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Domoic Acid Poisoning as a Possible Cause of Seasonal Cetacean Mass Stranding Events in Tasmania, Australia

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2 Abstract

3 The periodic trend to cetacean mass stranding events in the Australian island state of
4 Tasmania remains unexplained. This article introduces the hypothesis that domoic acid
5 poisoning may be a causative agent in these events. The hypothesis arises from the previously
6 evidenced role of aeolian dust as a vector of iron input to the Southern Ocean; the role of iron
7 enrichment in *Pseudo-nitzschia* bloom proliferation and domoic acid production; and
8 importantly, the characteristic toxicosis of domoic acid poisoning in mammalian subjects
9 leading to spatial navigation deficits. As a pre-requisite for quantitative evaluation, the
10 plausibility of this hypothesis was considered through correlation analyses between historical
11 monthly stranding event numbers, mean monthly chlorophyll concentration and average
12 monthly atmospheric dust loading. Correlation of these variables, which under the domoic
13 acid stranding scenario would be linked, revealed strong agreement ($r=0.80-0.87$). We
14 therefore advocate implementation of strategic quantitative investigation of the role of
15 domoic acid in Tasmanian cetacean mass stranding events.

16 **Keywords:** cetacean mass stranding, domoic acid, iron fertilisation, aeolian dust, Southern
17 Ocean

20 Introduction

21 Cetacean mass stranding events are a frequent occurrence along coastlines surrounding the
22 Tasmanian state of Australia (Gales et al. 2012). The Tasmanian stranding record details 680
23 stranding events over the past 200 years, with 67 of 380 stranding events over the past two
24 decades being considered mass strandings based on the classification of two or more
25 individuals (other than mother-calf pairs) (Evans et al. 2002). The majority of stranding
26 events comprise species of the cetacean sub-order Odontocetii or “toothed whales”,
27 especially long-finned pilot whale (*Globicephala macrocephalus*).

28
29 The causes of mass stranding events in odontocetes are currently unknown but numerous
30 environmental factors have been implicated, including coastal topography and oceanography
31 (Brabyn and McLean 1992), meteorological and geomagnetic conditions (Mazzariol et al.
32 2011), seismic activity and sonar noise (Fernandez et al. 2005). Most recently, the periodicity
33 of Tasmanian mass stranding events were shown to correlate closely with both zonal and
34 meridional winds (Evans et al. 2005). The authors posed that such persistent wind patterns
35 may be associated with nutrient-rich waters, and thereby cetacean foraging being forced
36 closer to shore. In turn, an increase in the concentration of cetaceans in continental shelf
37 waters would raise the probability of a random stranding event. This finding, whilst it did not
38 identify the proximal cause of stranding, represents a significant advance towards
39 understanding the potentially climate-related phenomenon in this geographic region.

40
41 Domoic acid (DA; $C_{15}H_{21}NO_6$; CAS 14277-97-5) is a neurotoxin produced by the pennate
42 diatom *Pseudo-nitzschia* spp., which has a cosmopolitan distribution (Kim et al. 2015). DA
43 can cause amnesic shellfish poisoning in humans, with symptoms including vomiting,
44 seizures, memory loss and disorientation (Perl et al. 1990). DA has also been shown to be the
45 causative agent in sea lion (*Zalophus californianus*) and northern fur seal (*Callorhinus*
46 *ursinus*) mass mortality events in central California (Lefebvre et al. 2010; Scholin et al.
47 2000). Sea lions and fur seals affected by DA poisoning exhibited neurological dysfunction
48 but were otherwise in good body condition. Clinical investigation of subjects revealed brain
49 lesions, particularly of the hippocampus (Lefebvre et al. 2010; Montie et al. 2012). The
50 hippocampus is a part of the cerebral cortex, which is associated with episodic memory and
51 spatial navigation (Eichenbaum et al. 1999; Burgess et al. 2002). In humans, it is commonly

52 subject to atrophy and degeneration in cognitive disorders such as Alzheimer's disease (Kold
53 and Wishaw, 1996). Recently, Cook et al., (2015) advanced this research through application
54 of functional Magnetic Resonance Imaging (MRI) of Californian Sea Lions undergoing
55 rehabilitation. Animals with hippocampal lesions showed significantly altered hippocampal
56 networks, which may lead to maladaptive navigational behaviour and subsequent mortality in
57 the wild.

