of damage in both hemispheres considered equal) or asymmetrical (severity of damage in the hemispheres differed) damage. Temporal horn enlargement was also used as confirmation of hippocampal atrophy. Pathological T2 hyperintensity was a reflection of gliosis or edema in the affected tissue.

A brain damage index (BDI) quantifying severity of brain damage was derived for statistical purposes. Brain damage was weighted as follows: mild unilateral lesions were rated as mild, 1; moderate, 2; or severe, 3. Bilateral damage was a simple addition of the right- and left-sided severity; that is, damage classified as asymmetrical bilateral damage, left moderate/right severe, would have a BDI of 5. Hippocampal atrophy had a multiplier of 5; temporal horn enlargement, a multiplier of 1; and parahippocampal atrophy, a multiplier of 7. If the T2 hyperintensity signal was present there was an addition of 10 and if absent 0. A larger BDI number, therefore, was indicative of greater overall brain damage. For example, CSL 6024 was diagnosed with asymmetrical bilateral (left mild/right moderate) hippocampal atrophy $[(1 + 2) \times 5 = 15]$, with severe right-sided temporal horn enlargement $(3 \times 1 = 3)$, unilateral right-sided severe parahippocampal atrophy $(3 \times 7 = 21)$, and positive for T2 hyperintensity signal (+10); therefore, the BDI for CSL 6024 was 49.

Satellite Telemetry

CSLs recovering from acute neurologic cases were fitted and released with a satellite depth recording tag (SDR-T16; $n = 3$) or a smart position or temperature transmitting tag (SPOT; $n = 9$) manufactured by Wildlife Computers, Redmond, WA. Data from the SDR-T16 included ARGOS positions, summaries of dive depth and duration in 6-h bins, and histograms of time at depth. SPOT tags recorded ARGOS position and temperature (-40°C to 60°C ; $\pm 0.2^{\circ}\text{C}$). Animals classified as chronic neurologic cases were fitted with either an SDR-T16 ($n = 1$), a SPOT ($n = 3$) tag, or a Series 7000 Satellite Relayed Data Logger (SRDL; $n = 18$) manufactured by Sea Mammal Research Unit at St. Andrews University in Scotland. Data collected by the SRDL included location, depth, water temperature, swim speed, time, summary data (haul-out, surface swimming, and dive), and diagnostic information. Transmitters were adhered to the dorsal pelage of the animals with Devcon 5-min epoxy or Loctite 422 pressure adhesive. To ensure proper placement of the tag, physical restraint and sedation with midazolam (0.1 mL/kg) and/or isoflurane by mask were used to minimize movement while the glue cured. Data were collected until molting occurred, the animal restranded, at which time the tag was removed, or the tag stopped transmitting prematurely.

Animal activities were organized into three categories: dive, surface swimming, and haul-out. A haul-out began when the tag was dry for a minimum of 10 min and ended when the tag had been continuously wet for 40 s (McConnell *et al.* 1992). Surface swimming was defined as time spent at less than 4 m depth for more than 9 min, and dives were categorized as animals exceeding 4 m depth (McConnell *et al.* 1992).

Positions collected at sea were filtered using ARGOS location quality, swim speed (maximum 3 m/s), and time (McConnell *et al.* 1992). Some points, however, had inland locations indicating those points had low positional accuracy that could potentially be greater than 1 km; therefore, a polygon mask was applied in ArcMap 9.0 to eliminate all data points that fell inland, ensuring distance calculations were not overestimated. Distances between points were calculated as a straight-line distance using the Hawth's tool extension in ArcMap 9.0 (Beyer 2004); distances were summed, and distance traveled per day (maximum, mean \pm SD) was calculated. Maximum distances the animals traveled from shore were calculated in ArcMap 9.0 as the shortest straight-line distance from shore to the furthest offshore position. The mean \pm SD and maximum dive depth and duration also were calculated.

