MATERIALS AND METHODS

A citrated plasma sample was obtained from a young (1-2 years) female California gray whale kept in captivity in San Diego, Calif. The sample was deep-frozen and shipped via air express to Philadelphia where the assays were made. The plasma sample was slightly lipemic. The prothrombin time, partial thromboplastin time, Factor V, Factor XI, and Factor XII assays were made in the Coagulation Laboratory at the Hospital of the University of Pennsylvania. It was not possible to do a fibrinogen assay on the sample.

Standard laboratory procedures employing commercial reagents were used to conduct the assays, with the exception of Factor XII where dolphin, *Tursiops truncatus*, plasma was used as the substrate. Plasma reagent from Factor XI deficient cattle was used for the Factor XI assay.

RESULTS AND DISCUSSION

The results of the assays on the gray whale plasma and some results on a few odontocete whales, from the literature, are shown in Table 1.

Table 1.—A comparison of some clotting factors between odontocete whales and a baleen whale. The numbers in parentheses indicate the number of samples.

	Tursiops truncatus[1,2,3]	*Orcinus orca*[2]	*Eschrichtius robustus*
Prothrombin time (sec)	17.0(14)	15.6(3)	26.5(1)
Partial thromboplastin time (ptt) (sec)	346 (15)	216 (3)	107 (1)
Factor V(%)	136 (14)	239 (3)	17 sec (1)
Factor XI (%)	92.7(14)	146 (3)	24.6(1)
Factor XII (%)	0 (15)	0 (3)	3.4(1)

[1] Lewis, Bayer, and Szeto (1969).
[2] Robinson, Kropatkin, and Aggeler (1969).
[3] Ridgway (1972).

The divergence of our results on the gray whale plasma for prothrombin time, partial thromboplastin time, Factor V, and Factor XI assays from those of the two species of odontocete whales can be explained perhaps on the elapsed time between sampling and assay. The presence of a low level of Factor XII in the gray whale plasma to the non-existence in odontocete plasma warrants some consideration. The significance of this difference teleologically is not known. One of the problems encountered by deep diving humans is decompression sickness. This sickness is attributed to the formation of microclots (disseminated intravascular coagulation) with resulting consequences. It is known that slow-moving acid blood has a propensity to clot faster. This property has been attributed to activation of Factor XII and subsequent clot formation.

Whales dive deeply and are not believed to suffer from decompression sickness. Perhaps the lack of Factor XII or low levels of it is nature's way of protecting the animals.

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ACKNOWLEDGMENTS

The author wishes to express appreciation to H. A. Wurzel, Director, Coagulation Laboratory, Hospital of the University of Pennsylvania, and his staff as well as to J. C. Sweeney, Naval Undersea Center, San Diego, Calif. for providing the opportunity to make this study on the gray whale.

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MFR Paper 1050. From Marine Fisheries Review, *Vol. 36, No. 4, April 1974. Copies of this paper, in limited numbers, are available from D83, Technical Information Division, Environmental Science Information Center, NOAA, Washington, DC 20235.*

MFR PAPER 1051

Fluorescent Karyotype of the California Gray Whale

DEBORAH A. DUFFIELD

ABSTRACT

The fluorescent karyotype of the California gray whale, Eschrichtius robustus, *is presented and the use of the fluorescent banding technique for distinguishing between various cetacean karyotypes is discussed.*

The California gray whale, *Eschrichtius robustus* (Gibbosus) has a diploid chromosome number of 44 (Benirschke's unpublished data cited in Kulu, 1972; Arnason, 1972). Since reporting of the gray whale karyotype,

