Clinical signs and mortality of non-released stranded California sea lions housed in display facilities: the suspected role of prior exposure to algal toxins

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Abstract
Stranded California sea lions considered unable to survive in the wild are often placed in public display facilities. Exposure to the biotoxin domoic acid (DA) is a common cause of stranding, and chronic effects are observed long after initial exposure. Medical records for 171 sea lions placed in US institutions between 2000 and 2016 were reviewed, including results from clinical examinations, histopathology, behavioural testing and advanced imaging. There was a statistically significant increase in neurological disease detected in neonates (24%) compared with other age classes (11%). Sixty per cent of all neurological cases died during the study period. In the 11 neurological neonate cases, six died (55%) and five are still alive with three of five developing epilepsy during placement. Of the six neurological neonate cases that died, one was attributed to DA toxicosis, one to seizures and four to acute unexplained neurological disease. This survey suggests delayed neurological disease can develop in sea lions after stranding as neonates. These data coupled with stranding records and epidemiological data on DA-producing algal blooms suggest further research into effects of neonatal exposure to DA on risk of neurological disease in later life is warranted. California sea lions offer a natural model of DA exposure to study such effects.

Introduction
Exposure to the marine algal toxin domoic acid (DA) is the most common cause of neurological abnormalities in California sea lions (Zalophus californianus) that strand along the coast of California, USA, and has become increasingly common since first documented in 1998.1-3 DA is a potent neurotoxin produced by diatoms of the genus Pseudo-nitzschia that causes disease and death in a wide variety of vertebrate species, including humans.4 California sea lions are commonly affected because they forage in areas of frequent Pseudo-nitzschia blooms and eat sardines and anchovies that consume the diatom.5 DA toxicosis was first described in California sea lions in 1998, when more than 400 animals died during a Pseudo-nitzschia bloom.1 Acute neurological signs range from ataxia, head weaving and scratching, to seizures and coma.6 DA exposure can also lead to epilepsy, behavioural abnormalities (ranging from abnormal migrations to changes in auditory responses), neuropathology and cardiomyopathy.7-13 DA binds to cellular glutamate receptors, causing excitation and, potentially, excitotoxicity.14 It has a particular affinity for the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of glutamate receptors, and thus clinical signs most frequently result from damage to...
tissues with high concentrations of AMPA receptors, such as the hippocampus and myocardium. Within the hippocampus, the dentate gyrus is a common site of initial damage resulting from DA exposure.

DA can cause reproductive failure in sea lions through mortality of pregnant females, abortion and premature parturition. DA, which is water soluble, has been found in amniotic and fetal fluids, showing that DA crosses the placenta of adult female sea lions. While DA is typically cleared within hours from the bloodstream of exposed sea lions, it has been found in fetal fluids up to a week after the adult female sea lions stranded and hence since last potential ingestion of toxin. As the blood–brain barrier is incompletely developed in the fetus and in neonates, DA in fetal fluids is likely readily bioavailable to neuronal tissues. Additionally, DA can be passed from the sea lion dam to her pup through milk. Thus, DA exposure of fetal and neonatal sea lions occurs in the wild, but the effects of in utero and neonatal exposure of sea lions to DA are unknown to date. Studies in mice suggest that in utero exposure of fetal mice to DA causes hippocampal damage, which results in alterations in locomotor activity and changes in spatial learning in mice pups. Early postnatal DA exposure also results in alterations in spontaneous behaviour of rats that persists into adulthood.

In the USA, stranded sick and injured marine mammals are rehabilitated with the ultimate goal of release back into the ecosystem under authorisation of the federal government’s National Marine Fisheries Service (NMFS). Prior to release, developmental, behavioural, ecological and medical criteria are used to evaluate individual animals to assess release suitability. Individuals may be deemed non-releasable for a variety of reasons, including those that affect the ability to forage successfully. Such animals can be permanently housed for public display or research. Neurological disorders can impact release suitability and may be secondary to a variety of causes, including biotoxin exposure, meningitis, neoplasia or trauma.