An ANCOVA was performed with condition (control, acute neurologic, or chronic neurologic) as the factor, covariate was mass of the animal at the time of release, and dependent variables were maximum and mean dive depth and duration, maximum distance traveled per day, maximum distance the animals traveled from shore, and percentage of time the animals spent diving, surface swimming, and hauled out. If the interaction term for the ANCOVA was significant, an ANOVA was performed using the same factor and dependent variables. A significance value of $P \leq 0.05$ was used for all statistical tests. Assumptions were tested using Levene's and F tests to ensure equal variances and Kolmogorov-Smirnov test was performed to ensure normality. If assumptions were not met data were log transformed. The Tukey *post hoc* test was used to determine which treatment was statistically different from the control. Control data consisted of adult males (Weise 2006), adult females (Kuhn 2006), and juveniles (Orr¹) that were CSLs caught in the wild, displaying no abnormal neurological signs at the time of tagging. All control animals were tagged during 2003–2006 using either SRDL, SPOT, or SDR-T16 tags.

Magnetic Resonance Imaging/Behavioral Data Analysis

For statistical purposes the BDI was divided into three categories: low (0–19), medium (20–39), and high (≥ 40). An ANOVA was performed with the BDI categories (mild, moderate, severe) as the independent variables and the dependent variables were maximum and mean dive depth and duration, maximum and mean distance traveled per day, maximum distance from shore, percentage time spent diving, surface swimming, and hauled out. The ANOVA was considered significant at $P \leq 0.05$. Levene's and Kolmogorov-Smirnov tests were used to determine whether the data for each variable had equal variances and were distributed normally. Nonnormal data were log-transformed before analyses. Mortality rate between the three BDI groups (mild, moderate, and severe) was tested with a chi-squared, with a critical $\chi^2 = 7.815$.

RESULTS

Diagnosis and Treatment

Of the animals included in the study, 12 were acute cases that stranded from 2004 to 2005 along the California coast. Eight of the acute cases were males (4 subadults and 4 adults) and 4 were adult females (Table 1). Twenty-two chronic neurologic cases stranded along the California coast from 2003 to 2006. Fourteen of the chronic neurologic cases were female (3 yearlings, 4 subadults, and 7 adults) and 8 were

Table 1. Animals affected by domoic acid toxicosis.

CSL #	Age class	Sex	Diagnosis	Release date	# Days/tag type	Survival	BDI
6039	Subadult	M	A	7/8/04	10/SP	Unknown	N/A
6053	Adult	M	A	9/16/04	11/SP	Unknown	N/A
6116	Subadult	M	A	10/13/04	47/SP	Unknown	N/A
6518	Adult	F	A	6/7/05	50/SP	Unknown	N/A
6521	Adult	M	A	6/7/05	5/SP	Unknown	N/A
6584	Adult	F	A	7/24/05	19/SP	Died postrelease	N/A
6608	Adult	F	A	7/24/05	25/SP	Unknown	N/A
6668	Adult	F	A	8/17/05	16/SP	Unknown	N/A
6674	Subadult	M	A	8/17/05	24/SP	Unknown	N/A
6677	Adult	M	A	8/17/05	33/SP	Unknown	N/A
6706	Adult	M	A	8/17/05	35/SP	Euthanized	N/A
6720	Subadult	M	A	8/25/05	33/SP	Unknown	N/A
5468	Yearling	M	CN	5/15/03	43/SM	Euthanized	16
5531*	Adult	F	CN	7/1/03	21/SM	N/A	28
			CN	9/26/03	21/SM	Euthanized	–
5810	Subadult	F	CN	9/19/03	32/SM	Died postrelease	38
5985	Subadult	F	CN	11/11/03	106/SM	Unknown	40
6012	Adult	F	CN	3/10/04	35/SM	Unknown	29
6018	Adult	F	CN	6/29/04	26/SM	Unknown	34
6024	Adult	F	CN	5/20/04	26/SM	Unknown	49
6433*	Yearling	F	CN	1/13/05	129/SM	N/A	33
			CN	6/1/06	61/SP	Unknown	–
6510	Juvenile	M	CN	6/7/05	38/SM	Euthanized	42
6640	Adult	M	CN	5/31/06	7/SM	Euthanized	13
6667	Yearling	M	CN	9/19/05	30/SP	Unknown	24
6673	Juvenile	M	CN	9/30/05	33/SM	Unknown	58
6710	Yearling	F	CN	9/26/05	<1/SP	Unknown	17
6731	Yearling	F	CN	10/28/05	25/SP	Euthanized	6
6740	Adult	F	CN	9/26/05	8/SD	Unknown	64
6887	Subadult	M	CN	7/5/06	8/SM	Euthanized	25
6902	Subadult	F	CN	8/1/06	<1/SM	Unknown	42
6904	Juvenile	M	CN	7/19/06	24/SM	Euthanized	29
7007	Subadult	F	CN	10/11/06	70/SM	Unknown	34
7028	Adult	F	CN	9/25/06	20/SM	Unknown	66
7077	Adult	M	CN	11/7/06	20/SM	Died postrelease	6
7096	Adult	F	CN	12/1/06	24/SM	Unknown	37