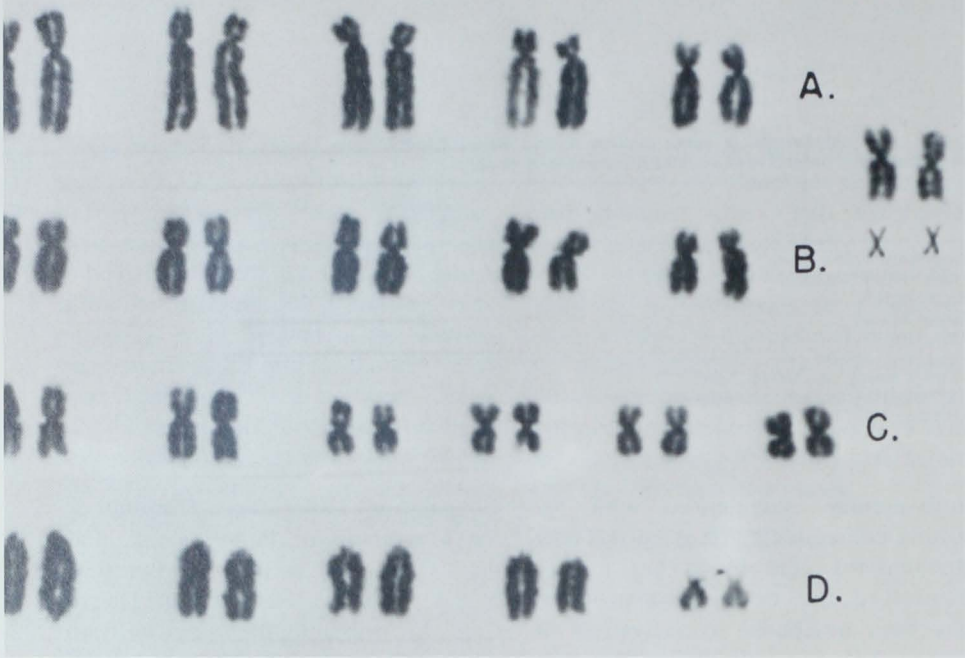

Figure 1.—Karyotype of the California gray whale, Gigi. The autosomes are arranged into four groups based on centromere position and relative size. The provisional X chromosomes are indicated.

prepared by standard homogeneous staining techniques, advances in the differential staining of chromosomes have added another dimension to karyotypic analysis by making it now possible to individually characterize each chromosome of the complement. Consequently, and as part of a larger cytotaxonomic study of marine mammals, evaluation of the gray whale karyotype by quinacrine mustard fluorescent banding was undertaken on Gigi, a captive female gray whale.

MATERIALS AND METHODS

Chromosome preparations were obtained by blood culture (Kulu, Veomett, and Sparkes, 1971). Exposure of the cells to 0.075 M KCl for 8 minutes was the preferred hypotonic treatment and cold, rather than flame-dried, slides were made. The slides were stained with Giemsa for normal karyotyping or with quinacrine mustard (50 micrograms/ml buffer for 30-40 minutes) for fluorescent karyotyping. Photographs of fluorescent metaphases were taken on Kodak[1] Tri-X film with an exposure time of 45-50 seconds. Ten Giemsa and eight fluorescent karyotypes were analyzed.

[1] Use of trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

RESULTS

Gigi's karyotype (Giemsa) is illustrated in Figure 1. The autosomes are provisionally arranged into four groups (designated A, B, C, and D). Group A is composed of five pairs of large submetacentric chromosomes, Group B of five pairs of medium-sized submetacentrics, Group C of six pairs of metacentrics, and Group D of five pairs of acrocentric chromosomes. Within each group the chromosome pairs are arranged by decreasing size. The presumptive X chromosomes are indicated in the karyotype.

The fluorescent karyotype of *Eschrichtius robustus* is presented in Figure 2. The arrangement of the chromosomes follows that of the standard karyotype. The banding pattern of each chromosome pair is distinctive and in addition to allowing positive identification of the homologues makes it possible to characterize each pair of the complement in order to facilitate karyotypic comparison with other species. The fluorescent banding pattern of the presumptive X chromosome is the same as that exhibited by the X chromosome of another of the baleen whales, the sei whale, *Balaenoptera borealis*[2], and a number of the smaller odontocete cetacean species (personal observation).

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DISCUSSION

The karyotype of the California gray whale appears to be very similar in number and gross morphology to that of a number of other cetaceans, both mysticete and odontocete[3] (Kulu, 1972; Arnason, 1972). The fluorescent karyotype of the gray whale was examined in the hope that the resolution of chromosome structure afforded by fluorescent banding would indicate differences between its karyotype and that of other cetaceans not obvious by regular staining methods. In order to illustrate the level of karyotypic comparison made possible by fluorescent banding, the larger submetacentrics which comprise Group A (pairs 1-5) in two mysticete and two odontocete species are shown in Figure 3. While there are certain similarities between the banding patterns of all four species, it is clearly possible to distinguish between the overall banding pattern of the mysticete (gray whale, sei whale) chromosomes and that of the odontocetes (*Tursiops truncatus*, *Lagenorhynchus obliquidens*). Less obvious differences are also present which further distinguish gray whale from sei whale and *Tursiops* from *Lagenorhynchus*. A detailed comparison of the fluorescent karyotypes of these cetaceans is beyond the scope of this report; however, it can be concluded

[2] Arnason (1972) has reported that the X chromosome of *B. borealis* is one of the larger chromosomes of the complement, such as found in other of the balaenopteran whales. However, both standard and fluorescent karyotypes of a male sei whale, tissue from which was made available to this author by the Richmond whaling station in California, indicate that the X chromosome of *B. borealis* is of medium size and similar both in relative size and banding pattern to the provisional X chromosome of the odontocetes (Kulu, 1972).

