Marine Mammal Science

Cetacean genome size diversity

Journal:	*Marine Mammal Science*
Manuscript ID	MMSCI-4563.R3
Manuscript Type:	Note
Date Submitted by the Author:	n/a
Complete List of Authors:	Oziolor, Elias; Baylor University Department of Environmental Science, Environmental Science Gregory, T. Ryan; University of Guelph, Biology Bickham, John; Texas A&M University St. Leger, Judy; SeaWorld Parks and Entertainment, Research and Science Matson, Cole; Baylor University Department of Environmental Science, Environmental Science
Keywords:	Cetacea, C-value, evolution, genome size, whale, dolphin, diversity

Cetacean genome size diversity

Elias M. Oziolor[1,a,*], T. Ryan Gregory[2,a,] John W. Bickham[3], Judy St. Leger[4], Cole W. Matson[1]

[1] Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, and the Institute for Biomedical Studies, Baylor University, Waco, TX 76798, USA

[2] Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

[3] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77845, USA

[4] Animal Care – Research and Pathology, SeaWorld Parks and Entertainment, San Diego, CA 92109, USA

[a] These authors contributed equally to this manuscript.

*Corresponding author: Elias M. Oziolor

Email: emoziolor@ucdavis.edu

Keywords: Body mass, brain size, Cetacea, C-value, encephalization quotient, evolution, genome size, whale

Sirenians and cetaceans are unique among mammals for exhibiting a purely aquatic lifestyle, resulting in dramatic morphological, physiological, and behavioral changes (Gatesy *et al.* 2013). Freed from the constraints of gravity by the buoyancy of water, cetaceans have evolved a range of body sizes, from the relatively diminutive Maui's dolphin (*Cephalorhynchus hectori maui*) at 1 m in length and 50 kg in mass to the largest animal ever known, the blue whale (*Balaenoptera musculus*) at ~30 m and almost 200,000 kg (Jefferson 1993, Montgomery *et al.* 2013). Modern dolphins, porpoises, and other whales (classically placed in the order "Cetacea") are nested phylogenetically within the order Artiodactyla of even-toed ungulates (Gatesy *et al*. 2013). A revised order Cetartiodactyla reflects these new phylogenetic insights and emphasizes the close evolutionary affinity of whales to their terrestrial relatives (Gatesy *et al*. 2013).

Cetacea contains 88 extant species, divided into the 70 toothed whales (Odontoceti) and the 18 baleen whales (Mysticeti). Due to the protected status of many whales combined with inherent challenges in sampling, several aspects of their biology remain poorly described. Large-scale genomic analyses have become available for cetaceans only quite recently and include members of both toothed and baleen whales (McGowen *et al.* 2012, Keane *et al.* 2016). However, estimates of total genome size are still quite rare. At present, the Animal Genome Size Database contains data for only five species of cetaceans, most of which come from a single study published more than 10 yr ago (Du and Wang 2006).

Genome size has been shown to correlate with various cellular, morphological, developmental, and physiological parameters in mammals and other animals (Gregory 2005, Smith and Gregory 2009), but it has not been possible to examine potential relationships such as these in cetaceans. In other taxa, it has been observed that genome size, through the intermediate

of cell size, is related to resting metabolic rate, diving ability, migration distance, cold tolerance, longevity, relative brain size, body size, and other such organism-level parameters (Gregory 2001, 2005; Andrews and Gregory 2009; Smith and Gregory 2009). We provide flow cytometric estimates for 18 species of cetaceans (primarily odontocetes) as well as the hippopotamus (*Hippopotamus amphibious*), the closest living relative of cetaceans, and another predominantly aquatic mammal, the bearded seal (*Erignathus barbatus*), to provide information for future comparisons that explore the evolutionary relationships of genome size in cetaceans.

Spleens were collected during postmortem evaluations from stranded or captive animals. Samples from stranded animals were collected under NOAA's responsibility to the MMPA 1972 under Section 109(h), and a Stranding Agreement as part of the Marine Mammal Health and Stranding Response Act. Samples from animals under managed care, or captivity, were collected during necropsy examinations from animals held under USDA/APHIS Registration No: 58-C-0077. All samples (Table S1, S2) were stored at -80˚C until analysis.