CSL # is the California sea lion identification number and * means the animal restranded and was rereleased with a tag. Age is the age class of the animal determined on admittance to TMMC; sex of the animal—M = male and F = female; diagnosis of the animal is either acute (A) or chronic neurologic (CN); release date is the date the animals were tagged and released from TMMC; # days is the total number of days the tag transmitted; tag types are the types of tag used, where SP = SPOT tag, SM = SMRU tag, and SD = SDR-T16 tag; and survival is the animals known survival at the conclusion of the project. Survival is unknown if that animal was not resighted dead or alive; euthanasia decisions were made by veterinarians at TMMC and presumed died postrelease means we have reason to believe the animal died; and BDI is the brain damage index number calculated on the basis of Magnetic Resonance Imaging interpretation.

males ranging in age class from yearling ($n = 2$) to juvenile ($n = 3$), subadults ($n = 1$), and adults ($n = 2$; Table 1).

Magnetic Resonance Imaging Interpretation

Brain damage induced by DA, for chronic neurologic cases diagnosed via MRI, was characterized by hippocampal and parahippocampal atrophy, temporal horn enlargement, and associated parenchymal T2 hyperintensity signal. All chronic neurologic cases ($n = 22$) were diagnosed with hippocampal atrophy: 55% of the animals had unilateral damage and 45% bilateral damage (50% symmetrical and 50% asymmetrical). Severity of hippocampal atrophy, including unilateral and bilateral (symmetrical and asymmetrical) damage, varied from mild ($n = 8$), through moderate ($n = 14$), to severe ($n = 10$). Unilateral temporal horn enlargement occurred in 59% (8 mild, 1 moderate, and 4 severe) of animals and bilaterally in 41% (10 mild, 5 moderate, and 3 severe) of the animals. Sixteen sea lions had detectable parahippocampal atrophy: 75% with unilateral damage and 25% with bilateral damage (2 animals had asymmetrical bilateral damage). Including unilateral, symmetrical, and asymmetrical bilateral damage, 11 animals were classified with mild parahippocampal atrophy, 7 with moderate, and 2 with severe. Fifty-nine percent of CSLs also exhibited T2 hyperintensity signal.

The numbers for the BDI ranged from 6 to 66. The numbers were arranged into categories of mild ($n = 5$), moderate ($n = 10$), and severe ($n = 7$) for statistical purposes (Table 1).

Satellite Telemetry

Acutely classified animals were fitted with SDR-T16 or SPOT tags, which transmitted for up to 50 days (Table 1). Dive parameters for those animals were not calculated because so few tags ($n = 3$) collected those data. The mean distance traveled per day for all animals was 28.8 ± 23.4 km (Table 2). The minimum distance an animal traveled per day was 8.1 km (CSL 6521), whereas CSL 6116 traveled the maximum distance per day of 241.0 km (Table 2). Eight of the 12 acute animals traveled less than 100 km from shore. The remaining 4 acute animals traveled greater than 148 km from shore, with CSL 6720 traveling 953.7 km during the course of transmission.

Survival for 10 of the 12 acute cases was unknown. CSL 6608 was, however, seen in Ensenada, Mexico, lethargic and unresponsive on May 2, 2006, 9 months after she stopped transmitting.² CSL 6584 was presumed to have died postrelease due to a head injury and massive shark wound on her back, which was documented photographically by TMMC volunteers. CSL 6706 reestranded 4 days after release, lethargic and seizing, and was euthanized (Table 1).

All chronic neurologic cases were fitted with an SRDL, SDR-T16, or SPOT tag, which transmitted up to 129 days (Table 1). Chronic neurologic cases had a mean dive depth of 31.0 ± 20.8 m (Table 3), females 31.6 ± 20.0 m and males 29.9 ± 23.8 m. Maximum dive depth for females was 345 m and for males it was 289 m. The mean dive duration for all chronic neurologic cases was 1.0 ± 0.6 min (Table 3);

²Personal communication from Hector Benet-Contreras, Inspector Federal, Profepa., Lopez Mateos 1306-16b, Zona Centro. Ensenada, B.C. Mexico c.p. 22800, May 2006.

