

University of Nebraska - Lincoln  
DigitalCommons@University of Nebraska - Lincoln

USGS Staff -- Published Research

US Geological Survey

8-10-2018

# Ancient convergent losses of Paraoxonase 1 yield potential risks for modern marine mammals

Wynn K. Meyer

*Department of Computational and Systems Biology, University of Pittsburgh*

Jerrica Jamison

*Dietrich School of Arts and Sciences, University of Pittsburgh*

Rebecca Richter

*Division of Medical Genetics, Department of Medicine, University of Washington*

Stacy E. Woods


*Bloomberg School of Public Health, Johns Hopkins University*

Raghavendran Partha

*Department of Computational and Systems Biology, University of Pittsburgh*

*See next page for additional authors*

Follow this and additional works at: <http://digitalcommons.unl.edu/usgsstaffpub>

 Part of the [Geology Commons](#), [Oceanography and Atmospheric Sciences and Meteorology Commons](#), [Other Earth Sciences Commons](#), and the [Other Environmental Sciences Commons](#)

Meyer, Wynn K.; Jamison, Jerrica; Richter, Rebecca; Woods, Stacy E.; Partha, Raghavendran; Kowalczyk, Amanda; Kronk, Charles; Chikina, Maria; Bonde, Robert K.; Crocker, Daniel E.; Gaspard, Joseph; Lanyon, Janet M.; Marsillach, Judit; Furlong, Clement E.; and Clark, Nathan L., "Ancient convergent losses of Paraoxonase 1 yield potential risks for modern marine mammals" (2018). *USGS Staff - Published Research*. 1050.

<http://digitalcommons.unl.edu/usgsstaffpub/1050>

This Article is brought to you for free and open access by the US Geological Survey at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USGS Staff -- Published Research by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Wynn K. Meyer, Jerrica Jamison, Rebecca Richter, Stacy E. Woods, Raghavendran Partha, Amanda Kowalczyk, Charles Kronk, Maria Chikina, Robert K. Bonde, Daniel E. Crocker, Joseph Gaspard, Janet M. Lanyon, Judit Marsillach, Clement E. Furlong, and Nathan L. Clark

## EVOLUTION

# Ancient convergent losses of *Paraoxonase 1* yield potential risks for modern marine mammals

Wynn K. Meyer<sup>1</sup>, Jerrica Jamison<sup>2</sup>, Rebecca Richter<sup>3</sup>, Stacy E. Woods<sup>4\*</sup>, Raghavendran Partha<sup>1</sup>, Amanda Kowalczyk<sup>1</sup>, Charles Kronk<sup>2</sup>, Maria Chikina<sup>1</sup>, Robert K. Bonde<sup>5</sup>, Daniel E. Crocker<sup>6</sup>, Joseph Gaspard<sup>7</sup>, Janet M. Lanyon<sup>8</sup>, Judit Marsillach<sup>3</sup>, Clement E. Furlong<sup>3,9</sup>, Nathan L. Clark<sup>1,10†</sup>

Mammals diversified by colonizing drastically different environments, with each transition yielding numerous molecular changes, including losses of protein function. Though not initially deleterious, these losses could subsequently carry deleterious pleiotropic consequences. We have used phylogenetic methods to identify convergent functional losses across independent marine mammal lineages. In one extreme case, *Paraoxonase 1* (*PON1*) accrued lesions in all marine lineages, while remaining intact in all terrestrial mammals. These lesions coincide with *PON1* enzymatic activity loss in marine species' blood plasma. This convergent loss is likely explained by parallel shifts in marine ancestors' lipid metabolism and/or bloodstream oxidative environment affecting *PON1*'s role in fatty acid oxidation. *PON1* loss also eliminates marine mammals' main defense against neurotoxicity from specific man-made organophosphorus compounds, implying potential risks in modern environments.

**A**s the ancestors of aquatic marine mammals adopted obligate aquatic lifestyles, they evolved many adaptive changes, such as those that improved locomotion and respiration in and perception of their new environment (1–3). Many of these morphological and physiological changes occurred in parallel in distinct lineages of marine mammals, including cetaceans, pinnipeds, and sirenians. Although convergent trait changes are frequently adaptive, environmental transitions can also result in non-adaptive convergent trait loss due to release from functional constraint. Examples of convergently reduced or lost traits include olfaction in marine mammals (4–6), bitter taste receptors in carnivorous tetrapods (7), and eyes in subterranean species (8–10). Any convergent evolutionary

change in the context of a given environment can carry negative consequences in a different environment as a result of pleiotropy (one genetic locus influencing multiple phenotypes).

