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PAPER

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

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tissues with high concentrations of AMPA receptors, such as the hippocampus and myocardium.[8 10] Within the hippocampus, the dentate gyrus is a common site of initial damage resulting from DA exposure.[15 16]

DA can cause reproductive failure in sea lions through mortality of pregnant females, abortion and premature parturition.[17] DA, which is water soluble,[18] 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,[19] 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.[17 20] 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.[21] 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.[22 23] Early postnatal DA exposure also results in alterations in spontaneous behaviour of rats that persists into adulthood.[24]

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.[25] Individuals may be deemed non-releasable for a variety of reasons, including those that affect the ability to forage successfully.[26] 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.[27 28]

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*.[2]

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 10 29] or other (eg, 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.[11] 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*.[13] 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*.[13] Left and right hippocampuses were traced separately, and the caudodorsal 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,[13] 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

Table 1 US rehabilitation centres and managed care institutions that participated in the survey on non-releasable California sea lions placed in display facilities between 2000 and 2016

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	Galveston, Texas
Aquarium of the Pacific	Long Beach, California
Atlantis Marine World	Riverhead, New York
Audubon Park and Zoological Garden	New Orleans, Louisiana
Birmingham Zoo	Birmingham, Alabama
Blank Park Zoo	Des Moines, Iowa
Brookfield Zoo	Brookfield, Illinois
Buffalo Zoo	Buffalo, New York
Cincinnati Zoo	Cincinnati, Ohio
Cleveland Metroparks Zoo	Cleveland, Ohio
Denver Zoo	Denver, Colorado
Dolphin Cove Research and Education Center (Dolphins Plus Key Largo)	Key Largo, Florida
Dolphin Research Center	Grassy Key, Florida
Fresno Chaffee Zoo	Fresno, California
Georgia Aquarium	Atlanta, Georgia
Gladys Porter Zoo	Brownsville, Texas
Gulf World Marine Park	Panama City Beach, Florida
Houston Zoo	Houston, Texas
Institute of Marine Mammal Science	Gulfport, Mississippi
Indianapolis Zoo	Indianapolis, Indiana
Kansas City Zoo	Kansas City, Missouri
Long Marine Lab	Santa Cruz, California
Louisville Zoo	Louisville, Kentucky
Miami Seaquarium	Miami, Florida
Miller Park Zoo	Bloomington, Illinois
Oceans of Fun (at the Milwaukee County Zoo)	Milwaukee, Wisconsin
Moss Landing Marine Lab	Moss Landing, California
Mystic Aquarium	Stonington, Connecticut
Smithsonian National Zoological Park	Washington, DC
New England Aquarium	Boston, Massachusetts
North Carolina Zoo	Asheboro, North Carolina
Ocean World	Crescent City, California
Oklahoma City Zoo	Oklahoma City, Oklahoma
Omaha's Henry Doorly Zoo	Omaha, Nebraska
Oregon Coast Aquarium	Newport, Oregon
Pittsburgh Zoo	Pittsburgh, Pennsylvania
Point Defiance Zoo	Tacoma, Washington
Riverbanks Zoo	Columbia, South Carolina
San Diego Zoo	San Diego, California
San Francisco Zoo	San Francisco, California
Sea Life Park Hawaii	Waimanalo Beach, Hawaii
SeaWorld San Diego	San Diego, California
SeaWorld Orlando	Orlando, Florida
Seneca Park Zoo	Rochester, New York
Shedd Aquarium	Chicago, Illinois
Six Flags Discovery Kingdom	Vallejo, California
Squalus, Inc (Sea Lion Splash)	Myakka City, Florida
St. Paul's Como Zoo	St. Paul, Minnesota

Continued

Table 1 Continued

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

WCS, Wildlife Conservation Society.

