# Health Assessment, Antibiotic Treatment, and Behavioral Responses to Herding Efforts of a Cow-Calf Pair of Humpback Whales (*Megaptera novaeangliae*) in the Sacramento River Delta, California

Frances M. D. Gulland,<sup>1</sup> Felicia B. Nutter,<sup>1</sup> Kristin Dixon,<sup>1</sup> John Calambokidis,<sup>2</sup> Greg Schorr,<sup>2</sup> Jay Barlow,<sup>3</sup> Teri Rowles,<sup>4</sup> Sarah Wilkin,<sup>4</sup> Trevor Spradlin,<sup>4</sup> Laurie Gage,<sup>4</sup> Jason Mulsow,<sup>5</sup> Colleen Reichmuth,<sup>5</sup> Michael Moore,<sup>6</sup> Jamison Smith,<sup>7</sup> Pieter Folkens,<sup>8</sup> Sean F. Hanser,<sup>8.9</sup> Spencer Jang,<sup>9</sup> and C. Scott Baker<sup>10</sup>

> <sup>1</sup>The Marine Mammal Center, Sausalito, CA 94965, USA; E-mail: Gullandf@tmmc.org <sup>2</sup>Cascadia Research Collective, Olympia, WA 98501, USA

<sup>3</sup>National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, CA 92037, USA

<sup>4</sup>National Marine Fisheries Service, Office of Protected Resources, Silver Spring, MD 20910, USA

<sup>5</sup>University of California–Santa Cruz, Santa Cruz, CA 95060, USA

<sup>6</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

<sup>7</sup>National Marine Fisheries Service, Protected Resources Division, Gloucester, MA 01930, USA

<sup>8</sup>Alaska Whale Foundation, Benicia, CA 94510, USA <sup>9</sup>University of California–Davis, Davis, CA 95616, USA

<sup>10</sup>Marine Mammal Institute and Department of Fisheries and Wildlife, Oregon State University,

2030 SE Marine Science Drive, Newport, OR 97365, USA

#### Abstract

A mother and female calf humpback whale (Megaptera novaeangliae) pair were observed at an atypical location, 72 nmi inland in the Port of Sacramento, California, on 16 May 2007. Sequencing of mtDNA from a skin biopsy showed the cow to be an E1 haplotype, which is common in the California feeding population. Both animals had lacerations, suggesting sharp trauma from a boat strike. Photographs taken over 11 d showed generalized deterioration of skin condition and necrotic wound edges. Behavioral responses were recorded during attempts to move the animals downriver to the Pacific Ocean. The attempts included playback of alarm tones, humpback and killer whale sounds, banging hollow steel pipes ("Oikami pipes"), spraying water from fire hoses on the water surface, and utilizing tug and power boat engine noise and movement. None of these deterrents resulted in significant, consistent downstream movement by the whales. Antibiotic therapy (ceftiofur) was administered by a dart, representing the first reported antibiotic treatment of free-ranging live whales. After 11 d, the animals swam downstream from fresh water at Rio Vista to brackish water, and their skin condition noticeably improved 24 h later. The animals followed the deep-water channel through the Sacramento

Delta and San Francisco Bay, reaching the ocean at least 20 d after first entering the Sacramento River.

Key Words: freshwater, antibiotics, playbacks, biopsy, humpback whale, *Megaptera novaeangliae* 

### Introduction

In the past century, reports of cetaceans in coastal river systems have attracted considerable attention from the public and media due to concern over their distance from typical migratory routes and the potential for collision with ship traffic. Further concerns are raised due to the lack of suitable prey and the potential for adverse effects of fresh water on the osmoregulation and skin of cetaceans (Geraci & Bruce-Allen, 1987). Attempts to return whales swimming in river systems to the ocean have had mixed results. For example, a bottlenose whale (*Hyperoodon ampullatus*) in the Thames River in London was placed on a float to return it to sea, although it died during the effort (P. Jepson, pers. comm., 15 November 2007).

Killer whales (*Orcinus orca*) are the whale species most often observed in fresh water. In 1931, a killer whale swam 161 km up the Columbia River and was shot by a human opposite Vancouver, Washington, USA. In 1940, two killer whales were observed in the same area (Scheffer & Slipp, 1948). Nine killer whales were observed in Barnes Lake, southeast Alaska, in 1994 (a brackish tidal water body) and were herded out using boats and Oikami pipes after one animal died (D. Bain, pers. comm., 14 November 2007). Anecdotal reports of mysticete whales in fresh or brackish water are rarer than those of odontocetes. They include a northern right whale (Eubalaena glacialis) in the Delaware River, USA, in 1994 (D. Mattila, pers. comm., 1 November 2007); a Brydes whale (Balaenoptera edeni) up the Manning River, Australia, in 1994 (D. Coughran, pers. comm., 4 November 2007); and a humpback whale (*Megaptera novaeangliae*) in the Annapolis River, Canada, in 2004 (D. Jones, pers. comm., 25 November 2007). The most wellknown mysticete to stray into fresh water was a male humpback whale, "Humphrey," that swam up the Sacramento River in California and eventually returned to the Pacific Ocean after a variety of interventions, having spent 16 d in fresh water (see "Discussion").