Spleen samples were used to establish genome size with a flow cytometry protocol originally developed by Vindelov and Christensen (1994) and modified by Oziolor *et al*. (2014). A minimum of 10,000 cells were used to establish a mean genome size of each sample. Information about the available cetaceans with previously established genome sizes by flow cytometry (7 individuals; 5 species) was added to this analysis from the Animal Genome Size Database. Chicken blood was used as a standard (accepted as 1.25 picograms (pg)) and was co-prepared with samples.

Haploid nuclear DNA contents in mammals range approximately 5-fold from 1.63 pg in Carriker's round-eared bat (*Lophostoma carrikeri*) to 8.40 pg in the polyploid red viscacha rat (*Tympanoctomys barrerae*), averaging 3.21 pg ± 0.85 SD (Gregory 2017). Some groups, most

notably bats, have particularly small and constrained genomes, whereas others, such as rodents, have genomes that cover a much wider range (Gregory 2017) (Fig. 1). The expanded data set presented here suggests that cetaceans have genome sizes that are typical for mammals. As shown in Figure 1, the cetaceans studied to date have genome sizes that fall within the range observed among terrestrial artiodactyls, though they exhibit a larger average size than other members of the Cetartiodactyla (3.68 pg ± 0.40 SD for cetaceans *vs*. 3.39 pg ± 0.55 SD in terrestrial artiodactyls). The hippopotamus, which is the closest living relative of cetaceans, was found in the present study to have a genome size of 3.39 pg – *i.e*., average for an artiodactyl.

The smallest genomes so far reported for Cetartiodactyla (~2.7 pg) are found in species such as giraffes (*Giraffa camelopardalis*), camels (*Camelus bactrianus*), and llamas (*Lama glama*) (Gregory 2017). Interestingly, camels and llamas have erythrocytes that are not only enucleate like those of other adult mammals but are atypical in shape. Whereas most mammals (including cetaceans) have erythrocytes that are shaped as biconcave discs, those of some terrestrial artiodactyls may be tiny, spherical, oval, or crescent-shaped (Gulliver 1875). Previous relationships have shown the strong relationship between enucleate cell size and genome size in mammals (Gregory 2000, 2001). Thus, it is possible that achieving these unique erythrocyte morphologies requires smaller than average nuclei in progenitor cells, and thus smaller than average genomes. Unfortunately, data on cetacean genome sizes and cell sizes are insufficient for a detailed assessment of any correlation between these two parameters, but across mammals (Gregory 2000) and vertebrates more broadly (Gregory 2001), there is a strong positive relationship between genome size and overall cell size. However, it can be said that cetacean erythrocyte sizes are in the range that would be expected given their genome sizes (Hawkey 1975, Gregory 2000).

Aside from cetaceans, only a few other aquatic mammals have been studied for genome size thus far (Gregory 2017). However, even with this limited information, it appears that there is significant variability in genome size across species that share an aquatic lifestyle. For example, the bearded seal genome size was estimated in the present study as 2.96 pg, which is similar to published estimates for the spotted seal (*Phoca largha*; 2.94 pg) and California sea lion (*Zalophus californianus*; 3.15 pg) (Du and Wang 2006). All are smaller than the mammalian average. By contrast, the Florida manatee (*Trichechus manatus*) has a much larger estimated genome size of 4.67 pg (Redi *et al.* 2007) .

Cetaceans are remarkable for many reasons, though they remain a challenge to study. Very little information has been available on the genome sizes of cetaceans, in part because of the difficulty in obtaining material for analysis. The addition of 18 new estimates for cetaceans, as well as their closest living relative, the hippopotamus, represents an important step toward filling this gap in the animal genome size dataset. It is important to note that it is difficult to make any informed phylogenetically independent contrasts in cetaceans with the data we have available because of the necessity to obtain large enough sample sizes of each species represented. This is a direction that should be prioritized for future investigations.

ACKNOWLEDGEMENTS

We thank the Baylor University Molecular Biosciences Center for providing access to the flow cytometer used in this study. We thank Lexi Mena and Nancy Stedman of SeaWorld Parks and Entertainment, Megan Stolen and Wendy Durden of the Hubbs-SeaWorld Research Institute and Frances Gulland from The Marine Mammal Center for their contributions in sample collection and management of samples. EMO performed the genome size estimates. EMO and TRG analyzed the data and wrote the manuscript. JWB advised on analysis and edited

manuscript. JSL organized and provided samples as well as edited manuscript. CWM organized study reviewed the manuscript and troubleshot data acquisition.

