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Movement, dive behavior, and survival of California sea lions (Zalophus californianus) post-treatment for domoic acid toxicosis

Kate Thomas
California State University, Monterey Bay

MOVEMENT, DIVE BEHAVIOR, AND SURVIVAL OF CALIFORNIA SEA LIONS *(ZALOPHUS CALIFORNIANUS)* POST-TREATMENT FOR DOMOIC ACID TOXICOSIS

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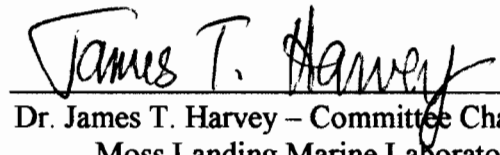
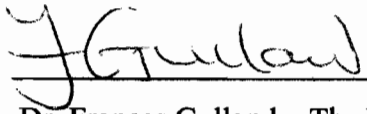


Dr. James T. Harvey – Committee Chair, Professor
Moss Landing Marine Laboratories



Dr. Frances Gulland – The Marine Mammal Center

Dr. Rikk G. Kvitek – Professor, CSU Monterey Bay

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. Post-treatment, animals were fitted with satellite transmitters and their movement and dive behaviors compared with those of a control group. There were no significant differences in behavior between acute cases and controls. There were, however, significant differences between chronic neurologic cases and controls: chronic neurologic cases dove shallower for shorter durations, traveled greater distances per day and 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.

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Introduction

Domoic acid (DA) is a water-soluble, neuroexcitatory toxin produced by the diatom *Pseudo-nitzschia* spp.. Structurally, domoic acid is related to kainic acid, an analogue of glutamate, which causes neuronal excitation (Teitelbaum *et al.* 1990, Lefebvre 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. Teitelbaum *et al.* (1990) conducted neuropathological studies on four human individuals who died seven days to four months following consumption of the toxic cultivated mussels grown on Prince Edward Island in 1987. These individuals had neuronal necrosis or loss and astrocytosis, mostly in the hippocampus and amygdaloid nucleus.

Since the 1987 outbreak in Canada, 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 1999, Gulland 2000,

Scholin *et al.* 2000). In 2000, 184 California sea lions (CSLs) stranded displaying clinical signs of domoic acid toxicosis (Gulland *et al.* 2002) again along the California coast.

Since 1998, the number of California sea lions 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.* 1999, 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 re-learning previously learned tasks, and an inability to form new memories adequately (Clayton *et al.* 1999, Scotville and Milner 2000). California sea lions with domoic acid toxicosis, therefore, also may have difficulty performing everyday tasks such as foraging and migrating.

California sea lions 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 non-breeding seasons, adult males disperse north as far as Washington State, whereas most females remain in the Southern California Bight (Weise 2006, Reidman 1990). Lactating females and juveniles tagged on San Nicholas Island and adult males tagged in Monterey, CA traveled less than 65 km from shore in search of prey (Kuhn 2006, Weise 2006, Orr Unpublished data). 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 depths of 475 m. Juveniles, however, were reported diving to shallower depths (167 m; Orr unpublished data).

Kuhn (2006) also determined lactating females spend 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 California sea lions could be successfully rehabilitated following DA exposure, and whether acquired neurological damage influenced movement and dive behaviors. California sea lions in rehabilitation suffering from chronic effects of previous DA toxicosis were evaluated neurologically using MR imaging. Satellite telemetry was then used to evaluate their movements and dive behavior post-release and parameters 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 California sea lions suffering from chronic neurologic effects of DA exposure.

Methods

Diagnosis and Treatment:

From 2003 to 2006, stranded California sea lions with clinical signs of DA toxicosis (unresponsiveness, head weaving, scratching, flipper biting, ataxia, and/or seizures; Figs. D1 and D2) were treated at The Marine Mammal Center (TMMC) in Sausalito, California. As described in Greig *et al.* (2005), sex and age classes were determined by genital morphology, body length, weight, and stage of sagittal crest development. Upon 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 5 other animals admitted to TMMC with neurological signs within a 48-hr 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 re-stranded exhibiting neurological signs, or 3) the animal exhibited intermittent seizures at least 2 weeks apart and/or two weeks after admission to the TMMC.

Affected California sea lions 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 domoic acid symptoms included controlling seizures with diazepam, lorazepam, and/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. Magnetic resonance (MR) imaging was then used to assess brain morphology.

Magnetic Resonance Imaging Interpretation:

California sea lions with chronic neurological impairment were sedated in preparation for MR imaging using a combination of medetomidine (0.07 mg/kg) and tiletamine-zolazeopam (1 mg/kg; Haulena and Gulland 2001). Imaging procedures followed were described in 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 three 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 also was used as confirmation of hippocampal atrophy. Pathological T2 hyperintensity was a reflection of gliosis and/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 valued at 1, moderate 2, and severe 3. Bilateral damage was a simple addition of the right and left sided severity, i.e. damage classified as asymmetrical bilateral damage, left moderate/right severe, would have a BDI of five. 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)*5 = 15), with severe right sided temporal horn enlargement (3*1 = 3), unilateral right sided severe parahippocampal atrophy (3*7 = 21), and positive for T2 hyperintensity signal (+10), therefore, the BDI for CSL 6024 was 49.

Satellite Telemetry

California sea lions recovering from acute toxicosis 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 in Washington, USA. Data from the SDR-T16 included ARGOS positions, summaries of dive depth and duration in 6 hours bins, and histograms of time at depth. SPOT tags recorded ARGOS position and temperature (-40ºC to 60ºC; ±0.2ºC). Animals classified as chronic neurologic cases were fitted with either a 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 (SMRU) 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 minute 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 re-stranded at which time the tag was removed, or the tag/battery 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 seconds (SMRU). 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 (SMRU).

Positions collected at sea were filtered using ARGOS location quality, swim speed (maximum 3m/s), and time. 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 which fell inland, ensuring distance calculations were not overestimated. Distances between points were calculated using the Hawth's Tool extension in ArcMap 9.0 (Beyer 2004); distances were summed, and distance traveled per day (mean, max, ±SD) 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. Dive depth and duration (mean, max, ±SD) also were calculated.

An ANCOVA was performed with condition (control, acute, 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 $P = 0.05$ 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 meet 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 (Orr unpublished data) which were California sea lions caught in the wild displaying no abnormal neurological signs.

Magnetic Resonance Imaging/Behavioral Data Analysis:

For statistical purposes the brain damage index was divided into three categories: low (0-19), medium (20-39), and high (≥40). BDI categories were analyzed by ANOVA using the following behavioral variables: 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 a $P \leq 0.05$. Levene's test was used to ensure equal variances and Kolmogorov-Smirnov test performed to ensure normality. Non-normal data were log transformed before analyses.

Results

Diagnosis and Treatment:

Of the animals included in the study, twelve were acute cases that stranded from 2004 to 2005 along the California coast. Eight of the acute cases were males (sub-adults = 4, adults = 4) and four 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 (yearling = 3, sub-adult = 4, adult = 7) and 8 were males ranging in age class from yearling (n = 2) to juvenile (n = 3), sub-adults (n = 1), and adults (n = 2; Table 1).

Magnetic Resonance Imaging Interpretation:

Brain damage induced by domoic acid, for chronic neurologic cases diagnosed via MR imaging, was characterized by hippocampal and parahippocampal atrophy, temporal horn enlargement and associated parenchymal T2 hyperintensity signal (Fig. A 1). All chronic neurologic cases (n = 22) were diagnosed with hippocampal atrophy: 55% of the animals had unilateral damage and 45% bilateral damage (50% symmetrical, 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% (mild = 8, moderate = 1, and severe = 4) of animals and bilaterally in 41% (mild = 10, moderate = 5, and severe = 3) 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: eleven animals were classified with mild parahippocampal atrophy, 7 with moderate, and 2 with severe. Fifty-nine percent of California sea lions also exhibited T2 hyperintensity signal.

The numbers for the brain damage index (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-affected 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 few tags (n = 3) collected those data. The mean distance traveled per day for all animals was 31.4 ± 28.6 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 470.5 km (Table 2). Eight of the 12 acute animals traveled less than 100 km from shore. The four remaining acute cases traveled greater than 148 km from shore with CSL 6720 traveling 953.7 km during the course of transmission.

Survival for ten of the twelve acute cases was unknown. CSL 6608 was, however, seen in Ensenada, Mexico lethargic and unresponsive on 2 May 2006, nine months after she stopped transmitting (Benet, pers com.). CSL 6584 was presumed to have died post-release due to a head injury and massive shark wound on her back, which was documented photographically by TMMC volunteers. CSL 6706 re-stranded 4 days after release, lethargic and seizuring and was euthanized (Table 1).

All chronic neurologic cases were fitted with a 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); 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 41.4 ± 19.8 km with a maximum distance per day of 713.3 km by CSL 6731 (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 also were documented although not statistically tested. CSL 7007, a female, released in Sausalito, California in early October 2006 stopped

transmitting on 24 Dec 2006 in Washington State (Fig. 2), and CSL 5985 swam 4 km up a river where she spent ten days and was documented continuously swimming in circles.

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

Survival of fifteen of the twenty-two 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 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; indicating the animal (with tag) washed out to sea and sank. Seven sea lions re-stranded seizuring, 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 of the variables were log transformed to meet the assumptions of normality and/or equal variances before the ANCOVA was performed. Interaction terms for the ANCOVA were significant for four of the variables (maximum

dive depth, maximum distance traveled per day, maximum distance from shore, and percentage of time surface swimming); ANCOVAs were, therefore, performed on the remaining variables. Mean dive depths ($P = 0.051$) of California sea lions 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 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 the remaining four variables that had significant interaction terms 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). Control animals swam greater distances per day (121.3 ± 77.9 km) than animals suffering from domoic acid toxicosis (89.5 ± 146.8 km; $P = 0.386$), however, animals from the latter group swam further from shore ($P = 0.008$). 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.

Magnetic Resonance Imaging Behavioral Analysis:

Categories for the brain damage index (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 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 values were greater than 0.206). The mortality for animals in the mild BDI group was 80%, for the moderate group was 20%, and for the severe group was 14%.

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, three of which showed severe navigational impairments. CSL 5985 had moderate right-sided parahippocampal atrophy (Table A 1) and spent 10 days 5 km up the Salinas River after her release (Fig. C 3). CSL 7007 was the female that abnormally transited to Washington (Fig. 2) in winter, likely as a result of her bilateral mild parahippocampal atrophy (Table A 1). 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 (Figs. C 1 and C 15) with no apparent abnormal movements during the time of transmission. CSL 6904 and 5468, however, restranded 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 (Scotville and Milner, 2000). Fortin *et al.* (2002) determined the hippocampus was essential for remembering a sequence of events, although it is not necessarily important for remembering the items within a unique series. 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 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, California sea lions suffering from chronic neurologic effects and morphological changes in the brain acted significantly different than control California sea lions. Control animals dove deeper and for greater durations than chronic neurologic animals. Due to the shorter shallower dives chronic neurologic cases my not be as successful foragers as the control animals. This was supported by the fact when animals re-stranded 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 California sea lions traveling to a cold water front 450 km from shore during a 2005 warm water anomaly (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 movements 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 affects than control or acute cases.

Although there were no statistical significant associations between the severity 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. MR imaging 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 be detected by MR imaging. 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.

California sea lions classified as having acute neurological symptoms did not receive MR imaging, 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 post-mortem 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, 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 four to eight weeks before molting, so this likely decreased the transmission time substantially. The tags used for acute animals were location only 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. It is unknown if significant behavioral differences would have been detected if SRDLs were used on acute animals instead of location only tags.

Several behaviors displayed by animals affected by domoic acid were not quantifiable. For example, CSL 7096 displayed varying post-release 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 (Fig. D3 and D4). Other animals that re-stranded apparently disorientated were found sitting on a police car on the San Francisco waterfront (CSL 6887; Fig. D 5) and 3 km inland, in the city of San Francisco (CSL 5531).

In summary, post-release behaviors for California sea lions 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 MR imaging. In this study the mortality for California sea lions with domoic acid 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, shorter durations resulting probably in less foraging). Many probably had a prolonged death while at sea.

California sea lions 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 are needed to more accurately assess the effects of hippocampal and parahippocampal atrophy on this species.

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Table 1: Animals affected by domoic acid toxicosis. CSL # is the California sea lion identification number and * indicates the animal restranded and was re-released with a tag. Age is the age class of the animal determined upon 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 data is the date the animals were tagged and released from TMMC, last transmission is the date the tag stopped transmitting, # days is the total number of days the tag transmitted, and survivability is the animals know 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 post-release means we have reason to believe the animal died, and BDI is the brain damage index number calculated based on MR interpretation.

CSL #	Age class	Sex	Diagnosis	Release date	Last transmission	# Days	Survival	BDI
6039	Sub-adult	M	A	7/8/04	7/17/2004	10	Unknown	N/A
6053	Adult	M	A	9/16/04	9/26/2004	11	Unknown	N/A
6116	Sub-adult	M	A	10/13/04	11/28/2004	47	Unknown	N/A
6518	Adult	F	A	6/7/05	7/26/05	50	Unknown	N/A
6521	Adult	M	A	6/7/05	6/11/05	5	Unknown	N/A
6584	Adult	F	A	7/24/05	8/11/05	19	Died post-release	N/A
6608	Adult	F	A	7/24/05	8/17/05	25	Unknown	N/A
6668	Adult	F	A	8/17/05	9/1/05	16	Unknown	N/A
6674	Sub-adult	M	A	8/17/05	9/9/05	24	Unknown	N/A
6677	Adult	M	A	8/17/05	8/18/05	33	Unknown	N/A
6706	Adult	M	A	8/17/05	8/20/05	35	Euthanized	N/A
6720	Sub-adult	M	A	8/25/05	9/26/05	33	Unknown	N/A
5468	Yearling	M	CN	5/15/03	6/26/03	43	Euthanized	16
5531*	Adult	F	CN	7/1/03	7/22/03	21	N/A	28
			CN	9/26/03	10/16/03	21	Euthanized	-
5810	Sub-adult	F	CN	9/19/03	10/20/03	32	Died post-release	38
5985	Sub-adult	F	CN	11/11/03	2/24/04	106	Unknown	40
6012	Adult	F	CN	3/10/04	4/13/04	35	Unknown	29
6018	Adult	F	CN	6/29/04	7/24/04	26	Unknown	34
6024	Adult	F	CN	5/20/04	6/14/04	26	Unknown	49
6433*	Yearling	F	CN	1/13/05	5/21/05	129	N/A	33
			CN	6/1/06	7/31/06	61	Unknown	-
6510	Juvenile	M	CN	6/7/05	7/14/05	38	Euthanized	42
6640	Adult	M	CN	5/31/06	6/6/06	7	Euthanized	13
6667	Yearling	M	CN	9/19/05	10/18/05	30	Unknown	24
6673	Juvenile	M	CN	9/30/05	10/14/05	33	Unknown	58
6710	Yearling	F	CN	9/26/05	9/26/05	<1	Unknown	17
6731	Yearling	F	CN	10/28/05	11/21/05	25	Euthanized	6
6740	Adult	F	CN	9/26/05	10/3/05	8	Unknown	64
6887	Sub-adult	M	CN	7/5/06	7/13/06	8	Euthanized	25
6902	Sub-adult	F	CN	8/1/06	8/1/06	<1	Unknown	42
6904	Juvenile	M	CN	7/19/06	7/30/06	24	Euthanized	29
7007	Sub-adult	F	CN	10/11/06	12/19/06	70	Unknown	34
7028	Adult	F	CN	9/25/06	10/14/06	20	Unknown	66
7077	Adult	M	CN	11/7/06	12/6/06	20	Died post-release	6
7096	Adult	F	CN	12/1/06	12/25/06	24	Unknown	37

Table 2: Movement variables calculated from the satellite tags. Condition is the animals classification (acute, chronic neurologic, control), mean distance traveled per day is the mean of all animals km±SD (range), max distance traveled per day is the mean of all animals km±SD (range), and max distance traveled from shore is the mean of all animals km±SD (range).