Table 2. Movement parameters calculated from the satellite tags.

Condition (n)	Mean distance traveled per day in km \pm SD (range)	Max distance traveled per day in km \pm SD (range)	Max distance from shore in km \pm SD (range)
Acute (12)	28.8 \pm 23.4 (5.1–75.2)	85.4 \pm 75.7 (8.1–241.0)	163.2 \pm 266.7 (2.9–953.7)
Chronic neurologic (22)	39.0 \pm 20.6 (11.8–97.6)	130.3 \pm 67.9 (34.2–251.1)	186.0 \pm 397.8 (7.5–1,862.2)
Control (67)	30.7 \pm 20.5 (7.2–79.9)	115.9 \pm 77.9 (22.7–284.0)	34.7 \pm 11.8 (11.6–64.3)

Condition is the animal's illness classification; mean distance traveled per day is the mean of all animals in km \pm SD (range); max distance traveled per day is the mean of all animals in km \pm SD (range); and max distance traveled from shore is the mean of all animals in km \pm SD (range). Max = maximum.

Table 3. Dive parameters calculated from the satellite tags.

Condition (<i>n</i>)	Mean dive depth in m \pm SD (range)	Mean/max dive depth in m \pm SD (range)	Mean dive duration in min \pm SD (range)	Max dive duration in min (range)	Haul-out% \pm SD (range)	Surface swimming% \pm SD (range)	Dive% \pm SD (range)
Chronic neurologic (22)	31.0 \pm 20.8 (8.5–81.6)	199.3 \pm 88.4 (20.5–345.0)	1.0 \pm 0.6 (0.3–2.8)	4.9 \pm 1.7 (1.7–9.0)	32.8 \pm 10.9 (4.1–50.6)	47.1 \pm 11.1 (30.9–71.7)	20.1 \pm 7.0 (10.7–32.9)
Control (67)	41.0 \pm 32.6 (8.0–171.3)	285.2 \pm 127.4 (41.0–481.5)	1.4 \pm 0.8 (0.2–3.8)	7.6 \pm 2.1 (3.5–14.7)	38.8 \pm 15.6 (1.1–82.3)	39.0 \pm 15.3 (5.8–89.3)	22.3 \pm 8.1 (2.5–35.5)

Condition is the animal's illness classification; mean dive depth is the mean of all animals within the classification group in m \pm SD (range); maximum dive depth is the mean of all animals in m \pm SD (min/max); mean dive duration is the mean of all animals in min \pm SD (range); max dive duration is the mean of all animals in min \pm SD (range); haul-out is the mean percentage of all animals% \pm SD (range); surface swimming is the mean percentage of all animals% \pm SD (range); and dive is the mean percentage of all animals% \pm SD (range). Max = maximum.

CSL 7077 had the greatest dive duration for males (9.0 min) and CSL 5531 had the greatest dive duration for females (7.1 min).

The mean distance chronic neurologic animals traveled per day was 39.0 ± 20.6 km with a maximum distance per day of 251.1 km by CSL 5985 (Table 2). The maximum distance any chronic neurologic animal traveled from shore was 1,862 km, which was half the distance from California to Hawaii before transmission terminated (Fig. 1, Table 2). The next furthest distance from shore was 953 km (CSL 6720), whereas CSL 6521 and 6706 traveled a maximum of 2.9 km from shore (Table 2). Abnormal northern and easterly movements were also documented, although not statistically tested. CSL 7007, a female, released in Sausalito, California, in early October 2006 stopped transmitting on December 24, 2006, in Washington State (Fig. 2), and CSL 5985 swam 4 km up a river where she spent 10 days and was documented as continuously swimming in circles.

Behavioral summaries were calculated by SRDLs, and consisted of percentage of time hauled out, surface swimming, and diving. The mean percentage of time spent hauled out was $32.8\% \pm 10.9\%$, surface swimming was $47.1\% \pm 11.1\%$, and diving was $20.1\% \pm 7.0\%$.