DATA ACCESSIBILITY

All our data are accessible in Supplementary Information.

FUNDING

Funding for this project was provided by start-up funds to CWM from Baylor University.

FIGURES

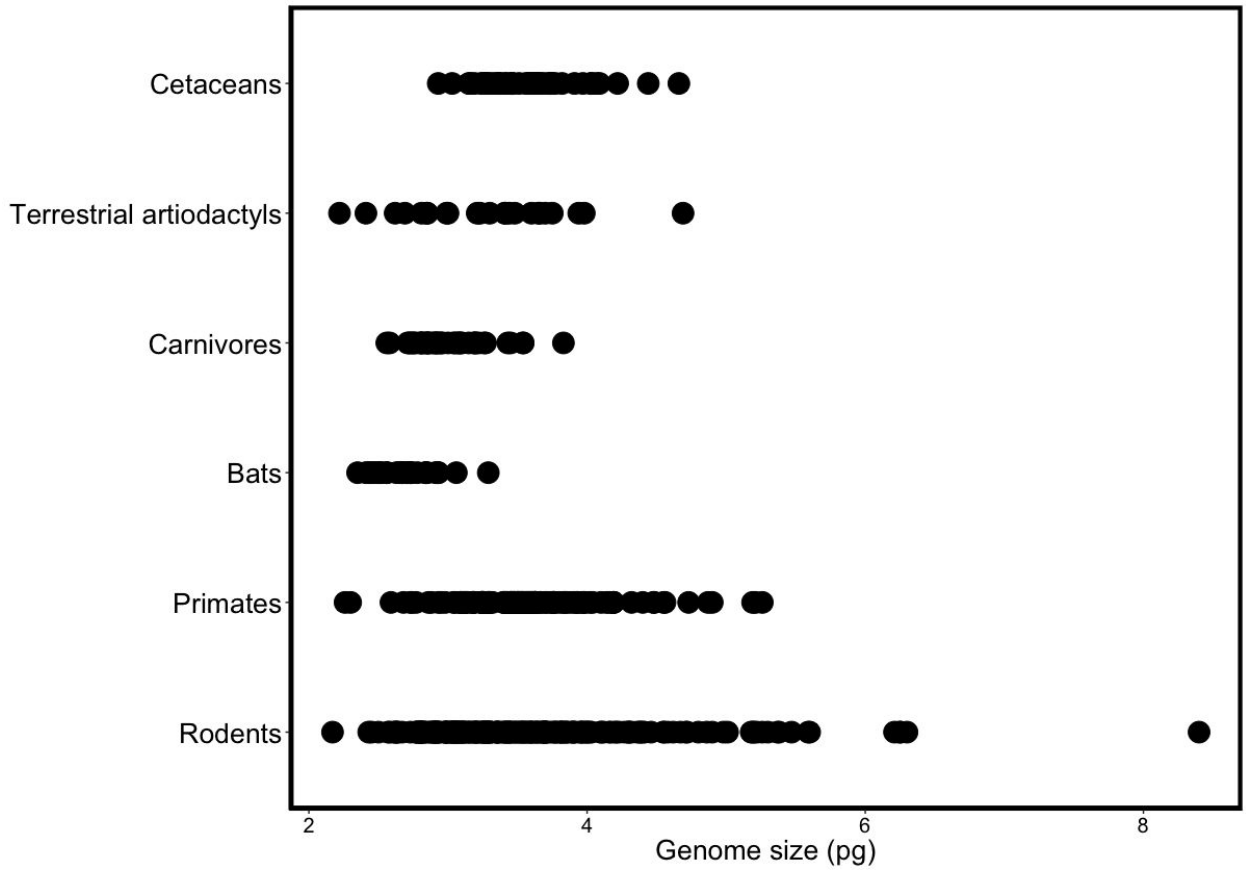

Figure 1. Diversity in genome size estimates for some major groups of mammals, based on data from the Animal Genome Size Database (Gregory 2017) and the present study (Table S1, S2).

Each dot represents a single estimate. Numbers of species/individuals used are as follows: cetaceans (n=24/n=47); terrestrial artiodactyls (n=20/n=24); carnivores (n=28/n=43); bats (n=25/n=28); primates (n=66/n=110); rodents (n=90/n=218)

REFERENCES

Andrews, C. B. and T. R. Gregory. 2009. Genome size is inversely correlated with relative brain size in parrots and cockatoos. Genome 52:261-267.