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 toxicosis.[29]

Table 2 Reason for non-releasability, presence of neurological signs and disposition of California sea lions deemed non-releasable and placed in managed care at US institutions between 2000 and 2016 (individuals that survived following placement remain in public display facilities)

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

Neonates

A significant difference was found (x^2 (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

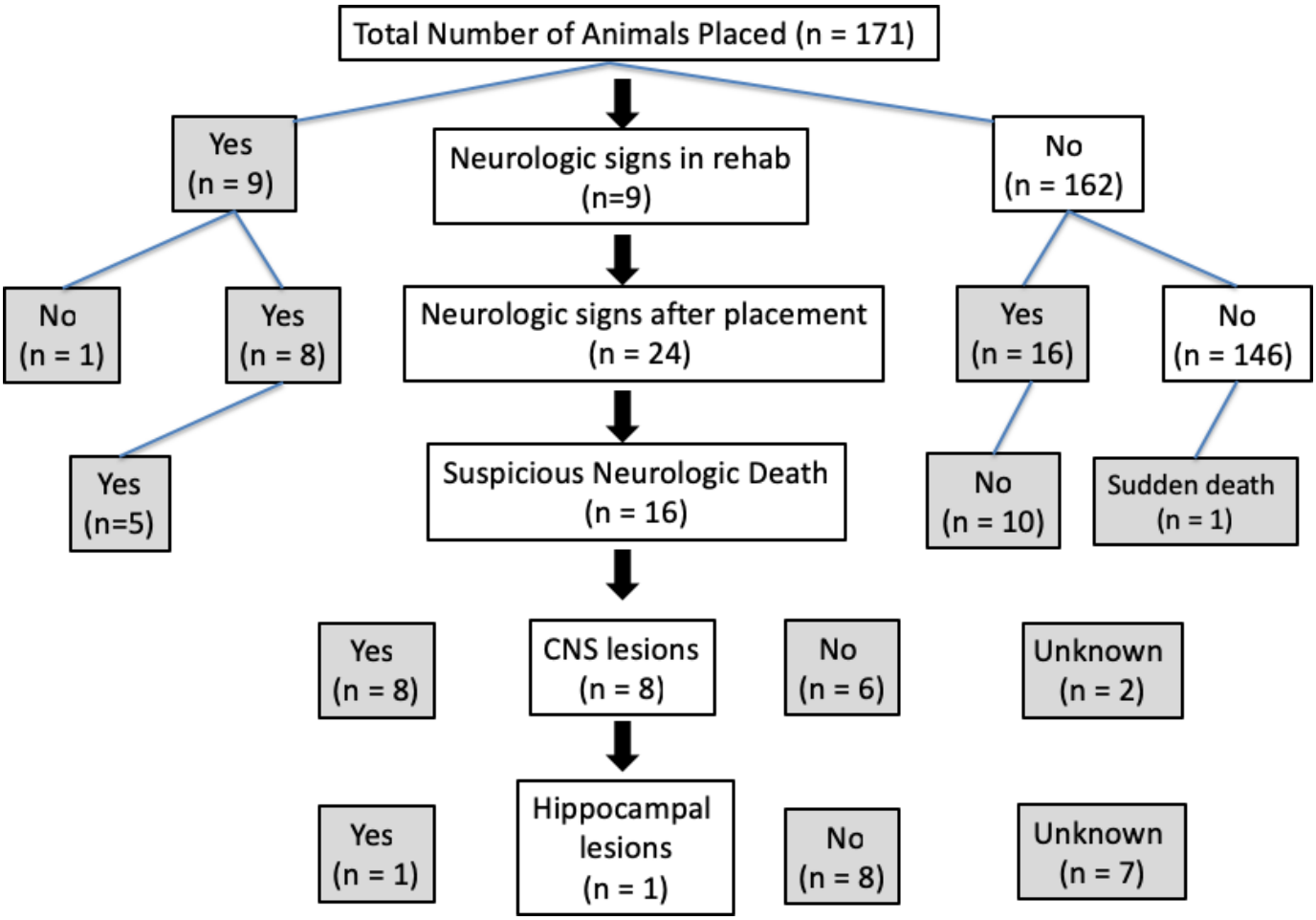

Figure 1 Case progression for neurological cases (25/171), describing California sea lions that developed neurological signs following placement, and their findings on necropsy and histopathology. Neurological cases are highlighted in grey. CNS, central nervous system.