58
59 DA is a water soluble, tricarboxylic acid with a very short half-life in most tissue
60 compartments (Fuquay et al. 2012). Further, it has a very poor ability to penetrate the blood-
61 brain barrier (Edebo et al. 1998; Pulido 2008). Toxicosis therefore appears to occur through
62 acute exposure, enhanced by rapid biomagnification during DA-producing *Pseudo-nitzschia*
63 bloom events, rendering higher trophic predators, such as pinnipeds and odontocetes, at the
64 greatest risk. The transfer of DA through the food web to cetaceans has previously been
65 evidenced with the detection of DA in fecal samples from humpback whales (*Megaptera*
66 *novaeangliae*) and blue whales (*Balaenoptera musculus*) feeding in Monterey Bay, California
67 (Lefebvre et al. 2002). Whilst less is known about the long term effects of chronic exposure
68 (Pulido 2008), it is clear that the characteristic toxicologic pathology of DA in mammalian
69 subjects could contribute substantially to cetacean disorientation leading to stranding.

70
71 Until recently, oceanic forms of *Pseudo-nitzschia* were thought to be non-toxic. However,
72 iron fertilization experiments in the Southern Ocean revealed that not only does iron
73 enrichment stimulate growth of *Pseudo-nitzschia* spp., one of the dominant diatom species in
74 the region (Gervais and Riebesell 2002; Kopczynska et al. 2007), but the species also
75 responds to iron addition by producing DA (Trick et al. 2010). The sub-Antarctic Southern
76 Ocean is characterized as a high-nutrient, low-chlorophyll region and it has been postulated
77 that the system is iron-limited (Sedwick et al. 1999). A major natural source of iron input to
78 the ocean surface is deposition of land-derived aeolian dust (Jickells et al. 2005). Research
79 within the author team has previously evidenced the role that episodic input of iron-rich
80 Australian dust plays in increasing primary productivity in the sub-Antarctic Southern Ocean
81 (Gabric et al. 2002). This raises the possibility of a link between Australian dust events and
82 Tasmanian cetacean strandings through the production of DA by *Pseudo-nitzschia*.

83
84 Establishing an unambiguous connection between DA production and odontocete mass
85 stranding events is not a simple endeavour and requires a multidisciplinary approach
86 including histopathological investigation of subjects; knowledge of foraging prior to
87 stranding; chemical or biochemical verification of DA in the tissues of subjects and
88 taxonomic and DA analysis of phytoplankton in the region. In the absence of such multi-
89 dimensional data-sets, retrospective investigations have previously performed time-series
90 cross-correlation between *Pseudo-nitzschia* bloom activity and stranding events (Torres de la
91 Riva et al. 2009), finding correlative relationships for several stranding species and lending
92 support for DA as an active agent in these events.

93
94 Here we introduce the potential role of domoic acid as a causative agent in Tasmanian
95 cetacean mass stranding events. We consider the plausibility of the hypothesis as a first step
96 towards the facilitation of resource-intensive quantitative analysis. To do so, we performed
97 multi-annual, correlation analyses of mass-stranding events along the Tasmanian coastline
98 with monthly satellite derived chlorophyll data and dust activity as a natural source of iron
99 enrichment. The potential role of DA as a causative agent in mass-stranding events is
100 discussed and a framework for strategic investigations to test the hypothesis is proposed.

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Methods and Materials

We performed correlation analyses of variables that, under the DA related stranding scenario would be related and contributing factors. The frequency of stranding events by month was cross correlated with both proximate ocean chlorophyll-*a* (CHL-*a*) and dust activity.