Over the past decade, neonate California sea lions have stranded with DA-intoxicated mothers. Sea lions have also stranded for unknown reasons at times of harmful algal blooms; in these cases, in utero or lactational exposure to DA could have occurred. To investigate development, clinical signs and survival of these young sea lions, medical records for individuals that were not released and were maintained under human care post-stranding were reviewed.

Materials and methods
Stranded California sea lions included in this study were deemed non-releasable by a veterinarian at a NMFS-authorised rehabilitation facility and subsequently placed in public display facilities in the USA between January 2000 and December 2016. A survey requesting information about neurological signs, diagnostic imaging, treatment and disposition was sent to 60 of these organisations. When neurological signs were noted, complete medical records were reviewed. If an animal died, complete medical and pathology records were obtained from attending veterinary staff and reviewed. A single board-certified pathologist reviewed all pathology records and/or H&E-stained slides (Colegrove). Stranding network facilities that originally rehabilitated these animals were contacted, and medical records were reviewed to determine whether neurological issues played a role in the stranding. All records were anonymised, and individual cases reported here are referred to by their blinded study number.

Age classes
Sea lions of all age classes were included in the study. A neonate was defined as a sea lion that was either born in a rehabilitation facility or estimated to be less than 1 week old at the time of stranding based on external characteristics (umbilicus, tooth eruption). Because of a particular interest in neonate survival, all other age classes were grouped as non-neonates and included pups (1 week–1 year), yearling males and females (1–2 years), juvenile males (2–4 years), subadult males (4–8 years), juvenile and subadult females (2–5 years), adult males (5+ years) and adult females (5+ years). Age classes were defined as in Greig et al. Reasons for non-releasability
Reasons for non-releasability included young age (preweaned), chronic untreatable illness (including illnesses neurological in origin), habituation to humans, restrand (stranded more than twice and was likely to continue to restrand for behavioural reasons with no signs of disease) and trauma (anthropogenic and natural sources).

Neurological signs
Clinical signs that were considered potentially neurological in origin included seizures, tremors, head weaving, ataxia or abnormal behaviour for the species, such as repetitive regurgitation, and perceived learning delays.

Causes of death
Causes of death were classified as unknown: acute death (animals that died acutely, with no observed neurological signs and with no specific postmortem findings to explain the death), suspect drowning (animals in which drowning was suspected based on lesions, such as pulmonary oedema, or observations surrounding death), seizures and their sequelae (eg, drowning or aspiration), DA toxicosis (clinical signs consistent with neurological disease, as well as lesions to the limbic system characteristic of DA toxicosis determined via either MRI, gross examination or histopathology including but not restricted to atrophy,
gliosis and neuronal necrosis in the hippocampus\(^6\)\(^\text{12}\)\(^\text{29}\) or other (e.g., metastatic neoplasia, infarcts and infectious meningoencephalitis).

**Advanced imaging and behavioural study**

A subset (n=6) of sea lions (nos. 23, 28, 30–33) was part of a previous study that compared behavioural performances in controlled spatial memory tests with hippocampal volumes measured using MRI, and hippocampal integrity was assessed by a veterinary radiologist blinded to the animal’s condition.\(^1\)\(^1\) These six cases had the most comprehensive pre-placement evaluations for comparison with subsequent survey data and included one neonate and five non-neonates.