Du, B. and D. Wang. 2006. C-values of seven marine mammal species determined by flow cytometry. Zoological Science 23:1017-1020.

Gatesy, J., J. H. Geisler, J. Chang, *et al.* 2013. A phylogenetic blueprint for a modern whale. Molecular Phylogenetics and Evolution 66:479-506.

Gregory, T. R. 2000. Nucleotypic effects without nuclei: Genome size and erythrocyte size in mammals. Genome 43:895-901.

Gregory, T. R. 2001. The bigger the C-value, the larger the cell: Genome size and red blood cell size in vertebrates. Blood Cells Molecules and Diseases 27:830-843.

Gregory, T. R. 2005. Genome size evolution in animals. Elsevier Science Bv, Sara Burgerhartstraat 25, Po Box 211, 1000 Ae Amsterdam, Netherlands.

Gregory, T. R. 2017. Animal genome size database. www.genomesize.com.

Gulliver, G. 1875. Observations on the sizes and shapes of the red corpuscles of the blood of vertebrates, with drawings of them to a uniform scale, and extended and revised tables of measurements. Proceedings of the Zoological Society of London, Part I: 474-495.

Hawkey, C. M. 1975. Comparative mammalian haematology. The Whitefriars Press Ltd., Great Britain.

Jefferson, T. A., Leatherwood S., Webber, M.A. 1993. FAO Species identification guide. Marine mammals of the world., Food and Agriculture Organization of the United Nations or of the United Nations Environment Programme. Rome.

Keane, M., J. Semeiks, A. E. Webb, *et al.* 2016. Insights into the evolution of longevity from the bowhead whale genome. Cell Reports 10:112-122.

McGowen, M. R., L. I. Grossman and D. E. Wildman. 2012. Dolphin genome provides evidence for adaptive evolution of nervous system genes and a molecular rate slowdown. Proceedings of the Royal Society B-Biological Sciences 279:3643-3651.

Montgomery, S. H., J. H. Geisler, M. R. Mcgowen, C. Fox, L. Marino and J. Gatesy. 2013. The evolutionary history of cetacean brain and body size. Evolution 67:3339-3353.

Oziolor, E. M., E. Bigorgne, L. Aguilar, S. Usenko and C. W. Matson. 2014. Evolved resistance to PCB- and PAH-induced cardiac teratogenesis, and reduced CYP1A activity in Gulf killifish (*Fundulus grandis*) populations from the Houston Ship Channel, Texas. Aquatic Toxicology 150:210-219.

Redi, C. A., S. Garagna, M. Zuccotti and E. Capanna. 2007. Genome size: A novel genomic signature in support of Afrotheria. Journal of Molecular Evolution 64:484-487.

Smith, J. D. L. and T. R. Gregory. 2009. The genome sizes of megabats (Chiroptera: *Pteropodidae*) are remarkably constrained. Biology Letters 5:347-351.

Vindelov, L. L. and I. J. Christensen. 1994. Detergent and proteolytic enzyme-based techniques for nuclear isolation and DNA content analysis. Methods in Cell Biology, Vol 41 41:219-229.

Cetacean genome size diversity - *Electronic supplemental material*

Table 1. Genome size estimates for cetaceans and other species of interest from the present study and previously included in the Animal Genome Size Database.