To characterize how mammals responded to selective pressures imposed by the marine environment, we identified genes that convergently lost function in marine mammals. We identified candidate pseudogenes with observed early stop codons and/or frameshifts (genetic lesions) in 58 eutherian mammals' genomes in a 100-way vertebrate alignment (11). Using our predicted pseudogene calls, we then tested, for each gene, whether its pattern of functional loss was better explained by a model with one loss rate throughout the mammalian phylogeny or by a model in which the loss rate was dependent upon the ter-

restrial or marine state of a given branch in a likelihood ratio test (LRT) (12). To ensure that our results were not strongly influenced by errors in pseudogene calling, we performed manual checks of lesion calls against reference genomes for our top genes, along with comparisons of pseudogene calls at highly conserved genes for marine and terrestrial species (13). We used simulations to estimate empirical gene-specific *P* values and study-wide (multiple-test-corrected) false discovery rates (FDR) for all genes (13) (Table 1 and table S1). The set of genes with the strongest evidence for a higher loss rate on marine lineages was strongly enriched for functions related to chemosensation, driven by many olfactory and taste receptors (tables S2 to S5). These results are consistent with previous behavioral, anatomical, and genetic studies indicating a reduction of smell and taste in marine mammals (5, 14, 15).

We also observed a notable pattern of convergent loss in the marine environment at *Paraoxonase 1* (*PON1*) (Table 1) (13). *PON1* encodes a bloodstream enzyme that reduces oxidative damage to lipids in low- and high-density lipoprotein (LDL and HDL) particles, potentially preventing atherosclerotic plaque formation (16, 17) (Fig. 1A). *PON1* also hydrolyzes the oxon forms of specific organophosphate compounds, such that it is the main line of defense against some man-made pesticide by-products,

<sup>1</sup>Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, USA. <sup>2</sup>Dietrich School of Arts and Sciences, University of Pittsburgh, Pittsburgh, PA, USA. <sup>3</sup>Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA, USA. <sup>4</sup>Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA. <sup>5</sup>Wetland and Aquatic Research Center, U.S. Geological Survey, Gainesville, FL, USA. <sup>6</sup>Department of Biology, Sonoma State University, Rohnert Park, CA, USA. <sup>7</sup>Pittsburgh Zoo and PPG Aquarium, Pittsburgh, PA, USA. <sup>8</sup>School of Biological Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia. <sup>9</sup>Department of Genome Sciences, University of Washington, Seattle, WA, USA. <sup>10</sup>Pittsburgh Center for Evolutionary Biology and Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

\*Present address: Natural Resources Defense Council, Washington, DC, USA.

†Corresponding author. Email: nclark@pitt.edu

**Table 1. Top 10 manually validated genes with evidence for marine-specific loss.** Loss rates represent the inferred instantaneous rates of transition from functional gene (1) to pseudogene (0) per unit branch length under the relevant model in BayesTraits (12, 13), restricted to a maximum value of 100 (the default).

Gene	Loss rate (independent)	Marine loss rate (dependent)	Terrestrial loss rate (dependent)	LRT statistic	Empirical <i>P</i> value	FDR	Description of gene product
<i>PON1</i>	0.672	49.7	0	22.24	$3.08 \times 10^{-6}$	0.0154	Paraoxonase 1
<i>OR10Z1</i>	1.15	100	0.467	19.99	$7.25 \times 10^{-6}$	0.0201	Olfactory receptor
<i>OR8D4</i>	1.25	100	0.510	19.21	$1.60 \times 10^{-5}$	0.0201	Olfactory receptor
<i>TAS2R1</i>	1.32	100	0.535	19.20	$1.60 \times 10^{-5}$	0.0201	Taste receptor
<i>OR1F2P</i>	2.03	100	1.18	15.86	$5.40 \times 10^{-5}$	0.0831	Olfactory receptor
<i>GSTM1</i>	1.48	100	0.762	15.82	$3.90 \times 10^{-5}$	0.0831	Glutathione S-transferase mu 1
<i>OR6K2</i>	2.02	100	1.22	15.79	$4.50 \times 10^{-5}$	0.0831	Olfactory receptor
<i>OR51D1</i>	1.13	49.3	0.466	15.59	$8.60 \times 10^{-5}$	0.0831	Olfactory receptor
<i>TAAR5</i>	1.17	48.2	0.484	15.16	$9.90 \times 10^{-5}$	0.0936	Trace amine-associated receptor 5
<i>OR4C13</i>	1.77	100	0.915	14.88	$7.00 \times 10^{-5}$	0.0972	Olfactory receptor

