

Mass Stranding of Marine Birds Caused by a Surfactant-Producing Red Tide

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22 **Abstract**

23 In November-December 2007 a widespread seabird mortality event occurred in
24 Monterey Bay, California, USA, coincident with a massive red tide caused by the
25 dinoflagellate *Akashiwo sanguinea*. Affected birds had a slimy yellow-green material on
26 their feathers, which were saturated with water, and they were severely hypothermic.
27 We determined that foam containing surfactant-like proteins, derived from organic
28 matter of the red tide, coated their feathers and neutralized natural water repellency and
29 insulation. No evidence of petroleum or other oils or biotoxin was found. This is the
30 first documented case of its kind, but previous similar events may have gone
31 undetected. The frequency and amplitude of red tides have increased in Monterey Bay
32 since 2004, suggesting that impacts on wintering marine birds may continue or increase.

34 **Introduction**

35 Harmful algal blooms (HABs) caused by a variety of dinoflagellates occur worldwide
36 and can cause morbidity and mortality of fish, invertebrates, marine birds, marine
37 mammals and humans [1-2]. Morbidity and mortality may result from ingestion,
38 inhalation or absorption of toxins, including the potent neurotoxins domoic acid,
39 saxitoxin, and brevetoxin [3-6] or from hypoxia/anoxia that develops during bloom
40 respiration and senescence [1]. Herein we present the first report of harmful effects
41 caused by external coating of marine birds by a proteinaceous foam derived from a red
42 tide bloom that occurred November 2007 in Monterey Bay, CA (USA). Monterey Bay
43 is an important foraging and moulting area for a diversity of migratory birds, second
44 only to San Francisco Bay in importance to sea ducks and near shore species wintering

45 along the west coast of the United States. Although this red tide bloom was ostensibly
46 nontoxic, it was very harmful, causing morbidity and mortality of more than 700
47 seabirds. Affected birds had a slimy yellow-green material on their feathers, which were
48 saturated with water, and they were severely hypothermic. Foam containing surfactant-
49 like proteins, derived from organic matter of the red tide, coated their feathers and
50 neutralized natural water repellency and insulation. No evidence of petroleum, other oils
51 or biotoxin was found. This is the first documented case of its kind, but previous similar
52 events worldwide may have gone undetected. The frequency and amplitude of red tides
53 have increased in Monterey Bay since 2004 [7], suggesting that impacts on wintering
54 marine birds may continue or increase.

55

56 **Results**

57 Fourteen seabird species were affected during the 2007 red tide, particularly those
58 that feed in near shore habitat, such as surf scoters (*Melanitta perspicillata*), Pacific and
59 red-throated loons (*Gavia* spp.), and Clark's and western grebes (*Aechmophorus* spp.).
60 Some of these, including the loons, grebes and northern fulmar (*Fulmarus glacialis*),
61 had just completed their southward migration and were in poor nutritional condition.
62 Also, both species of loons and grebes undergo a catastrophic moult, simultaneously
63 losing their flight feathers shortly after arriving in Monterey Bay. As a result, many
64 birds were flightless and could not escape or compensate for loss of water repellency
65 and body heat. Although the proteinaceous material was relatively easy to remove from
66 the birds using abbreviated oil spill washing procedures, seabird mortality was still
67 substantial.

68 Live-stranded birds were severely hypothermic, wet and hypoglycaemic, with
69 variable patches of a slimy pale yellow-green material on their feathers (Figure 1) with a
70 “bathtub ring” distribution, primarily on the breast area and ventral surfaces of wings
71 and tail. Many had a pungent odour similar to that of linseed oil while still wet, but with
72 time, this material dried, leaving a fine crust with minimal smell. Upon washing,
73 rehydration, warming, and nutritional supplementation, many affected birds recovered,
74 suggesting that the product was minimally toxic or nontoxic. Gross post-mortem and
75 microscopic pathology examinations of affected birds revealed no significant lesions
76 other than mild to moderate emaciation, diffuse pallor suggestive of mild to moderate
77 anaemia, and a mild erythematous rash or mild, raised pox-like lesions. With
78 supportive care the skin lesions, cachexia and anaemia resolved spontaneously.