One animal (no. 3) that was not part of the previous behavioural study died following an unknown acute event during the current study period, and the brain was extracted post mortem. A frontal section was removed for histology, and the cerebellum and brainstem were also removed. The brain was fixed in formalin and then shipped to Emory University for MRI. Preparation for scanning and structural and diffusion tensor imaging (DTI—a scanning approach that allows imaging white matter tracts) protocols were the same as used in Cook et al.\(^1\)\(^3\) High-resolution (0.6×0.6×0.5 mm) structural images were used for assessing hippocampal volumes. Hippocampal tissue was identified and traced in the transverse plane, as in Cook et al.\(^1\)\(^3\) Left and right hippocampuses were traced separately, and the caudal dorsal and rostroventral volumes of each hippocampus were estimated by evenly dividing the hippocampuses by number of slices along that axis. Because of the missing frontal section, whole-brain volume (minus cerebellum) was computed by measuring the complete hemisphere and multiplying by two. DTI data were acquired using 52 directions and 1 mm isotropic voxels. The fornix, a primary tract connecting the hippocampus to the thalamus and mammillary bodies, was manually traced in both right and left hemispheres. Fractional anisotropy values, which are associated with white matter integrity,\(^1\)\(^3\) were extracted from each fornix and averaged.

**Results**

All 60 US institutions responded to the survey request (table 1). A total of 171 California sea lions were deemed non-releasable and placed at these institutions between 2000 and 2016 (table 2). Of these individuals, 54% were male and 46% were female. The most common cause for placement was a history of re-stranding (45%, n=77), followed by pre-weaning neonatal stranding (27%, n=46). When placed at an institution, 76% were pups/yearlings, 19% were juveniles/subadults and 5% were adults. Of all animals placed in managed care, 17% (29/171) had died at the time of the survey. Animals that died during the study period (n=29) were housed in managed care for a mean of 2.9 years (range

| Participating rehabilitation centres | US city, state |
|-------------------------------------|----------------|
| California Wildlife Center          | Calabasas, California |
| Channel Islands Marine Wildlife Institute | Santa Barbara, California |
| Marine Mammal Care Center Los Angeles | San Pedro, California |
| Northcoast Marine Mammal Center     | Crescent City, California |
| Oregon Coast Aquarium               | Newport, Oregon     |
| Pacific Marine Mammal Center        | Laguna Beach, California |
| Santa Barbara Marine Mammal Center  | Santa Barbara, California |
| SeaWorld San Diego                  | San Diego, California |
| The Marine Mammal Center            | Sausalito, California |

**Participating managed care institutions**

- Aquarium at Moody Gardens
- Aquarium of the Pacific
- Atlantis Marine World
- Audubon Zoo and Zoological Garden
- Birmingham Zoo
- Blank Park Zoo
- Brookfield Zoo
- Buffalo Zoo
- Cincinnati Zoo
- Cleveland Metroparks Zoo
- Denver Zoo
- Dolphin Cove Research and Education Center
- Dolphin Research Center
- Fresno Chaffee Zoo
- Georgia Aquarium
- Gladys Porter Zoo
- Gulf World Marine Park
- Houston Zoo
- Institute of Marine Mammal Science
- Indianapolis Zoo
- Kansas City Zoo
- Long Marine Lab
- Louisville Zoo
- Miami Seaquarium
- Miller Park Zoo
- Oceans of Fun (at the Milwaukee County Zoo)
- Moss Landing Marine Lab
- Mystic Aquarium
- Smithsonian National Zoological Park
- New England Aquarium
- North Carolina Zoo
- Ocean World
- Oklahoma City Zoo
- Olin’s Henry Doorly Zoo
- Oregon Coast Aquarium
- Pittsburgh Zoo
- Point Defiance Zoo
- Riverbanks Zoo
- San Diego Zoo
- San Francisco Zoo
- Sea Life Park Hawaii
- SeaWorld San Diego
- SeaWorld Orlando
- Seneca Park Zoo
- Shedd Aquarium
- Six Flags Discovery Kingdom
- Squalus, Inc (Sea Lion Splash)
- St. Paul’s Como Zoo