including chlorpyrifos oxon and diazoxon (Fig. 1B) (18). The *PON1* coding sequence contains genetic lesions in the cetacean, pinniped, and sirenian lineages but is intact in all 53 terrestrial mammal genomes surveyed (Fig. 1C and table S1).

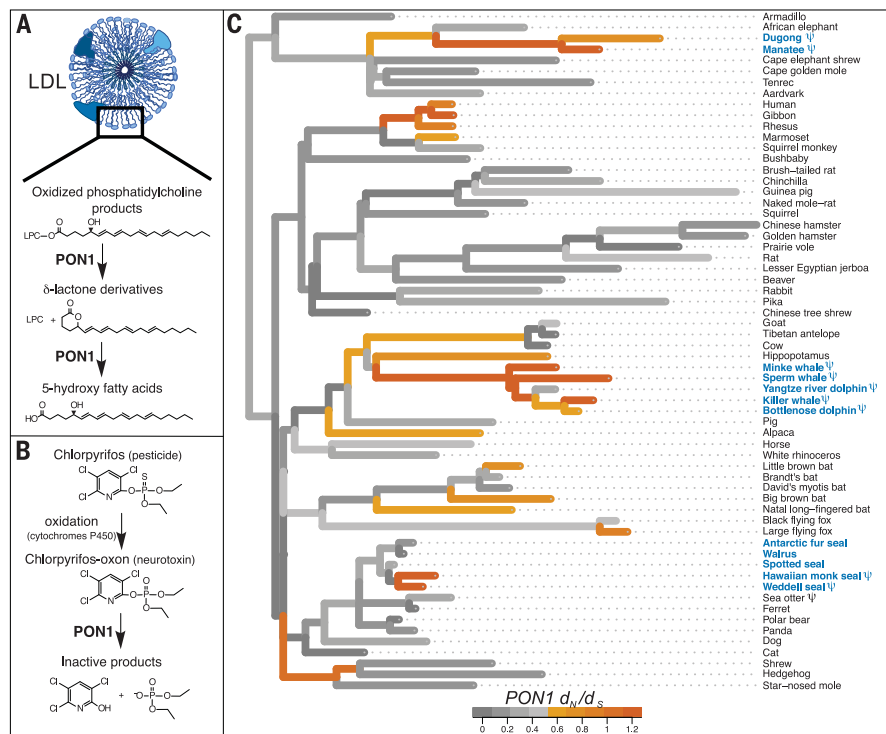
To estimate when *PON1* function was lost in the three marine mammal clades, we obtained *PON1* sequences for 14 additional species, including three cetaceans, the dugong, and two pinnipeds, and we estimated evolutionary

rates across the mammalian phylogeny (13) (Fig. 1C and fig. S1). We observed shared genetic lesions among all sequenced cetaceans and a different shared lesion in sirenians (fig. S2), and the inferred ratio of nonsynonymous to synonymous substitutions ( $d_N/d_S$ ) was not significantly different from one on the ancestral branches of both clades (cetacean ancestor  $d_N/d_S = 1.09$ ,  $P = 0.79$ ; sirenian ancestor  $d_N/d_S = 1.20$ ,  $P = 0.57$ ). This suggests that *PON1* lost functional constraint in the ancestral cetacean lineage soon after its split with the ancestral hippopotamid lineage, approximately 53 million years (Ma) ago [95% confidence interval (CI) lower bound, 34.5 Ma ago] (13, 19). In sirenians, functional loss occurred soon after the split with the ancestral elephantid lineage, approximately 64 Ma ago (lower bound, 41.7 Ma ago) (19).