79 Three distinct pulses of live and dead bird strandings occurred in spatial and
80 temporal coincidence with the development and circulation of an intense “red tide”. The
81 first pulse of red tide and bird strandings was most concentrated in northern Monterey
82 Bay (Figure 2A). The second pulse was marked by expansion of the red tide and
83 increased seabird strandings along the southern portion of bay (Figures 2B and 2E). The
84 third pulse occurred in both areas simultaneously (Figure 2C). Standardized periodic
85 surveys documented marine bird stranding rates 2 to 24 times greater than the 10-year
86 average for Western/Clark’s grebes on most northern beaches in Monterey Bay (Figure
87 2D). These species typically forage along the shoreline where dense patches of
88 senescing algae and foam were observed. In contrast, northern fulmar are a more
89 pelagic species that forage near the centre of Monterey Bay. Fulmar appeared to have
90 encountered the red tide and its attendant foam in the southern and central bay as
91 supported by the MERIS satellite data (Figure 2B). Fulmar stranded in numbers 2 to 4
92 times greater than the 10-year average for the months of November-December, but were
93 more highly localized to specific beaches (Figures 2E).

94 Plankton samples collected at various Monterey Bay nearshore and offshore
95 stations during November 2007 were consistently dominated (both numerically and by
96 biomass) by *Akashiwo sanguinea* (= *Gymnodinium sanguineum*), an autotrophic
97 dinoflagellate (Figure 3, circular inset). Significantly elevated levels of other biotoxin-
98 producing dinoflagellates were not detected in Monterey Bay during this event.
99 *Akashiwo sanguinea* is capable of producing large quantities of mycosporine-like amino
100 acids (MAAs), which are water-soluble and serve as powerful surfactants [8]. *Akashiwo*
101 *sanguinea*-associated blooms have also been linked to coral bleaching due to the
102 formation of proteinaceous material [9], similar to that found in this event. In this event
103 surface foam accumulated adjacent to the most densely concentrated portions of the
104 bloom along frontal edges (Figure 3) and in the surf zone where wave action
105 presumably produced foam through turbulent mixing of the organic-rich seawater.
106 Affected birds were observed swimming in the thick foam near the shoreline. When
107 normal seabird feathers were dipped in the foam, the contour feathers and underlying
108 down lost their normal water repellency (Figure 4D) and became soaked. Clean
109 seawater (Figure 4A, 4B, 4C) and deionised (Milli-Q) water did not have this effect.

110 Extracts of seawater from four areas in northern Monterey Bay heavily impacted
111 by the red tide were analyzed for polar and non-polar compounds by gas- and liquid
112 chromatography-mass spectroscopy and were found to be negative for petroleum
113 compounds, commercial surfactants, pesticides, domoic acid, okadaic acid, and
114 microcystin toxins. However, samples of the co-occurring surface foam present at these
115 same sites contained significant concentrations of an organic compound with a
116 predominant chromatographic peak at 1230 mw, corresponding to a m/z 616 dimer
117 composed of carbon, hydrogen, nitrogen and oxygen. Fragmentation of the m/z 616
118 parent compound resulted in loss of several m/z 59 fragments characteristic of peptide
119 chains. When foam samples were allowed to sit for several days at ambient temperature,
120 these peptides became difficult to detect. Analysis of the surface foam and feathers from

121 affected birds coated with the yellow-green proteinaceous material revealed high
122 concentrations of the same 616 m/z protein dimer, but seawater collected from
123 unaffected areas and normal feathers of healthy seabirds of the same species were
124 negative for this compound.

125 Samples of the foam were also analyzed by UV-VIS spectroscopy, and exhibited
126 characteristic UV-absorption spectra indicative of MAAs that are commonly associated
127 with dinoflagellate blooms. As with the proteins detected by mass spectrometry, the
128 MAA-like absorption features degraded (within 24 hours) when stored in the dark at
129 4°C. Uni-algal, xenic laboratory cultures of *A. sanguinea* isolated from Monterey Bay
130 produced compounds with the same UV-absorption signature (indicative of MAA
131 compounds) as the field-collected algal, feather and foam samples. When senescent
132 laboratory cultures of *A. sanguinea* were vigorously agitated for 30 seconds and then
133 left to rest for 2 hours, they produced surface foam (Figure 4E) that had the same
134 wetting properties on normal bird feathers as the naturally occurring foam (Figure 4F);
135 the foam was still intact at the end of the two hour rest period. In contrast, agitation of
136 healthy *A. sanguinea* cultures did not result in foam production beyond transient bubble
137 formation which did not persist for more than ca. 15 seconds, and exposing normal bird
138 feathers to vigorously shaken healthy cultures or to sterile seawater media did not result
139 in wetting.