Survival of 15 of the 22 chronic neurologic cases was unknown although two animals (CSL 5810 and 7077) probably died in the wild (Table 1). CSL 5810 traveled halfway to Hawaii from California, where waters are warm with low productivity and prey sources were scarce. It is likely the month-long transit and shallow diving (≤ 40 m) resulted in starvation. CSL 7077 likely died on shore while the tag was still attached, as the last 7 days of transmission occurred from shore, with no change in location. The body of CSL 7077 could not be recovered due to the inaccessibility of the location of the last transmission; however, after large seas washed through the area the tag stopped transmitting, suggesting the animal (with tag) washed out to sea and sank. Seven sea lions reestranded, seizing, lethargic, emaciated, or with shark wounds and were euthanized due to poor prognosis for survival in the wild (Table 1).

Because dive information and behavioral summaries were not collected for animals with acute signs, the only variables included in the statistical test for this group were distance variables. All the variables were log-transformed to meet the assumptions of normality or equal variances before the ANCOVA was performed. Mean dive depths ($P = 0.051$) of CSLs termed chronic neurologic cases (29.3 ± 17.1 m) were less than those of the control groups (41.0 ± 32.6 m). Mean ($P < 0.001$) and maximum dive durations ($P < 0.001$) were significantly less for chronic neurologic cases (9.0 min) than for control animals (14.7 min). Chronic neurologic cases spent less time hauled out ($P < 0.001$) and less time diving ($P < 0.001$) than control animals. ANOVAs were conducted on four variables (maximum dive depth, maximum distance traveled per day, maximum distance from shore, and percentage of time surface swimming) that had significant interaction terms for the ANCOVA and all were log-transformed to meet assumptions. Maximum dive depth ($P = 0.044$) for control animals was greater (285.2 m) than for chronic neurologic animals (202.8 m). There was no significant difference for distance traveled per day between control and chronic neurologic animals; however, there was a significant difference between acute animals and the other two groups ($P = 0.002$). Both acutely and chronically affected animals swam further from shore ($P = 0.008$) than control animals. Control animals also spent less time surface swimming than chronic neurologic animals ($P = 0.061$). There was no significant difference for all movement variables between control and acute ($P = 0.130$) animals except for the distance traveled per day mentioned above.