Details of cetacean stranding events around Tasmania were obtained from the Tasmanian stranding database (Department of Primary Industries, Parks, Water and Environment (DPIPWE) Marine Mammal Conservation Program). The earliest recorded event in this database occurred in 1911, however, due to uncertainty concerning the completeness of the earlier parts of the record, only mass stranding events from 1965 onwards were examined. The minimum number of animals affected in any single stranding event was 10, hence for the purpose of this investigation, a mass-stranding event was defined as an event that involved ≥ 10 animals, as opposed to the commonly accepted ≥ 2 individuals (Gales et al., 2012). If stranding events occurred within one week of each other on the same coastline, they were classed as a single event. This yielded a total of 64 events with up to 216 animals reported in a single event. Stranding frequencies summed by month over the 48-year period provided a monthly chronology of stranding events for correlative evaluation.

Quantitative taxonomic data on diatom distribution (both spatial and temporal) is highly scarce for this remote global region. As such, we used ocean CHL-*a* concentration, obtained by remote sensing, as a proxy for the investigation of ocean phytoplankton productivity, assumed to reflect diatom bloom occurrence, as previously applied in the literature (Wilson et al, 2015). The mean monthly CHL-*a* concentration product based on the combined record of Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and Moderate Resolution Imaging Spectroradiometer (MODIS) satellite data was obtained for a 14 year period, January 1998 - December 2011 from the NASA hosted Oceancolor website. The monthly variation in CHL-*a* was determined for an area of interest bounded by 140-160°E and 40-50°S (Figure 1). Authors have previously identified this area of the Tasman Sea/Southern Ocean as having a high likelihood of phytoplankton response to iron-rich dust inputs from the Australian continent (Cropp et al. 2013).