| US city, state |
|----------------|
| Galveston, Texas |
| Long Beach, California |
| Riverhead, New York |
| New Orleans, Louisiana |
| Birmingham, Alabama |
| Des Moines, Iowa |
| Brookfield, Illinois |
| Buffalo, New York |
| Cincinnati, Ohio |
| Cleveland, Ohio |
| Denver, Colorado |
| Key Largo, Florida |
| Grass Key, Florida |
| Fresno, California |
| Atlanta, Georgia |
| Brownsville, Texas |
| Panama City Beach, Florida |
| Houston, Texas |
| Gulfport, Mississippi |
| Indianapolis, Indiana |
| Kansas City, Missouri |
| Santa Cruz, California |
| Louisville, Kentucky |
| Miami, Florida |
| Bloomington, Illinois |
| Milwaukee, Wisconsin |
| Moss Landing, California |
| Stonington, Connecticut |
| Washington, DC |
| Boston, Massachusetts |
| Asheville, North Carolina |
| Crescent City, California |
| Oklahoma City, Oklahoma |
| Dinah, Nebraska |
| Newport, Oregon |
| Pittsburgh, Pennsylvania |
| Tacoma, Washington |
| Columbia, South Carolina |
| San Diego, California |
| San Francisco, California |
| Waimanalo Beach, Hawaii |
| San Diego, California |
| Orlando, Florida |
| Rochester, New York |
| Chicago, Illinois |
| Vallejo, California |
| Myakka City, Florida |
| St. Paul, Minnesota |

**Continued**
0–10 years), while animals that were alive at the time of the survey (n=142) were housed in managed care for a mean of 4.7 years (range 0–14 years).

**Neurological clinical signs**

Of the 171 animals in the study, 25 (15%) exhibited clinical neurological signs (figure 1). Nine animals exhibited neurological signs during rehabilitation, but 16 animals developed neurological signs only after placement. Fifteen of the 25 sea lions with signs of neurological disease (60%) died during the study period. In addition, one individual (no. 2) that had not previously exhibited any neurological signs died acutely with no identifiable cause.

**Pathology and cause of death**

Twenty-nine animals died during the study period, including eight neonates and 21 non-neonates. Every animal that died received a gross necropsy and partial histopathological evaluation and was assigned a cause of death (other=18, seizures=4, unknown acute death=4, suspect drowning=2, DA=1; table 3). Overall, the central nervous system (CNS) was evaluated in 22/29 (76%) of total cases and the hippocampus was evaluated in 12/29 (41%) of total cases; in neurological cases or cases of sudden, unexplained death, the CNS was evaluated in 14/16 (88%) of cases and the hippocampus was evaluated in 9/16 (56%) of cases. Of the 22 overall cases in which the CNS was evaluated, histopathological lesions were noted in the brain in 11 cases (50%). In five cases, these lesions were not related to DA exposure (coccidioidomycosis (no. 15), toxoplasmosis (no. 7), infarcts from previous trauma (no. 28) and neoplasia (no. 16)). Non-specific findings of cerebral oedema or multifocal haemorrhage were noted in six animals (nos. 6, 9, 10, 13, 14 and 22) that died acutely, drowned, died following seizures or had an unrelated, non-neurological cause.

Mild non-suppurative encephalitis centred over the hippocampus was observed in one case (no. 5), with chronic mild to moderate fibrosing cardiomyopathy, consistent with DA toxicity.29

**Neonates**

A significant difference was found (χ² (1, N=171)=4.35, p<0.05) in comparing the prevalence of neurological cases in neonates with all other age classes. The prevalence of neurological cases was higher among neonates (11/46 cases; 24%) than among non-neonates (14/125; 11%).

Of the 46 neonatal cases, 38 were alive at the time of this survey with 33 not experiencing neurological signs to date (figure 2). However, five live neonates were neurological cases; one individual (no. 34) experienced seizures during rehabilitation but had not exhibited any neurological signs in managed care to date and had not received any treatment; since placement, one individual (no. 35) experienced mild tremors, and three individuals (nos. 36–38) had chronic epilepsy that was managed with medication.