Condition (n)	Mean distance traveled per day km±SD (range)	Max distance traveled per day km±SD (range)	Max distance from shore km±SD (range)
Acute (12)	31.4±28.6 (5.1-93.4)	115.4±139.8 (8.1-470.5)	163.2±266.7 (2.9-953.7)
Chronic Neurologic (22)	41.4±19.8 (11.8-97.6)	174.4±162.1 (34.2-713.3)	186.0±397.8 (7.5-1862.2)
Control (67)	30.7±20.5 (7.2-79.9)	121.3±77.9 (22.7-445.6)	34.7±11.8 (11.6-64.3)

Table 3: Dive variables calculated from the satellite tags. Condition is the animals classification(acute, chronic neurologic, control), mean dive depth is the mean of all animals within the classification group in m±SD (range), maximum dive de th is the mean of all animals m±SD (range), mean dive duration the mean of all animals min±SD (range), max dive duration is the mean of all animals min±SD (range), haul-out is the mean percentage of all animals %±SD (range), surface swimming is the mean percentage of all animals %±SD (range), and dive is the mean percentage of all animals %±SD (range),

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

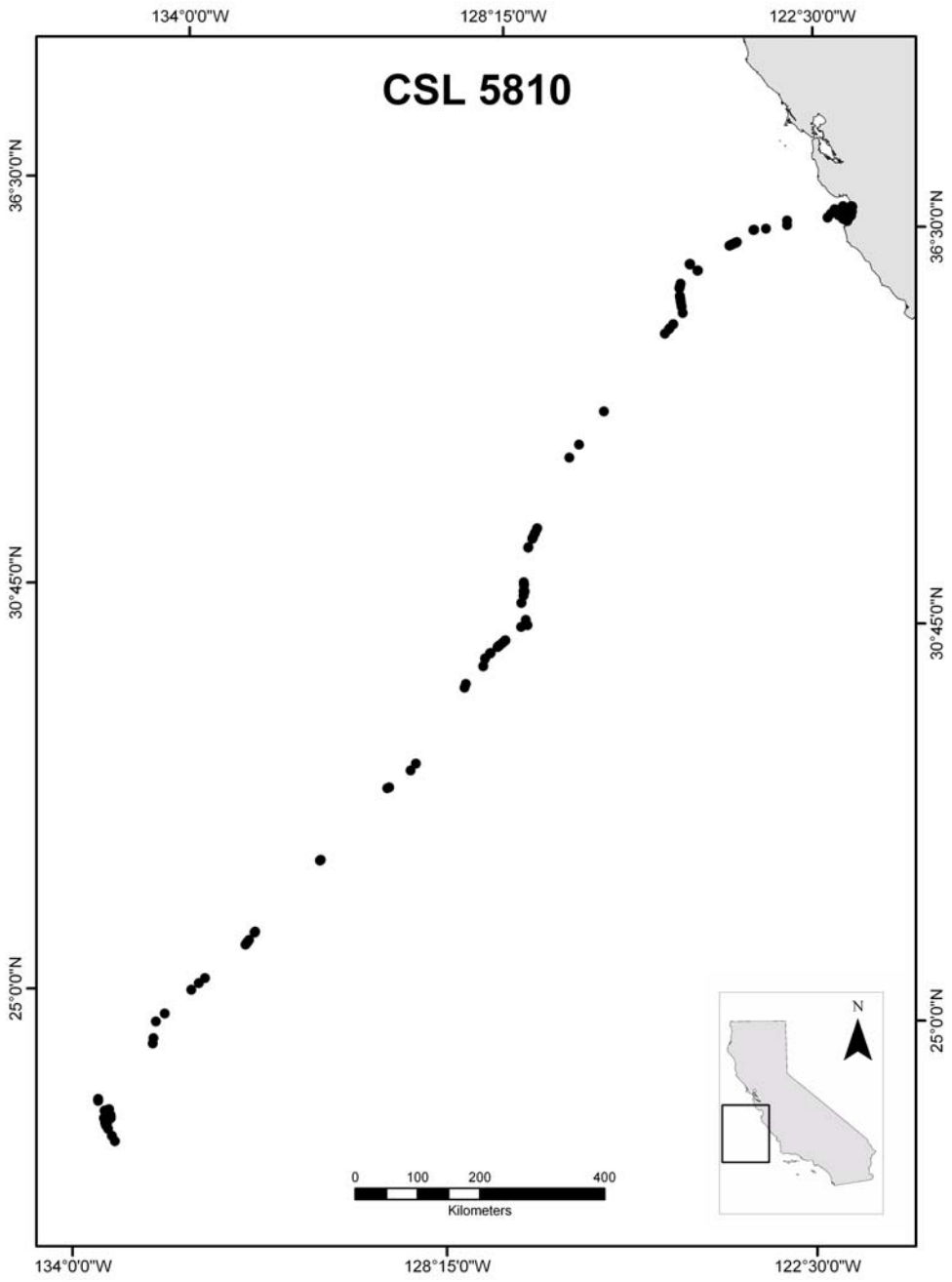


Fig 1: Location data (black dots) for CSL 5810 showing movement of the animal transiting halfway between California and Hawaii, abnormal for a near shore species.

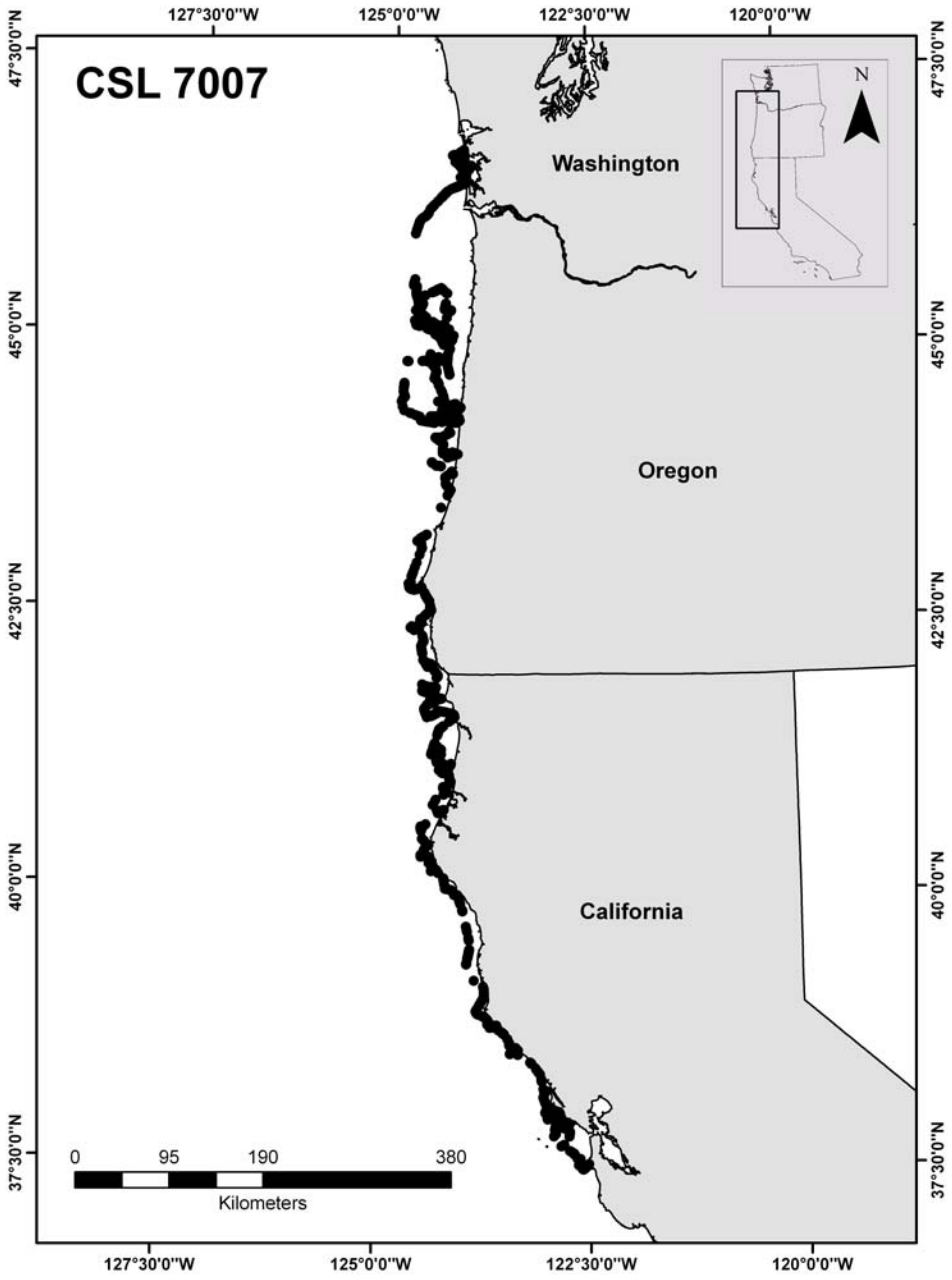


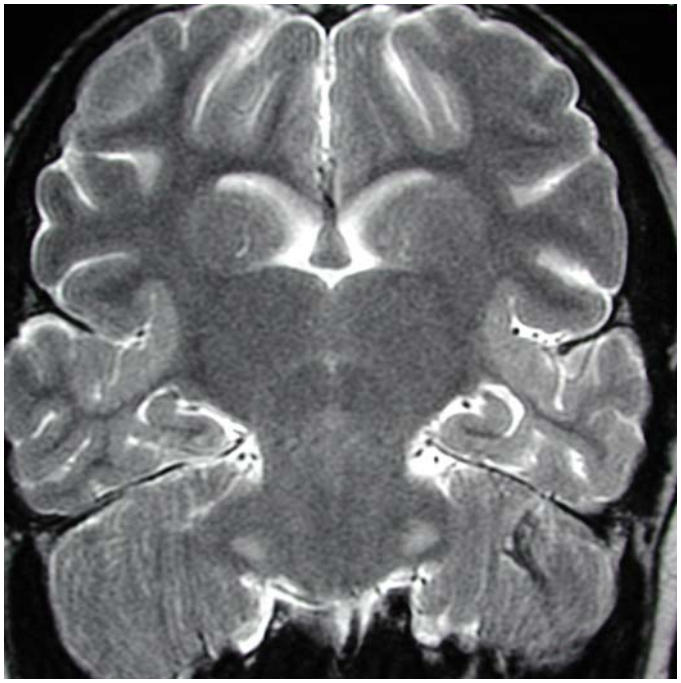
Fig 2: Location date (black dots) for CSL 7007 showing movement from California north to Washington. The transit is considered abnormal due to the fact this female stopped transmitting in Washington 24 Dec 2006 and females are not found in Washington during the winter months.

Appendix A: MRI results

Table A 1: MRI results for chronic neurologic California sea lions (CSL#) infected domoic acid toxicosis; L is for damage to the left hemisphere, R for damage to the right hemisphere, B is bilateral damage, Mi is for mild damage, Mo for moderate, and S for severe. If bilateral damage was asymmetrically the codes for left and right hemispheres are given. If bilateral damage was the same for both hemispheres then only B was used with the severity codes. T2 hyperintensity is present or absent.

CSL #	Hippocampal atrophy	Temporal Horn enlargement	Parahippocampal atrophy	T2 hyperintensity signal
5468	LMi	LMi	N/A	Present
5531	RMo	RMi	RMi	Present
5810	BMo	BMi	BMi	Absent
5985	RS	RMi	RMo	Present
6012	BMo	BMi	RMi	Absent
6018	LS	LMo	LMi	Present
6024	B - LMi/RMo	RS	RS	Present
6433	RS	RMi	RMi	Present
6510	RS	RS	RMo	Present
6640	LMi	LMi	LMi	Absent
6667	BMo	BMo	N/A	Absent
6673	B - LS/RMo	BMi	B-LMo/RMi	Present
6710	B - LMo/RMi	BMi	N/A	Absent
6731	LMi	LMi	N/A	Absent
6740	BMo	BS	BMo	Present
6887	RS	RS	RMi	Absent
6902	LS	LS	LMo	Present
6904	B - LMi/RMo	BMo	N/A	Present
7007	B - LMi/RMo	BMi	RMi	Present
7028	BS	B - LS/RMo	B - LMi/RMo	Present
7077	RMi	RMi	N/A	Absent
7096	LS	LMi	LS	Absent

(a)



(b)

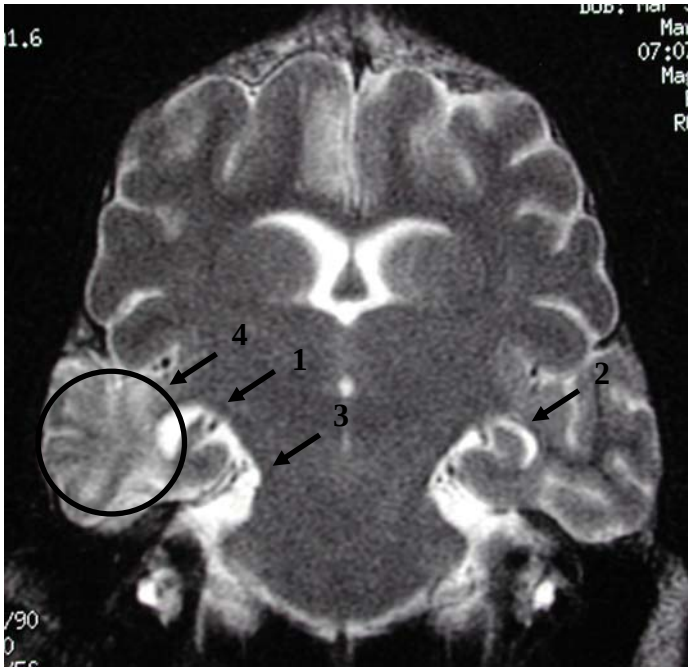


Fig A 1 :Examples of a normal (a) California sea lion brain and a sea lion brain affected by domoic acid (b) with 1 showing sever hippocampal atrophy, 2 a mild hippocampal atrophy, 3 severe parahippocampal atrophy, and 4 circling T2 hyperintensity signal.

Appendix B: Movements for Acute California sea lions

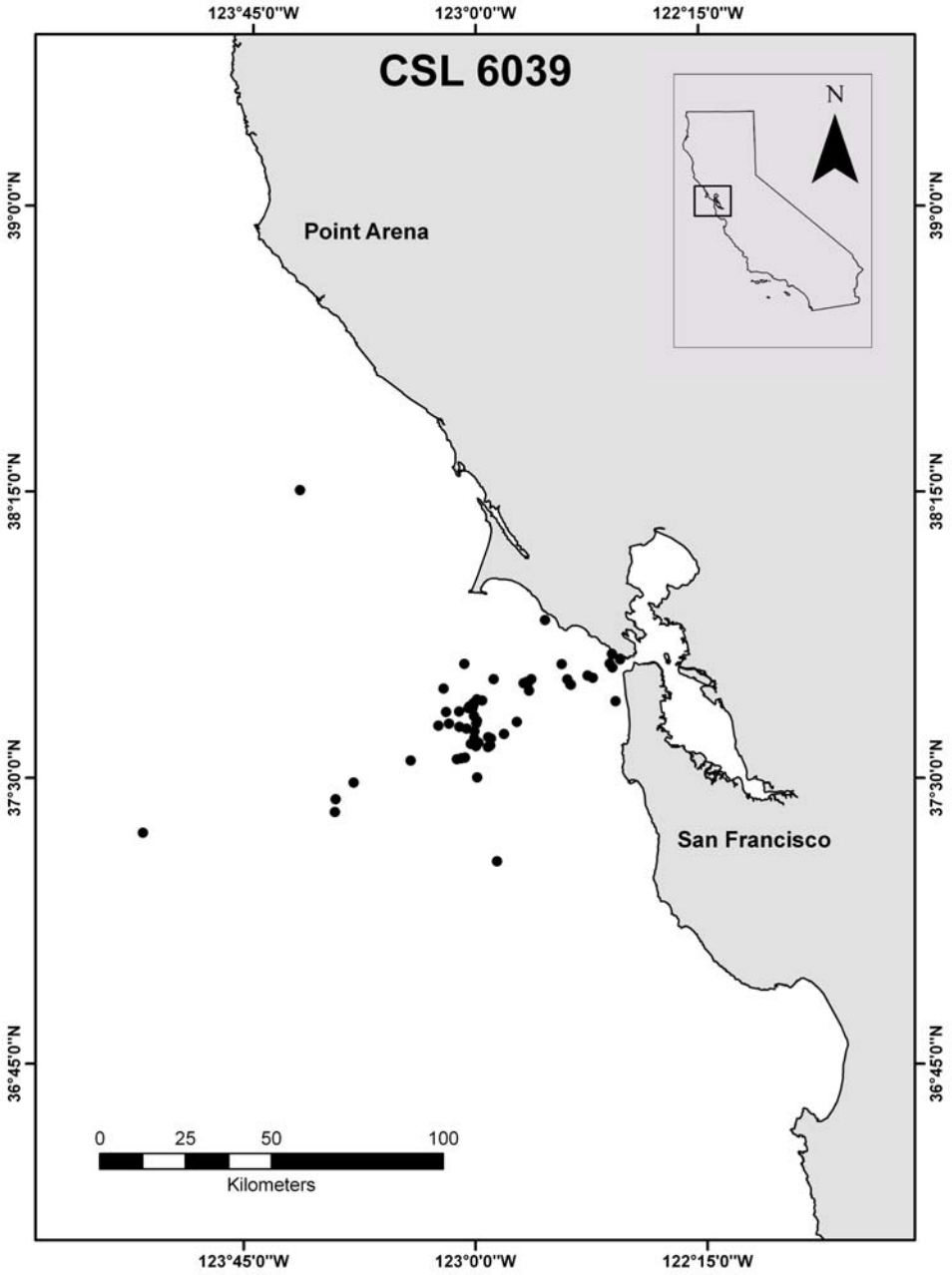


Fig B 1: Location data (black dots) showing movement for CSL 6093 outside San Francisco Bay.

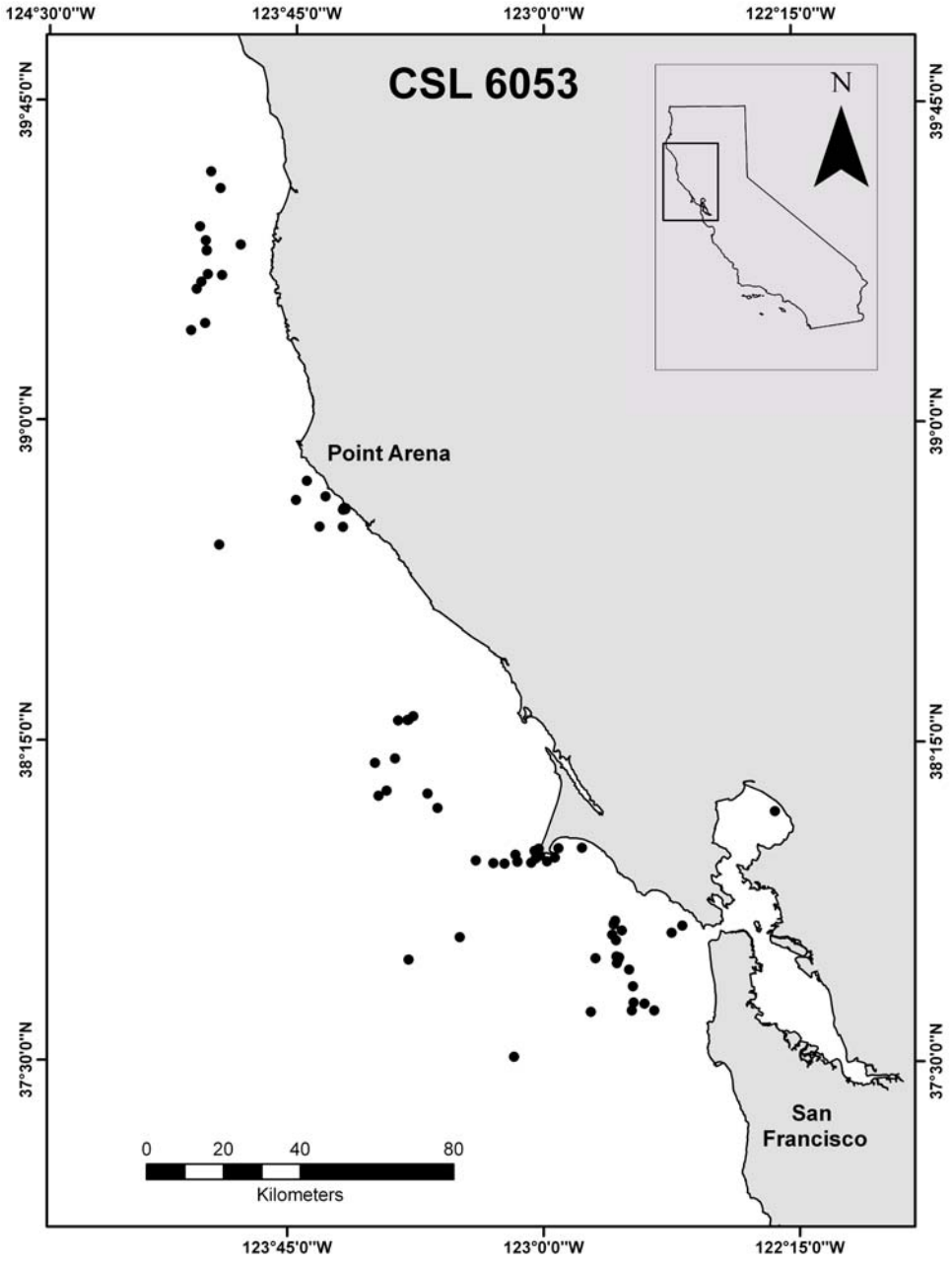


Fig B 2: Location data (black dots) for CSL 6053 showing northern movement patterns from San Francisco Bay.