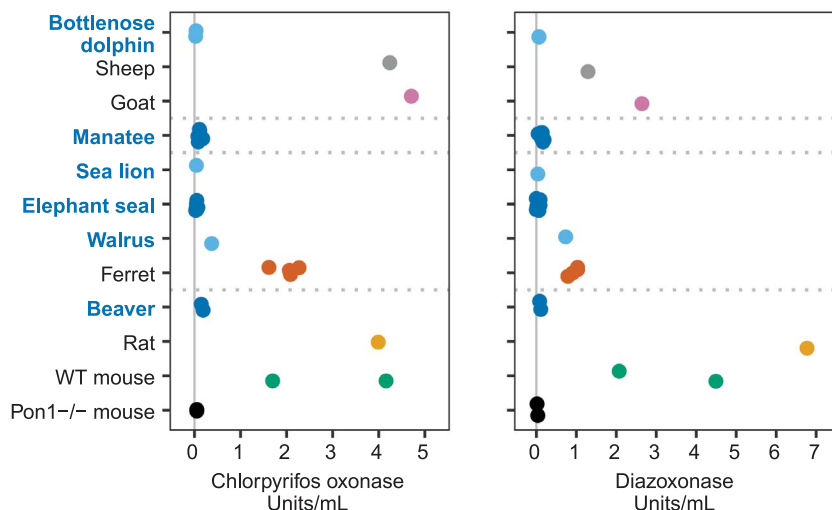
In pinnipeds, we observed clear evidence of *PON1* functional loss only among a subset of species within the family Phocidae, wherein Weddell seal and Hawaiian monk seal *PON1* sequences contained nonshared genetic lesions (fig. S2). Because these branches are short, it is difficult to estimate precisely when functional loss occurred in pinnipeds; however, there was likely at least one loss since the Phocidae-Otarioidea split approximately 21 Ma ago (95% CI, 0 to 21 Ma ago). This incomplete loss may reflect either a difference between the selective environments experienced by pinnipeds and those experienced by other marine mammals or pinnipeds' more recent colonization of the marine environment (pinnipeds, 24 Ma ago; cetaceans, 44.7 to 37.3 Ma ago; sirenians, 47.1 to 43.9 Ma ago) (20).

*PON1*'s functional loss in marine mammals may be related to its role in lipid metabolism via fatty acid beta-oxidation (21) (tables S6 and S7). The diets of both herbivorous and carnivorous aquatic mammals contain a higher proportion of  $\omega$ -3 relative to  $\omega$ -6 polyunsaturated fatty acids (PUFAs) than those of terrestrial mammals (22), and these PUFAs differ in their capacity to sustain oxidative damage (23). Marine and terrestrial mammals also have vastly different antioxidant profiles (24, 25), presumably because of the extreme oxidative stress experienced during diving, with repeated cycles of hypoxia and reperfusion. Rewiring of either lipid metabolism or antioxidant networks in ancient marine mammals may have obviated the function of *PON1*. Supporting the antioxidant hypothesis, the Weddell seal, which carries *PON1* lesions, is one of the longest-diving pinnipeds known, in contrast to the shorter-diving walrus and Antarctic fur seal, which lack lesions but share an aquatic diet (26). However, two semiaquatic mammals, the sea otter and the beaver, which are more moderate divers (26), also have either lesions or substitutions at sites predicted to be necessary for *PON1* function (fig. S2 and table S8).