Eight neonates died during the study period, with 75% (6/8) having cause of death compatible with neurological disease. One animal (no. 5) exhibited the mild hippocampal-focused encephalitis attributable to DA toxicosis, described above. This animal was born to a dam in rehabilitation that stranded during a documented harmful algal bloom. The dam died during rehabilitation, but no necropsy was performed. For five of the seven other neonatal cases (nos. 1–4, 6), either seizures or acute death were reported as the cause of death and were compatible with neurological disease. The CNS was evaluated in only 63% of neonate cases. Overall, in the 11 neurological neonatal cases, 55% died (6/11) while 45% (5/11) were alive at the end of the study period.

**Advanced imaging and behavioural testing outcomes**

Of the six animals that were part of a previous study on hippocampal volume and memory performed during their time in rehabilitation,11 two died during the study period and four were alive (table 4). Of the dead animals, one individual (no. 23) had no lesions detectable on MRI and did not develop neurological signs but died of haemorrhagic gastroenteritis. One individual (no. 28) had no hippocampal lesions detected by pre-placement MRI or by post-placement postmortem histology, but had cerebellar infarcts presumed secondary to trauma which were likely responsible for the extremely slow

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**Table 1**

| Participating rehabilitation centres | US city, state |
|-------------------------------------|----------------|
| Theater of the Sea                 | Islamorada, Florida |
| Tulsa Zoo                          | Tulsa, Oklahoma |
| Turtle Back Zoo                    | West Orange, New Jersey |
| U.S. Navy Marine Mammal Program    | San Diego, California |
| Utah's Hogle Zoo                   | Salt Lake City, Utah |
| Utica Zoo                          | Utica, New York |
| WCS (Bronx Zoo)                    | The Bronx, New York |
| WCS (Central Park Zoo)             | New York City, New York |
| WCS (New York Aquarium)            | Brooklyn, New York |
| WCS (Prospect Park Wildlife Center) | Brooklyn, New York |

**Table 2**

| Reason for non-releasability | Total placed | Clinical neurological signs | Died following placement |
|-----------------------------|--------------|---------------------------|--------------------------|
| Preweaned                   | 46           | 11                        | 8                        |
| Chronic illness             | 16           | 3                         | 5                        |
| Habituation                 | 7            | 0                         | 1                        |
| Restrand                    | 77           | 7                         | 9                        |
| Trauma                      | 25           | 4                         | 6                        |
| Total                       | 171          | 25                        | 29                       |
Figure 1  Case progression for neurological cases (25/171), describing California sea lions that developed neurological signs following placement, and their findings on necropsy and histopathology. Neurological cases are highlighted in grey. CNS, central nervous system.

Postmortem advanced imaging
Only one individual (no. 3) in the survey received postmortem brain imaging. This individual stranded as a neonate and exhibited ataxia prior to acute death. No pathology was noted in the CNS on histology. Postmortem MRI imaging was performed. Regional hippocampal volumes expressed as percentage of whole brain volume were as follows: right dorsal, 0.08%; right ventral, 0.14%; left dorsal, 0.15%; left ventral, 0.15%. Regional volumes were within the typical range for healthy sea lions previously measured with the exception of the right dorsal hippocampus, suggesting significant atrophy. Average fractional anisotropy (FA) values in the traced fornixes were right, 0.58 and left, 0.68. It is difficult to compare these FA values with prior findings (Cook et al) due to slight alterations in the image analysis methods used, but this suggests possible white matter pathology in the right fornix in the present case. Right dorsal hippocampal damage has previously been strongly associated with spatial memory deficits in sea lions, and fornix damage is associated with epilepsy and generalised memory deficits. These findings indicate significant potential cognitive impairment in this individual.
Table 3  Cause of death and histopathological lesions in the central nervous system of non-releasable California sea lions placed at US institutions between 2000 and 2016 that died during this time period (n=29)