Order	Unranked	Family	Species	Common Name	GS	STD	n	Source
Cetartiodactyla	Mysticeti	Balaenopteridae	*Megaptera novaeangliae*	Humpback whale	3.71	--	1	Present study
Cetartiodactyla	Mysticeti	Balaenidae	*Balaena mysticetus*	Bowhead whale	2.93			Keane et al. (2015)
Cetartiodactyla	Odontoceti	Delphinidae	*Cephalorhynchus commersonii*	Commerson's dolphin	3.20	0.057	2	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Delphinus delphis*	Common dolphin	3.56	0.170	5	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Globicephala macrorhynchus*	Short-finned pilot whale	3.56	0.269	2	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Grampus griseus*	Risso's dolphin	3.60	0.302	4	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Lagenorhynchus obliquidens*	Pacific white-sided dolphin	3.70	0.170	2	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Lissodelphis borealis*	Northern right whale dolphin	3.74	--	1	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Orcinus orca*	Killer whale	4.13	0.081	3	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Pseudorca crassidens*	False killer whale	3.56	--	1	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Stenella longirostris*	Spinner dolphin	3.45	0.275	2	Present study
Cetartiodactyla	Odontoceti	Delphinidae	*Tursiops truncatus*	Common bottlenose dolphin	3.51	0.240	6	Present study
			" "		3.03			Kato et al. (1980)
			" "		3.27			Du and Wang (2006)
Cetartiodactyla	Odontoceti	Delphinidae	*Sousa chinensis chinensis*	Indo-Pacific humpback dolphin	3.46			Du and Wang (2006)
Cetartiodactyla	Odontoceti	Kogiidae	*Kogia breviceps*	Pygmy sperm whale	3.48	0.261	4	Present study
Cetartiodactyla	Odontoceti	Kogiidae	*Kogia sima*	Dwarf sperm whale	3.51	--	1	Present study
Cetartiodactyla	Odontoceti	Monodontidae	*Delphinapterus leucas*	Beluga, white whale	3.55	0.120	2	Present study
			" "		3.29			Du and Wang (2006)
Cetartiodactyla	Odontoceti	Ziphiidae	*Mesoplodon densirostris*	Blainville's beaked whale	4.44	--	1	Present study
Cetartiodactyla	Odontoceti	Ziphiidae	*Mesoplodon europaeus*	Gervais' beaked whale	4.22	0.042	2	Present study
Cetartiodactyla	Odontoceti	Ziphiidae	*Ziphius cavirostris*	Cuvier's beaked whale	4.66	--	1	Present study
Cetartiodactyla	Odontoceti	Phocoenidae	*Neophocaena a. asiaeorientalis*	Yangtze finless porpoise	3.46			Du and Wang (2006)
Cetartiodactyla	Odontoceti	Physeteridae	*Physeter macrocephalus*	Sperm whale	3.44	--	1	Present study
Cetartiodactyla	Odontoceti	Lipotidae	*Lipotes vexillifer*	Baiji / Yangtze river dolphin	3.91			Du and Wang (2006)
Cetartiodactyla		Hippopotamidae	*Hippopotamus amphibius*	Hippopotamus	3.39	--	1	Present study
Carnivora		Phocidae	*Erignathus barbatus*	Bearded seal	2.96	--	1	Present study
Carnivora		Phocidae	*Phoca largha*	Spotted seal / Largha seal	2.94			Du and Wang (2006)
Carnivora		Otariidae	*Zalophus californianus*	California sea lion	3.15			Du and Wang (2006)