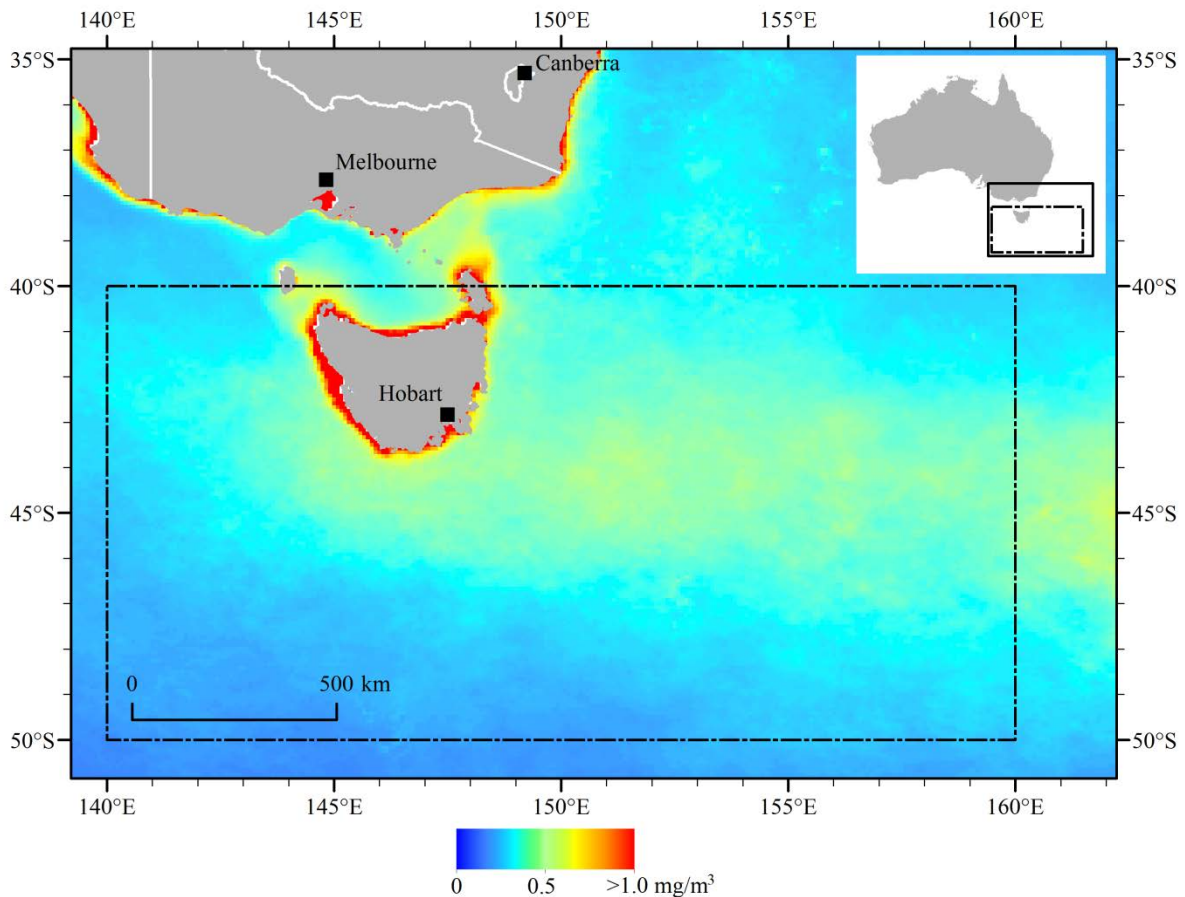


Fig 1 Long term (1998-2011) distribution of mean chlorophyll-*a* concentration from satellite sources in the regional area of interest.

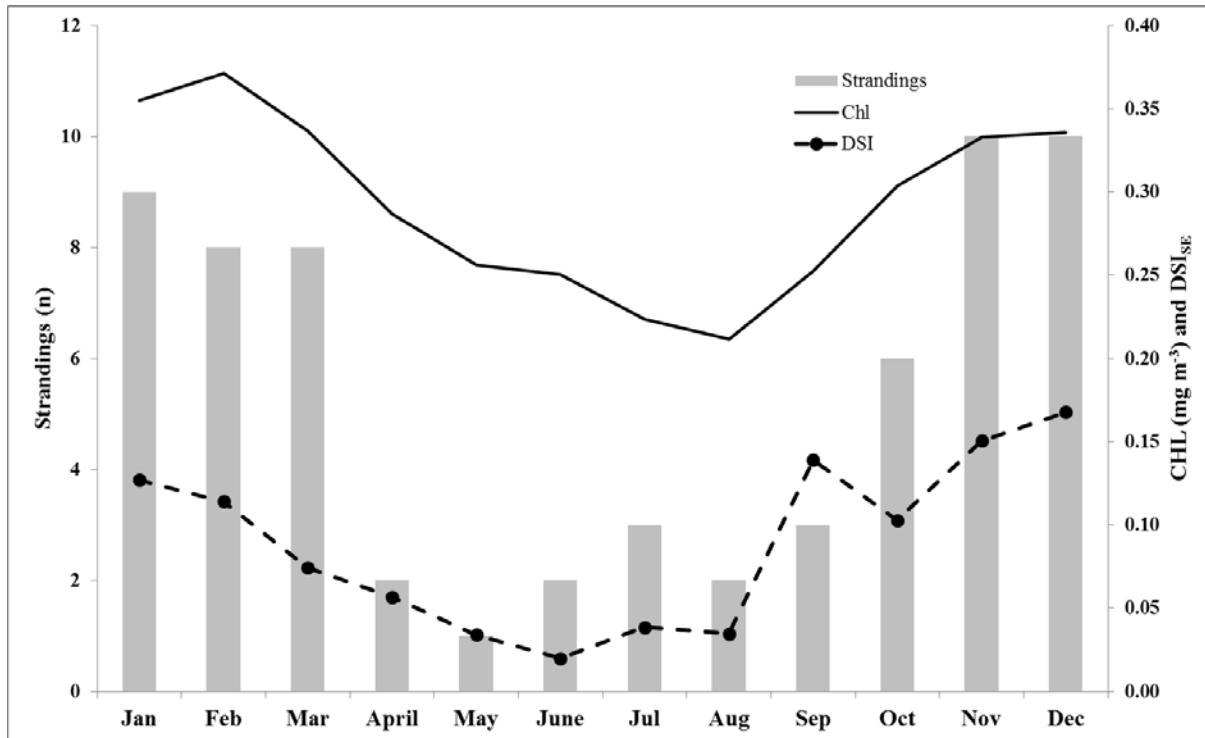
To characterise the variability in Australian dust activity for the period that stranding events were studied, a metric known as the Dust Storm Index (DSI) was examined. The DSI is based on the archive of Australian Bureau of Meteorology (ABM) observation records, and has been developed to analyse dust activity over the continent for historic timeframes (O’Loingsigh et al. 2014). The DSI value for a given period (e.g. month or year) for any observation location is derived from the daily weather codes reported at that meteorological station over the period. Beyond the scope of this paper, a full explanation of the DSI is provided by O’Loingsigh et al. (2014).