The underlying reasons for why these whales were in atypical locations are unknown, and the health status of the individuals involved has been poorly documented. Despite the concern over the health of such individual whales and the considerable resources that are often mobilized to rescue them, the efficacy of such efforts is unclear. Herein, the effectiveness of methods used to attempt herding of a mother-calf pair of humpback whales from the Sacramento River in May 2007 is documented. The deteriorating health condition of the whales during their stay in fresh water and the first therapeutic injection of antibiotics into freeswimming baleen whales are also described.

## **Materials and Methods**

#### Whales

On 15 May 2007, a mother and female calf humpback whale pair were reported 72 nmi from the Golden Gate Bridge in the Port of Sacramento, California, USA, in a fresh-water basin at the north end of the Sacramento River Deep Water Ship Channel, after first being sighted by the public at Benicia in the San Francisco Bay/River Delta system on 9 May 2007 (Figure 1). A team of observers maintained watch on these animals during daylight hours from 16 to 29 May using binoculars and high-resolution photographs taken from a rigid-hull inflatable, with additional observations made from one to ten additional vessels. Small samples of skin and blubber were collected from the cow on 21 May and from the calf on 29 May using a crossbow and biopsy dart (Lambertsen, 1987). During the whales' occupancy of the turning basin in Sacramento and while near Rio Vista, underwater sound was monitored using a Korg D1200 digital recorder (linear frequency response from 10.0 Hz to 20.0 kHz) and a Reson 1330 omni-directional hydrophone (linear frequency response from 10.0 Hz to 60.0 kHz).

#### Herding Attempts

From 17-27 May, a series of herding techniques were used in an attempt to move the whales south



Figure 1. Map of the San Francisco Bay/Sacramento-San Joaquin River Delta in California, USA, showing locations of the two humpback whales on different days; beginning at Benicia, numbered days from first sighting locate the whales at their last recorded sighting on each day.

down the Sacramento River (Table 1). Both potentially attractive and aversive approaches were used, starting with the least aggressive. From 15-20 May, recordings of humpback whale feeding and social sounds were played from an underwater speaker placed approximately 2 to 3 m under water at various locations in the port, including both up- and downstream of the animals. From 21-23 May, a series of herding operations were attempted using boats carrying 6- to 10-cm diameter steel pipes hanging over the side. The pipes were struck repeatedly with hammers to simulate a Japanese dolphin herding technique (Oikami pipes; see Ohsumi, 2001). On 23 May, recordings of sounds made by a killer whale attacking a gray whale (Eschrichtius robustus) and tonal alarm sounds were played 200 m upstream from the whales using a similar setup to that used to play the humpback feeding calls above. The tonal alarm sounds had previously proven useful in redirecting right whales on the Atlantic coast of the United States (D. Nowacek, pers. comm., 2 January 2007). On 25 May, a fire hose (applying 3,785 1 of water/ min) was sprayed on the water surface ahead of the whales when they were heading north (upstream) rather than south to the Pacific Ocean.

## Antibiotic Treatment

The antibiotic used was ceftiofur crystalline free acid sterile non-aqueous suspension (Excede, 200mg/ml, Pfizer Animal Health). This is a cephalosporin antibiotic with a broad spectrum of antibiotic activity, long duration of action following a single injection, consistent pharmacokinetics across several domesticated species, and availability in a highly concentrated form. Dosing based upon metabolic body weight is a mechanism to translate dosage regimens across species; use of body weight (in kg) raised to the power of 0.75 provides a reasonable approximation of metabolic kg (Mkg) in mammals (Riviere, 1999). The dose of ceftiofur in cattle is 6.6 mg/kg (for a 350-kg steer), and in swine is 5 mg/kg (for a 100-kg pig) administered subcutaneously by injection (Pfizer Animal Health). The 13-m long female humpback whale was estimated to weigh 25,000 kg and the 7-m long calf was estimated at 5,000 kg (Geraci & Lounsbury, 2005). Calculated metabolic body weights and metabolically scaled ceftiofur dosages are shown in Table 2. Dosages for the whales were chosen based on the mg/Mkg dosages for cattle and swine. The lower end of the dosage scale was selected for the mother due to her larger body size (17 mg/Mkg, 171 mL injection), whereas the higher dosage was selected for the calf due to the younger age and likely higher metabolic rate associated with growth (19 mg/ Mkg, 57 mL injection).