Cetacean genome size diversity - *Electronic supplemental material*

Table S1. Genome size estimate and source of sample for each individual specimen

Species ID	Species	Common Name	Sex	Age	Specimen	1c Genome Size (pg)	Location	Captive/Stranded
Hubbs-1209-Mn	*Megaptera novaeangliae*	Humpback whale	F	juvenile	Spleen	3.71	Atlantic	Stranded
SW031065	*Cephalorhynchus commersonii*	Commerson's dolphin	F	adult	Spleen	3.24		Managed care
SW070493	*Cephalorhynchus commerconii*	Commerson's dolphin	M	calf	Spleen	3.16		Managed care
SW100075	*Delphinus delphis*	Common dolphin	M	juvenile	Spleen	3.35	Pacific	Stranded
SW110953	*Delphinus delphis*	Common dolphin	M	calf	Spleen	3.42	Pacific	Stranded
SW070794	*Delphinus delphis*	Common dolphin	M	adult	Spleen	3.73		Managed care
SW110122	*Delphinus delphis*	Common dolphin	M	juvenile	Spleen	3.69	Pacific	Stranded
SW140476	*Delphinus delphis*	Common dolphin	M	yearling	Spleen	3.61	Pacific	Stranded
SW120472	*Globicephala macrorhynchus*	Short-finned pilot whale	M	adult	Spleen	3.37		Managed care
Hubbs-1036-Gm	*Globicephala macrorhynchus*	Short-finned pilot whale	F	adult	Spleen	3.75	Atlantic	Stranded
SW131056	*Grampus griseus*	Risso's dolphin	F	adult	Spleen	3.59	Pacific	Stranded
SWC150590	*Grampus griseus*	Risso's dolphin	M	calf	Spleen	4.03	Pacific	Stranded
Hubbs-1222-Gg	*Grampus griseus*	Risso's dolphin	F	adult	Spleen	3.37	Atlantic	Stranded
Hubbs-1266-Gg	*Grampus griseus*	Risso's dolphin	F	adult	Spleen	3.41	Atlantic	Stranded
SW060970	*Lagenorhynchus obliquidens*	Pacific white-sided dolphin	F	calf	Spleen	3.82		Managed care
SW070571	*Lagenorhynchus obliquidens*	Pacific white-sided dolphin	M	adult	Spleen	3.58		Managed care
SW060524	*Lissodelphis borealis*	Northern right whale dolphin	M	calf	Spleen	3.74	Pacific	Stranded
SW070782	*Orcinus orca*	Killer whale	M	adult	Spleen	4.22		Managed care
SW080429	*Orcinus orca*	Killer whale	F	juvenile	Spleen	4.07		Managed care
SW100830	*Orcinus orca*	Killer whale	F	adult	Spleen	4.09		Managed care
Hubbs-1304-Pc	*Pseudorca crassidens*	False killer whale	F	adult	Spleen	3.56	Atlantic	Stranded
Hubbs-1315-Tt	*Tursiops truncatus*	Common bottlenose dolphin	F	adult	Spleen	3.61	Atlantic	Stranded
Hubbs-1319-Tt	*Tursiops truncatus*	Common bottlenose dolphin	M	adult	Spleen	3.28	Atlantic	Stranded
Hubbs-1332-Tt	*Tursiops truncatus*	Common bottlenose dolphin	F	adult	Spleen	3.15	Atlantic	Stranded
Hubbs-13108-Tt	*Tursiops truncatus*	Common bottlenose dolphin	M	adult	Spleen	3.77	Atlantic	Stranded
Hubbs-13109-Tt	*Tursiops truncatus*	Common bottlenose dolphin	M	adult	Spleen	3.66	Atlantic	Stranded
SW101050	*Tursiops truncatus*	Common bottlenose dolphin	F	sub-adult	Spleen	3.59	Pacific	Stranded
SWFa1601B	*Feresa attenuata*	Pygmy killer whale	F	juvenile	Spleen	3.36	Atlantic	Stranded
SWSL1601B	*Stenella longirostris*	Spinner dolphin	F	adult	Spleen	3.64	Atlantic	Stranded
SWSL1602B	*Stenella longirostris*	Spinner dolphin	M	adult	Spleen	3.25	Atlantic	Stranded
Hubbs-1215-Kb	*Kogia breviceps*	Pygmy sperm whale	M	adult	Spleen	3.66	Atlantic	Stranded
Hubbs-1126-Kb	*Kogia breviceps*	Pygmy sperm whale	F	adult	Spleen	3.73	Atlantic	Stranded
Hubbs-1127-Kb	*Kogia breviceps*	Pygmy sperm whale	M	calf	Spleen	3.32	Atlantic	Stranded
Hubbs-1224-Kb	*Kogia breviceps*	Pygmy sperm whale	M	adult	Spleen	3.19	Atlantic	Stranded

Cetacean genome size diversity - *Electronic supplemental material*

Hubbs-1141-Ks	*Kogia sima*	Dwarf sperm whale	F	adult	Spleen	3.51	Atlantic	Stranded
SW120552	*Delphinapterus leucas*	Beluga, white whale	F	calf	Spleen	3.63		Managed care
SW140560	*Delphinapterus leucas*	Beluga, white whale	F	adult	Spleen	3.46		Managed care
Hubbs-1105-Md	*Mesoplodon densirostris*	Blainville's beaked whale	M	adult	Spleen	4.44	Atlantic	Stranded
Hubbs-0822-Me	*Mesoplodon europaeus*	Gervais' beaked whale	F	adult	Spleen	4.03	Atlantic	Stranded
Hubbs-1006-Me	*Mesoplodon europaeus*	Gervais' beaked whale	F	adult	Spleen	3.97	Atlantic	Stranded
Hubbs-1121	*Ziphius cavirostris*	Cuvier's beaked whale	F	juvenile	Spleen	4.66	Atlantic	Stranded
TMMC: C-233	*Physeter macrocephalus*	Sperm Whale	M	calf	Spleen	3.44	Pacific	Stranded
SWF-Eb-0705-B	Mammalia	*Erignathus barbatus*	M	adult	Spleen	2.96		Managed care
BGT54209	*Hippopotamus amphibius*	Hippopotamus	F	Adult	Spleen	3.39		Managed care