| Blinded study number | Age class at stranding | Reason for non-releasability | Neurological signs | CNS lesions | Hippocampal lesions | Cause of death |
|----------------------|------------------------|-----------------------------|-------------------|-------------|---------------------|----------------|
| 1                    | Neonate                | Preweaned                   | Seizures          | NE          | NE                  | Unknown—acute death |
| 2                    | Neonate                | Preweaned                   | NA                | NE          | NE                  | Unknown—acute death* |
| 3                    | Neonate                | Preweaned                   | Ataxia            | Normal      | Normal              | Unknown—acute death |
| 4                    | Neonate                | Preweaned                   | Seizures          | Normal      | Normal              | Unknown—acute death |
| 5                    | Neonate                | Preweaned                   | Repetitive regurgitation | Mild multifocal non-suppurative encephalitis focused on hippocampus | Perivascular cuffing | MF non-suppurative inflammation | Domoic acid toxicosis |
| 6                    | Neonate                | Preweaned                   | Seizures          | Acute cerebral oedema | Normal | Seizures |
| 7                    | Neonate                | Preweaned                   | Seizures          | Non-suppurative meningoencephalitis due to Toxoplasmosis infection | Unknown | Other |
| 8                    | Neonate                | Preweaned                   | NA                | NE          | NE                  | Other |
| 9                    | Non-neonate (juvenile) | Restrand                    | NA                | MF haemorrhage | Unknown | Suspect drowning |
| 10                   | Non-neonate (adult)    | Restrand                    | NA                | Meningeal oedema | Unknown | Suspect drowning |
| 11                   | Non-neonate (yearling) |慢性| Seizures          | Normal      | Normal              | Seizures |
| 12                   | Non-neonate (yearling) |慢性| Seizures          | MF haemorrhage | Normal | Seizures |
| 13                   | Non-neonate (yearling) |慢性| Seizures          | MF Purkinje cell necrosis | NE | Other |
| 14                   | Non-neonate (adult)    | Restrand                    | Seizures          | Other | Other |
| 15                   | Non-neonate (yearling) |慢性| Alaxia, head twitching | Meningitis due to Coccioides infection | Unknown | Other |
| 16                   | Non-neonate (juvenile) |慢性| Seizures          | Metastatic neoplasia | Normal | Other |
| 17                   | Non-neonate (juvenile) |慢性| NA                | Unknown | Other |
| 18                   | Non-neonate (yearling) |慢性| Seizures          | Normal      | Normal              | Other |
| 19                   | Non-neonate (yearling) |慢性| Obftundation, nystagmus | Normal | Unknown | Other |
| 20                   | Non-neonate (pup)      | Trauma                      | NA                | Normal      | NE                  | Other |
| 21                   | Non-neonate (pup)      | Restrand                    | NA                | Unknown      | Unknown | Other |
| 22                   | Non-neonate (pup)      | Trauma                      | NA                | Mild perivascular haemorrhage | Normal | Other |
| 23                   | Non-neonate (pup)      | Restrand                    | NA                | NE          | NE                  | Other |
| 24                   | Non-neonate (pup)      | Habitation                  | NA                | Normal      | Normal              | Other |
| 25                   | Non-neonate (pup)      | Chronic illness             | NA                | Normal      | Unknown              | Other |
| 26                   | Non-neonate (adult)    | Chronic illness             | NA                | NE          | NE                  | Other |
| 27                   | Non-neonate (pup)      | Trauma                      | NA                | NE          | NE                  | Other |
| 28                   | Non-neonate (yearling) |慢性| Ataxia, hypermetria | Infarctionts | Unknown | Other |
| 29                   | Non-neonate (pup)      | Restrand                    | NA                | Normal      | Normal              | Other |

*Sudden, unexplained death.
CNS, central nervous system; MF, multifocal; NA, not applicable; NE, not evaluated.

Discussion
Neurological disease is relatively common in non-releasable California sea lions, as 15% (25/171) of animals in the study exhibited neurological clinical signs, two-thirds of which developed neurological signs only after placement, exhibiting no signs during rehabilitation. Of the neurological cases, 60% died during the study period. For sea lions that exhibit acute neurological signs during rehabilitation, the most common cause currently is DA toxicosis. Animals that experience acute exposure and neuronal necrosis can develop a chronic epileptic syndrome that is characterised by behavioural changes, seizures, progressive neuronal stress and eventual neuronal loss and hippocampal atrophy. To date, chronic neurological disease has followed acute symptoms. However, here we document that the development of neurological signs and/or death can occur years after long periods of normal behaviour, often with no previously observed neurological disease. The results of this study suggest that delayed manifestation of neurological disease can occur following DA exposure during development and may lead eventually to death.