The antibiotic was administered by projectile dart using a custom-modified Paxarms rifle (Paxarms New Zealand Ltd.). The dart contained 57 ml and consisted of a 19-mm diameter aluminum tube threaded at both ends, machined to have an O-ring seat. The flight end cap had a rubber gasket with a marginal air hole to allow pressurization behind the piston. The stainless steel, carbon fiber lined needle (30-cm  $long \times 0.64$ -cm diameter with three ports) was glued into an end piece that threaded into the barrel and the side ports covered with a sleeve. A rubber buffer was screwed over the end piece. All of the O-rings, the syringe, and the projector barrel were coated with a silicon lubricant. The syringe was pressurized by pumping up the chamber behind the piston through a port in the axis of the flight to 130 psi. Once charged, a cap was placed on the flight port. The drug was released when needle penetration induced the port sleeve to slide up the needle, allowing the pressure to inject the drug.

#### Results

## Whales

On first assessment of the whales on 16 May, there was no consistent swim direction observed. dives were shallow, and whales surfaced every 4 to 8 min. Both animals appeared to be in good body condition, but they did have lacerations penetrating blubber and underlying muscle suggestive of sharp trauma from a boat strike (Figure 2). The adult female (estimated at 13-m long) had a straight-sided cut approximately 1-m long and 30-cm deep extending across the dorsum, cranial to the dorsal fin. The calf (estimated at 7-m long) had a vertical straight-sided cut on the right lateral thorax that was of unknown length due to the lack of visibility below the water. During this observation period, the remaining skin of both animals appeared in good condition, with no excess of cyamids or unusual lesions observed (Figure 2).

Only marginal identification photographs of the underside of the flukes of the animals could be obtained due to the infrequency with which they showed any portion of their flukes in the shallow water. Comparison of the limited views of the flukes that were obtained to the more than 1,800 humpback whales identified since 1986 off the U.S. West Coast (see, for example, Calambokidis et al., 1996; Calambokidis & Barlow, 2004) or to the broader collection of identification photographs from throughout the North Pacific for 2004 to 2006 from the Structure of Populations, Levels of Abundance and Status of Humpbacks (SPLASH) program did not reveal any matches.

The sex of the calf and cow were confirmed as female by amplification of a fragment of the

		ea of n prior t until	ods with ape. Ebb	, 1440 h.	idge, dge and	mpt; no			iged. 0700 h
	Comments	Overall tendency to move away from a transmission. Tugs active in turning bas to playbacks. Ebb current out of the Po ifter noon.	Playbacks of clips played for 5-min per 5-min intervals, then 45-min continual surrent out of Port until about 1400 h.	Whales left the turning basin on 20 Ma	Whales passed south under Rio Vista B then turned north and returned under by under the line of vessels with pipes.	Successful biopsy of cow on second att attempt to biopsy calf due to high wind conditions.	Pipe banged effort inconsistent.		19 vessels in flotilla; consistent pipe ba
VU 14144 2001	Effect(s) (	None	None	None until 20 May 1440 h at which time they left the turning basin.	None	None	Swam north under pipe- line twice, then turmed south and swam down river to 200 m north of Rio Vista Bridge. Turmed north at 2100 h.	Swam north under the pipeline three times.	Turned north under the
	Whales' movements prior to intervention	Swam slowly, no specific direction	Swam slowly, no specific direction	Swam slowly, no specific direction	Swam south down river.	Swam up- and downstream.	Swam north up river.	Moved downstream.	Calf tail lobbing; no consistent swim direction
mount rain pair of mumpoart	High tide location, time, height	Port of Sacramento 0524 h:2.52 m 1900 h:2.07 m	Port of Sacramento 0609 h:2.55 m 1959 h:2.1 m	19 May, Port of Sacramento 0658 h.2.52 m 2055 h: 2.07 20 May, Port of Sacramento 0743 h.2.46 m 2155 h.2.04 m Rio Vista 2059 h: 1.06 m	Rio Vista 0659 h:1.05 m	Rio Vista 0659 h:1.23 m 2155 h:1.05 m	Rio Vista 0659 h:1.23 m 2155 h:1.05 m	Rio Vista 0803 h:1.08 m	Rio Vista
ם חורוו רווייייט עווי	Date, Time	17 May 0900-1500 h	18 May 1100-1615 h	19, 20 May	21 May 0700-1045 h	21 May 1400-1530 h	21 May 1700-2100 h	22 May 1220-1445 h	23 May
Table 1. IIICI VOIDUN MI	Method	Playbacks of Alaskan humpback foraging sounds	Playbacks of California and Alaska humpback sounds	No stimuli; whales observed from deck of coastguard vessel	Banged hollow-steel pipes	Biopsied skin with a dart (cow)	Banged hollow-steel pipes	Banged hollow-steel pipes	Banged hollow-steel

