



Vaquitas (*Phocoena sinus*) continue to die from bycatch not pollutants

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Abstract

Background The vaquita of Mexico is critically endangered, with a population less than 19 individuals in 2018. The population continues to decline and gillnet use continues in vaquita habitat.

Methods Nine vaquita carcasses were examined from 2016 to 2018 to establish cause of death. Concentrations of polychlorinated biphenyls (PCBs), DDTs and polybrominated diphenyl ethers (PBDEs) in blubber (n=3) were determined using gas chromatography/mass spectrometry and faeces tested for domoic acid and saxitoxin.

Results Carcasses were in good nutritional status and had lesions and full stomachs consistent with fisheries bycatch. PCB, DDT and PBDE concentrations ranged between 94 and 180 ng/g, 500 and 1200 ng/g and 97 and 210 ng/g lipid weight, respectively, which are low compared with other marine mammals. No saxitoxin or domoic acid was detected.

Conclusion These findings support the conclusion that bycatch is the primary source of vaquita population decline and emphasise the need for stronger measures to eliminate gillnets from the Upper Gulf of California, Mexico.

Introduction

The vaquita (*Phocoena sinus*) is the world's most endangered marine mammal, with a current population estimate of fewer than 19 individuals.¹ It is endemic to the Upper Gulf of California (UGC), México, where it has been known to be caught in fishing nets since its discovery in 1958: in the early 1990s, 17 per cent of the population was estimated to be killed in gillnets based on direct observations of artisanal fishing.² Between 1997 and 2008, the population decline of 7–8 per cent

per year was attributed primarily to mortality in gillnets set for shrimp and finfish.³ Despite a ban on gillnet use in the vaquita range, an illegal totoaba fishery continues, driven by the black market for totoaba swim bladders in China.⁴ As continued efforts to save the vaquita from extinction focus on reducing mortality in gillnets, alternative explanations for the population decline are aired, despite comprehensive assessment and rejection of these hypotheses.⁵ Exposure to persistent organic pollutants (POPs) has been suggested as contributing to poor health and reproductive impairment, and harmful algal blooms killing other marine biota have occurred in the UGC.⁶ Here, the authors describe several recent carcasses collected from this rare species to provide data on nutritional status, lesions, biotoxin and blubber pollutant levels, to better inform management actions for conservation of the vaquita.

Materials and methods

All animals were found dead, so no ethics forms were required for this study. Carcasses were found on the beach of San Felipe, Baja California, Mexico, or floating in the Vaquita Refuge and collected under permit (PROFEPA No. SGPA/DGVS/07534/17). Blubber samples were exported from Mexico to the USA under CITES permits MX 89760 and 17US774223/9, and MMPA permit no 18706. One vaquita, V02F, died following capture

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for conservation efforts.⁷ All carcasses except V02F were frozen and thawed before examination. Carcass examination was conducted as described by Raverty and others, and 20 g blubber samples wrapped in Teflon and 20 g of faeces stored in a plastic vials at -20°C until analysis.⁸

Blubber samples from four vaquitas were analysed for lipids and POPs as described in Sloan and others.⁹ Briefly, ~ 0.5 g blubber was extracted using accelerated solvent extraction with methylene chloride. Before extract cleanup, two aliquots of each sample extract were removed for gravimetric lipid quantitation and lipid class determinations using a thin-layer chromatography/flame ionisation detection method using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan).⁹ Five classes of lipids (ie, sterol/wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated on silica rods using a chromatography tank containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v) based on polarity. The sample extract for POPs analysis was filtered through a gravity flow glass column packed with silica gel and alumina to remove polar compounds and concentrated for further cleanup using size exclusion liquid chromatography to remove lipids and other interfering compounds.

The cleaned extracts were analysed for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (eg, DDTs, chlordanes, dieldrin) by gas chromatography/mass spectrometry, with the mass spectrometer operated in selected ion monitoring mode. Summed PCBs (ΣPCBs) were calculated by summing the concentrations of 46 PCB congeners present as 40 chromatographic peaks (congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209). The summed DDTs (ΣDDTs) were calculated by summing the concentrations of *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT; $\Sigma\text{chlordanes}$ is the sum of oxychlordane, *gamma*-chlordane, nona-III-chlordane, *alpha*-chlordane, *trans*-nonachlor and *cis*-nonachlor; ΣHCHs (hexachlorocyclohexanes) is the sum of *alpha*-HCH, *beta*-HCH and *gamma*-HCH isomers and ΣPBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155 and 183, plus two pentabrominated, one hexabrominated and one heptabrominated congeners for which congener numbers are not known.