CNS lesions typically associated with DA toxicosis were only found in one sea lion placed in managed care as a neonate (no. 5), while in five neonatal cases a cause for seizures and/or acute death was not found (nos. 1–4, 6). Therefore, in 75% (6/8) of neonatal cases that died,
neurological disease may have been present, although detailed brain evaluation was not performed in all cases. Additionally, as was noted post mortem in five of the neurological cases, acute onset of perimortem seizures or neurological deficits may be secondary to diseases such as metastatic neoplasia, electrolyte disturbances from renal or hepatic dysfunction, or infectious diseases such as protozoal meningoencephalitis\(^{27,28}\), therefore, a definitive link to DA toxicosis in these neonate cases cannot be confirmed due to limited data.

Three neonatal sea lions (nos. 36–38) developed chronic epilepsy following placement, and their seizures are presently managed with medication. None of these individuals exhibited neurological signs during rehabilitation. However, because they stranded as neonates following a known DA bloom, their stranding history is consistent with DA exposure in utero.

Collectively, these findings support the hypothesis that developmental DA exposure may cause changes in the brain, but those changes are not consistent with the typical hippocampal atrophy or necrosis observed in older sea lions affected acutely by DA exposure. The abnormalities may not be readily observed on routine histopathology of the brain and advanced

### Table 4: Non-releasable California sea lions that were part of a previous study (Cook et al.) that compared behavioural performances in spatial memory tests with hippocampal volumes, measured using MRI (n=6)

| Blinded study number | Age class at stranding | Reason for non-releasability | MRI hippocampal findings | Behavioural testing results | Disposition | Cause of death |
|----------------------|------------------------|-------------------------------|---------------------------|-----------------------------|-------------|----------------|
| 23                   | Non-neonate            | Restrained                    | Normal                    | Normal                      | Died        | Other          |
| 28                   | Non-neonate            | Trauma                        | Normal*                   | Slow task acquisition       | Died        | Other          |
| 30                   | Non-neonate            | Restrained                    | Bilateral hippocampal atrophy | Poor delayed maze testing | Alive       | NA             |
| 31                   | Non-neonate            | Restrained                    | Initial MRI normal; post-placement MRI showed unilateral hippocampal atrophy | Normal | Alive | NA |
| 32                   | Non-neonate            | Restrained                    | Normal                    | Normal                      | Alive       | NA             |
| 33                   | Neonate                | Age                            | Left hippocampal atrophy | Poor maze and spatial memory performance | Alive | NA |

These assessments were performed following stranding and prior to placement. Neurological cases are shaded in grey.

*This animal had multiple cerebellar infarcts secondary to trauma, which were likely responsible for neurological signs and behavioural testing changes.

NA, not applicable.
techniques may be needed to characterise the lesions. DA toxicosis may also cause changes in connectivity and communication between different brain regions in sea lions, which may not always cause gross or histopathological lesions. In a previous study, 21% of animals that died with chronic neurological signs showed minimal or no typical DA-related lesions. All of these animals were immature animals, and developmental exposure to DA may have caused changes in the brain that were not identifiable or are yet to be characterised. Gross lesions (measured volumetrically via MRI or identified by the naked eye) are believed to be the result of repeated insult from chronic epilepsy. Some of the initial changes associated with DA exposure can be subtle, involving recursive synapsing in the dentate gyrus (excitotoxic driven plasticity), and may not be visible with routine H&E staining on histopathology. Further investigation is required to elucidate the effects of DA on the developing fetus, and non-releasable sea lions have the potential to play an important role in this research.