Table 1. Interventions and their effects on a mother-calf pair of humback whales in the Sacramento River May 2007

Method	Date, Time	High tide location, time, height	Whales' movements prior to intervention	Effect(s)	Comments
Tug boat engine noise nearby	23 May 1130-1200 h	Rio Vista 0922 h:0.96 m	Swam north under pipeline.	Headed towards tugs.	Engines were stopped when whales were 50 mm from tugs. Ebb current during this time.
Playbacks of record- ings of killer whale attack on gray whale	23 May 1500-1700 h	Rio Vista 0922 h:0.96 m			Ebb current during this time.
Playbacks of tonal sounds	23 May 1700-2000 h	Rio Vista 0922 h:0.96 m Rio Vista 2337 h:1.08 m	Swam in no consistent direction 1.6 km north of the Rio Vista Bridge.	Whales turned away from the upswing tone, downstream towards the bridge heading south, but turned back at the bridge and, once head- ing north, could not be turned south by the sounds. At end of ses- sion (at sunset), whales headed straight at speak- ers, passing within 3 m of the perceived sound of approximately 180 dB with no detectable response.	
Fire hose	25 May 1030-1120 h	Rio Vista 0022 h:1.14 m 1209 h:0.78 m	Swam north up Cache Slough.	Turned 90° from hose, but once 200 m south from the hose, turned back north.	Once away from the main volume of water from the hose, they returned to the original swim direction. A flood current was in effect.
Administered antibiotic by dart	26 May; first darts at 1030-1240 h then final dose for female at 1703 h	Rio Vista 0101 h:1.2 m 1317 h:0.78 m	Swam around north of Rio Vista Bridge; no consis- tent direction. Vocalized.	Calf did extensive flip- per slapping and rolling; mother exhaled under water several times.	Whales left the area 24 h later.
Scraped skin sample	27 May 0920-1220 h	Rio Vista 1416 h:0.81 m	Milling north of the Rio Vista Bridge.	No directed movement	Whales passed under the Rio Vista Bridge at 1330 h near the end of the flood current at Rio Vista.
Use of engine noise from inflatable by circling tightly behind whales	27 May	Decker Island 1415 h:0.75 m	Swam north back toward the Rio Vista Bridge.	Continued swimming south away from bridge	Unclear whether driving the inflatable in tight circles behind the whales caused an increase in swim speed or directionality to movement.
Biopsied skin (calf)	29 May 1830 h	Angel Island 2245 h:1.77 m	Milling in shallows off Tiburon	Deeper dive; more pur- poseful. Swam away from boat.	Flood current was in effect from about 1700 h until after 2200 h.

	Animal weight (kg)	Dose (mg/kg)	Total mg dose = BW(kg) × Dose (mg/kg)	Metabolic body weight (Mkg) = $BW(kg)^{0.75}$	Dose (mg/Mkg) = Total mg dose/Mkg
Cattle minimum dose	350	4.4	1,540	80.9	19.0
Cattle maximum dose	350	6.6	2,310	80.9	28.5
Swine	100	5.0	500	31.6	15.8
	Animal weight	Metabolic body weight (Mkg) = $BW(kg)^{0.75}$	Dose	Total mg dose = BW(Mkg) × Dose (mg/Mkg)	Dose mls
	(Kg)	D ((Kg)	(ing/wikg)	(ing/wikg)	(200 mg/m)
Humpback cow low	25,000	1,988.2	16.0	31,811	159*
Humpback cow high	25,000	1,988.2	19.0	37,775	189
Humpback calf low	5,000	594.6	16.0	9,514	48
Humpback calf high	5,000	594.6	19.0	11,297	56*

Table 2. Calculations for metabolically scaled Ceftiofur dosage injected in a mother and calf humpback whale

\*Indicates target dosage

sry gene multiplexed with fragments of the ZFY/ ZFX genes as positive control, and as described by Gilson et al. (1998). Sequencing of the mitochondrial (mt) DNA control region from skin showed both whales were an E1 haplotype, which is common in the California feeding population but not in the British Columbia/southeast Alaska feeding populations (e.g., Baker et al., 1990, 1998; Witteveen et al., 2004).

Lipid content of the cow's blubber in the biopsy plug was determined using TLC/FID via an Iatroscan Mark 6 (Ylitalo et al., 2005) and was 3.8%, consisting entirely of triglycerides. This value was below the range for lipid content of other humpback whales sampled off California in July (blubber lipid content mean was 14%) (G. Ylitalo, pers. comm., 30 June 2007).