The dust observation records from 180 long-term ABM stations are available nationwide for Australia. This study used a geographical subset of all stations in New South Wales, South Australia, Victoria and Tasmania (88 stations), to cover the south-eastern (SE) portion of the continent nearest to the ocean area of interest (DSI_{SE}). The selected stations for DSI_{SE} , whilst not representing major source regions themselves, are instead best placed to detect dust leaving the continent in transport over the Tasman Sea, Southern Ocean and the bounded area of interest.

Results and Discussion

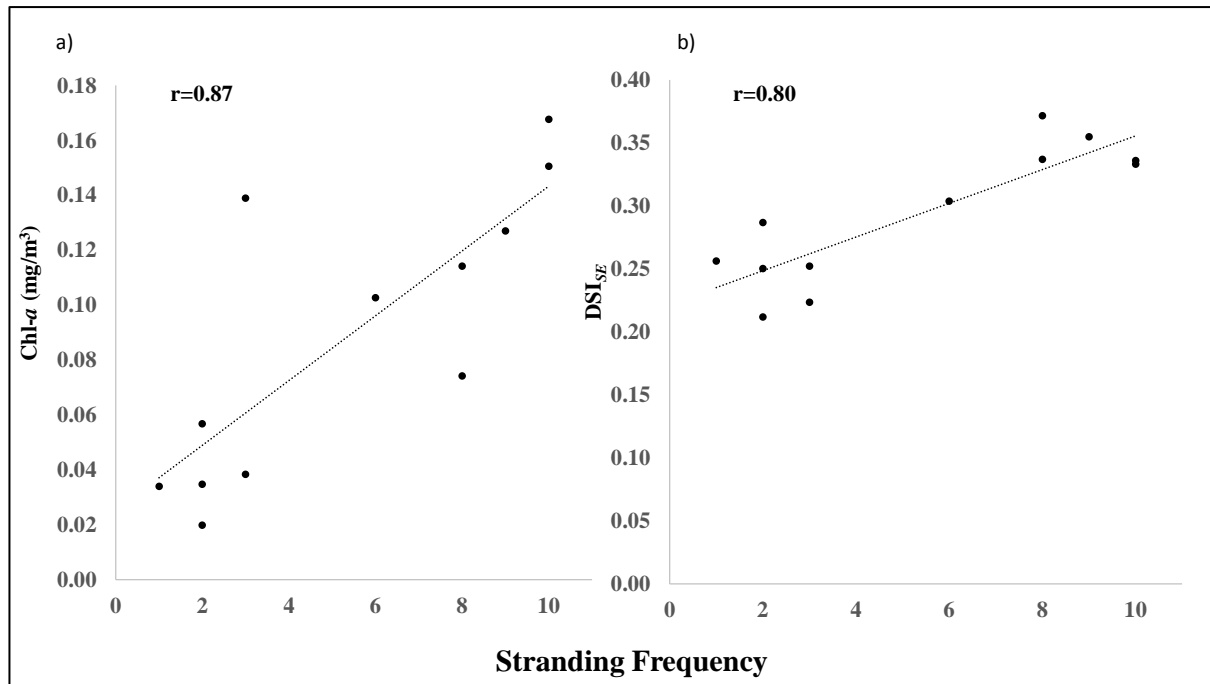
Stranding occurrences were strongly biased to the austral summer (Figure 2), with a peak of 10 events in each of the months of November and December recorded over the 48 year period. These two months alone involved 1289 of a total of 4215 animals. When monthly stranding frequencies were overlaid with multi-year CHL-*a* and DSI_{SE} climatologies, a clear