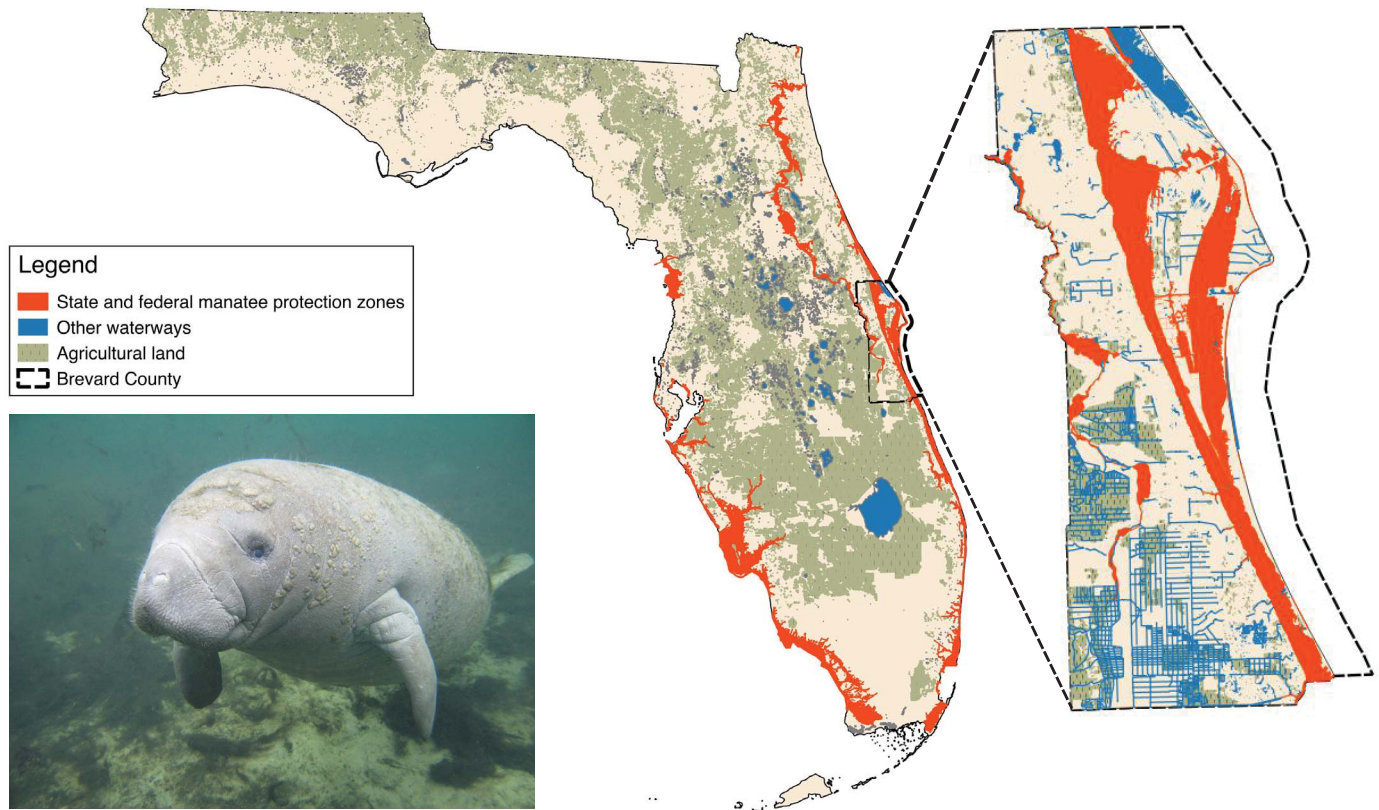
Whatever the cause, loss of *PON1* function may carry negative pleiotropic consequences for the health of marine mammals repeatedly exposed to man-made organophosphate compounds. *PON1* alone is protective against the



**Fig. 1. *PON1* functions and evolutionary history.** Illustration of *PON1*'s proposed roles in (A) preventing oxidative damage to LDL and HDL (16, 17) and (B) detoxifying the oxon by-product or metabolite of a common organophosphorus pesticide, chlorpyrifos (27). LPC, lysophosphatidylcholine. (C) Evolutionary rate of the *PON1* coding sequence across the phylogeny of 62 eutherian mammals. Branch lengths represent  $d_N$ , and colors represent  $d_N/d_S$  (see the color legend).  $d_N/d_S$  values greater than 1.2 were set to 1.2. Blue, marine species;  $\psi$ , genetic lesion(s) present.



**Fig. 2. Blood plasma enzymatic activity against two organophosphate-derived substrates.** Points represent rates of hydrolysis of chlorpyrifos oxon (left) or diazoxon (right) in micromoles per minute per milliliter for plasma from marine and semiaquatic species (in blue) and terrestrial out-groups (in grey) for sheep, goats, and rats are from Furlong *et al.* (35), who performed assays under the same experimental conditions used in this study. Vertical solid lines indicate no activity, and horizontal dashed lines separate species from different evolutionary clades. Control assays of alkaline phosphatase activity show that samples were not degraded (fig. S3). WT, wild-type.