#### Herding Attempts

The whales remained in the turning basin within the Port of Sacramento for 5 d (15-20 May) despite multiple attempts to lure them to the ocean using playbacks of humpback whale social sounds. During these 5 d in fresh water, the wounds on both animals appeared to widen, and the wound edges to become necrotic (Figure 2). On 20 May, the whales left the turning basin within 10 min of the movement of two tug boats across the basin, and they headed south down a 70-m wide channel towards a northbound freighter (192-m long, 30-m wide, 7-m draft). This movement may not have been a direct result of the activity of the tugboats as these tugs had been active at similar speeds within the basin for the previous 5 d. Tug movement was halted, and the whales swam around the incoming freighter and continued south downstream for 6 h at 5 to 9 km/h against a flood current, surfacing every 2 to 3 min (Figure 1). The whales were followed at a distance

of 150 m by 10 vessels, and 2 to 4 helicopters flew 500 m above the animals as they swam south. At dark (2100 h), the whales were last seen at the south end of the deep-water ship channel (Figure 1). The whales left the Port basin shortly before low tide at approximately 1430 h and swam into a flood tide that ended about 2100 h. The following morning at 0600 h, the whales were observed within 200 m of the previous evening's last sighting.

On 21 May, they swam downstream under the Rio Vista Bridge, then turned about 100 m beyond the bridge and returned up river, swimming under the bridge. During the first passage, the bridge was lifted, preventing traffic from driving over it. On the whales' return swim, however, the bridge was lowered, and road traffic was crossing the bridge continually. From 21-27 May, the whales remained within a 9.6-km stretch between the Rio Vista Bridge and the fork of Lindsey and Cache Sloughs during daylight hours (Figure 1). They swam up and down river, and often changed direction in association with changes in current, tending to swim into the current, although this was not a consistent observation. Typically, the southernmost point of their excursion was within 100 m north of the Rio Vista Bridge. The northernmost point they reached during this period was within Cache Slough (Figure 1); they were not observed entering the Sacramento Deep Water Ship Channel to the north.

The use of Oikami pipes did not result in consistent whale movements downstream. Although a line of up to 30 vessels of varying sizes was used, the whales swam upstream under the line of pipes on multiple occasions. On 23 May, playbacks of recordings of killer whales and tonal alarm sounds did not result in consistent movement of the whales, despite repeated playbacks in close



Figure 2. Changes in skin and wound conditions in the mother and calf humpback whale over 16 d

proximity to the whales at source levels exceeding 180 dB re: 1  $\mu$ Pa. After the use of a fire hose on 25 May, the whales turned 90° towards the bank of the river, and then turned back north at 100 m from the fireboat. This intervention was repeated three times, and the same response was observed.

On 26 May, the whales were darted with antibiotics (see the "Health Assessment and Antibiotic Therapy" section). Extensive digital recordings (16-bit, 44.1 kHz sampling rate) during herding attempts using underwater playbacks of humpback and killer whale vocalizations and noise from the pipes showed that neither the female nor the calf were heard to vocalize until 26 May after they were darted, when brief vocalizations were recorded from the calf during bouts of slapping pectoral fins on the surface of the water.

On 27 May, the whales finally swam under the Rio Vista Bridge at 1400 h, shortly before high

tide on the end of a flood current, and swam slowly downstream with an ebb current, for 6 h following the deep water channel to the San Joaquin River (Figure 1). On 28 May, the whales were relocated at dawn 20.9 km further downstream and about 200 m upstream of the Benicia Bridge in brackish water. They remained upstream of the bridge all day, and the calf performed multiple (> 100) chin slaps and breaches. The whales passed underneath the Benicia Bridge during the night of 28-29 May and were initially observed between the Benicia and Carquinez Bridges on the morning of 29 May at 0630 h. On 29 May, the whales moved under the Carquinez Bridge at 1020 h and under the Richmond Bridge at 1630 h, with an approximate swim speed of 5 to 9 km/h towards the Golden Gate Bridge. At 1645 h, they entered shallow water approximately 200 m beyond the Richmond Bridge and milled in this area for 2 h. Although

moving slowly towards the Golden Gate Bridge, purposeful movement appeared to stop in the shallow waters off Tiburon (Figure 1). The whales were last seen on 29 May at 2000 h at 5 nmi east of the Golden Gate Bridge heading west along the shore of the Tiburon Peninsula (Figure 1). Thorough searches of the delta and Golden Gate from water, land, and air over the following week did not reveal the whales. These surveys revealed the presence of other humpback whales in the open waters outside the Golden Gate typical of their distribution off northern California (Calambokidis et al., 1990; Barlow & Forney, 2007), including several whales feeding on dense schools of fish in the shipping lane at the entrance of San Francisco Bay. The presence of whales and prey at this location of high ship traffic could explain how the mother and calf were originally struck.