160 co-varying trend is evident (Figure 2). The highest CHL-*a* concentrations in the area of
 161 interest also occurred in the austral summer from November to February and were coincident
 162 with elevated dust activity and atmospheric loading at this time, with elevated relative values
 163 of DSI_{SE} evident (McTainsh et al. 1998). A rapid decline in stranding occurrence is noticeable
 164 for April, and remains low throughout the subsequent austral winter. All three variables
 165 showed good agreement with these lower values.
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 168
 169 **Fig 2** Monthly stranding frequency (1965-2013) presented alongside mean monthly
 170 chlorophyll-*a* and mean dust storm index (1998-2011)
 171

172 The calculated correlation coefficient (Pearson r) for the relationship between CHL-*a* and
 173 stranding frequency was 0.87 and 0.80 between DSI_{SE} and stranding frequency respectively
 174 (Figure 3). This very strong correlative relationship between related and contributing factors
 175 under the DA stranding scenario provides support for the validity of the hypothesis and
 176 suggests that further quantitative investigation is merited.



177
 178 **Fig 3** Scatterplots showing the correlative relationship between stranding frequency and
 179 CHL-*a* and DSI respectively
 180

181 The periodicity of southeast Australian cetacean stranding events has previously been
 182 demonstrated by Evans et al. (2005). The current work progresses the previous climate-
 183 related pathway of investigation, posing that a cause of stranding may be associated with DA
 184 poisoning, known to result in spatial disorientation in other mammalian subjects.
 185

186 The unequivocal verification of DA as a causative agent in seasonal stranding events requires
 187 long-term, multi-faceted monitoring programs. Reliable detection of DA in the biological
 188 fluids of subjects, either by High Performance Liquid Chromatography (HPLC) or Enzyme-
 189 Linked Immunosorbent Assay (ELISA) techniques, is an integral component of diagnosing a
 190 DA-related mortality (Lefebvre et al, 2010). When acceptable timeframes of sample
 191 acquisition are possible (immediately post-mortem), blood, urine, faeces, breast milk and
 192 aqueous humour represent readily accessible matrices for DA detection. The short half-life of
 193 domoic acid, however, often results in non-detection, even when there is strong evidence of a
 194 DA related mortality (Wilson et al. 2015). DA detection can be further complicated in strong
 195 socially bonded cetacean pods, such as those of pilot whales, where not all stranded
 196 individuals may display acute symptoms of the toxin. As such, histopathological examination
 197 of brain and heart and post-mortem brain imaging plays a critical role in the verification of
 198 acute effects of DA toxicosis. Such examination requires the removal and fixation of intact
 199 organs soon after death. Extensive post-mortem sampling efforts such as these
 200 simultaneously lend themselves to stomach content collection and analysis for the purpose of
 201 exposure pathway investigation. A comprehensive bio-monitoring program would similarly
 202 aim to confirm the presence of DA acid producing *Pseudo-nitzschia* in the proximate ocean
 203 sector in response to any stranding event.
 204

205 In practice, such comprehensive sampling campaigns are unrealistic without considerable
 206 resource allocation. The frequent remoteness of Tasmanian stranding locations, combined
 207 with the large number of animals affected during these events, and importantly, the priority

208 allocated to animal rescue at such times, presents the reality that attending stranding officers
209 are stretched in their capacity. The additional task of logistically-demanding post-mortem
210 tissue harvesting, sample fixation and transport of skulls and organs of large cetaceans is
211 therefore not a trivial consideration. Presentation of this correlative evidence in support of the
212 potential role of DA in cetacean mass stranding events in this region therefore represents a
213 vital first step towards facilitation of robust quantitative investigation. Quantitative
214 investigation remains imperative for advancing our understanding of the role of DA in these
215 cyclical mass stranding events and in turn our understanding of how climate change stands to
216 impact the periodicity and severity of these events (Havens et al. 2015; Cook et al, 2015).

217

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224 events in the region.

225

226

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