task acquisition exhibited during behavioural testing, and hypermetric ataxia throughout his life.[30] Of the live animals, one individual (no. 32) had no lesions detectable on MRI, did not exhibit any learning delays during behavioural testing and has not developed neurological signs to date. One animal (no. 31) had no evidence of hippocampal atrophy on MRI and no evidence of memory delays during behavioural testing prior to placement. Six years later, however, after placement in managed care, this individual began experiencing seizures. At that time, a second MRI was performed, and unilateral hippocampal atrophy was noted. This individual was alive at the time of writing and was treated with anticonvulsant therapy. One individual (no. 33) was a neonate that was born to a seizing dam, had left hippocampal atrophy on MRI and poor learning results on behavioural testing. It has not exhibited other clinical neurological signs to date.

One individual (no. 30) had bilateral hippocampal atrophy, but relative hippocampal volumes were above the group mean. During behavioural testing, this animal learned the basic task most quickly among all of the animals tested, but performed poorly during delayed maze testing, when hippocampal damage was likely to be relevant. This animal is alive with no apparent clinical signs.

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[11] 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*[13]) 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,[11] and fornix damage is associated with epilepsy and generalised memory deficits[31 32] 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 MF 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 *Toxoplasma* 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)	Trauma	Seizures	Normal	Normal	Seizures
12	Non-neonate (yearling)	Chronic Illness	Seizures	Normal	Normal	Seizures
13	Non-neonate (yearling)	Restrand	Seizures	MF haemorrhage	Normal	Seizures
14	Non-neonate (adult)	Restrand	Seizures	MF Purkinje cell necrosis	NE	Other
15	Non-neonate (yearling)	Trauma	Ataxia, head twitching	Meningitis due to *Coccioides* infection	Unknown	Other
16	Non-neonate (juvenile)	Restrand	Seizures	Metastatic neoplasia	Normal	Other
17	Non-neonate (juvenile)	Chronic Illness	NA	Normal	Unknown	Other
18	Non-neonate (yearling)	Restrand	Seizures	Normal	Normal	Other
19	Non-neonate (yearling)	Chronic illness	Obtundation, nystagmus	Normal	Unknown	Other
20	Non-neonate (juvenile)	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)	Habituation	NA	Normal	Normal	Other
25	Non-neonate (yearling)	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)	Trauma	Ataxia, hypermetria	Infarcts	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.[2 3] 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.[7 33] 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,