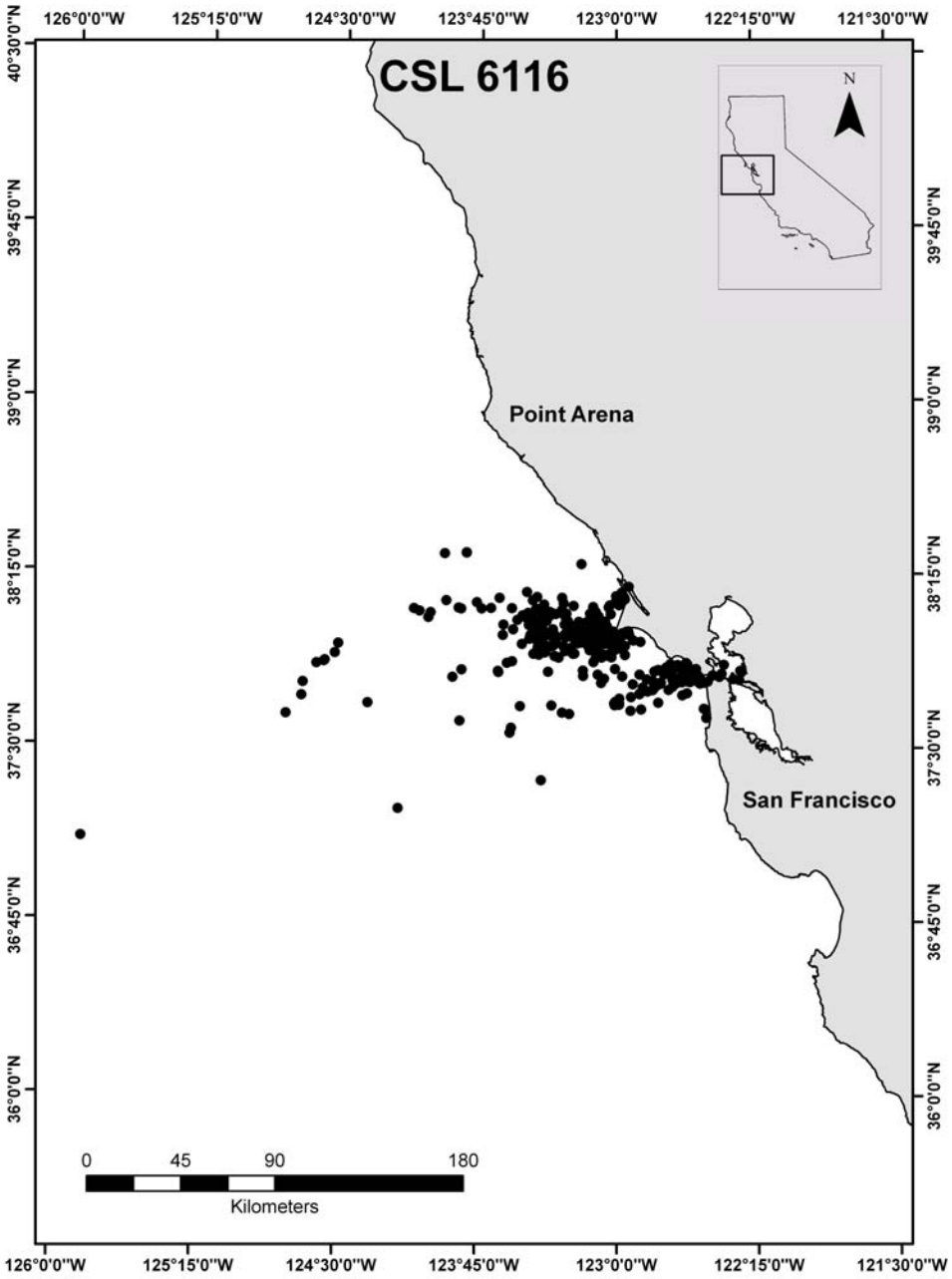


Fig B 3: Location data (black dots) for CSL 6116.

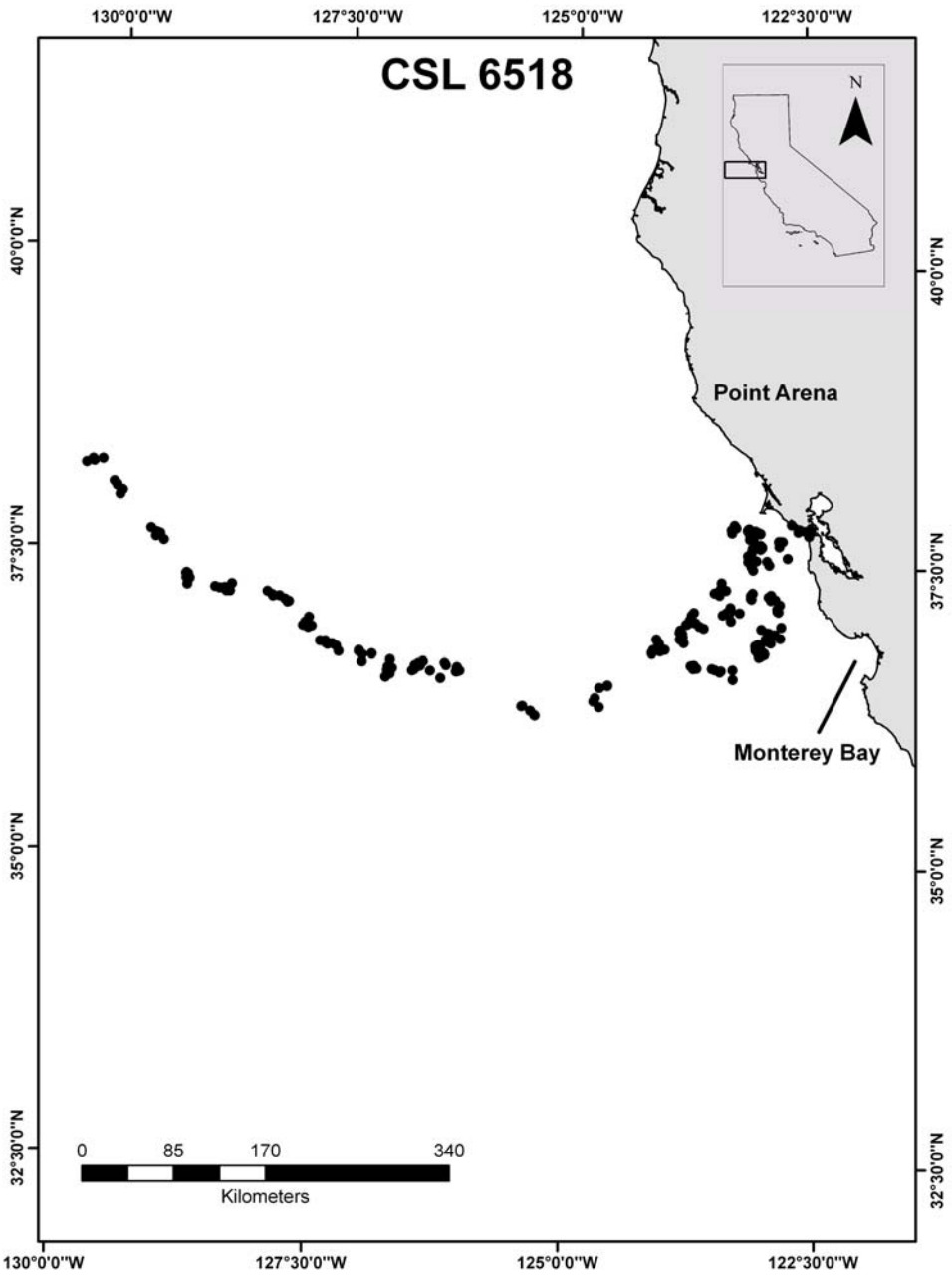


Fig B 4: Location data (black dots) for CSL 6518. The last location was 225 km from shore.

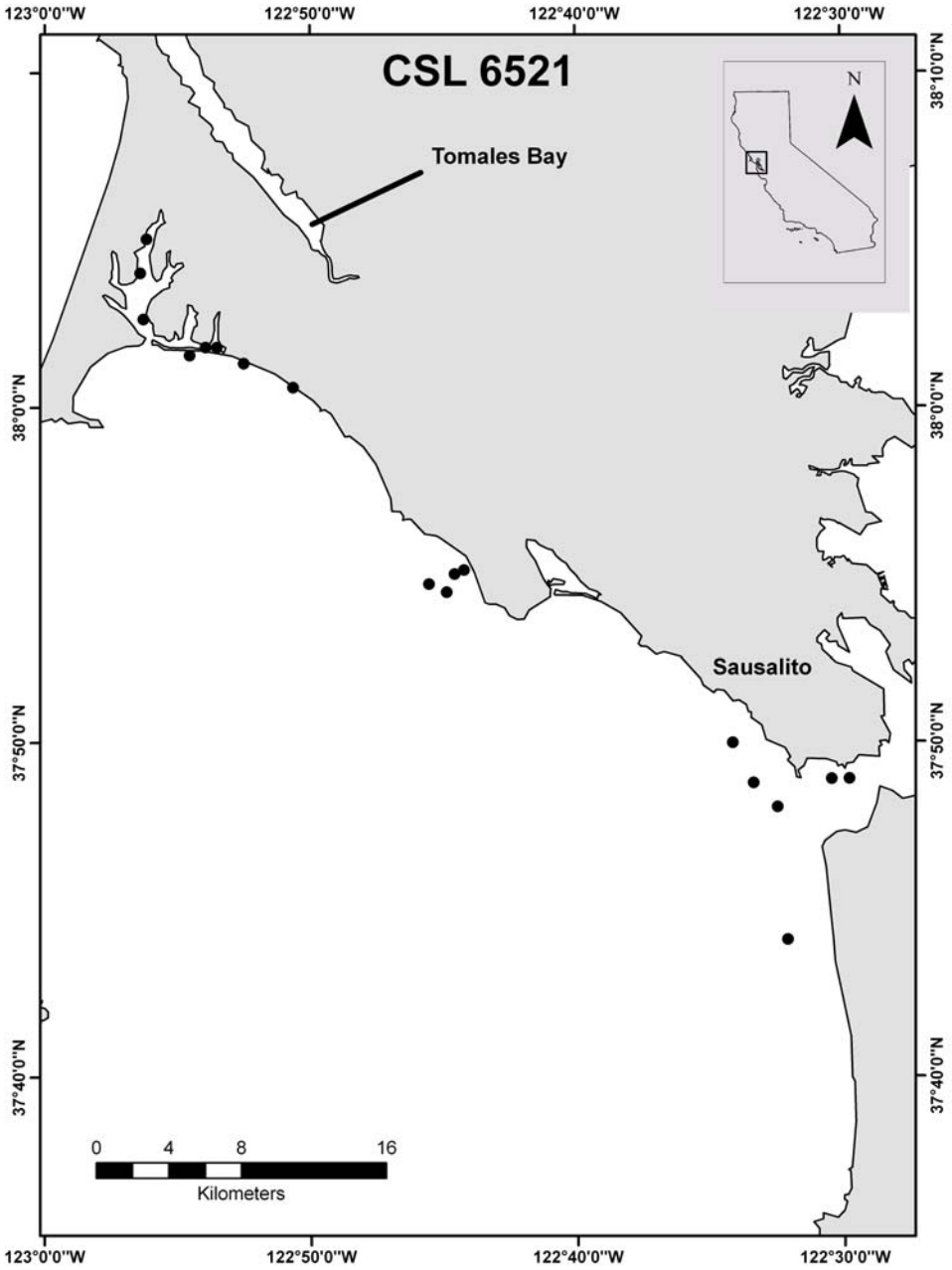


Fig B 5: Location data (black dots) for CSL 6521 displaying northern movement from Sausalito.

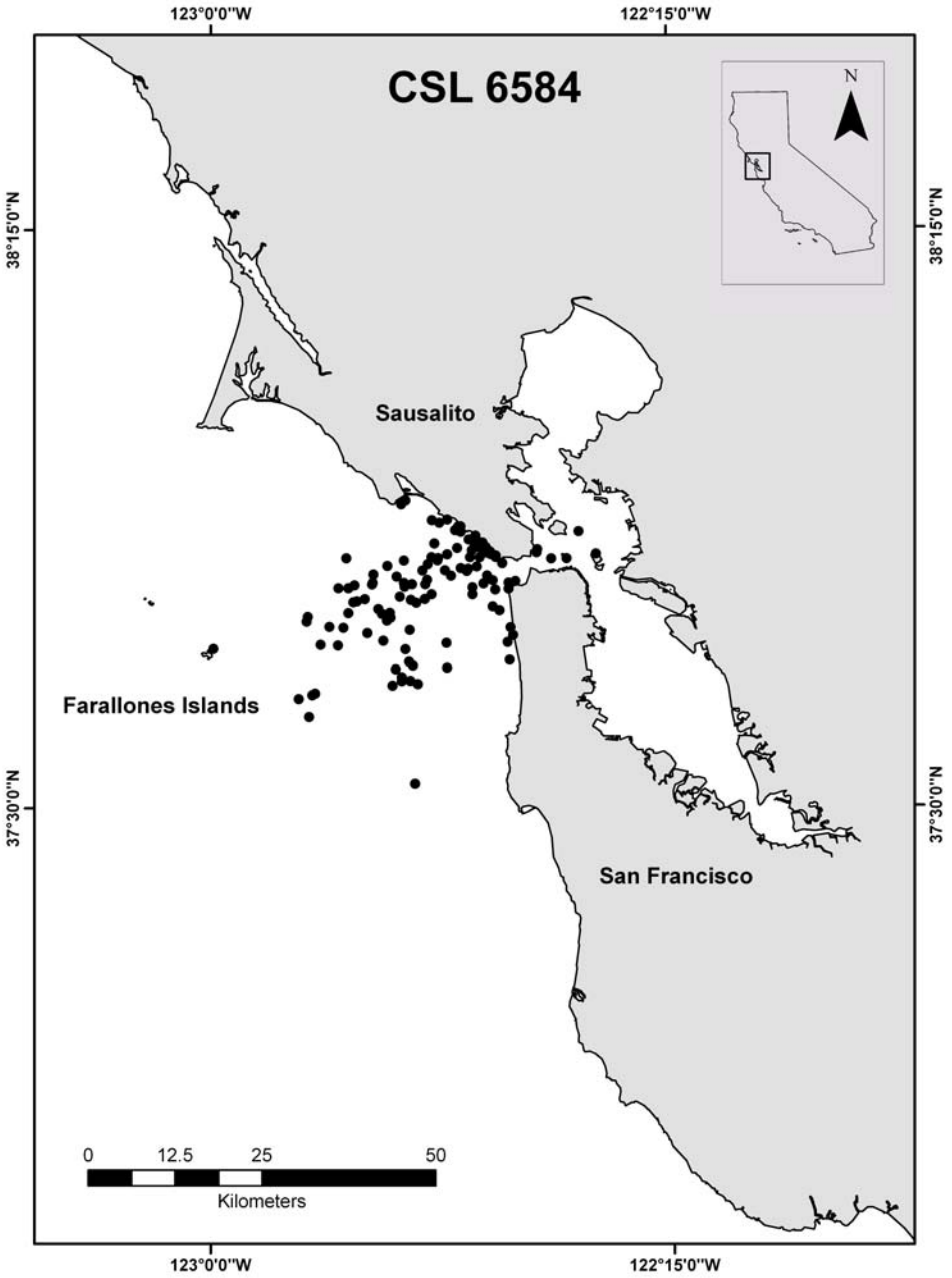


Fig B 6: Location data (black dots) displaying movement between the Farallone Islands and San Francisco Bay for CSL 6584.

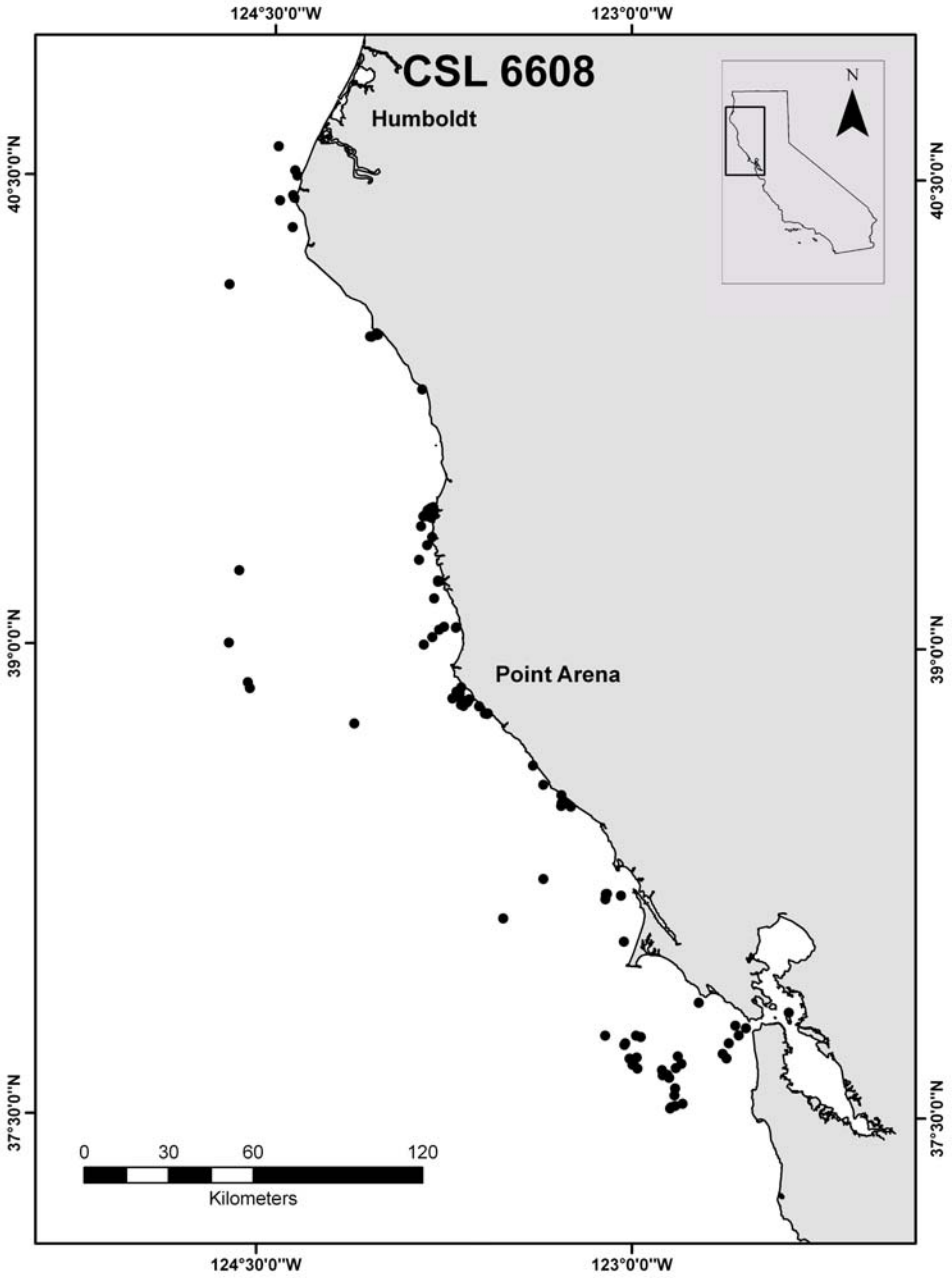


Fig B 7: Location data (black dots) for CSL 6608 displaying the northern movement for San Francisco.