[3] Of all cetaceans studied to date, only the sperm, pigmy sperm, and killer whales are karyotypically distinct by standard staining techniques.

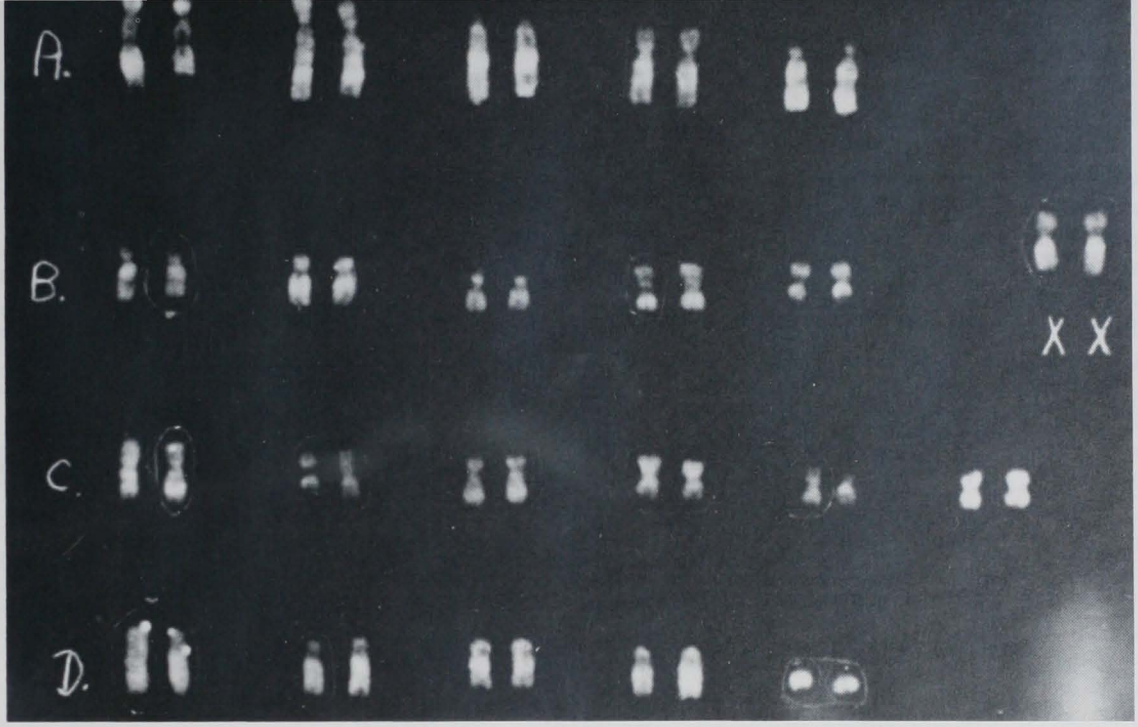

Figure 2.—The fluorescent karyotype of the California gray whale. Note that the members of each pair have identical bands while one pair can be distinguished from any other by its characteristic banding pattern.