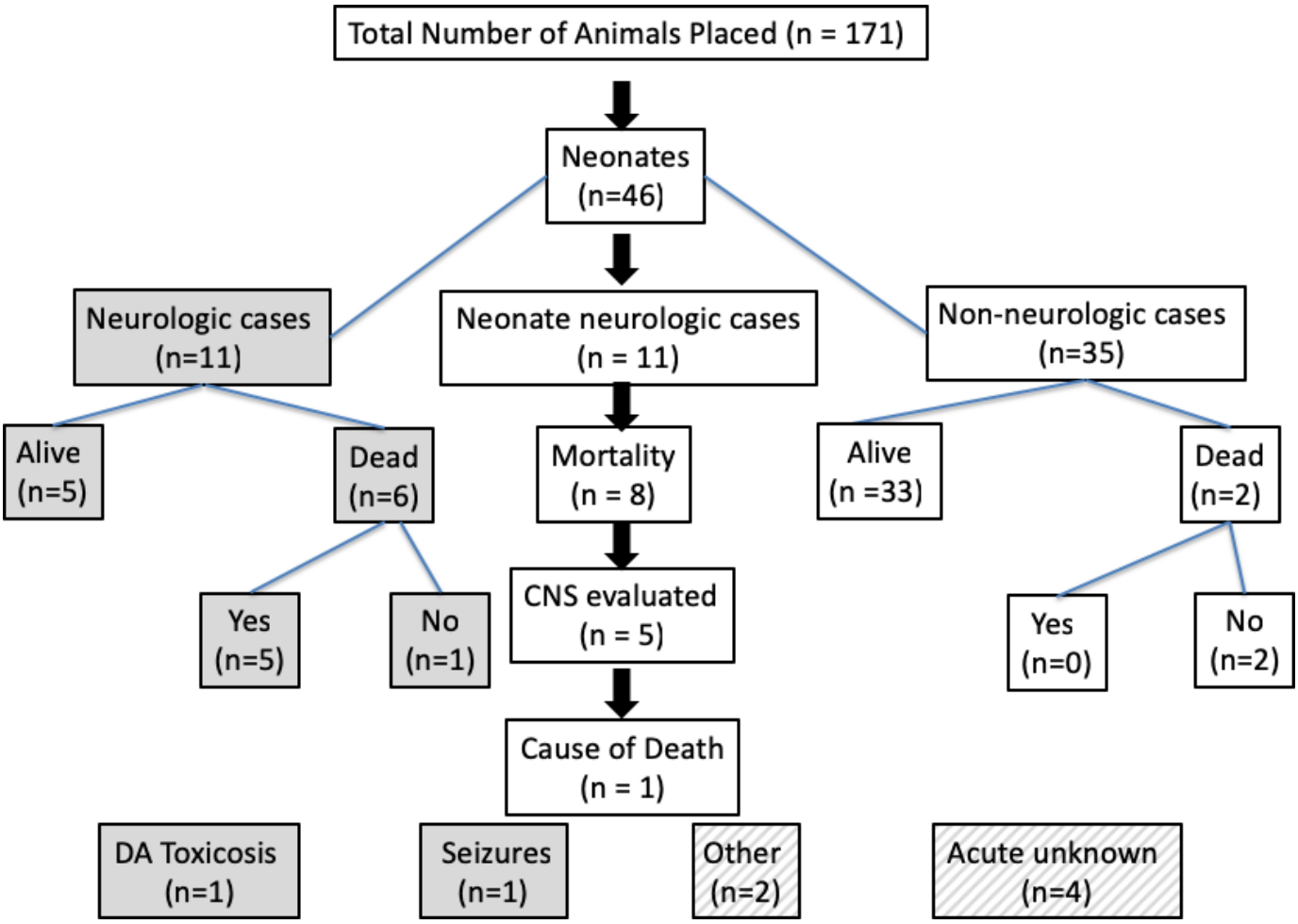

Figure 2 Case progression for all California sea lions that stranded as neonates (46/171), describing development of neurological signs, survival and cause of death. Neurological cases are highlighted in grey. Causes of death affecting both neurological and non-neurological cases are highlighted in grey stripes. CNS, central nervous system.

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	Restrand	Normal	Normal	Died	Other
28	Non-neonate	Trauma	Normal*	Slow task acquisition	Died	Other
30	Non-neonate	Restrand	Bilateral hippocampal atrophy	Poor delayed maze testing	Alive	NA
31	Non-neonate	Restrand	Initial MRI normal; post-placement MRI showed unilateral hippocampal atrophy	Normal	Alive	NA
32	Non-neonate	Restrand	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,[11] which may not always cause gross or histopathological lesions.[13] In a previous study, 21% of animals that died with chronic neurological signs showed minimal or no typical DA-related lesions.[7] 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.[16] 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.[32 34] Rodent data indicate that foraging errors in spatial choice tasks track hippocampal damage,[35] which is consistent with the well-established contribution of the hippocampus to spatial memory in multiple species.[36 37]

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,[38] and delayed epilepsy onset has been observed in early exposure rodent models.[22 39] 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.[40] 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.

► Additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/vetrec-2019-105371).

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