## Health Assessment and Antibiotic Therapy

Daily photographs showed generalized deterioration of skin condition and necrosis of wound edges in both whales as they remained in fresh water (Figure 2). After the first week in fresh water, the calf's wound was not further photographed because the animal rarely surfaced sufficiently for the wound to be seen. Histology of a skin biopsy from the cow collected on 21 May showed hydropic degeneration of epithelial cells and a mild eosinophilic dermatitis. A culture of skin scrapings taken from the cow on 27 May grew mixed bacteria, including Acinetobacter sp., Moraxella sp., Klebsiella oxytoca, Comamonas sp., Pseudomonas fluorescens, and Aeromonas hydrophila. No protozoa or diatoms were observed on microscopic examination of a smear from a skin scraping. Based on these observations and the prolonged duration of exposure to fresh water, the whales were treated with antibiotics to reduce the risk of septicemia following infection of the necrotic lacerations.

A single syringe was delivered to the calf at 1047 h on 26 May 2007. The dart was aimed at the dorsal fin area, but it fell short and entered the water about 2 m from the animal, then the needle penetrated the blubber ahead of the dorsal fin, to a depth of 3 to 6 cm. It was estimated that this was at or just below the subdermal sheath. The animal showed a degree of surging in the water 1 min later. The needle was observed to be absent by 1300 h on the same day after an extensive bout of the calf slapping her pectoral flipper against the water surface. Five syringes were shot at the mother on 26 May, three of which penetrated the skin. At 1105 h, a dart was fired at the right dorsal flank but glanced off the body. At 1121 h, a second dart was fired. The shot was low, skewed in flight, and did not penetrate the cow's skin. At 1140 h, a

dart was shot and penetrated the right flank, anterior to the dorsal fin to 80% of the needle length. At 1240 h, a dart penetrated to 80% of the needle ~30 cm anterior to the first injection. At 1710 h, a third syringe penetrated the left flank to 80% of the needle. After the first night, the needles appeared bent at a 90° angle such that the syringe barrel lay along the surface of the whale. Over the following 3 d (27-29 May), all of the darts fell out. The day after darting, the animals swam to brackish water, and their skin condition noticeably improved 24 h later (Figure 2). The whales had been in fresh water at least 16 d and had been in the river delta system at least 20 d.

#### Discussion

This report of two humpback whales in the Sacramento River describes the longest period this species has been observed in fresh water and documents their movements and condition during considerable human activity in their vicinity. Interestingly, their route up the Sacramento River was similar to one taken by a male humpback, "Humphrey," in 1985 (Figure 3). In both incidents, the humpback whales swam upstream deep into the Sacramento River Delta to a "dead end" location; spent several days in those locations; subsequently spent several days in a general location within an unrestrained environment; spent similar lengths of time in fresh water before exiting the delta; showed general resistance to herding attempts by humans; made self-initiated long and purposeful swims in their travel, regardless of human-made stimuli; and spent 2 to 3 d in transition from fresh water to salt water during their exits.

Although bridges appeared to limit the whales' movement (an observation that was also noted with Humphrey), other factors at these sites could have been important. The shadows of the bridges may have been perceived as obstacles by the whales as they most often moved under bridges when the sun was at a low angle or hidden by clouds. The Sacramento River widens about 100 m north of the Rio Vista Bridge where the whales often turned. Although road traffic over this bridge was often suggested to be limiting the whales' movement, the whales swam south under the bridge and then turned north back under it on 21 May while traffic was passing over it, in addition to the initial trip upriver. Cessation of road traffic, a drilling operation which was using compressed air to clear a shaft 500 m from the bridge, and an underwater Doppler current recorder under the bridge did not result in immediate movements past these potential obstructions. Interestingly, on the two occasions the whales left a site after 5 d of



Figure 3. Map of the movements of a male humpback whale in 1985, showing the temporal and spatial similarities to the movements of the mother and calf humpback whales in 2007

milling, no attempts to herd the animals had been made for 24 h.

Despite use of a variety of stimuli and herding techniques, there was no clear association between when whales moved past apparent hurdles in the river and the use of different interventions. The final movement of the animals the day after administration of antibiotics could have been a result of improved health, repeated harassment from the dart and observation teams, the influence of a flood tide, lack of food supply, or enough time having been spent at the Rio Vista Bridge to have explored the area sufficiently to identify a departure route.