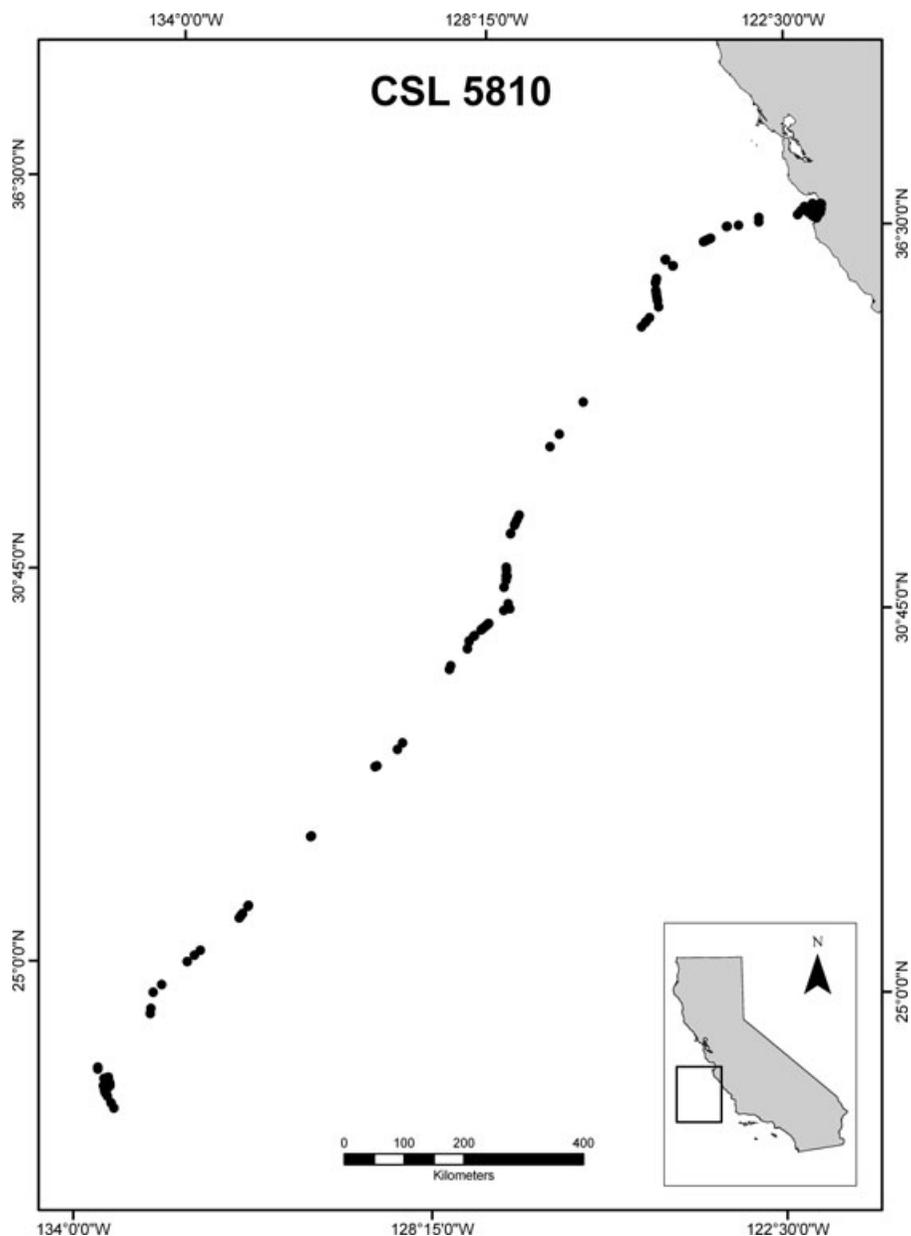


Figure 1. Location data (black dots) for CSL 5810 showing movement of the animal traveling halfway between California and Hawaii, abnormal for a nearshore species.

Magnetic Resonance Imaging/Behavioral Data Analysis

Categories for the BDI included mild ($n = 5$), moderate ($n = 10$), and severe ($n = 7$; Table 2). The BDI was compared with the satellite behavioral data using an