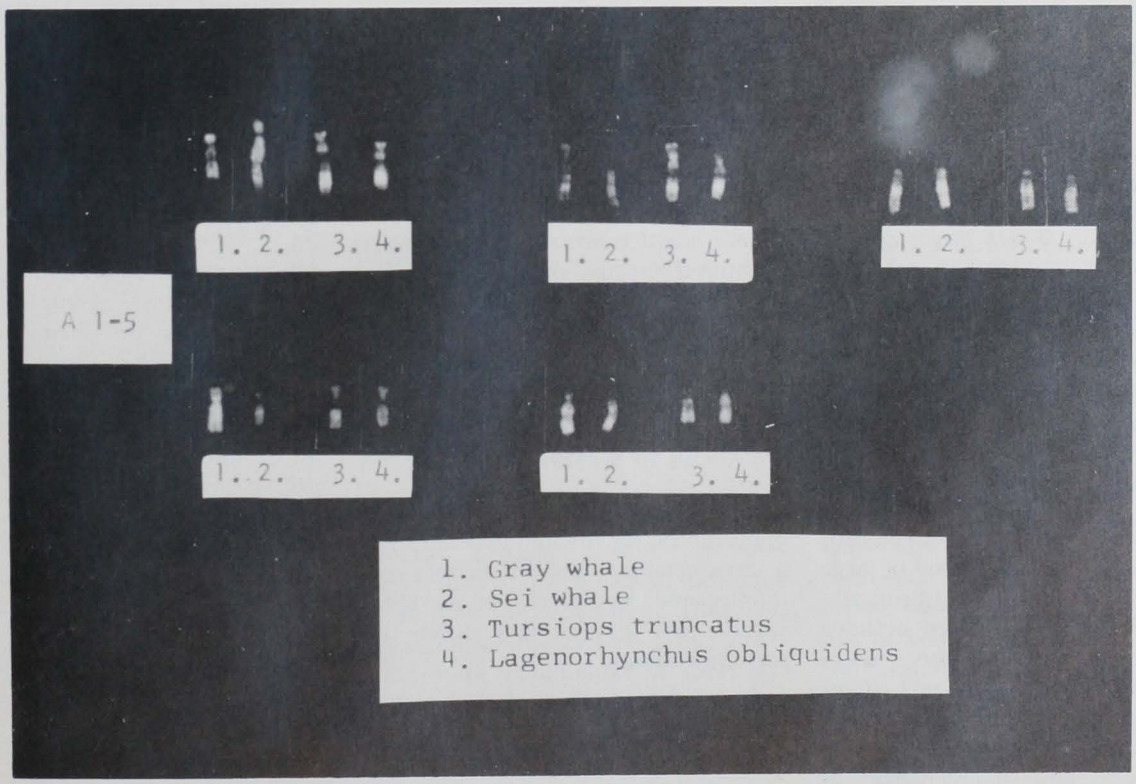

Figure 3.—Comparison of the banding patterns of four species. Only group A chromosomes are shown and only one chromosome from each pair per species. Species 1 and 2 are mysticetes, 3 and 4 are odontocetes.

from these initial observations that the comparison of fluorescent banded karyotypes will significantly enhance the potential contribution which karyotypic analyses can make to the resolution of the phyletic interrelationships of the modern Cetacea.

ACKNOWLEDGMENTS

My thanks to Sea World and Jay Sweeney of the Naval Undersea Center, San Diego, Calif. for making the gray whale samples available to me. The fluorescent technique followed in the preparation of the karyotypes is that of Helga Muller, UCLA Medical Genetics Unit, Los Angeles, California. This work was supported by a Mental Retardation Training Grant from the Departments of Psychiatry and Medicine, UCLA Medical School, Los Angeles, California.

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MFR Paper 1051. From Marine Fisheries Review, *Vol. 36, No. 4, April 1974. Copies of this paper, in limited numbers, are available from D83, Technical Information Division, Environmental Science Information Center, NOAA, Washington, DC 20235.*

MFR PAPER 1052

Some Physiological Parameters of the Blood of the California Gray Whale

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ABSTRACT

Hematocrit, a O_2-Hb dissociation curve, and blood volume have been determined for a California gray whale, Eschrichtius robustus, *and the results are compared to some physiological blood properties of other cetaceans. The* E. robustus *has a blood volume that is similar to values estimated for large whales by other authors. This is the first time isotopic techniques have been used to determine a large cetacean's blood volume.*

Large cetaceans do not appear to follow the trend of most terresterial mammals when the body size and P_{50} *are compared. The* P_{50} *for the* E. robustus *was 36.5 mm Hg and is the highest reported for any cetacean.*

The determination of the physiological properties of the blood of large cetaceans has been confined primarily to animals that are stranded or have been dead for many hours before blood samples can be drawn. Lenfant (1969) has summarized most of the data available on marine mammals. The capture and maintenance of Gigi, a California gray whale, *Eschrichtius robustus*, has given us the opportunity, for the first time, to study a large cetacean under definable conditions and to determine its blood volume and oxygen-hemoglobin dissociation curves.

METHODS

On two separate occasions the whale was given 10 μCi of radioiodinated human serum albumin (Risa)[1]. The labeled compound was administered to the animal in one of the brachial vessels in the right pectoral fin. Two blood specimens were taken following each determination to insure that mixing was complete and the albumin was not being eliminated rapidly from the serum. In the first test (27 December 1971) blood volume determination samples were taken at 14 and 20 minute intervals and in the second test (6 March 1972) were collected at 10 and 18 minute intervals after administration of the labeled compound. The blood samples removed for counting were taken from one of the brachial vessels of the left pectoral fin and put into well-heparinized tubes. Three ml of the heparinized whole blood was added to 3 ml of 1

[1] Abbott Laboratories, Chicago, Illinois. Use of trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.