The male humpback in the Sacramento River in 1985 was reported to have moved toward the playback of sounds of foraging humpback whale vocalizations on the final 2 d (L. Gage, pers. comm., 20 May 2007). The different reactions observed in these two situations may reflect individual or sex differences in responses to such playbacks of such sounds as well as differences in ambient noise as the wide repertoire of humpback vocalizations suggests a variety of likely uses for different calls (Dunlop et al., 2007). Observations in Hawaii indicate that male humpback whales move toward playbacks of foraging humpback whale sounds, although females do not, possibly due to sexually active males seeking mates (Mobley et al., 1988). The lack of response to the noise of banging pipes, a method which has been shown to be effective in moving killer whales (D. Bain, pers. comm., 14 November 2007) and dolphins, may be due to physiological differences between baleen and odontocete whale hearing (Wartzok & Ketten, 1999), although it is difficult to imagine that the noise was not detectable by the whales. Sound from the pipes recorded at a distance of 1 km averaged 14.4 dB re: 1 µPa above ambient underwater environmental noise in the range of 2.0 to 18.0 kHz. Although mysticetes are probably more sensitive to lower frequency sound, 2.0 kHz is well within the range of frequencies that humpback whales use to communicate (Wartzok & Ketten, 1999; Au, 2000). Boats with people banging pipes were typically within 500 m of the whales. Recordings of the pipe sounds from 0.9 km showed that there was a broad spectrum of frequencies in pipe noise. The frequencies with the highest amplitude (about 14 dB above ambient noise at 1 km) fell between approximately 3.0 and 11.0 kHz for the pipes used here. If pipe noise were to be used in future whale herding maneuvers, the pipes could be "tuned" to lower frequencies by cutting the length longer and using larger diameter pipes.

The skin changes observed in these animals likely reflected osmotic effects on the epithelium and colonization by atypical microorganisms from being in fresh water. Although skin sloughing has been reported in bottlenose (Tursiops truncatus) and common (Delphinus sp.) dolphins within 24 h of placement in fresh water, and salinity below 2% is considered unsuitable for maintenance of captive odontocete cetaceans (Greenwood et al., 1974), excessive skin sloughing was not observed in these whales. This could have been masked by the cream-colored plaques and film on the skin, however. The speed of improvement observed in the whales' skin upon re-entry to saline water suggests the plaques observed were superficial infestations of the skin, such as algae or protozoa, sensitive to increased salinity, rather than deep

bacterial infections. The flora of bowhead whale (Balaena mysticetus) skin can include diatoms and protozoa as well as bacteria (Henk & Mullan, 1996). The use of antibiotics was intended to prevent septicemia resulting from increasing necrosis of the lacerations and is unlikely to have been effective in improving skin conditions within 24 h. As this report describes the first use of antibiotics in free-ranging whales, it would have been ideal to monitor blood levels of ceftiofur following administration as well as the site of dart penetration to observe any potential post-darting side effects such as tissue necrosis. This was logistically unfeasible, although this study does show that such administration is possible and that no anaphylactic response was observed following the use of ceftiofur. Thus, further studies of the pharmacokinetics of this drug in cetaceans are warranted. Improvements could also be made to the form of drug administration. The 30-cm needle used in these animals was designed for use in right whales to penetrate the blubber and reach the underlying muscle. Penetration to the muscle was likely achieved in these whales by reducing the pressure in the delivery system. In the future, a variety of darts should be designed with shorter needles for species with relatively thinner blubber thicknesses.

Based on the observations of the responses of these two whales to intervention, and a review of anecdotal descriptions of other mysticete whales in unusual locations, management of similar events in the future should focus on protection of the animals from disturbance and ship strikes, rather than attempting to herd them. The whale(s) should be allowed time to explore their habitat and discover exit routes without efforts to drive them out. Regular observations using high resolution photography should be used to monitor skin integrity, and the use of satellite telemetry (without compromising the health of the animal) would be useful for long-term monitoring of animals' movements after leaving the inland location as re-sighting at sea is opportunistic and unlikely to provide detailed follow-up information.

### Acknowledgments

We thank California Lieutenant Governor John Garamendi for his efforts in saving these whales and his concern for ocean protection, and the U.S Coast Guard, California Office of Emergency Services, California Department of Fish and Game, California Highway Patrol, National Marine Fisheries Service, and the staff and volunteers of The Marine Mammal Center for their efforts in this response. We especially thank Bernie Kraus for initial playbacks of humpback sounds; David Rotstein for histology of the skin biopsy; Gina Ylitalo for blubber lipid analysis; Bob Wilson and Tom Kieckhefer for photography; Tenaya Norris and Denise Greig for assistance with figures; Pfizer Animal Health for their generous donation of Excede and Scott Brown for dosage advice; Bayer for the donation of Baytril; Grandpa's Compounding Pharmacy for concentrating drugs; and Drs. Jim McBain, Sam Ridgway, and Sue Thornton for advice on antibiotic treatment. Funding for this response was provided through the National Marine Fisheries Service John H. Prescott Marine Mammal Rescue Assistance Program.