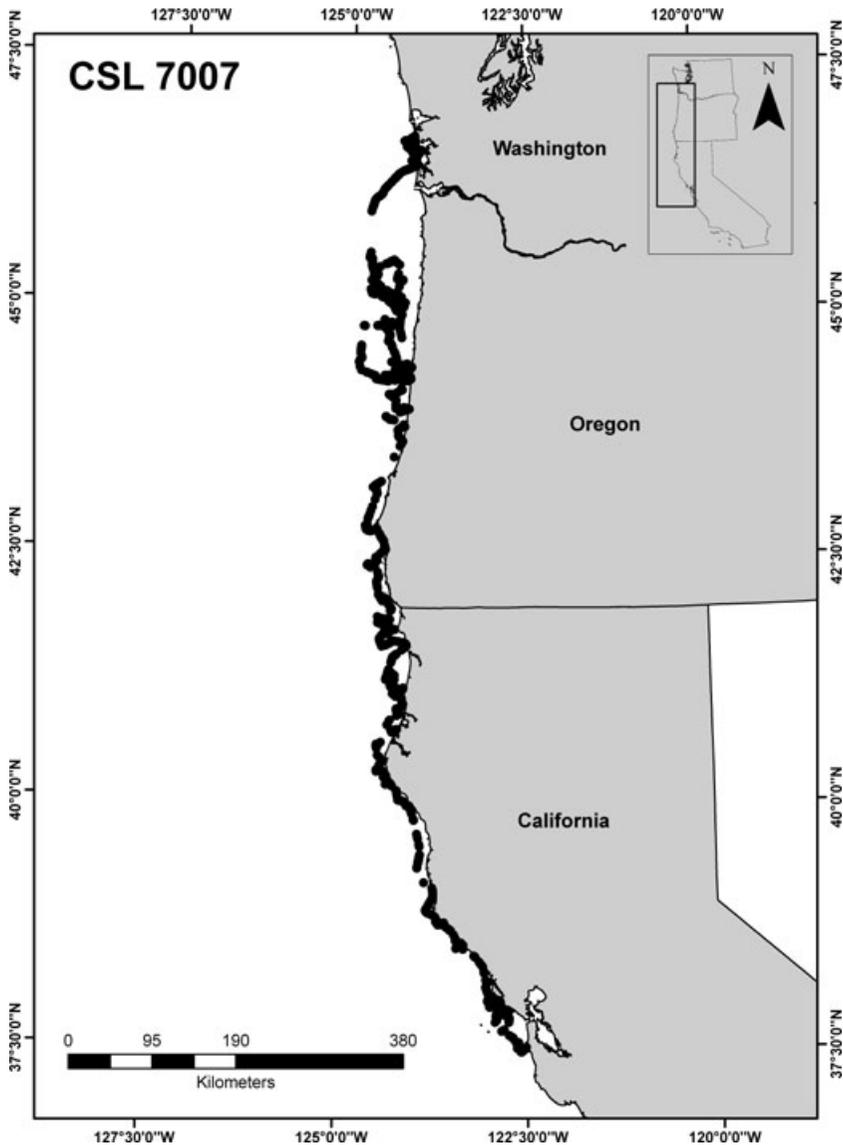


Figure 2. Location date (black dots) for CSL 7007 showing movement from California north to Washington. The transit is considered abnormal because this female stopped transmitting in Washington on December 24, 2006, and females are not found in Washington during the winter months.

ANOVA. Two of the nine variables tested did not meet the assumptions of normality and equal variances and were log-transformed (maximum distance traveled per day and maximum distance traveled from shore). No significant differences were found when comparing BDI categories with behavioral variables ($P > 0.206$). Mortality

rate for the BDI categories (mild 80%, moderate 20%, and severe 14%) was not significantly different ($\chi^2 = 2.0$, $df = 2$, $P = 0.368$).

DISCUSSION

All of the sea lions defined as chronic neurologic cases had some degree of hippocampal atrophy. Such brain damage limited the sea lions' ability to navigate and dive compared with controls. Sixteen of the 22 animals suffering from chronic neurologic effects also were diagnosed with parahippocampal atrophy of varying degrees of severity, 3 of which showed severe navigational impairments. CSL 5985 had moderate right-sided parahippocampal atrophy and spent 10 days 5 km up the Salinas River after her release. CSL 7007 was the female that abnormally transited to Washington (Fig. 2) in winter, likely as a result of her bilateral mild parahippocampal atrophy. The third case was the previously described animal (CSL 5810) that transited halfway to Hawaii from California (Fig. 1) after being diagnosed with bilateral mild parahippocampal atrophy. Two examples of animals not diagnosed with parahippocampal atrophy (CSL 6904 and 5468) stayed near or within Monterey Bay, with no apparent abnormal movements during the time of transmission. CSL 6904 and 5468, however, reestranded with seizures and other impairments too severe for treatment and were euthanized.

In humans, the hippocampus is important for factual learning and memory (Sprenger 1999). For example, two human patients that underwent a therapeutic bilateral medial temporal lobe resection, causing severe bilateral damage to the hippocampus, developed severe retrograde amnesia (Scoville and Milner 2000). The hippocampus also plays an important role in spatial navigation and topographic learning. Studies of rodents by O'Keefe and Dostrovsky (1971) and O'Keefe and Nadel (1978) indicated the hippocampus maintains a cognitive map that consists of place cells, indicating the current position of the animal in space. Rats with hippocampal lesions were unable to perform topographic learning tasks as predicted by the cognitive map theory (Morris *et al.* 1982). Broadbent *et al.* (2004) found rats with bilateral hippocampal lesions encompassing 30%–50% of the total hippocampal volume exhibited impaired spatial memory. For recognition memory to be impaired, however, bilateral lesions in the hippocampus had to be larger, encompassing 75%–100% of the total volume. These results not only indicated that the hippocampus was important for spatial and recognition memory but that spatial memory requires more hippocampal tissue. Conversely, DeRenzi (1982) showed that unilateral hippocampal lesions in humans did not impair topographical learning, suggesting the function of the hippocampus between rodents and primates may differ significantly. Aguirre *et al.* (1998) did find, however, that cells in the parahippocampus were activated when humans performed maze learning and recovery test. There was no simultaneous activation of cells within the hippocampus indicating the parahippocampus was the key structure for topographical learning in humans.