140

141 **Discussion**

142 Northeastern Monterey Bay has previously been identified as a “red tide
143 incubator” [10]. Analysis of remote sensing data (ocean color, temperature and currents)
144 suggests relatively weak circulation, and presumably enhanced retention of the

145 prolonged red tide bloom during the marine bird stranding event. The bloom was first
 146 detected by satellite in this area before the harmful effects on the marine birds were
 147 detected, and it persisted throughout the event. The first and third pulses of marine bird
 148 morbidity and mortality occurred primarily within this incubator region (Figure 2).

149 Because the gross lesions in affected birds were vague and nonspecific, and the
 150 worldwide distribution of *A. sanguinea* is broad, similar mortality events may have
 151 previously been unrecognized. Mass stranding of recently moulted grebes, loons and
 152 scoters with wet, “apparently oiled” feathers also occurred in Monterey Bay in 1997 and
 153 was temporally associated with an uncharacterized red tide. Red tides of similar
 154 magnitude were observed in Monterey Bay in August-September 2004 [11] and in
 155 September 2006, with no substantial increase in reports of bird mortalities. The lateness,
 156 duration and intensity of the 2007 *A. sanguinea* red tide and the natural history of the
 157 most heavily affected bird species evidently exacerbated the impacts of the bloom.

158 Prediction of the harmful effects of harmful algal blooms requires a better
 159 understanding of linkages between climate variability, phytoplankton blooms and
 160 natural history of higher animals. An “unprecedented” red tide produced by *A.*
 161 *sanguinea* in San Francisco Bay during September 2004 was attributed to an upper-
 162 atmosphere high-pressure anomaly off the U.S. west coast, following a summer of weak
 163 coastal upwelling [12]. The circulatory, nutrient, weather and surf conditions that
 164 existed in November 2007 favoured an especially intense and prolonged event that
 165 produced large amounts of foam from a very large, senescing dinoflagellate bloom by
 166 extending the red tide into a period of increased winter swell. It remains to be seen
 167 whether the combination of events we describe for 2007 is a result of climate change,
 168 but the frequency, amplitude and duration of “red tides” have increased substantially
 169 within Monterey Bay since 2004 [7] and continue to increase globally [4], suggesting
 170 that the harmful effects of dinoflagellate blooms may become more common.

171 **Materials and Methods**

172 **Bird data.** Live and dead bird stranding data were spatially binned according to where
173 the stranding locations formed clusters, and were temporally binned according to three
174 distinct pulses of stranding activity. Two species (western/Clark's grebes and northern
175 fulmars) were examined in more detail. The Beach Coastal Ocean Mammal and Bird
176 Education and Research Surveys program [13] record dead strandings during monthly
177 beach surveys. Data from November and December 2007 surveys are presented in
178 relation to the monthly mean (1997-2006) stranding data from the same beaches for the
179 same months.

180 **Description of the red tide.** We primarily used 300 m and 1200 m images from the
181 MERIS satellite sensor. Average MCI [14-15] corresponding to the three pulses of bird
182 strandings was calculated. For the first stranding period (7-18 November 2007), two
183 images preceding (2-3 Nov) and one image during this pulse (12 Nov) were averaged.
184 Three MCI images (19, 21, 22 Nov) within the second period (19-23 Nov) were
185 averaged. For the third period (24 Nov – 2 Dec), MCI coverage was not adequate, and
186 we used 500-m resolution MODIS true-color images.

187 **Chemical analysis.** Foam collected by boat and from shore and material from live and
188 dead stranded birds was analyzed following standard protocols for identification of
189 marine toxins, contaminants, and petroleum products. This included analysis by gas
190 chromatography with ion trap detector (ITD), electron capture detector (ECD), and
191 flame photometric detector (FPD), as well as liquid chromatography/mass spectrometry
192 (LC/MS), LC/MS/MS, and UV/VIS absorption.

193 **Foam reproduction.** A monospecific strain of *Akashiwo sanguinea* (AkaMB1106) was
194 grown on L1 media. The culture was allowed to enter stationary phase and senesce. 500
195 mL each from senescent and healthy cultures was agitated for approximately 30-60

seconds, until foam was produced in the senescent culture. L1 media with no cells was used as a control.