**Fig. 3. The manatee and the adjacency of its habitat to agricultural land use.** (Left) Florida manatee. (Center) Manatee protection zones and agricultural land in Florida. (Right) Manatee protection zones, waterways, and agricultural land in Brevard County.

highly toxic oxon forms of the heavily used pesticides chlorpyrifos and diazinon; these oxons are formed from the parent compounds in the environment and in vivo by cytochromes P450 (27) (Fig. 1B). We tested blood plasma from six marine and semiaquatic species for the capacity to hydrolyze these and other PON1 substrates (Fig. 2 and fig. S3). The plasma from all but one of the assayed marine and semiaquatic species showed activity levels against the PON1 substrates that more closely resembled those of the *Pon1* knockout (*Pon1*<sup>-/-</sup>) mouse than those of terrestrial out-groups. Thus, the genetic deterioration of *PON1* has left these species without a mechanism to break down specific neurotoxic compounds.

Given the sensitivity of *Pon1*<sup>-/-</sup> mice to organophosphate exposure (28), the inability of most marine mammal plasma to detoxify organophosphates suggests the potential for neurotoxicity if sufficient levels of these compounds accumulate in these animals' habitats or food sources. In Florida, agricultural use of organophosphate pesticides is common, and runoff can drain into manatee habitats. In Brevard County, where an estimated 70% of Atlantic coast manatees migrate or seasonally reside (29, 30), agricultural lands frequently abut manatee protection zones and waterways (Fig. 3). Limited sampling upstream of Manatee Bay has measured levels of chlorpyrifos as

high as 0.023 µg/liter (31), and levels could be much higher directly after pesticide applications (32). Dugongs may be at risk of exposure to organophosphorus pesticides that are used in the sugarcane industry along the Queensland coast of Australia and have been detected at 5 to 270 pg/liter in coastal river systems (33). Carnivorous marine mammals may also ingest these compounds through their diets of invertebrates and fish, which have shown evidence of bioaccumulation of organophosphates in Arctic populations (34). In order to improve our understanding of the extent of exposure and attendant risk marine mammals face, we recommend increased monitoring of marine mammal habitats, as well as the testing of tissues from deceased animals for biomarkers of organophosphate exposure.

The presence of these potential risks to many marine mammals due to their loss of PON1 function provides a clear example of the trade-offs possible in evolution: although PON1 functional loss was not deleterious and may even have been beneficial in ancestral marine environments, it may carry detrimental fitness consequences in modern environments.

#### REFERENCES AND NOTES

1. F. E. Fish, L. E. Howle, M. M. Murray, *Integr. Comp. Biol.* **48**, 788–800 (2008).
2. D. Wartzok, D. R. Ketten, in *Biology of Marine Mammals*, J. Reynolds, S. Rommel, Eds. (Smithsonian Institution Press, 1999), pp. 117–175.

3. V. Sharma *et al.*, *Nat. Commun.* **9**, 1215 (2018).
4. M. R. McGowen, C. Clark, J. Gatesy, *Syst. Biol.* **57**, 574–590 (2008).
5. M. L. Bills, thesis, University of Florida, Gainesville, FL (2011).
6. M. Chikina, J. D. Robinson, N. L. Clark, *Mol. Biol. Evol.* **33**, 2182–2192 (2016).
7. D. Li, J. Zhang, *Mol. Biol. Evol.* **31**, 303–309 (2014).
8. M. Protas, M. Conrad, J. B. Gross, C. Tabin, R. Borowsky, *Curr. Biol.* **17**, 452–454 (2007).
9. W. R. Jeffery, *Annu. Rev. Genet.* **43**, 25–47 (2009).
10. R. Partha *et al.*, *eLife* **6**, e25884 (2017).
11. UCSC Genome Browser, <http://genome.ucsc.edu/>.
12. M. Pagel, A. Meade, *BayesTraits* (2013).
13. Materials and methods are available as supplementary materials.
14. L. Marino, *Anat. Rec.* **290**, 694–700 (2007).
15. M. R. McGowen, J. Gatesy, D. E. Wildman, *Trends Ecol. Evol.* **29**, 336–346 (2014).
16. M. Rosenblatt, M. Aviram, *Biofactors* **35**, 98–104 (2009).
17. M. I. Mackness, S. Arrol, P. N. Durrington, *FEBS Lett.* **286**, 152–154 (1991).
18. W. F. Li *et al.*, *Pharmacogenetics* **10**, 767–779 (2000).
19. R. W. Meredith *et al.*, *Science* **334**, 521–524 (2011).
20. J. Thewissen, S. Nummela, in *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates*, J. Thewissen, S. Nummela, Eds. (University of California Press, 2008), pp. 1–28.
21. C. E. Furlong, J. Marsillach, G. P. Jarvick, L. G. Costa, *Chem. Biol. Interact.* **259** (Pt B), 51–62 (2016).
22. A.-M. Koussoroplis *et al.*, *Lipids* **43**, 461–466 (2008).
23. K. Miyashita, E. Nara, T. Ota, *Biosci. Biotechnol. Biochem.* **57**, 1638–1640 (1993).
24. J. P. Vázquez-Medina, T. Zenteno-Savín, R. Elsner, R. M. Ortiz, *J. Comp. Physiol. B* **182**, 741–750 (2012).
25. N. Cantú-Medellín, B. Byrd, A. Hohn, J. P. Vázquez-Medina, T. Zenteno-Savín, *Comp. Biochem. Physiol. A* **158**, 438–443 (2011).
26. S. Mirceta *et al.*, *Science* **340**, 1234192–1234192 (2013).
27. C. E. Furlong, *J. Biochem. Mol. Toxicol.* **21**, 197–205 (2007).