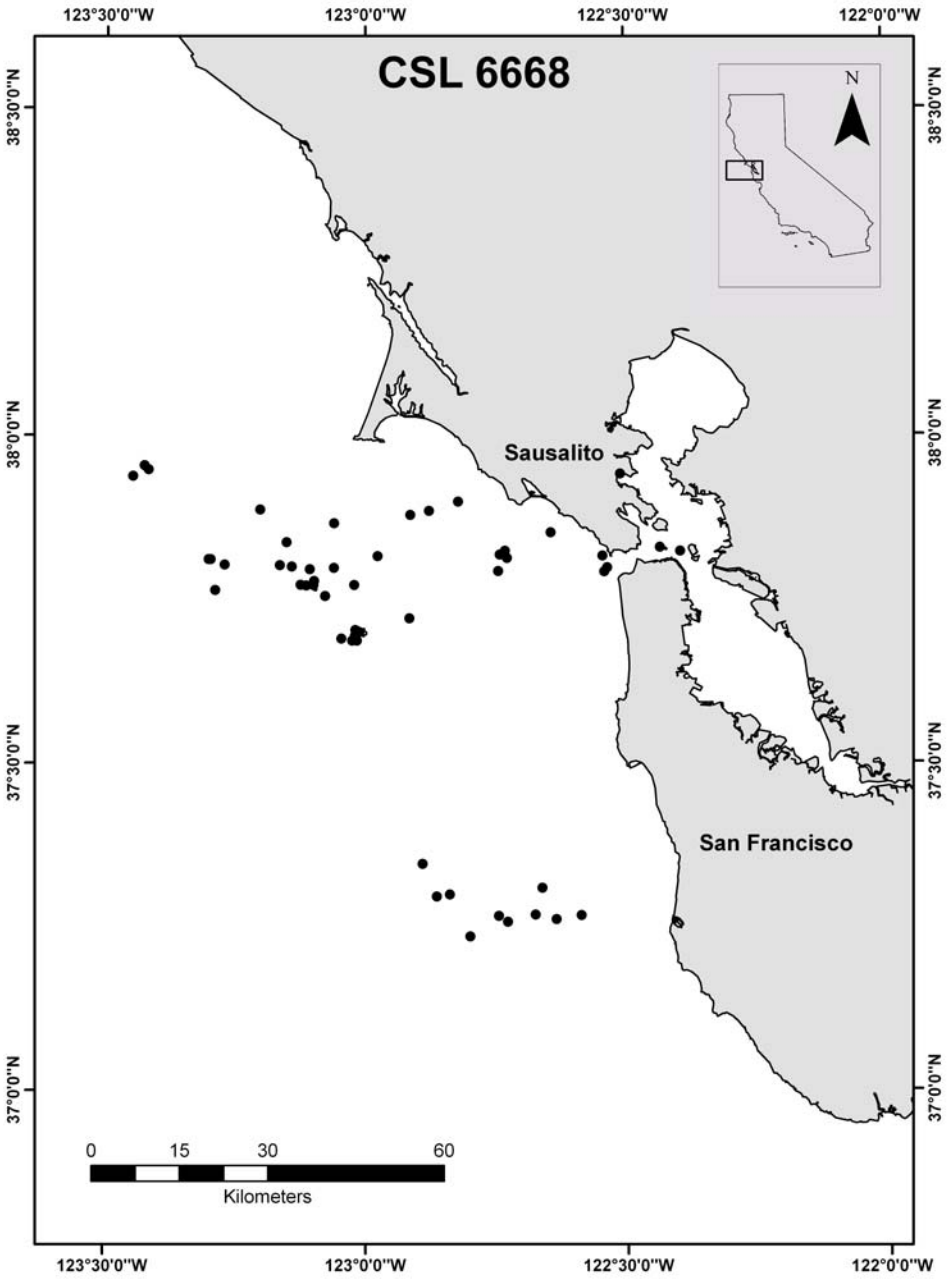


Fig B 8: Location data (black dots) for CSL 6668.

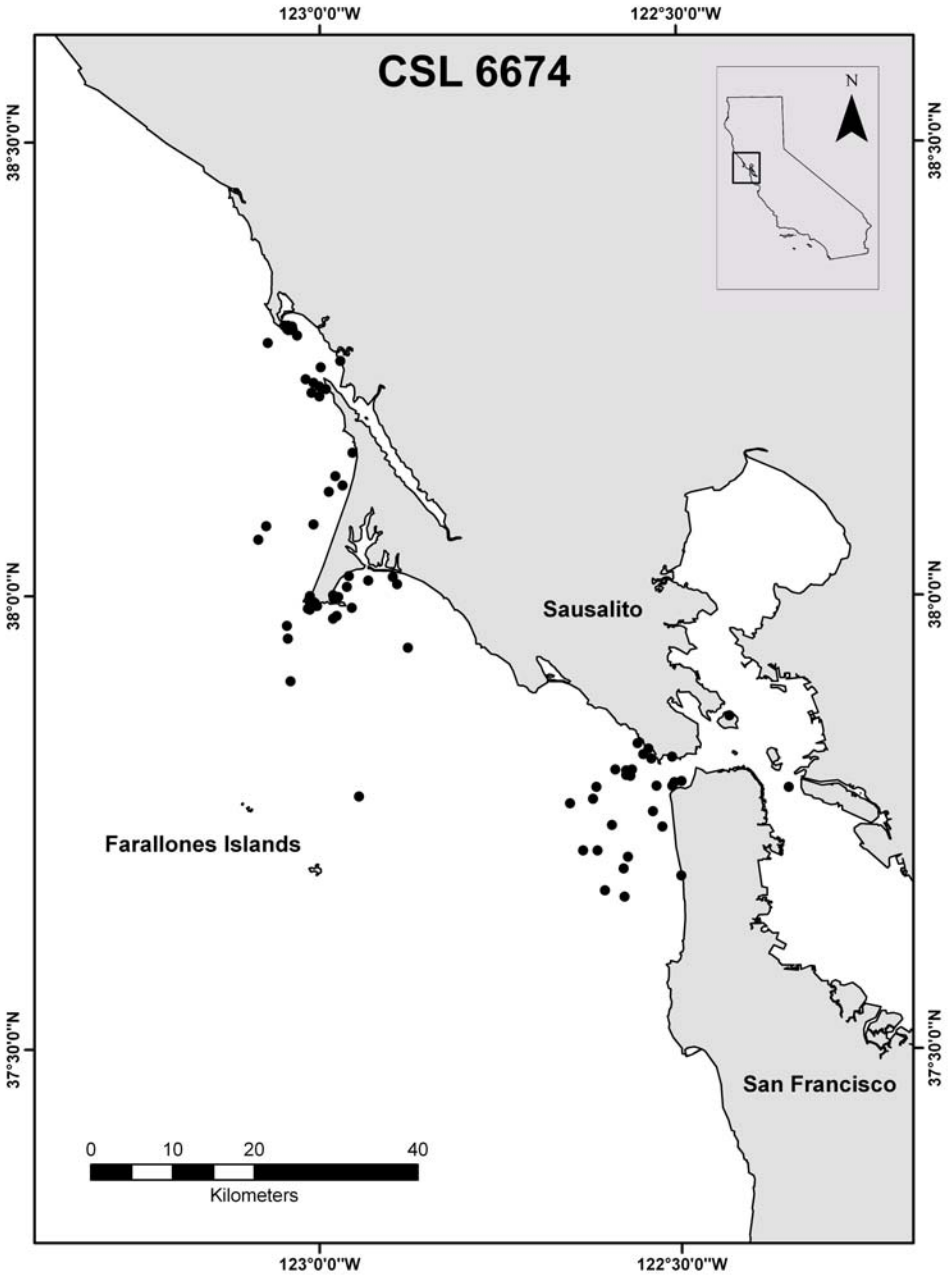


Fig B 9: Location data (black dots) for CSL 6674 traveling north from San Francisco.

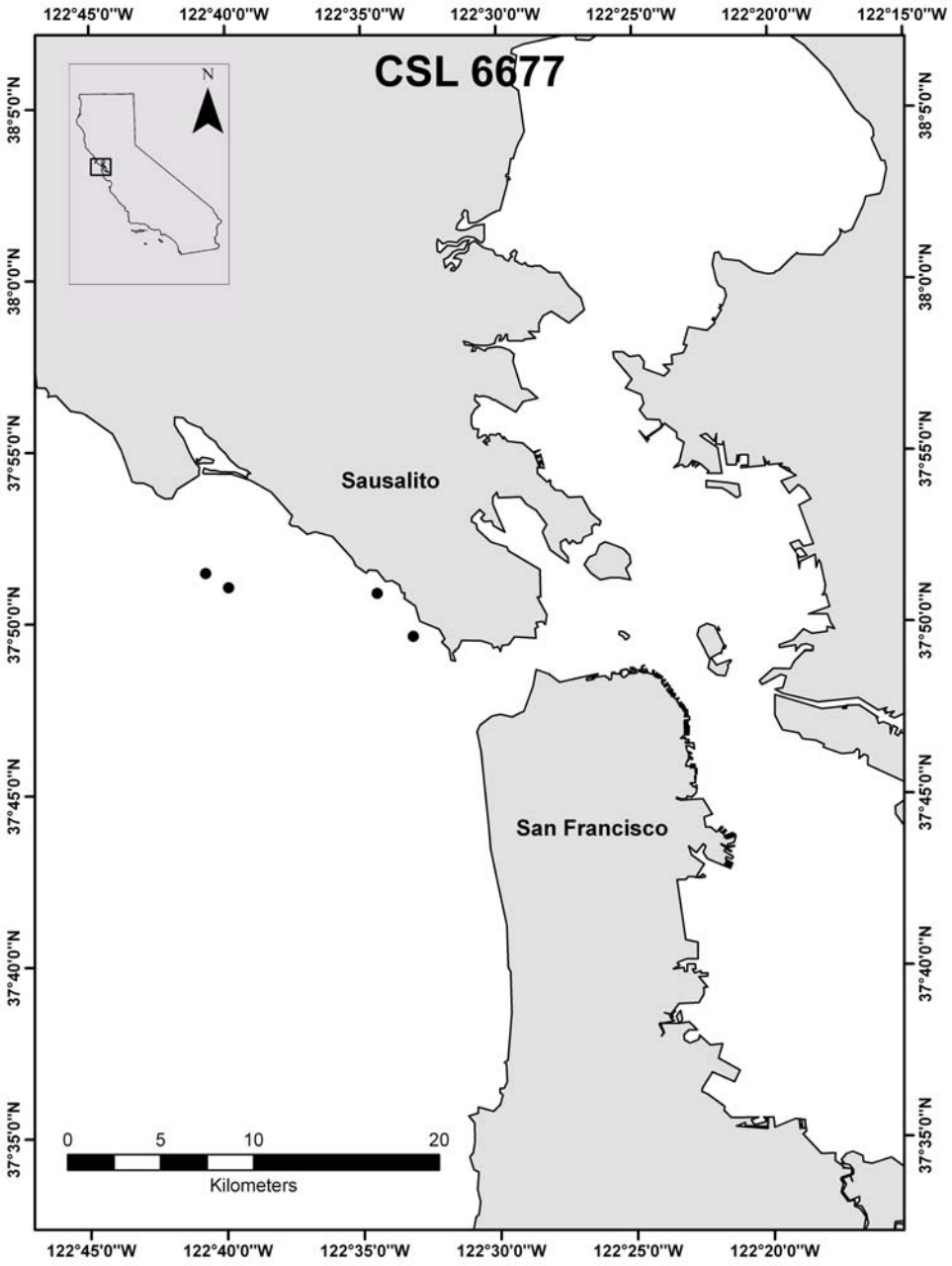


Fig B 10: Location data (black dots) for CSL 6677. Transmission was < 1 day and the animal traveled 7.7 km from shore.

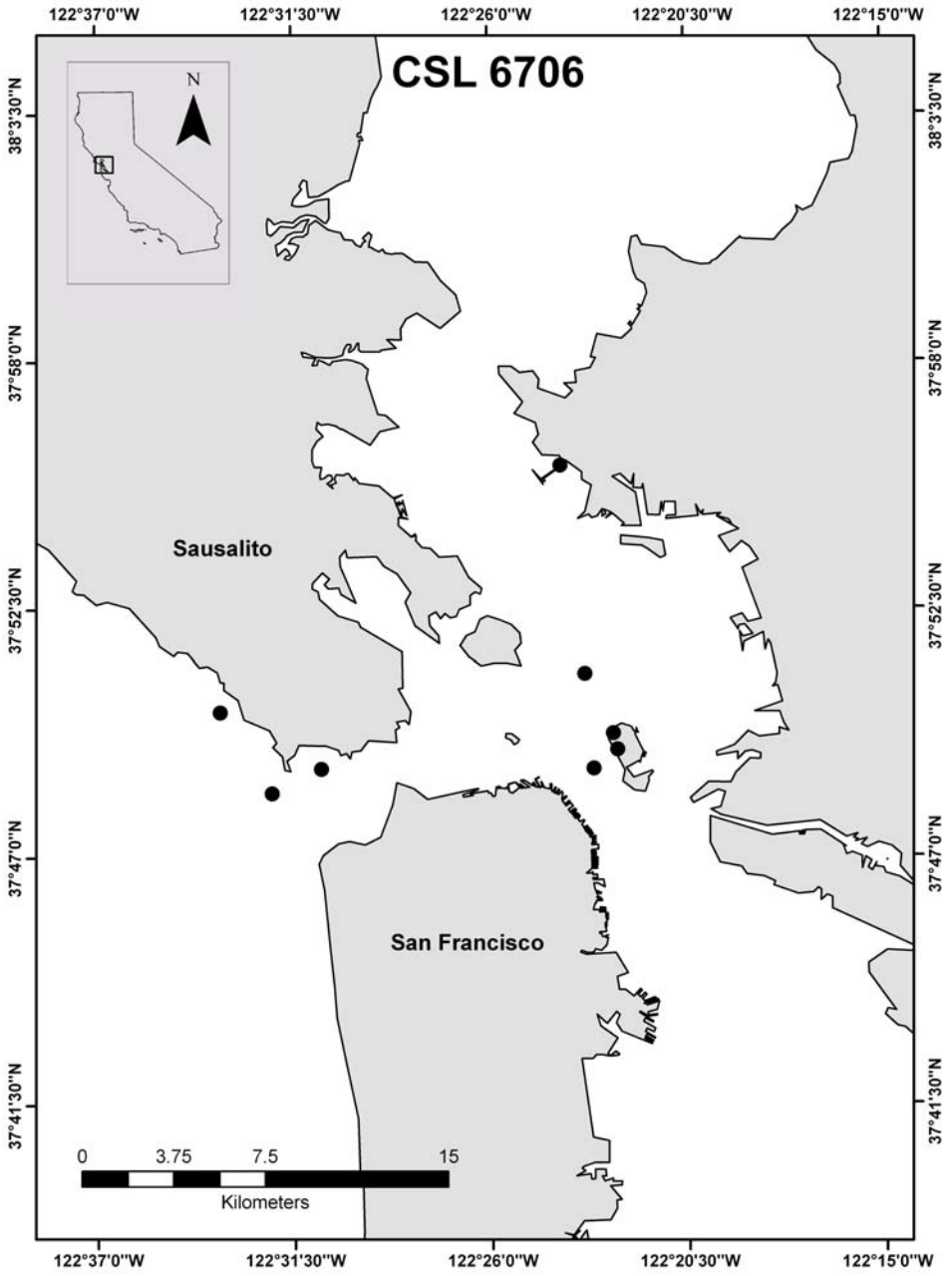


Fig B 11: Location data (black dots) for CSL 6706 showing movements in San Francisco Bay.