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248

249 **Figure legends**

250 Figure 1: Dead seabirds with signs of protein-induced loss of waterproofing. A) A
 251 Pacific loon found dead in Monterey Bay, CA in November, 2007 with yellow-green
 252 staining of ventral breast feathers typical of over 700 marine birds affected by this
 253 event. B) Closer view of the stained feathers from (A), illustrating the yellow-green
 254 discoloration and the oily appearance of affected feathers. C) Discolored, wet and
 255 matted feathers on the ventral wing of a western grebe recovered during the same
 256 stranding event.

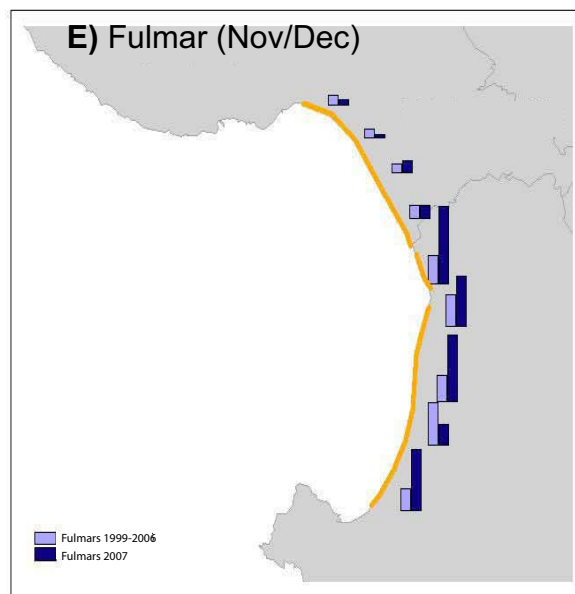
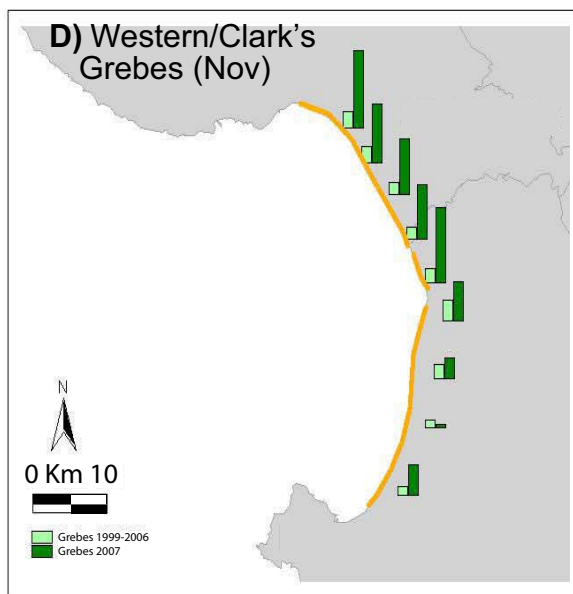
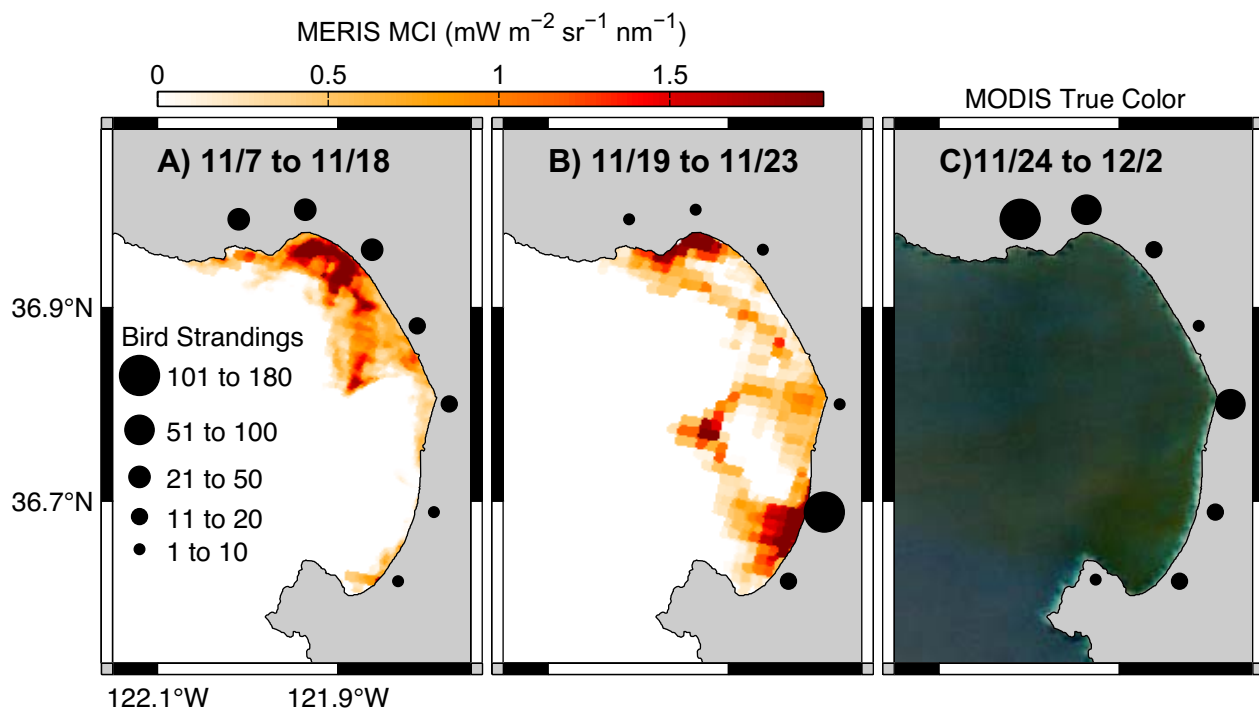
257 Figure 2. Correspondence between the spatial and temporal patterns of seabird
 258 strandings and red tide during November-December 2007 in Monterey Bay. Black
 259 circles represent the approximate location and magnitude of morbidity and mortality,
 260 and satellite data represent the red tide for panels (A-C). Three pulses of seabird
 261 stranding were observed: A) focused along the northern bay, B) focused in the south-
 262 central portion of the bay, and C) in both northern and central portions of the bay. In (A)
 263 and (B) the MERIS data indicate extreme bloom conditions above MCI level of ~0.3.
 264 Although ideal for red tide detection, MERIS coverage was not adequate for the third
 265 pulse. In (C) the reddish discoloration on the MODIS true color image during the third
 266 pulse (28 Nov) shows the spatial extent and location of the red tide. Panels (D) and (E)
 267 show increased seabird stranding during the November-December 2007 red tide event.
 268 Light bars are 10-year average (1997-2006) standardized monthly stranding counts on
 269 nine Monterey Bay beaches. Dark bars are 2007 counts for western/Clark's grebes and
 270 Northern fulmar, the species most affected during the mass-stranding event. Panel (D)