Faecal samples from three animals were tested for domoic acid and saxitoxin as described in Lefebvre and others.¹⁰

Results

Eight vaquita carcasses (sex, age and weights in table 1) were found in March and April 2016–2018, while one adult female (V02F) died in November 2017 following

capture for translocation. All animals except V02F were moderately to severely decomposed, with all tissues too decomposed for useful histological or microbiological examination. Postmortem findings in V02F have been previously reported and include cardiomyopathy and nephropathy consistent with capture stress.⁷

The eight animals that were found dead had severe sloughing of the skin. Blubber thicknesses at the lateral axilla varied from 0.4 to 1.4 cm. One (Ps4) had multifocal circular to ovoid 1–3 cm long lacerations with clean margins over the left side of the body consistent with stab wounds (see figure 1A). Two animals (Ps1, Ps4) had missing flukes at the time of postmortem examination, but photographs of one of these on the beach showed intact flukes, indicating removal was postmortem. Five animals had marks on the skin consistent with net or monofilament abrasion (see table 1 and figure 1B,C,D). These marks were cross hatchings reflective of a stretch mesh size of approximately 15 cm in two animals (figure 1B). Six animals had severe subcutaneous bruising, oedema and congestion mostly around the head, neck and axilla (see figure 1E). These six animals had stomachs containing partially digested fish, indicating recent ingestion (see figure 1F). The one adult female carcass had a distended uterine horn consistent with recent parturition.

Blubber lipid was 100 per cent triacylglycerols in three animals (Ps2, Ps7, V02F), while blubber from Ps9 had relatively high proportions of free fatty acids (22.9 per cent) in addition to triacylglycerols (77.1 per cent), indicating that the quality of the blubber was insufficient for pollutant determinations.

Blubber concentrations of POPs from three necropsied vaquita are shown in table 1. Overall, ranked concentrations of POPs were $\Sigma\text{DDTs} > \Sigma\text{PCBs} \sim \text{PBDEs} > \Sigma\text{chlordanes} > \text{HCB} > \Sigma\text{HCHs}$. Concentrations of POPs in the two juvenile females (Ps2, Ps7) were approximately twice that in the adult female (V02F), on a lipid-weight basis. Blubber ΣDDT concentrations ranged from 500 to 1200 ng/g, lipid weight (lw), whereas lower levels of ΣPCBs (94–180 ng/g lw) and ΣPBDEs (97–210 ng/g lw) were determined in these blubber samples.

No saxitoxin or domoic acid was detected in faecal samples from the three animals tested (Ps1, Ps3, Ps7).

Discussion

The seasonality of carcass discovery likely reflects the seasonality of totoaba runs and illegal gillnet fishing in the UGC. Although all carcasses were decomposed with sloughing skin so that evaluation of nutritional status was difficult, each had areas of the body with an intact blubber layer that was oozing fat and did not appear atrophied. The fresh adult, V02F, was in good nutritional condition with a thick blubber layer. The lesions in seven of the eight animals found dead were consistent with criteria for per-acute underwater

Table 1 (A) Details of nine vaquita carcasses examined in Baja California, Mexico, 2016–2018 (X=estimate) and (B) toxin levels in vaquita carcasses (BDL: below detectable limit)

(A)							
ID	Date found/date examined	Age class	Wt (kg)	Length (cm)	Sex	Gross lesions	
Ps1	March 4/March 24, 2016	Subadult		126*	M	Oedema, congestion and haemorrhage around neck and axilla, left side skin and blubber missing, flukes missing, stomach full of partially digested fish	
Ps2	March 13/March 24, 2016	Subadult, ovaries smooth, no corpora evident	29	127	F	Cross-hatched marks on skin of right flank, oedema, congestion and haemorrhage around neck, stomach full of partially digested fish	
Ps3	March 24/March 25, 2016	Adult, testes 500 and 550 g	41.7	140*	M	Linear circumferential skin marks around body, haematoma of entire head, stomach full of partially digested fish	
Ps4	March 19/March 29, 2017 floating	Adult, testes 20 cm long	36.7	140*	M	Stab wounds 1–3 cm long left side (upper side when floating), oedema and haemorrhage in axilla and ventral neck, flukes missing (removed postmortem), stomach full of partially digested fish	
Ps5	March 9/March 29, 2017	Fetus		61	F	Umbilicus patent, dorsal fin folded	
Ps7	April 20/July 11, 2017	Juvenile, ovaries smooth with no corpora evident		109.5	F	Cross-hatched marks with parallel lines 15 cm apart on skin of body, haemorrhage in musculature, partially digested fish in stomach	
Ps8	April 25/April 27, 2017	Juvenile		113	F	Linear impressions and cross-hatch marks on skin, bruising of melon fats	
Ps9	March 28/April 24, 2018	Adult	40	140	F	Abrasion of rostrum, bruising of neck and axilla, notch of skin missing from trailing edge dorsal fin, stomach full of partially digested fish, severely distended uterine horn (approximately 50 cm diameter)	
V02F	November 4, 2017	Adult, 15 years	41	136	F	Skin lesion on head typical of monofilament cut	
(B)							
ID	HCB	∑HCHs	∑CHLDs	∑PCBs	∑PBDEs	∑DDTs	Biotoxins
Ps1							Saxitoxin, DA: BDL
Ps2	29	14	55	180	170	1200	
Ps3							Saxitoxin, DA: BDL
Ps7	37	13	47	160	210	1200	Saxitoxin, DA: BDL
V02F	12	BDL	21	94	97	500	