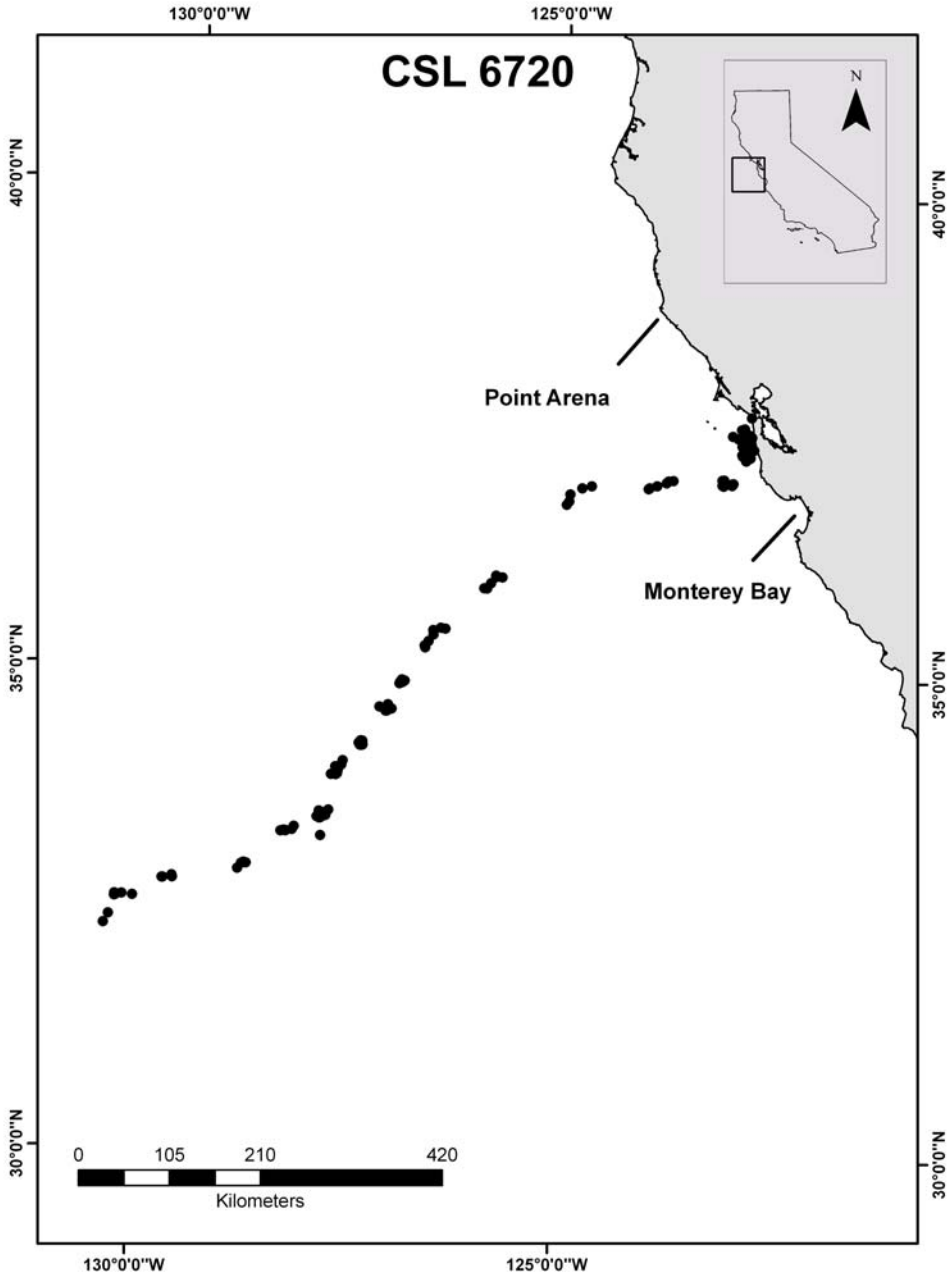


Fig B 12: Location data (black dots) displaying movement 953.7 km south west of California for CSL 6720.

Appendix C: Movements for chronic Neurologic

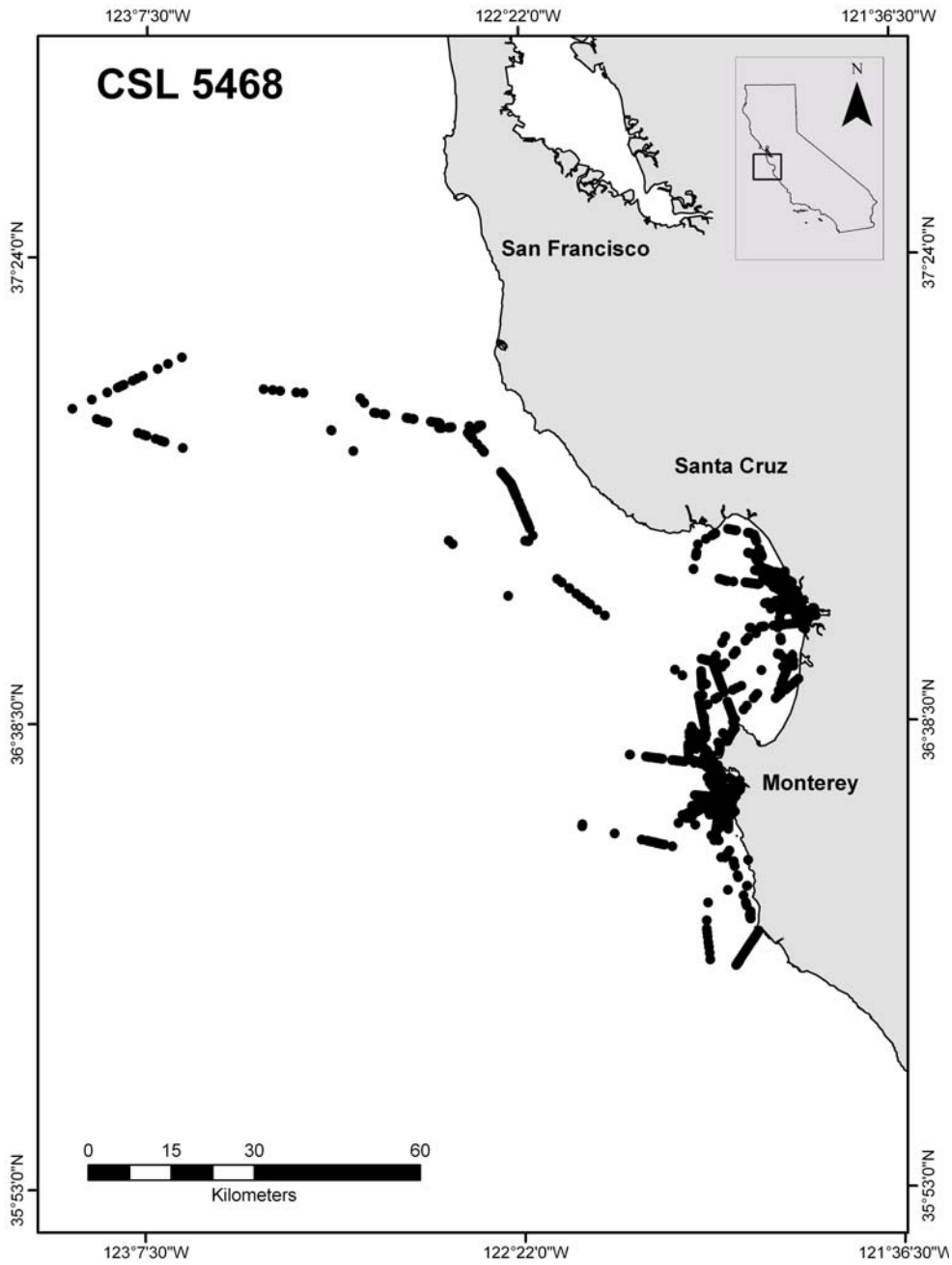


Fig C 1: Location data (black dots) for CSL 5468.

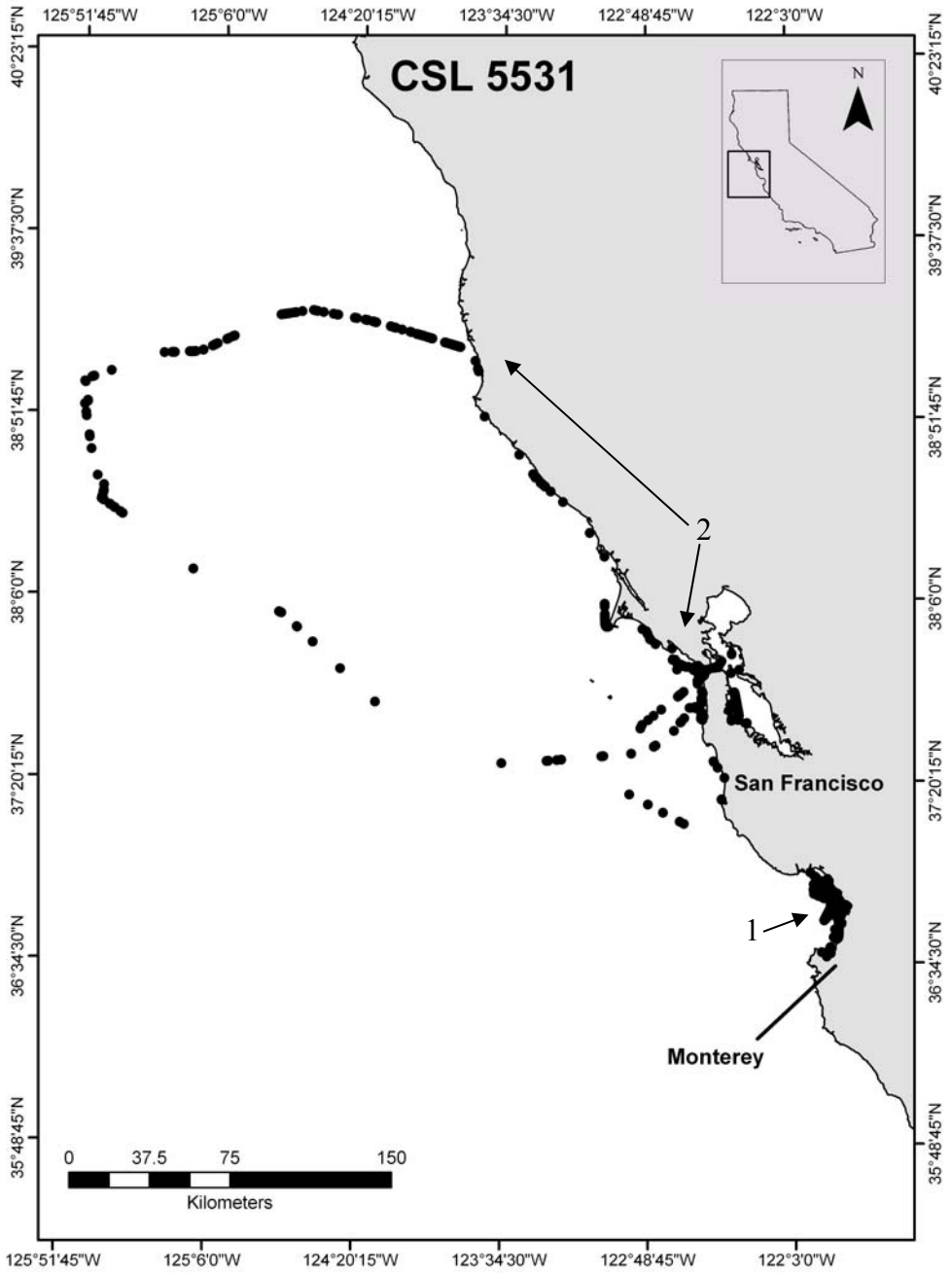


Fig C 2: Location data (black dots) for CSL 5531. Locations in Monterey Bay (1) are movements from the first release and locations near San Francisco (2) are the movement patterns for the second release.

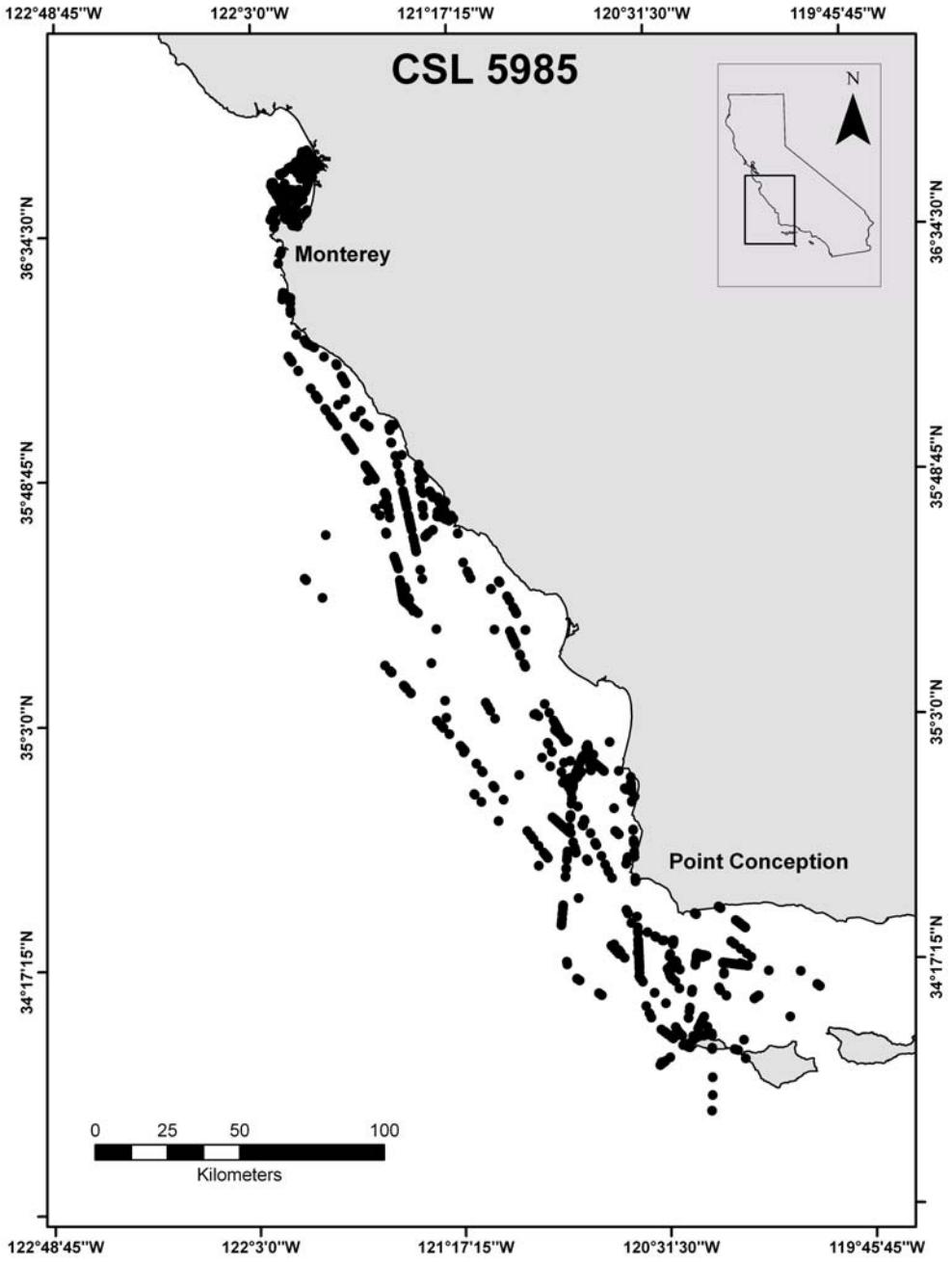


Fig C 3: Location data (black dots) for CSL 5985. CSL 5985 spent the first 10 days post-release 4 km up the Salinas River near Monterey.

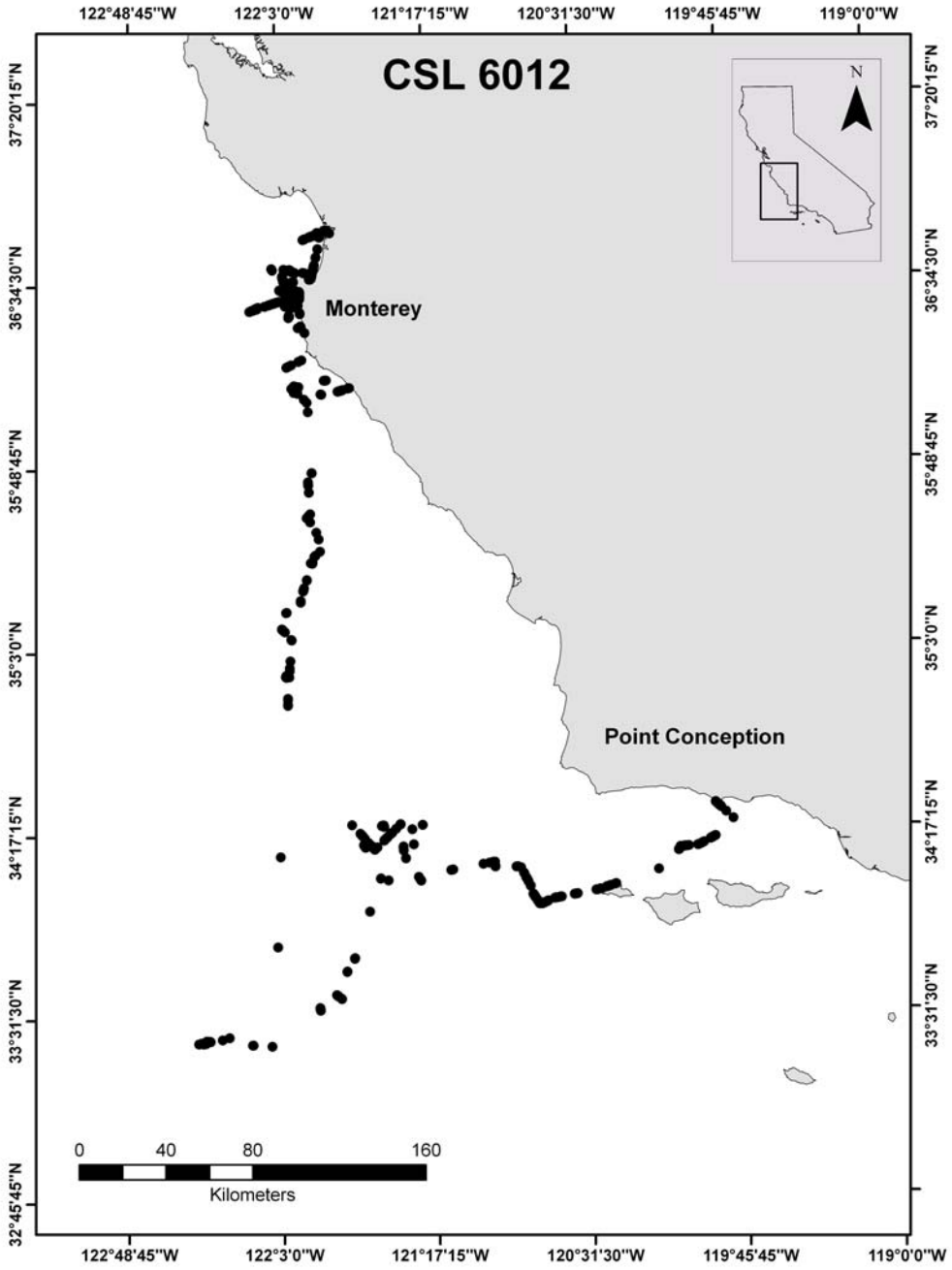


Fig C 4: Location data (black dots) for CSL 6012 traveling southwest from Monterey with maximum distance from shore 291.7 km.