271 corresponds to the first stranding event, while panel (E) corresponds to the second and
272 third waves of strandings.

273 Figure 3. Aerial photograph taken 26 November, 2007 off Pajaro Dunes Colony
274 (rectangular inset) in central Monterey Bay. A dense front of red tide is visible in the
275 left foreground. A distinct line of surface foam is spatially associated with the red tide,
276 along with aggregations of marine birds. Circular inset: a single non-senescent
277 *Akashiwo sanguinea* cell.

278 Figure 4. Natural and laboratory produced wetting of normal feathers. A) Normal
279 breast feather from a brown pelican after dipping in clean seawater. Note the minimal
280 wetting and matting of feather barbs. B) Normal seawater after agitation for 30 seconds,
281 then resting for 2 hours. Note the absence of surface foam. C) Normal-appearing breast
282 feathers from a Northern fulmar after dipping in the test tube containing normal
283 seawater. D) Normal breast feather from a brown pelican after dipping in surface foam
284 collected near an *A. sanguinea* bloom in Monterey Bay. Contact with the foam resulted
285 in severe wetting and matting of feather barbs. E) Senescent laboratory culture of *A.*
286 *sanguinea* after agitation for 30 seconds, then resting for 2 hours. Note the persistence
287 of a thick surface layer of foam. F) Normal breast feathers from a Northern fulmar after
288 dipping in the surface foam derived from the senescent *A. sanguinea* culture, resulting
289 in severe wetting and matting of feather barbs.







Seawater



A



B



C



D



E



F

Surface foam