Behaviorally, CSLs suffering from chronic neurologic effects and morphological changes in the brain acted significantly different than control CSLs. Control animals dove deeper and for greater durations than chronic neurologic animals. Due to the shorter shallower dives chronic neurologic cases may not be as successful foragers as the control animals. This was supported by the fact when chronic neurologic animals reestranded many were underweight or emaciated. Chronic neurologic animals traveled further from shore than control animals. There have been two documented

cases, however, of adult male CSLs traveling to a cold water front 450 km from shore during a warm water anomaly in 2005 (Weise 2006). Although the analysis of ocean conditions was not a part of the current study, none of the chronically neurologic animals traveled greater than 350 km from shore during 2005, with only three animals traveling greater than 130 km from shore during the same year. Thus, it is not likely the offshore movement of chronic neurologic animals in 2005 was due to their searching for the cold water front, but instead due to DA-associated brain lesions that affected their navigational abilities. There were no significant differences in the movement patterns of acute and control animals, indicating animals with chronic neurologic problems had greater brain damage, thus more effects than control or acute cases.

Although there were no statistical significant associations between the severities of brain damage with the behaviors of chronic neurologic cases, it is possible that the BDI was too crude a measure of overall neurologic damage. MRI has a low sensitivity for detecting neuronal loss, and by the time a mild hippocampal atrophy could be visualized on the image, significant damage may have already occurred in the brain, therefore, affecting the animals behaviorally. This is supported by findings by Silvagni *et al.* (2005) that documented histological changes in sea lions following acute DA exposure were not detected by MRI. It is also possible the behaviors tested in the current project were too coarse and unable to detect some memory or behavioral abnormalities caused by increased brain damage. In the future, detailed cognitive function tests should be performed on cases suffering from chronic neurologic effects from DA exposure to determine whether animals have memory and spatial navigation problems.

CSLs classified as having acute neurological symptoms did not receive MRI; therefore, we do not know the degree of brain damage in these animals. Silvagni *et al.* (2005) and Goldstein *et al.* (2008), however, documented hippocampal necrosis and atrophy on postmortem examinations of sea lions that died during a toxic bloom. It is possible the reason we did not determine a significant difference when comparing behaviors between control and acute animals was due to the small sample size, length of transmission while attached to the animal, or the type of data acquired for acute animals. The longest transmission time for acute animals was 50 days, with the majority of transmissions lasting less than 25 days. Unfortunately, many of these animals stranded and were released 4–8 weeks before molting, so this likely decreased the transmission time substantially. The tags used for acute animals were only location tags; therefore, we did not have dive or behavioral summary (dive, haul-out, or surface swimming) data, thus the only behaviors tested were mean and maximum distance traveled per day and maximum distance from shore.

Several behaviors displayed by animals affected by DA were not quantifiable. For example, CSL 7096 displayed varying postrelease behaviors. At one point she was extremely aggressive, challenging surfers at a surfing competition as they entered the water; the next night she was lethargic when interacting with people at a hot springs near the same beach. Other animals that restranded apparently disorientated were found sitting on a police car on the San Francisco waterfront (CSL 6887) and 3 km inland, in the city of San Francisco (CSL 5531).

In summary, postrelease behaviors for CSLs diagnosed as acutely intoxicated with DA were not significantly different from control animals. Behaviors of all chronic neurologic animals, however, were significantly different from control animals for maximum dive depths, maximum and mean dive durations, maximum distance traveled from shore, and percentage of time hauled out and diving. There were no

significant associations between behaviors and severity of brain damage assessed by MRI. In this study the mortality for CSLs with DA toxicosis was 32% (acute = 16% and chronic neurologic = 40%). Acute animals were disoriented, lethargic, and ataxic, but seemed to recover within a few days of treatment at TMMC and were considered releasable. In contrast, chronic neurologic individuals sometimes spent months at TMMC and once released continued to have seizures and act abnormally (*e.g.*, less diving, lesser depths, and shorter durations resulting probably in less foraging). Many probably had a prolonged death while at sea.

CSLs classified as chronic neurologic cases did not dive or migrate normally, can be a nuisance and a possible danger to humans and themselves, and have lesser probability of survival, indicating that these animals are not good candidates for rehabilitation. These findings highlight the need to explore alternative options for handling the increasing number of chronically affected animals. Development of specific cognitive function tests for sea lions is needed to more accurately assess the effects of hippocampal and parahippocampal atrophy on this species.

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