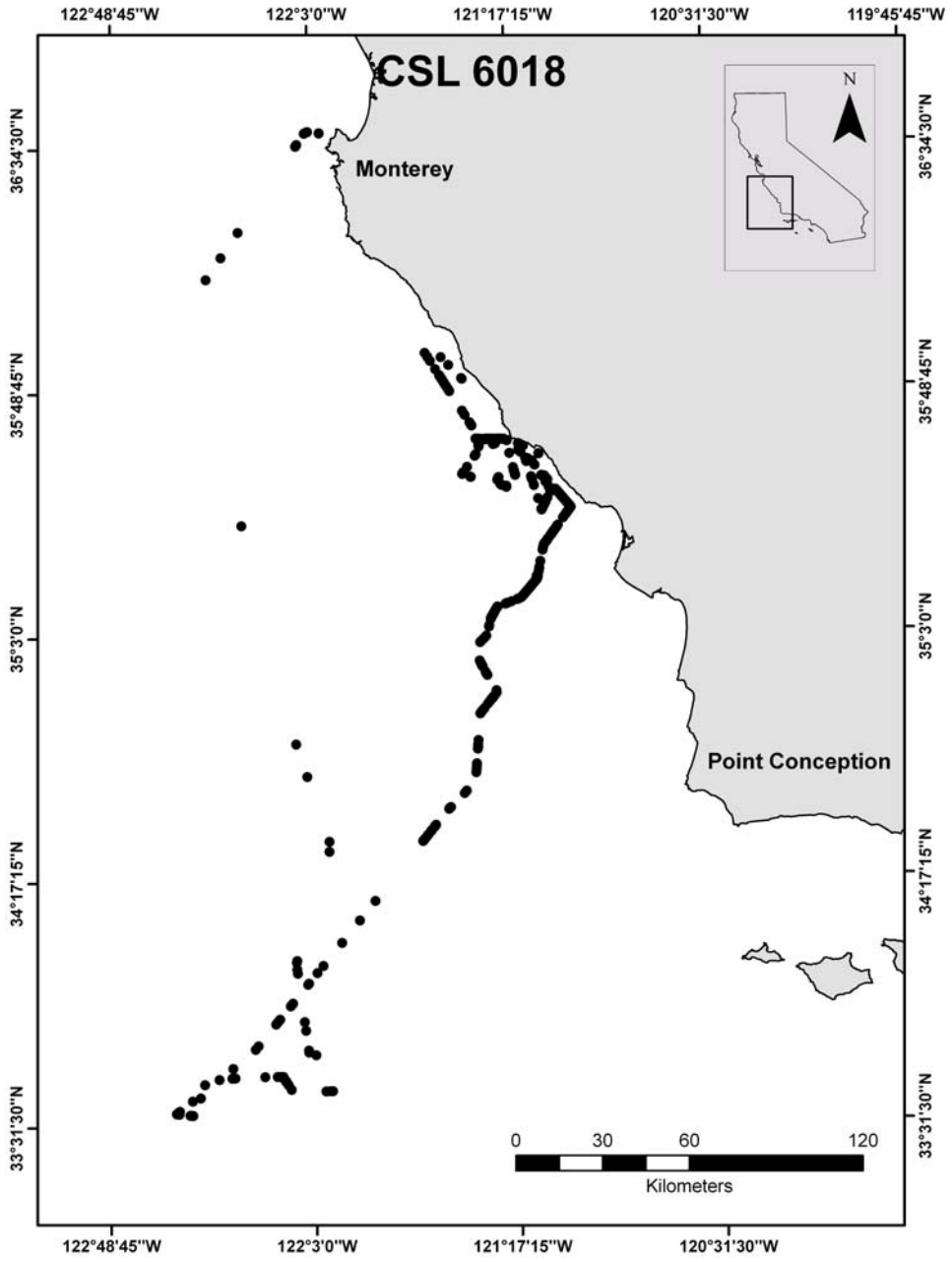


Fig C 5: Location data (black dots) showing movements from Monterey to 199.0 km from shore and back for CSL 6018.

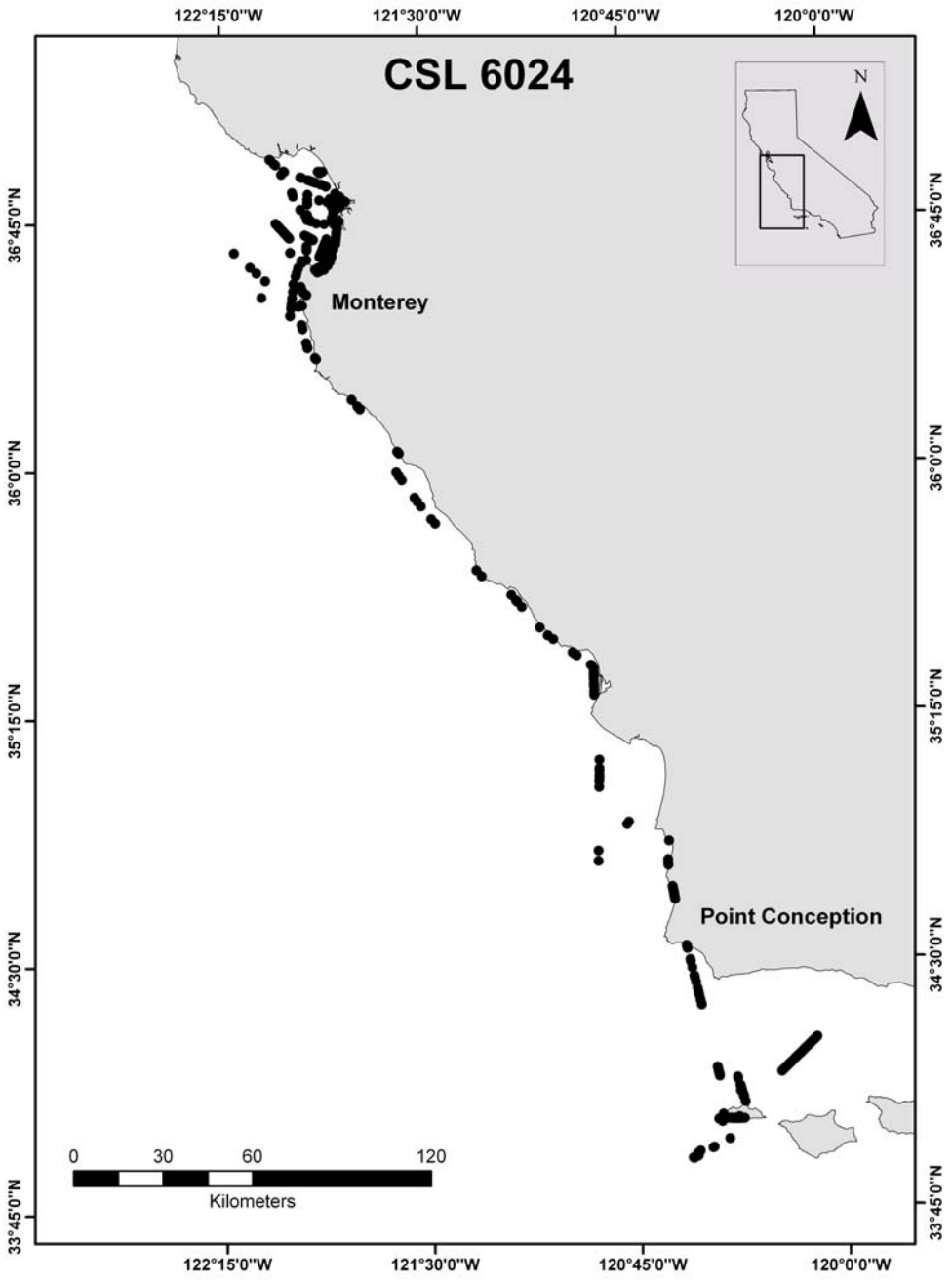


Fig C 6: Location data (black dots) for CSL 6024 displaying movements form Monterey Bay to San Miguel Island.

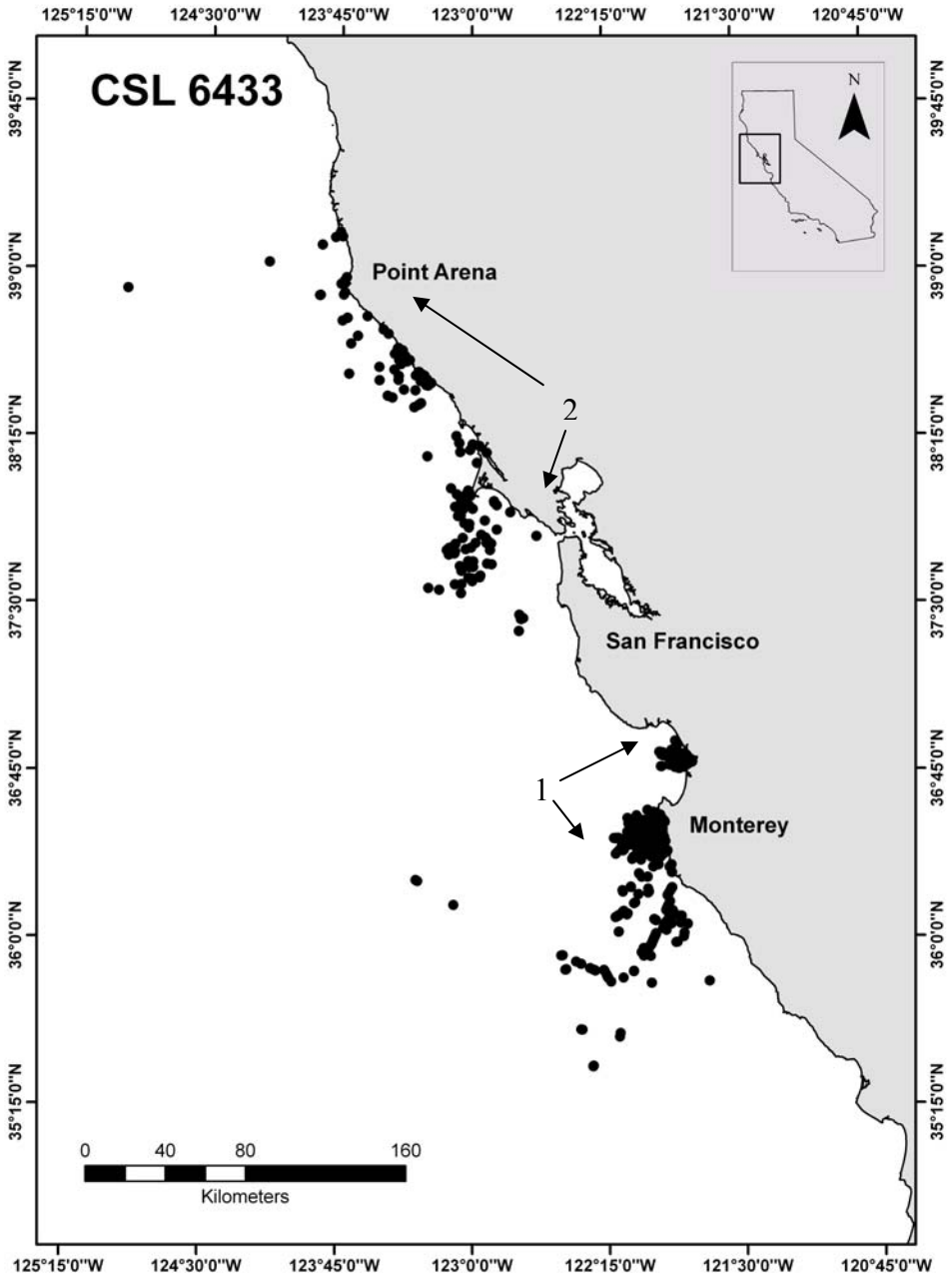


Fig C 7: Location data (black dots) for CSL 6433. The first release (1) was near Monterey Bay and the second release (2) was near San Francisco.

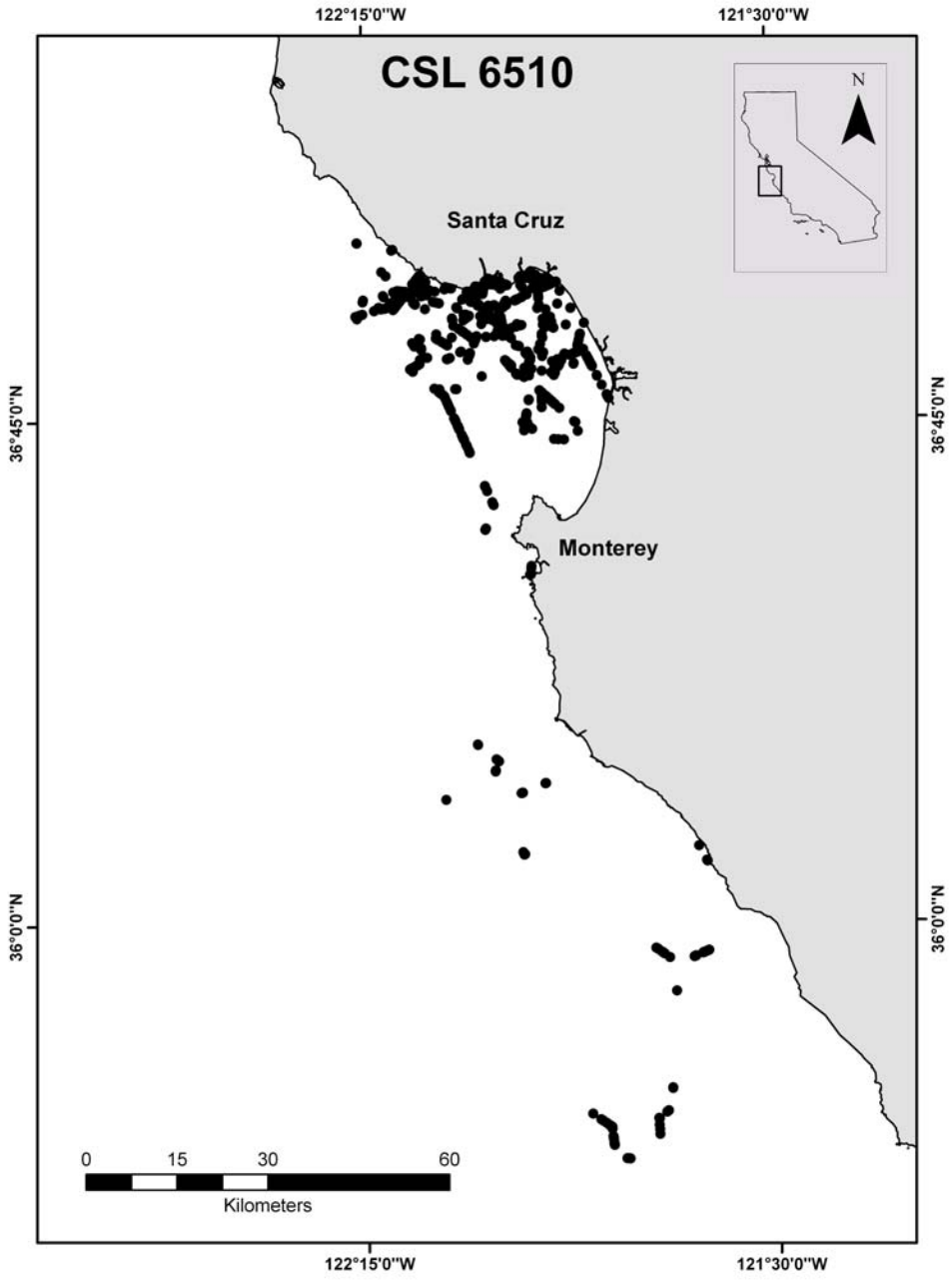


Fig C 8: Location data (black dots) displaying movement south from Monterey for CSL 6510.

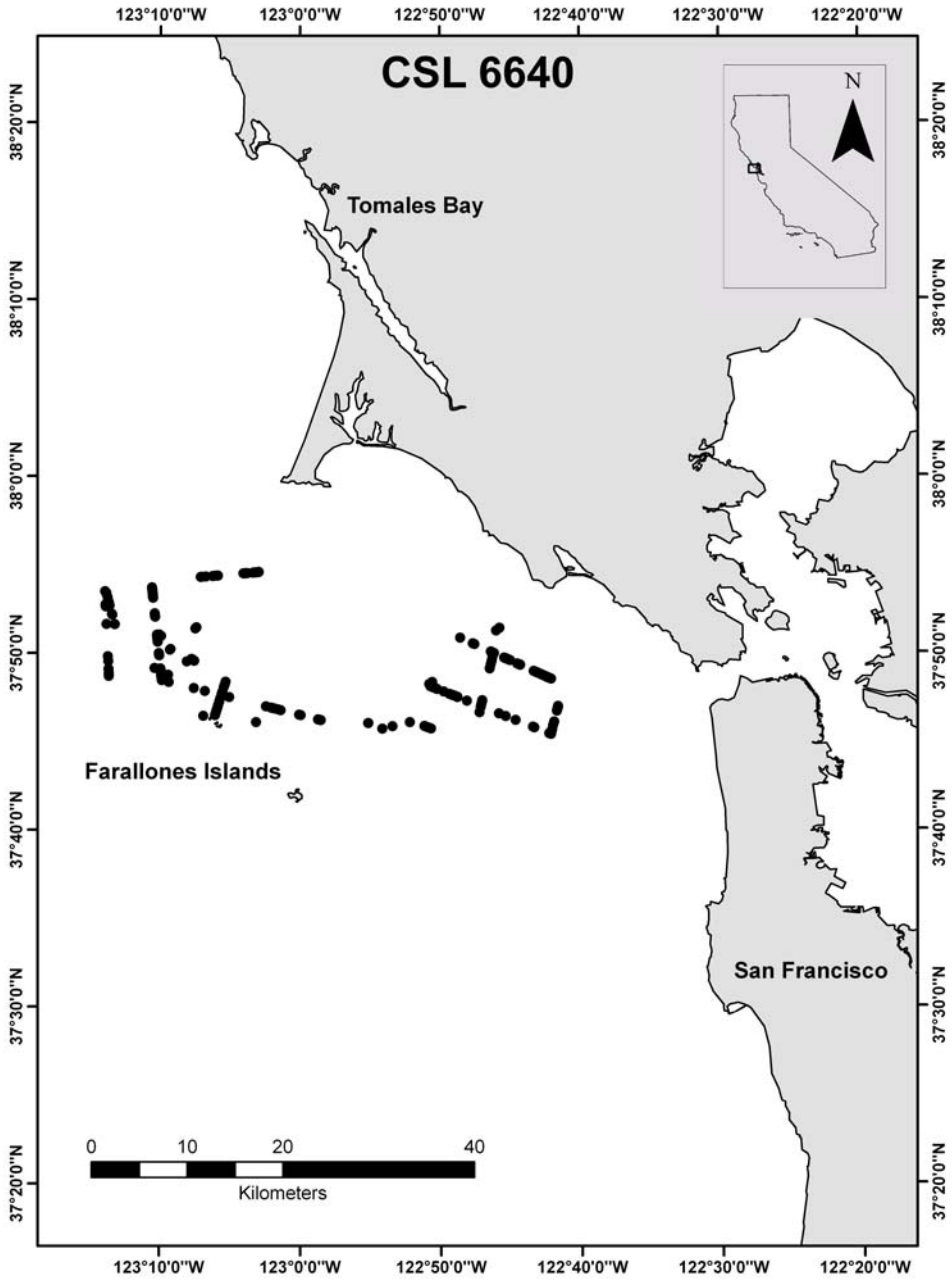


Fig C 9: Location data (black dots) for CSL 6640 between the mouth of San Francisco Bay and the Farallone Islands.