All persistent organic pollutants are reported in ng/g lipid weight.
PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl.

entrapment.¹¹ Skin lesions were consistent with net, rope or monofilament entanglements, although skin sloughing and decomposition made these lesions hard to detect. The stomachs with recently ingested prey observed in six of these seven are one of the most

consistent findings in cetacea bycaught in fishing nets.¹² No lesions were observed in the fetus which was markedly autolysed, so whether it was aborted or died because its mother also died and her carcass was not found is unknown. Although decomposition precluded

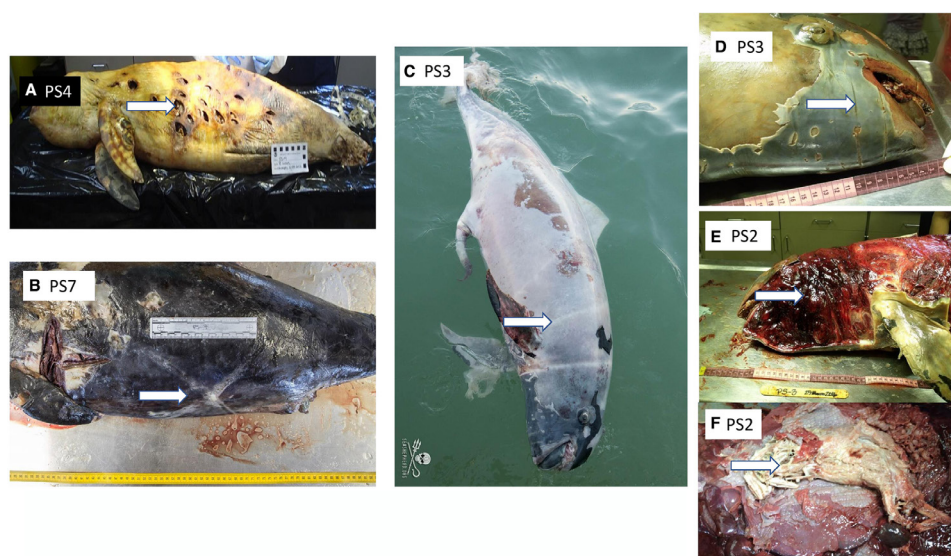


Figure 1 Photographs of vaquita carcasses, with arrows indicating (A) stab wounds, (B) cross-hatch marks, (C) net impressions (D) linear lacerations, (E) haemorrhage, head region and (F) partially digested prey in stomach.

useful microbiological analyses, no gross lesions suggestive of fatal infectious disease were evident.

Levels of POPs measured in vaquita blubber are lower than those in most other fish-eating odontocetes and pinnipeds and well below the toxicity threshold proposed by Kannan of 9000 ng/g PCB lw.¹³ For example, a recent review of PCB levels in European cetaceans included animals with levels at least 1000 times the values in these vaquita carcasses.¹⁴ The PCB and DDT concentrations are also lower than those in blubber of other marine mammals sampled further south in the Gulf of California. Adult female California sea lions (*Zalophus californianus*) sampled in 2005–2006 had mean blubber levels of PCBs and DDTs of 1400 and 3400 ng/g, lw, respectively, and fin whales (*Balaenoptera physalus*) biopsied in 2004–2005 had Σ PCBs from 40 to 290 ng/g lw and Σ DDT concentrations ranging from 300 to 2400 ng/g lw.^{15 16} Furthermore, DDT levels in the recently examined vaquitas were approximately half the levels measured in eight vaquita from 1985.¹⁷ The DDT levels in vaquitas have therefore declined considerably over the last 30 years, a trend that has been observed in other marine mammals.¹⁸ The levels of POPs present in blubber of the vaquita are thus below concentrations that would cause concern for toxicological endpoints such as reproductive effects.

Although these data are from a small sample size due to the few remaining animals in the population, they exclude POPs and biotoxins as the source of mortality and support the conclusion that bycatch in gillnets as the continued cause of the population decline. Furthermore, in March this year (2020), a video of a fresh dead vaquita in a gillnet was posted to the IUCN website, confirming bycatch continues.¹⁹ Thus, if vaquita are to remain extant, all efforts must be made to remove gillnets from their habitat.

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Competing interests None declared.

Patient consent for publication Not required.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Data available from Frances M D Gulland: fmdgulland@ucdavis.edu.

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