Assessment of hippocampal volume on MRI appears to be a potentially useful tool to predict future development of neurological signs, and behavioural testing may be able to identify animals with hippocampal pathology. In addition to potential memory deficits, previous studies have shown animals with DA habituate more slowly to auditory stimuli than do those sea lions with no apparent neurological deficits. Rodent data indicate that foraging errors in spatial choice tasks track hippocampal damage, which is consistent with the well-established contribution of the hippocampus to spatial memory in multiple species. One (no. 31) of the six individuals in the pre-placement behavioural study is of particular interest since the animal did not exhibit hippocampal atrophy on an original brain MRI (prior to the development of neurological signs), but later developed seizures and hippocampal atrophy. This animal that stranded as a yearling was housed at a facility where fish were routinely tested for the presence of DA, and water was tested for Pseudo-nitzschia spp. when reports of nearby blooms were a concern. Thus, it is highly unlikely that the development of hippocampal lesions was due to recent acute exposure to the biotoxin. Following acute DA toxicosis, a latent period of silent toxicity and structural damage characterises the transition to epileptic disease, and delayed epilepsy onset has been observed in early exposure rodent models. This last case supports the idea that detectable damage can present much later than the initial insult, and highlights the importance of advanced imaging at the onset of clinical signs, regardless of the level of suspicion for DA exposure.

Given the limited understanding of developmental effects of DA exposure in sea lions, and the fact that neurological disease can have a variety of aetiologies, systematic evaluation both premortem and postmortem is critical. One hundred forty-two placed sea lions are alive to date with no neurological signs. We suggest that animals that develop neurological signs—whether they survive or die—should receive an MRI to characterise brain lesions and potentially function. Combined with brain imaging, longitudinal neurobehavioural assessment of even a small subset of animals believed to be exposed in utero could provide important information about the developmental effects of in utero DA exposure in sea lions. Post mortem, neurological cases in particular should have a standard suite of CNS tissues collected for histopathological examination, and frozen for ancillary diagnostics if necessary. Because DA toxicosis is the leading cause of neurological disease in California sea lions, the hippocampus should be evaluated in all neurological cases. See online supplementary file 1 for further details on diagnostic protocols.

It is important to note that in six dead cases (including one neurological case and one case of sudden death, nos. 1, 2, 8, 23, 26, 27), the CNS was not evaluated. One individual (no. 14) was deemed non-releasable after a suspicion of having chronic DA toxicosis. It experienced seizures both in rehabilitation and after placement, and died during the study period. The cause of death was lymphosarcoma, and only non-specific multifocal Purkinje cell necrosis was reported in the CNS. The hippocampus was not examined, highlighting the particular need to evaluate the hippocampus in individuals for whom DA exposure is suspected. Given the lack of CNS testing in these individuals, the confirmed 11 neurological cases of the 171 individuals evaluated may be somewhat conservative for this sample.

As a result of environmental changes and anthropogenic impacts on the marine ecosystem, the size and frequency of DA-producing Pseudo-nitzschia algal blooms are increasing. Many wild California sea lions are likely to be exposed to at least low levels of DA at some time in their lives, and the combination of potential developmental, acute and/or chronic exposure to DA may continue to alter the clinical presentation of the disease in these animals in the future. Rehabilitation facilities, permitting agencies and managed care institutions must recognise the risk of neurological disease for each animal that is deemed non-releasable and placed in permanent care, and continue to investigate behavioural, clinical and morphological changes in these animals to improve our understanding of the impacts of DA exposure on mammals. Additional research and investigation into this topic may allow veterinarians to predict long-term outcomes which can lead to enhanced survival and success with long-term placement. At this time, the significant increase in neurological signs detected in neonates (24%) compared with other age classes.
(11%) after placement, including the development of epileptic seizures and the increased mortality in neonates due to neurological disease (75%), indicates that future placement of neonates may not be in the best welfare interest for this age class due to increasing environmental exposure to DA in utero or during nursing.

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Data availability statement
Data are available on request.

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