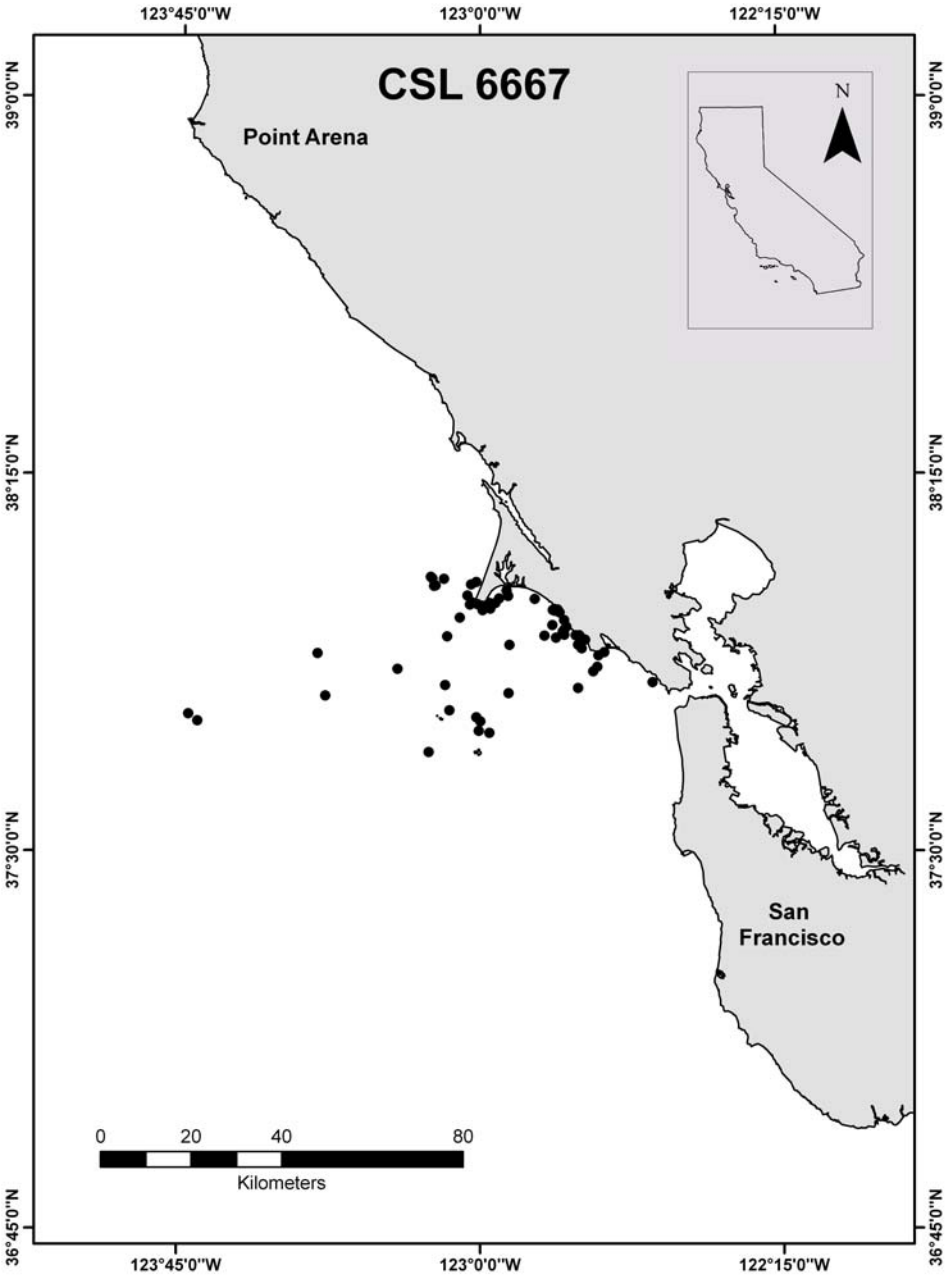


Fig C 10: Location data (black dots) for CSL 6667.

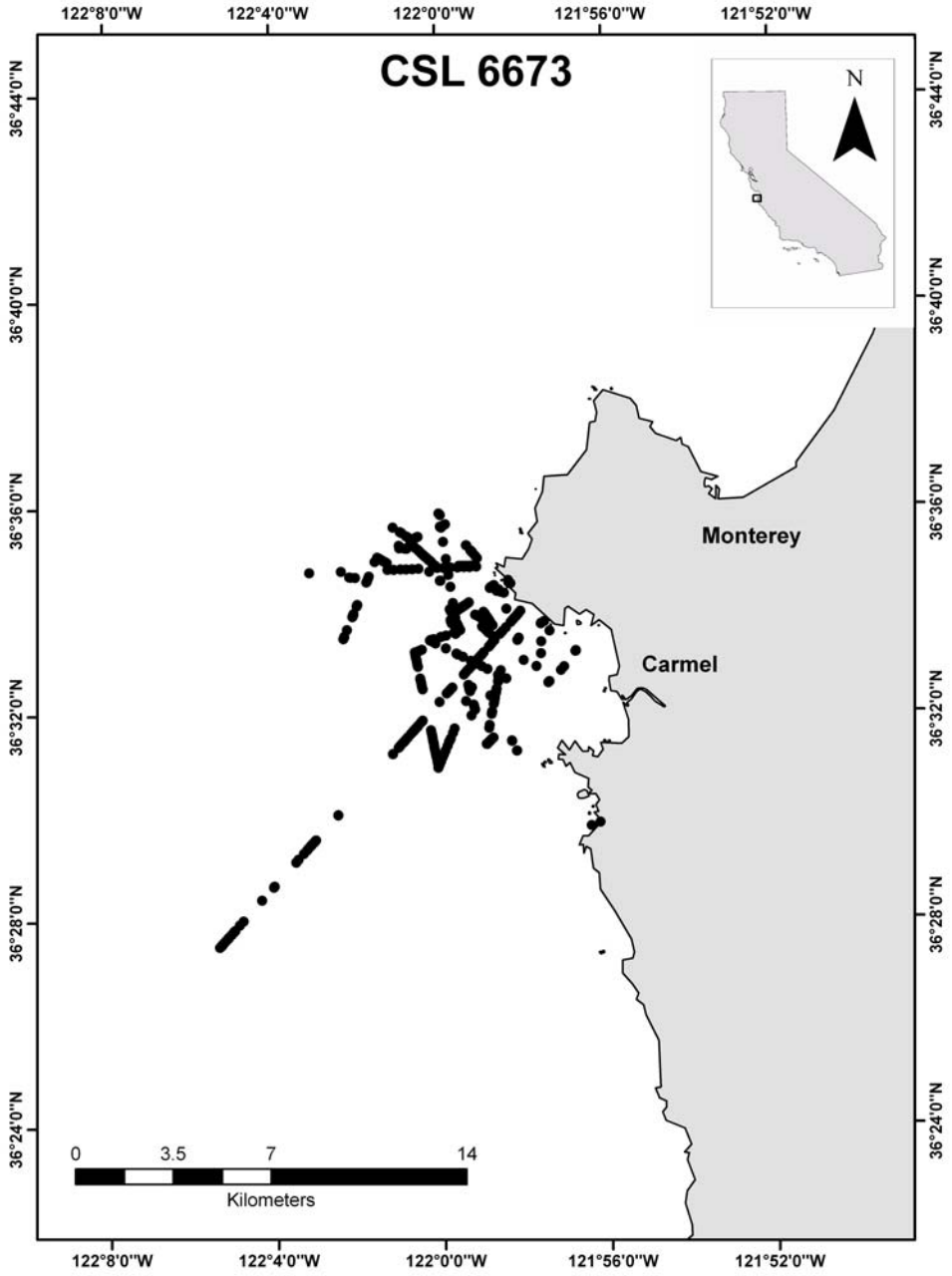


Fig C 11: Location data (black dots) for CSL 6673 offshore Carmel.

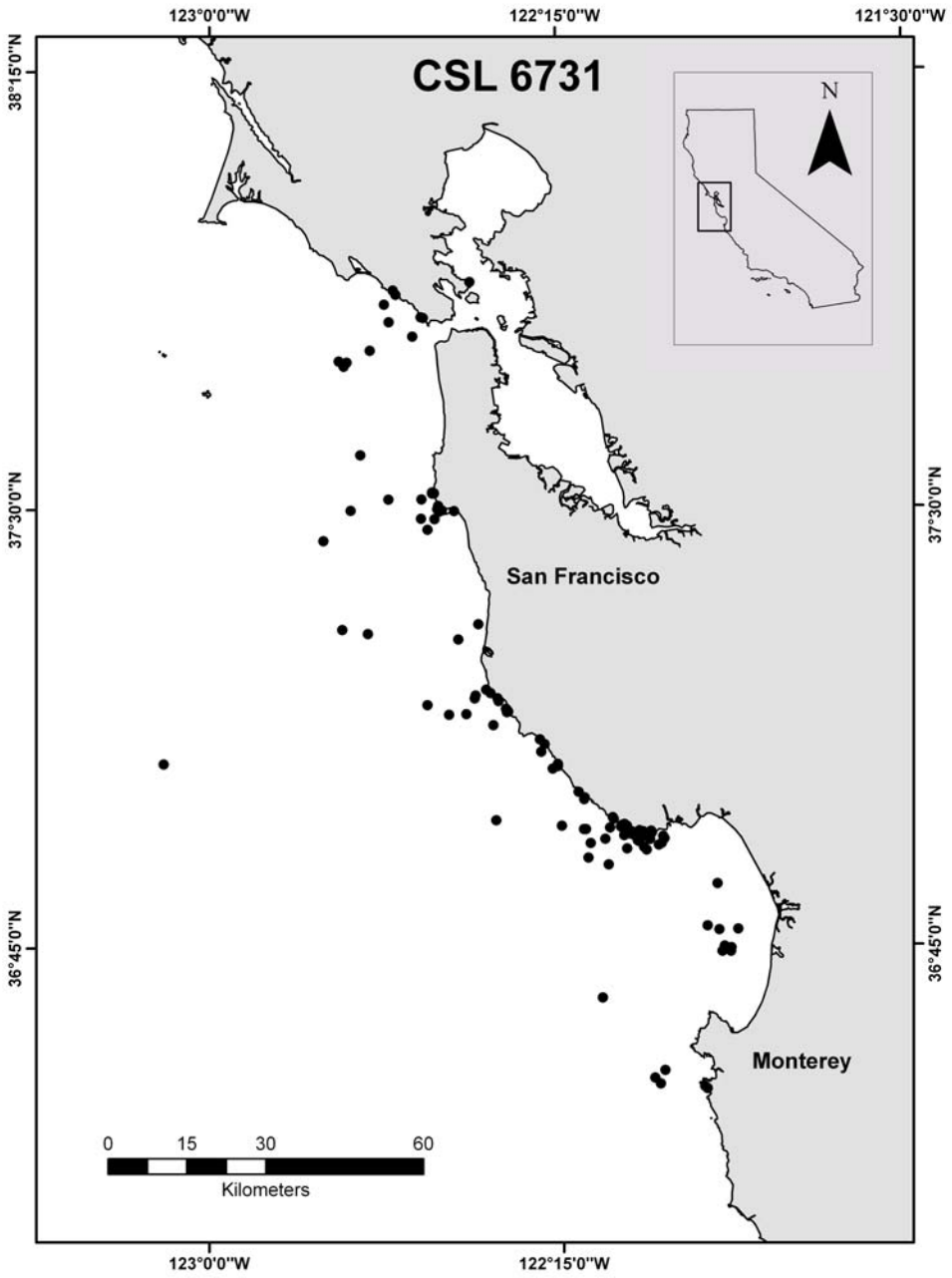


Fig C 12: Location data (black dots) for movements between Monterey and San Francisco for CSL 6731.

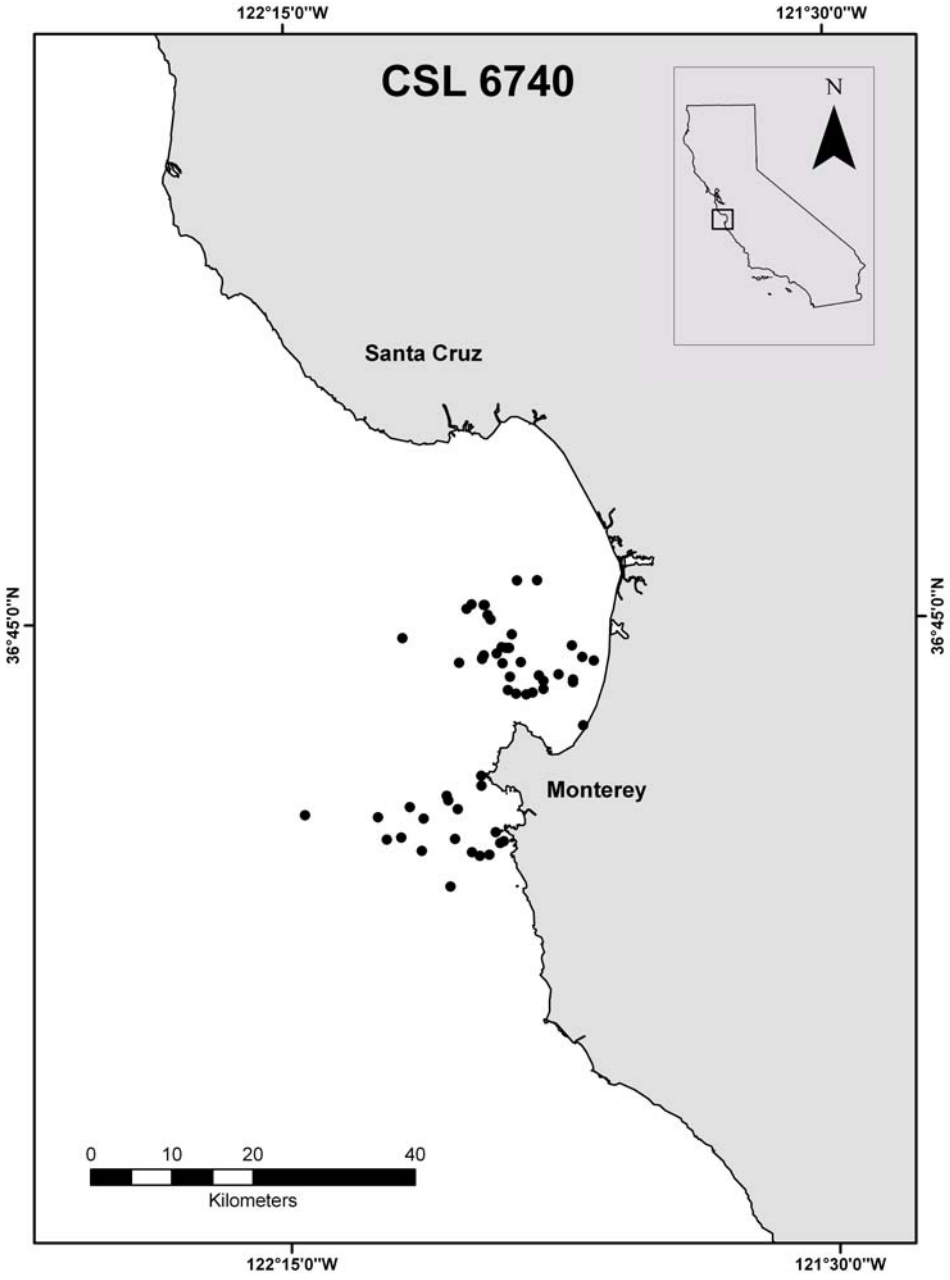


Fig C 13: Location data (black dots) for CSL 6740.