## Literature Cited

- Au, W. W. L. (2000). Hearing in whales and dolphins: An overview. In W. W. L. Au, R. R. Fay, & A. N. Popper (Eds.), *Hearing by whales and dolphins* (pp. 1-42). New York: Springer-Verlag.
- Baker, C. S., Palumbi, S. R., Lambertson, R. H., Weinrich, M. T., Calambokidis, J., & O'Brien, S. J. (1990). Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature*, 344, 238-240.
- Baker, C. S., Medrano-Gonzalez, L., Calambokidis, J., Perry, A., Pichler, F., Rosenbaum, H., et al. (1998). Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, 6, 695-707.
- Barlow, J., & Forney, K. A. (2007). Abundance and population density of cetaceans in the California Current ecosystem. *Fishery Bulletin*, 105, 509-526.
- Calambokidis, J., & Barlow, J. (2004). Abundance of blue and humpback whales in the eastern North Pacific estimated by capture-recapture and line-transect methods. *Marine Mammal Science*, 20, 63-85.
- Calambokidis, J., Cubbage, J. C., Steiger, G. H., Balcomb, K. C., & Bloedel, P. (1990). Population estimates of humpback whales in the Gulf of the Farallones, California. *Report to the International Whaling Commission, Special issue 12*, 325-333.
- Calambokidis, J., Steiger, G. H., Evenson, J. R., Flynn, K. R., Balcomb, K. C., Claridge, D. E., et al. (1996). Interchange and isolation of humpback whales off California and other North Pacific feeding grounds. *Marine Mammal Science*, 12, 215-226.
- Dunlop, R. A., Noad, M. J., Cato, D. H., & Stokes, D. (2007). The social vocalization repertoire of east Australian migrating humpback whales (*Megaptera* novaeangliae). Journal of the Acoustical Society of America, 122, 2893-2905.
- Geraci, J. R., & Bruce-Allen, L. (1987). Slow process of wound repair in beluga whales, *Delphinapterus leucas*. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 1661-1665.

- Geraci, J. R., & Lounsbury, V. J. (2005). Marine mammals ashore: A field guide for strandings (2nd ed.). Baltimore: National Aquarium in Baltimore. 372 pp.
- Gilson, A., Syvanen, M., Levine, K., & Banks, J. (1998). Deer gender determination by polymerase chain reaction: Validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, 84, 159-169.
- Greenwood, A. G., Harrison, R. J., &. Whitting, H. W. (1974). Functional and pathological aspects of the skin of marine mammals. In R. J. Harrison (Ed.), *Functional anatomy of marine mammals* (pp. 71-110). London: Academic Press.
- Henk, W. G., & Mullan, D. L. (1996). Common epidermal lesions of the bowhead whale, *Balaena mysticetus*. *Scanning Microscopy*, 10, 905-916.
- Lambertsen, R. H. (1987). A biopsy system for large whales and its use for cytogenetics. *Journal of Mammalogy*, 68, 443-445.
- Mobley, J. R., Herman, L. M., &. Frankel, A. S. (1988). Responses of wintering humpback whales (*Megaptera* novaeangliae) to playback of recordings of winter and summer vocalizations and of synthetic sound. Behavioral Ecology and Sociobiology, 23, 211-223.
- Ohsumi, S. (1972). Catch of marine mammals, mainly of small cetaceans, by local fisheries along the coast of Japan. Bulletin of the Far Seas Fisheries Research Laboratory, 7, 137-166.
- Riviere, J. E. (1999). Comparative pharmacokinetics. Ames: Iowa State University Press. 307 pp.
- Scheffer, V. B., & Slipp, J. W. (1948). The whales and dolphins of Washington State with a key to the cetaceans of the west coast of North America. *The American Midland Naturalist*, 39, 257-337.
- Wartzok, D., & Ketten, D. R. (1999). Marine mammal sensory systems. In J. E. Reynolds III & S. A. Rommel (Eds.), *Biology of marine mammals* (pp. 117-175). Washington, DC: Smithsonian Institution Press.
- Witteveen, B. H., Straley, J. M., Von Ziegesar, O., Steel, D., & Baker, S. (2004). Abundance and mtDNA differentiation of humpback whales (*Megaptera novaeangliae*) in the Shumagin Islands, Alaska. *Canadian Journal of Zoology*, 82, 1352-1359.
- Ylitalo, G. M., Yanagida, G. K., & Hufnagle, M. M. (2005). Determination of lipid classes and lipid content in tissues of aquatic organisms using a thin layer chromatography/flame ionization detection (TLC/FID) microlipid method. In G. K. Ostrander (Ed.), *Techniques in aquatic toxicology, Vol. 2* (pp. 227-237). Boca Raton, FL: CRC Press.