28. D. M. Shih *et al.*, *Nature* **394**, 284–287 (1998).
29. C. J. Deutsch *et al.*, *Wildl. Monogr.* **151**, 1–77 (2003).
30. J. Martin *et al.*, *Biol. Conserv.* **186**, 44–51 (2015).
31. J. F. Carriger, G. M. Rand, *Ecotoxicology* **17**, 660–679 (2008).
32. J. R. Aguirre-Rubi *et al.*, *Environ. Sci. Pollut. Res. Int.* **25**, 13396–13415 (2018).
33. M. Shaw *et al.*, *Mar. Pollut. Bull.* **60**, 113–122 (2010).
34. A. D. Morris *et al.*, *Environ. Toxicol. Chem.* **35**, 1695–1707 (2016).
35. C. E. Furlong *et al.*, *Neurotoxicology* **21**, 91–100 (2000).

#### ACKNOWLEDGMENTS

We thank B. Small for assistance with molecular work; K. Goulet and V. Fravel for supplying marine mammal samples; S. Lakdawala and the Lakdawala lab for supplying ferret samples; and A. Lusic, D. Shih, and A. Tward for supplying *Pon1* knockout mice; as well as all members of the Clark and Chikina labs and K. Dolan for feedback. Any use of trade, firm,

or product names is for descriptive purposes only and does not imply endorsement by the U.S. government. **Funding:** This study was funded by NIH grants R01HG009299 and U54 HG008540 to N.L.C. and M.C. A.K. was supported by NIH T32 training grant T32 EB009403 as part of the HHMI-NIBIB Interfaces Initiative. C.E.F., J.M., and R.R. were supported by funds from the Biotechnology Research Gift Fund, University of Washington, Division of Medical Genetics. J.M. was supported by grant 16SDG30300009 from the American Heart Association. Dugong samples were collected with funds from the Winifred Violet Scott Foundation and the Sea World Research and Rescue Foundation. The collection of manatee samples was funded by the U.S. Geological Survey. **Author contributions:** N.L.C., M.C., C.E.F., and W.K.M. designed the study. R.K.B., D.E.C., J.G., J.M.L., and C.E.F. provided samples and reagents. J.J., J.M., and R.R. performed laboratory experiments. W.K.M., J.J., R.P., A.K., C.K., and N.L.C. performed analyses. W.K.M., S.E.W., R.P., A.K., and N.L.C.

generated figures. W.K.M., C.E.F., and N.L.C. wrote the paper. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** The data reported in this paper are tabulated in the supplementary materials. Resequencing data for the *PON1* coding sequence in dugongs is available in GenBank (accession number MF197755). Scripts used in analyses are available at <https://github.com/nclark-lab/MarineFXLoss>.

#### SUPPLEMENTARY MATERIALS

[www.sciencemag.org/content/361/6402/591/suppl/DC1](http://www.sciencemag.org/content/361/6402/591/suppl/DC1)  
Materials and Methods  
Figs. S1 to S6  
Tables S1 to S13  
References (36–105)

25 August 2017; accepted 29 June 2018  
10.1126/science.aap7714