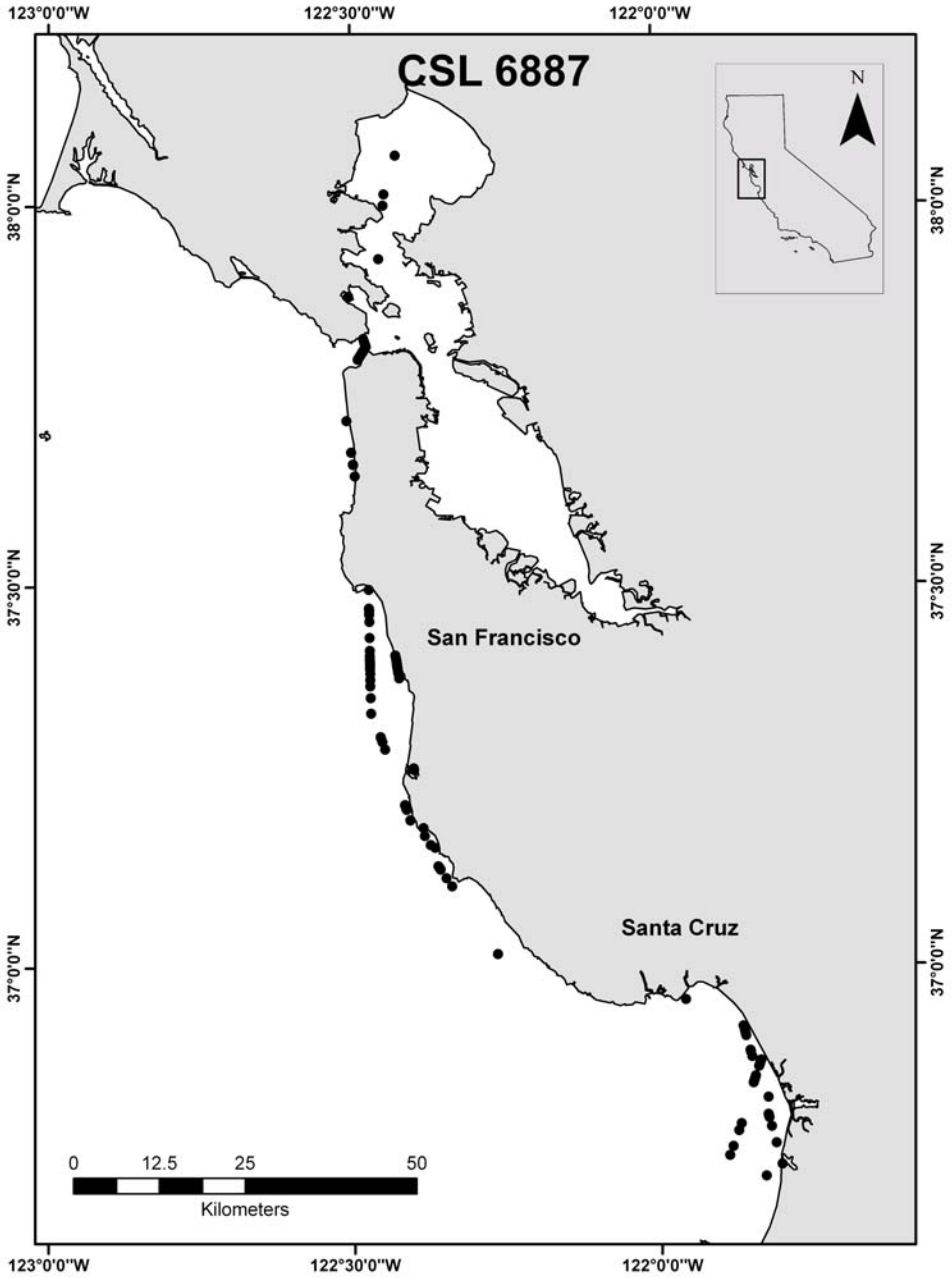


Fig C 14: Location data (black dots) for CSL 6887. Movement was from Monterey to San Francisco.

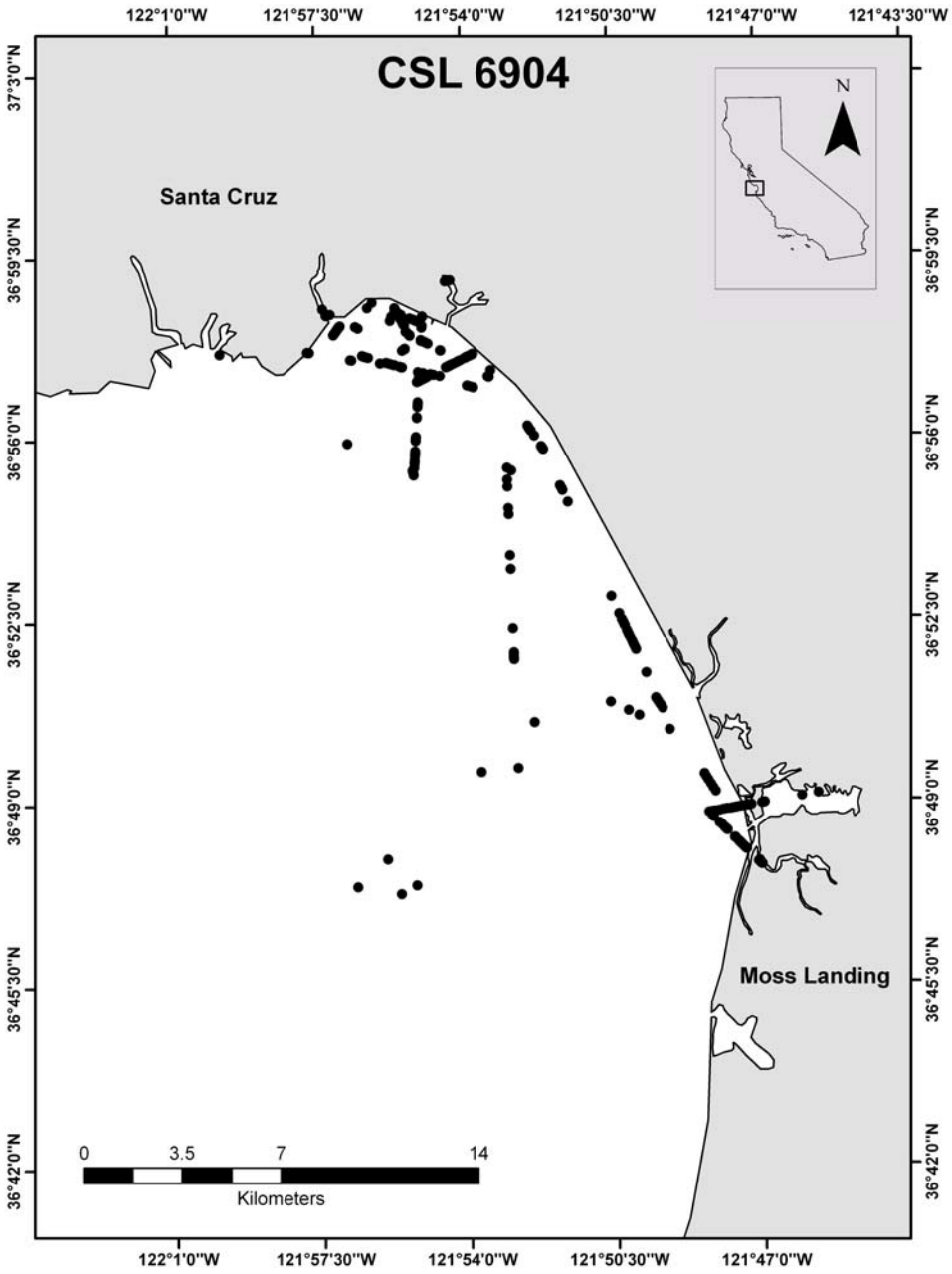


Fig C 15: Location data (black dots) in Monterey Bay for CSL 6904.

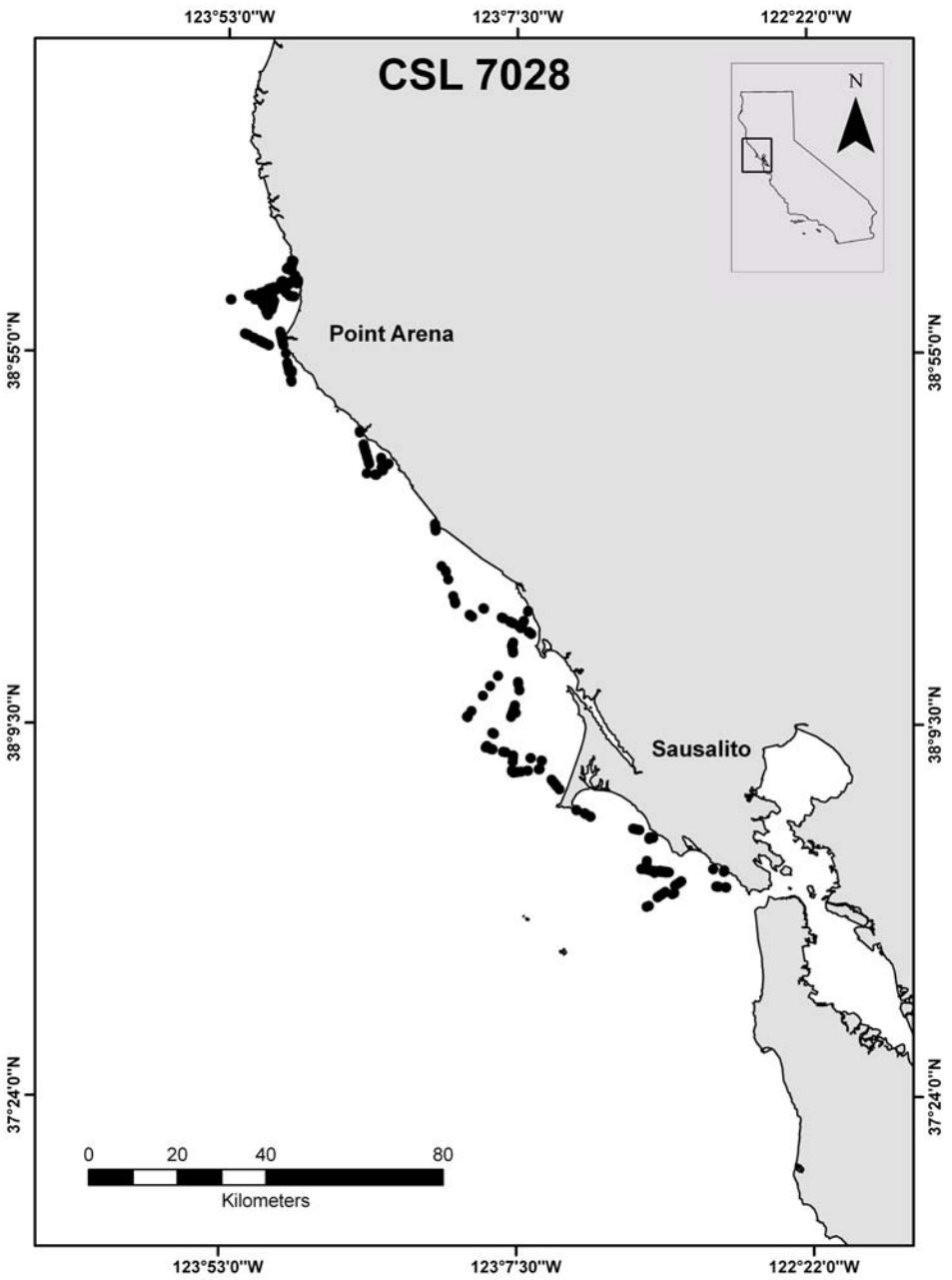


Fig C 16: Location data (black dots) for CSL 7028. Movement was north from Sausalito to Point Arena.

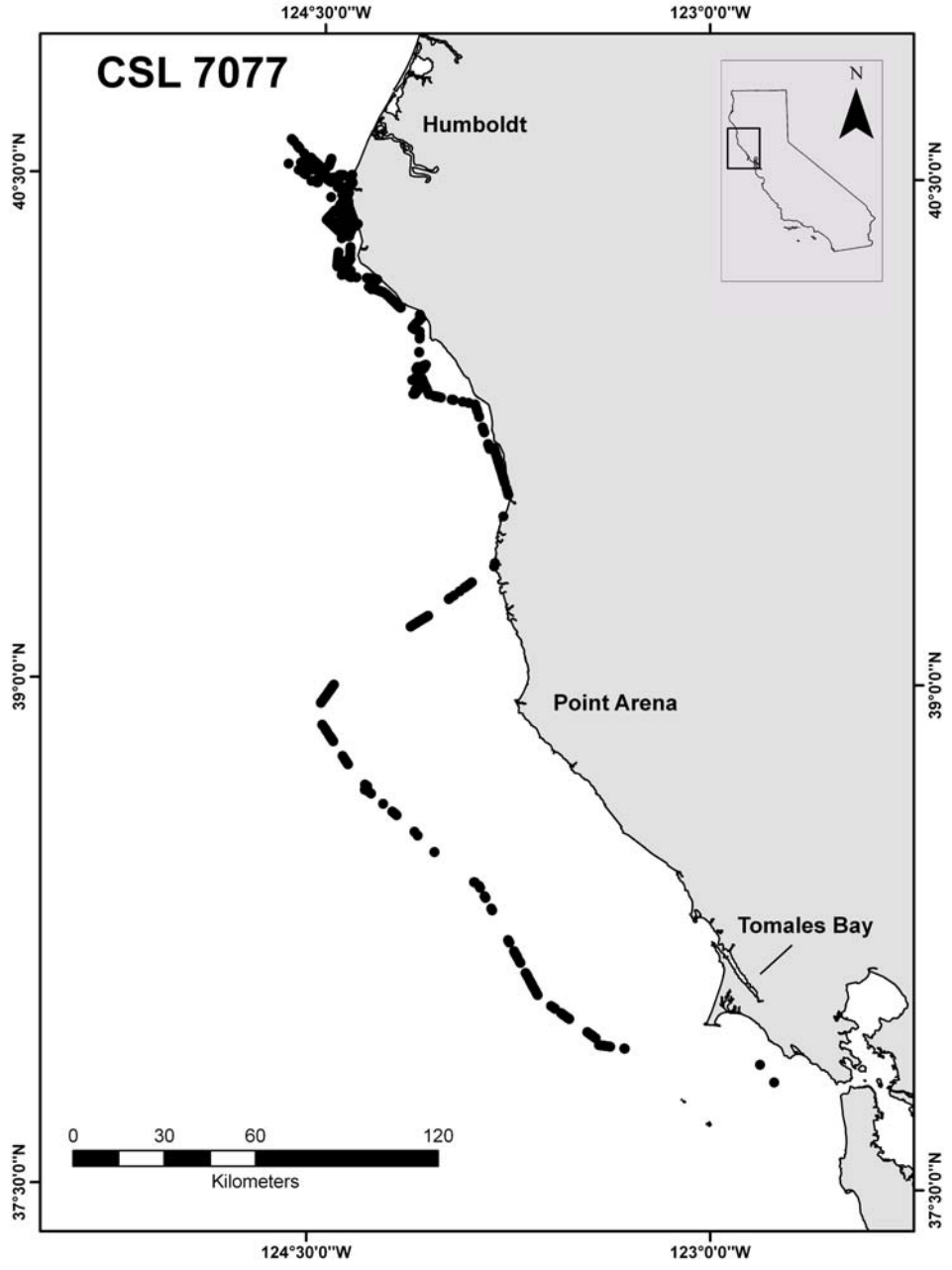


Fig C 17: Location data (black dots) displaying movements San Francisco to Humboldt for CSL 7077.

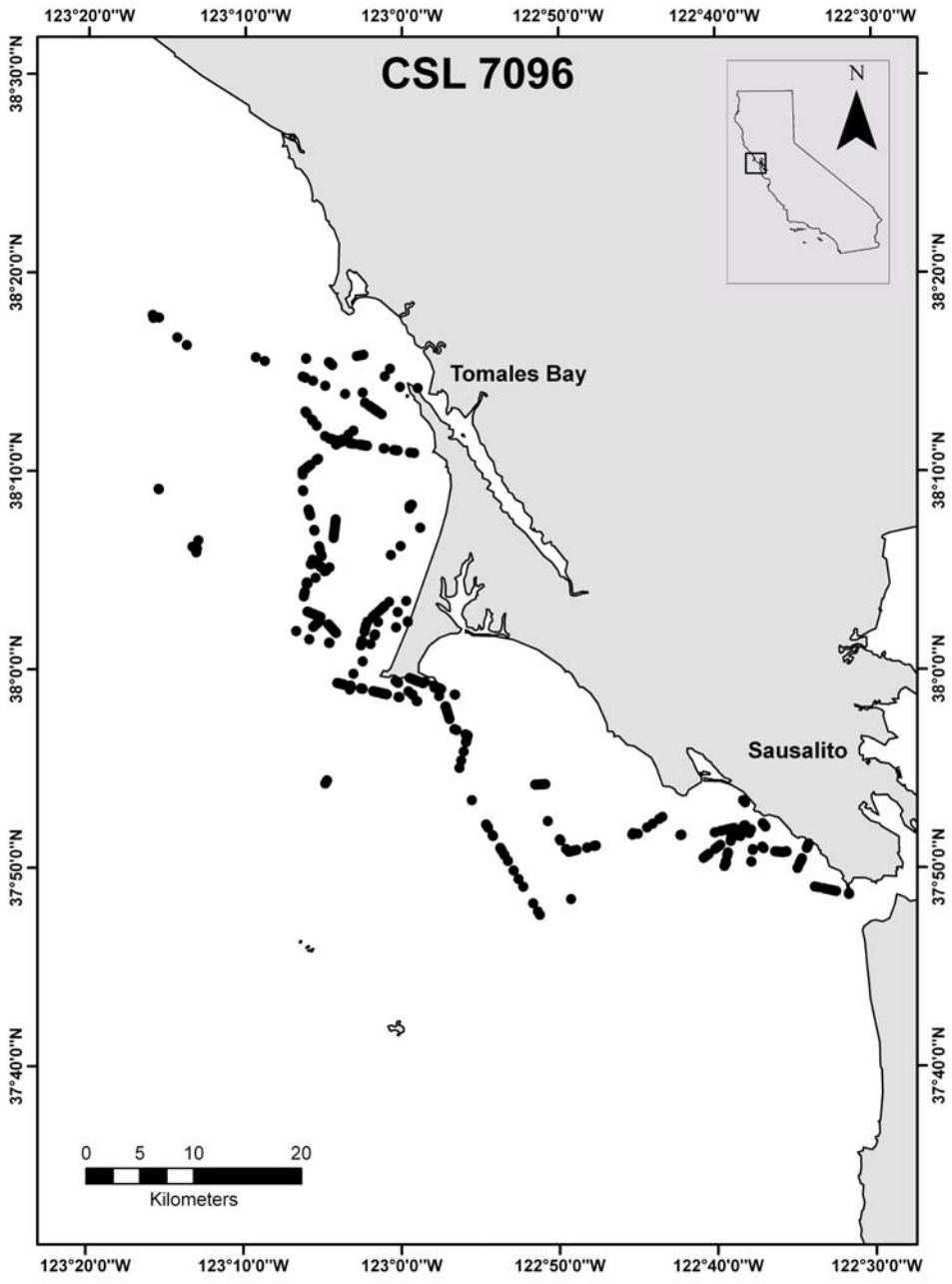


Fig C 18: Location data (black dots) showing northerly movements for CSL 7096.

Appendix D: Photo documentation of abnormal behaviors

Fig D 1: A California sea lion displaying flipper biting.

Fig D 2: A California sea lion displaying abnormal circular swimming.

Fig D 3: CSL 6584 with maggot infested head wound and a shark wound on her back.

Fig D 4: CSL 7096 chasing a surfer out of the water at Stinson Beach near San Francisco, California.

Fig D 5: CSL 7096 lethargic while interacting with people at a hot springs in central California.

Fig D 6: CSL 6887 disoriented in San Francisco, California.